Fine-Tuning the Superparamagnetic Properties of FeO@Fe₃O₄ Core/ Shell Nanoparticles and Superclusters by Controlling Size and Shape

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ABSTRACT: Fine-tuning the superparamagnetic (SPM) properties of iron oxide nanoparticles (NPs) through precise control over size, shape, and assembly into superclusters is essential for advanced biomedical and electronic applications. We first analyzed the sizedependent magnetic properties of FeO@Fe₃O₄ core/shell NPs in both spherical and cubic shapes prepared via the thermal decomposition of iron(III) oleate. The detailed analyses of structure, composition, and crystallinity confirmed the presence of both FeO and Fe₃O₄ phases and the formation of the core/shell structure, with an increasing FeO/Fe₃O₄ phase ratio correlated with larger particle size. Overall, the SPM properties of these core/shell NPs were maintained, although saturation magnetization and varied with size, shape, and FeO/ Fe₃O₄ ratio. Notably, iron oxide nanocubes exhibited enhanced saturation magnetization compared to their spherical counterparts. Next, we introduced a unique strategy to enhance



and fine-tune the SPM properties of $FeO@Fe_3O_4$ NPs by assembling them into supercluster particles to promote interparticle interaction. By controlling the size and shape of the primary nanocrystals, we demonstrated the creation of SPM superclusters of consistent sizes, including the 150 and 240 nm superclusters reported here, which exhibit different SPM behaviors. Our research presents a synthetic strategy for optimizing the SPM properties of iron oxide NPs and their superclusters across a wide range of magnetically driven applications, especially useful for biomedical technologies.

KEYWORDS: superparamagnetic properties, superclusters, superparticles, FeO@Fe₃O₄ core/shell, biomedicine, biosensing

INTRODUCTION

Magnetic iron oxide nanoparticles (IONPs) possessing superparamagnetic (SPM) properties and exceptional biocompatibility offer enormous potential in advanced biomedical applications, including magnetic resonance imaging (MRI), drug delivery, magnetic hyperthermia, and biosensing.^{1,2} The utility of SPM properties lies in their ability to be manipulated and the capability to switch on and off under a magnetic field. In contrast to ferrimagnetic (FiM) or ferromagnetic (FM) properties, SPM properties excel in minimizing interparticle interactions, thereby reducing particle clustering and aggregation. This feature enhances the dispersibility of nanoparticles (NPs) in colloidal solutions and facilitates their redispersion after magnetic separation. Consequently, tailoring the magnetic properties of IONPs by controlling their structure emerges as a crucial strategy for understanding nanomagnetism and optimizing NPs for specific biomedical applications.^{3,4} Practical approaches to tune the SPM properties of IONPs include controlling particle size, tailoring particle shape, and forming superclusters or superparticle structures.

Typically, IONPs with SPM properties are single-domain particles with sizes smaller than 25 nm.^{1,5} Although the transition size from SPM to FM behavior is generally considered to be around 25 nm, this size can vary depending

on the shapes (spheres, cubes, rods) and compositions of the NPs (Fe₃O₄, FeO, γ -Fe₂O₃, α -Fe₂O₃). Additionally, SPM properties can be observed in larger IONPs, above 100 nm, if these particles are secondary structures formed through the stacking of single-domain-size crystals in polycrystalline particles⁶⁻⁸ or through the assembly of SPM IONPs.⁹⁻¹¹ Indeed, large-size SPM IONPs (size >100 nm) have been created from polycrystalline NPs with small crystallite sizes,^{6,12} and from superclusters (also known as "superparticles") of single-domain IONP assemblies,9,11,13 or from clusters of single-domain SPM IONPs encapsulated in a polymer matrix or an inorganic shell.¹⁴ Among these three types of large-size SPM IONPs, supercluster particles (assemblies of singledomain IONPs) offer flexibility in manipulating particle structures compared to polycrystalline particles, while minimizing the nonmagnetic component, unlike the encapsulated

Received:March 6, 2025Revised:April 16, 2025Accepted:April 18, 2025Published:May 5, 2025







Figure 1. TEM images of (a-e) iron oxide nanospheres and (f-i) iron oxide nanocubes with tunable sizes. Size of nanoparticles (diameter for the nanospheres and edge length for the nanocubes) in nm.

particles. Consequently, tuning the size, shape, and composition of primary crystals is key to forming unique superparticle structures with desirable properties for advanced biomedical applications.

While maintaining the benefits of SPM properties, large-size SPM IONPs offer advantages such as stronger saturation magnetization through the collective close interactions of component SPM nanocrystals, facilitating efficient magnetic separation.^{10,15} This advantage holds immense potential for biomedical applications, positioning large-size SPM IONPs as a viable alternative to smaller SPM IONPs with sizes less than 30 nm. Despite the extensive use of SPM IONPs in biomedicine, concerns about toxicity persist due to their small size, which may lead to deep penetration and retention in sensitive areas of the body.^{16–18} Therefore, utilizing large SPM IONPs with improved magnetic properties, such as enhanced saturation magnetization (M_S) , presents an alternative approach with significant potential in biomedicine, deserving of more attention and further exploration. Dynabead M280 SPM particles, with a size of 2.8 μ m (clusters of SPM IONPs dispersed in a polymer matrix), are a prominent example of such large SPM particles useful in biodetection and biopurification.^{19,20} Recent studies have demonstrated the high efficiency of large SPM IONPs as magnetic agents in biosensing and MRI.^{13,21,22} Interestingly, the Pellegrino group reported the improved hyperthermia properties of clusters comprising small iron oxide nanocubes, suggesting the use of large SPM IONPs as an efficient approach to hyperthermiabased cancer treatment.²³ Our recent study has also revealed the large magnetic hyperthermia responses of large polycrystalline IONPs, with sizes ranging from 160 to 400 nm at a small dose of 0.5 mg/mL.²⁴

To date, two methods have been developed for producing SPM IONPs with tunable sizes and shapes: the thermal decomposition of iron(III) acetylacetonate in benzyl ether, and the thermal decomposition of iron(III) oleate in nonpolar solvents (hexadecane, octadecene, tetradecane, docosane).^{25–28} The former method offers advantages in producing a highly pure Fe₃O₄ phase but faces challenges due to the instability of benzyl ether at high temperatures, which negatively impacts reproducibility and scalability.^{27,29} On the other hand, the thermal decomposition of iron(III) oleate tends to form the antiferromagnetic (AFM) FeO phase within the core of Fe₃O₄ particles; however, its higher reproducibility makes it more suitable for larger-scale synthesis of particles.³⁰⁻³² Due to its AFM nature, the presence of the FeO phase usually reduces the overall $M_{\rm S}$ of the system; however, magnetic coupling with the FiM Fe₃O₄ phase can enhance magnetic anisotropy, rendering it useful for specific biomedical applications. The exchange coupling between the AFM FeO core and the FiM Fe₃O₄ shell has been shown to improve magnetic anisotropy, leading to enhanced magnetic hyperthermia responses in FeO@Fe₃O₄ core/shell NPs.^{33,34} However, to the best of our knowledge, the effect of varying FeO/Fe₃O₄ ratio on the magnetic and hyperthermia properties of the FeO@Fe₃O₄ core/shell system and their superclusters has not been systematically studied for both spherical and cubic shapes. From a different perspective, the exchangecoupled FeO@Fe₃O₄ core/shell systems have also been reported to exhibit an exchange-bias (EB) phenomenon, making them attractive for applications in spintronics and magnetic recording.^{35,36} These observations have led us to propose employing the thermal decomposition of iron(III) oleates as a method to produce core/shell IONPs with tunable



Figure 2. XRD patterns of (a) iron oxide nanospheres and (b) iron oxide nanocubes. Size of nanoparticles in nm.

sizes and shapes, followed by the formation of supercluster structures from these NPs. A clear understanding of the structure–property (magnetism) relationship in these nanosystems will help in tuning the SPM properties for diverse applications.

In the work reported here, we synthesized iron oxide nanospheres and iron oxide nanocubes through the thermal decomposition of iron(III) oleate, enabling tunable sizes for a comprehensive study of magnetic properties. The composition of these NP systems included $FeO@Fe_3O_4$ core/shell structures, with larger particles tending to exhibit a more pronounced FeO phase. Two critical parameters— M_s and $(T_{\rm B})$ —for different sizes of nanospheres and nanocubes were investigated for comparison. Experimental data revealed stronger $M_{\rm S}$ in the iron oxide nanocubes and provided guidance on selecting IONPs with desirable SPM properties. To further fine-tune the SPM properties, superclusters were fabricated using these selected IONPs (both iron oxide nanospheres and nanocubes) of various sizes and shapes. A systematic analysis of the magnetic properties of the resulting supercluster particles, relative to their primary IONPs, shows a significant improvement in $M_{\rm S}$ and a shift in the $T_{\rm B}$ toward higher temperatures. We attributed these enhanced magnetic properties to enhanced magnetic interactions among the primary IONPs that formed the superclusters, desirable for advanced biomedical applications. Interestingly, the magnetic properties of superclusters of similar sizes can be tuned by varying the size, shape, and FeO/Fe₃O₄ ratio of their constituent primary crystals. This finding underscores the great potential for fine-tuning the SPM properties of core/shell IONPs and their supercluster structures.

RESULTS AND DISCUSSION

Structural and Magnetic Characterization of Iron Oxide Nanoparticles. The structure and magnetic properties of IONPs with cubic and spherical shapes were studied by characterizing the NPs using transmission electron microscopy (TEM), X-ray diffractometry (XRD), superconducting quantum interference device (SQUID) measurements, and physical property measurement system (PPMS) measurements. In the context of this paper, the size of particles is defined as the diameter for spherical shapes and edge length for cubic shapes. Figure 1 presents TEM images of iron oxide nanospheres (IONSs) with average sizes ranging from 10 to 28 nm (Figure 1(a-e)) and iron oxide nanocubes (IONCs) from 10 to 18 nm (Figure 1(f-i)). All NP samples, both spherical and cubic, exhibited uniform sizes with narrow size distributions, where the standard deviation was controlled to be around 10% of the average size. The morphology of both the spherical and cubic NPs was uniformly controlled. Therefore, it is reasonable to assert that the synthesized NPs, whether spherical and cubic, are of identical quality with narrow size distributions and uniform morphologies. This uniformity enables subsequent studies focusing on the effects of size and shape on the magnetic properties of IONPs.

Figure 2 presents the XRD patterns of IONSs (Figure 2a) and IONCs (Figure 2b). Two major phases of iron oxide, FeO and Fe₃O₄, were observed, consistent with results from previous studies using a similar synthesis method.^{37,38} The formation of the FeO phase at the core of particles, followed by the Fe₃O₄ phase as the shell, was attributed to the fast growth rate of nanocrystals, which overwhelmed the oxidation rate of the FeO core.³⁹ To grow larger NPs, a fast growth rate is preferred, leading to a more pronounced formation of the FeO phase. Notably, the larger the size of the NPs, whether spherical and cubic, the more the FeO phase was identified. The largest IONSs, approximately 28 nm in size, prominently matched with the FeO phase, with the center position of peaks corresponding to FeO crystal planes (111), (200), (220), and two minor peaks at (311) and (222) being observable. Three regions in the XRD patterns were crucial for analysis: 2θ from 33 to 38°, 2θ from 41 to 44°, and 2θ from 59 to 64°.⁴⁰ In the first region, the diffraction peak of (111) in the FeO phase at $2\theta = 36.2^{\circ}$ was flanked by the diffraction peaks of (311) at 35.5° and (222) at 37.2° from Fe₃O₄. The maxima of the diffraction peaks were centered at 35.5° of (311) in the magnetite (Fe₃O₄) phase for IONSs size 9.8 nm and shifted toward $2\theta = 36.2^{\circ}$ (FeO (111) planes) for larger particles. For sample sizes of 21.8 and 28.3 nm for IONSs and 18 nm for IONCs, the maxima in this region were centered at 36.2° for FeO (111). In the region from 41 to 44° , the maxima of the peaks were centered at 43.2° for the (400) planes in Fe₃O₄ and shifted toward the lower diffraction angle at 42.0° for (200) in FeO. In the region from 61 to 63° , similar trends were observed, where the peak maxima for smaller particles (size 9.8 nm for IONSs and 11.8 nm for IONCs) were positioned at 62.7° and shifted toward the lower diffraction angle of 60.9° for (220) planes in FeO.

From the analysis of the XRD results, we observed an increase in the size of IONPs with a higher ratio of the FeO phase, which detrimentally impacts the $M_{\rm S}$ of the NPs. The (200) peak of FeO is the most prominent, with the highest

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Figure 3. High-resolution TEM and FFT of (a) nanospheres size 28.3 nm and (b) nanocubes size 18 nm.



Figure 4. (a) High-resolution Fe 2p XPS spectra and (b) Raman spectra of iron oxide nanospheres (NSs) and nanocubes (NCs).

peak intensity observed in the largest nanospheres (size 28.3 nm) and nanocubes (size 18 nm). By deconvoluting the (200) peak of FeO at around 42.0° and the (400) peak of Fe₃O₄ at 43.2°, the crystallite size of FeO could be estimated, 40,41 as shown in Table S1. Due to the insignificant amount of the FeO phase in smaller NPs, the crystallite size of the FeO phase in samples sized 9.8 nm (nanospheres), 10.2 nm (nanocubes), and 11.8 nm (nanocubes) could not be determined from XRD deconvolution. The peak positions of these samples mostly coincide with the (400) of the Fe₃O₄ phase; therefore, the crystallite size of Fe₃O₄ along (400) was calculated. The calculated Fe₃O₄ crystallite size closely matches the size of the NPs. Combining the features of XRD patterns with the shift of peak maxima at three regions (2 θ from 33 to 38°, 2 θ from 41 to 44°, and 2 θ from 59 to 64°), as discussed above, and the estimated crystallite sizes specified in Table S1, it is apparent that larger FeO@Fe₃O₄ core/shell NPs could possess a larger volume fraction of the FeO phase in the core/shell structure. It is reasonable to assume that larger particles, spherical or cubic in shape, would have lower $M_{\rm S}$ due to the AFM nature of FeO. It is also reasonable to assume that the $T_{\rm B}$ will shift toward higher temperatures for larger single-phase Fe_3O_4 particles, since T_B is a size-dependent parameter.^{26,42,43} However, this trend might not occur in biphasic systems like our FeO@

 Fe_3O_4 core/shell NPs. Changes in T_B are not solely driven by changes in the size and shape of NPs, but also by the FeO/ Fe_3O_4 ratio in the entire nanoparticle structure.³¹

Two samples of the largest size, nanospheres (size 28.3 nm) and nanocubes (size 18.0 nm), were further characterized using atomic resolution TEM, and the FFT were simulated for crystallographic analysis. From Figure 3, core/shell structures were observed, with the FeO size closely matching the calculated crystallite size values in Table S1. FFT diffraction patterns confirmed the existence of FeO as the core, with visible (111) and (200) planes, and Fe_3O_4 as the shell, with visible (220), (311), (222), and (400) planes. As indicated in the XRD results, the FeO phase is more dominant in the larger NPs. Therefore, the smallest sample, nanospheres with an average size of 9.8 nm, was further characterized. Highresolution TEM and FFT of the nanospheres (size 9.8 nm), shown in Figure S1, revealed no core/shell structures, with the entire particle indexed to the Fe_3O_4 phase, showing observable (220) and (311) planes of Fe_3O_4 . The atomic-resolution TEM and FFT results are consistent with the analysis from the XRD results.

The XRD patterns can be used to index and identify the presence of different iron oxide phases, such as FeO, Fe₃O₄, or γ -Fe₂O₃ (maghemite), and α -Fe₂O₃ (hematite). However,

particle shape	particle size (nm)	$M_{\rm S}~({\rm emu/g})$	$T_{\rm B}$ (K)	estimated volume (nm ³)	equivalent size (nm)
nanospheres	9.8 ± 0.8	50.4	108	493	7.9 (cubes)
nanospheres	13.1 ± 1.0	46.3	215	1177	10.6 (cubes)
nanospheres	15.6 ± 1.3	51.7	231	1987	12.6 (cubes)
nanospheres	21.8 ± 2.0	39.5	237	5422	17.6 (cubes)
nanospheres	28.3 ± 2.5	33.5	272	11,861	22.8 (cubes)
nanocubes	10.2 ± 0.9	68.0	187	1061	12.7 (spheres)
nanocubes	11.8 ± 1.3	65.6	252	1643	14.6 (spheres)
nanocubes	15.9 ± 1.4	51.6	285	4020	19.7 (spheres)
nanocubes	18.0 ± 1.4	44.9	271	5832	22.3 (spheres)

Table 1. Magnetic Properties of Iron Oxide Nanospheres and Iron Oxide Nanocubes^a

^aSize of nanospheres is the diameter of the particle, the size of nanocubes is the edge length of the particle. Equivalent size for the nanospheres means the size of nanocubes (edge length) with similar volume. Equivalent size for the nanocubes means the size of nanospheres (diameter) with similar volume. $M_{\rm S}$ recorded at 4 T and $T_{\rm B}$ collected under the applied field of 100 Oe.

 Fe_3O_4 and γ -Fe₂O₃ are indistinguishable in XRD patterns due to their similar cubic crystal structures.^{1,36} Consequently, the shell composition could be Fe_3O_4 , γ - Fe_2O_3 , or a mixture of these two phases. XPS, with its surface sensitivity, was used to further confirm the composition of the shells. Figure 4a shows the high-resolution XPS spectra of the Fe 2p region, confirming the presence of the Fe₃O₄ phase with no evidence of any γ -Fe₂O₃ phase.⁴⁴ The absence of a satellite peak around 718 eV is commonly used as an indicator of the absence of γ -Fe₂O₃.^{45,46} Therefore, the surface-sensitive data from the XPS measurements support the conclusion that the nanoparticle shell consists of exclusively (or at least highly predominantly) the Fe_3O_4 phase. To further confirm the phase composition of NPs, analysis using Raman spectroscopy was conducted with more details on the phase composition in a bulk scale. For Raman spectroscopy, γ -Fe₂O₃ typically exhibits three major peaks centered around 365, 510, and 700 cm⁻¹. And Raman peaks for Fe_3O_4 appear at 310, 540, and 670 cm⁻¹. For the FeO phase, FeO crystallizes as a rock-salt structure in the $Fm\overline{3}m$ space group, which is predicted to be a weak Raman scatterer without proper Raman active modes.^{47,48} All distinct peaks of the Fe₃O₄ phase, especially two major peaks at around 670 and 540 cm⁻¹, were clearly observed in Figure 4b, indicating the highly pure Fe₃O₄ phase in NPs.^{47,49} For the smallest sample size of 10.2 nm nanocubes, a slight shoulder around 700 cm⁻¹ suggests the presence of a small amount of γ - Fe_2O_3 . While the coexistence of γ - Fe_2O_3 and Fe_3O_4 may be more pronounced in smaller particle sizes,²⁶ overheating due to laser exposure during Raman characterization could also result in the formation of a tiny amount of γ -Fe₂O₃ in the smallest sample (which has a higher surface-to-volume ratio).^{50,51} Overall, the pure Fe_3O_4 phase remains the dominant phase in the nanoparticle shell. While the predominant phase is Fe₃O₄, the possible coexistence of γ - Fe_2O_3 and Fe_3O_4 phases, particularly a small fraction of γ -Fe₂O₃, cannot be completely ruled out at small nanoparticle sizes.²⁶ Deconvolution of the major Raman peak at approximately 670 cm⁻¹ can be used to estimate the content of the γ -Fe₂O₃ phase in the overall composition of the NPs.⁵² Our deconvolution results indicate approximately 16.0, 4.7, 9.2, and 7.1% γ -Fe₂O₃ content for nanocubes of 10.2 nm size and 18.0 nm size, and nanospheres of 13.1 nm size and 28.3 nm size, respectively. These results can perhaps be attributed to partial transformation during Raman analysis under laser exposure, effects from the sample drying process, defects in the Fe_3O_4 shell, or the inherent coexistence of the two phases, as previously reported.²⁶

The detailed field dependence of magnetization (M(H)) and temperature dependence of magnetization (M(T)) data of IONPs are presented in Figures S2 and S3. Table 1 summarizes the magnetic parameters derived from these data for IONPs of different sizes and shapes. For comparison, an equivalent size was calculated as described in the following sentences. For nanospheres, the equivalent size represents the edge length of nanocubes with a similar volume. Similarly, the equivalent size for nanocubes represents the diameter of nanospheres with a similar volume. The shape of the M(H)curves presented in Figures S2a and S3a indicates the need for a high magnetic field to saturate the magnetic moment of NPs due to the presence of the AFM FeO core.48,53 Here, we determine the $T_{\rm B}$ as the temperature at which the maximum magnetization in the zero-field-cooled (ZFC) curve is observed, the experimental method that is arguably the most widely accepted to determine the average $T_{\rm B}$ of SPM NPs.^{26,28,53-55} The magnetic nanoparticle sample was cooled without an applied magnetic field to freeze the Brownian rotations, and a small field (tickling field, typically 10 to 100 Oe) was applied as the magnetization was recorded during warming.⁵⁴ As the temperature increases, thermal energy disturbs the system, allowing more magnetic moments to acquire sufficient energy to align with the applied magnetic field. Consequently, magnetization increases, reaching a maximum when the number of unblocked (aligned) moments peaks at the $T_{\rm B}$. For temperatures higher than the $T_{\rm B}$, the thermal energy becomes strong enough to randomize the magnetic moments, resulting in a reduction in magnetization. Some researchers, however, define the $T_{\rm B}$ as the bifurcation point of the ZFC and field-cooled (FC) M(T) curves or by calculating the T derivative of the difference between ZFC and FC curves (d(ZFC-FC)/dT).⁵⁶ While the former is not widely accepted and depends on sample stacking in the powder phase, both alternative methods remain the subjects of debate and controversy.

Several important features can be observed in the M(T) curves shown in Figures S2(b-f) and S3(b-e). First, the Verwey transition (T_V) , occurring around 125 K, is characteristic of a pure magnetite phase with good crystalline quality and is observable in the M(T) curves.⁵⁷ However, due to the overlap with other magnetic interactions, T_B effects, or dependence on the applied field during M(T) measurements, T_V typically manifests as a subtle kink at approximately 110–125 K (e.g., Figure S2c) or as a steep increase in magnetization in the ZFC curve around 100–150 K (e.g., Figure S3(c,d)). Second, the Néel temperature (T_N) of the FeO phase, known

as the transition from AFM to a paramagnetic spin configuration of wüstite core, is clearly identified as a steep increase in magnetization starting at approximately 190 K,^{28,38,40,58} as illustrated in Figures S2(c,d,e,f) and S3(d,e). The $T_{\rm N}$ feature in the ZFC curve is typically sharp and largely size-independent, a behavior frequently also observed in other AFM-FM exchange bias systems.⁵⁹ Samples with smaller sizes, which are estimated to have negligible FeO cores (e.g., spherical particles with a size of 9.8 nm and cubic NPs with sizes of 10.2 and 11.8 nm), do not exhibit this behavior in their ZFC curves. The M(T) curves further corroborate the structural and compositional analysis of the nanoparticles. The biphase AFM/FiM nature of FeO@Fe₃O₄ core/shell NPs has been further confirmed by the observed shapes of the M(H) curves measured at 10 K under both ZFC and FC (3T) measurement protocols, as shown in Figure S4(a,b). It is worth noticing that the FiM Fe₃O₄ shell dominates the low-field behavior of the M(H) loops, while the open-up of the loops arises from the AFM FeO core.

Two trends can be drawn from our magnetic data: first, the $T_{\rm B}$ increases with the size of the particles, as expected; second, the $M_{\rm S}$ appears to decrease in particles with larger sizes. Figure 5 presents the $M_{\rm S}$ versus size relation, showing a trend of $M_{\rm S}$



Figure 5. Saturation magnetization (M_s) versus particle size relation for core/shell IONPs: (a) nanospheres and (b) nanocubes.

decreasing for larger particles. This reduction might be due to the AFM enhancement of the FeO core at the expense of the FiM Fe₃O₄ shell, thus reducing the total magnetic moment in larger particles. In this data set, the 15.6 nm nanospheres slightly deviate from the expected trend of $M_{\rm S}$ versus particle size. While the spherical samples have nearly similar FeO core sizes—4.0 nm for the sample size 15.6 and 4.3 nm for the sample size 13.1 nm—the volume fraction of FeO is more dominant in the 13.1 nm sample, resulting in weaker $M_{\rm S}$. Although it was initially expected that the FeO core size in the 15.6 nm sample would be larger than 4.3 nm—ensuring a more systematic and perfectly trended data set—this minor deviation is realistic and falls within the acceptable margin of error for nanoparticle synthesis and characterization. The $M_{\rm S}$ values of IONCs appear to be higher than those of IONSs, probably due to the enhanced crystallinity in the former. The data in Table 1 provides good guidance on selecting NPs with desired SPM properties for biomedical applications and for forming their supercluster structures. The reduction in $M_{\rm S}$ due to an increasing volume of the FeO phase, as estimated in Table S1, illustrates the relationship between the $M_{\rm S}$ and particle size in these core/shell IONPs.

From Table 1, we can compare the properties of IONCs with IONSs of comparable volumes. Three pairs of samples were compared: (a) 13.1 ± 1.0 nm (nanospheres) versus 10.2 ± 0.9 nm (nanocubes), (b) 15.6 ± 1.3 nm (nanospheres) versus 11.8 ± 1.3 nm (nanocubes), and (c) 21.8 ± 2.0 nm (nanospheres) versus 18.0 ± 1.4 nm (nanocubes). The comparisons show that the $M_{\rm S}$ values of IONCs are higher than those of IONSs, largely due to the dominance of the FiM Fe₃O₄ phase compared to the AFM FeO phase in the former (see Table S1). Calculating the $M_{\rm S}$ (nanocubes)/ $M_{\rm S}$ (nanospheres) ratio for the three pairs of samples, the ratios are 1.47, 1.27, and 1.14, respectively. This analysis confirms the superior magnetic properties of core/shell IONCs compared to their IONS counterparts for biomedical applications.

SPM Properties of Superclusters. The superclusters were formed through the self-assembly of IONPs, using oil droplets in a microemulsion as templates to form the supercluster structures.¹⁵ Figure 6 presents SEM images of superclusters fabricated from primary crystals of various sizes and shapes. The size distribution histogram is presented in Figure S5. The superclusters have rounded shapes with a broad size distribution, which can be attributed to the diverse size distribution of droplets formed in the emulsion. Our synthesis results showed that forming supercluster particles requires a specific concentration of NP colloidal solution in chloroform with good dispersibility. The larger the size of the primary crystals, the higher the concentration of colloidal solution needed to form the superclusters, see in Methods section for more details. Therefore, fabricating superclusters from primary crystals larger than 22 nm remains challenging.

The M(H) and M(T) data of the primary crystals and their corresponding superclusters are presented in Figures S6 and S7. Table 2 summarizes the magnetic parameters deduced from these data for the superclusters and their primary crystals. The data show that the formation of superclusters significantly enhances the $M_{\rm S}$ values compared to those of their constituent crystals. The enhancement of magnetization in the supercluster particles relative to their primary IONPs is remarkable, especially at low fields (Figures S6 and S7). This property is extremely useful for enhancing magnetic biosensing, MRI contrast, and hyperthermia efficiency.^{1,3,5,20} The increase in $M_{\rm S}$ in the superclusters can be rationalized by considering the reduction of spin misalignment or disordered spins on the surfaces of the primary crystals and at the interfaces between the FeO core and the Fe₃O₄ shell due to enhanced interparticle interactions when assembled. In addition, the core FeO may partially transform into Fe₃O₄ during the formation of the superclusters, which could contribute to the observed increase in $M_{\rm S}$. However, an enhancement in $M_{\rm S}$ is also observed in samples composed of small primary crystals (e.g., 13.1 nm nanospheres and 10.2 nm nanocubes), where the presence of FeO is negligible. The presence of FeO and the exchange coupling between the FeO core and the Fe₃O₄ shell in the FeO@Fe₃O₄ core/shell nanospheres (size 21.8 nm) are observed to be preserved in their superclusters with a size of 249 nm, as shown in Figure S8. The formation of the



Figure 6. (a) Schematic illustration of the formation of superclusters, and SEM images of superclusters formed by (b) nanocubes (10.2 nm), (c) nanocubes (11.8 nm), (d) nanospheres (13.1 nm), (e) nanospheres (15.6 nm), and (f) nanospheres (21.8 nm). The supercluster size was calculated based on a size distribution analysis and is specified in the top right corner of each image, while the size of the primary crystals is specified in the bottom right corner.

		magnetic field (0.3 T)		magnetic field (4 T)			
structures	size (nm)	M (emu/g)	% increase (%) ^a	$M_{\rm S}~({\rm emu/g})$	% increase (%) ^a	$T_{\rm B}$ (K)	shift of $T_{\rm B}$ (K)
superclusters	156 ± 34	44.3	59.4	56.5	22.0	223	8
nanospheres	13.1 ± 1.0	27.8		46.3		215	
superclusters	241 ± 57	35.2	15.0	56.4	9.3	252	21
nanospheres	15.6 ± 1.3	30.6		51.6		231	
superclusters	249 ± 40	24.1	42.6	48.4	22.5	282	45
nanospheres	21.8 ± 2.0	16.9		39.5		237	
superclusters	150 ± 37	58.1	10.7	69.6	2.4	231	44
nanocubes	10.2 ± 0.9	52.5		68.0		187	
superclusters	235 ± 80	57.5	13.2	71.5	9.0	265	13
nanocubes	11.8 ± 1.3	50.8		65.6		252	

Table 2. Magnetic Properties of Primary Crystals and Their Corresponding Superclusters

"The percentage (%) increase was calculated by comparing the $M_{\rm S}$ values of the superclusters to those of their constituent primary crystals. $T_{\rm B}$ measured under an applied field of 100 Oe.

superclusters also shifts the $T_{\rm B}$ to higher temperatures. The $T_{\rm B}$ is defined here as the temperature that separates the hightemperature SPM state $(T > T_B)$ from the low-temperature FiM state $(T < T_B)$. The shift of T_B toward higher temperatures and the flattening feature of ZFC M(T) curves both imply stronger magnetic interactions among particles.³⁶ However, the average values of $T_{\rm B}$ for the superclusters are well below 300 K, preserving the excellent SPM properties of their primary nanocrystals. Interestingly, the supercluster particles in Figure 6(c,e,f), which are approximately 240 nm in size, exhibit different values of $T_{\rm B}$ and $M_{\rm S}$, demonstrating the possibility of fine-tuning the SPM properties of supercluster particles by controlling the size and shape of their primary core/shell IONPs, as well as the FeO/Fe_3O_4 phase ratio. Notably, different magnetic properties were observed in supercluster particles approximately 150 nm in size, which were made from

nanospheres with a size of 13.1 nm and nanocubes with a size of 10.2 nm.

In this study, we demonstrate for the first time that the SPM properties of superclusters (also referred to as "magnetic assemblies" or "magnetic nanoclusters") can be finely tuned in terms of both $M_{\rm S}$ and $T_{\rm B}$. These parameters significantly influence the performance of NPs in biomedical applications, such as MRI, hyperthermia, and biosensing, due to the synergistic interplay between these parameters and their effects on magnetization capability, magnetic relaxation, interparticle interactions, and the Brownian motion of particles.^{3,54,60} Given the growing interest in utilizing this class of materials for diverse biological and biomedical applications, ^{13,21,22,61} this approach offers an effective strategy to optimize nanoparticle configurations for maximum efficiency or to tailor their properties to achieve a specific range of magnetic responses or performances. For instance, magnetic particles of similar

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particle size (nm)	iron(III) oleate (g)	sodium oleate (g)	oleic acid (g)	1-octadecene (mL)	docosane (g)	temperature (°C)
10.2 ± 0.9	1.6	0.3	0.282	20	0	320
11.8 ± 1.3	1.8	0.3	0.282	15	0	320
15.9 ± 1.4	1.8	0.3	0.282	7.5	5.0	345
18.0 ± 1.4	1.8	0.3	0.282	2.5	8.5	360
^{<i>a</i>} Particle size means t	he edge length of the	nanocubes.				

Table 3. Synthetic Conditions for Iron Oxide Nanocubes of Varying Sizes^a

Table 4. Formation Conditions of Superclusters with Primary Crystals of Varying Sizes and Shapes

size of primary crystals (nm)	nanoparticle colloidal solution	DTAB in H ₂ O	size of superclusters (nm)	vortex time (s)
10.2 ± 0.9 (nanocubes)	5 mg/mL, 4.0 mL	20 mg/mL, 4.0 mL	150 ± 37	90
11.8 ± 1.3 (nanocubes)	10 mg/mL, 2.0 mL	20 mg/mL, 2.0 mL	235 ± 80	90
13.1 ± 1.0 (nanospheres)	5 mg/mL, 4.0 mL	20 mg/mL, 4.0 mL	156 ± 34	90
15.6 ± 1.3 (nanospheres)	10 mg/mL, 2.0 mL	20 mg/mL, 2.0 mL	241 ± 57	90
21.8 ± 2.0 (nanospheres)	50 mg/mL, 1.0 mL	15 mg/mL, 3.3 mL	249 ± 40	90

sizes but with different magnetic properties can serve as magnetic labeling agents for distinct biomolecules or antibodies, and can be integrated into detection platforms and microfluidic devices, enabling differentiation in magnetic signals for efficient biodetection.⁶² Furthermore, these applications can extend to electronic devices, sensing technologies, and spintronics, where the utilization of magnetic properties (SPM or FM) can be adjusted to align with the operating temperature range of the devices.

CONCLUSIONS

We present a practical approach to fine-tuning the superparamagnetic properties of iron oxide nanoparticles through the selection of specific structures and the formation of superclusters, which are desirable for advanced biosensing and biomedical applications. We have found that FeO@Fe₃O₄ NPs, in both spherical and cubic shapes, synthesized via thermal decomposition of iron(III) oleate, show reduced $M_{\rm s}$ as particle size increases due to the larger fraction of the AFM FeO phase. Compared to their nanosphere counterparts, the FeO@Fe₃O₄ nanocubes exhibit superior SPM properties. The formation of superclusters from these primary IONPs significantly enhances both the $M_{\rm S}$ and the $T_{\rm B}$ while retaining SPM characteristics. By tailoring the size and shape of primary crystals, it is possible to create supercluster structures of comparable sizes with distinct magnetic responses. Our research underscores the potential of manipulating the magnetic functionalities of IONPs through structural and morphological modifications, providing a new pathway to develop nanomaterials with desirable magnetic properties for specific uses in sensing, diagnostics, and therapy. Understanding the magnetic properties of these nanosystems also advances the fields of nanotechnology and materials science.

METHODS

Materials. For the synthesis of IONPs, the following chemicals were used: iron(III) chloride hexahydrate (FeCl₃·6H₂O; 97%; Alfa Aesar), sodium oleate (97%; TCI), oleic acid (90%; Sigma-Aldrich), 1-octadecene (90%; Sigma-Aldrich), and docosane (90%; Sigma-Aldrich). Common solvents employed included deionized water with a resistance of 18 M Ω ·cm (Academic Milli-Q Water System; Millipore Corporation), ethanol (200 proof; Decon Laboratories), chloroform (99%; Sigma-Aldrich), and *n*-hexane (99%; Oakwood). Dodecyltrimethylammonium bromide (DTAB) (98%; Sigma-Aldrich) was used in the synthesis of the supercluster particles.

Synthesis of Iron Oleate Precursors. Iron(III) oleate precursors were synthesized as previously reported.²⁶ First, a mixture of 10.8 g of FeCl₃·6H₂O, 36.5 g of sodium oleate, 80 mL of ethanol, 60 mL of deionized water, and 140 mL of *n*-hexane was refluxed at 70 °C for 4 h. The mixture was then transferred to a separatory funnel, where the top organic layer containing iron(III) oleate precursors was separated, and the bottom aqueous solution was removed. The iron(III) oleate in the organic layer was extracted at least five times with 200 mL of Milli-Q water each time. Subsequently, *n*-hexane was evaporated using a rotary evaporator. The iron(III) oleate product was then dried in an oven at 80 °C for 48 h.

Synthesis of Spherical Iron Oxide Nanoparticles. IONSs with tunable sizes were synthesized by the thermal decomposition of iron(III) oleate in the presence of oleic acid and 1-octadecene, as previously described with modifications.^{26,41,63} Prior to the reaction, the solution was degassed with N₂ for 2 h, and minimal N₂ bubbling was maintained throughout the reaction. The mixture was refluxed at 310 $^{\circ}\mathrm{C}$ for 30 min, with a heating rate of approximately 30 $^{\circ}\mathrm{C/min}.$ The concentration of iron oleate precursors was varied to control the size of the NPs. A mixture of 1.8 g of iron(III) oleate and 0.285 g of oleic acid in 12.57 g of 1-octadecene solvent produced IONPs approximately 10 nm in size. By increasing the concentration of iron(III) oleate, adjusting the amount of oleic acid surfactant, and controlling the heating rate, various sizes of IONSs could be produced, ranging from 10 to 28 nm. The amount of iron(III) oleate used (1.8 to 2.8 g) was systematically varied to tune the size of NPs. Notably, adjusting the oleic acid concentration from 15 to 20% could produce rounder NPs, albeit with a slight shift to larger sizes. After synthesis, the reaction was allowed to cool naturally to approximately 60 °C before starting the washing process. The cooling process from the reaction temperature to this range took about 1 to 1 h and 15 min. The round-bottom flask was cleaned with aqua regia, followed by piranha solution, and dried overnight in a 150 °C oven. To wash the NPs, ethanol was added to the colloidal solution (in n-hexane), followed by centrifugation to remove the supernatant. The NPs were redispersed in *n*-hexane with the addition of 50 to 100 μ L of oleic acid to enhance colloidal stability and prevent aggregation. The NPs were washed three times before being stored in a 4 °C refrigerator.

Synthesis of Cubic Iron Oxide Nanoparticles. IONCs of varying sizes were synthesized through the thermal decomposition of 1.8 g of iron(III) oleate in a solvent mixture of 1-octadecene and docosane, facilitated by sodium oleate and oleic acid surfactants. This method was adapted from previous work, with modifications.^{28,37} The solvent compositions and reaction temperature, detailed in Table 3, were used to control the size of the nanocubes. Before refluxing, the mixture was held at 100 °C for 30 min, then heated to 200 °C and maintained for an additional 30 min. All reactions were subjected to 30 min of reflux. Similarly, a natural cooling protocol for postsynthesis of spherical NPs was applied. After synthesis, the glassware and NPs

underwent a cleaning process similar to that used for spherical NPs. Finally, the NPs were stored in a 4 $^\circ C$ refrigerator.

Formation of Superclusters. Superclusters were prepared using a method reported in previous publications, with modifications.¹⁰ The NPs were separated from the stock solution by centrifugation at 6000 rpm for 5 min. Ethanol was added to the stock colloidal solution prior to centrifugation to facilitate separation. The volume ratio of stock colloidal solutions to ethanol was set at 1:1 for a size of 13 nm, 3:1 for 16 nm, and 7:1 for 21 nm. The supernatant was removed after centrifugation, and the pellet was redispersed in chloroform. To ensure that the NPs formed a stable colloidal solution in chloroform without aggregating, the solution was sonicated thoroughly in an ultrasonication bath with the addition of 10 μ L of oleic acid surfactant. The NP colloidal solution was then added to the DTAB solution, followed by vortexing for exactly 90 s. The organic solvent was evaporated by heating at 55-60 °C for 4 h under magnetic stirring. The supercluster particles were collected by centrifugation (3000 rpm, 5 min) and washed with ethanol at least 4 times. The conditions for fabricating superclusters with varying sizes of primary crystals are specified in Table 4. We observed that the concentration of the NP colloidal solution was the most critical parameter in determining the formation of superclusters.

Materials Characterization. The iron oxide nanospheres and nanocubes were characterized using transmission electron microscopy. The lower resolution/lower magnification images were collected using a TEM (JEOL 2010F) at an acceleration voltage of 200 kV. The atomic resolution images were collected using a Titan/Themis at an acceleration voltage of 300 kV. In both cases, the particles were deposited on holey carbon Cu grids 300 mesh. The TEM image analysis was carried out using DigitalMicrograph and Velox software. A powder X-ray diffractometer (PXRD) (Smart Lab, Rigaku), operating with Cu K α irradiation at 40 mA and 44 kV with a scanning rate of 2°/min, was used for crystallographic analysis. To prepare a sample for PXRD analysis, the powder of NPs was dropped onto cleaned glass substrates. The superclusters were imaged using a scanning electron microscope (SEM, JSM-6330F) at an acceleration voltage of 15 kV. X-ray photoelectron spectroscopy (XPS) analyses were performed using a PHI 5700 X-ray photoelectron spectrometer with a monochromatic Al K α X-ray source to characterize the iron oxide nanoparticles drop-casted on a cleaned silicon wafer. The C 1s peak at a binding energy of 284.8 eV was used for calibration. The Raman scattering spectra of the samples were measured with a Horiba JY T64000 triple spectrometer coupled with an Olympus optical microscope. The microscope focused a 488 nm laser beam onto the sample using ×100 objectives. Magnetic properties were characterized by a superconducting quantum interference device (SQUID, MPMS3, Quantum Design) and a physical property measurement system (PPMS) from Quantum Design. Magnetic field-dependent magnetization measurements (M(H)) were conducted at 300 K in the range of ± 40 kOe. Temperature-dependent magnetization measurements (M(T)) were conducted over a broad temperature range (2 to 400 K) following a standard zero-field-cooled/field-cooled (ZFC/FC) measurement protocol at a field strength of 100 Oe.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.5c04288.

Crystallite sizes of FeO (200) and Fe₃O₄ (400) determined by XRD (Table S1); atomic resolution TEM image and FFT of nanospheres sized 9.8 nm; field dependence of magnetization M(H) at 300 K of nanospheres (Figure S2) and nanocubes (Figure S3); M(H) ZFC and 3T-FC at 10 K of nanocubes size 18.0 and nanospheres size 28.3 nm (Figure S4); size distribution of supercluster particles (Figure S5); field dependence of magnetization at 300 K of supercluster particles and their primary crystals (Figures S6 and S7);

M(H) ZFC and 3T-FC at 10 K of superclusters made by nanospheres size 21.8 nm (PDF)

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Author Contributions

M.D.N. conceptualized, synthesized nanoparticles, collected data, and wrote the manuscript. S.H. synthesized nanoparticles. M.F., L.Z.D., D.D.T., C.-W.C. analyzed magnetic properties. V.G.H. conducted Raman spectroscopy. P.C. revised the manuscript. F.C.R.H. conducted the TEM analysis. M.-H.P. revised the manuscript and discussed the nanomagnetism. T.R.L. supervised the study, acquired funding, wrote the manuscript, conceptualized, and discussed the experimental results. All authors read and reviewed the manuscript.

Notes

The authors declare no competing financial interest.

M.D.N. thanks Dr. Priscilla F. Pieters for valuable advice in nanoparticle synthesis. T.R.L. thanks the Air Force Office of Scientific Research (FA9550-23-1-0581; 23RT0567) and the Robert A. Welch Foundation (Grant Nos. E-1320 and V-E-0001) for generous financial support. F.C.R.H. expresses gratitude to the Robert A. Welch Foundation (Grant No. V-E-0001) for generous funding. C.-W.C. and L.Z.D. acknowledge support from US Air Force Office of Scientific Research Grants (FA9550-15-1-0236 and FA9550-20-1-0068), the T.L.L. Temple Foundation, and the John J. and Rebecca Moores Endowment. M.H.P. acknowledges support from the US Department of Energy, Office of Basic Energy Sciences, Division of Materials Science and Engineering under Grant No. DE-FG02-07ER46438.

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