DEVELOPMENT OF INORGANIC-ORGANIC HYBRID NANOMATERIALS FOR BIOLOGICAL AND BIOMEDICAL APPLICATIONS

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ABSTRACT

William J. Rieter: Development of Inorganic-Organic Hybrid Nanomaterials for Biological and Biomedical Applications
(Under the direction of Wenbin Lin)

Despite the great potential of combining inorganic and organic components to afford materials with unique properties, the development of so-called “hybrid” nanomaterials is a relatively new area of materials science. In this work, we outline the syntheses and characterization of several new classes of hybrid nanomaterials with potential applications in biomedical imaging and therapy. First, we use a microemulsion-based method to prepare gadolinium-based nanoscale metal-organic frameworks (NMOFs) as novel Magnetic Resonance Imaging (MRI) contrast enhancing agents. We have also developed a method to coat the NMOFs with shells of amorphous silica, and illustrate the potential utility of these core-shell structures as probes for the ratiomeric luminescence detection of analytes in solution and as templates for new composite nanomaterials. Second, we use a general strategy to precipitate nanoscale coordination polymer (NCP) particles composed of platinum-based anticancer drugs from a solution of the components via the addition of a poor solvent. The release of the drug species is controlled by varying the thickness of an amorphous silica shell, and the anticancer efficacies of the drug formulations are demonstrated against multiple cancer cell lines in vitro. Lastly, we have developed several unique silicon oxide-based nanoparticle formulations, including core-shell and
polysilsesquioxane structures, which have multifunctional imaging capabilities superior to agents that are currently used in the clinic.
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LIST OF ABBREVIATIONS

°C degrees Celsius

$\lambda_{\text{em}}$ wavelength of emission

$\lambda_{\text{ex}}$ wavelength of excitation

$\mu$ micro-

ala $L$-alanine

AOT bis(1-ethylhexyl)sulfosuccinate

AR aspect ratio

ATCC American Type Culture Collection

BDC 1,4-benzenedicarboxylic acid

calc calculated

c(RGDfK) cyclic(arginine-glycine-aspartic acid-phenylalanine-lysine)

CTAB cetyltri(methyl)ammonium bromide

DCP-AES Direct Current Plasma-Atomic Emission Spectrometry

DLS Dynamic Light Scattering

DMF dimethylformamide

DOTB 1,4,7,10-tetraazacyclododecane-$N,N',N''$-$N''$-tetramethyl-$p$-benzoic acid

DOTB-ME 1,4,7,10-tetraazacyclododecane-$N,N',N''$-$N''$-tetramethyl-$p$-methyl benzoate

DPA dipicolinic acid

DSCP disuccinato cisplatin

DTPA diethylenetriaminepentaacetic acid

DTPA-BSCA diethylenetriaminepentaacetic acid-bis[tri(ethoxy)silylpropyl cystamide]

DTPA-BSiA diethylenetriaminepentaacetic acid-bis[tri(ethoxy)silylpropyl amide]
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<td>ethylenediaminetetraacetic acid</td>
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<td>EDTA-MSiA</td>
<td>ethylenetriaminetetraacetic acid-mono[tri(ethoxy)silylpropyl amide]</td>
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<tr>
<td>ESI</td>
<td>electrospray ionization</td>
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<td>Ln</td>
<td>lanthanide: specifically used to denote Eu³⁺, Gd³⁺, and Tb³⁺</td>
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<td>Nanoscale Coordination Polymer</td>
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<td>Tb₂(DSCP)₃(H₂O)₁₂ Nanoscale Coordination Polymer</td>
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<td>NMOF 1’’</td>
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<td>Silica Nanoparticle</td>
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<td>Gd-SiDTTA functionalized Silica Nanoparticles</td>
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SNP 2  Gd-DTPA-BSiA functionalized Silica Nanoparticles
SPI-77 sterically stabilized liposomal cisplatin formulation
T  Tesla
$\frac{1}{2}$ half-life
$T_1$ longitudinal relaxation
$T_2$ transverse relaxation
TEM Transmission Electron Microscopy
TEOS tetraethyl orthosilicate
TGA Thermogravimetric Analysis
Triton X-100 $t$-octylphenoxy polyethoxyethanol
v/v volume to volume
W water to surfactant ratio
XRD X-Ray Diffraction
CHAPTER 1
GADOLINIUM-BASED NANOSCALE METAL-ORGANIC FRAMEWORKS AS
POTENTIAL MULTIMODAL CONTRAST ENHANCING AGENTS

1.1 Introduction

1.1.1 Nanoscale Metal-Organic Frameworks

Metal-organic frameworks (MOFs) are a class of crystalline solids constructed from metal ions, or metal ion clusters, having two or more vacant coordination sites and polydentate organic bridging ligands. Owing to the choice of a wide selection of metal ions and an infinite number of organic bridging ligands, MOFs have the potential to be engineered with a limitless range of structures, compositions, and properties. This high degree of tailorability has led the syntheses of a variety of bulk MOF materials, displaying promising characteristics for a number of applications, such as gas sorption, catalysis, and nonlinear optics. Despite the great potential of combining metal and organic components to afford crystalline materials with unique properties, there have been no reported attempts to downsize MOFs into the nano-regime. A number of reports have, however, appeared outlining the syntheses of cyano-bridged coordination polymer, or cyano-metallate, nanoparticles over the last decade. While these nanoparticles do not strictly adhere to the conventional definition of MOF materials, their preparation has provided valuable insight into to how one might synthesize similar inorganic-organic hybrid materials on the nanometer-scale.
1.1.2 Microemulsion-Based Syntheses

Among the many techniques that have been explored to prepare cyanometallate nanoparticles, water-in-oil, or reverse, microemulsions have emerged as the most versatile and reproducible method. Reverse microemulsions are composed of water droplets stabilized by a surfactant in a predominant organic phase. The water to surfactant ratio, or $W$ value, is the most important parameter to consider in the syntheses since it largely determines the number and size of the reverse micelles within the microemulsion. Particles prepared via this method often display size dependent behavior with respect to the $W$ value. Other parameters one should also consider in the syntheses include the choice of surfactant, surfactant concentration, choice of co-surfactant, co-surfactant concentration, choice of reactant species, reactant concentration, reactant ratio, and temperature. The extent to which each of these parameters influences particle size and morphology is largely dependent on the material under investigation.\(^{17}\)

1.1.3 Fundamentals of Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a non-invasive imaging technique whereby images are generated based on the nuclear magnetic resonance signals of the water proton ($^1$H) nuclei located in different tissue environments throughout the specimen. While MRI is a rather versatile tool, capable of yielding information regarding the physico-chemical state of tissues, flow diffusion, and motion, it is predominantly used to obtain $T_1$- or $T_2$-weighted anatomical images. $T_1$-weighted MR images are generated based on the rate of longitudinal relaxation ($T_1$) of the water $^1$H nuclei. When the protons resonating at an equilibrium frequency dependent upon the magnitude of the applied magnetic field are excited with a
radiofrequency pulse, a change in their net magnetization will occur. Protons having more rapid longitudinal relaxation, or a shorter $T_1$, will relax back to their equilibrium state fast, yielding higher net electromagnetic signals transmitted back to the spectrometer, and thus show up as more intense signals in the MR image. $T_2$-weighted MR images, on the other hand, are generated based on the rate of transverse relaxation ($T_2$) of the water $^1$H nuclei. Because a faster transverse relaxation results in more rapid dephasing of individual spins, the corresponding signal decreases as the value of $T_2$ becomes smaller, ultimately decreasing the signal intensity in the MR image. Owing to its incredibly high spatial resolution, excellent soft tissue contrast, and limitless depth of penetration, MRI has become a very powerful diagnostic tool in medicine. The main drawback of MRI, however, is its intrinsically low sensitivity.\textsuperscript{18}

\textbf{1.1.4 Contrast Agents in $T_1$-Weighted Magnetic Resonance Imaging}

Contrast agents are often administered to enhance the rates of water proton relaxation and increase the sensitivity of MRI as an imaging technique. Efficient $T_1$-enhancing contrast agents generally have a large spin number, at least one vacant site on the metal for the rapid inner-sphere exchange of water molecules, slow electron relaxation, and slow rotational diffusion. They are typically composed of a highly paramagnetic metal, such as Gd$^{3+}$, and are administered as metal-chelate complexes (several commercially available $T_1$-weighted contrast agents are shown in Figure 1-1) to reduce the potentially high toxicity attributed to the free metal ions. The relaxivity value ($r_1$ for $T_1$-enhancing agents), or $1 / T_1$ per mM of Gd$^{3+}$, of a contrast agent is a measure of its ability to increase the rate of relaxation, and is given in units of mM$^{-1}$ s$^{-1}$. Small-molecule Gd-complexes typically have $r_1$ values of $\sim$4-5
It has recently been shown that by incorporating Gd-complexes into nanoparticle formulations, such as micelles and liposomes, the $r_1$ values on a per mM of Gd$^{3+}$ basis are significantly higher.$^{20,21}$ More importantly, each particle is capable of delivering hundreds to thousands of Gd-complexes, resulting in unprecedented relaxivities on a per mM of particle basis, which, in turn, enables them to be targeted to receptors expressed on the surfaces of cells at concentrations below the enhancement limits of molecular Gd-chelates for molecular imaging purposes.

![Figure 1-1. Examples of commercially available $T_1$-weighted Gd-based MRI contrast agents.]

**1.1.5 Motivation for This Work**

The ability to tailor the compositions, structures, and properties of MOF materials has prompted us to develop new multimodal imaging probes by incorporating suitable metal ions and organic moieties into nanoscale MOFs. In this work, we use a general water-in-oil microemulsion-based methodology for the synthesis of Gd-based NMOFs. We hypothesized that by incorporating Gd$^{3+}$ metal ions into NMOFs, we would effectively decrease the rates of rotational diffusion, thereby increasing the relaxivities of the nanometer-scale contrast
agents. Furthermore, each NMOF would have the ability to carry hundreds to thousands of the Gd$^{3+}$ ions, affording contrast agents with enormous relaxivities on a per particle basis.

1.2 Results and Discussion

1.2.1 Synthesis of Nanoscale Metal-Organic Frameworks

Nanoscale metal-organic frameworks (NMOFs) were synthesized in reverse microemulsions composed of the surfactant cetyltrimethylammonium bromide (CTAB), the co-surfactant 1-hexanol, iso-octanes or n-heptanes, and water. In a typical experiment, an aliquot of an aqueous solution of the metal ion species was added to a magnetically stirred CTAB/1-hexanol/iso-octanes mixture, yielding a microemulsion corresponding to a particular $W$ (water to surfactant molar ratio). An equivalent microemulsion was prepared by adding an aliquot of an aqueous solution of the methylammonium salt of the bridging ligand to a separate CTAB/1-hexanol/iso-octanes mixture. After stirring the separate microemulsions until visibly clear, they were combined and stirred for an additional period of time. The particles were subsequently isolated via centrifugation, washed with ethanol, and redispersed in ethanol via sonication. The NMOFs were characterized by Scanning Electron Microscopy (SEM), Powder X-Ray Diffraction (PXRD), and Thermogravimetric Analysis (TGA). The yields, compositions, morphologies, and sizes for most NMOF formulations were highly reproducible under identical microemulsion conditions.

The use of reverse microemulsions allowed us to precisely tune specific variables of the reaction system in order to alter the nucleation and growth kinetics of the NMOF particles. For instance, we chose CTAB as the stabilizing surfactant on account of its nature as a non-coordinating amphiphile. Other commonly used surfactants, such as negatively.
charged bis(1-ethylhexyl)sulfosuccinate (AOT) and neutral t-octylphenoxy polyethoxyethanol (Triton X-100), often have a tendency to coordinate to metal ions involved in the reaction. While they too may one day prove useful in the syntheses of NMOFs, it was our initial aim to use a microemulsion system without the chemical involvement of the surfactant. Other key variables of the reaction system included the surfactant concentration, the concentrations of the reactants, the reactant ratio, the pH of the reactant solutions, and most importantly, the $W$ value. The $W$ value was such a vital parameter since it largely determined the size and quantity of reverse micelles within the microemulsion system.

1.2.2 Synthesis and Characterization of $\text{Gd}_2(\text{BDC})_3(\text{H}_2\text{O})_4$

The first NMOF system we successfully synthesized using reverse microemulsions had the composition $\text{Gd}_2(\text{BDC})_3(\text{H}_2\text{O})_4$ (1). NMOFs of 1 were prepared by magnetically stirring aqueous solutions of GdCl$_3$ and di(methylammonium) 1,4-benzenedicarboxylate (BDC) together at a 2:3 molar ratio in the microemulsion mixture. After 2 h of vigorous stirring, NMOFs of 1 were isolated as a white powder in greater than 90 % yield. SEM micrographs of 1 showed that rods of uniform size and shape were synthesized from a single reaction (Figure 1-2 and 1-3). Interestingly, we found the aspect ratios (ARs) of 1 could be reproducibly tuned by varying the $W$ of the microemulsion. NMOFs of 1 with dimensions 100 to 150 nm in length by ~40 nm in width (AR ~3) were obtained at $W = 5$ (Figure 1-2). As the $W$ was increased from 5 to 10, NMOFs of 1 with dimensions of up to ~2 µm in length by ~100 nm in width were obtained under otherwise identical conditions (Figure 1-3). The particle sizes and ARs were also affected by the reactant concentration and the reactant ratio.
Deviations in the concentration of reactants from 0.050 M:0.075 M (Gd: BDC), or of the metal to ligand molar ratio from 2:3, typically resulted in a decrease of particle size.

**Figure 1-2.** SEM micrographs of NMOFs of 1 synthesized at $W = 5$ in the cationic reverse microemulsion reaction mixture.

**Figure 1-3.** SEM micrographs of NMOFs of 1 synthesized at $W = 10$ in the cationic reverse microemulsion reaction mixture.

We believe the rod-like shape of the nanoparticles obtained under these conditions may have resulted from the anisotropies associated with the growth of the nanocrystals. We also speculate that longer particles obtained as a result of increasing the $W$ were due to a decrease in sites of nucleation within the microemulsion. Previous calculations suggest that by increasing $W$, one not only increases the hydrodynamic radii of the individual micelles
within the microemulsion, but also decreases the percent of micelles occupied by the reactants. In support of this hypothesis, we found the particle size decreased with increases to the concentration of reactants within the microemulsion, presumably because we were increasing the percent of micelles occupied by the reactants, and thus increasing the sites of nucleation.

Powder XRD data showed that NMOFs of 1 were crystalline and corresponded to the known Tb$_2$(BDC)$_3$(H$_2$O)$_4$ phase reported by Reineke et al.$^{22}$ Crystallographic data for Tb$_2$(BDC)$_3$(H$_2$O)$_4$ is given in Table 1-1 and the crystal structure is shown in Figure 1-4. The composition of 1 was further supported by TGA data (Figure 1-5B). An initial TGA weight loss of 7.9 % in the range of 125 to 140 °C corresponded to the loss of the 4 coordinated water molecules (calc. 8.2 %). The next and final weight loss of 50.9 % in the range of 430 to 550 °C corresponded to the loss of the remaining 3 benzenedicarboxylate moieties (calc. 50.6 %).

| Table 1-1. Crystallographic Data for Tb$_2$(BDC)$_3$(H$_2$O)$_4$.$^{22}$ |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| empirical formula | Tb$_2$(BDC)$_3$(H$_2$O)$_4$ |  |
| formula weight  | 882.26           |  |
| crystal system   | triclinic        |  |
| space group      | P1              |  |
| Z                | 1               |  |
| a                | 6.1420(2) Å      |  |
| b                | 10.0694(1) Å     |  |
| c                | 10.0956(3) Å     |  |
| α                | 102.247(2) °     |  |
| β                | 91.118(1) °      |  |
| γ                | 101.518(2) °     |  |
| V                | 596.63(3) Å$^3$  |  |
| $d_{calc}$ (g/cm$^3$) | 2.455           |  |
Figure 1-4. (A) The building block unit including the asymmetric unit present in crystalline Tb$_2$(BDC)$_3$(H$_2$O)$_4$. (B) Crystal structure for Tb$_2$(BDC)$_3$(H$_2$O)$_4$ where hydrogen atoms have been omitted for clarity.

Figure 1-5. (A) The experimental powder XRD pattern for NMOFs of 1 (blue) and the calculated powder XRD pattern from the bulk crystalline phase of Tb$_2$(BDC)$_3$(H$_2$O)$_4$ (red). (B) TGA curve for NMOFs of 1.

1.2.3 Synthesis and Characterization of [Gd(iBTC)(H$_2$O)$_3$]$\cdot$H$_2$O

To probe the generality of NMOF syntheses using reverse microemulsions, we have prepared NMOFs of the composition [Gd(iBTC)(H$_2$O)$_3$]$\cdot$H$_2$O (2). In a typical synthesis, aqueous solutions of GdCl$_3$ and tri(methylammonium) 1,2,4-benzenetricarboxylate (iBTC) at a molar ratio of 2:1 were magnetically stirred in a microemulsion mixture at $W = 15$. 

9
Irregularly-shaped nanoplates with a diameter of ~100 nm and a length of ~35 nm (Figure 1-6) were isolated after 8 h as a white powder in ~75 % yield. In contrast to NMOFs of 1, increases in the metal to ligand ratio in the syntheses of 2 did not affect particle size or morphology; however, decreases in the metal to ligand ratio instead gave sheets and flower-like micro-particles (Figure 1-7).

The experimental PXRD pattern for 2 matched that of the known \([\text{Nd}(i\text{BTC})(\text{H}_2\text{O})_3]\cdot\text{H}_2\text{O}\) phase reported by Jin et al.\(^{23}\) Crystallographic data for \([\text{Nd}(i\text{BTC})(\text{H}_2\text{O})_3]\cdot\text{H}_2\text{O}\) is given in Table 1-2 and the crystal structure is shown in Figure 1-8. As shown in Figure 1-9B, TGA gave an initial weight loss of 16.3 % from r.t. to ~300 °C, which corresponded to the loss of 1 lattice water molecule and 3 coordinated water molecules (calc. 16.5 %). A final weight loss of 41.5 % was observed from ~400 °C to 580 °C, which corresponded to the loss of organic moieties (calc. 41.9 %).

Figure 1-6. SEM micrographs of NMOFs of 2 synthesized at \(W = 15\) in the cationic reverse microemulsion reaction mixture.
**Figure 1-7.** SEM micrographs of flower-like microparticles of 2 synthesized at $W = 15$ in the cationic reverse microemulsion reaction mixture with a 2:1 ligand to metal ratio.

**Table 1-2.** Crystallographic Data for $[\text{Nd}((\text{BTC})\text{(H}_2\text{O})_3)]\text{H}_2\text{O}$.\(^{23}\)

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Figure 1-8. (A) The building block unit present in crystalline [Nd(iBTC)(H₂O)₃]·H₂O. C = gray, O = red, and Nd = blue. (B) Crystal structure for [Nd(iBTC)(H₂O)₃]·H₂O where hydrogen atoms have been omitted for clarity.

Figure 1-9. (A) The experimental powder XRD pattern for NMOFs of 2 (blue) and the calculated powder XRD pattern from the bulk crystalline phase of [Nd(1,2,4-iBTC)(H₂O)₃]·H₂O (red).²³ (B) TGA curve for NMOFs of 2 (identical to the bulk phase).

1.2.4 Synthesis and Characterization of Gd₄(DOTB)₅(H₂O)

Inspired by the initial success we had in synthesizing NMOFs using reverse microemulsions, we also attempted to design new polydentate bridging ligands which could potentially result in NMOFs of significantly higher stabilities than 1 and 2 \( (K_{sp} < 10^{-6} \text{ M}) \). We synthesized 1,4,7,10-tetraazacyclododecane-\( N,N',N'' \cdot N'' \cdot \)-tetramethyl-p-methyl benzoate
DOTB), an analogue of the DOTA molecule used to chelate Gd\(^{3+}\) in some commercially available contrast agents. The ligand was synthesized in >95% overall yield by first reacting cyclen with methyl 4-bromomethylbenzoate in a basic biphasic mixture, and then hydrolyzing the resulting methylester to the tetra-acid with sodium hydroxide. We designed this polydentate ligand under the assumption Gd\(^{3+}\) would be coordinated by the four nitrogen donor atoms in the pocket created by the 1,4,7,10-tetraazacyclododecane (cyclen) backbone. Due to the inability of the benzoate groups to bite back and coordinate to the Gd\(^{3+}\) ion, as in the case of the commercially available contrast agent Dotarem (Figure 1-1), they would be free to coordinate to adjacent Gd\(^{3+}\) ions, creating a more stable framework on account of extensive metal chelation by the ligands.

![Scheme illustration the synthesis of DOTB of from the cyclen backbone.](image)

**Figure 1-10.** Scheme illustration the synthesis of DOTB of from the cyclen backbone.

NMOFs of the composition Gd\(_4\)(DOTB)\(_3\)(H\(_2\)O) (3) were synthesized by mixing aqueous solutions of GdCl\(_3\) and tetra(methylammonium) DOTB at a molar ratio of 2.5:1 in a microemulsion mixture at \(W = 15\) for 18 h. We initially obtained square plates with sides \(\sim 100\) nm in length (Figure 1-11) using the standard microemulsion procedure previously outlined; however, the synthesis was somewhat irreproducible for each new DOTB salt solution that was prepared. The lack of reproducibility may have resulted from slight inconsistencies in the pH of the tetra(methylammonium) DOTB solution. Given the DOTB molecule has both carboxylic acid and amine functionalities, the neutral ligand could also exist as the zwitterion in aqueous solution, creating problems when attempting to synthesize the NMOFs. We have thus attempted to prepare NMOFs using the potassium DOTB salt.
(Figure 1-12), using low (Figure 1-13 and 1-14) and high temperature (Figure 1-15) surfactant-assisted methods, and via the direct injection of the metal solution to a precursor microemulsion containing the ligand salt (Figure 1-6 and 1-17). While each method resulted in nanoparticles of vaguely similar morphology, the system has again proven to be somewhat irreproducible for each new DOTB salt solution that was prepared.

**Figure 1-11.** SEM micrographs of NMOFs of 3 synthesized under the standard microemulsion conditions at $W = 15$.

**Figure 1-12.** SEM micrographs of NMOFs of 3 synthesized at $W = 20$ in the cationic reverse microemulsion reaction mixture using the tetrapotassium DOTB salt.
Figure 1-13. SEM micrographs of NMOFs of 3 synthesized at $W = 20$ in the cationic reverse microemulsion reaction mixture at 0 °C.

Figure 1-14. TEM micrographs of NMOFs of 3 synthesized at $W = 20$ in the cationic reverse microemulsion reaction mixture at 0 °C.

Figure 1-15. SEM micrographs of NMOFs of 3 synthesized at $W = 20$ in the cationic reverse microemulsion reaction mixture at 120 °C.
Figure 1-16.  SEM micrographs of NMOFs of 3 synthesized at (A) $W = 20$ and (B) $W = 25$ in the cationic reverse microemulsion reaction mixture using a rapid injection method.

Figure 1-17.  TEM micrographs of NMOFs of 3 synthesized at (A) $W = 20$ in the cationic reverse microemulsion reaction mixture using a rapid injection method.

We were able to roughly determine the composition of the particles via TGA. An initial weight loss of 0.58 % was observed from approximately 100 °C to 110 °C, corresponding to the loss of one water molecule (calc. 0.65 %). A final weight loss of 71.5 % from approximately 400 °C to 580 °C corresponded to the loss of three DOTB ligands (calc. 73.9 %). The data was consistent with the charge-balanced formula $\text{Gd}_4(\text{DOTB})_3(\text{H}_2\text{O})$. 
Figure 1-18. (A) Powder XRD pattern for NMOFs of 3. (B) TGA curve for NMOFs of 3 synthesized under standard microemulsion conditions.

Powder XRD data for the isolated material suggested the NMOFs were crystalline; however, we have not yet been able to prepare single, diffraction quality, crystals for the phase of the NMOF obtained in the microemulsion reaction. We have, however, obtained crystals for a 1-d MOF containing the ligand with the formula \([\text{Gd}_2(\text{DOTB-H})(\text{CHO}_2)_3(\text{DMF})(\text{H}_2\text{O})_3]\cdot2\text{H}_2\text{O}\). Crystallographic data for \([\text{Gd}_2(\text{DOTB-H})(\text{CHO}_2)_3(\text{DMF})(\text{H}_2\text{O})_3]\cdot2\text{H}_2\text{O}\) is given in Table 1-3 and the crystal structure is shown in Figure 1-19. Interestingly, the ligand does not adopt the expected planar configuration, indicating the \(\text{Gd}^{3+}\) ion does not coordinate to the cyclen backbone in the MOF nanocrystals as we hoped.

<table>
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Figure 1-19. (A) The building block unit including the asymmetric unit (less the lattice water molecules) present in crystalline [Gd$_2$(DOTB-H)(CHO$_2$)$_3$(DMF)(H$_2$O)$_3$]·2H$_2$O. C = gray, O = red, N = blue, and Gd = blue. (B) 1-d chain and (C) packing structures for [Gd$_2$(DOTB-H)(CHO$_2$)$_3$(DMF)(H$_2$O)$_3$]·2H$_2$O where hydrogen atoms have been omitted for clarity.

1.2.5 Nanoscale Metal-Organic Frameworks as MRI Contrast Agents

We subsequently evaluated the Magnetic Resonance Imaging (MRI) contrast enhancing capabilities of the Gd-based NMOFs. Relaxivity values ($r_1$ and $r_2$) were determined on a Bruker 3.0 Tesla MR scanner by plotting the reciprocals of the longitudinal ($T_1$) and transverse ($T_2$) relaxation times of aqueous NMOF suspensions against Gd$^{3+}$ ion concentration. NMOFs of 1 with dimensions ~100 nm in length by ~40 nm in width (~4.5×10$^5$ Gd$^{3+}$/particle) gave $r_1$ values of 35.8 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$ and ~1.6×10$^7$ mM$^{-1}$ particle s$^{-1}$. 
These nanoparticles exhibited $r_2$ values of 55.6 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$ and $\sim 2.5 \times 10^7$ mM$^{-1}$ particle s$^{-1}$. NMOFs of 1 with dimensions $\sim$400 nm in length by $\sim$70 nm in width ($\sim 7.4 \times 10^6$ Gd$^{3+}$/particle) gave $r_1$ values of 26.9 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$ and $\sim 2.0 \times 10^8$ mM$^{-1}$ particle s$^{-1}$; and $r_2$ values of 49.1 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$ and $\sim 3.6 \times 10^8$ mM$^{-1}$ particle s$^{-1}$. A further increase in particle size to $\sim$1 $\mu$m in length and $\sim$100 nm in width ($\sim 5.6 \times 10^7$ Gd$^{3+}$/particle) resulted in $r_1$ values of 20.1 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$ and $\sim 1.1 \times 10^9$ mM$^{-1}$ particle s$^{-1}$; and $r_2$ values of 45.7 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$ and $\sim 2.6 \times 10^9$ mM$^{-1}$ particle s$^{-1}$. Given that the rapid inner sphere exchange of water molecules coordinated to the metal ion is a predominant mechanism in longitudinal relaxation, we believe that the Gd$^{3+}$ metal ions at or near the surface of the NMOFs were primarily responsible for the observed relaxivities. In support of our hypothesis, the inverse size dependence of the ion relaxivities observed for 1 was consistent with the decreasing surface to volume ratios associated with increases in particle size.

As shown in Figure 1-21, NMOFs of 1 are much more efficient in enhancing the water signals in $T_1$-weighted images than the clinically used contrast agent OmniScan. Furthermore, the very high $r_1$ and $r_2$ relaxivities exhibited by 1 would potentially allow their use as both $T_1$- and $T_2$-weighted contrast agents depending on the MR pulse sequence employed.

![Figure 1-20](image.png)

**Figure 1-20.** Relaxivity curves for NMOFs of 1 with dimensions of (A) $\sim$ 100 nm by 40 nm, (B) $\sim$ 400 nm by 70 nm, and (C) $\sim$ 1000 nm by 100 nm.
Figure 1-21. $T_1$-weighted MRI phantom images of NMOFs of 1 and omniscan suspended in water containing 1% xanthan gum as a dispersing agent.

We also measured the relaxivities of 2 with dimensions ~100 nm in diameter and ~35 nm in length and of square plates of 3 with sides measuring ~100 nm in length. NMOFs of 2 exhibited $r_1$ values of 13.0 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$ and ~3.25×10$^7$ mM$^{-1}$ particle s$^{-1}$ (with ~2.5×10$^6$ Gd$^{3+}$/particle). The $r_2$ values were determined to be 29.4 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$ and ~7.35×10$^7$ mM$^{-1}$ particle s$^{-1}$. NMOFs of 3 had an $r_1$ value of 10.88 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$ and an $r_2$ value of 47.4 mM$^{-1}$ Gd$^{3+}$ s$^{-1}$. We were unable to accurately determine the relaxivities on a per particle basis for 3 because we have yet to determine the crystalline density of the NMOFs formed in the synthesis. Regardless, the measurements clearly showed that NMOFs of 2 and 3 also exhibited relaxivities superior to those of clinically used $T_1$-enhancing contrast agents on both a per Gd and per particle basis.
The modular design of NMOFs has enabled us to dope them with luminescent lanthanide ions during the syntheses. Luminescent NMOFs of 1 with the composition Eu_{0.10}:Gd_{1.90}(BDC)_3(H_2O)_4 (1a) and Tb_{0.10}:Gd_{1.90}(BDC)_3(H_2O)_4 (1b) were prepared by substituting 5 mol % GdCl_3 with Eu(NO_3)_3 or Tb(NO_3)_3 during the synthesis. NMOFs of 1a and 1b were similar in size and shape to those of 1. Dispersions of the doped NMOFs were highly luminescent upon UV excitation, with the characteristic red and green luminescence from the Eu^{3+} and Tb^{3+} species, respectively (Figure 1-23).

Emission and excitation spectra were obtained using a dilute ethanolic dispersion of the doped NMOFs at λ_{em} = 250 nm and λ_{ex} = 615 nm for 1a, and at λ_{em} = 325 nm and λ_{ex} = 543 nm for 1b. The spectra corresponded well with previously determined luminescence emission lines found in the literature. For Eu^{3+}, emission peaks were observed at wavelengths of 592, 615, 652, and 700 nm, which corresponded to transitions from the long-lived 5D_0 excited state to the 7F_J states (where J = 1, 2, 3, and 4). In the case of Tb^{3+}, transitions from the 5D_4 to the 7F_J states (where J = 6, 5, 4, and 3) occurred at the corresponding wavelengths of 487, 546, 587, and 621 nm, respectively.\textsuperscript{19,24}
Figure 1-23. Luminescence of ethanolic suspensions of 1a (5 % Eu-doped Gd$_2$(BDC)$_3$(H$_2$O)$_4$), 1, and 1b (5 % Tb-doped Gd$_2$(BDC)$_3$(H$_2$O)$_4$) under UV excitation at 254 nm (right).

Figure 1-24. Excitation (red) and emission (blue) spectra for 1a (A) and 1b (B).
1.3 Concluding Remarks

We have developed a general reverse microemulsion-based methodology for the syntheses of NMOFs. These NMOFs exhibit large $r_1$ and $r_2$ relaxivities on a per mM of Gd$^{3+}$ basis and extraordinarily large $r_1$ and $r_2$ relaxivities on a per mM of nanoparticle basis (Table 1-4). Doping of luminescent lanthanide ions was also demonstrated. The generality of this approach should allow for the design of NMOFs with a wide range of new properties.

Table 1-4. MRI Relaxivity Values for NMOFs 1-3.

<table>
<thead>
<tr>
<th></th>
<th>Size (nm)</th>
<th>Gd/particle</th>
<th>Ion $r_1$</th>
<th>Ion $r_2$</th>
<th>Particle $r_1$</th>
<th>Particle $r_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omniscan</td>
<td>molecular</td>
<td>1</td>
<td>4.1</td>
<td>negligible</td>
<td>4.1</td>
<td>negligible</td>
</tr>
<tr>
<td>Gd$_4$(BDC)$_3$(H$_2$O)$_4$</td>
<td>100 x 40</td>
<td>4.5 x 10$^5$</td>
<td>35.8</td>
<td>55.6</td>
<td>1.6 x 10$^7$</td>
<td>2.5 x 10$^7$</td>
</tr>
<tr>
<td>Gd$_4$(BDC)$_3$(H$_2$O)$_4$</td>
<td>400 x 70</td>
<td>7.4 x 10$^6$</td>
<td>26.9</td>
<td>49.1</td>
<td>2.0 x 10$^8$</td>
<td>3.6 x 10$^8$</td>
</tr>
<tr>
<td>Gd$_4$(BDC)$_3$(H$_2$O)$_4$</td>
<td>1000 x 100</td>
<td>5.6 x 10$^7$</td>
<td>20.1</td>
<td>45.7</td>
<td>1.1 x 10$^9$</td>
<td>2.6 x 10$^9$</td>
</tr>
<tr>
<td>[Gd(BTC)(H$_2$O)$_3$]-H$_2$O</td>
<td>100 (d) x 35</td>
<td>2.5 x 10$^6$</td>
<td>13.0</td>
<td>29.4</td>
<td>3.3 x 10$^7$</td>
<td>7.4 x 10$^7$</td>
</tr>
<tr>
<td>Gd$_4$(DOTB)$_3$(H$_2$O)</td>
<td>100 x 100</td>
<td>-</td>
<td>10.8</td>
<td>47.4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All relaxivity values are given in mM$^{-1}$s$^{-1}$ (3.0 T).
1.4 Experimental Section

1.4.1 General Procedures and Instrumentation

Materials and Methods. All reagents and solvents were obtained from commercial sources and used without further purification. Thermogravimetric analysis (TGA) was performed using a Shimadzu TGA-50 equipped with a platinum pan and heated at a rate of 3 °C/min under air. Powder X-ray diffraction (XRD) intensity data was collected on a Rigaku Multiflex powder diffractometer using Cu radiation. Excitation and emission spectra were acquired using a Shimadzu RF-5301PC Spectrofluorophotometer. A Hitachi 4700 Field Emission Scanning Electron Microscope (SEM) or a JEM 100CX-II Transmission Electron Microscope was used to determine particle size and morphology. A Cressington 108 Auto Sputter Coater equipped with a Au/Pd (80/20) target and MTM-10 thickness monitor was used to coat the sample with a thin (~5 nm) conductive layer before taking SEM micrographs on glass substrate. The samples for TEM were prepared on amorphous carbon coated copper grids. Metal ion concentrations were measured using an Applied Research Laboratories (ARL) SpectraSpan 7 Direct Current Plasma (DCP) Spectrometer. Longitudinal relaxivities ($T_1$) were determined using a multi-flip angle 3D-gradient echo pulse sequence on a Bruker 3.0 Tesla Magnetic Resonance Imaging (MRI) scanner. The transverse relaxivities ($T_2$) were determined with a CPMG-type spin echo sequence (10 echoes) on a Bruker 3.0 Tesla MRI scanner.

1.4.2 Ligand and Complex Syntheses

Preparation of the Methylammonium Salts of the Ligands. Ligands were dissolved in a minimal volume of 40 % aqueous methylamine. Excess methylamine and
solvent were removed at ~50 °C on a rotary evaporator to yield an off white solid or, in some cases, an oily residue. The remaining residue was subsequently dissolved in distilled water and quantitatively transferred to a volumetric flask so-as-to reach the predetermined concentration.

**Synthesis of 1,4,7,10-tetraazacyclododecane-\(N,N',N''\)'\(,'N''\)'-tetramethyl-p-methyl benzoate (DOTB-ME).** A mixture of cyclen (1.72 g, 10 mmol), methyl 4-bromomethylbenzoate (11.5 g, 50.0 mmol), tetrahexylammonium iodide (4.8 g, 10 mmol), NaOH (4.0 g, 0.10 mol), water (50 mL), and methylenechlordide (150 mL) was vigorously stirred at room temperature for 48 h. Ethyl acetate (100 mL) was added to the reaction mixture, which was subsequently concentrated to 100 mL. The resultant solid was isolated by vacuum filtration, and washed with water and ethyl acetate to yield a white solid. Yield: 7.23 g (95 %). \(^1\)H NMR (CDCl\(_3\), 400 MHz, ppm): 7.91 (d, \(^3\)J\(_{H-H}\) = 8.3 Hz, 8H), 7.39 (d, \(^3\)J\(_{H-H}\) = 8.3 Hz, 8H), 3.92 (s, 12H), 3.44 (s, 8H), 2.66 (s, 16H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 167.1, 145.5, 129.7, 128.8, 128.6, 59.8, 53.3, 52.0.

![Figure 1-26](image.png)

**Figure 1-26.** Scheme illustrating the synthesis of DOTB-ME via the nucleophilic addition of methyl 4-bromomethylbenzoate to cyclen from a basic mixture with tetrahexylammonium iodide as a phase transfer catalyst.

**Synthesis of 1,4,7,10-tetraazacyclododecane-\(N,N',N''\)'\(,'N''\)'-tetramethyl-p-benzoic acid (DOTB).** A mixture of DOTB-ME (7.23 g, 9.50 mmol), NaOH (10 g, 0.25 mol), water (50 mL) and methanol (50 mL) was refluxed overnight. The mixture was concentrated to ~ 30 mL and carefully neutralized via the addition of dilute HCl. The solid was isolated by vacuum filtration, and washed with water and acetone to yield a white
powder. Yield: 7.0 g (100%). $^1$H NMR (D$_2$O, 400 MHz, ppm): δ 7.82 (d, $^3$J$_{H-H}$ = 7.8 Hz, 8H), 7.14 (br, 8H), 3.34 (br, 8H), 2.63 (br, 16H). $^{13}$C NMR (D$_2$O, 100 MHz, ppm): δ 175.2, 140.8, 135.2, 130.2, 129.6, 59.2, 47.8. (The NMR was taken with the for corresponding sodium salt because the free acid is not soluble in common NMR solvents.)

Figure 1-27. Scheme illustrating the synthesis basic hydrolysis of DOTB-ME to DOTB.

1.4.3 Microemulsion Syntheses of Nanoscale Metal-Organic Frameworks

General Microemulsion Synthesis. A cationic microemulsion composed of cetyltrimethylammonium bromide (CTAB), heptanes or iso-octanes, 1-hexanol, and water was used in the NMOF syntheses. 1-Hexanol (51.09 g, 0.50 mol) was diluted to 1.0 L with iso-octanes in a volumetric flask. A round bottom flask was charged with CTAB and the respective volume of 0.50 M 1-hexanol stock solution resulting in a 50.0 mM CTAB/0.50 M 1-hexanol/iso-octanes milky white mixture. An aliquot of an aqueous solution of a single reactant was then added to the above surfactant mixture with vigorous magnetic stirring to a yield a visibly clear, isotropic microemulsion corresponding to a particular $W$ (water to surfactant molar ratio). For example, 45.0 µL of water was added to yield 10 mL of a $W = 5$ microemulsion. A separate, equivalent microemulsion was prepared using the aqueous solution of the other reactant. After stirring the separate microemulsions until visibly clear, they were combined and stirred for an additional period of time before isolating the precipitate by centrifuge, washing it with ethanol, and redispersing it in ethanol via sonication.
Synthesis of Gd$_2$(1,4-benzenedicarboxylate)$_3$(H$_2$O)$_4$, 1, $AR \sim 3$. A $W = 5$ microemulsion was prepared by adding 225 $\mu$L of a 50.0 mM GdCl$_3$ solution and 225 $\mu$L of a 75.0 mM di(methylammonium) benzenedicarboxylate (BDC) salt solution to separate 50 mL aliquots of a 50.0 mM CTAB/heptane/1-hexanol mixture. The separate microemulsions were stirred vigorously for at least 10 min at room temperature, or until visibly clear. The microemulsion containing the metal salt was quickly added to that of the ligand. The resultant 100 mL microemulsion of $W = 5$ was stirred vigorously for at least an additional 2 hr. Shortly after the addition, the resultant microemulsion gained a slight bluish hue. The nanoparticles were isolated via centrifugation at 13,000 rpm. The supernatant was removed and the remaining pellet was redispersed in ethanol via ultrasonication. The particles were washed up to 3 times using this isolation/redispersion method.

Synthesis of Gd$_2$(BDC)$_3$(H$_2$O)$_4$, 1, $AR \sim 20$. A $W = 15$ microemulsion was prepared by adding 675 $\mu$L of a 50.0 mM GdCl$_3$ solution and 675 $\mu$L of a 75.0 mM di(methylammonium) BDC salt solution to separate 50 mL aliquots of a 50.0 mM CTAB/heptane/1-hexanol/iso-octanes mixture. The microemulsions were combined and the material was worked up as previously described. Yield: 12.5 mg (84 %) of an off-white powder.

Synthesis of Eu$_{0.10}$:Gd$_{1.90}$(BDC)$_3$(H$_2$O)$_4$, 1a, and Tb$_{0.10}$:Gd$_{1.90}$(BDC)$_3$(H$_2$O)$_4$, 1b. NMOFs of 1 were doped with the photoluminescent lanthanide ions Eu$^{3+}$ or Tb$^{3+}$ by preparing the NMOFs as previously described and using 5 mol % of the corresponding Eu$^{3+}$ or Tb$^{3+}$ nitrate during the synthesis. For example, 112.5 $\mu$L of a 100.0 mM (GdCl$_3$)$_{0.95}$:[Eu(NO$_3$)$_3$]$_{0.05}$ aqueous solution and 112.5 $\mu$L of a 100.0 mM di(methylammonium) BDC salt solution were added to separate 25 mL aliquots of a 50.0
mM CTAB/0.50 M 1-hexanol/iso-octanes microemulsion mixture with vigorous stirring at room temperature. The microemulsions were combined and the material was worked up as previously described.

**Synthesis of Gd[(1,2,4-benzenetricarboxylate)(H$_2$O)$_3$]-H$_2$O, 2.** NMOFS of 2 were prepared by adding 675 µL of a 50.0 mM tri(methylammonium) 1,2,4-benzenetricarboxylic aqueous solution and 675 µL of a 100.0 mM GdCl$_3$ solution to separate 50 mL aliquots of a 50.0 mM CTAB/1-hexanol/iso-octane microemulsion mixture with vigorous stirring at room temperature. After 10 min. the microemulsions were combined and stirred for an additional 8 hours, during which the mixture gains a slightly bluish hue. The NMOFs were isolated, washed, and resuspended in ethanol as previously described. Yield: 10.4 mg (73.7 %) of an off-white powder.

**Synthesis of Gd$_4$(1,4,7,10-tetraazacyclododecane-$N,N',N''$-$N'''$-tetramethyl-p-methyl benzoate)$_3$(H$_2$O), 3, via the standard microemulsion synthesis.** NMOFs of 3 were prepared by adding 63.5 µL of a 10.0 mM tetra(methylammonium) DOTB aqueous solution and 63.5 µL of a 25.0 mM GdCl$_3$ solution to separate 5.0 mL aliquots of a 50.0 mM CTAB/1-hexanol/iso-octane microemulsion mixture ($W = 15$) with vigorous stirring at room temperature. After 10 min. the microemulsions were combined and stirred for an additional 18 hours. The nanoparticles were isolated, washed, and resuspended in ethanol as previously described.

**Synthesis of Gd$_4$(DOTB)$_3$(H$_2$O), 3, via the standard microemulsion synthesis with the potassium salt.** NMOFs of 3 were prepared by adding 90.0 µL of a 25.0 mM aqueous tetrapotassium DOTB solution and 90.0 µL of a 25.0 mM GdCl$_3$ solution to separate 5.0 mL aliquots of a 50.0 mM CTAB/1-hexanol/iso-octane microemulsion mixture ($W = 20$)
with vigorous stirring at room temperature. After 10 min the microemulsions were combined and stirred for an additional 24 hours. The nanoparticles were isolated, washed, and resuspended in ethanol as previously described.

**Low temperature synthesis of Gd$_4$(DOTB)$_3$(H$_2$O), 3.** NMOFs of 3 were prepared by adding 90.0 µL of a 12.5 mM aqueous tetra(methylammonium) DOTB aqueous solution and 90.0 µL of a 25.0 mM GdCl$_3$ solution to separate 5.0 mL aliquots of a 50.0 mM CTAB/1-hexanol/isoctane microemulsion mixture with vigorous stirring at 0 °C. The microemulsions were combined and stirred for an additional 2 h at 0 °C. The ice bath was removed and stirring was continued for 6 hours. The nanoparticles were isolated, washed, and resuspended in ethanol as previously described.

**High temperature synthesis of Gd$_4$(DOTB)$_3$(H$_2$O), 3.** NMOFs of 3 were prepared by adding 90.0 µL of a 50.0 mM aqueous tetra(methylammonium) DOTB solution and 90.0 µL of a 100.0 mM GdCl$_3$ solution to separate 5.0 mL aliquots of a 50.0 mM CTAB/1-hexanol/isoctane microemulsion mixture (W = 20) with vigorous stirring at room temperature. After 10 min the microemulsions were combined, stirred together for an additional 10 min, placed in a Teflon-lined digestion bomb, and heated at 120 °C in an oven for 4 h. The nanoparticles were isolated, washed, and resuspended in ethanol as previously described.

**Synthesis of Gd$_4$(DOTB)$_3$(H$_2$O), 3, via a rapid injection method.** A precursor microemulsion of the tetra(methylammonium) DOTB salt was prepared by adding 90.0 µL of a 12.5 mM tetra(methylammonium) DOTB aqueous salt solution to a 10.0 mL aliquot of a 50.0 mM CTAB/1-hexanol/isoctane microemulsion mixture (W = 10) with vigorous stirring at room temperature. After 10 min, 90.0 µL of a 25.0 mM GdCl$_3$ solution was rapidly
injected into the microemulsion (now $W = 20$), which was subsequently stirred at room temperature for an additional 2 hours. The nanoparticles were isolated, washed, and resuspended in ethanol as previously described.
1.5 References


CHAPTER 2
SYNTHESIS AND CHARACTERIZATION OF COMPOSITE NANOMATERIALS
FROM NANOSCALE METAL-ORGANIC FRAMEWORKS AND SILICA

2.1 Introduction

2.1.1 Silica-Based Nanocomposites

Coating nanostructures with a silica shell via sol-gel or microemulsion-based methods has enabled the synthesis of an array of core-shell nanocomposites, such as silica-coated superparamagnetic metal oxides,\textsuperscript{1,2} quantum dots,\textsuperscript{3-9} gold,\textsuperscript{10-13} carbon nanotubes,\textsuperscript{14} and organic polymers.\textsuperscript{15-17} The silica shell as a surface coating offers numerous advantages, including enhanced water dispersibility, biocompatibility, and the ability to further functionalize the core-shell nanostructures via the co-condensation of silyl-derived molecules. Furthermore, the properties exhibited by these core-shell particles are often unique to the composite material. For example, they often have increased stabilities when compared against the templated core, they can have higher surface areas, and they can be engineered to have different optical properties. Core-shell nanoparticles are thus attractive candidates for a wide range of applications, including drug delivery, catalysis, and as templates for new composite materials.
2.1.2 Coating Nanostructures with Silica

Two methods are routinely used to coat nanostructures with silica. The first is a sol-gel procedure known as the Stöber method. In a typical reaction, a dispersion of the nanoparticles in an ethanol/ammonia mixture is treated with tetraethyl orthosilicate (TEOS), a silica precursor. Ammonia initiates the condensation of TEOS to SiO$_2$, which adsorbs onto the surfaces of the particles and grow into smooth shells at a molecular level. Several factors influence the coating efficiency, including the size, aspect ratio, and surface charge of the nanoparticles, as well as the ratio of TEOS to nanoparticles in the mixture and the duration of the reaction. To increase the coating efficiency, nanoparticles are often functionalized with small molecule coupling agents such as 3-aminopropyltri(ethoxy)silane or macromolecules such as the polymer polyvinylpyrrolidone (PVP). The surface coatings increase solvent dispersibility and allow manipulation of the interaction potential. Another method for coating nanostructures is to carry out the condensation of the silica shell in a reverse microemulsion. Like the Stöber method, TEOS is condensed onto the surfaces of particles in a basic mixture; however, the microemulsion reaction itself disperses the particles instead of using modified particle intermediates. The microemulsion method has been shown to work exceptionally well in coating discrete nanoparticles with a silica shell.

2.1.3 Motivations for This Work

In the previous chapter, we illustrated the potential utility of nanoscale metal-organic frameworks in multimodal imaging. Owing to their tunable nature, we also believe that NMOFs may be used in a variety of other imaging, biosensing, biolabeling, drug delivery, and templating applications. The successful utility of NMOFs in these areas will, however,
critically depend on our ability to modify and functionalize their surfaces to engender stability, biocompatibility, and specific functionality. We have thus developed a general method for synthesizing a new class of nanocomposites with an NMOF core and a silica shell. We demonstrate the ability to control the release of metal constituents from silica-coated NMOFs and to further functionalize them for the luminescence sensing of dipicolinic acid (DPA), which is a major constituent of many pathogenic spore-forming bacteria. Furthermore, we illustrate the utility of NMOFs in templating new silica-based materials, such as metal-oxide@silica and hollow nanostructures.

2.2 Results and Discussion

2.2.1 Surface Modification of Nanoscale Metal-Organic Frameworks

We have developed a general method to coat NMOFs with shells of amorphous silica. The silica shell as a surface coating offers several advantages, including enhanced water dispersibility, biocompatibility, and the ability to further functionalize the core-shell nanostructures via the co-condensation of silyl-derived molecules. We have best illustrated the coating method using NMOFs of the composition Ln₂(BDC)₃(H₂O)₄, 1, but it has been applied to many other NMOF formulations with equal success.

NMOFs of 1 were first synthesized using the cationic microemulsion system as previously described. The synthesis allowed for the aspect ratios (ARs) of 1 to be reproducibly tuned from ~2.5 to 40 by adjusting the water to surfactant molar ratio, W. As shown in Figure 2-1, we then used a strategy developed by van Blaaderen et al. to deposit silica on NMOFs whose surfaces had been modified with polyvinylpyrrolidone (PVP).² Treatment of as-synthesized 1 with PVP (MW=40000, up to 2 mol %) in situ over several
hours led to highly dispersible PVP-coated nanorods. TEM showed that PVP-functionalized \( \text{Ln}_2(\text{BDC})_3(\text{H}_2\text{O})_4 \) nanoparticles (denoted as \( 1' \)) synthesized at \( W = 5 \) had a rod-like morphology with dimensions approximately 100 nm in length by 40 nm in width (Figure 2-2). The polydispersity of \( 1' \) was low, and their particle size and morphology corresponded well to those of \( 1 \). Nanoparticles of \( 1' \) with an aspect ratio as high as 40 were also synthesized in high yield at \( W = 15 \). The NMOFs were isolated, washed with ethanol, and redispersed in ethanol.

![Figure 2-1](image.png)

**Figure 2-1.** Scheme illustrating the general procedure to coat NMOFs with silica via the basic condensation of TEOS onto PVP-modified intermediate particles.

![Figure 2-2](image.png)

**Figure 2-2.** TEM micrographs of \( 1' \) (PVP-modified \( \text{Ln}_2(\text{BDC})_3(\text{H}_2\text{O})_4 \)).

Nanoparticles of \( 1' \) were subsequently coated with silica shells of variable thickness using a sol-gel procedure. Nanoparticles of \( 1' \) were first primed with silica by treating them
with tetraethyl orthosilicate (TEOS, 5 µL per mg of 1′ synthesized at \(W < 10\)) in a solution of 4% (v/v) aqueous ammonia in ethanol (~0.2 mg of 1′/mL) for 2 h. An additional aliquot of TEOS (5 µL per mg of 1′) was then added, and the mixture was stirred for a period of time (up to 7 h) to afford NMOFs with a silica shell of desired thickness (1′′). For instance, an uneven silica shell of 2-3 nm in thickness was deposited on the surface of 1′ after 3 h (Figure 2-3), whereas a silica shell thickness of 8-9 nm was achieved simply by prolonging the reaction time to 7 h (Figure 2-4). For 1′ with high aspect ratios (\(W > 10\)), the reaction conditions were slightly modified to minimize the formation of secondary silica nuclei. For a thin noncontiguous silica shell, an aliquot of TEOS (1.25 µL per mg of 1′ synthesized at \(W > 10\)) was added to a dispersion of 1′ in 4% (v/v) aqueous ammonia in ethanol (~0.2 mg of 1′/mL). After 2 h, an additional aliquot of TEOS (5.0 µL per mg of 1′) was added and the reaction was continued for an additional 3 h. The silica-coated NMOFs (1′′) were then isolated by centrifuge, washed with ethanol, and redispersed in ethanol via ultrasonication. A thicker silica shell (8-9 nm) on high AR 1′′ was obtained by reintroducing the isolated silica-primed NMOFs into a separate sol-gel reaction with a TEOS concentration of 5 µL/1 mg of 1′′, and stirring the resultant mixture for at least 6 h at room temperature. The nanoparticles were then isolated and washed as previously described. Representative TEM images for 1′′ synthesized at \(W = 15\) with thin (2-3 nm) and thick (8-9 nm) silica coatings are shown in Figure 2-5 and Figure 2-6, respectively. Generally speaking, prolonged reaction times and larger aliquots of TEOS resulted in thicker silica shells.
Figure 2-3. TEM micrographs of low AR 1” with a 2-3 nm amorphous silica shell.

Figure 2-4. TEM micrographs of low AR 1” with an 8-9 nm amorphous silica shell.

Figure 2-5. TEM micrographs of high AR 1” with a 2-3 nm noncontiguous silica shell.
The compositions of $1'$ and $1''$ were further established using TGA (Figures 2-7A and 2-7B) and Powder XRD (Figure 2-8). TGA data showed that $1'$ had a slight increase of the total weight loss over that of $1$ as a result of the PVP coating (5.5 % for NMOFs synthesized at $W = 5$ and 1.5 % for NMOFs synthesized at $W = 15$). In contrast, the weight loss for $1''$ decreased significantly due to the presence of the silica shell. For $1''$ synthesized at $W = 5$, a 2-3 nm silica coating led to a 9.6% reduction in weight loss, whereas an 8-9 nm coating of silica reduced the total weight loss by 28.5 %. For $1''$ synthesized at $W = 15$, a 2-3 nm silica coating led to a 9.4 % reduction in weight loss, whereas an 8-9 nm coating of silica reduced the total weight loss by 25.7 %. The smaller changes to total weight loss for $1'$ and $1''$ synthesized at $W = 15$ were consistent with the decreased surface areas associated with the larger particles. PXRD studies showed that $1'$ and $1''$ exhibited the same pattern as macroscopic $1$, further proving the presence of the crystalline NMOF core in both PVP- and silica-coated $1$. 

**Figure 2-6.** TEM micrographs of high AR $1''$ with an 8-9 nm amorphous silica shell.
Figure 2-7. TGA curves for NMOFs of 1 (black), 1’ (blue), 1’’ (thin shell, red), and 1’’ (thick shell, green) for NMOFs synthesized at (A) \( W = 5 \) and (B) \( W = 15 \).

Figure 2-8. Powder XRD patterns for the known crystalline phase (black), 1’ (red), 1’’ (blue).

### 2.2.2 Dipicolinic Acid Detection with Silica-Coated Nanoscale Metal-Organic Frameworks

To illustrate a potential utility of NMOF-based core-shell nanostructures, we have prepared low AR Eu-doped Gd\(_2\)(BDC)\(_3\)(H\(_2\)O)\(_4\)@SiO\(_2\) nanoparticles (1a’’) at \( W = 5 \) and have further functionalized the silica surface with a Tb ethylenediaminetetraacetic acid mono(silylpropyl)amide derivative, Tb(EDTA-MSiA), to function as ratiometric probes for
the luminescence detection of dipicolinic acid (DPA) in solution. Tb$^{3+}$-ions and, more recently, molecular Tb$^{3+}$ complexes have been used as highly sensitive luminescence probes for Anthrax and other bacterial spores by forming complexes with DPA, which can constitute up to 15% of the spores’ dry mass.$^{18-22}$ The Tb$^{3+}$ ions luminesce via a mechanism relying upon sensitized emission, which requires the presence of a suitable chromophore, such as DPA, to absorb light at a convenient wavelength and transfer its excitation energy to the metal ion.$^{17}$

![Scheme illustrating the functionalization of silica-coated Eu-doped NMOFs with Tb-EDTA, and the subsequent activation of the Tb$^{3+}$ luminescence upon dipicolinic acid complexation.](image)

**Figure 2-9.** Scheme illustrating the functionalization of silica-coated Eu-doped NMOFs with Tb-EDTA, and the subsequent activation of the Tb$^{3+}$ luminescence upon dipicolinic acid complexation.

In the case of our probe, under UV excitation at 278 nm, 1a$'''$-Tb(EDTA) only gives Eu luminescence because the Tb(EDTA) moiety is essentially non-emissive. As DPA is added to an ethanol or ethanol:water dispersion of 1a$'''$-Tb(EDTA), the Tb luminescence becomes clearly visible due to the formation of the Tb(EDTA)-DPA complex. The Tb luminescence signal thus provides a sensitive probe for DPA detection while the Eu emission from the NMOF core acts as non-interfering internal calibration. Such a sensing scheme is insensitive to perturbations of the system caused by analyte addition and thus obviates tedious and often unreliable external calibration.
As shown in Figure 2-10B, the relationship between the ratio of Tb to Eu emission intensities and DPA concentration displayed normal saturation behavior. At low DPA concentrations, the ratio of signal intensities increased linearly; however, the ratio of intensities began to level off as the Tb(EDTA) complexes became coordinatively saturated with DPA. The DPA detection limit for this system was estimated to be ~48 nM. Such a ratiometric sensing scheme works equally well in a Tris buffer solution and is able to selectively detect DPA in the presence of biologically prevalent interfering molecules, such as amino acids.

Figure 2-10. (A) Luminescence vs DPA concentration for 1a’’-Tb-EDTA with 20 % monolayer Tb-EDTA coverage ($\lambda_{ex} = 278$ nm). (B) Ratio of Tb to Eu emission intensities vs DPA concentration for 1a’’-Tb-EDTA with 20 % monolayer Tb-EDTA coverage (red = Tb$_{544nm}$:Eu$_{592nm}$; black = Tb$_{544nm}$:Eu$_{615nm}$).
**Figure 2-11.** (A) Luminescence vs DPA concentration for 1a''-Tb-EDTA with 5% monolayer Tb-EDTA coverage ($\lambda_{ex} = 278$ nm). (B) Ratio of Tb to Eu emission intensities vs DPA concentration for 1a''-Tb-EDTA with 5% monolayer Tb-EDTA coverage (red = Tb$_{544nm}$:Eu$_{592nm}$; black = Tb$_{544nm}$:Eu$_{615nm}$).

**Figure 2-12.** (A) Dependence of the ratio of Tb$_{544nm}$ to Eu$_{592nm}$ emission intensities for 1a''-Tb-EDTA on DPA in a 10 mM Tris buffered ethanol (1:1) solution (black) and in the presence of 0.6 mM alanine (red). (B) Ratio of Tb$_{544nm}$ to Eu$_{592nm}$ emission intensities vs Ala concentration for 1a''-Tb-EDTA on DPA in a 10 mM Tris buffered ethanol (1:1) solution at 5 µM (black) and 150 µM (red) DPA.

### 2.2.3 Nanoscale Metal-Organic Frameworks for Controlled Release Applications

More importantly, however, we wanted to demonstrate the potential utility of NMOFs and silica-coated NMOFs for controlled release purposes. Traditionally, macroscopic metal-organic framework crystals have been investigated for a variety of applications, including
heterogeneous asymmetric catalysis, nonlinear optics, and gas sorption. Their use for these applications is often dependent upon their solvent and/or atmospheric stability. The most common method investigators have used to judge the solvent stability of MOFs is by placing them in various solvents and observing if they have completely dissolved into solution. Yet, rarely have they accounted for the fact that MOFs, like most metal-complexes, have an associated solubility product constant ($K_{sp}$), particularly in aqueous solution. For instance, we measured the aqueous solubility of most of the Gd-based NMOFs synthesized by our group to be in the micromolar range. NMOFs of 1 were shown to be one of the most stable systems, with an equilibrium $[\text{Gd}] < 1 \times 10^{-6}$ M. The stabilizing effect of an 8-9 nm silica shell on the NMOF core was also investigated. Figure 2-14 shows the dissolution (dissociation) curves for 1 and $1''$ when dialyzed against water at 37 °C. The dissolution curves for both 1 and $1''$ at pH = 4 can be modeled as a zeroth order rate law with an apparent rate constant of 0.1791 and 0.091 percent Gd$^{3+}$/h, respectively. The silica shell of $1''$ has thus stabilized the NMOF core against dissolution. A similar stabilizing effect of the silica shell was also observed at pH = 5, with an apparent rate constant of 0.1602 and 0.0557 percent Gd$^{3+}$/h for 1 and $1''$, respectively. These results indicate that the rates of cargo release from such core-shell nanostructures can be readily controlled, presumably by taking advantage of slow diffusion of metal and organic constituents through the silica shell.

Figure 2-13. Scheme illustrating the controlled release of constituents from the NMOF core of $1''$ and formation of hollow silica shells upon complete removal of the core.
Figure 2-14. Time-dependent dissolution curves for high AR 1 (red) and 1'' (black) with an 8-9 nm shell at (A) pH = 4 and (B) pH = 5 and 37 °C.

Additionally, the NMOF core of 1'' could be completely removed (via dissolution) at low pH to afford hollow silica shells with varied thickness and aspect ratios (Figures 2-15 to 2-17). Since the morphologies of NMOFs can be controlled by exploiting the energetics of different crystallographic faces, we believe the present approach can be used to produce a diversity of nanoshell morphologies that are not accessible with presently available templates.

Figure 2-15. TEM micrographs of hollow silica shells (8-9 nm thickness) prepared via acid digestion of low AR 1'' synthesized at $W = 5$. 
2.2.4 Nanoscale Metal-Organic Frameworks as Sacrificial Templates for New Composite Materials

Nanoparticles of 1” were used to synthesize a new class of lanthanide-oxide@silica composite materials. Calcination of 1” at 600 °C gave nanoparticles equivalent in size and morphology to their predecessors; however, the organic molecules used to construct the NMOFs were completely removed and the remaining metal ions were transformed into the

Figure 2-16. TEM micrographs of hollow silica shells (2-3 nm thickness) with a noncontiguous wall prepared via acid digestion of high AR 1” synthesized at $W = 15$.

Figure 2-17. TEM micrographs of hollow silica shells (8-9 nm thickness) prepared via acid digestion of high AR 1” synthesized at $W = 15$. 
corresponding metal-oxide species during the process. TEM micrographs showed the Ln\(_2\)O\(_3\) species preferentially formed along the surfaces of the silica shell or as small particles within the silica matrix (Figures 2-18 and 2-19). Powder XRD confirmed that the NMOF core was completely removed and transformed into the known lanthanide oxide phase (Figure 2-20).

**Figure 2-18.** TEM micrographs of composite nanoparticles after high AR NMOFs of 1″ with a 2-3 nm amorphous silica shell were calcined at 600 °C.

**Figure 2-19.** TEM micrographs of composite nanoparticles after high AR NMOFs of 1″ with an 8-9 nm amorphous silica shell were calcined at 600 °C.
We had also made various attempts to coat the surfaces of the NMOFs with silica directly in the microemulsion without the need for, or prior isolation of, PVP-modified NMOFs. However, the NMOFs acted as sacrificial templates for the growth of siliceous composite materials similar in morphology to the preexisting NMOFs instead of forming the absolute core-shell structures seen in 1″. In a typical experiment, NMOFs of 1 were first synthesized using the cationic microemulsion system as previously described. After 2 h to 4 h, aliquots of aqueous NH$_3$ and TEOS were added to the microemulsion. The reaction was subsequently stirred for up to 48 h before the isolation of the siliceous particles.

As shown in Figure 2-21, it appeared that for NMOFs of 1 with low ARs (synthesized at values of $W < 10$) the NMOF core was only partially etched away during the reaction. TEM images clearly showed an area of higher electron density within the core of the nanoparticles. The presence of the NMOF structure was also confirmed by an observed TGA weight loss of ~7.5 % from ~500-550 °C, in the case of particles synthesized at $W = 8$. As the value of $W$ was increased to 15, the electron density of the core not only became less

**Figure 2-20.** Powder XRD patterns for the composite nanoparticles after high AR NMOFs of 1″ with a (A) 2-3 nm and an (B) 8-9 nm amorphous silica shell were calcined at 600 °C.
uniform, but the TGA weight loss for the organic species at ~500 °C was not observed. For comparison purposes, a definitive weight loss of ~20 % was observed for the high AR 1″ at ~500 °C. These observations suggested that the NMOFs synthesized in the microemulsion were acting as templates for the growth of silica particles by replacing the organic species within the NMOFs. It was our belief that the metal ions used to construct the NMOFs were themselves being incorporated into the particles during the condensation of amorphous silica. The negatively charged silicon oxide moieties may have preferentially coordinated the metal ions over the carboxylate functionalities of the bridging ligands. In support of our hypothesis, the electron density in the TEM images could be made more uniform by washing the particles with dilute acid to remove the adsorbed lanthanide ions, leaving a more homogeneous structure of amorphous silica.
Figure 2-21. TEM micrographs of silica-based composite nanoparticles prepared by adding NH$_3$ and TEOS directly to NMOF microemulsion reaction mixture at (A) $W = 5$, (B) $W = 6$, (C) $W = 7$, (D) $W = 8$, (E) $W = 10$, and (F) $W = 15$. 

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Figure 2-22. TGA curves for silica-based composite nanoparticles prepared by adding NH$_3$ and TEOS directly to the NMOF microemulsion reaction mixture at $W = 8$ (black) and $W = 15$ (red).

2.3 Concluding Remarks

We have developed a general method to coat NMOFs with silica shells of variable thickness. These shells increase NMOF core stability and allow for the controlled release of metal constituents. Additionally, silica-coated NMOFs were functionalized for the detection of nM concentrations of DPA, an important molecular marker in spore-producing bacteria. Owing to the tunability of NMOF composition and morphology, the present approach should allow for the synthesis of novel core-shell hybrid nanostructures for future imaging, sensing, drug delivery, and templating applications.

2.4 Experimental Section

2.4.1 General Procedures and Instrumentation

Materials and Methods. All reagents and solvents were obtained from commercial sources and used without further purification. Thermogravimetric analysis (TGA) was performed using a Shimadzu TGA-50 equipped with a platinum pan and heated at a rate of 3
°C / min under air. PXRD data was collected on a Bruker SMART APEX II single crystal
diffractometer using Cu radiation. The data was subsequently processed with the APEX 2
package using the phase ID plug-in. Excitation and emission spectra were acquired using a
Shimadzu RF-5301PC Spectrofluorophotometer. A Hitachi 4700 Field Emission Scanning
Electron Microscope (SEM) was used to determine particle size and morphology on glass
substrate. A Cressington 108 Auto Sputter Coater equipped with a Au/Pd (80/20) target and
MTM-10 thickness monitor was used to coat the sample with a thin (~5 nm) conductive layer
before taking micrographs. Likewise, a JEM 100CX-II Transmission Electron Microscope
(TEM) was used to determine particle size, morphology, and silica shell thickness. The
samples for TEM were prepared on amorphous carbon coated copper grids. Metal ion
concentrations were measured using an Applied Research Laboratories (ARL) SpectraSpan 7
Direct Current Plasma (DCP) Spectrometer.

Measuring the Rates of Constituent Release. Approximately 15 mg of dry
uncoated NMOFs were dialyzed against 500 mL of an aqueous solution with magnetic
stirring at 37 °C using 3500 MW cutoff cellulose tubing. The pH of the solution was
adjusted with dilute HNO₃ and/or NaOH prior to submerging the sample. Aliquots of the
dialyzing solvent were removed at predesignated times and the solvent was evaporated under
reduced pressure. The sample was then diluted to 3 mL with 1M HNO₃ for DCP analysis.
Initially, larger volumes were removed to increase the detection limits (up to 30 mL of the
solution was taken to increase the Ln detection sensitivity by a factor of 10). For silica-
coated NMOFs an amount of sample was added corresponding to ~15 mg of the NMOF core
based on TGA (e.g. ~22 mg were added for NMOFs with an 8-9 nm coat synthesized at W =
15). The experiment was again carried out as previously described.
2.4.2 Ligand and Complex Syntheses

Preparation of the Methylammonium Salts of the Ligands. Ligands were dissolved in a minimal volume of 40 % aqueous methylamine. Excess methylamine and solvent were removed at ~50 °C on a rotary evaporator to yield an off white solid or, in some cases, an oily residue. The remaining residue was subsequently dissolved in distilled water and quantitatively transferred to a volumetric flask so-as-to reach the predetermined concentration.

Synthesis of ethylenediaminetetraacetic acid monoanhydride. Ethylenediamine tetraacetic acid monoanhydride was synthesized from the dianhydride following a procedure published by Ebright et al.23 A mixture of EDTA-dianhydride (1.000 g, 3.903 mmol) in 5 mL of anhydrous DMF was heated to 100 °C with magnetic stirring under an inert atmosphere. Once the mixture had become a clear solution, its temperature was reduced to 75 °C. A solution of water (70.0 µL, 3.885 mmol) in 1 mL of anhydrous DMF was added to above solution dropwise over 30 min with magnetic stirring, and the reaction was continued for an additional 2 hours at 75 °C. The heat was removed and the reaction was continued at room temperature overnight to allow the product to precipitate, which was collected by vacuum filtration to yield a white solid. Yield: 0.9259 g (86.5%). 1H NMR (DMSO, 300 MHz, ppm): 3.71 (s, 4H), 3.42 (s, 4H), 2.79 (t, \(J_{H-H} = 6.0\) Hz, 2H), 2.57 (t, \(J_{H-H} = 6.0\) Hz, 2H).

Figure 2-23. Scheme illustrating the synthesis of EDTA monoanhydride via the hydrolysis of ETDA dianhydride with 1 equivalent of water.
Synthesis of ethylenediaminetetraacetic acid mono[tri(ethoxy)silylpropyl]amide (EDTA-MSiA). EDTA-MSiA was synthesized by suspending 0.2500 g (0.9116 mmol) of EDTA-monoanhydride in 8 mL of anhydrous pyridine. 0.21 mL (0.20 g, 0.90 mmol) of 3-aminopropyltri(ethoxy)silane was then added, and the reaction was stirred at room temperature under N\textsubscript{2} for 16 hours. The product was precipitated upon the addition of hexanes, and collected by centrifuging at 3300 rpm. The product was then washed with additional hexanes, and dried under vacuum. Yield: 0.2528 g (56.0%). \textsuperscript{1}H NMR (DMSO, 400 MHz, ppm):  8.08 (s, 1H), 3.70 (q, \textit{J}_{\text{H-H}} = 7.5 Hz, 12H), 3.35 (s, 4H), 3.19 (s, 4H), 3.04 (br., 4H), 2.69 (s, 4H), 1.44 (m, 4H), 1.12 (t, \textit{J}_{\text{H-H}} = 7.0 Hz, 18H), 0.51 (t, \textit{J}_{\text{H-H}} = 8.2 Hz, 2H).

![Figure 2-24](image)

**Figure 2-24.** Scheme illustrating the synthesis of EDTA-MSiA by reacting EDTA monoanhydride with 3-aminopropyltri(ethoxy)silane.

Synthesis of Tb(EDTA-MSiA). To prepare the terbium complex, EDTA-MSiA (0.1000 g, 0.2018 mmol) was dissolved in 3 eq of NaOH (1.21 mL of a 0.5 M solution) at room temperature with magnetic stirring. One equivalent of TbCl\textsubscript{3} (1.35 mL of a 0.15 M solution) was then added dropwise, and the solution was stirred for an additional 30 minutes. Approximately 5 mL of ethanol was then added to the solution to precipitate the complex, which was isolated by centrifuging at 3200 rpm for 10 minutes. The complex was then washed with additional ethanol and dried under high vacuum. Yield: 0.0555 g (39.0%). The complex was dissolved in distilled water, and diluted to a total volume of 5 mL to give a ~0.016 M solution.
A separate, equivalent microemulsion was prepared using the aqueous solution of the other reactant. After stirring the separate microemulsions until visibly clear, they were combined and stirred for an additional period of time before the modification or isolation.

**2.4.3 Surface Modification of Nanoscale Metal-Organic Frameworks**

**General Microemulsion Syntheses.** A cationic microemulsion composed of cetyltrimethylammonium bromide (CTAB), heptanes or iso-octanes, 1-hexanol, and water was used in the NMOF synthesis. 1-Hexanol (51.09 g, 0.50 mol) was diluted to 1.0 L with iso-octanes in a volumetric flask. A round bottom flask was charged with CTAB and the respective volume of 0.50 M 1-hexanol stock solution, resulting in a 50.0 mM CTAB/0.50 M 1-hexanol/iso-octanes milky white mixture. An aliquot of an aqueous solution of a single reactant was then added to the above surfactant mixture with vigorous magnetic stirring to a yield a visibly clear, isotropic microemulsion corresponding to a particular $W$ (water to surfactant molar ratio). For example, 45.0 $\mu$L of water was added to yield 10 mL of a $W = 5$ microemulsion. A separate, equivalent microemulsion was prepared using the aqueous solution of the other reactant. After stirring the separate microemulsions until visibly clear, they were combined and stirred for an additional period of time before the modification or isolation.

**Synthesis of PVP-modified Ln$_2$(BDC)$_3$(H$_2$O)$_4$ NMOFs, 1', AR ~2.5.** A $W = 5$ microemulsion was prepared by adding 225 $\mu$L of a 0.15 M LnCl$_3$ aqueous solution to 50 mL of a 50.0 mM CTAB/0.50 M 1-hexanol/iso-octane mixture. Another microemulsion was prepared by adding 225 $\mu$L of a 0.20 M di(methylammonium) benzenedicarboxylate (BDC)
solution to 50 mL of a 50.0 mM CTAB/0.50 M 1-hexanol/isooctane mixture. Both microemulsions were vigorously stirred for at least 10 min, or until optically transparent, and then they were combined. The resultant microemulsion was stirred for an additional 2 h before adding 675 µL of a 5.0 mM PVP (MW = 40000) solution. After stirring the reaction mixture for an additional 12 h at room temperature, the PVP-modified NMOFs were isolated via centrifugation, washed with ethanol, and re-dispersed in ethanol via ultrasonication. Yield: 12.8 mg (96 % based on NMOF core) of an off-white powder.

**Synthesis of PVP-modified Ln\(_2\)(BDC)\(_3\)(H\(_2\)O)\(_4\) NMOFs, 1', AR ~20-40.** A W = 15 microemulsion was prepared by adding 450 µL of a 0.15 M GdCl\(_3\) aqueous solution to 100 mL of 50.0 mM CTAB/0.50 M 1-hexanol/isooctane mixture, followed by the addition of 900 µL of distilled H\(_2\)O. Another microemulsion was prepared by adding 450 µL of a 0.20 M di(methylammonium)benzenedicarboxylate solution to 100 mL of a 50.0 mM CTAB/0.50 M 1-hexanol/isooctane mixture, followed by the addition of 900 µL of distilled H\(_2\)O. Both microemulsions were vigorously stirred for at least 10 min, or until optically transparent, and then they were combined. The resultant microemulsion was stirred for an additional 2 h before adding 1.35 mL of a 5.0 mM PVP (MW = 40000) solution. After stirring the reaction mixture for an additional 12 h at room temperature the PVP-modified NMOFs were isolated via centrifugation, washed with ethanol, and re-dispersed in ethanol via ultrasonication. Yield: quantitative.

**Synthesis of PVP-modified Eu\(_{0.04}\):Gd\(_{1.96}\)(BDC)\(_3\)(H\(_2\)O)\(_4\) NMOFs, 1a', AR ~2.5.** Gd\(_2\)(BDC)\(_3\)(H\(_2\)O)\(_4\) NMOFs were doped with the Eu\(^{3+}\) by preparing the NMOFs as previously described using 2 mol % of EuCl\(_3\) in the place of an equivalent amount of GdCl\(_3\) in the synthesis.
Synthesis of Silica-Coated Ln$_2$(BDC)$_3$(H$_2$O)$_4$, 1′′. Ln$_2$(BDC)$_3$(H$_2$O)$_4$ NMOFs@PVP, 1′, were coated with shells of amorphous silica using established literature procedures. Briefly, an aliquot of 1′ in ethanol was diluted to a concentration of 0.2 mg of 1′/mL in a solution of 4 % (v/v) aqueous NH$_3$ in ethanol. With magnetic stirring, tetraethyl orthosilicate (TEOS, 5 µL/1 mg of 1′ synthesized at $W < 10$ and 1.25 to 2.5 µL/1 mg of 1′ synthesized at $W > 10$) was added to the dispersion and the reaction was continued for at least 2 hours. Silica shell thickness would typically increase with reaction time and the volume of TEOS added to the reaction mixture.

For a silica shell approximately 3 nm thick on 1′ synthesized at $W = 5$, 11.2 mg of 2 was dispersed in 60 mL of 4 % aqueous NH$_3$ in ethanol. 56 µL of TEOS was added to the dispersion with magnetic stirring. After 2 h, another 56 µL of TEOS was added to the dispersion, and the reaction was continued for 3 h. Ln$_2$(BDC)$_3$(H$_2$O)$_4$ NMOFs@silica, 1′′, were isolated by centrifuge, washed with ethanol, and re-dispersed in the desired solvent via sonication. A 8-9 nm thick shell with minimal secondary nucleation was obtained simply by increasing the reaction time for the second step from 3 h to 7 h.

For a thin amorphous, discontinuous silica shell on 1′ synthesized at $W = 15$, 20.0 mg of 1′ was dispersed in 100 mL 4 % aqueous NH$_3$ in ethanol. 25.0 µL of TEOS was added to the dispersion with magnetic stirring. After 2 h, another 100 µL of TEOS was added to the dispersion, and the reaction was continued for an additional 3 h. Nanoparticles of 3 were then isolated by centrifuge, washed twice with EtOH, and redispersed in ethanol. An 8-9 nm silica shell for the high AR nanorods was obtained by reintroducing the isolated silica-primed NMOFs into a separate sol-gel reaction with a TEOS concentration of 5 µL/1 mg of 1′′, and
stirring the resultant mixture for 6 h at room temperature. The nanoparticles were then isolated and washed as previously described. All yields were quantitative.

2.4.4 Dipicolinic Acid Detection with Silica-Coated Nanoscale Metal-Organic Frameworks

Synthesis of Tb(EDTA-MSiA) terminated 1a′′. Eu-doped PVP-functionalized NMOFs of 1 were synthesized at W = 5 and coated with an 8-9 nm silica shell as previously described. An aliquot of a stock suspension of 1a′′ in ethanol was diluted to a total volume of 4 mL with ethanol to give a particle concentration of 2.2 mg/mL. The pH of the suspension was adjusted to approximately 10 via the addition of aqueous ammonia. 42 µL of a 0.0176 M solution of the Tb(EDTA-MSiA) complex was then added, and the reaction was stirred at room temperature for 16 hours. The particles were isolated by centrifuge and were washed with distilled water and ethanol before their redispersement in ethanol.

Luminescence Detection of Dipicolinic Acid. An aliquot of a stock suspension of Tb(EDTA-MSiA) terminated 1a′′ was diluted to a total volume of 2 mL with additional ethanol, giving a final particle concentration of 0.50 mg/mL. Emission intensity data (λ<sub>ex</sub> = 278 nm) was collected. An aqueous solution of disodium dipicolinate (DPA) was then added incrementally, increasing the DPA concentration from 0.10 µM to 200 µM. After each addition, emission intensity data was collected. Intensities at 544 nm, 592 nm, and 615 nm were measured 4 times each at the individual DPA concentrations, and the averages were used to determine the Tb<sup>3+</sup>:Eu<sup>3+</sup> intensity ratios.

The DPA detection limit was estimated using the background intensity plus three standard deviations. A plot of the intensity ratio (544 nm/592 nm) versus [DPA] from 0 to
6.0 μM gave a linear fit with the equation: \( y = 0.1501x + 0.0436 \). The background intensity ratio was measured to be 0.04426 ± 0.00216, so the background plus three standard deviations was equal to 0.05074. By plugging this value into the equation for the linear fit, a [DPA] of 47.6 nM was determined to be the detection limit.

**Luminescence Detection of DPA in a Buffer Solution.** An aliquot (8 μL) of a stock suspension of Tb(EDTA-MSiA) functionalized 1a′′ (21 mg/mL) was added to 2 mL of 10 mM Tris-buffered ethanol in water (1:1) at pH 7.6. Emission intensity data (\( \lambda_{ex} = 278 \text{ nm} \)) was collected. An aqueous solution of disodium DPA was then added incrementally, increasing the DPA concentration from 0.10 μM to 200 μM. After each addition, emission intensity data was collected. Intensities at 544 nm, 592 nm, and 615 nm were measured 4 times each at each DPA concentration, and the average was used to determine the Tb\(^{3+}\):Eu\(^{3+}\) intensity ratios. A very similar dependence of Tb-DPA to Eu signal intensity ratio on DPA concentration was observed for the Tb(EDTA-MSiA) functionalized NMOFs in the buffered solution. The DPA detection limit was estimated to be 56.6 nM in the buffered solution using the above method.

**Luminescence Detection of DPA in the Presence of L-Alanine.** The luminescence experiment was similarly carried out as previously described in the presence of 0.6 mM alanine (ala), a biologically relevant carboxylic acid containing molecule, in a 10 mM Tris buffered ethanol:water (1:1) solution at pH 7.6. To further determine if the Tb(EDTA-MSiA) complex was selective for the DPA ligand against other biologically relevant carboxylic acid containing molecules, 4 μL of the nanoparticle stock suspension was added to 2 mL of the 10 mM Tris buffered ethanol:water (1:1) solution at pH 7.6. To the suspension was added an aliquot of a disodium DPA aqueous solution to reach the desired
DPA concentration. One sample was brought to 5 mM in DPA while another was brought to 150 mM. The luminescence was allowed to reach equilibrium over several minutes, and was subsequently recorded. Aliquots of 100 mM Ala to obtain Ala concentrations of 0.3, 0.6, 0.9, and 1.2 mM were added to the DPA nanoparticle suspensions. The luminescent intensities were recorded approximately 5 minutes after each addition and the ratio of Tb to Eu emission intensities were plotted against the [ala].

2.4.5 Composite Materials Via Nanoscale Metal-Organic Frameworks

Synthesis of Lanthanide-Oxide@Silica Nanoparticles. Silica-coated NMOFs were prepared as previously described. The material was isolated, dried in vacuo, and calcined at 600 °C for 4 h to yield lanthanide-oxide@silica composite nanostructures.

Synthesis of Silica Nanostructures via the Sacrificial Templating of NMOFs in a $W = 5$ Microemulsion. NMOFs of 1 were prepared using cationic microemulsions as previously described. Briefly, a $W = 5$ microemulsion was prepared by adding 22.5 µL of a 0.133 M GdCl₃ solution and 22.5 µL of a 0.20 M di(methylammonium) benzenedicarboxylate (BDC) salt solution to separate 5.0 mL aliquots of a 50.0 mM CTAB/heptane/1-hexanol mixture. The separate microemulsions were stirred vigorously for at least 10 min at room temperature, or until visibly clear. The microemulsion containing the metal salt was quickly added to that of the ligand. After stirring the resultant microemulsion for 4 h, an aliquot of aqueous NH₃ (90 µL) and an aliquot of tetraethyl orthosilicate (50 µL) were added to the reaction mixture, which was subsequently stirred at r.t. for up to an additional 48 h.
Synthesis of Silica Nanostructures via the Sacrificial Templating of NMOFs in a $W = 8$ Microemulsion. NMOFs of 1 were prepared using cationic microemulsions as previously described, but an additional 13.5 µL of water were added to each of the separate microemulsion mixtures. After 12 h of TEOS treatment, the particles were isolated and washed. A large number of secondary silica nuclei could be seen in the TEM micrographs of the material. To reduce the extent of secondary nucleation, less TEOS could be added to the microemulsion mixture or the time of treatment could be reduced.

Synthesis of Silica Nanostructures via the Sacrificial Templating of NMOFs in a $W = 15$ Microemulsion. NMOFs of 1 were prepared using cationic microemulsions as previously described, but an additional 45.0 µL of water were added to the each of the separate microemulsion mixtures. After 3 to 4 h of TEOS treatment, the rods were isolated and washed.
2.5 References


CHAPTER 3
NANOSCALE COORDINATION POLYMERS FOR PLATINUM-BASED ANTICANCER DRUG DELIVERY

3.1 Introduction

3.1.1 Nanoscale Coordination Polymers: A Historical Overview

Nanoscale coordination polymers (NCPs) are a class of “soft” materials constructed from metal ions and polydentate bridging ligands. Although the definition technically includes most classes of inorganic nanoparticles, such as oxides, carbonates, and phosphates, the term has traditionally been used to describe materials with organic linkers. Owing to an essentially limitless choice of building blocks, they have the potential to be engineered for a wide range of applications, including heterogeneous catalysis, imaging, and sensing. Yet, challenges remain in the design of functional NCPs that meet the stringent requirements of biological systems, such as stability in an aqueous environment and at high concentrations of competing ligands. Although the stability of NCPs can be enhanced via a judicious choice of metal connectors and bridging ligands, they can alternatively be designed for the controlled release of biologically functional species by exploiting their inherent solubility in an aqueous environment. Advancements in our understanding of how the size, charge, and composition of nanoparticles influence their in vitro and in vivo efficacies have led us to believe this latter approach has great potential, and the key to its success lies in our ability to stabilize the
NCPs so that the sustained release of functional cargoes can be achieved upon their delivery to the intended tissues.

3.1.2 Nanoparticles in Medicine

Over the last decade, the clinical value of materials on the nanometer-scale has become increasingly evident, predominantly in the context of particle-mediated drug delivery for cancer therapy.\textsuperscript{5-7} Studies have routinely shown that nanoparticle-based drug formulations offer numerous advantages over their small molecule counterparts, including enhanced drug accumulation in tumor tissue, reductions in systemic toxicity, and the ability to be surface-functionalized with passivating and targeting moieties. The particle-mediated approach has been validated in the clinic with the approval of a variety of nanoparticle-based therapeutics, notably doxorubicin-loaded pegylated liposome formulations (DOXIL) and albumin-bound paclitaxel (ABRAXANE), and it has recently been shown to be particularly promising for a class of anticancer drugs known as platins.\textsuperscript{8,9}

3.1.3 Platinum-Based Anticancer Therapeutics

Molecular platinum-based therapeutics, such as cisplatin (\textit{cis}-diammine dicholoro platinum(II)), have been extensively used in the clinic since the late 1970s to treat many forms of cancer, including testicular, ovarian, cervical, head and neck; however, they are known to have notoriously severe systemic, dose-limiting toxicities and are often inactivated by irreversible protein binding. A challenge thus remains to increase their tumor specificity and reduce detrimental side reactions that typically limit their therapeutic efficacy. To address these issues, two noteworthy strategies for platinum delivery have been proposed
and are currently under clinical investigation: 1) the coordination of platinum drugs to biocompatible copolymers such as hydroxypropylmethacrylamide (HPMA), in the case of AP5346 (ProLindac);\textsuperscript{10} and 2) the encapsulation of platinum drugs into sterically stabilized liposomes, in the case of the SPI-77 (STEALTH);\textsuperscript{11} Interestingly, while the polymer-bound platins have been shown to significantly enhance the amount of platinum delivered to the tumor and tumor DNA, liposome-encapsulated platins show only modest tumor response rates in comparison to their molecular counterparts, presumably due to slow drug release and activation.

\subsection*{3.1.4 Motivations for this Work}

In this work, we have developed a novel and general strategy for the delivery of Pt-based drugs to cancer cells via their inclusion into NCPs. The NCPs are stabilized with shells of amorphous silica to prevent rapid dissolution and to effectively control the release of the Pt species. We hypothesized that the NCPs would dissolve at rate dependent upon silica-shell thickness, releasing the Pt species which would have inherent cytotoxicity against cancer cell line, or could be functionalized with organic moieties to target receptors overexpressed on the cells, resulting in the internalization of the NCPs via receptor mediated endocytosis, degradation, and subsequent activation to the active Pt species intracellularly.
3.2 Results and Discussion

3.2.1 Synthesis and Characterization of Pt-Based Nanoscale Coordination Polymers

Nanoscale coordination polymers constructed from metal ions and $c,c,t$-(diammine-dichlorodisuccinato)Pt(IV) (DSCP = disuccinatocisplatin) were synthesized by precipitating the particles from an aqueous precursor solution of the components via the addition of a poor solvent. In a typical experiment, an aqueous solution of the metal salt (chloride or nitrate) and the di(methylammonium) DSCP salt was prepared in a beaker, and its pH was adjusted using dilute NaOH or HCl. A poor solvent was then added to the precursor solution with vigorous magnetic stirring to induce particle formation. The NCPs were isolated by centrifuge, washed with methanol and ethanol, and redispersed in ethanol via ultrasonication. The nanoparticles were subsequently characterized by inductively coupled plasma-mass spectrometry (ICP-MS), thermogravimetric analysis (TGA), transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), and powder X-Ray diffraction (PXRD).

This general synthetic approach is based on the premise that the NCP particles are significantly less soluble in the precipitating solvent than the individual components. For instance, neither the metal salt nor the di(methylammonium) DSCP salt precipitates out of aqueous solution upon addition of methanol; however, when in solution together, a clear dispersion with a distinct hue immediately forms, indicating the formation of nanoparticles. Analogous precipitation methods have recently been used by several other groups to formulate a small variety of coordination polymer nanoparticles.$^{12}$
The first NCPs we prepared were constructed from Tb\textsuperscript{3+} ion connectors and DSCP bridging ligands, NCP-1 (Figure 3-1). We arbitrarily chose a lanthanide as the metal ion connector because carboxylates tend to bind relatively strongly ions of the lanthanide series, and we previously had success synthesizing a variety of lanthanide-based nanoscale metal organic frameworks in reverse microemulsions.\textsuperscript{3} To synthesize NCP-1, the pH of a solution of TbCl\textsubscript{3} (15 mM) and DSCP (10 mM) was adjusted to 5.5 with dilute NaOH. Methanol, or a methanol/ethanol mixture (1:1), was quickly poured over the precursor solution with vigorous magnetic stirring, inducing nanoparticle formation. NCP-1 was subsequently isolated in >70% yield via centrifugation and washed with ethanol.

The key variables affecting the synthesis of discrete NCP-1 particles were the reactant concentration, the reactant ratio, and the pH of the precursor solution. Increases in the reactant concentration or decreases in the metal to ligand ratio would typically result in an amorphous material with gel-like morphology. We hypothesized the excess metal ions in the reaction mixture were terminating the particles with positively charged Tb ions, and thus stabilizing the particles from aggregation via interparticle electrostatic repulsion. The most important variable in the synthesis, however, was the pH of the precursor solution. This method relies heavily on the solubilities of both the reactants and the products, and the equilibrium existing between the two. According to LeChâtelier’s Principle, the equilibrium would shift to compensate for perturbations of the system, one being a change in the pH. At pH values below 5, the equilibrium was essentially being forced to the side of the free DSCP diacid species. Therefore, we were unable to precipitate or isolate NCPs at pH values below 5. On the other hand, by increasing the pH above 6, the product would prematurely precipitate, resulting again in an amorphous gel-like material instead of individual
nanoparticles. We also hypothesized that the slightly acidic environment (pH 5-6) assisted in stabilizing the particles from aggregation, as evidenced by a drop in pH as a result of the formation of HCl or HNO$_3$ during the reaction.

Figure 3-1. Schematic illustrating the general procedure to formulate NCP-1 from an aqueous precursor solution of TbCl$_3$ and DSCP via the addition of a poor solvent.

TEM, DLS, and PXRD were used to determine the morphology, size, and structure of the NCPs. As shown in Figure 3-2, as-synthesized NCP-1 particles were spherical in morphology. DLS measurements gave a diameter of 58.3 ± 11.3 nm for the particles in ethanol (Figure 3-3). Unfortunately, there were no PXRD peaks that would indicate a crystalline phase for NCP-1, which prohibited a detailed understanding of its structure.

We used ICP-MS and TGA to roughly determine the chemical composition of the NCPs. ICP-MS measurements consistently gave a Tb:Pt molar ratio of slightly higher than 2:3 for NCP-1, suggesting the nanoparticles were Tb-terminated. These results also suggested the formula for NCP-1 was Tb$_2$(DSCP)$_3$(H$_2$O)$_x$. TGA was used to determine the number of water molecules and to confirm Tb$_2$(DSCP)$_3$(H$_2$O)$_x$ as the correct formula. A 9.2 % weight loss was observed from r.t. to ~150 °C, corresponding to 12 water molecules (calc. ~9.7 %). A 36.0 % weight loss from ~150 °C to 600 °C was observed for the succinate and
amine moieties (calc. ~36 %), resulting in an empirical formula of Tb$_2$(DSCP)$_3$(H$_2$O)$_{12}$ for NCP-1. This formula was expected because the metal ions and bridging ligands were present in a charge balanced ratio, and also for the reason that the twelve water molecules would occupy the remaining vacant sites of coordination around the Tb metal ions.

Figure 3-2. TEM micrographs of NCP-1 particles obtained via rapid precipitation from an aqueous precursor solution with methanol.

Figure 3-3. DLS curves for NCP-1, PVP-functionalized NCP-1, NCP-1'-a, and NCP-1'-b obtained by plotting the number % against particle size.
To demonstrate the generality of the method, we also attempted to prepare NCPs built from other metal ions. **NCP-2** was built from Zn$^{2+}$ metal ion connectors and **NCP-3** was built from Cu$^{2+}$ metal ion connectors. To synthesize **NCP-2**, the pH of a solution of Zn(NO$_3$)$_2$ (20 mM) and DSCP (5 mM) was adjusted to ~5.0 with dilute HCl. Methanol was poured over the precursor solution with vigorous magnetic stirring, inducing particle formation. Again, the particles appeared to be spherical in morphology (Figure 3-5), but the exact conditions for a reproducible synthesis of **NCP-2** have yet to be determined. It was clear, however, that the same key variables affecting the formation of **NCP-1** could also be applied to **NCP-2**. Increases to the reactant concentrations, decreases in the metal to ligand ratio, and increases in the pH above 5 all resulted in the formation of an amorphous gel-like material.
Figure 3-5. SEM (A) and TEM (B) micrographs of NCP-2.

To synthesize NCP-3, the pH of a solution of Cu(NO₃)₂ (10 mM) and DSCP (5 mM) was adjusted to ~5.0 with dilute HCl. Methanol, or a 1:1 methanol/ethanol mixture (4 volume equivalents), was added dropwise to the precursor solution with vigorous magnetic stirring over a period of 1 min, slowly inducing particle formation. In the case of NCP-3, the addition of the poor solvent was prolonged to decrease particle nucleation and allow adequate time for the particles to grow and form the energy minimized spherical morphology. As expected, this slight modification to the synthesis resulted in larger spheres of up to 100 nm in diameter (Figure 3-6). It also demonstrated how the precipitation method can and must be specifically tailored to produce each separate NCP formulation.
3.2.2 Surface Modification of Nanoscale Coordination Polymers

While the NCPs were stable and readily dispersible in most organic solvents, nanoparticle formation was reversible if excess water was added to the reaction mixture or to the isolated material. In order to stabilize the NCPs against rapid dissolution in water, we encapsulated them in shells of amorphous silica. Silica as a surface coating offers numerous advantages, including enhanced water dispersibility, biocompatibility, and its ease of further functionalization with a variety of silyl-derived molecules. To coat the NCPs, we first modified their surfaces with polyvinylpyrrolidone (PVP) according to established literature procedures (Figure 3-7). As shown in Figure 3-8, PVP-modified NCP-1 particles retained their characteristic spherical morphology, but they exhibited an increased DLS diameter of 67.7 ± 14.3 nm. The PVP-modified nanoparticle intermediates were then treated with tetraethyl orthosilicate (TEOS) in a 4% (v/v) aqueous ammonia/ethanol mixture to yield silica-coated particles, NCP-1′ (Figure 3-7). The silica shell thickness could be tuned by varying the reaction time or the amount of TEOS added to the reaction mixture. For example, 2 h of TEOS treatment (2.5 µL/mg NCP at a dilution of 0.2 mg NCP/mL basic ethanol) afforded NCP-1′-a with a silica shell thickness of ~2 nm (Figure 3-9). These
particles had a DLS diameter of 52.8 ± 8.1 nm. 4 h of TEOS treatment under otherwise identical conditions afforded NCP-1′-b, which had a silica shell thickness of ~7 nm (Figure 3-10) and a DLS diameter of 68.6 ± 10.2 nm. The TGA curves shown in Figure 3-4 gave a 7.0 and 8.5 % reduction in the organic weight loss for NCP-1′-a and NCP-1′-b (adjusted to compensate for variations in the water content), confirming the presence of the silica shell. The shell thicknesses were highly reproducible, varying to only a small degree when the same reaction conditions were used from sample to sample.

![Diagram](image)

**Figure 3-7.** Schematic illustrating the general procedure to coat NCP-1 with shells of amorphous silica via PVP-modified particle intermediates.

![TEM micrographs](image)

**Figure 3-8.** TEM micrographs of PVP-functionalized NCP-1.
Figure 3-9. TEM micrographs of NCP-1'-a.

Figure 3-10. TEM micrographs of NCP-1'-b.

Figure 3-11. SEM micrographs of NCP-1'-b.
3.2.3 Properties of Nanoscale Coordination Polymers In Vitro

To investigate the controlled-release behavior of the silica-coated NCPs, we dialyzed samples against HEPES buffer (pH 7.4) at 37 °C. Aliquots were removed from the dialyzing solvent at predefined time intervals and the metal concentrations were measured via ICP-MS analysis. As shown in Figure 3-13, we were able to efficiently control the release of the Pt species by varying the silica shell thickness. The half-lives of dissolution for NCP-1'-a and NCP-1'-b were determined to be ~5.5 h and ~8.5 h, respectively, whereas the as-synthesized NCP-1 particles gave a $t_{1/2}$ of ~1.0 h. The rates of release for NCP-1' would, in principle, allow sufficient time for the Pt-based NCPs to circulate throughout the body and accumulate in tumor tissue via the EPR effect. The NCPs could be further engineered to have significantly longer half-lives of release by increasing shell thickness if necessary.

![Diagram](image)

**Figure 3-12.** Schematic illustrating the aqueous release of DSCP from NCP-1', or the functionalization of NCP-1' with c(RGDfK) and subsequent aqueous release of DSCP.
Figure 3-13. Release profiles for as-synthesized NCP-1, NCP-1′-a, and NCP-1′-b obtained by plotting the % Pt released against time.

Our abilities to formulate NCPs from Pt compounds and to control the release of the DSCP species from the silica-coated NCPs prompted us to evaluate their anticancer efficacies. First, we performed *in vitro* cytotoxicity assays on the angiogenic human colon carcinoma cell line HT-29. Treatment of HT-29 with DSCP, NCP-1, and NCP-1′ did not lead to any appreciable cell death after 72 h of incubation, presumably because the DSCP species released from NCP-1 and NCP-1′ did not have a pathway to enter the cells effectively, and there were no reductants in the media under the standard *in vitro* conditions to transform DSCP into the active Pt(II) species. Interestingly, released DSCP would theoretically become active *in vivo* via reduction to the Pt(II) species by endogenous biomolecules. Recent studies have shown that satraplatin, a similar Pt(IV) complex under phase III clinical investigation, has a very short half-life of only 6.3 min in whole blood as a result of rapid reduction to the Pt(II) species by reducing agents associated with red blood cells (Figure 3-14).
In order to enhance the cellular uptake of NCP-1′ \textit{in vitro}, we grafted silyl-derived c(RGDfK) onto its surface (Figure 3-12). Cyclic(RGDfK) is a small cyclic peptide sequence exhibiting high binding affinity for the $\alpha_v\beta_3$ integrin upregulated in many angiogenic cancer cells (such as HT-29). As shown in Figure 3-15, c(RGDfK)-targeted NCP-1′-a and NCP-1′-b gave IC$_{50}$ (50 % Inhibitory Concentration) values of 9.7 µM and 11.9 µM, respectively, while our cisplatin standard had an IC$_{50}$ value of only 13.0 µM. These results suggest the targeted NCPs are sufficiently internalized via receptor-mediated endocytosis. Once inside the cells, the DSCP species released from the silica-coated NCPs could then be reduced to the active Pt(II) species by intracellular reductants, such as glutathione, that are present in concentrations up to 10 mM.

![Scheme illustrating the reduction of Satraplatin (JM216) by red blood cells (RBCs) to its primary metabolite JM118.](image)

\textbf{Figure 3-14.} Scheme illustrating the reduction of Satraplatin (JM216) by red blood cells (RBCs) to its primary metabolite JM118.
Figure 3-15. *In vitro* cytotoxicity assay curves for HT-29 cells obtained by plotting the % cell viability against the Pt concentration.

As a further control, we also performed *in vitro* cytotoxicity assays on a human breast carcinoma cell line (MCF-7) that does not overexpress the $\alpha_v\beta_3$ integrin. In contrast to HT-29, the Pt(IV) complex was active against MCF-7. Thus, as expected, DSCP and the silica-coated NCPs gave IC$_{50}$ values on the same order of magnitude as the cisplatin standard in our studies. When incubated for 72h at 4000 cells/well, DSCP gave an IC$_{50}$ value of 11.1 $\mu$M whereas cisplatin gave an IC$_{50}$ value of 10.7 $\mu$M (Figure 3-16A). At 2000 cells/well NCP-1'-b gave an IC$_{50}$ value of 1.8 $\mu$M while cisplatin gave an IC$_{50}$ value of 2.1 $\mu$M (Figure 3-16B). These results are an important positive control because they suggest the DSCP species is released from NCP-1' over time and has a cytotoxic efficacy roughly equivalent to DSCP alone when compared against a cisplatin standard.
3.3 Concluding Remarks

We have developed a general strategy to formulate highly soluble nanoparticles based on Pt-containing nanoscale coordination polymers. We can control the release of the Pt drug by encapsulating the NCPs in shells of amorphous silica, and we have demonstrated their anticancer efficacies \textit{in vitro}. The generality of this approach should allow for the design of NCPs as effective delivery vehicles for a variety of biologically functional cargoes such as therapeutic and imaging agents.

3.4 Experimental

\textit{3.4.1 General Procedures and Instrumentation}

Materials and General Methods. All reagents and solvents were purchased from commercial sources and used without further purification unless otherwise indicated. Thermogravimetric analysis (TGA) was performed using a Shimadzu TGA-50 equipped with a platinum pan, and the sample was heated at a rate of 3 °C/min under air. Powder X-ray diffraction (PXRD) patterns were collected on a Bruker SMART APEX II diffractometer.
using Cu radiation. The PXRD patterns were processed with the APEX 2 package using phase ID plugin. A Hitachi 4700 Field Emission Scanning Electron Microscope and a JEM 100CX-II Transmission Electron Microscope were used to determine particle size and morphology. A Cressington 108 Auto Sputter Coater equipped with an Au/Pd (80/20) target and MTM-10 thickness monitor was used to coat samples with a conductive layer before obtaining SEM micrographs. SEM micrographs were obtained on glass substrate. TEM micrographs were obtained on carbon-coated copper grids. A Beckman Coulter N5 Submicron Particle Size Analyzer was used to determine the sample’s hydrodynamic diameter and polydispersity. A Varian 820-MS Inductively Coupled Plasma-Mass Spectrometer was used to determine metal concentration.

**Dissolution Studies.** Approximately 3 mg of NCPs dispersed in 2 mL of 2 mM HEPES buffer (pH 7.4) were dialyzed against 248 mL 2 mM HEPES buffer (pH 7.4) using cellulose dialysis tubing (3500 MW cutoff). Aliquots were removed from outside the dialysis bag to determine the moles of Pt released into the dialyzing buffer by ICP-MS. The percentage of the initial Pt dose released into the dialyzing buffer due to NCP dissolution was calculated using the equation:  

\[
\% \text{ Released} = \frac{[V_{\text{tot}} \times C] + Y}{Z} \times 100;
\]

where \( V_{\text{tot}} \) = Total solvent volume remaining, \( C \) = Pt concentration as determined by ICP-MS, \( Y \) = total mole Pt removed from the solution, and \( Z \) = mole Pt added to dialysis tubing.

### 3.4.2 Ligand Syntheses

**Synthesis of \( c,c,t-\text{Pt(NH}_3\text{)}_2\text{Cl}_2(\text{OH})_2 \).** Disuccinatocisplatin was prepared according literature procedures.\(^{13}\) A mixture of \( \text{cis-Pt(NH}_3\text{)}_2\text{Cl}_2 \), a.k.a. cisplatin, (2.00 g, 6.67 mmol) and \( \text{H}_2\text{O}_2 \) (30 wt%, 11.37 mL, 100.0 mmol) in \( \text{H}_2\text{O} \) (90 mL, pH 7) was heated at 70 °C with
vigorously stirring for 5 h in the dark. The heat was removed and stirring was continued overnight. After concentrating the mixture to ~10 mL, the product was allowed to precipitate at 4 °C over several hours. The product was collected via vacuum filtration, washed with ice cold H₂O, ethanol and diethyl ether, and vacuum dried. The product was obtained as a bright yellow powder. Yield: 1.911 g (85.8 %).

Figure 3-17. Scheme illustrating the two step synthesis of DSCP. First, cisplatin is reduced to Pt(NH₃)₂Cl₂(OH)₂ via aqueous oxidation with hydrogen peroxide. The dihydroxocisplatin then reacted with succinic anhydride to form DSCP.

Synthesis of c,c,t-Pt(NH₃)₂Cl₂(OOCCCH₂CH₂CO₂H)₂, DSCP. A mixture of c,c,t-Pt(NH₃)₂Cl₂(OH)₂ (1.00 g, 3.00 mmol) and succinic anhydride (1.20 g, 12.0 mmol) in dimethyl sulfoxide (DMSO) (3.00 mL) was heated at 70 °C in the dark for 24 h with magnetic stirring. The solvent was subsequently removed via lyophilization. The pale yellow product was recrystallized from acetone at -20 °C, isolated via vacuum filtration, and washed with ice cold acetone. Yield: 1.350 g (84.2 %). NMR: ¹H (DMSO, 500 MHz, ppm): 2.31 (t, 4H), 2.44 (t, 4H), 6.44 (broad s, 6H), 12.04 (s, 2H).

Synthesis of tri(ethoxy)silylpropyl carbamoyl c(RGDFK). Cyclic(arginine-glycine-DfK), c(RGDFK), (2.0 mg, 3.313 µmol) was added to a small roundbottom and dried under high vacuum. Anhydrous DMSO (500 µL) and triethylamine (0.20 µL) were added to the roundbottom and the mixture was magnetically stirred under argon gas for 24 h. The solution (4 mg c(RGDFK)/mL DMSO) was placed in a freezer for later use.
Yield: 235 mg (73 % isolated based on DSCP).

3.4.3 Syntheses of Nanoscale Coordination Polymers

Synthesis of Tb$_2$(DSCP)$_3$(H$_2$O)$_{12}$ NCPs, NCP-1. A solution of di(methylammonium) DSCP salt (0.50 mmol) and TbCl$_3$ (0.75 mmol) in 50 mL distilled water was prepared in a 600 mL beaker. The pH of the solution was subsequently adjusted to 5.5 with dilute NaOH. A 1:1 mixture of methanol and ethanol (200 mL) was rapidly added to the magnetically stirred precursor solution, which resulted in the formation of a clear dispersion with a bluish-white hue (Figure 3-19). The resulting mixture was magnetically stirred in the dark for an additional 1 h before isolating the product via centrifugation, washing it with methanol and ethanol, and redispersing it in ethanol via ultra-sonication. Yield: 235 mg (73 % isolated based on DSCP).

Synthesis of Zn$_x$(DSCP)$_3$(H$_2$O)$_{12}$ NCPs, NCP-2. A solution of di(methylammonium) DSCP salt (5.0 μmol) and Zn(NO$_3$)$_2$ (20.0 μmol) in 1.0 mL distilled water was prepared in a
small vial. The pH of the solution was subsequently adjusted to 4.75-5.0 with dilute HCl. Methanol was rapidly added to the magnetically stirred precursor solution, which resulted in the formation of a clear dispersion with a whitish hue. The resulting mixture was magnetically stirred in the dark for an additional 2 h before isolating the product via centrifugation, washing it with methanol and ethanol, and redispersing it in ethanol via ultrasonication.

**Synthesis of Cuₙ(DSCP)ₙ(H₂O)ₙ NCPs, NCP-3.** A solution of di(methylammonium) DSCP salt (5.0 µmol) and Cu(NO₃)₂ (10.0 µmol) in 1.0 mL distilled water was prepared in a small vial. The pH of the solution was subsequently adjusted to 5.5 with dilute NaOH. A 1:1 mixture of methanol and ethanol (4 mL) was added dropwise to the magnetically stirred precursor solution over a period of 1 min, resulting in the formation of a clear dispersion with a bluish-white hue. The resulting mixture was magnetically stirred in the dark for an additional 1 h before isolating the product via centrifugation, washing it with methanol and ethanol, and redispersing it in ethanol via ultrasonication.

**3.4.4 Surface Modification of Nanoscale Coordination Polymers.**

**Synthesis of Polyvinylpyrrolidone-Functionalized NCP-1.** NCP-1 particles were diluted with ethanol to a final concentration of ~2 mg/mL. To this dispersion was added 0.02 mole equivalents of polyvinylpyrrolidone (PVP, MW 40000) and the resultant mixture was magnetically stirred in the dark for an additional 24 h. The PVP-modified NCPs were isolated via centrifugation, washed with ethanol, and redispersed in ethanol via ultrasonication.
Synthesis of Silica-Coated NCP-1, NCP-1’. The NCPs were coated with shells of amorphous silica using a well-established sol-gel methodology.$^{14}$ An aliquot of the ethanolic PVP-coated NCP dispersion was diluted to a concentration of 0.20 mg/mL in 4 % (v/v) NH$_3$ in ethanol. With magnetic stirring, tetraethyl orthosilicate (TEOS, 2.5 $\mu$L/mg NCPs) was added to the dispersion and the reaction was continued for at least 2 hours. Silica shell thickness would typically increase with reaction time and the volume of TEOS added to the reaction mixture. The silica-coated NCPs were subsequently isolated via centrifugation, washed with ethanol, and redispersed in ethanol via ultra-sonication.

Synthesis of c(RGDfK)-Functionalized NCP-1’. An aliquot of the c(RGDfK) DMSO solution (~5 mass %) was added to a dispersion of NCP-1’ in 4 % NH$_3$ in ethanol (~2 mg/mL). The mixture was magnetically stirred at room temperature for 24 h. The c(RGDfK)-functionalized NCP-1’ particles were isolated via centrifugation, washed with ethanol and DMSO, and redispersed in DMSO via ultra-sonication.

3.4.5 In Vitro Protocols

Cell Lines. All cell lines were purchased from the Tissue Culture Facility of the UNC Lineberger Comprehensive Cancer Center, and cultured as per ATCC recommendations. HT29 cells (ATCC# HTB 38) were propagated in McCoy’s 5A (Cellgro) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. MCF-7 cells (ATCC# HTB 22) were propagated in MEM Alpha (Cellgro) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 1% sodium pyruvate, and 10 $\mu$g/mL insulin.

HT-29 Viability Assays. HT-29 cells were grown in 96-well plates at 2000 cells/well with a total volume of 100 $\mu$L. After 24 hr incubation, the media was replaced with 100 $\mu$L
of drug solution containing 0.5% DMSO and 0.5% PBS in media. All concentrations were performed in quadruplicate. Cell viability was measured after 72 hr using CellTiter 96 Aqueous One Solution Assay (Promega) according to the manufacturer’s protocol.

MCF-7 Viability Assays. MCF-7 cells were assayed under the same conditions as HT-29. Additionally, the cells were assayed at 4000 c/w and 72hr.
3.5 References


CHAPTER 4
SILICA AND SILSESQUIOXANE-BASED NANOPARTICLES AS FUNCTIONAL PLATFORMS FOR MULTIMODAL BIOMEDICAL IMAGING

4.1 Introduction

4.1.1 Nanoparticle-Based Magnetic Resonance Imaging Contrast Agents

Magnetic resonance imaging (MRI) has emerged as the most powerful diagnostic tool owing to its non-invasive nature, intrinsically high spatial resolution, and reliance on non-radioactive contrast agents. Although many MRI contrast agents are currently available for clinical use, the vast majority of them (with the exception of iron oxide nanoparticles) are Gd- or Mn-based coordination complexes that need to be administered in high concentrations in order to provide satisfactory image contrast enhancements.\textsuperscript{1-3} With the ability to carry large payloads of active magnetic centers, nanoparticulate MRI contrast agents can work at much lower concentrations and thus be rendered target-specific by labeling desired cells through phagocytic pathways or via conjugation to affinity molecules specific to cell surface markers.\textsuperscript{4}

Pioneering work by Weissleder et al. and others has demonstrated the utility of iron oxide nanoparticles as target-specific MRI contrast agents for tumor angiogenesis, inflammation, and gene expression.\textsuperscript{5-6} Owing to the enhanced transverse relaxation of water protons, iron oxide nanoparticles are typically used in $T_2$-weighted MR imaging, which leads
to the reduction of signal intensity. It is desirable to have new nanomaterials that enhance signal intensities in $T_1$-weighted MR imaging. Gd$^{3+}$-containing microemulsions developed by Lanza, Wickline, and co-workers have provided an interesting platform for designing nanoscale $T_1$ contrast agents. Further development of nanoscale MRI contrast agents will, however, require new nanomaterials that are able to carry high payloads of magnetic centers.

### 4.1.2 Synthesis and Functionalization of Silica Nanoparticles

Silica nanoparticles (SNPs) synthesized via sol-gel and microemulsion-based methods provide a robust platform for the design of multimodal imaging agents. The sol-gel, or Stöber, method entails rapidly stirring tetraethyl orthosilicate (TEOS) in a mixture of ethanol and ammonia. Ammonia acts as a catalyst to initiate the condensation of TEOS into SiO$_2$ nuclei. Once the nuclei form, they evolve into spherical silica particles whose sizes are dependent upon the amount of TEOS in the reaction mixture and the duration of the reaction. Because the Stöber synthesis involves nucleation and growth of particles in bulk solution at a molecular level, it yields samples whose polydispersity decreases over time and with increases to particle size. Microemulsions are typically used to prepare monodisperse samples with smaller mean diameters (<60 nm). Reverse micelles within the microemulsion act themselves as sites of nucleation for the silica nanoparticles. Both methods allow for the incorporation of silyl-derived molecules such as fluorophores into the silica matrix; however, the microemulsion method appears to more versatile because it also allows for the efficient incorporation of charged molecules such as [Ru(bpy)$_3$]Cl$_2$ into the matrix. More importantly, the surfaces of SNPs can be modified with a limitless variety of silyl-derived molecules,
leading to materials with specific functionalities, such as target specificity and MRI contrast enhancement capabilities.\textsuperscript{8-10}

### 4.1.3 Polysilsesquioxane Nanoparticles

Polysilsesquioxane nanoparticles are another class of materials that are attractive candidates as novel multimodal imaging agents. Polysilsesquioxanes are a class of hybrid materials synthesized via the polymerization of molecular building blocks composed a variable organic fragment and two or more polymerizable trifunctional silyl groups, or \([ (R' \text{OSiSiOR}'_3] \) for the building block with two silyl groups.\textsuperscript{11,12} Although silsesquioxanes and polysilsequioxanes have been extensively studied on the bulk scale, their synthesis and potential application on the nanometer-scale is a new area of materials science. Polysilsesquioxane nanoparticles have been synthesized using reverse microemulsions. In the syntheses, the molecular building blocks are polymerized in a mixture of water, surfactant, co-surfactant, and organic solvent via their co-condensation to \([ \text{O}_{1.5}\text{SiR} \text{SiO}_{1.5} ]_n \) with dilute acid or ammonia. Interestingly, the resulting particles have consistently displayed xerogel-like behavior when dried. In other words, they are composed of up to 95\% solvent in solution, and swell to volumes orders of magnitude greater than those observed under the electron microscope. These results have significant implications with respect to the design of contrast agents for magnetic resonance imaging. For instance, they suggest Gd-complexes located on the interiors of the particles may be just as accessible for the rapid exchange of water molecules as those on the surfaces of the particles. Additionally, free Si(OH) moieties would allow for the conjugation of a variety of other silyl-derived molecules, such as fluorophores, targeting moieties, and passivating molecules.
4.2 Results and Discussion

4.2.1 Synthesis of Silica Nanoparticles

Silica nanoparticles (SNPs) were synthesized using a water-in-oil microemulsion system composed of the surfactant t-octylphenoxypolyethoxylate (Triton X-100), the co-surfactant 1-hexanol, cyclohexane, and water. In a typical experiment, an aliquot of water was added to a magnetically stirred solution of Triton X-100, 1-hexanol, and cyclohexane. To the resulting microemulsion was added, in turn, an aliquot of tetraethyl orthosilicate (TEOS) and an aliquot of aqueous ammonia to initiate the condensation of TEOS. The total volume of the aqueous components corresponded to the desired $W$ value of the microemulsion. The reaction was continued for up to 48 h before isolating the SNPs by centrifuge, washing them with ethanol and water, and redispersing them in the desired solvent via ultra-sonication.

We could control the sizes of the nanoparticles by varying the $W$ value of the microemulsion. Generally, we observed that smaller nanoparticles were obtained with increasing values of $W$. As shown in Figure 4-1, SNPs with diameters of ~60 nm, ~40 nm, and ~20 nm were isolated from microemulsions with $W$ values of 10, 15, and 20, respectively. Hypothetically, this is because the extent of nucleation relies heavily upon the number of reverse micelles within the microemulsion. The organic soluble TEOS molecules are believed to undergo basic hydrolysis at the water/oil interface of the reverse micelles. Increases in the $W$ value not only result in larger micelles, but more micelles, there are more sites for the TEOS molecules to undergo hydrolysis. Over time, additional molecules condense onto the silica nuclei forming in the water phase until the supply of TEOS is essentially exhausted.
Figure 4-1. TEM micrographs of silica nanoparticles synthesized via reverse microemulsions at $W = 10$, 15, and 20 (left to right).

Additionally, the use of reverse microemulsions allowed us to incorporate water soluble molecules, such as the luminophore $[\text{Ru(bpy)}_3]\text{Cl}_2$, into the silica matrix. For SNPs $\sim 40$ nm in diameter, we were able to efficiently load each SNP with several thousand $[\text{Ru(bpy)}_3]\text{Cl}_2$ molecules. TGA for the $[\text{Ru(bpy)}_3]\text{Cl}_2$-doped SNPs consistently gave a weight loss of 2-3 % for the organic species of the luminophore. Furthermore, it appeared that by incorporating the luminophore into the silica matrix, its luminescent properties were significantly enhanced, as indicated by an increase in the ratio of the emission intensity relative to the excitation intensity (Figure 4-2).

Figure 4-2. Excitation (dashed) and emission (solid) curves for $[\text{Ru(bpy)}_3]\text{Cl}_2$ in aqueous solution (blue) and incorporated in the silica nanoparticle matrix (red).
4.2.2 Core-Shell Hybrid Silica Nanoparticles as Multifunctional Imaging Agents

In our initial studies, we prepared hybrid SNPs containing a luminescent [Ru(bpy)$_3$]Cl$_2$ core and functionalized them with roughly a monolayer of the silyl-derived Gd complex, Gadolinium tri(hydroxy)silylpropyldiethylenetriamine tetraacetate (abbreviated as Gd-SiDTTA) (Figure 4-3). The Gd-SiDTTA functionalized SNPs (1) were prepared by adding 0.80 mL of ammonia to a microemulsion consisting of water (2.28 mL), 0.16 mL of a 0.10 M aqueous [Ru(bpy)$_3$]Cl$_2$ solution, and 0.40 mL of TEOS in 40.0 mL of a 0.30 M Triton X-100/1.5 M 1-hexanol/cyclohexane solution to initiate the hydrolysis process. After stirring the microemulsion at room temperature for ~12 h, 1.0 mL of a 0.12 M aqueous Gd-SiDTTA solution was added and the reaction mixture was stirred for an additional 24 h. Ethanol was poured over the microemulsion to precipitate the particles, which were subsequently isolated by centrifuge, washed with ethanol and water, and redispersed in the desired via ultrasonication.

![Figure 4-3. Scheme illustrating nanoparticles of 1 (Gd-SiDTTA functionalized SNPs).](image)

As shown in Figures 4-4 and 4-5, nanoparticles of 1 from the above synthesis were monodisperse spheres with an approximate diameter of 37 nm. The synthesis was highly reproducible and the nanoparticle diameters were tunable by varying the $W$ value of the microemulsion. Nanoparticles of 1 with an average diameter of 37 nm synthesized at $W = 15$ were used for subsequent studies. The SiDTTA ligand used in the study provided seven
binding sites for Gd$^{3+}$ so that the toxicity of the nanoparticles (due to leaching of Gd$^{3+}$ centers) could be minimized.

**Figure 4-4.** SEM micrographs of nanoparticles of 1 (Gd-SiDTTA functionalized SNPs) synthesized at $W = 15$.

**Figure 4-5.** TEM micrographs of nanoparticles of 1 (Gd-SiDTTA functionalized SNPs) synthesized at $W = 15$.

TGA analysis of 1 gave an initial weight loss of 12 % from room temperature (r.t.) to ~180 °C and a further weight loss of 11 % from ~180 to ~450 °C, which corresponded to the adsorbed solvent species and the organic components of Gd-SiDTTA, respectively. Direct Current Plasma (DCP) measurements indicated that nanoparticles of 1 contained ~4.3 wt%
Gd. The TGA and DCP results indicated that there was a loading of ~10200 Gd-SiDTTA/particle. These results suggest that Gd-SiDTTA has a footprint of approximately 0.42 nm$^2$ on the surface of the silica nanoparticles. This figure is smaller than expected presumably because nanoparticles of 1 gain slightly more than a monolayer coat of Gd-SiDTTA on their surfaces under the conditions employed.

![Figure 4-6. TGA curve for nanoparticles of 1 (Gd-SiDTTA functionalized SNPs) synthesized at $W = 15$.](image_url)

The MR contrast enhancing efficacy of 1 was measured using a Bruker 3.0 T MRI scanner. Nanoparticles of 1 had a longitudinal relaxivity ($r_1$) of 19.7 s$^{-1}$ and a transverse relaxivity ($r_2$) of 60.0 s$^{-1}$ per mM of Gd$^{3+}$ (Figure 4-7). These relaxivity values are much higher than those of the Gd-SiDTTA complex ($r_1$ of 4.9 s$^{-1}$ and $r_2$ of 9.1 s$^{-1}$ per mM Gd$^{3+}$), presumably as a result of increases to the rotational correlation rate ($\tau_r$) of 1 in solution. On account of the high payload of Gd-SiDTTA complexes, nanoparticles of 1 displayed $r_1$ and $r_2$ values of $2.0 \times 10^5$ s$^{-1}$ and $6.1 \times 10^5$ s$^{-1}$ on a per mM of particle basis, respectively. These relaxivity values are significantly higher than those of the clinically-used $T_1$-weighted MRI contrast agents. Additionally, a dispersion of 1 in water gave the characteristic ligand to
metal charge transfer absorption peak at ~450 nm and an emission peak at 595 nm ($\lambda_{ex} = 488$ nm) for the incorporated [Ru(bpy)$_3$]Cl$_2$. Nanoparticles of 1 were thus highly luminescent upon UV excitation, allowing its optical detection using fluorescence microscopy.

Figure 4-7. Longitudinal ($r_1$, black) and transverse ($r_2$, red) MR relaxivity curves for nanoparticles of 1 synthesized at $W = 15$ with ~10200 Gd$^{3+}$ per SNP.

Although nanoparticles of 1 are attractive candidates as multimodal imaging agents themselves, we have made attempts to increase the Gd$^{3+}$ loading per particle so that we might obtain relaxivity values superior to those of the best experimental nanometer-sized imaging agents currently under clinical investigation (microemulsion formulations with $r_1 > 10^6$ mM$^{-1}$ particles s$^{-1}$). We have thus developed a bis(silyl)-derived Gd complex, Gadolinium diethylenetriaminepentaacetic acid bis(tri(ethoxy)silylpropylamide) (abbreviated as Gd-DTPA-BSiA), to polymerize onto the surface of the SNPs (Figure 4-8). The Gd-DTPA-BSiA coated SNPs (2) were obtained using a similar microemulsion procedure as in the synthesis of 1.

As shown in Figure 4-9, nanoparticles of 2 had a spherical morphology with an average TEM diameter of 40 nm. TGA analysis of 2 showed an initial weight loss of 13.5 %
from r.t. to ~180 °C for the adsorbed solvent species and a further weight loss of 33.2 % from 280–450 °C for the organic components of Gd-DTPA-BSiA (Figure 4-10). These results, coupled with DCP measurement data, suggested that nanoparticles of 2 contained ~30000 Gd-DTPA-BSiA/particle. The loading for 2 was significantly higher than 1 because the Gd-DTPA-BSiA complex in 2 can form multilayers on the SNPs to lead to a thick coating of polysilsesquioxane polymer. Relaxivity measurements, however, indicated that 2 had an $r_1$ of 7.8 s$^{-1}$ and $r_2$ of 12.3 s$^{-1}$ on per mM of Gd$^{3+}$ basis. For comparison purposes, the Gd-DTPA-BSiA complex had an $r_1$ of 6.2 s$^{-1}$ and $r_2$ of 8.0 s$^{-1}$ per mM Gd$^{3+}$. The relaxivity values exhibited by 2 were unfortunately lower than those of 1, presumably due to the fact that the Gd$^{3+}$ centers closer to the SNP core were not readily accessible to water molecules. Nonetheless, 2 still had an impressive $r_1$ of 2.3×10$^5$ s$^{-1}$ and $r_2$ of and 3.7×10$^5$ s$^{-1}$ on a per mM of particle basis.

**Figure 4-8.** Illustration representing nanoparticles of 2 (Gd-DTPA-BSiA functionalized SNPs).
Figure 4-9. TEM micrographs of nanoparticles of 2 (Gd-DTPA-BSiA functionalized SNPs) synthesized at \( W = 15 \).

Figure 4-10. TGA curve for nanoparticles of 2 (Gd-DTPA-BSiA functionalized SNPs) synthesized at \( W = 15 \).
Figure 4-11. Longitudinal ($r_1$, black) and transverse ($r_2$, red) MR relaxivity curves for nanoparticles of 2 synthesized at $W = 15$ with $\sim30000$ Gd$^{3+}$ per SNP.

Given the high relaxivities and stabilities exhibited by 1 and 2, we tested the efficacy of nanoparticles of 1 in vitro as agents for multimodal imaging. An immortalized monocyte cell line was used for the study because of the phagocytic capacity of monocytes, and also on account that they play an important role in autoimmune diseases such as rheumatoid arthritis. Figure 4-12 shows that monocyte cells incubated for 30 min with medium containing nanoparticles of 1 efficiently internalized the particles. The luminescence of [Ru(bpy)$_3$]Cl$_2$ was clearly visible in the laser scanning confocal microscopic z-section images. Flow cytometric measurements indicated that the efficiency of labeling monocyte cells with 1 was greater than 98% (Figure 4-13D). More importantly, we successfully observed MR image contrast enhancement of the labeled monocytes when compared to a control of unlabeled monocytes. To prepare the cells for MR imaging, we incubated $\sim18\times10^6$ cells in 1.4 mL of media containing 10.65 mg of 1 for 1 h. The cells were isolated by centrifuge, washed twice with PBS buffer, pelleted, and covered with a layer of PBS buffer (200 µL). As shown in Figure 4-14, significant positive signal enhancement in the $T_1$-
weighted image and negative signal enhancement in the $T_2$-weighted image were observed for the labeled cells, depending on the MR pulse sequence employed. Finally, viability assays showed that nanoparticles of 1 were not toxic to monocyte cells, even after incubation with a nanoparticle loading as high as 0.123 mg per 5000 monocyte cells (Figure 4-15).

Figure 4-12. Confocal microscopic images of monocyte cells labeled with nanoparticles of 1 (Gd-SiDTTA functionalized SNPs).
Figure 4-13. Flow cytometry results for the unlabeled monocyte cells (red) and monocyte cells labeled with 1 (blue) under the following incubation conditions: (A) 0 mg of 1/1×10^6 cells in mL media; R1 = 95.5% of total events; (B) 0.004 mg of 1/1×10^6 in 2 mL media; 0.6% NP labeling efficiency, R2 = 94.0% of total; (C) 0.042 mg of 1/1×10^6 in 2 mL media; 10.8% NP labeling efficiency, R2 = 94.2% of total events; (D) 0.418 mg of 1/1×10^6 in 2 mL media; 98.0% NP labeling efficiency, R2 = 90.9% of total events; (E) 2.140 mg of 1/1×10^6 in 2 mL media; 99.4% NP labeling efficiency, R2 = 91.3% of total events. The insets show the purity of the cell populations.
4.2.4 Polysilsesquioxane Nanoparticles as Multifunctional Imaging Agents

We have also developed a method to formulate polysilsesquioxane nanoparticles (PNPs) from bis(silyl)-derived Gd(DTPA) complexes using reverse microemulsion procedures similar to those described above. By doing so, we have eliminated the need for an SNP core to graft the complexes onto. This strategy yielded nanoparticles composed almost entirely of active imaging cargoes. Initially, we attempted to prepare PNPs from Gd-DTPA-BSiA, the same complex that we used to formulate nanoparticles of 2. In the synthesis, an aliquot of aqueous ammonia was added to a magnetically stirred mixture of an
aqueous Gd-DTPA-BSiA, Triton X-100, 1-hexanol, cyclohexane, and TEOS to initiate the condensation of the alkoxy silane moieties. After stirring the reaction mixture for up to 12 h, ethanol was added to the microemulsion to precipitate the particles, which were subsequently isolated by centrifuge, washed with ethanol and water, and redispersed in the desired via ultra-sonication.

We found that the sizes and the degree of morphological irregularity could be controlled by varying the $W$ value of the microemulsion and the amount of TEOS used in the reaction. As shown in Figure 4-16, PNP s synthesized at $W = 10$ using ~10 molar equivalents of TEOS had an irregular morphology and were ~30 nm in diameter. By increasing the amount of TEOS to ~20 molar equivalents, smooth spherical PNP s with an approximate diameter of 50 nm were obtained under otherwise identical conditions (Figure 4-17). Increases to $W$ value of the microemulsion typically resulted in samples with larger particles; however, they also had a significantly higher degree of polydispersity.

![Figure 4-16. TEM micrographs of Gd-DTPA-BSiA PNP s synthesized in the neutral microemulsion at $W = 10$ using ~10 molar equivalents of TEOS.](image-url)
Figure 4-17. TEM micrographs of Gd-DTPA-BSiA PNPs synthesized in the neutral microemulsion at $W = 10$ using $\sim 20$ molar equivalents of TEOS.

Figure 4-18. TEM micrographs of Gd-DTPA-BSiA PNPs synthesized in the neutral microemulsion at $W = 15$ using $\sim 10$ molar equivalents of TEOS.

TGA was used to determine the degree of Gd-DTPA-BSiA incorporation. For PNPs synthesized at $W = 10$ using $\sim 10$ molar equivalents of TEOS, an initial weight loss of 10.8 % was observed for the adsorbed solvent species from r.t. to $\sim 150 \, ^\circ$C and an additional weight loss of 28.6 % was observed for the organic components from $\sim 150$-600 $^\circ$C. The adjusted weight loss for the organic species was thus determined to be 32.1 %, which corresponded precisely to the calculated weight loss based on the sum total of the reactants added to the
microemulsion. For PNPs synthesized at \( W = 10 \) using \( \sim 20 \) molar equivalents of TEOS, an initial weight loss of 10.0 % was observed for the adsorbed solvent species from r.t. to \( \sim 150 \) \(^\circ\)C and an additional weight loss of 20.6 % was observed for the organic components from \( \sim 150-600 \) \(^\circ\)C. The adjusted weight loss for the organic species was determined to be 22.9 %, which again was very accurate when compared against the expected weight loss.

![TGA curves for Gd-DTPA-BSiA PNPs synthesized in the neutral microemulsion at \( W = 10 \) using \( \sim 10 \) molar equivalents of TEOS (blue) and \( \sim 20 \) molar equivalents of TEOS (red).](image)

**Figure 4-19.** TGA curves for Gd-DTPA-BSiA PNPs synthesized in the neutral microemulsion at \( W = 10 \) using \( \sim 10 \) molar equivalents of TEOS (blue) and \( \sim 20 \) molar equivalents of TEOS (red).

We also evaluated the MRI contrast enhancing capability of Gd-DTPA-BSiA PNPs synthesized at \( W = 10 \) using \( \sim 20 \) molar equivalents of TEOS. The longitudinal relaxivity \( (r_1) \) of the PNPs was determined to be 8.6 mM\(^{-1}\) Gd\(^{3+}\) s\(^{-1}\), while the transverse relaxivity \( (r_2) \) was determined to be 24.0 mM\(^{-1}\) Gd\(^{3+}\) s\(^{-1}\). These values were again greater than those of clinically used \( T_1 \)-weighted contrast enhancing agents. More importantly, the high Gd-DTPA-BSiA loadings would yield large relaxivities on a per particle basis, though the precise values were difficult to determine without knowing the bulk density of the particles.
In an attempt to make nanometer-scale contrast agents clinically viable, we developed a method to formulate biodegradable polysilsesquioxane nanoparticles from a novel Gd complex, Gadolinium[diethylenetriaminepentaacetic acid-bis(triethoxysilylpropylcystamide)] (abbreviated as Gd-DTPA-BSCA). The monomer unit was synthesized via a multistep procedure. First, we prepared bis(2-pyridyldisulfanylethylamido) diethylene-triaminepentaacetic acid dihydrochloride (abbreviated as (PDSEA)$_2$DTPA·2HCl) by reacting DTPA dianhydride with 2 equivalents of 2-pyridyldisulfanylethylamine hydrochloride in anhydrous pyridine. We then formed the Gd complex by adjusting the pH of an aqueous (PDSEA)$_2$DTPA·2HCl solution with NaOH, and subsequently adding GdCl$_3$ to the mixture. Finally, [(PDSEA)$_2$Gd(DTPA)]·2HCl was reacted with 3-mercaptopropyl tri(ethoxy)silane in methanol to yield the bis(silyl)-derived Gd complex, Gd-DTPA-BSCA. By forming the Gd complex before the addition of the silane moieties, we obviated the need to introduce any water or base that might hydrolyze the alkoxysilane groups to the corresponding hydroxide during the synthesis, which we believed was crucial to our success in formulating PNPs.
composed solely of co-condensed monomer. It was, however, necessary to add TEOS into the reaction mixture in the synthesis of the Gd-DTPA-BSiA PNPs on account that the Gd-DTPA-BSiA had most likely undergone hydrolysis or partial condensation in its aqueous solution before the synthesis.

**Figure 4-21.** Scheme illustrating the synthesis of the Gd(DTPA-BSiA) polysilsesquioxane monomer and the subsequent formation of Gd(DTPA-BSiA) PNPs via basic co-condensation of the monomer in a microemulsion.

Gd-DTPA-BSiA PNPs were synthesized using microemulsion procedures similar to those previously described. Briefly, an aqueous solution of Gd-DTPA-BSiA was added to a mixture of Triton X-100, 1-hexanol, and cyclohexane with magnetic stirring. It is important to note that the Gd-DTPA-BSiA complex was typically kept under high vacuum until its use, and the aqueous Gd-DTPA-BSiA solution was prepared immediately prior adding it to the microemulsion. Shortly after its addition to the microemulsion mixture, an aliquot of aqueous ammonia was added to initiate the co-condensation of the monomer units into PNPs. After ~24 h, the PNPs were precipitated, isolated, washed and redispersed as previously described.

According to DLS measurements, the sizes of the particles could be tuned by adjusting the amount of Gd-DTPA-BSiA added to the reaction mixture. PNPs with hydrodynamic diameters of 81.2 ± 6.4 nm and 123.1 ± 13.5 nm were isolated from a \( W = 15 \) microemulsion using 5.0 µmol and 15.0 µmol of Gd-DTPA-BSiA/10 mL of the mixture,
respectively (Figure 4-22). Interestingly, the PNPs did not exhibit the same diameters when vacuum dried. For example, PNPs synthesized at $W = 15$ using 15.0 µmol of Gd-DTPA-BSiA/10 mL of the microemulsion mixture were ~30 nm in diameter when vacuum dried (Figure 4-23). The changes in particle size corresponded to a 70:1 ratio of volumes for the wet and dry samples. The xerogel-like behavior exhibited by the PNPs suggests that they have a highly irregular and porous structure in solution. Our observations are in accordance with similar results published in the literature.

![Figure 4-22](image1.png)

**Figure 4-22.** DLS results for Gd-DTPA-BSCA PNPs synthesized in a $W = 15$ microemulsion using 5 µmol and 15 µmol of Gd-DTPA-BSCA/10 mL of the microemulsion mixture.

![Figure 4-23](image2.png)

**Figure 4-23.** SEM micrographs of Gd(DTPA-BSCA) nanoparticles synthesized in a $W = 15$ microemulsion at 15 µmol Gd(DTPA-BSCA)/10 mL.
To examine the degradability of the Gd-DTPA-BSCA PNPs, we dialyzed our samples against PBS buffer at 37 °C in the presence of the biologically relevant reducing agent, cysteine. The particles were found to be stable in buffer before the addition of cysteine to the reaction mixture; however, immediately upon its addition the particles would rapidly degrade until a steady state dependent upon the concentrations of redox species within the system was reached. For instance, at an intracellular thiol concentration of 10 mM, the PNPs completely degraded and displayed a half-life of ~2.5 h (Figure 4-24). However, when the extracellular thiol concentration of 15 µM was used, a small fraction of the particles rapidly degraded before a steady state was reached, after which the particles degraded very slowly. Under \textit{in vivo} conditions, we believe the low extracellular thiol concentrations would still degrade the particles relatively fast since there would be a constant reservoir of reductant species in the bloodstream.

\textbf{Figure 4-24.} Degradation curves for Gd-DTPA-BSCA nanoparticles in the presence of 10 mM cysteine (black) and 15 µM cysteine (red).

The Gd-DTPA-BSCA PNPs exhibited MRI contrast enhancing efficacies superior to those of the clinically-used small molecule chelates. The $r_1$ and $r_2$ relaxivities of PNPs
synthesized at $W = 10$ using 5 $\mu$mol of Gd-DTPA-BSiA/10 mL of the microemulsion mixture were determined to be 11.7 s$^{-1}$ and 18.6 s$^{-1}$ on a per mM of Gd basis, respectively. Monocyte cells incubated with the Gd-DTPA-BSCA PNPs clearly showed the MRI contrast enhancing capability of the nanoparticles. The $T_1$-weighted and $T_2$-weighted MR images in Figure 4-26 shows the respective positive and negative signal enhancement for the PNP loaded monocyte cells. These results once again suggest that PNPs can be used as both $T_1$- or $T_2$-enhancing contrast agents, depending on the MR pulse sequence used in the study.

![Figure 4-25.](image)

Longitudinal ($r_1$, black) and transverse ($r_2$, red) MR relaxivity curves Gd-DTPA-BSCA PNPs synthesized in $W = 10$ microemulsion.

![Figure 4-26.](image)

MR phantom images of monocytes incubated with no particle (A) and as-synthesized Gd-DTPA-BSCA nanoparticles (B). TR=160 in the $T_1$-weighted image and the TE=130 in the $T_2$-weighted image.
4.3 Concluding Remarks

We have developed robust hybrid silica nanoparticles containing a luminescent core and a paramagnetic coating as optical and MRI contrast agents. We have demonstrated their efficacy in vitro by labeling monocyte cells with the core-shell nanoparticles. Furthermore, we have developed method to formulate polysilsesquioxane from silyl-derived Gd-based molecular MRI contrast agents. In attempt to improve the clinical safety of the agents, we have formulated disulfide-derived degradable polysilsequioxane nanoparticles. The hybrid nanoparticles developed in this study all provide MR image enhancement superior to that obtained with clinically contrast agents, and should pave the way for the development of new biologically useful nanomaterials.

4.4 Experimental Section

4.4.1 General Procedures and Instrumentation

Materials and Methods. All reagents and solvents were purchased from commercial sources and used without further purification. Thermogravimetric analysis (TGA) was performed using a Shimadzu TGA-50 equipped with a platinum pan and heated at a rate of 3 °C / min under air. A Hitachi 4700 Field Emission Scanning Electron Microscope (SEM) and a JEM 100CX-II Transmission Electron Microscope (TEM) were used to determine particle size and morphology. Glass and carbon-coated copper substrates were used for SEM and TEM image acquisition, respectively. A Cressington 108 Auto Sputter Coater equipped with an Au/Pd (80/20) target and MTM-10 thickness monitor was used to coat the sample with a thin conductive layer (~5 nm) before taking SEM images. Gd\(^{3+}\) ion concentration was measured using an Applied Research Laboratories (ARL) SpectraSpan7...
Direct Current Plasma (DCP) Spectrometer. Emission and excitation data were collected on a Shimadzu RF-5301PC Spectrofluorophotometer. Longitudinal relaxivities \( (T_1) \) were determined using a multi-flip angle 3D-gradient echo pulse sequence on a Bruker 3.0 Tesla Magnetic Resonance Imaging (MRI) scanner. The transverse relaxivities \( (T_2) \) were determined with a CPMG-type spin-echo sequence (10 echoes). Confocal laser scanning microscope images were taken with a Zeiss LSM5 Pascal Confocal Laser Scanning Microscope or a Leica SP2 Laser Scanning Confocal Microscope with 488 nm excitation and a 530 LP emission filter.

**Simulation of Gd-DTPA-BSCA Nanoparticle Degradation.** 250 mL of 10 mM phosphate buffer (pH 7) at 37 °C was sparged with argon gas for 30 min and kept under an argon atmosphere for the remainder of the experiment. Ten milligrams of the nanomaterial dispersed in ~1 mL of the buffer was dialyzed against the above solution using 3500 MW cutoff cellulose dialysis tubing. After at least a 2 h equilibration period, an aliquot of the solution was withdrawn to mark \( t_0 \). Cysteine (303 mg, 2.50 mmol) was added to the solution and additional aliquots were removed at predetermined intervals to determine the \([\text{Gd}^{3+}]\) released into the dialyzing buffer by direct current plasma (DCP) spectrometry. The percentage of the initial \([\text{Gd}^{3+}]\) dose released into the dialyzing buffer due to disulfide cleavage was calculated using the equation: % Released = \(\left\{\frac{(V_{\text{tot}} \times C) + Y}{Z}\right\} \times 100\); where \(V_{\text{tot}}\) = Total solvent volume remaining, \(C\) = Gd concentration as determined by DCP, \(Y\) = total mole Gd removed from solution, and \(Z\) = mole Gd added to dialysis tubing.

A similar procedure was used to obtain a degradation curve at 15 μM cysteine, except that 5 mg of nanomaterial was dialyzed against 400 mL of phosphate buffer to minimize the perturbation to the system when removing larger sample aliquots.
4.4.2 Ligand and Complex Syntheses

Synthesis of tri(hydroxy)silylpropyl diethylenetriaminetetraacetic acid (SiDTTA). Bromoacetic acid (0.5558 g, 4.00 mmol) and 3-tri(methoxy)silylpropyldiethylene triamine (0.2654 g, 1.00 mmol) were dissolved in 1.0 mL of distilled H₂O and 2.0 mL 2M NaOH (4.00 mmol) with magnetic stirring. The reaction solution was subsequently heated to 50°C, and an additional 3.0 mL of 2M NaOH were added dropwise over approximately 30 minutes. After stirring the mixture for 2 h at 50°C, the solvent was removed under reduced pressure to yield a viscous yellow oil. An off-white hygroscopic powder was isolated from the oil in high yield (>90%) by precipitation with EtOH, and subsequent drying in vacuo. MS (ESI negative ion): m/z 542.2 [M-H]⁻ for the silanetriol from a basic solution. NMR: ¹H (D₂O, 300 MHz, ppm): 0.47 (2H), 1.55 (2H), 2.62-2.78 (10H), 3.14-3.21 (8H).

Figure 4-27. Scheme illustrating the synthesis of SiDTTA via the basic addition of bromoacetic acid to tri(methoxy)silylpropyldiethylene triamine.

Synthesis of Gd-SiDTTA. The gadolinium complex was prepared by dissolving the isolated SiDTTA product (108.6 mg, 0.2 mmol) in 4 mL H₂O with magnetic stirring at room temperature. GdCl₃ (380 µL of a 0.50 M solution, 0.19 mmol) was slowly titrated into the solution until the formed precipitate would no longer dissolve back into solution, while maintaining a pH of ~9 with the dropwise addition of 2M NaOH. After stirring the above reaction for 2 h, Chelex 100 (Na⁺ form) was added to remove excess Gd³⁺, which
was removed via filtration after 30 min. The resultant solution was then concentrated to 1 mL to yield a ~0.20 M solution of the mono-silyl derived Gd complex (Gd-Si-DTTA).

**Figure 4-28.**Scheme illustrating the synthesis of Gd-SiDTTA.

**Synthesis of diethylenetriaminepentaacetic acid bis[tri(ethoxy)silylpropyl]amide (DTPA-BSiA).** Diethylenetriamine pentaacetic acid dianhydride (5.000 g, 13.995 mmol) was dissolved in 110 mL of anhydrous pyridine under a steady flow of nitrogen. Using standard Schlenk line techniques 3-aminopropyl triethoxysilane (6.85 g, 31.00 mmol) was added and the resultant reaction mixture was magnetically stirred under nitrogen for 24 hours. The product was then precipitated with copious amounts of hexane, isolated via centrifuge, washed with additional aliquots of hexanes, and dried to yield 10.436 g (93.2 %) of the desired compound (DTPA-BSiA). MS (ESI negative ion): m/z 631.3 [M-H]⁻ for the silanetriol from a basic solution. NMR: ¹H (DMSO, ppm): 0.52 (t, 4H), 1.14 (t, 18H), 1.44(p, 4H), 2.81 (t, 4H), 2.92 (t, 4H), 3.04 (q, 4H), 3.22 (s, 6H), 3.34 (s, 4H), 3.73 (q, 12H), 8.06 (t, 2H). ¹³C(¹H) (DMSO, ppm): 8.0 (2C), 18.8 (18C), 23.4 (2C), 41.8 (2C), 51.2 (2C), 52.8 (2C), 55.9 (2C), 56.7 (2C), 58.3 (1C), 58.4 (6C), 170.7 (2C), 173.4 (3C).

**Figure 4-29.**Scheme illustrating the synthesis of DTPA-BSiA via the addition of 3-aminopropyl tri(ethoxy)silane to a mixture of DTPA-dianhydride in anhydrous pyridine.
Synthesis of Gd-DTPA-BSiA. To prepare the gadolinium complex, DTPA-BSiA (1.77 g, 2.22 mmol) was dissolved in ~3 equivalents of NaOH (6.0 mL of a 1.0 M solution) with magnetic stirring for 30 minutes. To this solution was added 0.90 equivalent of GdCl₃ (4.0 mL of a 0.5 M solution, 0.002 mol) and the mixture was magnetically stirred at room temperature for several hours, the volume of the solution was adjusted to 10 mL to yield a visibly clear yellow 0.20 M solution of the modified gadodiamide complex.

Figure 4-30. Scheme illustrating the synthesis of Gd-DTPA-BSiA.

Synthesis of 2-pyridyldisulfanyl ethylamine hydrochloride, PDSEA·HCl. A solution of aminoethanethiol hydrochloride (1.132 g, 10.0 mmol) in methanol (10 mL) was added dropwise to a magnetically stirred mixture of 2,2'-dipyridyl disulfide (4.4062 g, 20.0 mmol) and acetic acid (800 µL) in methanol (20 mL) over a period of 30 min. After magnetically stirring the solution an additional 24 hours, the solvent was removed by rotary evaporation. The product was isolated by dissolution of the bright yellow oil in a minimal volume of methanol, precipitation with diethyl ether, and isolation via centrifuge. This washing procedure was repeated up to 10 times before drying the white crystalline product in vacuo. Yield: 1.935 g (87 %). NMR: ¹H (300 MHz, MeOD, ppm): 3.13 (t, 2H), 3.29 (t, 2H), 7.31 (t, 1H), 7.67 (d, 1H), 7.80 (t, 1H), 8.53 (d, 1H).
Scheme illustrating the synthesis of PDSEA-HCl by reacting 2,2′-dipyridyl disulfide with aminoethanethiol hydrochloride in an acidic methanolic solution.

**Synthesis of bis(2-pyridyl disulfanyl ethylamido) diethylenetriamine pentaacetic acid dihydrochloride, (PDSEA)$_2$DTPA·2HCl.** A mixture of PDSEA·HCl (0.804 g, 3.60 mmol) and DTPA dianhydride (0.516 g, 1.44 mmol) in anhydrous pyridine (10 mL, distilled over CaH$_2$) was magnetically stirred under a nitrogen gas atmosphere for 24 h. The product was precipitated with diethyl ether and isolated via centrifuge. The residue was subsequently dissolved in a minimal volume of methanol, precipitated with diethyl ether, and isolated via centrifuge. This procedure was repeated up to 4 more times before drying the off-white product *in vacuo* overnight. Yield: quantitative. MS (ESI positive ion): observed 365.5 for [M-2Cl]$^{2+}$ and 730.1 for [M-2Cl-H]$^+$, *m/z* 801.1 (Expected 801.3). NMR: $^1$H (300 MHz, D$_2$O, ppm): 2.97 (t, 4H), 3.30 (s, 8H), 3.52 (t, 4H), 3.69-3.78 (10H), 7.52 (t, 2H), 8.00 (d, 2H), 8.10 (t, 2H), 8.45 (d, 2H).

Scheme illustrating the synthesis of (PDSEA)$_2$DTPA·2HCl via the addition of PDSEA·HCl to a mixture of DTPA-dianhydride in anhydrous pyridine.

**Synthesis of [(PDSEA)$_2$Gd(DTPA)]·2HCl.** The pH of a solution of (PDSEA)$_2$DTPA·2HCl (0.730 g, 1.00 mmol) in water (10 mL) was adjusted from ~2 to ~10
via the addition of dilute NaOH (4.0 mL of 1 M, 4.0 eq). GdCl$_3$ (0.364 g, 0.98 mmol) in water (1 mL) was added dropwise to the solution, which (pH ~3.5) was thereafter magnetically stirred for an additional 6 h. The solvent was removed via rotary evaporation. The residue was subsequently dissolved in a minimal volume of methanol, precipitated with diethyl ether, and isolated via centrifuge. This procedure was repeated up to 4 more times before drying the off-white product in vacuo overnight. Yield: quantitative. MS (ESI positive ion): observed 443.0 for [M-2Cl]$_2^+$, 454.0 for [M-2Cl-H+Na]$_2^+$, 885.1 for [M-2Cl-H], and 906.9 for [M-2Cl-2H+Na]$_2^+$, m/z 956.0 (Expected 956.03).

Figure 4-33. Scheme illustrating the synthesis of [(PDSEA)$_2$Gd(DTPA)]·2HCl.

**Synthesis of Gadolinium[diethylenetriamine pentaacetic acid-bis(triethoxysilylpropyl cystamide)], Gd-DTPA-BSCA.** A solution of [(PDSEA)$_2$Gd(DTPA)]·2HCl (0.200 g, 0.222 mmol) was prepared via the addition of enough methanol (~3.5 mL) to completely dissolve the starting material. 3-mercaptopropyl triethoxysilane, MPTES, (0.132 mg, 0.555 mmol) was added to the solution and magnetic stirring was continued under a nitrogen gas atmosphere for ~12 h. Diethyl ether was added to the solution to precipitate the product, which was subsequently isolated by centrifuge. The residue was dissolved in minimal methanol, precipitated with diethyl ether, and isolated by centrifuge. This procedure was repeated up to 4 more times before drying the off-white
product *in vacuo* overnight. The product was extremely moisture sensitive, so it was keep under high vacuum or in the dessicator until it was used. Yield: 185 mg (73 %).

![Scheme illustrating the synthesis of Gd-DTPA-BSCA](image)

**Figure 4-34.** Scheme illustrating the synthesis of Gd-DTPA-BSCA by reacting \([(\text{PDSEA})_2\text{Gd(DTPA)}\)-2HCl with MPTES in methanol.**

### 4.4.3 Microemulsion Syntheses of Silicon Oxide-Based Nanoparticles

**General Syntheses of Silica Nanoparticles.** Silica nanoparticles (SNPs) were synthesized via the neutral Triton X-100/1-hexanol/cyclohexane microemulsion system. Initially, Triton X-100 (15.625 g, 0.075 mol) and 1-hexanol (38.318 g, 0.375 mol) were dissolved in cyclohexane and diluted to 250 mL to make a 0.3 M Triton X-100 stock microemulsion solution with 5 molar equivalents of the co-surfactant 1-hexanol. A typical synthesis using a $W = 15$ ($W = [\text{H}_2\text{O}]/[\text{surfactant}]$) microemulsion system consisted of adding 3.05 mL distilled H$_2$O and 500 µL TEOS to 50 mL of a 0.3 M Triton X-100/1.5 M 1-hexanol/cyclohexane stock solution while vigorously stirring at room temperature. After 10 min of vigorous stirring, or until the microemulsion mixture became optically transparent, 1 mL of aqueous NH$_4$OH was added to initiate hydrolysis, and the resultant visibly clear microemulsion mixture was stirred for another 24 hrs before workup, which consisted of precipitating the nanoparticles with an equivalent volume (with respect to the total microemulsion volume) of ethanol, isolating the nanoparticles via centrifuge at 13000 rpm,
and subsequently washing them with methanol and H$_2$O before redispersion in the desired solvent.

**Synthesis of [Ru(bipy)$_3$]Cl$_2$-Doped Gd-SiDTTA Functionalized SNPs, 1.**

Ru(bipy)$_3^{2+}$-doped SNPs were prepared by adding 2.28 mL distilled H$_2$O, 160 µL of a 0.1 M Ru(bipy)$_3^{2+}$ aqueous solution, and 400 µL TEOS to 40 mL of a 0.3 M Triton X-100/1.5 M 1-hexanol/cyclohexane stock solution while vigorously stirring at room temperature. After 10 min of vigorous stirring at room temperature, 0.8 mL of aqueous NH$_4^+$OH was added to initiate hydrolysis. The resultant optically transparent red microemulsion mixture was stirred for another 20 h before adding 1.0 mL of a 0.12 M Gd-Si-DTTA aqueous solution to the reaction mixture and stirring for an additional 24 h. The functionalized SNPs were then precipitated with an equivalent volume of ethanol and isolated via centrifugation at 13000 for ~30 min. The SNPs were subsequently washed with ethanol and water by redispersing them via sonication and isolating them via centrifugation before their redispersment in water. Approximately 150 mg of functionalized SNPs were isolated from this procedure.

**Synthesis of Gd-DTPA-BSiA Functionalized Ru(bipy)$_3^{2+}$-Doped Silica Nanoparticles, 2.**

Ru(bipy)$_3^{2+}$-doped SNPs were prepared by adding 2.85 mL distilled H$_2$O, 200 µL of a 0.1 M Ru(bipy)$_3^{2+}$ aqueous solution, and 500 µL TEOS to 50 mL of a 0.3 M Triton X-100/1.5 M 1-hexanol/cyclohexane stock solution while vigorously stirring at room temperature. After ~10 min, 1 mL of aqueous NH$_4^+$OH was added to initiate hydrolysis, and the resultant optically transparent red microemulsion mixture was stirred for another 24 h at room temperature. To a 10 mL aliquot of the above reaction mixture was added 385 µL of a 0.2 M Gd-DTPA-BSiA solution and the reaction mixture was stirred for an additional 12 h. The Gd-DTPA-BSiA functionalized SNPs were then precipitated with an equivalent volume
of ethanol and isolated via centrifuge at 13000 rpm for 30 min. The SNPs were subsequently washed with ethanol and water before redispersing them in 5 mL of water. Approximately 55 mg of functionalized SNPs were isolated from this procedure (using 10 mL of the above microemulsion reaction). Results suggest that when care is taken in isolating and washing the SNPs > 300 mg of 2 can be isolated from a ~54 mL microemulsion reaction.

**Synthesis of Gd-DTPA-BSiA Polysilsesquioxane Nanoparticles, ~10 Molar Equivalents of TEOS.** For a \( W = 10 \) microemulsion, an aliquot of Gd-DTPA-BSiA (100 µL of a 0.22 M solution) was added to a magnetically stirred mixture of water (340 µL), 10 mL of a 0.3 M Triton X-100/1.5 M 1-hexanol/cyclohexane solution, and TEOS (50 µL). Shortly after, aqueous \( \text{NH}_4^+\text{OH}^- \) (100 µL) was added to initiate hydrolysis, and the resultant microemulsion mixture was stirred for an additional 5 h. The PNPs were precipitated with an equivalent volume of ethanol and isolated via centrifugation. The PNPs were subsequently washed with ethanol before redispersing them in ethanol.

**Synthesis of Gd-DTPA-BSiA Polysilsesquioxane Nanoparticles, ~20 Molar Equivalents of TEOS.** As per the synthesis above using 100 µL of TEOS.

**Synthesis of Gd-DTPA-BSCA Polysilsesquioxane Nanoparticles.** For a \( W = 15 \) microemulsion, a solution of Gd-DTPA-BSCA (57.0 mg, 0.05 mmol) in water (7.6 mL) was prepared IMMEDIATELY prior to adding it to a magnetically stirred solution of 0.3 M Triton X-100/1.5 M 1-hexanol/cyclohexane (100 mL). Shortly after, aqueous \( \text{NH}_4^+\text{OH}^- \) (0.50 mL) was added to initiate hydrolysis and the resultant microemulsion mixture was stirred for an additional 24 h. The PNPs were precipitated with an equivalent volume of ethanol and isolated via centrifugation. The PNPs were subsequently washed with ethanol before redispersing them in ethanol. Contaminants and low molecular weight species were
removed by dialyzing the PNPs against dilute phosphate buffered saline (pH 7) using 3500 MW cutoff cellulose dialysis tubing. The remaining nanoparticles were dispersed in distilled water.

### 4.4.5 In Vitro and In Vivo Protocols

**Cell Culture.** An immortalized monocyte cell line was generated using literature procedures.\(^1\)\(^-\)\(^3\) Briefly, bone marrow progenitor cells from C57Bl/6 mice were harvested and grown in conditioned medium containing 10% heat-inactivated fetal calf serum, 1% L-glutamine, and 20% LADMAC (catalog no. CRL 2420; American Type Culture Collection) supernatant in Minimal Essential Medium. Once immortalized, cells were grown in the aforementioned conditioned medium, which provided the isolated monocytes with colony-stimulating factor-1. Cell lines were matured over 9 months to achieve a homogeneous population expressing the macrophage/monocyte marker MOMA-2 (data not shown) with phagocytic capacity.

**Confocal Imaging.** Monocyte cells were incubated in 2.0 mL of media with 17.0 µL of nanoparticle suspension (24.6 mg/mL) for 30 minutes at 37 °C with 5% CO\(_2\). The cells were isolated from the media via centrifugation at 1000 RPM for 10 min at 4 °C, and subsequently washed with a fresh aliquot of medium. The isolated pellet was suspended in 100 µL of PBS and imaged using confocal microscopy: excitation at 488 nm, emission using 530 LP filter settings, and 252×zoom (63× oil immersion optical, 4× digital).

**Cell Viability Assay.** Monocyte cells were counted by the trypan blue exclusion assay and distributed into a 96-well plate at a concentration of 5000 cells per 100 µL per well. Cells were incubated with various concentrations of nanoparticles of \(1\): 123 µg, 12.3
µg, 1.23 µg, 0.123 µg, 0.0123 µg, and 0 µg in 5 µL of distilled H₂O. After 20 hours of incubation, 20 µL of MTS solution was added to each well and it was allowed to incubate for an additional 4 hours. The microplate was read for an absorbance peak at 492 nm at \( t = 0 \) h and \( t = 4 \) h after MTS addition. The changes in absorbance (from \( t = 0 \) h \( \rightarrow \) \( t = 4 \) h) were necessary to subtract nanoparticle background from the viability assay.

**MRI Cellular Phantom Studies for SNPs of 1.** Monocyte cells were trypsinized for 5 minutes at 37 °C and 5% CO₂ before collection by low speed centrifugation. The cell concentration was determined by the trypan blue exclusion assay. Approximately 18.1x10⁶ monocytes were placed in a culture dish with 1 mL of media and 0.433 mL of nanoparticle solution (24.6 mg/mL). After 1 hour of incubation, the cells were washed with fresh media twice and pelleted. A final layer of PBS (200 µL) was added on top, careful not to disturb the pellet, for MR imaging of the cells. Upon completion of MR imaging, the cells were digested in 1.0 M HNO₃ for DCP measurements of the total Gd^{3+} taken in by the cells.

**MRI Cellular Phantom Studies for Gd-DTPA-BSCA PNPs.** Monocyte cells labeled with Gd-DTPA-BSCA PNPs were prepared as previously described using the following conditions: 200 µL of Gd-DTPA-BSCA PNP dispersion (0.100 mg) incubated with 2x10⁶ cells for 1 h in 2 mL of media.
4.5 References


