Nanodisco Balls: Control over Surface versus Core Loading of Diagnostically Active Nanocrystals into Polymer Nanoparticles

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ABSTRACT Nanoparticles of complex architectures can have unique properties. Self-assembly of spherical nanocrystals is a high yielding route to such systems. In this study, we report the self-assembly of a polymer and nanocrystals into aggregates, where the location of the nanocrystals can be controlled to be either at the surface or in the core. These nanospheres, when surface decorated with nanocrystals, resemble disco balls, thus the term nanodisco balls. We studied the mechanism of this surface loading phenomenon and found it to be Ca2+ dependent. We also investigated whether excess phospholipids could prevent nanocrystal adherence. We found surface loading to occur with a variety of nanocrystal types including iron oxide nanoparticles, quantum dots, and nanophosphors, as well as sizes (10–30 nm) and shapes. Additionally, surface loading occurred over a range of polymer molecular weights (∼30–3000 kDa) and phospholipid carbon tail length. We also show that nanocrystals remain diagnostically active after loading onto the polymer nanospheres, i.e., providing contrast in the case of magnetic resonance imaging for iron oxide nanoparticles and fluorescence for quantum dots. Last, we demonstrated that a fluorescently labeled protein model drug can be delivered by surface loaded nanospheres. We present a platform for contrast media delivery, with the unusual feature that the payload can be controllably localized to the core or the surface.

KEYWORDS: directed assembly · nanoparticles · polyphosphazene · MRI · multimodal · theranostic

Methods to create complex nanoparticle architectures such as stars, shells, cubes, and frames have led to structures with unique properties.1,2 However, direct syntheses of such structures are frequently low yielding and difficult to scale up. An alternative approach is to form complex architectures from self-assembly of simpler, high-yielding components. One such route is incorporation of inorganic nanocrystals into polymeric nanoparticles. Such assemblies have a wide range of applications including uses in catalysis,3 energy,4 drug delivery,5,6 and medical imaging,7 Often, the structural organization of these nanoparticles will dictate its function and behavior in a given application. For example, previous research has shown that gold nanoparticles are capable of quenching the emission of nearby fluorescent sources when closely arranged. Jennings et al. showed that fluorescence could be recovered by separating the 1.5 nm gold nanoparticles and fluorophore a distance as little as 15 nm.8 Hence, separate core and surface loading of gold cores and fluorophores could maintain fluorophore fluorescence. Therefore, the ability to control localization of nanocrystals via self-assembly would be a valuable tool in the synthesis of nanocrystal/polymer composite particles. Control over nanocrystal distribution within the core of polymer constructs has previously been demonstrated.9,10 For instance, Luo et al. found that by modifying the surface ligands of...
gold nanoparticles, their incorporation could be focused to specific regions (i.e., the core center or at the edge of the core) within nanoparticles formed from amphiphilic copolymers of polystyrene and poly-(acrylic acid).18

In this study, we report the serendipitous discovery of a system where nanocrystals can be selectively loaded onto the surface of polymer nanospheres, as opposed to the core. This forms a structure reminiscent of a disco ball, which we term a nanodisco ball. We have found that phospholipid coated inorganic nanoparticles can be used to surface decorate a polymer nanosphere formed from poly(bis(4-carboxyphenoxy)-phosphazene), or PCPP, whereas carboxylic acid coated nanocrystals localize to the core. PCPP is a part of a larger class of phosphorus–nitrogen backbone based polymers called polyphosphazenes.19 These polymers have drawn interest for use in biomedical applications due to their tunable functionality and biocompatibility.20.22 By modifying the polyphosphazene side chains and molecular weight, the biodegradation rate of polymers can be controlled, making this a promising platform for drug and contrast agent delivery.23,24 Specifically, PCPP has been used to form ionically cross-linked hydrogels,25,26 polymer films,27 and more recently microencapsulating spheres.23,28,29 This is the first report of a system that integrates polyphosphazenes and nanocrystals. Furthermore, this is a rare instance of a system where nanocrystals can be preferentially loaded onto the surface of a particle as opposed to internal loading. We believe that this novel system has excellent potential as a contrast agent or theranostic delivery platform.

Herein, we investigate the conditions for surface localization of nanocrystals onto PCPP nanospheres (Figure 1a, b). We report the synthesis of these nanodisco balls and their characterization via transmission electron microscopy, scanning electron microscopy, and electron microscopy tomography. They can be loaded with separate core and surface payloads, maximizing contrast for two imaging methods. We have probed the synthetic process for the key steps in the formation of the nanodisco balls. We evaluated the effects of varying the physical and chemical properties of the nanocrystals used on surface loading. We also varied phospholipid tail length for micelle formation and PCPP molecular weights for synthesis. In addition, we have investigated how competing micelles might affect the adherence of nanocrystals. Lastly, we assessed the diagnostic, drug delivery, and biological application of nanodisco balls.

**RESULTS**

**Surface Decoration of PCPP Nanospheres.** PCPP is a biocompatible polymer, which we have used to create a novel platform to exploit for nanocrystal delivery...
in biomedical applications. PCPP nanospheres were synthesized using a modified method that was previously developed to make microspheres. The polycarboxylate PCPP is cross-linked with the polycation spermine to form polymer spheres. The self-assembled nanospheres were dispersed in CaCl2, which we found necessary to halt nanoparticle growth. This process resulted in polymer nanoparticles of 508 nm (Figure 2a). As an initial step in loading these polymer nanoparticles, we added dimercaptosuccinic acid coated iron oxides (IO-DMSA) to the synthesis prior to adding the spermine cross-linker. We found that IO-DMSA were included in the core of the polymer spheres (Figure 2b). We then decided to explore the incorporation of iron oxide nanoparticles with alternative coatings.

Lipid coatings are commonly used to make nanocrystals biocompatible. Oleic acid coated nanocrystals were rendered water-soluble through encapsulation in micelles formed by MHPC. We mixed these phospholipid coated nanocrystals with PCPP before the addition of spermine. In the final product, we found that, surprisingly, the nanocrystals were exclusively localized to the surface of the nanospheres in a structure we found reminiscent of a disco ball (Figure 2c,d). As an initial step in loading these polymer nanoparticles, we added dimercaptosuccinic acid coated iron oxides (IO-DMSA) to the synthesis prior to adding the spermine cross-linker. We found that IO-DMSA were included in the core of the polymer spheres (Figure 2b). We then decided to explore the incorporation of iron oxide nanoparticles with alternative coatings.

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demonstrated the theranostic potential of the nanoparticles.

**Surface Adhesion Occurs during Stabilization with CaCl₂.** The surface loading of PCPP nanospheres occurs via self-assembly during synthesis. To investigate the crucial step in the formation of surface loaded nanospheres, we added IO-MHPC at different points in the process. Initially, we formed IO-NB by mixing IO-MHPC with a PCPP solution and then immediately adding spermine as schematically outlined in Figure 1b. The mixture was quickly transferred to a CaCl₂ solution and incubated at room temperature for 30 min. Afterward, the solution was washed through centrifugation and resuspended in water. To determine if longer incubations of IO-MHPC and PCPP solution together would result in internal loading of IO-MHPC, the reagents were incubated to- gether at room temperature for 30 min and 24 h before the addition of spermine. Results in Figure 3a–c show no visible differences between our standard synthesis, 30 min preincubation, or 24 h preincubation, indicating that longer incubation times did not affect spatial distribution of IO-MHPC in PCPP nanospheres (Figure 3c).

Next, we examined the effect of mixing PCPP with spermine before the addition of IO-MHPC. As shown in Figure 3d, IO-MHPC surface adherence was unaffected when added after spermine addition, demonstrating that surface loading does not occur during the PCPP and spermine cross-linking process. To further explore the mechanism of surface loading, nonloaded spheres were completely formed before the addition of IO-MHPC. The PCPP solution and spermine were first mixed and then transferred to CaCl₂ for 30 min at room temperature. Afterward, IO-MHPC was added into the solution and stirred for an additional 30 min. The results seen in Figure 3e showed no surface loading of IO-MHPC in the sample. These data demonstrate that surface loading of MHPC lipid coated IONP (IO-MHPC) is achievable at any stage before CaCl₂ stabilization. We also found fully synthesized nonloaded nanospheres (nanospheres formed in the absence IO-MHPC) could be surface loaded by incubating the nonloaded spheres with IO-MHPC and then transferring to 0.88% CaCl₂ solution for 30 min Figure 3f). These data suggest a calcium-dependent mechanism for surface loading. Presumably, there is some form of calcium-mediated binding between the PCPP and phospholipid head group (Ca\(^{2+}\) binding to phospholipids is well-known). Other divalent cations could likely produce similar phenomenon as Ca\(^{2+}\).

With our standard synthesis method, we studied the IO-NB stability over time when stored in Milli-q water at room temperature. After 24 h, the IO-NB were found to loosely settle but could easily be redispersed with gently mixing. The surface loading and structure of these nanospheres was unaffected over the observed time frame of 7 months as determined by TEM (Figure S2, Supporting Information). These data indicate that the particles are robustly stable when stored at room temperature for extended periods of time.

**Surface Loading Is Independent of Polymer Size, Lipid Length, Core Size, and Core Type.** Exclusive surface loading was found with the use of MHPC lipids encapsulating 15.6 nm diameter iron nanoparticles. We examined the flexibility of the reaction by varying parameters such as PCPP molecular weight, phospholipid length, and nanocrystal core size and type. Surface loaded nanospheres were synthesized using our standard method with PCPP in a range of molecular weights (35.6 kDa, 1.0 MDa, 3.8 MDa MW). Surface loading of IO-MHPC was found with each PCPP MW, demonstrating that the process is independent of polymer size (Figure S3, Supporting Information). The effect of phospholipid tail length was studied by encapsulating 15.6 nm diameter IONP with 12 (LHPC), 14 (MHPC), 16 (PHPC), and 18 (SHPC) carbon tail chain length phospholipids. PCPP nanospheres were successfully synthesized and surface loaded with each micelle formation (Figure S4, Supporting Information, note that LHPC is a relatively poor amphiphile and therefore formed aggregates as opposed to individually dispersed nanocrystals). Additionally, oleate-coated IONP...
of increasing sizes (10, 15, 20, 25, 30 nm diameters) were encapsulated in micelles using MHPC/DMPC mixtures (IO-MHPC-DMPC). Micelles synthesized using only MHPC to encapsulate the larger 25 and 30 nm IONP were not stable; however, we found that they could be stably coated with a 1:1 mixture of MHPC/DMPC and that nanoparticles with such coatings adhered to PCPP nanospheres also. The PCPP nanospheres were synthesized using our standard method with the various IONP core sizes. TEM revealed surface loading occurred for each core size (Figure S5, Supporting Information).

To further explore the breadth of applicability of this surface loaded process, a variety of diagnostically active nanocrystals were investigated for adherence to PCPP nanospheres. Oleic acid coated quantum dots (CdS spheres) and nanophosphors (β-NaGdF₄ spheres, rods, LaF₃ discs) were all found to incorporate into MHPC micelles using the same procedure as for IONP. Surface loading of each core variant was achieved through our standard method of PCPP nanosphere synthesis. TEM verified surface adsorption to be present in each sample (Figure 4). From these data, it seems that nanocrystals encapsulated into phosphocholine headgroup lipid micelles can be successfully surface adsorbed onto PCPP nanospheres regardless of the core type, size, or shape.

**Excess Empty Micelles Disrupt Nanosphere Formation but not Surface Adherence.** We examined whether an excess of empty micelles would reduce surface adsorption of IO-MHPC on PCPP nanospheres. Empty MHPC micelles were mixed together with IO-MHPC at increasing ratios (1:1, 5:1, 10:1, 25:1, based on phospholipid content). PCPP nanosphere synthesis was performed using our standard method with these empty micelles:IO-MHPC ratios. From TEM, lower ratio mixtures did not appear to affect surface adsorption of IO-MHPC onto the PCPP nanospheres (Figure 5). However, at larger ratios of 10:1 and 25:1, disruption of nanosphere formation was observed. These particles appeared amorphous but the surface adsorption of the IO-MHPC was still present. The increased concentration of empty micelles appeared to interfere with the spermine-PCPP cross-linking. We hypothesized that performing the cross-linking before the addition of IO-MHPC and empty micelles would allow for the observation of competition without disrupting the PCPP nanosphere formation. We found that by adding the spermine before the IO-MHPC, we were able to surface load IO-MHPC in the presence of high concentrations of empty micelles. Ratios of 10:1, 15:1, 20:1, 25:1 were used in the synthesis, and surface loading of IO-MHPC appeared similar in all samples (Figure S6, Supporting Information). This phenomenon could be due to the larger area of interaction for the IO-MHPC with the PCPP nanospheres creating stronger attachments, therefore preferentially binding IO-MHPC as compared to empty MHPC micelles.

**Particles Remain Diagnostically Active after Surface Loading.** With the ability to load a variety of diagnostically active nanoparticles, we evaluated the contrast generating properties of nanocrystal loaded polymer particles. Magnetic moment curves measured for IO-MHPC and IO-NB did not indicate magnetic hysteresis (Figure 7, Supporting Information). IO-MHPC and IO-NB had almost identical magnetization saturation values of 67.81 and 65.01 emu/g, respectively. The longitudinal and transverse relaxivity (measures of MRI contrast) of surface loaded IO-NB were examined (Table 2) at 1.41 T and 37 °C. Surface loaded particles, IO-NB, were found to have a lower transverse ($r_2$) relaxivity than free
IO-MHPC, although still at a substantial value of 68.2 mM/C0/1 s/C0/1. The decreased relaxivity could be caused by some loss of interaction between the surrounding water due to the absorption of IO-MHPC to the PCPP nanospheres.37 However, the ratio of transverse to longitudinal relaxivity (r2/r1) was higher for IO-NB indicating good properties for T2-weighted imaging. Additionally, for further evaluation, contrast generation for IO-NB was evaluated with a MRI scan. Iron oxide increases the rate of transverse relaxation therefore leading to a decrease in signal intensity, or darkening in T2/T2*-weighted MR images. An MR image of a phantom containing IO-NB (0.05 and 0.16 mM Fe), DPBS, free IO-MHPC (0.16 mM Fe), and nonloaded PCPP spheres are displayed in Figure 6a. The DPBS and nonloaded PCPP nanospheres were of relatively similar signal intensity. As expected, the IO-NB were much darker, with a concentration-dependent intensity. Although the IO-NB and IO-MHPC samples were at the same concentration, the IO-NB produced less signal. This effect could be due to the increased relaxivity (r2/r1) ratio seen in the relaxation measurements or perhaps is due to the different field strengths used for imaging and to determine relaxivities. Overall, IO-NB retained relaxation properties that allow for contrast generation in MRI. Moreover, PCPP nanospheres loaded with quantum dots (QD-NB) exhibit similar fluorescent properties as free quantum dots (QD-MHPC). The fluorescence of the quantum dots persisted after encapsulation in micelles and PCPP surface loading (Figure 6b). The phantom image in Figure 6c indicates the strong CT contrast produced by gold loaded PCPP particles.

**TABLE 2. Relaxation Measurements for Free IO-MHPC and IO-NB at 1.41 T**

|        | r1 (mM⁻¹ s⁻¹) | r2 (mM⁻¹ s⁻¹) | r2/r1 |
|--------|---------------|---------------|-------|
| IO-MHPC| 3.95          | 107.66        | 27.3  |
| IO-NB  | 1.81          | 68.22         | 37.6  |

**In Vitro Evaluation of Surface Loaded Nanospheres.** The robustness of surface attachment of IO-MHPC to PCPP nanospheres was briefly evaluated for potential biological applications. We incubated nanodisco balls for 1, 4, and 24 h in cell culture media at 37 °C. IO-NB were then collected through centrifugation, and iron oxide content was measured through ICP-OES (Figure 7a). The loss of iron content in the samples appeared similar at each time point, suggesting that after some initial IO-MHPC release the remainder was retained over the time frame tested. TEM performed on these nanoparticles revealed that many IO-MHPC were still attached to the surface of PCPP nanospheres after 4 h incubation (Figure 7b). Next, we incubated IO-NB with RAW 264.7 monocytes for 4 h. Remarkably, TEM performed on these cells revealed IO-MHPC organized in ring shapes located within endosomes (Figure 7c). We believe this demonstrates that the IO-MHPC still remain surface bound to PCPP nanospheres after undergoing cellular uptake.

**Delivery of Protein Loaded IO-NB.** With exclusive surface loading of polymer nanospheres, the internal core of the nanospheres can be used for drug loading. We used bovine serum albumin conjugated with fluorescein isothiocyanate (FITC-BSA, 0.25 mg) as a model drug. This was loaded into PCPP nanospheres (FITC-PCPP). Additionally, FITC-BSA was loaded into the core of nanospheres with simultaneous surface loading of IO-MHPC (FITC-NB, 0.1, 0.25 mg). TEM and fluorescence imaging were used to characterize FITC-PCPP and FITC-NB. Both particles demonstrated successful loading of FITC-BSA into the PCPP nanosphere as evident from the fluorescence of the purified material. TEM of FITC-NB revealed that IO-MHPC were surface loaded in the presence of the additional FITC-BSA payload, without alteration of the NB structure (Figure S8, Supporting Information).

To evaluate the ability of these particles to deliver FITC-BSA to cells, they were incubated with monocytes for 4 h. Afterward the cells were stained with DAPI and imaged with fluorescence microscopy. Phase images of treated cells reveal areas of particle uptake within the cells (Figure 8). Additionally, FITC can be seen within the cells as defined by DAPI (nuclei stain). The added surface loaded IO-MHPC did not prevent the
delivery of FITC-BSA to the cells. These data demonstrate that “drug” (FITC-BSA) loading can be achieved with additional exclusive surface loading. Furthermore, the loaded nanospheres remained stable for \textit{in vitro} tracking of delivery into cells.

**CONCLUSIONS**

As we have described above, PCPP nanospheres can be loaded with nanocrystals in a controlled fashion to result in exclusive localization of the nanocrystals to the surface of the sphere rather than the typically
observed core loading of polymer nanoparticles. We found that simultaneous surface and core loading is possible. A range of diagnostically active nanocrystals could be surface loaded onto the PCPP nanospheres regardless of their individual core size, shape, or chemical composition. The nanocrystals loaded on the spheres retained their contrast generating properties, i.e., MR contrast for iron oxide particles and fluorescence for quantum dots. The point of nanocrystal attachment was also probed through the investigation of each synthesis step. We observed that surface loading was successful prior to CaCl₂ addition. In addition, we found that surface loading was achievable using a variation of polymer sizes and phospholipid tail lengths, demonstrating multiple feasible approaches to surface loading. We showed the stability of the nanodisco balls in cell culture media and the potential for drug delivery applications. In summary, we have presented a robust and unique platform for surface localization of nanoparticles onto a polymeric sphere to form “nanodisco balls”. Selective loading into the core and surface presents new opportunities in the areas of drug delivery and theranostics.

### MATERIALS AND METHODS

**Materials.** Poly(bis(4-carboxyphenyloxy)phosphazene) disodium salt (PCPP, 1 MDa) was purchased from Sigma-Aldrich (St. Louis, MO). PCPP polymers of 3.8 MDa and 36.4 kDa molecular weight were synthesized at the Pennsylvania State University. All phospholipids including 1-myristoyl-2-hydroxy-sn-glycero-3-phosphocholine (MHPG) and chain length variants were purchased from Avanti Polar Lipids (Alabaster, AL). Oleic acid capped cadmium sulfide quantum dots were purchased from Oleic acid capped iron oxide nanoparticles (IONP) of various sizes (10, 15, 20, 25, 30 nm) were purchased from Ocean NanoTech (Springdale, AR), and some IONP were synthesized at the University of Pennsylvania (vide infra). All other chemicals of analytical grade were purchased through Sigma-Aldrich (St. Louis, MO) with the exceptions of sodium fluoride (Acros Organics, NJ), trifluoroacetic acid (Alfa Aesar, MA), Tetrahydrofuran (EMD, PA), dichloromethane (EMD, PA), and ethyl ether (EMD, PA). Gadolinium or lanthanum trifluoroacetate precursors were prepared using a literature method by refluxing gadolinium or lanthanum oxide in trifluoroacetic acid/water mixture (50 vol %). A monocyte cell line, RAW 264.7, was purchased from ATCC. Cells were cultured in Dulbecco’s Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (10,000 units/mL, 10,000 µg/mL) from Life Technologies Invitrogen (Grand Island, NY).

**Polyphosphazene Synthesis.** For 35.6 kDa PCPP preparation, PCl₃ (0.11 g) was dissolved in 50 mL of anhydrous dichloromethane (DCM) for 10 min. Chlorophosphoranimine (6.00 g), less viscous living oligo(dichlorophosphazene). The polymer DCM was then removed under reduced pressure to give color-rapidly, and the mixture was stirred at room temperature for 4 h. The mixture was stirred at room temperature for 3 days. Afterward, the solution was concentrated and precipitated into water (300 mL) and precipitated into water (300 mL). Potassium tert-butoxide (7.51 g) and water (1.34 g) were added to the polymer solution. The reaction was stirred at room temperature for 3 days and was then concentrated. This was dialyzed versus water for 2 days, (1:1) methanol/water for 2 days, and then (4:1) methanol/water for 1 day. The solution was then removed under reduced pressure to yield a white solid that was obtained in an 80% yield (molecular weight: 3882000 g/mol; PDI: 1.62; repeat units: 9600). For the deprotection reaction, the polymer (3.00 g) was dissolved in anhydrous THF (10 mL). Potassium tert-butoxide (7.51 g) and water (1.34 g) were added to the polymer solution. The reaction was stirred at room temperature for 3 days and was then concentrated. This was dialyzed versus water for 2 days, (1:1) methanol/water for 2 days, and then (4:1) methanol/water for 1 day. The solution was then removed under reduced pressure to yield a white solid that was obtained in an 80% yield (molecular weight: 3882000 g/mol; PDI: 1.62; repeat units: 9600).

**Iron Oxide Synthesis.** Iron oxide nanoparticles (IONP) were synthesized using oleic acid as the capping ligand following a modified literature method. Typically, 1.5 g of iron chloride and 5.2 g of sodium oleate were first added in a 100 mL flask. Subsequently, 20 mL of hexane, 11.5 mL of ethanol, and 8.8 mL of distilled water were added to the flask, and the mixture was sonicated. The two-phase mixture was heated to reflux (~70°C) for 4 h, which produced iron oleate in the organic layer. The upper organic layer was washed three times with 30 mL of water and separated by centrifugation (5000 rpm, 10 min). After washing, the hexane was evaporated from the dark brown organic layer and stored under vacuum. The synthesis of 15.6 nm iron oxide nanoparticles was carried out by reacting 5.5 g of iron oleate and 1.5 g of oleic acid in 31 g of 1-octadecene in a 250 mL round-bottom flask. The reaction mixture was heated to 320°C at a rate of 200°C/h and kept at that temperature for 30 min. The color of the solution turned from dark brown to black upon the formation of nanoparticles. The resulting solution was cooled to room temperature, and nanoparticles were precipitated by adding ethanol (35 mL). The precipitated nanoparticles were collected by centrifugation (5000 rpm, 10 min) and then redispersed in hexane (10 mL). The nanoparticles were further purified by precipitation with acetone (35 mL), centrifuging at 5000 rpm for 10 min, and redispersing the collected nanoparticles in hexane (10 mL). This washing step was repeated two more times. After the final washing step, the IONP were redissolved in chloroform (10 mL) and centrifuged at low speed (3000 rpm, 5 min) to remove aggregates. A sample of these IONP was rendered water-soluble through encapsulation with dimercaptosuccinic acid (Figure S9a, Supporting Information) following previous literature methods (IOMDSA). A high molecular weight (MW) PCPP (3.8 MDa) was synthesized by first dissolving poly(dichlorophosphazene) (2.00 g) in 200 mL of THF. Poly(dichlorophosphazene) was prepared by the thermal ring-opening polymerization of precursors and sublimed hexachlorocyclopentaphosphazene (Fushimi Chemical Co., Japan) in evacuated Pyrex tubes at 250°C. Propyl-4-hydroxybenzoate (9.33 g) was dissolved in THF (100 mL) and then added to the polymer solution. Solid cesium carbonate (16.9 g) was then immediately added to the reaction mixture. The reaction proceeded at room temperature for 3 days. Afterward, the solution was concentrated and precipitated into water 3 times and hexane once. The solvent was removed under reduced pressure to yield a white solid that was obtained in an 80% yield (molecular weight: 3882000 g/mol; PDI: 1.62; repeat units: 9600). For the depolymerization reaction, the polymer (3.00 g) was dissolved in anhydrous THF (300 mL). Potassium tert-butoxide (7.51 g) and water (1.34 g) were added to the polymer solution. The reaction was stirred at room temperature for 3 days and was then concentrated. This was dialyzed versus water for 2 days, (1:1) methanol/water for 2 days, and then (4:1) methanol/water for 1 day. The solution was then removed under reduced pressure to yield the product with an 81% yield. Poly(bis(4-carboxyphenoxy)phosphazene) disodium salt (PCPP, 1 MDa) was purchased from Sigma-Aldrich (St. Louis, MO). Poly(bis(4-carboxyphenoxy)phosphazene) dipotassium was obtained by dialysis against water/methanol (1:1) for 2 days, and then methanol for 2 days (Spectra/Por dialysis membrane, MWCO: 1000). Poly(bis(4-carboxyphenoxy)phosphazene) dipotassium was obtained by the removal of all solvent under vacuum at 35°C (overall yield: 46%). 31P NMR (D2O): δ = -18.75 (s). 1H NMR (D2O): δ 7.31 (d, 2H), 6.50 (d, 2H).
Gold Nanoparticle Synthesis. Carboxylate-acid coated gold nanoparticles (AuNP) were synthesized using the Turkevich method with slight modifications. Briefly, 85 mg of chloroauric acid (HAuCl₄) was dissolved in 250 mL of Milli-Q water for a final concentration of 0.05 M. After 38.8 mM solution of sodium citrate (285 mg in 25 mL Milli-Q water) was added, causing the color of the solution to become dark red. The solution was heated for another 15 min and then allowed to cool to room temperature. For carboxylic acid ligand exchange, an 11.9 mM solution of 11-mercaptoundecanoic acid (11-MUA, 2.6 mg in 1 mL of EtOH) was added to the citrate AuNP as prepared above. The solution was stirred for 24 h. Afterward, the AuNP were purified and collected by centrifuging at 12.5 kcf for 45 min. The pellet was resuspended with Milli-Q water and repeated twice. The AuNP solution was finally resuspended in 1 mL of Milli-Q water and filtered through a 0.45 μm syringe filter.

Polymer and Particle Characterization. IH and 31P NMR spectra were recorded on a Bruker WM-360 NMR spectrometer operated at 360 and 145 MHz, respectively. IH NMR spectra were referenced to solvent signals, while 31P NMR chemical shifts were relative to 85% phosphoric acid as an external reference, with positive shift values downfield from the reference. Molecular weights were estimated using a Hewlett-Packard HP 1090 gel permeation chromatograph (GPC) equipped with an HP-1047A refractive index detector, American Polymer Standards AM gel 10 mm and AM gel 10 mm 104 Å columns, and calibrated versus polystyrene standards.

Transmission electron microscopy images were acquired on a FEI Tecnai T12 microscope at 120 kV. Scanning electron microscopy was performed with a Philips XL20 at 10 kV. Fluorescent imaging for both the quantum dots and the FITC-BSA was performed with an IVIS Spectrum system using 465 nm excitation and 520 nm emission filters. Relativities were measured using a Bruker Minispec mq relaxometer at 1.41 T (60 MHz) and 40 °C. Freely suspended IO-MHPC were prepared in DPBS for measurements. PCPP samples were prepared in a 1% agar gel in DPBS to prevent sedimentation of nanoparticles. Iron oxide concentrations were determined through inductively coupled plasma-optical emission spectroscopy (ICP-OES) on a Spectra Genesis system.

Magnetic hysteresis measurements were performed on a MicroMag Magnetometer (Westerville, OH) at a field range of 10 kOe. Samples (5 μL) were prepared on a coverglass for measurements. Magnetic resonance imaging (MRI) was used to evaluate IO-NB contrast generating properties. IO-NB were prepared in a 1% agar gel at concentrations of 0.05 mM and 0.16 mM of Fe. Additionally, control samples of DPBS, non-loaded PCPP, and 0.16 mM of Fe IO-MHPC in 1% agar gel were scanned. For MRI phantom preparation, samples were placed in a 2% agar gel doped with 0.35 mM manganese chloride. The samples were scanned using a head coil on a Siemens Magnetom Trio with a 3 T magnet. A 2D spin echo sequence was used. Relevant imaging parameters were as follows: echo time (TE), 15 ms; repetition time (TR), 10 s; 1 slice with thickness 3 mm, 1 average, flip angle (FA), 90 deg; acquisition matrix, 184 × 256; in-plane spatial resolution 0.546 mm², field of view (FOV) 140 mm². The resulting images were processed using OsiriX v.3.0.1 32-bit (Geneva, Switzerland; www.osirix-viewer.com). CT images were acquired with a Siemens Definition DS 64-slice clinical CT scanner at 140 kV (245 mA) with a matrix size of 512 × 512, field of view 37 × 37 cm, reconstruction kernel B30f, and slice thickness of 0.6 cm. Images were analyzed using OsiriX 64 bit (v3.7.1).

In Vitro Incubations. RAW 264.7 cells were cultured on 6-well plates at a concentration of 2 million cells/mL at 37 °C. After 24 h, the cells were treated with IO-NB at a concentration of 50 μg of Fe/mL (0.89 mM) for 24 h. The cells were washed 3 times with DPBS and then collected by gentle scraping. The cells were

Quantum dots and nanophosphors were encapsulated using the similar methods as β-NaGdF₄ spheres, rods, LaF₃ discs, or iron oxides of varying cores sizes and coatings were substituted for IO-MHPC for inclusion in the PCPP nanosphere synthesis. PCPP of additional molecular weights (35.6 kDa and 3.88 MDa) were also used to form surface loaded nanoparticles. Additionally, FITC-BSA (0.1, 0.25 mg) was core loaded into PCPP nanoparticles using the standard synthesis method with and without the simultaneous surface loading of IO-MHPC (0.15 mg) to form FITC-PCPP and FITC-NB, respectively.
fixed in a solution of 2% glutaraldehyde and 5% paraformaldehyde in DPBS. Fixed cells were embedded in resin and prepared for TEM using standard methods.11 Cell sections were imaged using a FEI Tecnai T12 electron microscope.

For fluorescent microscopy, RAW 264.7 cells were cultured on glass-bottomed well dishes (20 mm diameter) at a concentration of 800K cells per well. After 24 h, the cells were treated with 50 μg Fe/ml (0.89 mM) of FITC-NB or FITC-PCPP for 4 h. The cells were then washed with DPBS 3 times and stained with DAPI. Imaging was performed on a Nikon Eclipse fluorescence microscope (Nikon Eclipse Ti-U, Melville, NY). The DAPI stain was imaged using a 358 nm excitation filter and a 461 nm emission filter. FITC was imaged using a 494 nm excitation filter and a 516 nm emission filter.

Conflict of Interest: The authors declare no competing financial interest.

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Supporting Information Available: TEM of gold nanocrystal loaded particles, the TEM results of IO-NB stability over time, synthesis for varied polymer molecular weight, phospholipid help with magnetic moment measurements, and Therapeutics. We thank A. Andrianov for helpful suggestions with regard to FCPP sphere synthesis and M. Chorny for his help with magnetic moment measurements.

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