I. THE BINDING OF Cu(II) and Zn(II) TO POLY-N-ISOPROPYLACRYLAMIDE AND ITS EFFECTS ON THE PHASE TRANSITION and II. THE SYNTHESIS AND CHARACTERIZATION OF NICKELALACTONES CHELATED BY P-N-P PINCER LIGANDS

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I. THE BINDING OF Cu(II) and Zn(II) TO POLY-N-ISOPROPYLMAMIDE AND ITS EFFECTS ON THE PHASE TRANSITION

and

II. THE SYNTHESIS AND CHARACTERIZATION OF NICKELALACTONES CHELATED BY P-N-P PINCER LIGANDS

BY

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Bachelor of Science, Wheaton College (IL), 2013

DISSERTATION

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Chemistry

December, 2019
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November 14, 2019

Approval signatures are on file with the University of New Hampshire Graduate School.
DEDICATION

To The Lighthouse Student Ministries and all the men who lived there alongside me. Ours was a special time.
ACKNOWLEDGEMENTS

I would like to thank my family and friends for helping me throughout this long and rewarding journey. To my parents Steven and Nancy, for showing interest in my work, reveling in my successes, and offering encouragement whenever I stumbled. To my siblings, for making sure I got out of the lab to see some sunshine every now and again. To my friends scattered across the country, for putting up with selfish whinging and never letting me fall into loneliness.

I must also mention my heartfelt appreciation to the other Planalp research group members. Lea and Mahmoud gave me strong examples on how to pursue research. Those who followed after afforded a long awaited chance to learn from the other end of the totem pole. Ryan, Evan, Leo, and Noah, be sure to emulate my merits while shunning my vices.

The many professors of the UNH Chemistry Department demonstrated patience and investment when fostering my growth over the years. Due to my research’s eclectic nature I consulted with, and received guidance from, nearly all of you, for which I am grateful. My thanks to everyone willing to scrap with me about data, interpretation, and the big questions in life. I love a good argument.

To my advisor, Roy Planalp. I couldn’t have asked for a better research experience. In hindsight, I would do it all again, just maybe with a few less experimental mishaps on my part.

Lastly, my thanks to our beloved chemistry department staff members. I couldn’t keep my shoes tied without Cindi Rower watching out for me.
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ABSTRACT

I. THE BINDING OF Cu(II) and Zn(II) TO POLY-N-ISOPROPYLACRYLAMIDE AND ITS EFFECTS ON THE PHASE TRANSITION

and

II. THE SYNTHESIS AND CHARACTERIZATION OF NICKELALACTONES CHELATED BY P-N-P PINCER LIGANDS

By

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The responsive material poly-N-isopropylacrylamide phase-transitions from hydrophilic to hydrophobic conformations above 32 °C in aqueous media. Researchers have successfully tied that responsive behavior to other dissolved species such as pH, metal concentrations, and even glucose concentration. From our interests in developing sensor technologies we ascertained that direct interactions between metal ion and polymer had received little to no attention. Our investigations into this literature gap discovered that dissolved metal ion only slightly shifts the lower critical solution temperature. However, metal ion greatly increases the size of polymer aggregates, and is found to bind directly to the polymer. We present a metal crosslinking scheme that fully rationalizes our observations. Due to difficulties in examining our system, we also present a second order scattering method of original design for determining particle size during dynamic aggregation processes.
Global consumption creates a market demand for cheaper more available materials such as polyacrylates. For decades chemists have sought a catalytic route for producing the acrylic acid monomer from C₂H₄ and CO₂ precursors. Very recently that goal was realized, but the system requires considerable improvements before becoming appropriate for large scale production. We investigated the cycle’s most troublesome catalytic intermediate, a nickelalactone chelated by bidentate phosphine ligands. Using the Dubois motif, we synthesized a family of PNP nickelalactones and the corresponding carbonyl complexes. These compounds we thoroughly characterized by NMR spectroscopy. Based on thermal stabilities the cyclohexyl phosphine derivatives were better suited for catalysis than their phenyl analogues.
I. THE BINDING OF Cu(II) and Zn(II) TO POLY-N-ISOPROPYLACRYLAMIDE AND ITS EFFECTS ON THE PHASE TRANSITION
CHAPTER 1

BUILDING RATIOMETRIC FLUORESCENT SENSORS FOR METAL ION USING POLY-N-ISOPROPYLACRYLAMIDE

Introduction

This project explored the interaction between free metal ion and the thermoresponsive polymer poly-N-isopropylacrylamide (PNIPAm). Preliminary scattering studies discovered evidence that dissolved metal ion could alter PNIPAm’s behavior. However, the scattering technique utilized lacked established methodologies to interpret the data. To our knowledge these data indicated the first example of metal ions altering pure PNIPAm’s behavior, and we could neither explain the metal’s role in the process nor even pinpoint the parameter changing. We aimed to rationalize these scattering data and understand how metal ions influence PNIPAm. Pursuing these goals led to two largely separate investigations. The first aimed to create an interpretive model that could quantitatively relate the scattering measurements to some physical property. That model proved its merits in pursuit of the second goal: identifying possible PNIPAm-metal interactions and rationalizing how they informed behavior.

While both of these goals led in separate directions they each emerged from a metal sensing objective. Such sensors aim to create a ratiometric fluorescent response to bioavailable metal ion and identify trace concentrations in aqueous media. Ironically, many of the necessary sensor components never appeared in our experiments or the findings detailed herein. However,
the foundational concepts and features used for sensing formed the contextual background for this project. Data interpretation and hypothesis formation constantly occurred in relation to that foundation.

These foundational subjects are contained in this introductory section rather than repeat similar concepts for multiple chapters. We shall discuss a variety of topics required for building sensors. The discussion begins with the need for specialized metal binding, and then moves on to fluorescent techniques and the problems associated with applying them to paramagnetic metals. Then we describe the sensor’s overall design and the preferred fluorescent mechanism. After establishing general concepts the discussion turns to specifics regarding PNIPAm preparation, the polymer’s thermoresponsive behavior, tracking conformation changes via fluorescence, and the fully assembled metal sensor. Finally, a discussion of light scattering theory concludes the background topics. The research presented in the following chapters obviously diverges from many background elements included here. Therefore the chapter ends with the motivations which prompted the study.

**Aqueous Metal Ion**

Aquatic environments naturally contain a variety of dissolved metal species. Expected mineral assays differ from region to region according to geographical features, but industrial activity such as mining facilities can dramatically alter local conditions. Conditions can shift naturally through erosion processes. The natural changes are accelerated by industrial accidents or negligent disposal practices, but even abandoned equipment can oxidize with time and passively leach into the ground and enter the water table. Dissolved transition metals oftentimes act as Lewis Acids to substantially lower pH and can stress biological organisms by participating
in redox chemistry. Excessive metal concentrations raise concern for an ecosystem’s long term survival.\textsuperscript{2}

Uncontrolled metal presence can significantly damage living systems. Metals will accumulate in biological organisms either from ingestion as a part of the food chain or absorption from repeated environmental exposure. Interactions between metal ion and proteins can disrupt normal biological functions, usually to the organism’s overall detriment. These problems arise from two basic mechanisms. A biomolecule’s function depends strongly on its tertiary and quaternary configurations. Chelating a free metal typically triggers a shape change that renders the original function inoperable. Metals will also participate in redox chemistry. Electron transfer reactions can destructively change bonds directly after binding, as well as generate reactive oxygen species that go on to cause oxidative stress. Although many transition metals can damage biological systems, their usefulness towards industrial applications ensures continuing opportunity for contamination. Lead and mercury cause numerous neurological symptoms and are now infamous for once widespread everyday exposures,\textsuperscript{3} in more recent times they are used far more sparingly. Chromium is a common electroplating material, but its water soluble forms ravage DNA given the chance.\textsuperscript{4} Modern construction relies on iron for steel frameworks and copper for plumbing and electronic wiring. While less infamous, excessive iron produces oxidative stress through Fenton chemistry\textsuperscript{5} and unregulated copper is linked to Wilson’s disease.\textsuperscript{6} Metal ions are fundamental components to biology but have the potential to bring those systems crashing down.

Aquatic species are more susceptible than humans to complications from dissolved metal. Fish and other submerged organisms exist at the mercy of the ambient environment, and in many cases cannot migrate to other environs to mitigate stress. The massive volume of water
available, particularly for oceans, helps to minimize the impact of acute changes to pH, temperature, and other dissolved solutes. Many fish have adapted to largely stable conditions and respond poorly to even subtle changes caused by human activity. For example, fresh water fish begin to deteriorate as dissolved copper ion becomes more concentrated. Fish gills enable respiration by absorbing dissolved molecular oxygen. These gills operate through equilibrium with alkali and alkaline earth cations. Copper ion binds more strongly to binding sites across those gills, and higher concentrations cause tissue damage and eventual death. Ubiquitous copper use raises concerns for long term contamination of fresh water lakes and rivers. Assessing environmental health and identifying changes requires an accurate means to measure quantities of dissolved metal ion.

Specialized ligands are required to interact only with “bioavailable” copper to help gather more actionable measurements. Copper toxicity towards fish species stems from competitive binding events on gills. Because not all copper can engage in such reactions, we draw distinction between total concentration and bioavailable concentration, a lower value which indicates the amount of copper free to interact with the fish’s binding sites. According to the biotic ligand model, bioavailable copper concentration more accurately indicates toxicity, but is also a more difficult value to determine. Freshwater environments also contain a variety of flora at different stages of life and decay. Organic fragments from decaying plant life, such as humic acid, can also bind copper. However, their many varying structures make it difficult to accurately predict binding even after quantifying the amount of organic matter present. Existing methods do not account for these distinctions. Currently, the sample is collected on site and the analysis performed elsewhere using ICP-MS (inductively coupled plasma mass spectroscopy). This provides a highly accurate measurement of the total copper present, but poorly predicts the actual
toxicity towards the native fish life. Therefore, an appropriate sensor strategy for monitoring copper concentration should selectively respond only to bioavailable copper species.

**Ligand Design Principles**

In addition to the nuances of competitive binding several other concerns complicate the ligand design. Binding copper is usually a trivial problem, numerous ligand motifs exist which perform that function excellently, but selectively binding copper becomes more difficult. Cu(II) is a slightly harder Lewis acid than Zn(II), and most O and N ligands prefer Cu(II) thermodynamically by approx. two orders of magnitude. However, zinc’s higher natural abundance counterbalances that advantage. Also, ligand designs should not bind the metal too strongly. With increasing binding strength the ligand begins to out-compete dissolved organic matter (DOM) which blurs the distinction between total and bioavailable metal. The binding event needs to be reversible lest the measured response behave more as a dosimeter rather than the intended dynamic sensor. In practical terms, this discourages extensive chelation, and ambient water should occupy some binding sites instead of additional ligand(s). Good ligand design can create a means to interact with the target metal, but it remains meaningless without also having a strategy to observe that interaction.

Native equilibria present in fresh water environments necessitate a highly sensitive response mechanism for accurate copper detection. Copper ion experiences multiple equilibrium processes which influence its aqueous concentration. Under alkaline conditions an insoluble hydroxide forms and at fixed pH the hydroxide species becomes more abundant with higher copper concentrations. Copper can also bind to DOM but to what degree is notoriously difficult to predict even after quantifying DOM, though binding generally occurs through hydroxyl and carboxylic acid groups. It follows from LeChatelier’s principle that measuring
ambient copper via binding mechanisms in relatively large quantities will disturb preexisting equilibria, and previously bound nontoxic metal will release into solution. Within sensor contexts this translates to creating a hidden bias which alters the final measurement. Since the target copper ion is already at relatively low concentrations this means that the sensor can only be used in extremely low quantities. Because of these low quantities the detection method needs to be highly sensitive to get any metal dependent signal at all. Fluorescence techniques have such sensitivity, but unfortunately also has compatibility problems with copper which force innovative designs.

**Fluorescence**

All molecules participate in a rapid exchange of energy with their surroundings. Molecules most commonly store and transmit this energy kinetically, colloquially understood as heat. Apart from translational movement, molecules can also hold energy within rotations and vibrations. These three energy modes all relate to molecule’s motion in space, but even higher in energy are the electronic energy levels. Higher electronic levels are considered excited states compared to the unexcited ground state. Excited states exist only briefly and after shedding their excess energy will return to more thermodynamically stable ground state. That energy can leave through several mechanisms, including fluorescence.

Fluorescence refers to an emissive relaxation, that is, an excited molecule returns to its ground state by ejecting a photon. Fluorescence usually occurs either in the UV or visible region, owing to the band gaps between electronic energy levels, for both the absorption and emission. In most cases, the total energy absorbed exceeds the energy emitted because of two consequences of thermal relaxation. The first is the more rapid relaxation of rotational and vibrational states than electronic states. The available vibrational and rotational energy levels
for both the ground state and excited state dictate the quantized transitions, and also broaden the observed spectra. While the absorption can excite directly between these modes, the emission occurs after a slight relaxation to that electronic state’s lowest energy level. The small loss in energy creates the observed red shift in spectra. In some cases, the excited state can partially relax to a different spin state through intersystem crossing, before returning to ground state energy levels.

In its most basic form fluorescent is simply a relaxation. Multiple relaxation pathways exist, which accounts for the second major reason for energy loss. The excited state can redistribute the absorbed energy elsewhere without ejecting a photon. The sum total of these effects dictates a fluorophore’s “quantum efficiency”. The quantum efficiency expresses the average number of photons emitted per absorption, somewhere between 0 and 1. Thermal redistribution is the simplest culprit. Collisions with other molecules can transfer the energy, essentially releasing it to the surroundings. Also, by shifting conformation or changing velocity the molecule can transform electronic energy into kinetic energy. These effects occur more readily for flexible molecules which will generally have lower quantum efficiency than their more rigid counterparts.

**Fluorescent quenching**

Various environmental factors can “quench” fluorescence by promoting non-emissive relaxations thereby lowering emission intensity. As already mentioned collisions with surrounding molecules can relax the excited state and circumvent the emission. The energy and frequency of these collisions will increase at higher temperature, thus dubbed thermal quenching. However, each molecule has its own performance curve to describe thermal sensitivity. Thermal quenching implies kinetic energy exchange with solvent molecules. Concentration quenching
refers to the interactions between multiple fluorophore molecules. At low concentrations, observed intensities will directly relate to the amount of fluorophore. Raising concentrations eventually has diminishing returns towards intensity as the isolation breaks down and fluorophores interact with each other. Collisions between excited and unexcited fluorophores can transfer the stored energy without producing emissive events. At high concentrations this effect can deactivate enough fluorophores to reverse the trend, and observable intensities decrease with additional fluorophore. Some species can quench fluorescence without direct interaction.

The most important form of quenching to this work, paramagnetic quenching, is best understood through the phosphorescent mechanism. In fluorescence, both excitation and relaxation occur within the same singlet spin system. Alternatively, the singlet excited state can access a new spin system. Through an intersystem crossing, the excited state can perform a forbidden transition to access a triplet state at slightly lower energy. That triplet can still relax to the ground state by ejecting a photon. This phosphorescence process is distinct from fluorescence as it goes through a forbidden spin transition, and has a slower time scale given its low probability. The intersystem crossing can also occur intermolecularly, meaning that non-emissive species with higher spin multiplicities can quench the fluorescent signal. The most infamous aqueous quenchers are paramagnetic metal species, such as Cu(II) or Fe(III), which makes it difficult to quantify such species at low concentrations via fluorescence strategies.

**Sensor Design Principles**

Copper quenches fluorescence and lowers the overall emission intensity. Copper(II) is paramagnetic, meaning it has one or more unpaired electrons. With a d⁹ electron configuration Cu(II) has one unpaired electron and a doublet spin state. An energy transfer can occur between
a fluorophore’s singlet excited state and copper’s doublet ground state, though the exact mechanism is unclear. Copper’s excited state relaxes nonradiatively thereby quenching fluorescent intensity. Quenching has spatial dependence, the smaller the distance between metal ion and fluorophore the greater the effect. This means quenching occurs more at higher metal concentrations since the collisional frequency is higher. A fluorescence technique can theoretically function as a signal source for copper measurements since the environments intended for application have low overall copper concentrations. However, a functioning fluorescent sensor must produce some change in connection to interaction with the metal ion. Traditionally this has resulted in copper binding sites covalently linked to fluorophores over such small distances that quenching inevitably occurs after binding the metal. Such sensors have the undesired monicker “turn off” because the presence of target analyte nullifies the observable signal. Absence of signal could also correspond to instrument failure or non-ideal sample conditions making “turn on” sensors far preferred over “turn off” alternatives. Recently, new sensor designs have emerged to overcome Cu(II)’s spatial quenching of fluorophore and create improved fluorescent “turn on” sensors.

A new strategy has emerged using polymer chemistry to craft fluorescent sensors to limit quenching. Fluorophore and ligand are covalently linked and their close proximity ultimately lowers signal intensity, but that limitation can be lifted by abandoning the small molecule regime. Using an extended polymeric component as the linker can distance the fluorophore from metal sufficiently to prevent quenching. Of course bound metal ion still needs to alter fluorescent behavior which becomes more difficult after effectively isolating the two species. Choosing a responsive material for the polymer spacer solves this problem.
Responsive polymers can respond to environmental stimuli by changing their conformation and physical behavior. Because polymers typically have such a large number of freely rotating bonds, they exhibit a greater range of flexibility, and have more varied conformations, than their small molecule counterparts. Bulk solubility trends are generally driven by the subtle differences between polymer-solvent and polymer-polymer interactions. For some polymer-solvent combinations these two types of interaction offer similar energy stabilizations. In these cases opportunity exists for environmental changes, such as pH or temperature, to reverse the relative stability between polymer-solvent and polymer-polymer interaction. These changes affect solubility and make polymers adopt new shapes and sizes.\textsuperscript{15}

Such responsive behavior is attractive for tuning fluorescent emissions via metal binding over large distances. Two key features accompany the metal ion after binding to bifunctional ligand comonomers. First, regardless of ligand design the polymer’s overall charge will change upon binding, for uncharged ligands,

\[ L + M^{n+} \rightarrow LM^{n+} \]

and charged ligands,

\[ LY^- + M^{n+} \rightarrow LM^{(n-y)^+} \]

Because like charges repel each other, a polymer bearing multiple charges will have less conformational freedom and favor larger and more expanded configurations. Likewise polymer systems without charge will have a greater range of motion, and on average exhibit smaller more compact arrangements. Second, metals have a solvation sphere that electrostatically orienters nearby water. The bound metal forces the polymer to accommodate the necessary water to stabilize its charge. Overall this causes the polymer to experience increased exposure to solvent localized at the metal ligand complex. These two qualities together direct the polymer towards
different geometries in the presence and absence of target metal ion. Elsewhere, spaced far enough away to stymie quenching, the fluorophore also experiences a new local environment owing to bulk changes across the entire polymer. Within this new environment the fluorophore emits a new quality of fluorescent signal. The strategy involves numerous synthetic components to refine, but by design this type of sensor produces a metal dependent response, notably without needing a “turn off” mechanism.

**FRET**

One of the more interesting energy transfers quenches the initial excited state but still leads to photon emission. Close contact between two of the same fluorophore can release the stored energy nonradiatively. Proximity between two different fluorophores can perform a direct energy transfer. Forster Resonance Energy Transfer (sometimes inappropriately called Fluorescent Resonance Energy Transfer), or FRET, describes an indirect excitation and emission process using different fluorophores. Two fluorophores are considered a compatible donor/acceptor pair if the emission spectrum of one overlaps with the absorbance spectrum of the other. For such pairs, absorbance by the donor fluorophore, even if the chosen excitation energy lies outside the acceptor fluorophore’s absorbance range, will result in emission spectra characteristic of each fluorophore. The energy transfer occurs through a quantum tunneling effect, and therefore depends on the average spatial distance between the two fluorophores,

\[ E = \frac{1}{1 + \left(\frac{r}{R_0}\right)^6} \]

Where **E** is the transfer efficiency, **r** is the distance between fluorophores, and **R₀** is the distance corresponding to 50% transfer efficiency. Shorter distances lead to more efficient energy transfer and a relatively more pronounced acceptor emission peak. Conversely, longer distances
give a higher donor to acceptor ratio, with the acceptor peak eventually disappearing entirely. FRET’s clear indication of small scale changes, in addition to being a highly sensitive technique, makes it an ideal strategy for creating sensor technologies.

**PNIPAm and RAFT**

The Planalp and Seitz collaboration pursues ratiometric fluorescent sensors using the thermoresponsive poly-N-isopropylacrylamide (PNIPAm). PNIPAm is a water soluble polymer with lower critical solution temperature (LCST) behavior. The polymer owes its solubility to the hydrophilic amide groups that form favorable hydrogen bonds with solvent. At elevated temperature the overall energy stabilization from H-bonding weakens and hydrophobic interactions at isopropyl groups begin to compromise solubility. The polymer chain reorients itself in order to limit interactions between the isopropyl groups and water. A phase transition from open coil to globule occurs, shrinking the hydrodynamic radius and increasing overall entropy due to expelling previously organized water molecules. The LCST is at ~32° for PNIPAm, though the value can shift several degrees based on molecular weight, and can change dramatically with different copolymer compositions. Upon crossing the LCST linear chains collapse from a random coil into a globule, and according to concentration globules will assemble into aggregates and precipitate.

PNIPAm chains were synthesized using radical polymerization mechanisms. Radical polymerization proceeds through three reaction steps. Initiation, a radical initiator attacks an olefin to generate a 2° radical. During propagation, the still reactive radical attacks another olefin, because the radical survives each addition the overall reaction continues and the chain grows longer. In termination, the radical quenches, such as through head to head recombination, and the polymer stops growing. Initiation reactions do not all occur simultaneously, and the
propagation step has much faster reaction kinetics than initiation. Depending on when chains started growing they will finish the reaction at a wide range of sizes. Chain length homogeneity is expressed using a polydispersity index (PDI) defined as a ratio of weight average molecular weight, \( M_w \), and number average molecular weight, \( M_n \),

\[
PDI = \frac{M_w}{M_n}
\]

\( M_w \) favors larger polymers and except for a perfectly monodisperse sample will always exceed \( M_n \). PDI values close to 1 indicate fairly monodisperse materials, and conversely larger values indicate broad or multimodal distributions.

Early polymer preparations began with an uncontrolled free radical approach before adopting a controlled radical mechanism. At the time the department lacked suitable characterization methods compatible with the PNIPAm system. Peak broadening present in \(^1\)H NMR suggested polymerization reaction had taken place, but to what extent or quality remained unknown. Long term investigations required a more reliable foundation.

In response, synthetic strategies evolved to a reverse addition fragmentation chain transfer (RAFT) method, a controlled radical mechanism. RAFT uses a chain transfer agent (CTA) to interrupt the propagation stage. A CTA has: (1) a reactive dithioester which can momentarily trap the propagating radical, (2) a Z group, bound to the sp\(^2\) carbon, chosen to tune the dithioester’s electronic properties, and (3) a S bound R group that can continue propagation with monomer after radical fragmentation.

![Figure 1. Radical transfer mechanism using CTA](image-url)
In solution, a propagating radical reacts with the thiocarbonyl to form a new carbon-sulfur bond and the radical moves to the now sp\(^3\) carbon. A thioether will then homolytically fragment thereby restoring the dithioester. Because either of the thioethers can fragment, the active radical can now reside with the CTA’s R group, which will begin polymerization as a new growing chain. The original chain remains dormant until another radical reacts with the CTA. In uncontrolled radical mechanisms the polymer continues growing until termination, but during RAFT mechanism a chains growth is repeatedly paused and resumed throughout the reaction process. Constantly swapping the active radical between multiple chains slows overall chain growth. Slowing propagation in this way ensures that the many chains grow at a similar pace, resulting in more similar chains and a narrower PDI than for uncontrolled radical polymerizations. Note that this requires a small number of radicals relative to CTA and molecular weights are controlled by tuning monomer to CTA ratios instead of monomer to initiator ratios.

The CTA remains attached to the polymer after the reaction concludes. Monomer \(^1\text{H}\) NMR signals typically broaden in polymers because of their overall flexibility, and motion, numerous possible conformations, and distribution of molecular weights. The CTA fragments are attached to the polymer chain ends and retain their sharp peak definition. By comparing integration ratios between monomer signals and CTA signals the \(M_n\) is calculable. This requires resolvable signals from the CTA without overlap from monomer. Increasing molecular weight makes CTA more difficult to discern from baseline noise, and practically, once molecular weights exceed the range of 15-20 KDa CTA signals becomes too small to judge molecular weight.

**PNIPAm and its LCST behaviors**
Poly-N-isopropylacrylamide (PNIPAm) is perhaps the most famous thermoresponsive polymer to date. It owes its functionality to the monomer’s two distinct structural motifs: a hydrophobic isopropyl group and a hydrophilic amide. These two regions have starkly contrasting stabilities in aqueous solvent, and their competition for energy minimization creates useful solubility properties. The material has LCST characteristics; elevating temperature triggers a phase transition from hydrophilic to hydrophobic conformations, and depending on structure, the polymer can become insoluble. Rising temperatures continuously lowers the net stabilization afforded by hydrogen bonding and eventually, it becomes too weak to remain soluble.

Two properties then drive the polymer’s conformational shift. The first is fairly intuitive; shrinking inwardly the polymer can “hide” the isopropyl from bulk solvent and minimize the unfavorable hydrophobic interaction. This will occur regardless of macroscale structural design. For example, a surface-attached linear chain will extend into expanded and open forms below the LCST, but after crossing the LCST will wrap about itself close against the surface. Similarly, freely suspended linear chains undergo a coil to globule transition about the LCST which lowers their hydrodynamic radii. More rigid structures have less conformational freedom to orient the isopropyl groups, but the same phenomena are still observed. Even crosslinked PNIPAm gels will swell and shrink below and above the LCST respectively. The behavior remains consistent because of the second driving factor behind LCST transitions, entropy. Below the LCST the polymer prioritizes the amide’s H-bonding over segregating the isopropyl groups. This in turn organizes a larger volume of water molecules in and around the polymer. While the polymer may seem more restricted and ordered above the LCST, the smaller size actually expels large
amounts of organized water molecules which causes a net entropic increase. The resulting changes to overall shape depend on the polymer’s synthetic design.

The balancing act between hydrophobicity and hydrophilicity controls the LCST. At sufficient molecular weights, pure PNIPAm transitions near 32°C. Oligomers have more exposed isopropyl groups than larger chains and have a slightly lower LCST. Similarly, small chains are more susceptible to hydrophobic contributions from chain end groups, such as those left over from RAFT preparations. PNIPAm also takes on new LCST behaviors in mixed MeOH/H$_2$O solvent systems, though the present work investigated only pure water environments. Swapping out NIPAm with alternative monomers achieves more dramatic shifts in LCST. Less water soluble choices, such as styrene, promote precipitation and will lower LCST, whereas more hydrophilic monomers promote solvation and raise the LCST. This largely requires the substitute monomers to interrupt extended NIPAm sequences, as achievable through random copolymerization. When mostly segregated, i.e. block copolymers, the NIPAm block behaves quite similarly to pure PNIPAm, and thermally inactive blocks remain unaffected.

Polar monomer choices oftentimes introduce an additional far greater influence to the LCST. Many polar monomers owe their water solubility to hydrogen bonding, NIPAm included. Some participate in fully fledged acid/base chemistry and readily take on positive or negative charge; examples include polyacrylic acid and polyvinyl pyridine. Such copolymers obviously exhibit pH dependence, and the resulting localized charge greatly increases affinity for solvent and introduces like charge repulsion onto the polymer backbone. Both effects raise the LCST and conversely, neutralizing or shielding charged species lowers the LCST. In this example the polymer adopts new behavior at varying H$^+$ concentration, and controlling polymer composition can achieve the same type of response using numerous other dissolved species. The ability to
alter the polymer’s behavior in different environmental conditions makes PNIPAm ideal for crafting sensitive materials.

**PNIPAM sensors and FRET**

By combining both PNIPAM’s environmental responses and fluorescent spectroscopy’s high sensitivity one can create sensor technologies. The binding of target analyte onto the polymer backbone directs PNIPAm towards either its hydrophobic (closed) or hydrophilic (open) conformations.\(^\text{23}\) Fluorophores also bound to the polymer produce a different emission spectrum based on their new local conditions. Such designs allow for selective sensing while keeping the analyte and fluorophore separated.\(^\text{14}\)

Traditional small molecule designs use a more direct approach. The binding region is often closely located to the fluorophore, changing fluorescent signal through redox mechanisms, altering the electronic π system, and even covalent ring openings. The close proximity makes the strategy less suitable when the bound analyte stifles fluorescence. Many biologically-relevant metal species have unpaired electrons and will quench fluorescent emissions owing to their paramagnetism. One can utilize quenching effects to design “turn off” sensors, but these require additional experimental care since low signal could occur during high copper concentration (quenching), interference from matrix effects, and general instrument failure.

The PNIPAm scaffold separates the paramagnetic metal ion from the fluorophore and diminishes quenching effects. Since the metal ion and fluorophore lack interaction the sensor has less mechanistic options for producing signal changes. However, the separation also means that the chosen mechanism functions appropriately across a wider variety of targeted species.\(^\text{24}\) FRET ratios change with the distance between the two fluorophores. Given proper polymer
composition, a small number of bound metal ions can shift the polymer’s conformation without crowding the fluorophores and quenching emissions.\textsuperscript{25}

FRET technique also offers one of the most desired features for sensing, ratiometric measuring. The fluorescent spectra contain intensities from two separate fluorophores. Absolute intensities change with concentration, and for comparing different samples it’s preferable to analyze the relative ratio between donor and acceptor peaks. Outside factors can easily impact overall intensities, but these proportional influences can’t alter the intensity ratio. This means that measurements are more resilient against the negative effects of instrumental fluctuation and sample matrix.

**PNIPAm sensors and free metal ion**

Despite PNIPAm’s repeated role in creating metal sensors the literature makes little mention of interactions between the dissolved metal and the polymer backbone. Targeted sensing requires a ligand comonomer to facilitate selective binding, and bound vs. unbound forms adopt different conformations, which in turn produce different fluorescent signals. Control experiments have varied the metal ion, but seemingly only for ligand bearing copolymers, not pure PNIPAm. They demonstrate large shifts in LCST attributed to metal binding. However, such findings require only qualitative scattering data, and leave subtle conformational impacts unexplored. Many sensors are concerned with low concentration ranges, and their control studies gauge the effect of trace spectator ions, but do not test performance in more extreme quantities. The lack of available data suggests a widespread assumption that either metal ion does not interact with the PNIPAm chain, or, interacts too weakly to change physical behavior. We chose to test these assumptions using a simplified PNIPAm system. The polymer contained none of the usual ligand or fluorescent components used for sensing, and the simplest
possible linear structure. The interactions were hypothesized to have subtle manifestations, so experiments focused on the LCST transition itself by way of light scattering.

**Light Scattering**

It is difficult to overstate the amount of detail and knowledge generally involved in light scattering. Much of this field requires mathematical backgrounds more commonly found in physicists than chemists. Thankfully, many of the numerous subcategories have limited interdependence. The research described here focuses on a small portion of scattering theory, the scattering of light by large molecules suspended in liquid solution.

Changing environments can redirect electromagnetic radiation. Light’s velocity will depend on the medium containing it, and all mediums regardless of phase will lower its speed in comparison to a vacuum. As long as the medium remains constant, light will continue on its vector course without deviating. At the interface between two different refractive indeces (RI), i.e. the medium changes, opportunity arises to scatter light, and the greater the change the higher the probability. Because the interface between RI’s initiates scattering it follows that the geometry of that interface informs scattering behavior. More practically, light scattering depends on the size and shape of the scattering object.

Rayleigh theory marked the first solution for scattering of light by small particles. As described by Lord Rayleigh, light scattering becomes more likely at higher energy. Scattering scales with $\lambda^{-4}$ therefore diminishing rapidly with longer wavelengths. The traditional example to illustrate this phenomenon is the clear blue daytime sky, where high energy blue light scatters more than low energy red light. Orange and Red sunsets appear because of an increase in atmospheric pathlength giving more opportunity for the low energy red light to scatter. The theory begins to breakdown however when wavelength and scattering particle become
comparable in size.\textsuperscript{26} Particles larger than $1/20^{\text{th}}$ the wavelength require more rigorous treatment.

In the early 20\textsuperscript{th} century Gustav Mie presented an improved light scattering solution. The Raleigh solution works for relatively small particles because photons also act mostly like particles at those relative size differences. At more comparable sizes light’s wave properties play a more significant role. The scattering photon occupies small particles for a negligible amount of time. But larger particles contain the photon long enough to cause a phase shift compared to the original propagating wavelength. Wave dynamics now emerge as constructive and destructive interference, which in turn biases the scattering profile in the forward direction. The Rayleigh regime predicts isotropic scattering profiles while Mie theory can also account for anisotropic scattering profiles. Both theories must still assume spherical particles however. As of yet, no general solution exists for light scattered by different shapes.\textsuperscript{27}

**Light Scattering Techniques**

Two major strategies exist for sizing suspended particles using scattering data. The first is static light scattering (SLS). Static techniques utilize the unique scattering profile afforded by a given particle size and wavelength combination. If observed, the profile allows calculations to identify the original particle size. Accuracy improves by measuring the entire profile though this costs additional resources. One of the most prevalent static techniques is Multi Angle Light Scattering (MALS). As the name suggests scattered light is measured across multiple angles positioned to obtain an appropriate representation of the overall profile. The second major strategy, dynamic light scattering (DLS), uses a completely different approach.\textsuperscript{28} Particles in solution travel randomly in Brownian motion. Their movement will depend on size with smaller objects traveling faster than larger objects. Scattered light intensity varies over small time scales.
as scatterers randomly move into and out of the incident light. In contrast to SLS, which disregards this noise and signal averages, DLS analyzes the noise pattern directly and derives size information by also leveraging diffusion properties. Scattering generally increases with size causing an interpretation bias towards larger particles. Both SLS and DLS struggle to accurately determine particle sizes for samples with wide or multimodal particle distributions. For this reason scattering techniques ideally analyze suspensions with low polydispersity. Common sample preparations include pre-filtering solutions and size exclusion chromatography.

This project primarily employed a rarely used static light scattering technique called second order scattering (SOS). Traditionally, SOS represents an unwanted nuisance during fluorescence measurements. Fluorescent samples sometimes contain suspended particles. These particles will oftentimes elastically scatter the incident light intended to cause fluorescence. The sample’s “emission” then has both the scattered light and the expected fluorescence. This scattered light can damage the detector, which prompts users to choose measurement windows at lower (longer wavelength) energy than the excitation beam. Those measurement windows rely on an adjustable diffraction grating to control which wavelengths reach the detector. However, an unwanted optical effect can occur at the diffraction grating. The grating normally aims the first order diffraction towards the detector, but higher order diffractions at different angles also exist, though the intensity decreases greatly at higher orders. The angle for a given wavelength’s second order diffraction coincides with the first order diffraction of that wavelength’s double (Figure 2).
Because the fluorescent detector does not discriminate between energies, this means that the instrument could incorrectly report 300 nm light as 600 nm light. In practice, this only happens when a sample fluoresces near twice the excitation wavelength while containing suspended scatterers,

\[ 2\lambda_{\text{excitation}} = \lambda_{\text{emission}} \]

The phrase “second order scattering” is a conceptual misdirect since it refers to a combination of second order diffraction and elastic scattering phenomena. The studies performed here do not involve fluorescence and may therefore ignore the primary complication associated with SOS.

SOS offers several useful advantages over alternative scattering methods. Data are collected using a fluorometer, a highly pervasive instrument found in most university chemistry departments. More advanced scattering instruments that directly report particle size are by comparison usually: (1) costly, requiring significant capital investment or outside collaboration (2) specialized, and subsequently not compatible with all materials, functional groups, or solution conditions (3) experimentally restrictive, for example, when run in-line with
chromatographic separations sample changes can only be viewed through time lapse measurements as opposed to monitoring in real time.

Additionally, SOS measurements experienced less interference from extraneous particulate, namely dust. Samples are normally filtered to prevent large dust particles from contributing to the characterization. Initial tests discovered SOS intensities from PNIPAm samples plummeted after filtering, an effect attributed to removal of large portions of suspended PNIPAm. The lowered particle concentration rendered scattering data unobtainable through DLS, which suggests that SOS technique has a lower concentration limit than DLS. Measurements were thereafter performed using unfiltered suspensions. SOS intensity results from the combined effects of all scatterers, including dust. Because the signal from dust particles remains unchanged throughout the experiment, that contribution can be identified and removed using a blank. Therefore, the SOS method is capable of examining samples otherwise incompatible with filtration techniques.

**Initial Motivations**

Skepticism is a necessary quality when designing and building these target sensor technologies. From the above description it is tempting to oversimplify to “fluorescence changes because copper is bound”, an A -> B rationale, but the situation has more nuance to consider. A more accurate perspective: (a) fluorescence changes because the fluorophore’s environment is different, (b) the fluorophore’s environment is controlled by polymer conformation, which has likely shifted, (c) the polymer’s conformation was likely changed by a new overall charge and solvation, (d) charge and solvation differences were brought on by a newly bound metal ion, (e) the metal is likely copper because of the ligand’s selective binding affinity. A -> B -> C -> D -> E. Each of those steps introduces an opportunity for incorrect assumptions. However,
completely removing the “what if?” problem would require an unreasonable amount data, much of it unobtainable.

Conventional methods address the problem through a combination of calibration and control studies. Calibration identifies a useable relationship between measured response and analyte, while control experiments try to reveal circumstances which undermine the calibration. Control experiments are by nature tedious and nearly endless. Multivariable systems quickly accrue an untenable number of experimental combinations to explore. Limited resources demand highly relevant and efficient experiment choices.

The Planalp and Seitz collaboration has recent history of designing and optimizing metal sensors. This project has explored mostly targeted Cu(II) and Zn(II) across multiple avenues. Shortly before this current investigation began a new experimental trend emerged. The observed fluorescence behavior started to contradict prediction. Emission data continued to show dependence on copper concentration, but the changing peak intensities defied expectation and reproducibly moved in the opposite direction. These results prompted concern over the sensor’s underlying behavior. Perhaps an undiscovered property had just revealed itself that should merit a new design feature. The unexpected data was later explained through experiments unrelated to the projects described herein, but at the time it prompted a new series of control experiments to investigate uncharacterized fundamental interactions.

Sensor test samples had too many tunable factors to allow for more than a cursory assessment. These include: solution temperature, solution pH, and the concentrations of polymer, pH buffer, and metal salt. The polymer mostly contains an acrylamide monomer for its majority backbone but also has additional co-monomers for high affinity ligands and fluorophore tags. These can have different percent incorporations and distribution across the backbone.
Finally, the polymer’s molecular weight and synthetic arrangement, e.g. linear vs. branching vs. crosslinked etc., also contribute to overall behavior. The sheer quantity of possible variations discouraged lengthy in-depth investigations. Originally, simple assessments were proposed to identify evidence for unexpected interaction. The complicated sensor system was simplified to lower the number of variables, and then quickly find combinations that changed physical behavior. Any discovered interaction could then later inform the design process.

Performing these tests would require a different physical measurement technique. The sensor’s fluorescence signal depended on too many factors, and it could only indirectly provide insight into the polymers behavioral changes. Additionally, the presence of fluorophores across the polymer was itself one of the variables to be tested. This investigation started with exploring the possibility of metal interaction directly with the acrylamide backbone. Experiments were slated to use a linear homopolymer without fluorophores. Any samples lacking fluorophore would by definition require a different measurement technique. Light scattering techniques were used then as the primary resource through this project.
CHAPTER 2

A SECOND ORDER SCATTERING METHOD FOR DETERMINING PARTICLE SIZES
DURING AGGREGATION PROCESSES

Summary

The present chapter describes the creation of a new interpretation method for calculating particle sizes from light scattering data. The first major attempt to explain SOS measurements used Rayleigh scattering theory. The Rayleigh model needed large estimations and utilized a correction factor based on the inner filter effect. The model’s performance fell short of the quantitative goals and could not even provide a reliable qualitative explanation. The flaws were deemed too great to merit further investigation and the Rayleigh explanation was ultimately abandoned. The second model using Mie Theory eventually succeeded and generates quantitative particle data. An extensive validation using Polystyrene (PS) particles demonstrated good agreement between prediction and experiment.

Initial Second Order Scattering measurements and conditions

Before collecting SOS measurements the researcher must first select an appropriate wavelength. The chosen wavelength should maximize observable signal. Shorter wavelengths will tend to scatter more than longer wavelengths and increase intensity, and photo multiplier tubes (PMT) used in fluorometers are more sensitive to high energy photons. However, many
organic functional groups absorb in the UV (ultraviolet) range which will lower intensity, and the absorption events could trigger photochemical reaction depending on the sample. Ideally, identify and use the shortest wavelength without any overlap with the samples absorption spectrum. Certain physical parameters will further dictate the wavelength but those reasons will be discussed later on in this chapter.

A brief warning about wavelength selection before describing experimental conditions: SOS technique cannot utilize the full set of excitation wavelengths available during normal fluorescence measurements. Measurements settings for detecting SOS “emission” use double the incident wavelength, recall that despite the chosen wavelength difference the photon energy remains constant. However, the fluorometer has a maximum wavelength detection setting. The longest excitation wavelengths far exceed the available detection settings, making SOS unmeasurable. Therefore, one must confirm instrument compatibility towards the excitation and emission wavelengths before committing to experiment.

Experimental solutions held three reagents in addition to aqueous solvent. This study investigated possible interaction with one of two different metal salts, copper(II) nitrate and zinc(II) nitrate. Zinc ion is colorless and copper has a broad absorbance peak covering the longer portions of the visible spectrum. Also present is a morpholine-derived pH buffer from the Goods buffer series to prevent solution changes caused by metal reaction, and finally the PNIPAm polymer. Both the buffer and polymer absorb only in the UV and not the visible. The PNIPAm monomer has an absorption shoulder that trails off before reaching the near UV. The wavelength 400 nm was chosen to fully ensure no absorbance.
Figure 3. SOS intensities and PNIPAm concentration.

Sample preparations are fully detailed in the methods section. Combined stock solutions yielded prepared samples at target concentrations, and external temperature control reproducibly induced LCST phase transition. SOS measurements quickly showed the expected increase in scattering but intensity values were surprisingly unstable. Further experimentation revealed that intensities were highly time dependent and samples were not suited to performing quick individual scans. Kinetic type measurements showed sharp increases in intensity for the first
several minutes when heated above the LCST, followed by a sharp decrease that gave way to a gentle shoulder over 1 hour. Though intensity did not fully stabilize within this time subsequent measurements were stopped after 45 minutes due to time constraints. While varying system parameters it was discovered that intensity changed greatly with polymer concentration. By decreasing concentrations the intensity’s magnitude lessened and the “peak” became less pronounced, eventually disappearing completely at 0.0005 mg/mL. When experiments moved from uncontrolled free radical to RAFT prepared PNIPAm, the “peak” was rediscovered at a considerably higher concentration of 0.05 mg/mL.

Subsequent metal studies raised interest in understanding “peak” phenomena. Lower PNIPAm concentrations removed the peak, but samples including copper and zinc caused the peak to reappear. Metal quantity dictated the “peak’s” overall shape suggesting a link between metal ion and PNIPAm’s behavior. This finding’s novelty demanded an explanation, but extensive literature searches revealed no clear interpretive insights to rationalize the data. It was deemed worthwhile to create a new interpretive method and thereafter discover the underlying chemical behavior.

**Analyzing SOS Phenomena**
0.1 M pH 6.3 MOPS buffer, 0.01 M Zn(NO$_3$)$_2$, 0.05 g/L 10K M$_r$. (a, above) absolute values, (b, below) normalized values.

Figure 4. Time dependence on SOS peak appearance for different excitation wavelengths.
Two hypotheses were initially assessed to determine if the apparent decrease in observed scattering occurred because of compromised PNIPAm suspension. First, the metal was suspected of catalyzing hydrolysis of the polymer’s amide groups, thereby changing LCST phase properties and scattering behavior. Repeated heating and cooling cycles yielded identical scattering behavior indicating a reversible process inconsistent with a destructive hydrolysis reaction. Additionally, when the chosen excitation wavelength was increased from 400 nm to 425 nm and 450 nm, the “peak” position shifted to longer times with wavelength (Figure 4b). If the “peak” resulted from a discrete A to B reaction process it would be expected for the “peaks” to coincide with time rather than shift.

Second, particle settling could remove particles from suspension and lower overall scattering. Naked eye observations had not identified precipitation, but the size range of PNIPAm particles was unknown as well as whether substantial settling could occur below normal human detection limits.

In fact, benchtop observations seemed to indicate an increase in overall scattering despite the apparent decrease found in SOS measurements. This was qualitatively confirmed by simultaneously heating two PNIPAm samples, with only one containing dissolved metal. When placed in front of a hand held flashlight, the metal sample, having an SOS “peak” and lower intensity at longer times, displayed more scattering to the naked eye than the metal free analogue, which in contrast has no SOS “peak” and higher intensity at longer times.
0.1 M pH 6.3 MOPS, 0.01 M Zn(NO$_3$)$_2$, 0.05 g/L 10K M$_{w}$, 400 nm incident light. SOS at 90° (above) and UV-Vis 0°.

Figure 5. Angular dependence on scattering behavior.
This was further confirmed quantitatively by comparing SOS and UV-Vis (Figure 5). SOS indicates scattering at 90° whereas UV-Vis measures at 0°, meaning it directly measures the incident light after it passes through the sample. For samples that do not absorb the chosen wavelength, an increase in absorbance, i.e. lower transmittance, indicates scattering by the sample. UV-Vis measurements showed that total scattering increased during the entire measurement period, the exact opposite as predicted by a settling process. Instead, the intensity drop off in SOS measurements occurred because of the detection angle, though the reason remained elusive.
0.1 M pH 6.3 MOPS, 0.05 g/L 10K Mₙ. SOS (above) and DLS (below) measurements for equivalent samples.

Figure 6. SOS vs. DLS comparison.
In contrast to SOS findings, the UV-Vis data behaved more as expected for a simple PNIPAm system. Therefore it seemed unlikely that the SOS “peak” signified a never before seen physical behavior. PNIPAm is well known for LCST phase transition that collapses polymer chains and forms aggregates, and the distinct SOS profiles likely result from changes in conventional parameters such as aggregation kinetics or particle dimension. Dynamic Light Scattering (DLS) was utilized to look for quantifiable differences between PNIPAm samples with and without the “peak”. Although the heating protocols between the DLS and SOS could not be directly replicated the DLS measurements found that PNIPAm chains aggregated faster and formed larger particles when exposed to metal ion (Figure 6). It seemed clear from DLS data that the SOS intensities were merely responding to changes in particle size.

DLS could provide such data indefinitely, but it was highly preferable to use the SOS method in the long term. DLS measurements were sensitive to dust contamination and greatly benefit from prefiltering samples. However, after filtering samples with conventional pvdf (polyvinylidene fluoride) syringe disc filters the instrument failed to collect any usable data. SOS tests showed a dramatic decrease in intensity and new data profile after filtration. The filtration removed PNIPAm chains altering the sample. To circumvent this, DLS samples required laborious solvent purification techniques, with only intermittent success which resulted in frequent failed attempts when collecting DLS data. SOS measurements could be collected quickly and simply without the need for filtering, but unfortunately no established means existed for extracting particle size information from the data.

**Interpretation of SOS data: the Rayleigh model**

The first major attempt at an interpretive model to treat SOS data was built using Rayleigh scattering theory. The theory indicated that for particles suspended in a liquid the light
scattering would increase with particle size. The volume fraction of particles to solvent was assumed to be fixed, meaning that single polymer chains were treated as discrete particles and aggregates remained suspended.

\[ T = \frac{I}{I_0} = e^{\left[ -\frac{32\pi r^3 \phi d}{\lambda^4} \frac{r^4}{m^2+2} \right]} \]  

(1)

Rayleigh scattering where \( r \) = particle radius, \( \phi \) = volume fraction of suspended particles, \( d \) = pathlength, \( m \) = refractive index ratio of particle to solvent, and \( \lambda \) = incident wavelength.

The above formula is intended for treating transmittance data. Unfortunately, fluorometer type instruments cannot measure \( I_0 \) and therefore the transmittance value, \( I/I_0 \) ratio, cannot be determined experimentally. Instead, the measured SOS signal was assumed proportional to the light lost to scattering expressed as \( 1 - I/I_0 \). The Rayleigh model used RI values of 1.45 and 1.33 for PNIPAm and water, a 1cm pathlength, and 400 nm incident wavelengths. The particle’s volume fraction depends on their number and volume, which could change with overall particle composition. Because the composition was unknown the particles were estimated as hard spheres in order calculate \( \phi \) at 0.05 mg/mL PNIPAm.
Figure 7. Uncorrected Rayleigh Prediction

**Inner filter effect correction to the Rayleigh model**

The Rayleigh theory alone was insufficient to describe SOS measurements. Figure 7 suggests that as size increases the expected scattering also increases and Eq. 1 indicates that this trend does not eventually reverse. To explain the observed decrease in signal the inner filter effect was included to the model. If the passage of light through a sample is substantially blocked, either by absorption or scattering effects, the $I_0$ effectively decreases as a function of distance through the sample. A fluorometer’s detector views signal from only a small volume portion of the sample holder. Decreasing the effective $I_0$ diminishes that regions maximum capacity for scattering light. Furthermore, multiple scattering events could also lessen the
number of photons otherwise directed towards the detector. Adapting from the work of Zhang and coworkers, a correction factor was devised to account for the inner filter effect.\(^{37}\)

$$\left( K_{sc} \right)^{\frac{x+y}{d}}$$  \hspace{1cm} (2)

Eq. 2: inner filter effect correction

Where \( d \) is the distance between particles and \( x \) and \( y \) indicate the distance traveled by the incident light in the corresponding dimension. In this case \( K_{sc} \) is a value between 0 and 1 that indicates how much the passage of light is impeded; low values suggest significant interference and values close to 1 suggest photons continue mostly uninterrupted. Integrating over both the \( x \) and \( y \) dimensions gives a predicted scattering intensity correction described by eq. 3.

$$\frac{\left( (K_{sc})^{\frac{1}{d}} - 1 \right)^{2}}{\left( \ln(K_{sc}) \right)^{2}} d^{2}$$  \hspace{1cm} (3)

Eq. 3: integrated inner filter effect correction

The correction still needed values for the scattering efficiency \( K_{sc} \). The value indicates the likelihood of scattering events occurring. Since equation 1 also relates to the amount of scattering it was used to estimate \( K_{sc} \). Across different PNIPAm samples \( d, \lambda, \) and \( m \) remain static and \( r \) is the independent variable of interest. By adjusting the volume fraction \( \phi \), new \( I/I_{0} \) values were calculated to use as substitutes for \( K_{sc} \) in Eq. 3. From Eq. 1 it follows that scattering becomes more prevalent as \( r \) increases. The estimated \( K_{sc} \) values then must also increase with \( r \). The volume fraction was adjusted using numerous combinations of particle count and containing volume. The most successful iteration found occurred when 18,000 particles fill a rectangular prism with a 1 cm square cross section and height equal to the particle’s diameter. Because the combined particle volume grows faster with size than the total volume, the estimated \( K_{sc} \) also increases with particle size. The natural log terms in Eq. 3
necessitate software capable of using more than 15 degrees of precision to prevent, or else during calculations the numerals will truncate and produce a nonsensical result.

Figure 8. Inner filter effect correction.

The correction factor will later combine with the original Rayleigh model prediction. Values near 1 indicate small amounts of multiple scattering requiring only a slight correction; values near 0 indicate considerable multiple scattering events and a greater correction. As shown in Figure 8 the inner filter effect becomes more significant as the PNIPAm chains combine into larger aggregates.

**The Rayleigh model: comparison to SOS data**
A crude comparison to experimental data was achieved using both DLS and SOS measurements. DLS and SOS measurements were each collected as a function of time. Performing a 1 to 1 comparison between the two time axes gave a relationship between DLS size and SOS intensity. At best this should only be considered for rough qualitative purposes; the heating protocols are slightly different across the two methods, and the DLS collection contains an approx. 1 minute gap between initiating the experiment and actual data collection.

![Figure 9. Rayleigh model comparison against SOS/DLS data.](image)

The final model achieved an extremely poor means of relating SOS intensity to particle size. The DLS/SOS comparison included in Fig 6 is not high quality, but is still sufficient for making qualitative judgments. The model shows almost no agreement with experimental data.
even after attempting to optimize the fit by tuning $K_{sc}$. Several criticisms were levied against the model’s application of theory which likely accounts for the discrepancy:

1) The Rayleigh Theory used was designed for explaining transmission type measurements. It was assumed that the same behavior would remain proportionally similar at other detection angles.

2) Rayleigh Theory poorly describes scattering behavior when particle sizes are not small relative to incident light, and the experimental conditions modeled generate particles as large as $\frac{1}{2}$ excitation wavelength.

3) The Inner Filter Effect correction assumed that measured intensity would strictly decrease from multiple scattering events, but neglected to consider the possibility of multiple scattering redirecting “lost” photons towards the detector.

4) The scattering efficiency constant $K_{sc}$ was unknown and the utilized values were obtained only after making heavy estimations.

Ultimately this predictive model was deemed unsuccessful and abandoned in favor of higher levels of scattering theory.

**Mie Theory Model**

A far more successful prediction model may be constructed using Mie Theory. Named for Gustav Mie who pioneered the theory in the early 20th century, Mie Theory offers a more complete solution to light scattering. When considering particles larger than 5% the wavelength, the incident photon behaves as a wave and experiences significant interference, either constructive or destructive based on the scattering angle. This results in a highly anisotropic scattering profile not present in the Rayleigh treatment. Therefore, for larger particles relative to
light, the measured scattering intensity depends on the measurement angle. Mie calculations are far more complex than Rayleigh calculations because of these nuanced relationships between size, wavelength, and angle, and are typically performed using open source software packages. Mie calculations were first performed using the Mie Scattering Calculator, a web based program, and then the outputs were coded in Microsoft Excel to model scattering measurements.

The model’s basic form was assembled intuitively to address three simple questions. When an incident photon collides with a PNIPAm particle (1) will a scattering event occur, (2) where would a scattered photon go, and (3) how often might this happen.

\[ \frac{I}{I_{\text{max}}} \propto K_{\text{sc}} I_0 N \]  

(4)

\( K_{\text{sc}}, I_0, \) and \( N \) are terms related to probability, direction, and concentration respectively.

**The Mie Model: scattering efficiency and angular dependence**

The first term is an expression for the likelihood that the impact of photon upon particle causes a scattering event. Once again this term is the scattering efficiency \( K_{\text{sc}} \), however unlike in the failed Rayleigh model \( K_{\text{sc}} \) requires no estimation as it is directly available as an output of the Mie calculations. For a given wavelength of incident light, \( K_{\text{sc}} \) increases with both particle size and refractive index, signifying a higher propensity to scatter.
The second component accounts for the scattering profile anisotropy. What new direction will a photon take after scattering? This term is denoted $I_\theta$ for the angular dependence of scattering, and represents the fraction of the scattering profile directed in the specified direction. Only $90^\circ$ angle scattering was considered to match the fixed angle of the fluorometer, but this term could be adjusted for instruments capable of measuring at different angles. $I_\theta$ was calculated from Mie outputs by dividing the predicted scattering at $90^\circ$ by the sum of scattering from 360 total angles across the xy plane.
Figure 11. Angular dependence vs. size. $\lambda=400$ nm, RI=1.45
As particles become larger relative to incident wavelengths, scattering becomes increasingly anisotropic. $I_0$ then decreases with particle size as the overall scattering profile becomes biased towards forward scattering. Because $K_{sc}$ and $I_0$ undergo opposite trends with size, their product qualitatively already agrees with the anticipated SOS intensity peak as a function of particle size. This suggests that the peak observed in SOS measurements represents a fundamental size dependent property for scattering, instead of a new emerging physical property. The data in figure 9 considers a fixed quantity of particles, that is, it ignores the mechanism by which particles grow in actual samples. The final term ensures that the model obeys the law of mass conservation.
The Mie Model: relative particle concentration N

The final component for the Mie Theory model accounts for the variable particle quantity. When PNIPAm samples are heated above the LCST, the polymer solvent interactions become less favorable which manifests both as a coil to globule phase transition and interchain aggregation. Because the system is closed during measurements any size increase due to aggregation events requires the total particle count to decrease, which decreases measured scattering. The Rayleigh model accounts for this problem comparing volume occupied by particles against the solution volume, which remains constant regardless of particle size. The Mie model does not benefit from such a parameter since \(K_{sc}I_0\) treats the scattering profiles of individual particles. The relative number of scattering particles, or \(N\), accounts for the missing concentration component. In fact, both the relative number and the absolute number of scattering particles will give a mathematically equivalent result. However, this only occurs because the final product is normalized, a choice justified in the following section. The label is chosen to closer conform to the model’s intended purpose and limit confusion. \(N\) decreases rapidly with size since particle volume scales with the radius cubed.
Because the scattering efficiency, angular dependence of scattering, and relative number of scattering particles all depend on particle size, the intensity can be considered a function of particle diameter $I=f(d)$. From Figure 13 it is apparent that the reverse case does not hold; a single intensity value can indicate two different particle sizes. Each Intensity measurement cannot be treated in isolation and must be interpreted within the context of a dynamic system with trends on particle size. Detailed procedures for performing these calculations are included in the methods chapter.

The addition of the relative particle concentration $N$ term causes the predicted peak to shift towards smaller particles. Using these three terms in concert achieves a qualitative...
representation of SOS data sets, but an additional consideration is necessary before treating actual data.

**The Mie Model: intensity ratios vs. raw intensity**

The predictive model is incompatible with raw intensity data for two major reasons. First, the absolute intensity will be dictated by the overall particle concentration. $K_{sc}I_0N$ indicates relative scattering differences based on particle size. $N$ accounts for changing number of scatterers in a closed system but does not account for differences between systems at varying polymer concentration. Because of even slight variations between separate samples it becomes unreliable to compare intensity values from two different data sets. Second, SOS intensity measurements are performed without a reference. Unlike instruments collecting transmission measurements the fluorometer outputs are arbitrary and do not rely on an internal calibration. Without that calibration step SOS data are highly susceptible to variations in instrument condition.

To circumvent this issue, raw intensities are dropped in favor of an intensity ratio. An internal reference point within the measurement data is selected to obtain intensity ratio values which can be compared across multiple data sets. Samples with aggregation phenomena achieve different particle sizes at different rates. The most reliable choice for a reference occurs when particles grow large enough to reverse intensity trends, at the peak scattering intensity. According to the Mie model, the highest intensity value corresponds to a specific predictable particle size. All raw intensity values are therefore converted to a ratio value between 0 and 1, $I/I_{\text{max}}$, and calculated size outputs can be compared between different samples. By definition then, the original intensity measurements are abandoned upon normalizing $K_{sc}I_0N$ (eq. 4).
Under ideal conditions $I_{\text{max}}$ is retrieved directly from the analyzed data set, measurements lacking a defined peak require additional steps. Without a value for $I_{\text{max}}$ an intensity ratio cannot be obtained. Therefore the user must apply one of two approaches. The preferred option is to perform the experiment at a different excitation wavelength. Even though refractive index changes slightly with wavelength, while investigating polystyrene samples the $I_{\text{max}}$ occurred when particle diameter $d = 0.4\lambda_{\text{ex}}$ across a variety of wavelengths. As long as the refractive index is known (or reasonably estimated) for the intended wavelength, shorter wavelengths can accentuate $I_{\text{max}}$ for smaller particles and likewise longer wavelengths accentuate $I_{\text{max}}$ for larger particles. A problem with selecting a different wavelength is that organic functional groups typically absorb in the UV regions, while detector sensitivity plummets at lower energies; selecting a new wavelength may still be insufficient to enable the desired peak. A more drastic approach is possible but far from ideal. By slightly altering sample conditions the necessary particle size might be achieved to create a peak and the corresponding $I_{\text{max}}$ value measured, which would then be applied to interpreting the data set lacking a peak. Because the $I_{\text{max}}$ comes from a different sample extreme care must be taken not to alter key parameters such as particle concentration (mg/mL) and refractive index.

Finally, it should be noted that all sizes calculated by the Mie model are an average value. In practical terms macromolecules and the particles they form exist as a distribution of sizes and are never truly monodisperse, excepting biological examples such as proteins. Light scattering is biased towards larger particles making it difficult to reliably resolve experimental distributions. In depth modeling and calculations are routinely employed to address this dilemma and outputs should always be considered as an estimate. Our model cannot address size distributions. Sizes were assumed perfectly monodisperse to simplify calculations. This assumption was deemed
appropriate because any attempt to account for experimental distribution would itself require assumptions which could not be verified without alternative experimental techniques.

**SOS method validation using polystyrene beads**

The ultimate goal for this prediction model is to enable a direct accurate interpretation from SOS intensity measurements to calculate particle sizes. Achieving this aim requires a careful series of control experiments. We applied the method to Polystyrene (PS) particles to assess the model’s accuracy and to gauge the effect of concentration. The PS particles are well characterized standards with hard diameters spanning the range of sizes expected of our aggregating PNIPAm samples. These standards were graciously synthesized and provided by Pei Zhang of the Tsavalas research group. The PS particles used were prepared at narrow distributions and characterized at 52 nm, 101 nm, 151 nm, and 206 nm. These four PS sizes were used to simulate a controlled aggregation process. While the number of total scattering particles decreases throughout an aggregation process, as particles combine the overall concentration of scattering material remains constant. Aggregation is simulated by choosing a fixed total concentration, 0.05 mg/mL in this case, and varying the chosen particle size. The colloidal stability of PS is also ideal for quickly collecting data at numerous polymer concentrations and excitation wavelengths.
These data confirmed the long standing hypothesis that SOS peak data causally resulted from particle size trends. From smaller to larger particle sizes the measured SOS intensity...
increased and then decreased. After normalizing across each of the four chosen excitation wavelengths, 375 nm, 400 nm, 425 nm, and 450 nm, another qualitative trend appears. The relative SOS signal at longer wavelengths goes down for 101 nm particles conversely goes up for 206 nm particles (Figure 14), which agrees qualitatively with previously shown PNIPAm SOS data (Figure 4). The 375 nm series appears as an outlier in the data set due to the 151 nm particle data point. A measurement of 1,000 arbitrary units suggests the sample likely exceeded the instrument’s detection limit. This limitation influences the entire 375 nm wavelength data set when making normalized comparisons. For this reason this data point is omitted from subsequent comparisons. The sought after prediction model must account for observed changes in relative intensity across varying wavelengths. The expected link between particle size and wavelength combinations that generate $I_{\text{max}}$ would imply that the prediction curve should shift towards larger sizes at longer wavelengths.

To compare the PS measurements to model prediction the data was first normalized. The maximum value needed to perform the division was obtained by first assuming the highest intensity of the four available data points agreed with the model. The highest presumed intensity was then estimated according to the expected comparative ratio. That estimated value was used to normalize the four data points for each series, three in the case of 375 nm excitation series. By definition the highest measured intensity would appear to agree with the model, but deviation between prediction and experiment for the other three particle sizes would indicate failure on part of the model. When performed, the intensity ratios were inadequately described by $K_{sc}I_0N$. The calculated outputs from Eq. 4 showed that qualitative shift, but did not yet agree quantitatively.
The $K_{sc}I_0N$ would require further modification to accurately describe experimental results. An additional constant or mathematical operation could improve the model. The previous experience of optimizing the Rayleigh model made this approach unappealing however. Instead, we searched for an experimental or theoretical justification to adjust the model. In its current form the model contained two scattering terms, $K_{sc}I_0$, and one concentration term, $N$. The concentration term corrects for the changing number of particles as size changes, but does not account for bulk solution properties. Measurements at differing mg/mL concentration were gathered to scan for yet unconsidered qualities.
Figure 15. Concentration effects PS SOS intensity.

Two of the PS standards were measured at different wavelengths across a wide concentration range. Results are plotted on a log scale to demonstrate the severe nonlinearity
between concentration and intensity (Figure 15). These findings briefly prompted a logarithmic correction, \( \log(N) \). This correction proved insufficient; while the 52 nm particles showed some similarity, the 101 nm particles wildly depart from a log relationship over the same concentration range. Increasing particle concentration has diminishing returns on intensity, and can eventually lower overall intensity as shown by the 101 nm particles. After trying multiple exponential corrections, \( N^{1/6} \) gave the smallest deviation from PS measurements and therefore the most optimal result (Figure 16). We attribute this behavior generally to the inner filter effect, which implies that sufficiently low concentrations would restore linearity. This explanation is itself qualitative and does not readily rationalize the necessity of a \( 1/6^{th} \) power correction. For this reason the new prediction model is considered empirically derived.

\[
\frac{I}{I_{\max}} = K_{sc}I_0N^{1/6}
\]  

(5)
Figure 16. Mie model validation.

The final empirical adjustment to the Mie prediction model caused a small shift in predictions, but it was sufficient to the initial goals for accurately describing SOS intensities. The accuracy improves for larger particles, becoming accurate to within 10% where $I/I_{\text{max}} > 0.4$, but said value is influenced by the 375 nm series with a comparatively less complete characterization with only three intensity values. For the 400 nm, 425 nm, and 450 nm series the accuracy further improved to within 6%.
**The Mie Model: the role of refractive index**

Dependence of scattering upon the RI was investigated and found to minimally impact particle size predictions. Although the RI of PNIPAm is accepted as 1.45, the exact water content within PNIPAm particles is difficult to determine. The value 1.45 was compared against 1.38, an exaggerated value chosen to represent an unreasonable contribution from water, to gauge the relevance of differing RI by as much as 0.07. The change in RI caused, on average, a 3–4% difference in calculated size. The same trend was found when the model was computed using RI in the range of 1.45–1.628 for PS. This suggests that the dependency of RI on wavelength affects particle size predictions minimally, considering that between 400nm and 700nm, the RI of PS shifts by a smaller margin of 0.045.
CHAPTER 3

THE BINDING OF Cu(II) AND Zn(ii) TO POLY-N-ISOPROPYLACRYLAMIDE

Summary

This chapter details the discovered interaction between dissolved transition metal ions and the PNIPAm system. This work originated within sensor development research and while it did not attempt to build a sensor, the findings are largely relevant to that field. Using the method described in chapter 2, the physical effect of metal on PNIPAm is explored. The findings are conclusively linked to a direct binding phenomenon between metal ion and PNIPAm chain. Finally, a mechanism is proposed to rationalize the seemingly disparate experimental observations.

pH Effects

SOS experiments examined PNIPAm’s thermal transition alongside free metal ion. Samples included relatively short (approx. 100 repeating units) RAFT synthesized linear PNIPAm chains, a nitrate metal salt, and a pH buffer. By using an external temperature control unit the collected data viewed the heating from room temperature to 30°C, and then the more relevant ramp from 30° to 35°, for 40 minutes. Scattering intensities began increasing within seconds of modifying temperature settings. It became immediately apparent that samples with and without metal gave starkly different scattering profiles. Namely, those with added metal ion
gained a pronounced intensity peak with shape and position both dependent on the metal’s concentration. According to the interpretation method described in chapter 2, these scattering profiles relate to the PNIPAm aggregates forming above the LCST, with sharper peaks indicating larger particles. Control experiments found no evidence of scattering from insoluble metal or buffer compounds contributing to the profiles, only PNIPAm containing samples demonstrated any noteworthy intensity. We therefore concluded that metal ion promotes larger PNIPAm particles rather than producing a complementary, but independent, increase to the sample’s average particle size.

One of the earliest hypotheses on these phenomena considered bulk solution properties before moving onto direct interactions. Samples used the same stock solutions for preparation and experienced identical temperature changes. Both pH and ionic strength can shift with additional metal salt. Stronger Lewis acids cause similarly stronger decreases in pH. Experiments commonly included Cu$^{2+}$ or Zn$^{2+}$ because of the original sensor goal. Though Cu$^{2+}$ has greater acidity both metals readily form insoluble hydroxides near neutral pH. If the PNIPAm behavior substantially changes at different pH, then the observed metal dependencies could simply result from indirect effects, and be irrelevant for stable solutions. SOS measurements for pure PNIPAm with no metal present at pH 5.3 and 6.3 gave substantially different results. PNIPAm formed particles over 50% larger in the more acidic solution and no experiments explored the effect further.

Lower pH caused qualitatively similar results to higher metal concentration, larger PNIPAm particles. Because we were concerned primarily with metal dependence samples included a pH buffer to eliminate confusion between two separate influences. Planned experiments covered metal concentrations from micromolar to millimolar ranges. Thus, 0.1M
buffer, a relatively high concentration, ensured that any sample with Cu$^{2+}$ or Zn$^{2+}$ had at least 5 fold excess. Buffered samples continued to display metal dependence, eliminating pH as the driving factor.

Figure 17. PNIPAm SOS measurements. pH 6.3, 5.3
Ionic Strength Effects

The ionic strength experiments that followed further revealed the metal’s significance. Samples compared the impact of three different metal salts at equivalent ionic strength against the pure PNIPAm. Nitrate salts of Cu(NO$_3$)$_2$, Zn(NO$_3$)$_2$, and KNO$_3$ provide freely soluble metal ion for comparing cationic effects. Intentionally including K$^+$ gives an example with extremely weak binding compared to Cu$^{2+}$ and Zn$^{2+}$, while still coming from the same period. Metal acidification cannot alter pH in sufficiently buffered solutions but it can complicate ionic strength. Ionic strength scales with the square of the charge’s magnitude, so while the total
charge remains constant during hydroxide formation the ionic strength slightly decreases. This effect was however assumed negligible given the magnitude difference between sample’s metal salt and buffer concentrations.

Despite sharing the same ionic strength all four samples produced different behaviors. In agreement with hypothesis, KNO$_3$ had the smallest influence on PNIPAm’s LCST transition. Zn(NO$_3$)$_2$ produced greater impacts to scattering behavior, indicating an even bigger increase in size than shown by the previously discussed pH effects. The Cu(NO$_3$)$_2$ sample began precipitating a blue-green solid during data collection, making the scattering nonsensical and justifying its omission from figure 19. While we predict more potent metal effects for Cu$^{2+}$ than Zn$^{2+}$, the color and concentrations too easily align with precipitating Cu(OH)$_2$ to claim PNIPAm involvement. Most notably, Zn(NO$_3$)$_2$ altered PNIPAm’s transition considerably more than KNO$_3$ despite having only one third its concentration. This demonstrates the crucial role that metal identity has in influencing PNIPAm behavior.
The Effect of Metal Concentration

Both Cu\(^{2+}\) and Zn\(^{2+}\) cause PNIPAm to form larger particles when heated above the LCST. The sizes become progressively larger as metal concentration increases. In both cases the metal ion can more than double the size of PNIPAm aggregates, at 0.05 mg/mL aggregates originally barely over 100 nm eventually approached 250 nm. PNIPAm adopts the same type of new behavior for Cu\(^{2+}\) and Zn\(^{2+}\), and even K\(^{+}\), but the each metal requires varying quantities to achieve this. As metal concentration increases from zero, Zn\(^{2+}\) first alters particle size at 1 mM. Cu\(^{2+}\) needs only 0.09 mM to produce the same effect, a difference just slightly over 1 order of magnitude. PNIPAm also appears less sensitive to marginal increases in Zn\(^{2+}\) compared to Cu\(^{2+}\); a fivefold increase in Zn\(^{2+}\) produces similar results to when Cu\(^{2+}\) increases by less than half that
amount. Even at 30 mM, K⁺ failed to even promote particles larger than 160 nm. The collective evidence for unique metal dependent behavior prompted a search for a mechanistic explanation.

Figure 20. Calculated particle sizes at varying Zn(NO₃)₂.

0.1 M MOPS Buffer pH 6.3, 0.05 mg/mL PNIPAm 10,200 Mₙ, λ = 400 nm
Covalent changes via metal catalyzed reactions were initially considered but quickly rejected. Scattering profiles remained intact after repeated cycles of heating and cooling the sample. Less than 5 minutes elapse between preparing the sample from stock solution and crossing the LCST. Any covalent changes would have to conclude within that time since reproducible scattering profiles indicate chemical stability. Furthermore, three separate metals, one of them from the alkali family, would have to participate in highly similar reactions.

Figure 21. Calculated particle size at varying Cu(NO₃)₂

**Metal Removal Studies**
A non-covalent metal binding interaction seemed a more reasonable explanation. The difference between Cu$^{2+}$ and Zn$^{2+}$ matches Irving-Williams series predictions for a given ligand’s relative affinity for first row transition metals. If correct, then samples would behave similarly to intended sensor regimes; PNIPAm’s behavior would ignore total metal and respond only to free metal ion. Isolating the metal ion from the PNIPAm chain using a highly competitive ligand should cause scattering behavior to mimic samples containing no metal.

Figure 22. Zinc removal study using EDTA, raw SOS.

5 mM Zn(NO$_3$)$_2$, 0.1 M MOPS Buffer pH 6.3, 0.05 mg/mL PNIPAm 10,200 M$_n$, $\lambda$ = 400 nm. Black colored data show varying Zn(NO$_3$)$_2$ samples without EDTA for comparison.
Metal chelation experiments successfully reversed PNIPAm behavior, but also provided additional mechanistic insight. First, an initial heating cycle established a baseline comparison for 5 mM Zn(NO$_3$)$_2$. After cooling the sample to room temperature 1 equivalent of the high affinity chelator ethylenediaminetetraacetic acid (EDTA) joined the solution and immediately another heating cycle began. This process was repeated a second time with an additional equivalent of EDTA. The sample was then stored at room temp for 18 hr before performing one final heating cycle. As expected, after adding EDTA the scattering profile began to behave

Figure 23. Zinc removal study using EDTA, processed SOS.
similarly to samples with less than 5 mM Zn$^{2+}$. However, the time required for the sample to closely resemble 0 mM Zn$^{2+}$ far exceeded expectations. EDTA diffuses at a slower rate than its binding kinetics, and fully binding Zn$^{2+}$ should require merely a few seconds. It follows that something in the sample interfered with the chelator, slowing its ability to scavenge metal.

Figure 24. Copper removal study using EDTA, raw SOS

0.2 mM Cu(NO$_3$)$_2$, 0.1 M MES Buffer pH 6.3, 0.05 mg/mL PNIPAm 10,200 M$_n$, $\lambda = 400$ nm.
The sample only contains one component with sufficient steric bulk to interfere with EDTA, the PNIPAm chains. EDTA did successfully bind Zn$^{2+}$ and cause the sample to closely mimic samples without metal, but the effects did not manifest immediately. This prompts two viable explanations: (1) PNIPAm intrinsically interferes with EDTA and greatly slows its binding kinetics, or (2) PNIPAm shields the metal from EDTA while in its hydrophobic conformation. The Cu$^{2+}$ study gave the perfect chance to test these possibilities. Permitting an additional 15 minutes at room temperature after adding EDTA allows for the PNIPAm
aggregates to fully separate into their hydrophilic forms. The next heat cycle showed that size increases caused by metal had completely disappeared.

The apparent shielding against EDTA by PNIPAm suggests close association between the metal and polymer aggregates. Additionally, large portions of the associated metal likely reside inside the aggregates. If otherwise, the hydrophobic vs. hydrophilic conformations would give little resistance to EDTA. However, the SOS data supports a slowed metal chelation, but does not completely disprove EDTA binding above the LCST. The SOS technique excels at quickly characterizing changing particles and struggles with more stable samples. To explore this possibility, measurements turned to DLS instrumentation.

DLS measurements. Initial heating stage (left), EDTA addition at 35 °C (center) cool and reheat cycles (right). Samples are exposed to room temperature for 15 min between each heating. 0.05 mg/mL 8,400 Mn, 0.1 M pH 6.3 MES Buffer, 0.2 mM Cu(NO₃)₂, 0.36 mM EDTA.

Figure 26. Temperature effects on Cu²⁺ removal by EDTA.

DLS enabled viewing the PNIPAm system after it finished transitioning above the LCST. A Cu²⁺ containing sample was first raised above the LCST in similar fashion to the SOS. Instead of cooling the sample it instead received 1 equivalent of EDTA and maintained temperature for another 45 minutes. During that time the PNIPAm particles maintained a stable size. Notably, EDTA actually caused the particles to grow within the short gap between measurements. Metal
on the particle surface is removed by EDTA which partially destabilizes the PNIPAm, prompting further aggregation. This explanation requires several additional experiments to defend the claim, and receives further justification in the proposed mechanism section.

**Equilibrium Dialysis and Definitive Metal Binding**

Light scattering experiments continually gave hints of metal binding, but could not deliver irrefutable evidence. Most typical experimental approaches suffered from that same limitation. UV-Vis can view shifts in absorption peaks due different bound ligands, but Zn$^{2+}$ lacks visible absorption bands entirely. Though Cu$^{2+}$ has a broad d-d band, the low intensity and low metal concentration together make UV-Vis insufficient to establish binding. The polymer’s flexibility also hinders the NMR and crystallography technique’s reliability. An extensive search for definitive proof ended with equilibrium dialysis.

Equilibrium dialysis utilizes diffusion to demonstrate binding and calculate binding affinity. A semi-permeable membrane separates two chambers, one containing free ligand and the other free analyte, in this case polymer and Cu$^{2+}$ respectively. Crucially, the membrane prevents ligand from leaving its initial chamber, but the analyte can freely pass between them. Because of that requirement, the technique rarely finds application outside polymer and protein chemistry. The Cu$^{2+}$ analyte eventually diffuses across both barriers and eliminates the concentration gradient. However, the ligand can upset the balance by binding Cu$^{2+}$, essentially lowering free Cu$^{2+}$ within the ligand chamber. Additional Cu$^{2+}$ diffuses across the membrane to maintain even free analyte concentration in both chambers. Comparing the final free analyte to its initial concentration allows one to calculate the ligand’s binding affinity.

The determination required multiple considerations. Past experiments performed by members of the Seitz research group indicated that PNIPAm can require substantial time to reach
equilibrium. Samples therefore received over 48 hours before measuring Cu\(^{2+}\) concentration. Atomic Absorption spectroscopy identified the final metal concentration, which necessitates a calibration curve using Cu\(^{2+}\) standards. The first standard batch had an improper matrix which heavily inflated affinity calculations. The standards began incorporating the same buffer conditions present in experimental sample upon learning that extraneous ion sources dampen AA measurements.\(^{38}\)

The unknown bindings forced a concession in order to estimate binding affinity. Equilibrium dialysis presumes knowledge regarding the local binding environment. The calculation builds upon the stochiometrically determined formation,

\[ xM + yL \rightarrow zM_sL_y \]

which gives,

\[ K_f = \frac{[M_sL_y]^z}{[M]^x[L]^y} \]

This presents a dilemma. Measuring metal concentration can demonstrate that binding occurred, but it doesn’t reveal the actual coordination environment. One cannot perform the affinity calculation without selecting a coordination sphere to model. At this stage we settled for establishing binding as a proof of concept. Therefore our calculations assumed the weakest possible binding regime: a 1:1 ML ratio. Furthermore, each PNIPAm monomer was treated as a completely free and available ligand, without spatial constraints imposed by the polymer backbone.

\[ K_f = [ML][M]^{-1}[L]^{-1} \]

\[ [L] = [L]_i - [ML] \]

\[ [ML] = [M]_i - 2[M]_f \]
Table 1. Equilibrium dialysis results.

<table>
<thead>
<tr>
<th>[PNIPAM]</th>
<th>[M]_i</th>
<th>[M]_f</th>
<th>[L]_i</th>
<th>[L]_f</th>
<th>[M]_i : [L]_i</th>
<th>Log K_f</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/mL</td>
<td>2.48 mM</td>
<td>0.489 mM</td>
<td>8.48 mM</td>
<td>6.97 mM</td>
<td>0.293</td>
<td>2.65</td>
</tr>
<tr>
<td>0.05 mg/mL</td>
<td>0.127 mM</td>
<td>0.0223 mM</td>
<td>0.432 mM</td>
<td>0.350 mM</td>
<td>0.293</td>
<td>4.02</td>
</tr>
</tbody>
</table>

Though it simplifies the calculations the assumption demands too many concessions. Two experiments questioned the concept by using two different polymer concentrations while maintaining the same metal ligand ratio. The experiments definitively demonstrated binding of metal ion by PNIPAm, and as expected both experiments led to different calculated binding affinities. We attribute the differences to increased restrictions at higher polymer concentration. The individual amides are restricted by their location on the polymer and have less freedom than assumed for the sake of our calculations. H-bonding between PNIPAm chains and limited flexibility interfere with metal binding and cause effects similar to competitive ligand binding. The lower polymer concentration gave a higher binding strength because of less inter-chain association and more freely accessible amide groups.

**Revisiting LCST of Pure PNIPAm and [M]**

Literature shows that binding metals to PNIPAm using high affinity ligands dramatically shifts the LCST.²³ This study discovered metal binding without such ligands, but phase behavior still appeared between 30 and 35 °C. A question arose regarding the link between the ligand’s binding strength and impact on LCST. Sensor investigations tend to focus on low metal concentrations and may have overlooked behaviors appearing only above the intended trace levels.
DLS studies examined the PNIPAm system at varying temperatures and metal concentration. The transition itself occurred consistently within 0.5° of 31 °C. Aggregates grew in ascending quantities with each 0.5° increment after the transition began. DLS data became erratic after particles grew beyond 2 µm, which we attributed to settling. Consistent with SOS measurements the sizes further increased for samples containing metal salts. Although the bound metal promoted larger particles, the LCST seemed insensitive to any metal effects. This again confirms that metal ions play a major role in dictating PNIPAm dimensions even without high affinity ligands. Without high affinity ligands the bound metal has no noticeable impact on LCST thermodynamics.
Figure 28. LCST changes in copper samples.

Varying Cu(NO₃)₂, 0.1 M MES Buffer pH 6.3, 1 mg/mL PNIPAm 8,400 M₅.
Figure 29. LCST changes in copper samples, zoom.

High Cu$^{2+}$ concentrations gave an unanticipated result. Increasing Zn$^{2+}$ concentrations from 2.5 mM to 25 mM prompted more of the same, sizes further increased. From 0.25 mM to 2.5 mM, Cu$^{2+}$ produced an entirely new behavior. The transition again initiated at the same temperature, but with completely dissimilar particles. Sizes approx. tripled below the LCST and compared to the other samples grew more slowly upon crossing the LCST. Most surprisingly, near 40 °C the particles stopped growing and the system stabilized below 800 nm, and presented an apparent contradiction. The high metal conditions promoted larger particles below the LCST where PNIPAm is most well solvated, and arrested aggregation above the LCST where particles had otherwise grown large enough to render data questionable.
Proposed Binding Scheme of Metal Ion by PNIPAm

The previous sections presented evidence supporting metal ion’s non-innocent role in PNIPAm phase behavior without offering a mechanistic explanation. This final section combines each study’s findings into a single rationale. Equilibrium dialysis demonstrated binding interactions between PNIPAm and Cu\(^{2+}\). We surmise binding interaction with Zn\(^{2+}\) as well given the general agreement across the many scattering measurements, though instrumental limitation prevented confirmation. PNIPAm’s physical behavior changed for the metallic systems because metal ions repeatedly bind the amide functional group, likely via the carbonyl oxygen. The metal ion acted as a weakly bound noncovalent crosslinker, and its impact depends on concentration.

Our reasoning suggests the formation of three different binding motifs.

\[
\begin{align*}
M + L & \rightarrow ML & (1) \\
ML + L & \rightarrow ML_2 & (2) \\
ML + L' & \rightarrow MLL' & (3)
\end{align*}
\]

L and L’ represent NIPAm amide groups from two different polymer strands. ML is the metal associated polymer, and ML\(_2\) and MLL’ are the intra and inter strand linkages respectively. After forming ML, local concentrations of L exceed L’ and further binding is expected to favor intra strand linking. ML\(_2\) could possibly encourage a more tightly collapsed globule, and although we suspect its formation, there is no evidence for its presence via SOS and DLS reaction studies. ML\(_2\) formation restricts PNIPAm’s flexibility and is limited by the polymer’s molecular weight. Sufficient metal concentration will consume available L and the additional ML will then combine with L’. These inter molecular linkages cause the observed increase to
particle aggregation. Our scheme predicts several distinct binding regions according to the amount of metal.

I) Low Metal Ion Concentration.

Reactions (1) and (2) proceed to form ML$_2$ in limited quantity with available metal ion. Without ML, reaction (3) does not occur and there is no measurable change in size above or below the LCST. From the calculated binding constants it is clear that the metal is bound even when at low concentrations, but as shown in figure 20, scattering intensities do not change until enough metal is available.

II) Medium Metal Ion Concentration.

There is now enough metal to form both ML and ML$_2$ below the LCST. MLL’ possibly forms here but is unstable due to favorable solvent polymer interactions and cannot hold multiple chains together. MLL’ quickly reverts to ML and L’ causing no change in particle size below
the LCST. Above the LCST reaction (3) instead favors product due to the less favorable polymer solvent interaction. This promotes aggregation processes and leads to particle sizes greater than in region I (Figure 20).

III) High Metal Ion Concentration.

[ML] is now much higher below the LCST and MLL’ persists after formation. With additional MLL’ linkages able to form between chains, particle collisions can now result in aggregation events; size increases below the LCST. Above the LCST, the surface of growing particles contains available binding sites L and L’. These sites become saturated with metal forming ML and ML’. As [L’] diminishes reaction (3) arrests. With surfaces now covered in positive charge, collision events become unfavorable and aggregation arrests, resulting in particles far smaller than those in concentration regions I and II. This is demonstrated by two distinct observations: First, in Figure 26 the complexation of surface bound Cu$^{2+}$ by EDTA slightly destabilizes the suspension causing an increase in size. Second, the size trends reverse in Figure 28-29 when Cu$^{2+}$ concentration was increased to millimolar levels.

Zinc samples do not achieve region III even at concentrations as high as 25 mM. It is clear from the SOS results that Zn$^{2+}$ exhibits weaker binding to PNIPAm than Cu$^{2+}$. With more labile binding it likely cannot saturate the PNIPAm surface. Crosslinking reactions continue and lead to large particles.

We have considered an alternative possibility that metal presence could swell aggregates by increasing solvation. Since these PNIPAm chains lack covalent linkages the additional solvent could eventually lessen chain-chain interactions to the point of fragmenting the aggregate into smaller particles. This could explain, in part, the results shown in figure 28-29. The implication being that bound metal was able to make solvent-polymer interaction more favorable.
than polymer-polymer interaction above the LCST, effectively increasing the overall solubility. Similar behavior is common for PNIPAm copolymers that include high affinity ligands. However, these changes in solubility are accompanied by a substantial shift in LCST and this study found no such evidence for pure PNIPAm. Furthermore, it seems improbable for the effect to be capable of separating chains above the LCST, where interaction with solvent is least favorable, while simultaneously being incapable of producing the same result below the LCST despite the more suitable conditions. Indeed, the opposite is observed; particle sizes increase below the LCST at high metal concentration.
Conclusions

The preceding project set out to test a hypothesis of metal-polymer interactions between PNIPAm and late transition metal ions Cu(II) and Zn(II). We successfully demonstrated that metal can bind directly to acrylamide monomer, and that such binding influences LCST phase behavior with regards to observed particle sizes. To achieve that end we designed and verified a novel light scattering methodology capable of monitoring aggregating particles. The research focused on a single thermoresponsive polymer because of its widespread use in developing new sensor technologies. Within that context we see three available avenues for continuing this line of research.

1) Exploring additional metal species beyond Cu(II) and Zn(II), most notably Fe$^{2+/3+}$ given its biological relevance and natural abundance.

2) Examining how the metal’s influence on PNIPAm’s physical behavior changes when additional comonomers are included during synthesis.

3) Assessing the role of tertiary structure on the effects of metal binding by testing non-linear PNIPAm systems.
II. THE SYNTHESIS AND CHARACTERIZATION OF NICKELALACTONES CHELATED
BY P-N-P PINCER LIGANDS
FORWARD

Due to a tonal shift in research the following chapter deserves its own separate section from the previous chapters. The change in direction stems a practical reality of performing research; we did not anticipate the necessary time and effort needed to reach a satisfying conclusion to the PNIPAm research topic. We felt it necessary to take those findings to completion, and after managing it we had traveled considerably outside our normal chemical expertise. We therefore adopted a markedly different project to continue research within a more traditional inorganic discipline. The previous section focused on methodology and describing relatively large materials, and primarily employed light scattering techniques. This following section explores small molecule synthesis of inorganic metal complexes, and emphasizes NMR experiments and discussion.
CHAPTER 4

THE SYNTHESIS AND CHARACTERIZATION OF NICKELALACTONES CHELATED BY PNP PINCER LIGANDS

Summary

Herein we describe investigations on a catalytic nickel system for converting ethylene and carbon dioxide into acrylic acid. Presently, the field struggles with the conversion from the nickel(II)lactone intermediate to nickel bound acrylate. In order to better understand this reaction step, we isolated a series of nickelalactones chelated by varying L type ligands. The novel compounds were thoroughly characterized by NMR spectroscopy. Observations on the lactone’s general stability were used to infer preferred ligand characteristics for improving nickel’s catalytic output.

The chapter begins by establishing the motivations behind this research. A literature review will briefly detail the challenges facing the field and how it has progressed thus far. We explain the ligand design principles used to further our understanding of the nickel system. Because of its crucial role in characterizing the isolated compounds a discussion of NMR theory is also provided.

Ni catalyzed acrylic acid production

Chemical innovations take a variety of forms. Hydrocarbons store ample supplies of energy, but without the internal combustion engine they only find use as inefficient heat sources.
Before the advent of electronics, spectroscopic measurements relied on optical tools. Chemistry shares in the benefits from outside fields such as physics and engineering. When it comes to new avenues for manipulating bonds others look to chemist’s expertise. Synthetic chemistry achievements fall into several categories: discovering new reaction mechanisms, optimizing yields, developing improved synthetic pathways, and solving problems of scale. Each of these marks an important step for both fundamental understanding and application. Even so, nothing else excites chemists as much as that initial reaction discovery.

Every novel reaction conquers our current limitations. What was once inconceivable transforms into brand new possibilities. Sometimes this involves a creative answer to a practical constraint, such as solubility, pH, temperature, or lifetime. The difficulty lay not in underlying chemistry but rather the circumstances which surround it. Some reactions involve such a high energy barrier that practically speaking the product never appears. For such examples the only way to solve the problem directly requires a catalyst. Catalysts provide a unique scaffold that allow for more stable intermediates, which lower the activation energy and make the reaction proceed far more quickly. The Haber-Bosch process uses catalysts to perform the most important reaction in the world, nitrogen fixation. A desire to perform reactions involving large thermodynamic or kinetic difficulties inevitably leads to a search for effective catalysts.

Metals are uniquely suited for catalytic behavior. Catalysts need to perform specific tasks in sequence: promote association between reactants, permit otherwise unlikely transformations, release product to surrounding media, and finally return to its initial conditions ready to continue the cycle. Metals oftentimes can accommodate six or more binding sites with ligands attached at varying affinities. Through careful design one can tune the metal center’s electronic properties via ligand choice. By including open or labile binding positions the metal
can receive the intended reactants. Metals also have access to numerous oxidation states allowing them to help temporarily shuffle electrons and form new bonds. Each family has its own unique characteristics making it possible to pick the ideal metal for the intended task.

The 10th periodic family is well suited for C-C bond formation. Being at the latter end of the transition metals nickel, platinum, and palladium have a relatively high d-electron count. They can easily access both their 0 and 2+ oxidation states making them excellent candidates for promoting two-electron processes. Additionally, they are generally softer acids than earlier members of their row and readily form bonds with olefins. Ni, Pt, and Pd are all routinely used for a wide range of coupling reactions. A trend towards environmentally and geopolitically conscious chemistry has sparked interest in the highly abundant first row transition metals. Recently numerous reports have emerged using nickel to perform olefin insertion, cyclization, and carbon dioxide incorporation. This momentum is perfectly timed with new developments in a long standing catalytic challenge.

Our interests lie in using nickel to produce acrylic acid, a commodity feedstock for making polyacrylates. Acrylate monomers are currently synthesized from propylene and require multiple reaction steps. But propylene is itself an unwanted side product from ethylene production and will become less available as those processes optimize. Since the 1980s researchers have sought an alternative pathway to form acrylates from ethylene and carbon dioxide. Successfully scaling such a reaction would be highly lucrative and provide a means for recycling carbon dioxide, a highly stable waste product in no short supply. For several decades this goal went unrealized.

The specifics of the catalytic cycle frustrated chemists for some 30 years. It was first proposed that CO2 would insert into the nickel bound ethylene to form a nickelalactone
intermediate. An α proton hydride shift to the nickel center then forms the intended olefin. Ligand exchange with free ethylene followed by a reductive elimination liberates the acrylic acid and resets the catalyst. Unfortunately, the hydride shift never occurs. The lactone’s ring structure prevents the proton from orienting towards nickel to form necessary interactions. The distance between the two atoms is too great and the cycle arrests at the lactone stage. In 2012, Limbach reported the first major breakthrough for “catalytically” forming acrylic acid, though the cycle was divided into two distinct steps and turn over numbers (TON) barely entered double digits.\(^49\) Two years later Vogt demonstrated the cycle’s success in a true one pot reaction,\(^50\) only two months before Limbach followed through with the same achievement.\(^51\)

Their insights complement each other so effectively that they should receive dual credit for the discovery. In each case, they rely on stoichiometric equivalents of Brønsted–Lowry base to abstract the proton. Most bases strong enough to remove that proton are rendered useless under the reaction conditions because they competitively form stable carbonates with CO\(_2\). Aware of this potential setback, Limbach opted to avoid the side reaction by splitting his cycle into two steps and then investigate appropriate bases afterwards. That decision likely gave Vogt
the window of opportunity to achieve the one pot system first. But in the end, Limbach’s phenoxide bases achieved TON numbers up to 107, a fivefold increase over Vogt’s system. Vogt encounter initial success with trimethylamine and chose not to pursue it further. Both researchers present similar mechanistic schemes with one notable distinction.

Based on the evidence available Vogt provides the more accurate mechanism. Limbach hypothesizes that the added base deprotonates one of the lactones two α protons, prompting reductive elimination. Vogt instead predicts the lactone first undergoing Ni-O bond cleavage. Free of its restrictive ring strain the α protons can orient towards the nickel, form agostic interactions, and perform a β-hydride elimination. The added base then facilitates reductive elimination by abstracting the nickel bound proton. It all hinges on the liberated carboxylate. Vogt predicted that a hard Lewis acid, such as Li⁺ and to a lesser extent Na⁺, could competitively bind the carboxylate and discourage its reattachment to nickel. Preventing Ni-O bond formation encourages the hydride shift and allows the cycle to proceed. No direct evidence for the predicted intermediates exists, but the prediction agrees with Limbach’s observations. Limbach commented that Na⁺ improved catalytic activity when present. Additionally, he noted that as solvents became less polar the same catalyst performed far better (TON = 2 DMF, 10 THF, 13 dioxane, 17 toluene) but offered no further explanation. More polar solvents will stabilize dissolved cation and lower its affinity for free carboxylate, which according to Vogt’s mechanism stifles catalysis. The “dream reaction” had finally come true and by combining each of their findings we have an appropriate mechanism.

The landmark achievement occurred just a few years past and relatively few advances have occurred since. Manzini and coworkers did not advance TON numbers but did establish two important contributions. They designed a regeneration step into their procedure capable of
recycling nickel catalyst without needing sacrificial Zn\textsuperscript{0}, an improvement over both Limbach and Vogt’s methods. Additionally they demonstrated that carbonates formed from alkoxide + CO\textsubscript{2} reactions can still facilitate turn over if their decomposition temperature falls beneath that used during the catalytic reaction.\textsuperscript{52} In a 2018 conference paper Vavasori claims to have reached TON as high as 290 with a Ni(PPh\textsubscript{3})\textsubscript{2}Cl\textsubscript{2} precatalyst. They suggest that nickel is reduced either by the phosphine ligand or ambient phenoxide base. Unfortunately, they offer no experimental evidence pertaining to that claim, and give zero commentary rationalizing how the system outperforms past examples by such a margin.\textsuperscript{53} Earlier that same year, Bernskoetter applied findings from both Limbach and Vogt to optimize the system and achieve the highest reported TON recorded at 404. Curiously, cocatalytic additives such as NaI and Zn\textsuperscript{0} appear to have highly differing influences when applied to varying catalytic platforms.\textsuperscript{54}

**Ligand design**

Thus far reports have touched upon many relevant design parameters. Troublingly, observed effective conditions seem tied to specific catalytic platforms rather than easily discerned trends, making predicting worthwhile ligand structures extremely difficult. The sheer number of permutations makes it impractical to assess each combination. Despite those challenges experiments have already varied: CO\textsubscript{2} pressure, ethylene pressure, catalyst concentration, Lewis acid concentration, sacrificial electron donor concentration, temperature, solvent, Brønsted–Lowry base, equivalents of base, bite angle, and ligand bulk. While such a list may seem exhaustive it poignantly lacks ligand electronics. We set out to fill this apparent gap in the literature by preparing a family of electronically-nuanced ligands, and characterizing their influence on nickelalactone complexes.
We termed this family “PNP ligands” for their common PcNeP structure. The ligands bind in a bidentate fashion through their terminal phosphines to form a six membered ring with nickel. We selected two types of phosphine for our experiments, dicyclohexyl and diphenyl. The electron withdrawing phenyl rings contrast against the electron donating cyclohexyl rings. The dissimilar electronics cause different ligand behaviors while maintaining fairly similar steric bulk and mass. The nitrogen was chosen to serve both long term and short term goals.

The core PNP architecture takes direct inspiration from the Dubois ligand motif. Even in a boat conformation the nitrogen’s lone pair points slightly away from nickel and bears little to no binding character. It has been proposed that abstracting a proton from the nickel is a reductive elimination step necessary to close the cycle. The nitrogen can act as a proton shuttle, carrying bound protons to and from the metal center. Such functionality warranted investigation as a part of our long term goals. The specific R groups on nitrogen satisfied more short term interests.

We used three different R groups to tune the nitrogen’s basicity. The phenyl and benzyl versions vary by a methylene spacer that electronically isolates the nitrogen from the ring system. As indicated by their conjugate acid pKa’s, 4.87 vs 9.34 respectively, the benzyl derivative has far stronger basic character. These two groups allow us to compare a mostly unreactive nitrogen against a reactive analogue in terms of their acid base chemistry.
Additionally, aryl groups have packing characteristics preferable for growing crystals and for isolating solids instead of oils. The final group is more basic than benzyl, pKa = 10.71, but more importantly can immobilize the catalyst. Alkoxysilanes react with hydroxyl groups found on oxide surfaces, such as SiO$_2$ and TiO$_2$, to form stable covalent bonds and release water.$^{56}$ Surface attachment has advantages for separating the catalyst from products and spent reagents. Heterogeneous systems have also been shown to increase catalytic activity owing to the nearby electron reservoir.

**NMR: The Chemical Shift**

While many characterization techniques exist, NMR is by far the most useful method for revealing chemical structures in solution, and an indispensable tool for the synthetic chemist. NMR offers an extensive library of experiments for determining bond connectivity and spatial orientation. We relied upon two fundamental NMR concepts for characterizing our complexes, chemical shift and spin-spin splitting. We will provide sufficient background detail here to understand the conclusions drawn from our NMR experiments.$^{57}$

Given the level of complexity surrounding nuclear magnetic resonance the name itself is surprising forthcoming. Spin active nuclei will exist in multiple spin states. For the simplest case of $m_s = \frac{1}{2}$, two states are available, $+ \frac{1}{2}$ or $- \frac{1}{2}$, also called “spin up” and “spin down”. Under normal circumstances these two states orient randomly and are quite similar with only a small energy difference between them. However, an externally applied magnetic field will forcibly orient the two spins parallel to itself, though still opposite to each other. The external field restricts their orientation making it more difficult to resonate, or spin flip, from the ground state to the excited state. That is, the energy gap between the two states increases according to the strength of an external magnetic field. An NMR instrument imposes that magnetic field and
observes the energy required to induce resonance between a given nuclei’s spin states. Thus we have a simple name for a complicated process. If all nuclei behaved in the same fashion then the technique would be practically useless, thankfully, nuclei resonate at different energies due to multiple factors.

The biggest factor comes from the nuclei’s identity. The total nuclear spin depends on the combined effects of fundamental particles which comprise its protons and neutrons. This leads to an unavoidable annoyance; the total nuclear spin cannot be accurately predicted from an atomic number or mass, and we must commit them to memory or reference tables. Those nuclei with equal numbers of protons and neutrons have no net spin, those with differing yet even numbers have integer spins, and all other mixed combinations have half-integer spin. It follows then that different nuclear isotopes have differing nuclear spins. Relevant examples include $^1\text{H} = \frac{1}{2}$, $^2\text{H} = 1$, $^{12}\text{C} = 0$, $^{13}\text{C} = \frac{1}{2}$, $^{31}\text{P} = \frac{1}{2}$. Isotopic abundance directly informs signal intensities, most notorious in the case of carbon which is 99% NMR inactive. While many different nuclei can have the same nuclear spin that does not equate to equal energy gaps between spin states.

$$\Delta E = \gamma \left( \frac{h}{2\pi} \right) \beta_0 = \hbar \nu$$

Instead, the energy gap depends on the gyromagnetic ratio, $\gamma$, and the magnetic field strength, $\beta_0$. The gyromagnetic ratio depends on the nuclei’s magnetic moment and its angular momentum. Because angular momentum changes with mass no two nuclear isotopes behave identically, making it possible to measure a chosen NMR active isotope without worry of spectral overlap from other elements.

Atoms of the same isotope can still have subtle energy differences owing to the surrounding electron cloud. Moving electrons are themselves point charges capable of producing a magnetic field. The electrons generate an induced magnetic field in direct
opposition to the applied field. Magnetic fields are additive, and the nucleus experiences a smaller effective field than the applied field.

\[ \beta_0 = \beta_{\text{applied}} - \beta_{\text{induced}} \]

The effect is dubbed “shielding” since the induced field acts similar to a protective barrier against the external field’s full strength. The \( \beta_{\text{induced}} \) can be influenced by persistent magnetic fields generated close by, such as those coming from other atoms and functional groups located on the same molecule. Stronger induced fields are considered “upfield” and provide more shielding than weaker induced considered “downfield” and “deshielded”. \( \Delta E \) depends on neighboring atoms making it feasible to rationalize NMR signals according to chemical differences. \( \Delta E \) also changes with \( \beta_{\text{applied}} \) which varies across separate instruments, making absolute energy readings a poor basis for comparison. However, \( \beta_{\text{induced}} \) similarly changes across separate instruments because induced fields are proportional to the applied field.

\[ \beta_{\text{applied}} \propto \beta_{\text{induced}} \]

A given nuclei’s \( \Delta E \) is compared to an internal standard simultaneously experiencing the same external field, usually a small molecule such as tetramethylsilane but the residual solvent signal can also suffice. The difference between these two energies scales proportionally to the applied field,

\[ \frac{\Delta E_{\text{sample}} - \Delta E_{\text{standard}}}{\beta_{\text{applied}}} = \frac{\Delta Hz}{\text{MHz}_{\text{applied}}} = \text{ppm} \]

This new parts per million value is independent of magnetic field strength, allowing for intuitive comparisons between instruments. These values then depend only on \( \beta_{\text{induced}} \), or the surrounding chemical environment. Nuclei at different ppm are said to have different “chemical shifts”.

**NMR: Spin-Spin Coupling**
Independent of chemical shift, nuclei can take on a wide variety of intensity patterns. Though not exclusive, these patterns are predominantly created by spin-spin coupling effects. Nuclear spin states polarize the surrounding electron cloud. Sigma bonds effectively carry that spin information to other nuclei based on orbital overlap, which geometrically depends on dihedral angles. Two nuclei that share in each other’s spin states are considered coupled. The coupled nuclei A experience multiple magnetic environments owing to the available spin states on the coupling nuclei B. These subtle changes in environment very slightly shift A’s resonance frequency, and the original signal “splits” into multiple observed peaks. These energy differences decrease with number of intervening bonds and are small, usually less than 50 Hz for 3 bond- $^3$J -coupling. Notably, these values do not change with applied field strength and are true constants, called “J values”.

These J values combined with the number of spin active neighbors generate a distinctive splitting pattern. Every additional spin coupled nuclei multiplicatively increases the total amount of spin configurations by that nuclei’s number of spin states,

$$\text{# Spin configurations} = 2^n \cdot 3^m \cdot 4^l \ldots$$

where $n$, $m$, and $l$ are the number of coupled nuclei with 2, 3, 4, and so on available spin states. Despite exponential increase in configurations with coupled nuclei, under most circumstances the majority of the configurations are degenerate. Consider the small organic molecule propane, CH$_2$(CH$_3$)$_2$. The central two protons experience three bond spin coupling from all six of the neighboring protons, which have two spin states. The total number of spin configurations is therefore $2^n = 2^6 = 64$. However, each coupling has the same J value, and as such many of these configurations have identical resonance frequencies. The 64 states combine into only seven
energy values with observed signal intensities corresponding to Pascal’s triangle. For most simple cases the number of observed peaks is easily intuited from the molecules structure,

\[
\text{Peak multiplicity} = 2ni + 1
\]

Where \( n \) is the number of nearest neighbors (spin active) and \( i \) is total nuclear spin. Using the propane example: \( 2(6)(\frac{1}{2}) + 1 = 7 \). When discussing hydrocarbons this relationship is typically shortened to the so called \( n + 1 \) rule. As a warning the \( n + 1 \) “rule” is merely a matter of daily convenience since it breaks down for cases where spin \( \neq \frac{1}{2} \), such as deuterium. Additionally, nonequivalent \( J \) values do not adhere to such simple prediction patterns and demand more interpretive effort. Combined together, chemical shift and splitting data help elucidate the molecules overall structure. But, splitting can make spectra complicated enough to thwart interpretation.

To help keep spectra manageable the instrument can negate splitting effects by decoupling the nuclei. In fact, it is standard practice when collecting carbon data to decouple hydrogen. Otherwise the sheer quantity of H-C bonds in organic chemistry would make carbon spectra unmanageable. \(^1\)H nuclei are irradiated to constantly resonate between their two spin states. Instead of two distinct magnetic environments the bound carbon experiences an average environment and behaves like a singlet.

Finally, splitting patterns only occur for magnetically inequivalent nuclei. This fact exists as a consequence of quantum mechanics, which we will not endeavor to rationalize here. To summarize, magnetically equivalent nuclei will spin-spin couple to each other, but it produces no splitting. The configurations either produce degenerate resonance frequencies or invoke a forbidden spin transition and do not occur. In each case the NMR signal appears as a singlet. For interpreting spectra it becomes crucial then to identify symmetry elements and
determine magnetic equivalency.

**NMR: regarding $^{31}\text{P}$**

The inclusion of phosphorus in this project offers a rare opportunity to explore NMR data outside $^1\text{H}$ and $^{13}\text{C}$. Phosphorus has a nuclear spin of $\frac{1}{2}$ in near 100% natural abundance. $^{31}\text{P}$ shares many of the same properties as hydrogen. Notably, one can accurately compare molar equivalents from its integrated peak areas, and it participates in spin-spin coupling with nearby atoms. For each reaction performed, the $^{31}\text{P}$-NMR signals immediately revealed the relative amounts of product and starting material. Coupling effects also complicated carbon spectra. Unfortunately, most NMR instruments lack the hardware to perform phosphorus decoupled carbon measurements. Most notable for nickelalactone complexes, the carbon signals from both Ph and Cy groups on phosphorus atoms showed extensive splitting and overlap, a were indistinguishable.

Phosphorus chemical shifts are themselves consistently unintuitive. They of course adhere to the same principle as other nuclei, more deshielded nuclei have larger downfield shifts. It is quite difficult however to correctly predict deshielding effects from the structure. Consider the two synthetic building blocks used to make PNP ligands: Cy$_2$PH and Ph$_2$PH. Phenyl groups are electron withdrawing while cyclohexyl groups are electron donating. Cy$_2$PH has the more electron rich phosphorus atom, and indeed is a stronger Lewis base that reacts more violently towards oxidation. It is reasonable therefore to predict that Cy$_2$PH has stronger shielding and the more upfield shift. Reasonable, yet ultimately incorrect. In fact, the electron lone pair generates its own magnetic field that disrupts the phosphorus atom’s electron cloud, causing a downfield shift. The electron withdrawing effects on the phenyl rings draw electron density from the lone pair back towards the phosphorus nuclei, making it more spherically symmetrical and less
disruptive. This increases shielding and gives \( \text{Ph}_2\text{PH} \) a more upfield shift than \( \text{Cy}_2\text{PH} \).\(^{58}\) The intuitive misdirection continues even further.

Chemical shift trends amidst a phosphorus motif make poor predictors for differing motifs. For example, given the aforementioned behaviors for two \( \text{R}_2\text{PH} \) compounds, what should we expect of the PNP phosphine upon binding to nickel? The lone pair destabilizes the induced field’s shielding strength, but that electron density can also participate in shielding to some degree. So then, we can reach two plausible but contradictory predictions.

a) Downfield shift upon metal binding. The lone pair participates in shielding. Donating electron density away from the phosphorus lowers its contribution to shielding effects, causing a net loss in shielding.

b) Upfield shift upon metal binding. The lone pair’s magnetic field counteracts shielding effects. Donating electron density away distances the magnetic field from the phosphorus nucleus, lowering the disruptive effects and causing a net gain in shielding.

Upfield or downfield then? Without additional theory on hand or experimental data the answer remains elusive. To be clear, binding of phosphine to nickel produces a large downfield shift for every complex examined here without exception. The example is meant to illustrate phosphorus’ seemingly erratic behavior across different families. Phosphorus compounds manifest across a variety of geometries, oxidation states, and charge. It follows unfamiliar conventions compared to carbon and hydrogen. Phosphorus compounds of varying type have little in common with regards to chemical shift. We therefore warn against relying on trends to predict equivalent behavior in different families without first consulting literature examples.

**Synthesis Discussion**
The desired ligands are obtained through a Mannich type reaction. The Phosphine reacts first with formaldehyde to form a hydroxymethyl phosphine. The phosphine lone pair donates into the methylene bridge forming an equilibrium with the phosphonium ylide and its closely associated hydroxide. The ylide then serves as a suitable electrophile for attack from both primary and secondary amines. The liberated hydroxide scavenges the ammonium proton to form one water equivalent. Stoichiometry permitting, the reaction continues until it reaches a tertiary amine. With careful technique the reaction achieves excellent yields above 95%.

Figure 33. Mannich type mechanism for forming PNP ligands.

Experimental setup demands meticulous technique due to the phosphine’s propensity to oxidize. R₂PH phosphines readily react with molecular oxygen converting to a phosphine oxide. 10% dilutions in hexanes immediately form a white precipitate when exposed to atmosphere. The rapid decomposition is exothermic and presents a significant fire hazard. Prior to reaction, solvents are rigorously deoxygenated using multiple cycles of freeze pump thaw technique. Liquid phosphine reactants are added to the prepared reaction vessel via syringe through suba seal septa. The elevated reaction temperature at 80 °C decomposes paraformaldehyde into the necessary formaldehyde to combine with phosphine. The resulting hydroxymethyl phosphine is
isolable, but also troublesome to work with as it becomes viscous while remaining highly reactive towards oxygen. Multiple attempts to achieve the follow-up reaction with amine struggled with oxidation. Procedure moved to a one pot reaction to minimize transfers and opportunity for O$_2$ contamination.

Though PNP ligand formed as the major product, the mixture contained noticeably more unreacted phosphine than during the two step approach. We linked this outcome to the experimental apparatus. The reaction setup included a nitrogen bubbler to maintain an O$_2$ free environment and a chilled condenser to retain solvent at elevated temperature. We speculated that the hydroxymethyl phosphine could revert to starting materials, and more crucially, that free formaldehyde could escape the reaction vessel through the nitrogen bubbler. The condenser and bubbler were removed in favor of a sealed vessel to prevent formaldehyde loss. Removing the bubbler increases risk for oxidation as gases slowly exchange through small leaks in the glassware. To prevent O$_2$ from entering the vessel, the reactions were thereafter performed within a N$_2$ glovebox.

The synthesized ligands remain soluble in reaction solvents and are isolated under reduced pressure. The workup hinges upon the highly efficient reaction turnover to justify no separation efforts. A mixture will resolve itself naturally after the proceeding ligand exchange, but obtaining pure PNP ligand makes it easier to use the proper stoichiometry. The water byproduct also warrants concern. Water can oxidize phosphines and risks deactivating the nickelalactone. Toluene as a solvent ensures that water phase separates to bottom of the glassware. Removing the organic phase via pipette before reducing pressure minimizes chances for water to react with phosphine. The two ligands prepared using aminopropyltrimethoxysilane (APTMS) suffered from even this brief exposure and typically lost between 35-50% of their
methoxysilane groups. Thankfully, no extended Si-O-Si structures were observed and the ligands isolated as homogenous clear oils. As expected, the phenyl rings on aniline and benzyl amine derivatives offer improved packing over the propyl silane and isolate as fibrous solids.

New nickel carbonyl compounds using these PNP ligands formed without incident. At elevated temperature, PNP ligand readily undergoes ligand exchange reactions with Ni(PPh\textsubscript{3})(CO)\textsubscript{2}. These complexes were never isolated. Our motivation for this family extended solely towards gauging electronic differences on the metal according to ligand structure. We confirmed reaction progress by \textsuperscript{31}P-NMR and then collected IR absorbance data; and both techniques are compatible with liquid aliquots.

Unlike the nickel carbonyls, the nickelalactones involve additional attention. We first utilize Ni(COD)\textsubscript{2} as a convenient Ni(0) source which promptly exchanges COD for TMEDA. Succinic anhydride then performs an oxidative addition before eliminating CO to form the five membered nickelalactone. The final ligand exchange with PNP occurs fairly quickly over 1-2 hours. The final lactone remains mostly insoluble in THF allowing for a suction filtration to remove unreacted PNP and free TMEDA.

The difficulty comes from the complexes’ instability. Oxygen, water, and ambient temperature can all compromise the structure. This prompted us to use screw cap sealable NMR tubes and anhydrous solvents exclusively. If left in solvent, phenyl phosphine PNP ligands decompose in less than a day at room temperature, while cyclohexyl phosphines can survive for at least a week. For this reason, phenyl versions required fast hands and a prompt workup. PNP nickel lactones were placed into the glovebox freezer for long term storage.

**PNP Ligands: NMR spectroscopy**
1D NMR measurements confirmed successful syntheses. R₂PH starting material, R₂PCH₂OH intermediate, and PNP product all have sufficiently different $^{31}\text{P}$ chemical shifts without any overlap. The final ligand requires two sequential reactions with amine, and a cursory literature search indicated that the secondary and tertiary amine might have indistinguishable chemical shifts. Phosphorus measurements confirmed complete R₂PCH₂OH consumption. They could not however resolve possible mixtures of PNP and “PNH” due to incomplete reactions.

We elected to use carbon spectra instead of proton to confirm PNP purity. The identification occurs at the methylene spacer between N and P. The collected NMR data provide evidence for up to, but not exceeding, three bond couplings from $^{31}\text{P}$ for the various PNP compounds. The carbon signal splits from both $^1\text{J}$ and $^3\text{J}$ couplings. The proton is four bond lengths from the second P and experiences only a single $^2\text{J}$ coupling, making its multiplicity useless for assessing reaction progress. Overlap between aromatic rings on both the phosphine and amine groups made comparing peak integrations incompatible for some structures. Instead, we looked to the carbon spectra. The partially reacted PNH has only one P nuclei and should

![Figure 34. Example $^{13}\text{C}^{1\text{H}}$ splitting patterns.](image)
split the carbon signals into doublets. Fully formed PNP ligands have two carbon positions that split into a doublet of doublets, but still produce one signal due to $C_2$ symmetry through the N position. The PNP ligands with PTMS and benzyl have a third N bound methylene unit that also $^3J$ couples, though the different symmetry position gives it triplet multiplicity. The PNP ligand family has several reliable similarities within its NMR spectra.

Table 2. Relevant NMR assignments for PNP ligands.

<table>
<thead>
<tr>
<th>Abbrev.</th>
<th>$R$</th>
<th>$R'$</th>
<th>$^{13}C$, “$C_p$”</th>
<th>$^{13}C$, “$C_n$”</th>
<th>$^{31}P$</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>$[R'N(CH_2PR_2)_2]$</td>
<td>$[CN(CH_2PR_2)_2]$</td>
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<tr>
<td>L1</td>
<td>Ph</td>
<td>Ph</td>
<td>54.05, dd, (15, 8)$^a$</td>
<td>114.66, t, (3.0)</td>
<td>-27.42, s</td>
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<tr>
<td>L2</td>
<td>Ph</td>
<td>Bn</td>
<td>58.26, dd, (9.4, 5.2)</td>
<td>60.67, t, (9.5)</td>
<td>-27.56, s</td>
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<td>L3</td>
<td>Ph</td>
<td>PTMS</td>
<td>58.67, dd, (9.5, 5.6)</td>
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<td>L4</td>
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<td>Ph</td>
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<td>117.42, t, (1.6)$^b$</td>
<td>-15.52, s</td>
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<td>L5$^c$</td>
<td>Cy</td>
<td>Bn</td>
<td>52.33 dd, (8.5, 5.4))</td>
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<td>-17.31, s</td>
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<td>L6</td>
<td>Cy</td>
<td>PTMS</td>
<td>52.51, dd, (8.4, 5.7)</td>
<td>59.40, t, (9.2)</td>
<td>-17.70, s</td>
</tr>
</tbody>
</table>

a. The reduced precision reflects slightly asymmetric splitting. Peak intensities more resemble a quartet.
b. Peak had poor resolution due to low $J$ values. Splitting was measured from peak shoulders instead of maxima.
c. Synthesized and characterized by Taylir Bullick

We’ll first comment on phosphorus trends. The amine group appears to have only a small impact on phosphorus signals. L1-3 differ by less 1 ppm while L4-6 have slightly larger range of 2.18 ppm. The shifts seem to correlate to the same rankings as the nitrogen’s basicity, especially for L4-6. However, L3 is a clear outlier from L1 and L2 which have near identical shielding despite the wide gap in their electron donating ability. The Ph and Cy substituents on phosphorus have stronger influence forming two tight groupings. Before synthesis, $\text{Ph}_2\text{PH}$ has 12.37 ppm worth of shielding over $\text{Cy}_2\text{PH}$ at -40.31 ppm and -27.94 ppm respectively.

Exchanging hydride for alkylamine causes a similar downfield field shift for both starting materials, and the gap narrows slightly to 11.1 ppm on average. Carbon measurements had more features that require discussion.

Not surprisingly, carbon chemical shifts neatly sort according to two parameters:
1. The phosphorus groups, does R=Ph or R=Cy?

2. The amine groups, does R’ bind through an sp² or sp³ hybridized carbon?

Cₚ shifts ~6 ppm downfield for R=Ph vs. R=Cy, and an additional ~4.5 ppm for sp² vs sp³ R’ carbons. The increased sensitivity for R over R’ is explained through the net change, phosphorus switches two substituents and the nitrogen only one. Additionally, the Cₚ receives opposing influences from the N and P positions. As the two become more electron rich the amine drives signals downfield whereas phosphine directs upfield. Phosphorus R groups have practically no influence on Cₙ shifts given the distance between them. Excluding L1 and L4 with a <3 ppm difference the other two ligand pairs have a <1 ppm gap. The difference between aryl and alkyl group however amounts to a more than 50 ppm difference, though not unexpected.

Spin couplings were mostly consistent across the six ligands. Cₙ is symmetrically positioned with regards to both phosphines and cleanly splits into a triplet. Cₚ is directly bound to one phosphorus and three bond lengths from the other. The two inequivalent nuclei both couple and create a doublet of doublets. Coupling constants follow similar groupings as for chemical shifts. Constants decreased as expected with increasing bond count for Cₚ, ¹J > ³J. Relative ³J strengths between Cₚ and Cₙ varied with R’ but not R. This suggests that the increased bulk from Ph to Cy groups did not observably alter either of the dihedral angles. R’=Ph increased both ¹J and ³J for Cₚ by over half compared to R’=Bn and R’=PTMS. However, it also had an even greater decrease in Cₙ’s ³J making it difficult to ascribe a rationale without additional experiments. Strangely, when R’=Bn or R’=PTMS the Cₙ ³J nearly matched Cₚ ¹J for L2 and L3, and actually exceeded it for L5 and L6. This is particularly odd given the through bond nature of spin-spin coupling, though the differences are small. One ligand’s unique behavior set it apart from the rest.
L1 with R=R’=Ph had far more complex splitting than L2 and L3. We have so far chosen not to consider $^{13}$C signals from the PR$_2$ groups. Those signals are not necessary to confirm reaction success, and were deemed too burdensome to assign. Even R$_2$PH starting materials have nontrivial spectra. However the noticeable difference between L2 and L3 to L1 suggests an unignorable behavior change. C$_p$ had different peak intensities to all five other ligands, appearing more similar to a quartet. We considered a mixture of PNP and PNH from incomplete reaction, but the J values are too similar to L4 to accept that hypothesis. Additionally, PNH would have two symmetric peaks separated by ~14-15 Hz, which we do not observe.

L1, R=R’=Ph. Aromatic carbons (left, center) and C$_p$ (right).

Figure 35. Unique $^{13}$C splitting behaviors.

The aromatic region also has additional splitting not observed in L2 or L3. Two signals stand out, a doublet of triplets and a multiplet. Notably, these complex patterns are unique to the free ligand and do not appear in the nickelalactone. We suspect that observed couplings rely on the ligand’s rotation freedom about its N-C and C-P bonds, a feature that becomes restricted after binding to nickel. We believe this splitting behavior results from pi-stacking between phenyl groups on both the phosphorus and nitrogen. Intra molecular pi-stacking is only plausible for L1 and L2. From 3D modeling we found that only L1 can arrange into planar stacks. The
methylene spacer in L2’s benzyl group offsets the rings from each other and prevents orbital overlap.

**PNP Nickel Carbonyls: NMR and IR spectroscopies**

Nickel carbonyl complexes were prepared primarily to take advantage of CO as diagnostic tool. CO ligands act as both sigma-donors and pi-acceptors. As the metal becomes more electron rich it feeds excess e⁻ density into available ligand pi orbitals. Stronger pi backbonding “pushes” electrons towards oxygen’s partial positive charge. This weakens CO’s covalency, partially reducing it from a triple bond towards a double bond. CO bond strength directly relates to its IR vibrational frequency. Carbonyl ligands then can reveal the electronic properties of other ligands bound to metal.

We chose not to spend resources towards isolating these complexes. Both characterization methods were fully compatible with reaction solutions. Because we were uninterested in \(^1\)H and \(^{13}\)C spectra for these complexes, \(^{31}\)P measurements were gathered using reaction aliquots without performing a workup. We monitored four phosphorus intensities to determine reaction progress. As the reaction proceeded, the signals for unbound PNP and Ni(PPh)₃(CO)₂ starting materials decreased while free PPh₃ and Ni(PNP)(CO)₂ signals emerged.

IR measurements also used reaction aliquots in a liquid cell, which informed our choice of toluene as solvent. Liquid IR measurements ensured no complications from any Ni⁰ oxidation. The liquid cell was prepared in a glovebox.
PNP structural changes cause only small differences in the NMR spectra. Binding affects the free ligand’s shielding shift in a highly similar fashion. Even though the two phosphine lone pairs have noticeably different reactivities, that difference barely manifests here. The three

Table 3. Ni(PNP)(CO)\(_2\), \(^{31}\)P-nmr and IR

<table>
<thead>
<tr>
<th>Abbrev.</th>
<th>(R)</th>
<th>(R')</th>
<th>(\delta)</th>
<th>(\delta) vs. free ligand</th>
<th>CO vibrations cm(^{-1})</th>
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<tr>
<td>L1-CO</td>
<td>Ph</td>
<td>Ph</td>
<td>12.66</td>
<td>40.08</td>
<td>2005.21, 1951.21</td>
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<tr>
<td>L2-CO</td>
<td>Ph</td>
<td>Bn</td>
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<td>40.3</td>
<td>2003.28, 1947.35</td>
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<tr>
<td>L3-CO</td>
<td>Ph</td>
<td>PTMS</td>
<td>12.52</td>
<td>40.85</td>
<td>2003.28, 1946.87</td>
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<tr>
<td>L4-CO</td>
<td>Cy</td>
<td>Ph</td>
<td>25.25</td>
<td>40.77</td>
<td>1987.37, 1926.62</td>
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<tr>
<td>L5-CO(^a)</td>
<td>Cy</td>
<td>Bn</td>
<td>24.66</td>
<td>41.97</td>
<td>1984.55, 1923.73</td>
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<tr>
<td>L6-CO</td>
<td>Cy</td>
<td>PTMS</td>
<td>24.55</td>
<td>42.25</td>
<td>1985.44, 1924.21</td>
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\[\text{Ni(PPh)}_3(\text{CO})_2\]

<table>
<thead>
<tr>
<th>(\delta)</th>
<th>(\delta) vs. free ligand</th>
<th>CO vibrations cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.76</td>
<td>38.14</td>
<td>2002.32, 1944.94</td>
</tr>
</tbody>
</table>

\(^a\) Synthesis and characterization performed by Taylir Bullick
R=Ph and R=Cy maintain nearly the same relative shifts after chelating as they had before. Coordination effects seem surprisingly independent from phosphorus R groups. It is also unclear if the nitrogen groups impact $^{31}$P behavior. When R=Ph the variance seems entirely random with R’ choice, but when R=Cy signals shift downfield as the amine becomes more basic.

The IR data gives a better picture for PNP’s effect on the metal. These complexes have $C_{2v}$ symmetry allowing for two carbonyl IR bands. Unlike $^{31}$P data, the CO stretches show a clear difference in electron donation between the two R groups. The two CO peaks decrease by $\sim 18 \text{ cm}^{-1}$ and $\sim 24 \text{ cm}^{-1}$ between R=Ph and R=Cy. The greater donation from the alkyl phosphine gives the CO more double bond character and a less energetic vibration. The data also indicate that the amine partially influences the metal, but how it does so remains uncertain. The two alkyl amine pairs are near indistinguishable and differ by less than 1 cm$^{-1}$ in each IR band. In contrast, L1-CO and L4-CO peaks consistently show 2-3 cm$^{-1}$ higher energy. Clearly donation decreases when R’=Ph, but further structural analysis is required to determine the reason. The amine lone pair could possibly donate into the metal depending on ring strain, in which case the two alkyl amines form stronger interactions than phenyl amine. Alternatively, the aryl and alkyl amines have different sterics. Less bulk on the amine might allow the phosphine different bond angles and improve its orbital overlap with nickel. In either case, PNP ligands have stronger ligand bonding overall with alkyl groups at both the N and P positions.

**PNP Nickel Lactones: NMR Characterization**

(a) $^{31}$P

Nickelalactone spectra bring new challenges not found in free ligand and Ni(PNP)(CO)$_2$ compounds. First, the asymmetric lactone structure removes the C$_2$ axis which simplified previous spectra. The four phosphine R groups lose all equivalence and their signals become
impractical to assign. Luckily, the new lactone signals do not overlap with either the Ph or Cy regions. After multiple attempts at collecting usable spectra it was determined that nickelalactones can degrade over several hours at room temperature. $^{31}$P and $^{13}$C data were collected at 0 °C in sequential batches to prevent decomposition to paramagnetic species from ruining measurements. Due to this necessary precaution the lactones sp$^2$ carbon did not provide enough to S:N to register on many of the collected spectra.

Because of the lactone opposite the nickel center each phosphorus nuclei experiences different electronic conditions. The lactone’s two ends having different sigma donating strengths. Due to the trans effect on the metal’s $d_{x^2-y^2}$ orbital the two phosphorus donate different amounts to the nickel, breaking their magnetic equivalency. They have unique chemical shifts and also $^2$J spin couple through their nickel bonds. The mutual doublet splittings and 1:1 peak integrations confirm that both phosphorus donors successfully chelated the same nickel atom. The chemical shifts become more fascinating when taken in context with the free ligands and nickel carbonyls.

The nickelalactone complexes reveal a binding threshold that undoes the near identical shielding changes from binding. $P_{trans-C}$ nuclei appear to have weaker binding than in the nickel carbonyl, $\delta$ changes by ~25 ppm instead of ~41 ppm though with similar variance. These nuclei maintain the similar disparities found in their phosphine precursors. The $P_{trans-O}$ nuclei however show an even stronger binding and break the trend. $R=Cy$ causes ~47 ppm downfield shift but $R=Ph$ changes by the larger ~57 ppm shift. Notably, even though alkyl phosphines can donate more electron density to metals than aryl phosphines, the aryl phosphine still exhibited a larger change in shielding.
Recall that phenyl phosphines have higher relative shielding because the electron withdrawing groups partially pull the lone pair’s density towards the nuclei. That delocalization causes the increased shielding, however, Ni\textsuperscript{II} is stronger Lewis acid than Ni\textsuperscript{0}. We hypothesize that the nickel center overpowers the phenyl ring’s electron withdrawing effects. P\textsubscript{trans-O} no longer receives the shielding benefits from its R groups, which almost entirely erases the chemical shift gap between R=Ph and R=Cy compounds.
Table 4. $^{31}$P NMR data for PNP nickelalactones.

<table>
<thead>
<tr>
<th>Abbrev.</th>
<th>R</th>
<th>R'</th>
<th>$\delta_{R}$ (J Hz)</th>
<th>$\delta_{R}$ vs. free (J Hz)</th>
<th>$\delta_{R'}$ (J Hz)</th>
<th>$\delta_{R'}$ vs. free (J Hz)</th>
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<tbody>
<tr>
<td>L1-Ni$^{II}$Lac</td>
<td>Ph</td>
<td>Ph</td>
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<td>55.81</td>
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<td>L2-Ni$^{II}$Lac</td>
<td>Ph</td>
<td>Bn</td>
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<td>23.83</td>
<td>28.23, d, (34.9)</td>
<td>55.79</td>
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<tr>
<td>L3-Ni$^{II}$Lac</td>
<td>Ph</td>
<td>PTMS</td>
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<td>24.65</td>
<td>27.69, d, (35.0)</td>
<td>55.99</td>
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<tr>
<td>L4-Ni$^{II}$Lac</td>
<td>Cy</td>
<td>Ph</td>
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<td>24.45</td>
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<td>L5-Ni$^{II}$Lac$^a$</td>
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<td>Bn</td>
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<td>26.32</td>
<td>29.47, d, (37.8)</td>
<td>47.17</td>
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$^a$ Synthesis and characterization performed by Taylir Bullick

Table 5. $^{13}$C NMR data for PNP nickelalactones.

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<th>Abbrev.</th>
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<th>$\delta_{R}$ (J Hz)</th>
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<th>$\delta_{R'}$ (J Hz)</th>
<th>$\delta_{R'}$ (J Hz)</th>
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<tbody>
<tr>
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<td>Ph</td>
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<td>Bn</td>
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<td>49.98, d, (34.6)</td>
<td>65.83, t, (9.3)</td>
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$^a$ Synthesis and characterization performed by Taylir Bullick
(b) $^{13}$C, lactone positions

The additional lactone carbons do not overlap with PNP’s other aliphatic signals. The β position C3 gives two tightly grouped chemical shifts according to the phosphine. The alkide is the complex’s most shielded carbon. The opposing phosphine position informs the sigma bond’s strength, with the more highly donating R=Cy ligands forcing C3 to hold more of its electron density, thereby allowing it to maintain a stronger field than R=Ph ligands. Both phosphorus nuclei couple through the nickel, and form a consistent doublet of doublets for the entire ligand family. C2 is more removed from the ligand’s electronic effects. Its shielding remains virtually unchanged with ligand. Though present, its spin coupling is weak and behaves erratically across the six ligands.

(c) $^{13}$C: C4, C5, and C6 chemical shifts

The methylene spacers bound to phosphorus are most affected by PNP nickelalactones inequivalent phosphorus. Free ligand’s symmetry collapses the $C_p$’s into a single doublet of doublets. With unique phosphorus properties $C_p$ positions resolve into two different carbon signals, C4 and C5. They have their own splitting unique splitting behaviors. As R’ gains more conformational freedom the signals become less resolved. For R’=Ph signals are separated by nearly 2 ppm and show a clean doublet and doublet of doublets. When R’=Bn the signals swap their relative shifts by moving both upfield and downfield, and their separation shrinks to less than 1 ppm. R’=PTMs has more complicated behavior. When R=Cy the signals overlap with each other but remain legible. R=Ph signals stay resolved, but the non-ideal couplings warrant a dismissive multiplet designation.
C4 and C5 could not be assigned through one dimensional measurements, instead we turned to 2D-NMR. Ideally we would have directly performed 2D experiments using carbon and phosphorus nuclei. Due to limitations in available NMR hardware we instead took an indirect approach. HSQC experiments allowed us to identify which $^1$H signals coupled to $P_{\text{trans-O}}$ and $P_{\text{trans-C}}$. Then, a second experiment would link those $^1$H signals to their matching carbon...
position. In this way we determined that C4 is the doublet signal and C5 and the doublet of doublets.

Carbons opposite the amine are more deshielded after the ligand chelates nickel. The four ligands with aliphatic C-N bonds have the comparably small shift between 5 and 7 ppm. The two aromatic R’=Ph ligands shift by a far larger margin, over 35 ppm. The free ligand had rotational freedom for the R and R’ to impose steric hindrance on each other. After binding the ligand experiences ring strain and the R groups become mostly locked in place by their immobile phosphorus. We hypothesize that without interference the phenyl group can more easily align its p orbitals with the amine’s. With an additional lone pair participating in the pi system C6 is further deshielded.

(d) \(^{13}\)C: C4, C5, and C6 spin-spin couplings

The splitting behaviors for the three nitrogen bound carbon positions present a fascinating coincidence. The two phosphorus demonstrate their differences on multiple fronts. They have separate chemical shifts and couple each other. C4 and C5 have different \(^1J\) coupling constants. C5 has an observable \(^3J\) coupling and C4 does not. They are definitively inequivalent. Despite this, C6 without exception has a triplet splitting pattern, which would normally indicate coupling from two inequivalent magnetic fields. The C4 and C5 J values indicate that the phosphorus nuclei couple in surprising and different ways.

For instance, we predicted that the phosphorus which formed a higher \(^1J\) coupling would have more effective long range coupling. Experimental data showed the opposite, \(P_{\text{trans-O}}\) formed a higher \(^1J\) on the adjacent C5 but no observable \(^3J\) on C4, whereas \(P_{\text{trans-C}}\) has a \(^3J\) coupling to C5 despite its smaller \(^1J\) to C4. We hypothesized that the intervening bond angles tuned the \(^3J\)
couplings to C6 to varying degrees, and which coincidentally resulted in equivalent coupling constants. Because $^{3}J_{P1-C6}=^{3}J_{P2-C6}$ the expected doublet of doublets manifests as a triplet.

Table 6. Bond angles derived from DFT calculations.

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We attempted to affirm our coupling hypothesis via DFT calculations, but it met with failure. Nickelalactone structures were optimized and all pertinent dihedral angles measured. Coupling is expected to decrease from 0-90° and then increase from 90-180°. $^1J$ constants were used as a starting point to predict long range coupling. If the experimental $^3J$ values decreased by differing amounts for $P_{trans-O}$ and $P_{trans-C}$, then that change should be reflected in their dihedral angles. Calculated results did not match those predictions. At both the $C_n$ and $C_p$ positions $P_{trans-O}$ was found to have angles further from 90° than $P_{trans-C}$, which would indicate more effective long range coupling from $P_{trans-O}$. NMR experiments demonstrated the exact opposite. While the chemical shifts are accurately assigned the factors contributing to their observed spin couplings remain unknown.

(e) Stability studies

Data collection encountered difficulties post synthesis due to the lactone’s instability. Repeated attempts at characterization struggled with decomposition and unknown paramagnetic species. Usable measurements mandated reduced temperature to prevent thermal degradation.
We were interested in investigating these thermal properties and performed variable temperature $^{31}$P measurements, slowly raising temperature and observing chemical changes. From these experiments we discovered a remarkable trend; only the R=Ph complexes decomposed to paramagnetic end products. R=Cy complexes entirely survived the experimental conditions and did not degrade for over seven days while under nitrogen.
Figure 39. VT $^{31}$P-NMR experiments on nickelalactones.

Data was collected across multiple batches at 25, 35, 45, and 52 °C. Signal to noise ratios for R=Ph samples slowly eroded at higher T which we attribute to growing
paramagnetism. At 45 °C new $^{31}$P signals emerge near -6 ppm and -2 ppm, and an additional signal near 13 ppm at 52 °C. The original nickelalactone signals do not change with temperature. We conclude then that decomposition does not occur through P-Ni$^{II}$ bond changes. The -2 ppm signal has similar doublet characteristics to P$_{trans-C}$, and appears first. We suspect that the decomposition starts at the lactone ring. It is possible that it reverts to CO$_2$, C$_2$H$_4$, and Ni$^0$. Growing paramagnetism however suggests that any Ni$^0$ subsequently oxidizes, or an alternative reaction preserves Ni$^{II}$. Whatever the pathway, demetalization likely follows, a gray solid was observed within the sample tube upon exiting the instrument.

In stark contrast the R=Cy samples exhibited no evidence for decomposition. After extended exposure to elevated temperatures the nickelalactone retained its original properties when returned to ambient conditions. Both P$_{trans-C}$ and P$_{trans-O}$ at 9 ppm and 30 ppm respectively experience a slight deshielding effect as temperature increases. P$_{trans-C}$ shifted similarly for all three R’ groups, while P$_{trans-O}$ only moved downfield 0.1 ppm when R’=Bn. We attribute the lowered shielding to an increase P-Ni bond strength. The higher temperature is likely weakening the lactones Ni-O and Ni-C bonds which through the trans effect prompts a larger contribution from both phosphorus.

From our data we conclude that alkyl phosphines are better suited as catalysts from amongst the PNP ligand family. Phenyl phosphines could potentially turn over lactone into acrylate at lower temperatures, but the risk is too great. The ligand doesn’t form a strong enough bond to maintain the catalyst’s integrity. High reactivity and fast kinetics would be accompanied by low turn over numbers from poisonous side reactions. Alkyl versions better accommodate the Ni$^{II}$ increased electrophilicity. Under Vogt’s proposed mechanism this functionality is necessary to allow the Ni-O bond cleavage without degrading.
Conclusions

A family of six PNP ligands were synthesized and subsequently used to prepare corresponding nickelalactone and nickel carbonyl complexes. The ligand’s electronic effects on the metal center were assessed through IR spectroscopy, and while it demonstrated greater bonding from cyclohexyl phosphine ligands over phenyl phosphines, the differences were too small to demonstrate trends within either of those two groups. Nickelalactone structures were thoroughly examined through NMR spectroscopy. Spectra clearly showed that each phosphorus nuclei exhibits unique coupling behavior upon ligand exchange with TMEDA Nickelalactone. In addition, cyclohexyl phosphine ligands formed far more stable nickelalactones as shown by variable temperature $^{31}$P NMR experiments. From these findings we surmise that PNP ligands with more electron donating R groups make better candidates for catalytically producing sodium methacrylate. The rather obvious direction to continue this work is testing that hypothesis with the synthesized PNP ligands under catalytic conditions, and compare turn over numbers.
CHAPTER 5

Methods

I. Measurement Techniques

PNIPAm suspensions

Small quantities of isolated PNIPAm were dissolved in 100 mL volumetric glassware with ultra pure water. Despite PNIPAm’s reputation for water solubility, stock solutions repeatedly struggled to fully dissolve, possibly due to the low overall molecular weight. Multiple days with stirring were necessary for the solid PNIPAm to disappear from sight. Due to these solubility difficulties, the desired solutions were obtained in a timely fashion by employing multiple stock containers simultaneously.

I-a. Second Order Scattering

Scattering was measured on a Cary Eclipse Fluorescence Spectrophotometer using a 1 cm pathlength glass fluorescence cell. Emission wavelengths are always set to double the chosen excitation wavelength; 375 nm excitation and 750 nm emission, 400 nm excitation and 800 nm emission, 425 nm excitation and 850 nm emission, 450 nm excitation and 900 nm emission. Intensity readings were collected at 0.1 second intervals. Raw data was averaged over 10 second intervals before use in SOS prediction model calculations.

Cell temperature was controlled using a Cary single cell Peltier accessory. During each SOS run a 3 mL PNIPAm sample was held at 25°C for 2 minutes before adjusting control settings to 30°C and allowing 2.5 minutes for temperature stabilization. Temperature control was then modified to 35°C and data collection was commenced. Although cell temperature
could not be measured dynamically without disrupting scattering experiments, monitoring of cell temperature in control heating runs using a Fisher Scientific digital thermometer showed that the LCST temperature of 32°C was reached within 1 minute and stabilized within 4 minutes.

Early experiments tested the effect of changing instrument parameters, though the default settings proved adequate. Excitation and Emission slit widths were 5 nm. Excitation filter was set to “auto” and the Emission filter set to “open”. PMT detector voltage was 600 V. The temperature control accessory also allowed for magnetic stirring, and while a flea stir bar could freely spin within the cuvette, it dramatically increased noise and was not used during measurements.

I-b. Dynamic Light Scattering

We found that using 0.45 µm pvdf Acrodisc syringe filters partially removed polymer, which rendered the final concentration of 0.05 mg/mL samples too low to observe. To reduce interference from dust particles, stock solution volumes were instead prepared with ultra-pure water suction filtered through 0.2 µm polycarbonate membrane. All necessary glassware was prewashed with filtered water and kept upended prior to use to discourage dust collection. Even so, particle contamination remained a persistent difficulty which contributed to measured values. The impact on measured dispersity necessitated use of the “mean peak intensity size” rather than the preferred “Z-average diameter” for 0.05 mg/mL PNIPAM samples. Individual measurements with a PDI > 0.25, which in our samples indicated a broad dispersity index arising from contributions from contaminants, were rejected.

Fixed temperature experiments for phase transition and aggregation consisted of 180 consecutive collections of a single 20 second run. Sample volume of approx. 1 mL was placed from room temperature into the preheated 35°C sample holder and collection initiated. A system
optimization step occurs prior to the first collection which lasts for approximately one minute. Variable temperature experiments measured at 0.5°C increments from 27°C to 50°C. At each increment temperature was allowed to equilibrate for 600 seconds before 3 measurements consisting of 4 runs lasting 20 seconds each with a 0 second delay were taken.

I-c. Equilibrium Dialysis and Atomic Absorption Spectroscopy

Experiments were performed using a Scienceware equilibrium dialysis block. 3,500 molecular weight cutoff tubing, previously soaked in ultra pure water, was used as a semi permeable barrier between polymer and metal containing regions. Cells were prepared with 1 mg/mL PNIPAm against 2.5 mM Cu(NO₃)₂ and 0.05 mg/mL against 0.13 mM Cu(NO₃)₂. In each case, solutions contained 0.1 M pH 6.3 MOPS buffer and a NIPAM monomer to Cu²⁺ molar ratio of 3.4 was used. After assembly, cells were placed at room temperature for 48 hours to achieve equilibrium. Copper concentration changes on the metal side were determined using a Varian SpectrAA 220 Atomic Absorption spectrometer.

Extraneous ion interferes with AA measurements by lowering the observed absorbance; the initial attempt neglected this interaction rendering the calibration curve incompatible with dialysis samples. Subsequently, a 1mM Cu(NO₃)₂ solution was diluted with 0.1 M pH 6.3 MOPS buffer to prepare copper standards at 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. The low concentration range minimized risk of samples falling beneath the prepared calibration range, since higher sample concentrations can be diluted but lower concentrations require additional standards to extend the calibration. Measurements were collected in triplicate and between each copper sample the system was washed using ultra pure water. Monitoring the water rinses revealed a roaming baseline. The water measurements were used to perform a baseline correction in order to appropriately compare the dialysis samples against the calibration curve.
Figure 40. Equilibrium dialysis, AA measurements

\[ y = 2 \times 10^{-6}x^2 - 0.0002x - 0.0016 \]

\[ R^2 = 0.9092 \]
**I-d. Infrared Spectroscopy**

Infrared measurements were collected using a Thermo Nicolet Is10 FTIR. A liquid IR cell with KBr plates was used to analyze nickel carbonyl complexes dissolved in toluene. Samples were prepared within a N₂ atmosphere glovebox due to the complex’s oxygen sensitivity, which precluded a traditional background measurement to define a baseline. The baseline was defined subtracting a separate toluene spectrum collected within the same liquid cell. Nickel lactones were analyzed on a diamond Attenuated Total Reflectance (ATR) attachment rather than risk damaging the KBr plates with the more polar complex and solvents.
I-e. Nuclear Magnetic Resonance Spectroscopy

Spectra were gathered using Varian 400 MHz and Bruker 500 MHz nuclear magnetic resonance spectrometers. Due to solubility constrains TMEDA Nickelalactone samples were performed in DMF-d7, all other samples were performed in CDCl₃. Phosphorus containing compounds were prepared within a N₂ glovebox. Teflon sealed screw cap NMR tubes prevented oxidation reactions observed when using conventional polypropylene end caps. Due to thermal instability, PNP nickelalactone samples were measured at 0 °C. Multiple 256 scan measurements were combined to acquire ¹³C spectra for unstable nickelalactones.

Stability experiments began at 25 °C and continued through 35, 45, and 52 °C in order. Samples were allowed to equilibrate for 5 minutes at desired temperatures as indicated by the instrument’s sensors. Shimming protocols were repeated after equilibrating at each new temperature. Each spectrum contains 64 scans, requiring ~2 min to collect. Sequential spectra occur across equivalent time intervals. Between 10 and 15 minutes elapsed before acquiring data at the next temperature setting.

II. SOS Prediction Model: Outputs

What follows are the calculated values used to generate predictive models at different wavelength and refractive index. The predicted curve was assembled as a series of linear regressions between each data point [x=size and y= normalized Kₜₜ * I₀ * c¹/₆]. Experimental data was treated using these regressions to convert I/Iₘₐₓ into particle size.
400 nm wavelength and 1.378 refractive index

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<td>0.006562422</td>
</tr>
<tr>
<td>40</td>
<td>0.000911</td>
<td>0.00185</td>
<td>2.98E-05</td>
<td>2.96814E-07</td>
<td>0.017742441</td>
</tr>
<tr>
<td>50</td>
<td>0.002189</td>
<td>0.001848</td>
<td>1.53E-05</td>
<td>6.37199E-07</td>
<td>0.038089444</td>
</tr>
<tr>
<td>60</td>
<td>0.004448</td>
<td>0.001844</td>
<td>8.84E-06</td>
<td>1.17926E-06</td>
<td>0.070491619</td>
</tr>
<tr>
<td>70</td>
<td>0.008035</td>
<td>0.001837</td>
<td>5.57E-06</td>
<td>1.96494E-06</td>
<td>0.117457177</td>
</tr>
<tr>
<td>80</td>
<td>0.013289</td>
<td>0.001826</td>
<td>3.73E-06</td>
<td>3.0225E-06</td>
<td>0.180674323</td>
</tr>
<tr>
<td>90</td>
<td>0.020507</td>
<td>0.001811</td>
<td>2.62E-06</td>
<td>4.35945E-06</td>
<td>0.260592181</td>
</tr>
<tr>
<td>100</td>
<td>0.029901</td>
<td>0.001789</td>
<td>1.91E-06</td>
<td>5.95722E-06</td>
<td>0.35610084</td>
</tr>
<tr>
<td>110</td>
<td>0.041565</td>
<td>0.001758</td>
<td>1.43E-06</td>
<td>7.76193E-06</td>
<td>0.463979773</td>
</tr>
<tr>
<td>120</td>
<td>0.055455</td>
<td>0.001718</td>
<td>1.11E-06</td>
<td>9.68936E-06</td>
<td>0.579194565</td>
</tr>
<tr>
<td>130</td>
<td>0.071394</td>
<td>0.001666</td>
<td>8.69E-07</td>
<td>1.16193E-05</td>
<td>0.694562153</td>
</tr>
<tr>
<td>140</td>
<td>0.089111</td>
<td>0.001599</td>
<td>6.96E-07</td>
<td>1.34124E-05</td>
<td>0.801745765</td>
</tr>
<tr>
<td>150</td>
<td>0.10831</td>
<td>0.001515</td>
<td>5.66E-07</td>
<td>1.49258E-05</td>
<td>0.892208358</td>
</tr>
<tr>
<td>160</td>
<td>0.12879</td>
<td>0.001414</td>
<td>4.66E-07</td>
<td>1.60341E-05</td>
<td>0.958462442</td>
</tr>
<tr>
<td>170</td>
<td>0.1505</td>
<td>0.001295</td>
<td>3.89E-07</td>
<td>1.66493E-05</td>
<td>0.995237254</td>
</tr>
<tr>
<td>180</td>
<td>0.17365</td>
<td>0.00116</td>
<td>3.27E-07</td>
<td>1.6729E-05</td>
<td>1</td>
</tr>
<tr>
<td>190</td>
<td>0.19869</td>
<td>0.001015</td>
<td>2.78E-07</td>
<td>1.62951E-05</td>
<td>0.974061931</td>
</tr>
<tr>
<td>200</td>
<td>0.22624</td>
<td>0.000865</td>
<td>2.39E-07</td>
<td>1.54158E-05</td>
<td>0.921502908</td>
</tr>
<tr>
<td>210</td>
<td>0.25694</td>
<td>0.000719</td>
<td>2.06E-07</td>
<td>1.41926E-05</td>
<td>0.848382948</td>
</tr>
<tr>
<td>220</td>
<td>0.29124</td>
<td>0.000582</td>
<td>1.79E-07</td>
<td>1.2732E-05</td>
<td>0.761069969</td>
</tr>
<tr>
<td>230</td>
<td>0.32918</td>
<td>0.000461</td>
<td>1.57E-07</td>
<td>1.11426E-05</td>
<td>0.666065958</td>
</tr>
<tr>
<td>240</td>
<td>0.37029</td>
<td>0.000357</td>
<td>1.38E-07</td>
<td>9.50208E-06</td>
<td>0.567999949</td>
</tr>
<tr>
<td>250</td>
<td>0.41366</td>
<td>0.00027</td>
<td>1.22E-07</td>
<td>7.8803E-06</td>
<td>0.471055579</td>
</tr>
</tbody>
</table>
III. SOS Prediction Model Readme: constructing functional spreadsheet from scratch

This section shall detail how to construct a file capable of transforming raw data intensity values into average particle size values. While the original file may be preserved and circulated for some time, detailed spreadsheets can oftentimes be arcane to all but the original author. Without knowledge of its internal function users may be frustrated when adapting changes to system parameters and can easily render the file nonfunctional in the attempt. Each component will be discussed individually in terms of its contribution to the whole and how it completes the specific task. The operations mentioned were performed using Microsoft Excel 2010 and can likely be mimicked in later versions or alternative software as necessary. The descriptions assume only a slight familiarity with Excel and it is useful to read material elsewhere regarding the functions mentioned before modifying the syntax. Examples use column labels A, B, C etc. as needed, but note that this is only for purpose of description. Assembling an entire spreadsheet requires organizational choices across a wide a collection of distinct columns. Additionally, it is highly recommended to read an entire section before applying the commands described.

III-a. Smoothing (data averaging/noise reduction)

Before the raw data can be interpreted by the predictive model it must first undergo smoothing to lessen the impact of noise. The interpretation requires the cooperation of two separate regions or halves. These two regions are identifiable by their collective relationship, slope, between the x (average particle size) and y (normalized predicted intensity) axis. The “positive” half has a positive slope and has lower x values. The “negative” half in turn has a negative slope and has higher x values. As x increases the model must make a clean swap between using the “positive” and “negative” predictive outputs at the appropriate peak value. If possible (see section X.X) this value is assigned as the maximum value in the data set. However, depending on the amount
of noise in data collection the maximum value may be a poor choice to accurately describe the sample. Smoothing eases the value selection to improve the overall calculations.

10 second intervals were used for each averaging period. This was carried out using the AVERAGE and OFFSET functions

Example command:

=AVERAGE(OFFSET(A$1,ROWS(B$1:B1)*100-100,,100))

Where column “A” contains the input values (raw intensity measurements) and column “B” displays the output. Additionally, B1 refers to the cell containing the command. The command line value “100” ensures that the correct number of cells is included in the average. The value itself is chosen based on the data collection; a 10 second average period was desired and data was collected at 0.1 second intervals. The same command is applied to additional cells by clicking and dragging vertically down to the desired quantity depending on the volume of data.

=AVERAGE(OFFSET(A$1,ROWS(B$1:B2)*100-100,,100))

. .

=AVERAGE(OFFSET(A$1,ROWS(B$1:B5)*100-100,,100))

III-b. Data Normalization

The predictive model is based upon comparing each datum against a reference value. This lets samples of varying concentration or refractive index with large difference in intensity be treated in a similar fashion. Selecting this value is an interpretive exercise and requires a decision on part of the user (discussed in section X.X). Each value is divided by the reference to give outputs between 0 (or very close to 0 depending on noise) and 1.
\[ =A1/$B$1 \]

Where column “A” contains data to be normalized and B1 is the specific cell containing the chosen reference value, the “$” character’s keep B1 constant so that applying the command to other cells does not select a different cell to contain the reference value. If the reference is chosen from a different collection of data then it must be manually set. The same command is applied to additional cells by clicking and dragging vertically down to the desired quantity depending on the volume of data.

If the current data set contains a usable reference value then it can be conveniently found with the MAX function by placing the following command in $B$1.

\[ =\text{MAX}(C1:C1000) \]

Where Column “C” contains the entire data set. “1000” is an arbitrary value in this description and merely needs to be large enough to contain all of the data points.

*CAUTION*

Columns “A” and “C” appear to be the same as described here. The distinction is made to circumvent a mistake that is difficult to diagnose after the fact, though can be easily remedied. When using the MAX function to obtain the reference value it is crucial not to use raw measurement values. Instead, the data must have already undergone the Smoothing procedure. Failure to perform the smoothing first leads to inappropriate values being used further down the calculation process. Raw measurement values can only be used directly when the reference comes from a different data set and the MAX function is not used.

**III-c. Prediction Model to Mathematical Functions**
The remainder of the predictive model can be cynically described as brute force solutions to a simple problem: high order polynomial functions are difficult to solve and taxing to use. The theoretical model is itself a limited collection of individual data points. Unfortunately, an acceptable curve fit to the theory by a single function could only be achieved by 4\textsuperscript{th} order or higher polynomials, which meant using a single function extended too far beyond our expertise. Instead, a series of linear functions was applied to describe theoretical model.

The theoretical model is arranged as a collection of x and y values into Columns “A” and ”B” respectively. These are then given linear fits between each adjacent data value using the \texttt{LINEST} function. The \texttt{LINEST} function provides both the slope and intercept, both of which will be required shortly. In order to obtain both values as an array first highlight 2 horizontal cells to display the values. Next press the F2 key (for Windows users), which will change the display slightly of the 2 highlighted cells. Type the command line shown below and hit CTR + SHIFT + ENTER. If executed correctly the two selected cells will now display the slope and y-intercept

\[ =\text{LINEST}(A1:A2,B1:B2^\{1\},\text{TRUE,\text{FALSE}}) \]

Where A1:A2 represents the 2 y values arranged vertically, B1:B2 represents the 2 x values arranged vertically, \{1\} selects a 1\textsuperscript{st} order polynomial (linear) fit, \text{TRUE} calculates y intercept without setting value to 0, and \text{FALSE} chooses not to display regression statistics.

If executed correctly the two selected cells will now display the slope and y-intercept. The same procedure is repeated until a linear fit is obtained between each pair of adjacent points in the theoretical model.
The regression outputs are then copy pasted into new cells as numerical values in order to free the regression information from the array format. Because this transfer is manual it must be repeated each time the user modifies the theoretical model values.

### III-d. Function to Outputs

The previous sections have prepared raw data and set up the regressions needed to interpret it, while the rest of this chapter shall explain how to perform the interpretation. Ultimately the question is “which linear function most appropriately transforms a given intensity measurement into an average particle size value?” Unfortunately, although Intensity is a function of time, \( I(t) \), average particle size cannot be considered a function of intensity, \( S(I(t)) \). This is intuitively apparent when displayed graphically; each intensity value could reasonably have two different size outputs. Therefore, selection criteria are used to achieve the correct assignment based contextually by the surrounding data points as well as a fundamental assumption; particle sizes are strictly increasing, not decreasing. The model as it is described here should never be used when this assumption cannot be reasonably accepted.

Each linear regression generated thus far requires an initial and final point to define the curve. Therefore a predictive model of N data points will generate N-1 linear regressions. These regressions are separated into two regions. The first describes intensity measurements closer to \( t=0 \) that give smaller size outputs and uses regression with a positive slope. The second is the opposite, the latter portion of intensity measurements that give larger size outputs and have
negative slopes. The distinction between these regions is made at the models absolute maximum, the size which produces \( I_{\text{max}} \). For convenience the regions are referred to by their slopes, the “positive” and “negative” halves.

### III-e. “Positive” Half

Single input: multiple outputs

Every function in the “positive” half is treated simultaneously in context. At first many more outputs will be generated than are desired but these shall be quickly discarded in the next section. This description is the most complicated thus far. It assumes that regression data, slope and intercept, are stored as values in side-by-side columns, B (slope data) and C (corresponding intercept data), and are in descending order with the first regression and the top and the final regression at the bottom. Every regression present will require its own unique column. Arrange these in order with the first regression’s column on the left and the final regression’s column on the right. The first column is denoted “E” and the final Column “Z” for purpose of description. Column “D” represents normalized intensity values. Column “A” has the predictive model outputs and should contain one more data value than there are regressions.

\[
=\text{IF}($D1>$A$1,($D1-$C$1)/$B$1,0)
\]

This generates size outputs for a single regression in one column. Similar formulas are used in additional columns for the entire “positive” half. The “IF” clause marks the first selection step and turns unwanted outputs into a 0.

2\text{nd} column

\[
=\text{IF}($D1>$A$1,($D1-$C$2)/$B$2,0)
\]

100\text{th} column

\[
=\text{IF}($D1>$A$1,($D1-$C$100)/$B$100,0)
\]
Below is a representative example for when the formulas in all of the columns are selected and applied to every normalized intensity value:

<table>
<thead>
<tr>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.23061</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>43.67595</td>
<td>42.06526</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>63.69265</td>
<td>53.31124</td>
<td>52.13775</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>85.49686</td>
<td>65.56151</td>
<td>60.04657</td>
<td>60.03324</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>128.7701</td>
<td>89.87374</td>
<td>75.74264</td>
<td>71.23759</td>
<td>70.95681</td>
<td>0</td>
</tr>
<tr>
<td>178.9783</td>
<td>118.0822</td>
<td>93.95411</td>
<td>84.23752</td>
<td>81.00734</td>
<td>80.8344</td>
</tr>
</tbody>
</table>

Each row indicates possible size outputs for a single intensity value. Note that while many columns may contain a 0, each row requires further selection to assign each intensity value to a single size output. In fact, only the smallest value at the right hand side is desired, and all values not immediately adjacent to a 0 are easily discarded. Prepare a new collection of columns to receive the selected data.

Column E recipient

=IF(E1+F1>E1,0,E1)

Column F recipient

=IF(F1+G1>F1,0,F1)

Column Y recipient

=IF(Y1+Z1>Y1,0,Y1)

The final column does not strictly require this formula in place. If the user desires to apply it for convenience sake then ensure that the next immediate column contains empty cells or 0 values.
Otherwise the desired output data will be unintentionally rewritten to 0. The example table shown above should now appear in the new columns as follows:

<table>
<thead>
<tr>
<th>New E</th>
<th>New F</th>
<th>New G</th>
<th>New H</th>
<th>New I</th>
<th>New J</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.23061</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>42.06526</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>52.13775</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>60.03324</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>70.95681</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>80.8344</td>
</tr>
</tbody>
</table>

Finally, the original data gathered was intensity as a function of time. To graph the size outputs as a function of the time the data in the above table needs to be condensed into a single column. Because the unneeded values have been set to 0 an entire row is combined simply through addition:

1st row:
=SUM(E1:J1)

100th row:
=SUM(E100:J100)

Carrying this through each row gives the simplified collection of outputs:

34.23061
42.06526
52.13775
60.03324
70.95681
III-f. “Negative” Half

The linear regressions within the “negative” half are utilized in similar fashion to those in the positive half. The formulas used will appear to use the same apparent form. The “IF” clauses and comparisons in the “positive” half were arranged to treat ever increasing slopes. Because the slopes are instead now increasingly negative the formulas used may have slight variations.

*Take care* the column labels noted below use the same designations as in the previous section. This is done intentionally to keep cell choices clear in description. When actually assembling an Excel workbook to use both the “positive” and “negative” halves in concert the cell labels must kept distinct.

1st “negative” half linear regression

=IF($D1>$A$1,($D1-$C$1)/$B$1,0)

100th “negative” half linear regression

=IF($D1>$A$100,($D1-$C$100)/$B$100,0)

Where D1 is a measured time dependent intensity value, A1 is lower of two values used to form the linear regression in the prediction model (for the first regression in the “negative” half the higher of the higher of the two values is “1”), C1 is the linear regression’s y-intercept, and B1 is the linear regression slope and should be less than 0. A representative example is included below.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>169.6048</td>
<td>173.8611</td>
<td>177.7155</td>
<td>180.8281</td>
<td>182.5649</td>
</tr>
<tr>
<td>2</td>
<td>170.2284</td>
<td>174.2293</td>
<td>177.9958</td>
<td>181.0692</td>
<td>182.7917</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>180.1129</td>
<td>182.4742</td>
<td>184.9222</td>
<td>186.4152</td>
</tr>
</tbody>
</table>
Notice that in this example the “0” values appear on the left of the size outputs instead of the right. Therefore, a minor adjustment is made to the previously used selection equation.

Column E recipient

\[=IF(E1+F1>F1,0,F1)\]

Column F recipient

\[=IF(F1+G1>G1,0,G1)\]

Column Y recipient

\[=IF(Y1+Z1>Z1,0,Z1)\]

Which gives,

<table>
<thead>
<tr>
<th>New E</th>
<th>New F</th>
<th>New G</th>
<th>New H</th>
<th>New I</th>
<th>New J</th>
</tr>
</thead>
<tbody>
<tr>
<td>168.003</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>170.2284</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>180.1129</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>190.2172</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>200.1144</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>210.1115</td>
</tr>
</tbody>
</table>

And after the same addition as before, \(=\text{SUM(E1:J1)},\)

168.003
170.2284
180.1129
III-g. Synchronizing “Left” and “Right” Halves

At this point both the “positive” and “negative” halves have been prepared. What follows is how to correctly combine them to finally select a single size output per intensity value. But first, some clarification is in order. The example data sets shown above to describe the “positive” and “negative” half treatments were oversimplifications. In practicality both halves treat the entire data set before the appropriate region is chosen. A more complete example is below,

<table>
<thead>
<tr>
<th>left side</th>
<th>right side</th>
</tr>
</thead>
<tbody>
<tr>
<td>treatment</td>
<td>treatment</td>
</tr>
<tr>
<td>111 0 0 0 0</td>
<td>0 0 0 0 208</td>
</tr>
<tr>
<td>121 121 0 0 0</td>
<td>0 0 0 199 199</td>
</tr>
<tr>
<td>130 131 132 0 0</td>
<td>0 0 188 189 190</td>
</tr>
<tr>
<td>137 139 141 141 0</td>
<td>0 179 180 182 185</td>
</tr>
<tr>
<td>142 144 147 150 150</td>
<td>168 170 174 178 181</td>
</tr>
<tr>
<td>137 138 140 140 0</td>
<td>0 170 174 178 181</td>
</tr>
<tr>
<td>129 130 130 0 0</td>
<td>0 0 180 182 185</td>
</tr>
<tr>
<td>120 120 0 0 0</td>
<td>0 0 0 190 192</td>
</tr>
<tr>
<td>119 0 0 0 0</td>
<td>0 0 0 0 200</td>
</tr>
</tbody>
</table>
Each data set displays an inverting trend; “positive” half outputs increase to a maximum and then
decrease while “negative” half outputs decrease to a minimum and then increase. The mentioned
maximum and minimum occur when the normalized intensity value reaches 1. The “positive”
side outputs are retained from the start of measurements until the $I_{\text{max}}$ is reached at which point
the “negative” half values are used. The retained data is displayed in bold above as a visual aid
for realizing the data selection. In practice the outputs change in small increments and the union
between “positive” and “negative” halves is difficult to perceive even when displayed
graphically.

IV. Synthesis Reactions

IV-a. PNIPAm

\[ M_n \text{ theoretical} = \frac{[\text{Monomer}]}{[\text{Chain Transfer Agent}]} \times (\text{monomer M.W.}) + (\text{Chain Transfer Agent M.W.}) \]

N-isopropylacrylamide (2.3038 g, 20.4 mmol), 2-(Dodecylthiocarbonothioylthio)-2-
methylpropionic acid (0.0833 g, 0.228 mmol), azobisisobutyronitrile (0.00366 g, 0.0223 mmol),
and 5 mL dimethylformamide were combined in a 25 mL conical schlenk flask. The reaction
vessel was subjected to three cycles of freeze pump thaw technique, and then placed in preheated
80° C oil bath while under nitrogen for 24 hours. The flask was cooled to room temperature and
exposed to ambient atmosphere. The yellow solution was then transferred into 3,000 MW cutoff
dialysis tubing and submerged in 300 mL deionized water. The water bath was exchanged for
fresh solution at least 6 times over intervals no shorter than 6 hours. The dialysis tube’s contents
were transferred to a scintillation vial and NIPAm polymer isolated as a pale yellow solid under
vacuum. Molecular weight was calculated by comparing $^1\text{H-NMR}$ integration ratios.
Informal notes:
- AIBN yields two radicals per molecule. RAFT mechanisms commonly use an even smaller 1:10 radical to CTA molar ratio than the 1:5 example shown here.

- The small scale makes it difficult to accurately weigh AIBN. It’s more convenient to add small volumes of previously prepared DMF stock solutions than to weigh such small quantities of solid. Carefully consider the concentration’s shelf life before reusing any stock solutions over repeated experiments.

- Chemical manufacturers typically add a radical inhibitor to stabilize NIPAm stock containers. The inhibitor is easily removed by performing several recrystallizations in methanol. Purifying NIPAm necessitates cold storage to limit spontaneous reaction.

- Both NIPAm and PNIPAm are white solids. The yellow color results from the DDMAT CTA, and its prevalence in the final product lessens depending on molecular weight.

**IV-b. Polystyrene Standards**

The sole credit for preparing the polystyrene used for standardization belongs to Pei Zhang. She synthesized and characterized the particles while a member of the Tsavalas research group shortly before receiving a PhD in material science. The description here was provided by Dr. John G. Tsavalas for peer review, and it is included unaltered in this work for completeness sake.

Monodisperse polystyrene latex nanoparticles were prepared by seeded emulsion polymerization. The smallest particle size, 52 nm, was prepared by first performing a batch emulsion polymerization with 10% of the recipe monomer to develop “pre-seed” particles by micellar nucleation, followed by semi-batch feeding of the remaining monomer under starve-fed conditions so as to specifically grow those pre-seed particles without further particle nucleation.
This reaction was run in a 250 mL water-jacketed glass reactor at a reaction temperature of 70 °C under nitrogen and with condenser to prevent monomer evaporation. The initial batch portion of the reaction utilized 10% of 55 g total styrene monomer along with 1.6 g of sodium dodecyl sulfate (SDS) for colloidal stability and 0.61 g potassium persulfate (KPS) as the anionic radical initiator. After 30 minutes of batch reaction, the remaining portion of the 55 g of styrene monomer was fed over 4 hrs to the reactor, then held at temperature for another 2 hrs to complete monomer conversion, producing PS latex of 26.5% solids content and 52 nm diameter particles (by volume) measured by DLS. This 52 nm latex of PS nanoparticles served as one reference particle, yet also was utilized as the seed for further volumetric growth to prepare the subsequent three larger particle sizes. Those were produced by seeded emulsion polymerization with defined stage ratios (SR = mass ratio of new monomer fed to the existing seed particles relative to the seed particle mass) to target new diameters of 102 nm (SR 6.5), 155 nm (SR 2.7, based off of 102 nm seed), and 210 nm (SR 1.9, based off of 155 nm seed). All growth reactions were performed under conditions that ensured surfactant concentration was below the critical micelle concentration so as to prevent new particle formation and to exclusively grow existing particles. The resulting particle diameters (by volume) measured by DLS were in excellent agreement with targeted sizes (52, 101, 151, and 206 nm) with narrow polydispersity (1.20, 1.11, 1.15, 1.13, respectively). These particle sizes were also in agreement with analogous measurements by capillary hydrodynamic fractionation (CHDF), which is an analytical technique that does not rely on scattering.
IV-c. PNP Ligands

L1, R=Ph R’=Ph

Paraformaldehyde (104 mg, 3.4 mmol), diphenylphosphine (0.500 mL, 2.87 mmol),
aniline (0.130 mL, 1.44 mmol), and 4 mL toluene are added to a scintillation vial within a
nitrogen glovebox. The vial is firmly sealed and heated to 80 °C overnight. Product is isolated
under reduced pressure as an off white solid. $^{13}$C $\{^1$H$\}$ NMR (500 MHz, CDCl$_3$): $\delta$ 54.05
(PCH$_2$N, m), 114.66 (NCC$_5$H$_5$, t, $J_{PC}=2.98$ Hz) ppm. $^{31}$P $\{^1$H$\}$ NMR (500 MHz, CDCl$_3$): $\delta$ -
27.42 ppm.

L4, R=Cy R’=Ph

Paraformaldehyde (90 mg, 2.997 mmol), aniline (0.104 ml, 1.14 mmol), and
dicyclohexylphosphine (0.500 ml, 2.28 mmol), and toluene (5 ml) are added sequentially to a
Paraformaldehyde (106 mg, 3.52 mmol), diphenylphosphine (0.500 mL, 2.87 mmol), benzylamine (0.157 mL, 1.44 mmol), and 4 mL toluene are added sequentially to a scintillation vial within a nitrogen glovebox. The vial is capped firmly and heated to 80 °C overnight without stirring. Product is isolated under reduced pressure as a slightly yellow solid. $^{13}$C $^{1}$H NMR (500 MHz, CDCl$_3$): $\delta$ 58.26 (PCH$_2$N, dd, $J_{PC} = 9.4$, 5.2 Hz), 60.67 (NCH$_2$C$_6$H$_5$, t, $J_{PC} = 9.58$ Hz) ppm. $^{31}$P $^{1}$H NMR (500 MHz, CDCl$_3$): $\delta$ -27.56 ppm.
L5, R=Cy R'=Bn -> made by Taylir Bullick

$^{13}$C{$^{1}$H} NMR (500 MHz, CDCl$_3$): $\delta$ 52.33 (PCH$_2$N, dd, $J_{PC} = 8.5$, 5.4 Hz), 61.44 (NCH$_2$C$_6$H$_5$, t, $J_{PC} = 9.6$ Hz) ppm. $^{31}$P{$^{1}$H} NMR (500 MHz, CDCl$_3$): $\delta$ -17.31 ppm.

Paraformaldehyde (154 mg, 5.14 mmol), aminopropyltrimethoxysilane (0.400 ml, 2.29 mmol), diphenylphosphine (0.800 mL, 4.58 mmol), and 5 mL toluene are added sequentially to a scintillation vial within a nitrogen glovebox. The vial is capped firmly and heated to 80 °C overnight. The toluene layer is separated from water byproduct via pipette and under reduced pressure yields a colorless oil. $^{13}$C{$^{1}$H} NMR (500 MHz, CDCl$_3$): $\delta$ 58.67 (PCH$_2$N, dd, $J_{PC} = 9.5$, 5.6 Hz), 59.15 (NCH$_2$C$_2$H$_4$, t, $J_{PC} = 9.0$ Hz) ppm. $^{31}$P{$^{1}$H} NMR (500 MHz, CDCl$_3$): $\delta$ -28.43 ppm.
L6, R=Cy R’=PTMS

Paraformaldehyde (154 mg, 5.14 mmol), aminopropyltrimethoxysilane (0.400 ml, 2.29 mmol), dipphosphine (0.862 mL, 4.58 mmol), and 5 mL toluene are added sequentially to a scintillation vial within a nitrogen glovebox. The vial is capped firmly and heated to 80 °C overnight. The toluene layer is separated from water byproduct via pipette and under reduced pressure yields a colorless oil. $^{13}$C\{\textsuperscript{1}H\} NMR (500 MHz, CDCl\textsubscript{3}): δ 52.51 (PCH\textsubscript{2}N, dd, $J_{PC}$= 8.4, 5.7 Hz), 59.40 (NCH\textsubscript{2}C\textsubscript{2}H\textsubscript{4}, t, $J_{PC}$= 9.2 Hz) ppm. $^{31}$P\{\textsuperscript{1}H\} NMR (500 MHz, CDCl\textsubscript{3}): δ -17.70 ppm.

V-d. PNP Ni Carbonyl complexes

1 equivalent of bis(triphenylphosphine)nickel(0)dicarbonyl and PNP ligand were combined in dry toluene and heated to 80 °C overnight in a nitrogen glovebox. Aliquots were taken from the resulting solutions for $^{31}$P\{\textsuperscript{1}H\} NMR and liquid IR spectroscopies. $^{31}$P\{\textsuperscript{1}H\} NMR demonstrated reaction success through the disappearance of Ni(PPh\textsubscript{3})\textsubscript{2}(CO)\textsubscript{2} and unbound PNP ligand, and the appearance of both unbound PPh\textsubscript{3} and a new unique peak assigned to Ni(PNP)(CO)\textsubscript{2}.

\[\text{L1-Ni(CO)}_2, \text{R=Ph R'=Ph}\]

L1 (0.085 g, 0.148 mmol), Ni(PPh\textsubscript{3})\textsubscript{2}(CO)\textsubscript{2} (0.090 g, 0.141 mmol), 3 mL toluene.

$^{31}$P\{\textsuperscript{1}H\} NMR (500 MHz, CDCl\textsubscript{3}): δ 12.66 ppm. IR: (CO) 1951, 2005 cm\textsuperscript{-1}. 
L4-Ni(CO)₂, R=Cy R’=Ph

L4 (0.194 g, 0.378 mmol), Ni(PPh₃)₂(CO)₂ (0.252 g, 394 mmol), 3 mL toluene.

${}^{31}\text{P}\{^1\text{H}\}$ NMR (500 MHz, CDCl₃): δ 25.25 ppm. IR: (CO) 1927, 1987 cm⁻¹.

L2-Ni(CO)₂, R=Ph R’=Bn

L2 (0.102 g, 0.203 mmol), Ni(PPh₃)₂(CO)₂ (0.126 g, 0.197 mmol), 4 mL toluene.

${}^{31}\text{P}\{^1\text{H}\}$ NMR (500 MHz, CDCl₃): δ 12.74 ppm. IR: (CO) 1947, 2003 cm⁻¹.

L5-Ni(CO)₂, R=Cy R’=Bn -> made by Taylir Bullick
$^{31}$P{¹H} NMR (500 MHz, CDCl₃): δ 24.66 ppm. IR: (CO) 1924, 1985 cm⁻¹.

L₃-Ni(CO)₂, R=Ph R’=PTMS

L₃ (0.085 g, 0.148 mmol), Ni(PPh₃)₂(CO)₂ (0.090 g, 0.133 mmol), 3 mL toluene.

$^{31}$P{¹H} NMR (500 MHz, CDCl₃): δ 12.52 ppm. IR: (CO) 1947, 2003 cm⁻¹.

L₆-Ni(CO)₂, R=Cy R’=PTMS

L₆ (0.105 g, 0.175 mmol), Ni(PPh₃)₂(CO)₂ (0.098 g, 0.153 mmol), 3 mL toluene.

$^{31}$P{¹H} NMR (500 MHz, CDCl₃): δ 24.55 ppm. IR: (CO) 1924, 1985 cm⁻¹.

IV-e. PNP Ni Lactone complexes
TMEDA Nickel Lactone

Within a nitrogen glovebox Ni(COD)$_2$ (1.000 g, 3.636 mmol), succinic anhydride (344 mg, 3.44 mmol), and N,N,N’,N’-tetramethylethylenediamine (~5 mL, excess) were combined and stirred overnight. The resulting mixture was suction filtered using a glass frit and washed with dry THF until the filtrate appeared colorless. The green solid was then transferred to vial and washed 2x with 2-3 mL THF, allowing the solid to settle and removing the supernatant via pipette. The pure product was isolated under vacuum. $^{13}$C{$^1$H} NMR (DMF-d7): δ -1.04 (C3), 37.83 (C2), 47.10 (C4’), 49.25 (C4), 56.87 (C5’), 61.73 (C5), 186.83 (C1) ppm. IR: (C=O) 1617 cm$^{-1}$.

L1-Ni$^{II}$Lac, R=Ph R’=Ph

Solid TMEDA Nickel Lactone (52 mg, 0.211 mmol), L1 (99 mg, 0.202 mmol), and 5 mL THF were added sequentially to a scintillation vial within a nitrogen glovebox. The vial was stirred for 1 hr and then placed into a glovebox refrigerator. After 5 days, solvent is removed under vacuum to yield a faintly yellow solid. $^{13}$C{$^1$H} NMR (400 MHz, CDCl$_3$): δ 22.50 (C3, dd, $J_{PC}$= 57.2, 23.3 Hz), 37.65 (C2, m), 54.67 (C5, d, $J_{PC}$= 35.1 Hz), 56.71 (C4, dd, $J_{PC}$= 37.6, 3.8 Hz) ppm. $^{31}$P{$^1$H} NMR (400 MHz, CDCl$_3$): δ -3.17 (P$_{trans-C}$, d, $J_{PP}$= 33.3 Hz), 28.39 (P$_{trans-O}$, d, $J_{PP}$= 32.0 Hz) ppm. IR: (C=O) 1635 cm$^{-1}$.
L4-Ni^{II}Lac, R=Cy R’=Ph

Solid TMEDA Nickel Lactone (45 mg, 0.182 mmol), L4 (100 mg, 0.195 mmol), and 6 mL THF were added sequentially to a scintillation vial within a nitrogen glovebox. The vial was stirred overnight during which the color changed from green to yellow and then orange. After spending several hours within the glovebox refrigerator, the orange/red solution was placed under vacuum to yield a faintly pink colored solid. $^{13}$C($^1$H) NMR (400 MHz, CDCl$_3$): $\delta$ 13.26 (C3, dd, $J_{PC}$ = 57.6, 28.9 Hz), 37.49 (C2, dd, $J_{PC}$ = 4.5, 2.1 Hz), 48.44 (C4, d, $J_{PC}$ = 28.2 Hz), 50.60 (C4’, dd, $J_{PC}$ = 33.0, 6.0 Hz) ppm. $^{31}$P($^1$H) NMR (400 MHz, CDCl$_3$): $\delta$ 8.93 (P$_{trans-C}$, d, $J_{PP}$ = 36.4 Hz), 29.27 (P$_{trans-O}$, d, $J_{PP}$ = 36.4 Hz) ppm. IR: (C=O) 1626 cm$^{-1}$.

L2-Ni^{II}Lac, R=Ph R’=Bn

Solid TMEDA Nickel Lactone (52 mg, 0.211 mmol), L2 (99 mg, 0.197 mmol), and 5 mL THF were added sequentially to a scintillation vial within a nitrogen glovebox. The vial was stirred for 1 hr and then placed into a glovebox refrigerator. After 5 days, solvent is removed
under vacuum to yield a faintly yellow solid. $^{13}$C {$^1$H} NMR (400 MHz, CDCl$_3$): $\delta$ 22.32 (C3, dd, $J_{PC}$= 57.7, 23.2 Hz), 37.55 (C2, dd, $J_{PC}$= 4.7, 2.3 Hz), 55.94 (C4', dd, $J_{PC}$= 40.1, 3.6 Hz), 56.97 (C4, d, $J_{PC}$= 36.2 Hz), ppm. $^{31}$P {$^1$H} NMR (400 MHz, CDCl$_3$): $\delta$ -3.73 (P$_{trans}$-C, d, $J_{PP}$= 35.0 Hz), 28.23 (P$_{trans}$-O, d, $J_{PP}$= 34.9) ppm. IR: (C=O) 1626 cm$^{-1}$.

L5-Ni$^{II}$Lac, R=Cy R'=Bn -> made by Taylir

$^{13}$C {$^1$H} NMR (500 MHz, CDCl$_3$): $\delta$ 12.99 (C3, dd, $J_{PC}$= 58.7, 29.1 Hz), 37.58 (C2, d, $J_{PC}$= 3.3 Hz), 49.40 (C4', dd, $J_{PC}$= 33.9, 5.9 Hz), 49.80 (C4, d, $J_{PC}$= 29.2 Hz).40 ppm. $^{31}$P {$^1$H} NMR (500 MHz, CDCl$_3$): $\delta$ 8.82 (P$_{trans}$-C, d, $J_{PP}$= 37.8 Hz), 30.51 (P$_{trans}$-O, d, $J_{PP}$= 37.9 Hz) ppm.

L3-Ni$^{II}$Lac, R=Ph R'=PTMS

Solid TMEDA Nickel Lactone (80 mg, 0.324 mmol) and 5 mL THF were added to a scintillation vial within a nitrogen glovebox. L3 oil (186 mg, 0.324 mmol) was added via spatula and dissolved into the green suspension. The vial was firmly sealed placed into and placed into the glovebox refrigeration unit. After 5 days, solvent is removed under vacuum to yield a faintly
yellow solid. $^{13}$C{$^1$H} NMR (400 MHz, CDCl$_3$): $\delta$ 22.19 (C3, dd, $J_{PC}$ = 58.9, 22.6 Hz), 37.60 (C2, m), 56.35 (C4’, dd, $J_{PC}$ = 40.6, 3.1 Hz), 57.36 (C4, m) ppm. $^{31}$P{$^1$H} NMR (400 MHz, CDCl$_3$): $\delta$ -3.78 ($P_{trans-C}$, d, $J_{PP}$ = 35.1 Hz), 27.69 ($P_{trans-O}$, d, $J_{PP}$ = 35.0 Hz) ppm. IR: (C=O) 1627 cm$^{-1}$.

L6-Ni$^{11}$Lac, R=Cy R’=PTMS

Solid TMEDA Nickel Lactone (80 mg, 0.324 mmol) and 5 mL THF were added to a scintillation vial within a nitrogen glovebox. L6 oil (186 mg, 0.324 mmol) was added via spatula and dissolved into the green suspension. The vial was firmly sealed placed into and placed into the glovebox refrigeration unit. The vial was periodically allowed to stir at room temperature and returned to the refrigerator while reaction progress was determined by $^{31}$P NMR. After 1 hr, 20 min, and 15 min the mixture changed from a green to yellow/orange and reached 94% turnover. The vial was placed under reduced pressure for 9 hr at room temperature to yield an orange/red oil. $^{13}$C{$^1$H} NMR (400 MHz, CDCl$_3$): $\delta$ 37.48 (C2, m), 13.14 (C3, dd, $J_{PC}$ = 58.4, 29.0 Hz), 50.4 (C4, d, $J_{PC}$ = 30.5), 49.98 (C4’, d, $J_{PC}$ = 34.6 Hz) ppm. $^{31}$P{$^1$H} NMR (400 MHz, CDCl$_3$): $\delta$ 8.62 ($P_{trans-C}$, d, $J_{PP}$ = 38.1 Hz), 29.47 ($P_{trans-O}$, d, $J_{PP}$ = 37.8 Hz) ppm. IR: (C=O) 1620 cm$^{-1}$. 

![Diagram](image)
SPECTROSCOPIC DATA
Spectrum 1. $^1$H nmr, chain transfer agent
Spectrum 2. $^1$H nmr, n-isopropylacrylamide
Spectrum 3. $^1$H-nmr, PNIPAm $M_n = 10,200$
Spectrum 4. $^1$H nmr, PNIPAm zoom $M_n = 10,200$
Spectrum 5. $^1$H nmr, PNIPAm $M_n = 8,400$
Spectrum 6. $^1$H nmr, PNIPAm zoom $M_n = 8,400$
Spectrum 7. $^{31}$P nmr, PNP ligand R=R'=Ph
Spectrum 8. $^{13}$C nmr, PNP ligand $R=R'=\text{Ph}$
Spectrum 9. $^1$H nmr, PNP ligand R=R'=Ph
Spectrum 10. $^{31}$P nmr, PNP ligand R=Ph R'=Bn
Spectrum 11. $^{13}$C nmr, PNP ligand $R=\text{Ph}$ $R'=\text{Bn}$
Spectrum 12. $^1$H nmr, PNP ligand $R=Ph \ R'=Bn$
Spectrum 13. $^{31}$P nmr, PNP ligand $R=\text{Ph}$ $R'=\text{PTMS}$
Spectrum 14. $^{13}$C nmr, PNP ligand $R=\text{Ph}, R'=\text{PTMS}$
Spectrum 15. $^1$H nmr, PNP ligand $R=\text{Ph}$ $R'=\text{PTMS}$
Spectrum 16. $^{31}$P nmr, PNP ligand R=Cy R'=Ph
Spectrum 17. $^{13}$C nmr, PNP ligand R=Cy R'=Ph
Spectrum 18. $^1$H nmr, PNP ligand R=Cy R'=Ph
Spectrum 19. $^{31}$P nmr, PNP ligand R=Cy R'=Bn
Spectrum 20. $^{13}$C nmr, PNP ligand $R=\text{Cy} \quad R'=\text{Bn}$
Spectrum 21. $^1$H nmr, PNP ligand R=Cy R$'$=Bn
Spectrum 22. $^{31}$P nmr, PNP ligand R=Cy R'=PTMS
Spectrum 23. $^{13}$C nmr, PNP ligand $R=\text{Cy}$ $R'=\text{PTMS}$
Spectrum 24. $^1$H nmr, PNP ligand R=Cy R'=PTMS
Spectrum 25. $^{31}$P nmr, Ni(PNP)(CO)$_2$ synthesis R=Ph R'=Ph
Spectrum 26. IR, Ni(PNP)(CO)$_2$ R=Ph R'=Ph
Spectrum 27. $^{31}$P nmr, Ni(PNP)(CO)$_2$ synthesis R=Ph R'=Bn
Spectrum 28. IR, Ni(PNP)(CO)$_2$ R=Ph R'=Bn
Spectrum 29. $^{31}$P nmr, Ni(PNP)(CO)$_2$ synthesis $R=\text{Ph}$ $R'=\text{PTMS}$
Spectrum 30. IR, Ni(PNP)(CO)_2 R=Ph R'=PTMS
Spectrum 31. $^{31}$P nmr, Ni(PNP)(CO)$_2$ synthesis R=Cy R'=Ph
Spectrum 32. IR, Ni(PNP)(CO)₂ R=Cy R'=Ph
Spectrum 33. $^{31}$P nmr, Ni(PNP)(CO)$_2$ synthesis R=Cy R'=Bn
Spectrum 34. $^{31}$P nmr, Ni(PNP)(CO)$_2$ synthesis R=Cy R'=PTMS
Spectrum 35. IR, Ni(PNP)(CO)$_2$ R=Cy R'=PTMS
Spectrum 36. $^{13}$C nmr DMF-d7, TMEDA Ni Lactone
Spectrum 37. $^1$H nmr DMF-d7, TMEDA Ni Lactone
Spectrum 38. IR, TMEDA Ni Lactone
Spectrum 39. $^{31}$P nmr, PNP nickelalactone R=Ph R'=Ph
Spectrum 40. $^{13}$C nmr, PNP Nickelalactone $R=\text{Ph}$ $R'=\text{Ph}$
Spectrum 41. $^1$H nmr, PNP Nickelalactone R=Ph R'=Ph
Spectrum 42. HSQC nmr $^1$H vs $^{13}$C{$^1$H}, PNP Nickelalactone R=Ph R'=Ph
Spectrum 43. IR, PNP Nickelalactone R=Ph R'=Ph
Spectrum 44. $^{31}$P nmr, PNP Nickelalactone R=Ph R'='Bn
Spectrum 45. $^{13}$C nmr, PNP Nickelalactone $R=\text{Ph}$ $R'=\text{Bn}$
Spectrum 46. $^1$H nmr, PNP Nickelalactone R=Ph R'=Bn
Spectrum 47. HSQC nmr $^1$H vs $^{13}$C{$^1$H}, PNP Nickelalactone R=Ph R'=Bn
Spectrum 48. HSQC nmr $^1$H vs $^{31}$P, PNP Nickelalactone R=Ph $^{\prime}$=Bn
Spectrum 49. IR, PNP Nickelalactone $R=\text{Ph}$ $R'=\text{Bn}$
Spectrum 50. $^{31}$P nmr, PNP Nickelalactone R=Ph R'=PTMS
Spectrum 51. $^{13}$C nmr, PNP Nickelalactone R=Ph R'=PTMS
Spectrum 52. $^1$H nmr, PNP Nickelalactone R=Ph R'=PTMS
Spectrum 53. IR, PNP Nickelalactone R=Ph R'=PTMS
Spectrum 54. $^{31}$P nmr, PNP Nickelalactone R=Cy R'=Ph
Spectrum 55. $^{13}$C nmr, PNP Nickelalactone R=Cy R'=Ph
Spectrum 56. $^1$H nmr, PNP Nickelalactone R=Cy R'=Ph
Spectrum 57. IR, PNP Nickelalactone R=Cy R'=Ph
Spectrum 58. $^{31}$P nmr, PNP Nickelalactone R=Cy R'=Bn
Spectrum 59. $^{31}$C nmr, PNP Nickelalactone R=Cy R=Bn
Spectrum 60. $^1$H nmr, PNP Nickelalactone $R=$Cy $R'=$Bn
Spectrum 61. $^{31}$P nmr, PNP Nickelalactone R=Cy R'=PTMS
Spectrum 62. $^{13}$C nmr, PNP Nickelalactone R=Cy R'=PTMS
Spectrum 63. $^1$H nmr, PNP Nickelalactone R=Cy R'=PTMS
Spectrum 64. IR, PNP Nickelalactone R=Cy R’=PTMS
Spectrum 65. $^{31}$P nmr 25 °C, PNP Nickelalactone $R=\text{Ph}$ $R'=\text{Ph}$
Spectrum 66. $^{31}$P nmr 35 °C, PNP Nickelalactone R=Ph R'=Ph
Spectrum 67. $^{31}$P nmr 45 °C, PNP Nickelalactone $R=\text{Ph} \ R'=\text{Ph}$
Spectrum 68. $\text{^31P nmr 52 °C, PNP Nickelalactone } R=\text{Ph } R'=\text{Ph}$
Spectrum 69. $^{31}$P nmr 35 °C, PNP Nickelalactone $R=\text{Ph}$ $R'=\text{Bn}$
Spectrum 70. \(^{31}\text{P nmr} 45^\circ\text{C, PNP Nickelalactone } R=\text{Ph} R'=\text{Bn}\)
Spectrum 71. $^{31}$P nmr 52 °C, PNP Nickelalactone $R=\text{Ph} \ R'=\text{Bn}$
Spectrum 72. $^{31}$P nmr 25 °C, PNP Nickelalactone R=Ph R'=PTMS
Spectrum 73. $^{31}$P nmr 25 °C, PNP Nickelalactone R=Ph R'=PTMS
Spectrum 74. $^{31}$P nmr 45 °C, PNP Nickelalactone $R=\text{Ph}$ $R'=\text{PTMS}$
Spectrum 75. $^{31}$P nmr 52 °C, PNP Nickelalactone $R=$Ph $R'=$PTMS
Spectrum 76. $^{31}$P nmr 25 °C, PNP Nickelalactone R=Cy R'=Ph
Spectrum 77. $^{31}$P nmr 35 °C, PNP Nickelalactone $R$=Cy $R'$=Ph
Spectrum 78. $^{31}$P nmr 45 °C, PNP Nickelalactone R=Cy R'=Ph
Spectrum 79. $^{31}$P nmr 52 °C, PNP Nickelalactone R=Cy R'=Ph
Spectrum 80. $^{31}$P nmr 35 °C, PNP Nickelalactone R=Cy R'=Bn
Spectrum 81. $^{31}\text{P} \text{nmr } 45 \, ^\circ\text{C, PNP Nickelalactone } R=\text{Cy} \, R'=\text{Bn}$
Spectrum 82. $^{31}$P nmr 52 °C, PNP Nickelalactone R=Cy R'=Bn
Spectrum 83. $^{31}$P nmr 35 °C, PNP Nickelalactone R=Cy R'=PTMS
Spectrum 84. $^{31}$P nmr 45 °C, PNP Nickelalactone R=Cy R'=PTMS
Spectrum 85. $^{31}$P nmr 52 °C, PNP Nickelalactone $R=\text{Cy}$ $R'=\text{PTMS}$
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