Biological colloid engineering: Self-assembly of dipolar ferromagnetic chains in a functionalized biogenic ferrofluid

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We have studied the dynamic behavior of nanoparticles in ferrofluids consisting of single-domain, biogenic magnetite (Fe_3O_4) isolated from *Magnetospirillum magnetotacticum* (MS-1). Although dipolar chains form in magnetic colloids in zero applied field, when dried upon substrates, the solvent front disorders nanoparticle aggregation. Using avidin-biotin functionalization of the particles and substrate, we generated self-assembled, linear chain motifs that resist solvent front disruption in zero-field. The engineered self-assembly process we describe here provides an approach for the creation of ordered magnetic structures that could impact fields ranging from micro-electro-mechanical systems development to magnetic imaging of biological structures. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4742329>]

Over the past decade, the behavior of ferrofluids—colloidal suspensions of ferromagnetic nanoparticles—has generated substantial interest in fields ranging from applied physics to bioengineering.¹ In particular, the behavior of ferromagnetic nanoparticles in colloid suspensions is of particular interest because controlled assembly of these particles into ordered structures could impact applications such as ultrahigh density memory devices, micro-electro-mechanical systems (MEMS) actuators, bioengineered materials, and studies of cellular and molecular biophysics.² Although the zero-field formation of dipolar, linear chains in ferrofluids was postulated as early as 1970,³ experimental observations of these chains were only reported in the last decade using cryogenic transmission electron microscopy (cryo-TEM).⁴ The degree of order within these dried structures, and specifically their ability to form linear, dipolar self-assemblies, is governed by many factors. Dipolar structures should form when the dipolar potential is greater than thermal fluctuations (i.e., the dipolar coupling constant $\lambda > 2$),

$$\lambda = \frac{\mu_0 \mu^2}{4\pi k_B T \sigma^3}. \quad (1)$$

Here, $\mu_0 = 4\pi \times 10^{-7} \text{ J A}^{-2} \text{ m}^{-1}$, μ is the magnetic moment of one particle, k_B is the Boltzmann constant, T is the absolute temperature, and σ is the hard sphere diameter or average center-to-center particle separation.⁵ As a result, for particles from MS-1, where σ is $57 \mu\text{m}$,⁶ linear chain formation should be energetically favorable.

However, unlike in the cryo-TEM approach,⁴ where investigators vitrify nanoparticle assemblies, standard TEM studies frequently reveal diverse motifs of ordered and disordered

aggregation of nanoparticles in bulk assemblies that can depend on particle size.⁵ As a result, these approaches could be complemented by approaches to preserve linear dipolar structures that would be amenable to engineering magnetic structures as components of designed materials. Such methodologies could be useful in areas including engineering magnetic micro-devices as well as creating actuators in biomimetic systems to recapitulate natural cellular behavior.

For example, self-assembly phenomena, such as ferromagnetic particle alignment, are of great interest in both natural and synthetic systems. The parameters guiding self-assembly are especially important in biological systems, given that the inherent properties of biomolecules are utilized by nature to create cellular structures. There is significant interest in recapitulating such structures and the cellular behavior they enable within biomimetic systems.⁷ These artificial cell systems often consist of an artificial membrane system containing some type of bioengineered molecular assembly. Enabling such systems with more complex interior components would allow them to mimic dynamic cellular behaviors with diverse capabilities.

One such cellular behavior of interest is the ability to sense the environment and position oneself within it, which could be enabled in biomimetic systems using ferromagnetic dipolar chains. In nature, magnetotactic bacteria create dipolar magnetite chains⁸ to allow bacteria to use the geomagnetic field as a motility guide [Figure 1(a)]. In one previous application, an integrated MEMS-microfluidic system was used to isolate these internal chains of magnetite crystals (also known as magnetosomes) from MS-1.⁹ These isolated biogenic Fe_3O_4 chains sometimes formed ring-like structures often referred to as flux-closure rings,¹⁰ which preserves dipolar interactions between magnetic nanoparticles (MNPs) in one energetically favorable motif. In biomimetic systems, assembling larger linear magnetite structures with chemistry

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FIG. 1. (a) TEM photograph of magnetite chain inside *Magnetospirillum magnetotacticum* MS-1; (b) TEM photograph of bulk aggregate of isolated biogenic magnetite with the black arrow pointing toward structure shown in (c) TEM photograph of flux-closure ring.

amenable to integration in biomimetic structures would be useful to transfer this natural global positioning system.

In this letter, we describe an engineered self-assembly system for creating dipolar chains in zero-field with chemistry amenable to such integration. This self-assembly system was inspired by aforementioned previous work,^{4,9} well established analytical models of ferromagnetic particle dynamics^{3,5,11} that predict dipolar chain assembly, and our desire to engineer self-assembly of larger chains. Using the system, we were able to generate these ordered, self-assembled structures by functionalizing particles and substrates with an avidin-biotin molecular bonding approach. Functionalized particles were able to assemble into structures that firmly attached to a functionalized substrate and resisted displacement by the solvent front. In the absence of this bonding system, structures were immediately destroyed by the moving edge of evaporating solvent.

We initially began by isolating biogenic nanoparticles from MS-1 [Figure 1(a)]. These magnetotactic bacteria naturally form intracellular chains of single-domain, ferromagnetic crystals with center-to-center separations of approximately

57 μm .⁶ We isolated ferromagnetic particles from late-stationary phase cultures of MS-1 using a NaOH method similar to previously described approaches.¹¹ Isolated particles were then visualized with TEM [Figs. 1(b) and 1(c)] and observed to form aggregates. While generally disordered, in several places, we did note the formation of aggregate morphologies dominated by dipole interactions, such as dipolar chains and potential flux-closure ring structures as seen in Figure 1(c). However, the dried colloid morphology seemed to be dominated by labyrinthine and sometimes bulky aggregate patterns [Figure 1(b)]. Although our observations of chainlike queues of single particles, such as in the ring in Figure 1(c) suggested that ferromagnetic dipole interactions were directing morphology at the nanometer scale, the overall disorder at the micron-scale remained perplexing.

As a result, we examined the interactions of particles in suspension. After a brief sonication to disrupt dipole interactions between MNPs, colloid suspensions were placed on borosilicate glass and the microscope was focused just above the glass. We quickly saw the spontaneous, accretive assembly of long chains of MNPs in suspension. However, upon drying, the solvent front clearly disrupted these ordered structures. This process of disruption is shown in Fig. 2.

In order to prevent this disruption, we devised a reaction scheme whereby MNPs in solution would be able to self-assemble into dipolar chain subunits, and if close enough to the substrate to make contact, assembled structures would be able to bind tightly. As shown in Figure 3, we functionalized our biogenic Fe_3O_4 particles using the approach of Yamaura *et al.*¹² by aminating particles with (3-aminopropyl)triethoxysilane (APTES), followed by carbodiimide-enabled covalent bonding¹³ to avidin molecules using N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) [Fig. 3(a)]. Using the same chemical processes, we functionalized glass substrate with biotin [Fig. 3(b)]. Aggregates of magnetite particles functionalized with avidin conjugated to a Texas Red fluorophore are shown in Figs. 3(c) and 3(d) and reveal robust linking of avidin to magnetite (particles dried on non-functionalized glass). Droplets of avidin-functionalized magnetic colloids,

FIG. 2. (a) DIC micrograph of filamentous magnetic nanoparticle chains during formation in solution; (b–d) A sequence of DIC micrographs showing disruption of the filamentous chain by an advancing, drying solvent front. Scale bar = 40 μm (enhanced online) [URL: <http://dx.doi.org/10.1063/1.4742329.1>].

FIG. 3. (a) Scheme showing functionalization of biogenic magnetite with avidin and (b) functionalization of a borosilicate glass substrate with biotin, and final assembly of the glass-magnetite composite. The avidin functionalized surface is left exposed for binding with biotinylated polymers for biomimetic system integration; (c) Magnetite particles functionalized with avidin-Texas Red fluorophore shown with DIC microscopy and (d) fluorescent microscopy. Scale bar = 40 μm .

with phosphate buffered saline (PBS) as a solvent, were then placed on biotinylated glass substrates and particle behavior in the drying colloid was observed. The details of these steps are given in the supplementary information.¹⁴

After functionalizing these particles with avidin and placing suspensions of particles on biotinylated glass substrates, we created linear assemblies of nanoparticles. Drop-lets of biogenic magnetic colloids were placed on glass and continuous differential interference contrast (DIC) microscopy of particle assembly revealed single-domain nanoparticle dipoles aggregating into subunits while in suspension. These subunits further assembled into larger linear assemblies during the process of settling onto the substrate. Avidin-biotin binding was sufficient to resist disturbance of assemblies by solvent front displacement upon evaporation and assembled nanoparticle structures ordered themselves along the radii of droplets.

Furthermore, these structures bonded securely to the substrate and could not be disrupted by either the drying solvent front or vigorous washing in deionized H_2O . As shown in Figure 4, immediately upon drying, these structures were ordered outside the perimeter of a dried salt crystal extruded from the suspension [Figs. 4(a) and 4(b)]. Using NIH ImageJ software, we measured the length of 182 of these magnetic-particle structures [Figs. 4(c)–4(e)] from three different samples, which were $15.64 \pm 1.96 \mu\text{m}$ in length (standard error of the mean) [Fig. 4(f)].

Although the radial patterning of Fe_3O_4 MNP structures could potentially be caused by forces exerted by the drying

FIG. 4. (a) Phase-contrast micrograph showing dried ferrofluid aggregate with self-assembled dipolar chains (white arrow) positioned radially at the perimeter of an extruded (black arrow) salt crystal (scale bar = 400 μm); (b) Phase contrast micrograph showing self-assembled, dipolar chain using the described chemistry (scale bar = 25 μm); (c-e) Phase contrast micrographs of different size individual chain assemblies (scale bars = 15 μm); (f) size distribution of linear structures from three samples ($n = 182$) with mean length of $15.64 \pm 1.96 \mu\text{m}$ (standard error of the mean).

solvent front, using continuous DIC microscopy, we observed the formation of structures radiating from the center of the colloid suspension prior to solvent evaporation. Unlike our observations of non-functionalized particles, where entire self-assembled chains continued to oscillate in solution prior to displacement by solvent front, in experiments with functionalized MNPs and substrate some linear chain subunits quickly immobilized on substrate and, in an accretive process, connected to other linear chain subunits that did not immediately bond to substrate, ultimately forming a longer chain of particles.

As is apparent in Figure 1, magnetosome particles can interact with both the intended end-to-end interaction along the direction of the dipole or with an unintended side-to-side interaction aligning complementary poles of two dipoles. We propose that at first, the intended end-to-end interactions drive the assembly of filamentous chain subunits, while overall aggregation in the solution system is driven by the unintended side-to-side interactions. We model this behavior by making the simplifying assumption that end-to-end interactions equilibrate on a much slower timescale than side-to-side interactions. This assumption is justified by the expectation that end-to-end interactions are more energetically stable, and thus have slower dissociation rates. Yet, because there are fewer potential binding configurations for end-to-end interactions

between two chains (only two configurations, regardless of chain lengths), versus potential configurations for side-to-side interactions (multiple possible pairwise interactions, proportional to the product of chain lengths), our model implies a lower association rate. This model of more-frequent-but-weak side-to-side contacts, versus rarer-but-more-stable end-to-end contacts, leads to a prediction of rapid equilibration of side-to-side contacts and a relatively slow process of equilibration of end-to-end contacts. We can develop a simple model of the fast side-to-side process by assuming we have a set of chains of indeterminate lengths capable of aggregating by side-to-side interactions with a uniform on-rate, k_1 , and a uniform off-rate, k_2 . If we denote an aggregate of n free chains by F_n , then we can describe the behavior of the system in solution by the reversible reaction,

Here, we are assuming that the side-to-side bonds equilibrate in solution but that the end-to-end bonds are in some kinetically trapped state on the same time scale and thus the ensemble of linear chains is fixed. Then, we can derive a distribution of aggregate sizes for the model by noting that

$$[F_{n+1}] = \left(\frac{k_1}{k_2} \right) [F_1][F_n] \quad (3)$$

implying a geometric distribution of aggregate sizes in which isolated chains would be rare for sufficiently large k_1/k_2 . This competition between side-to-side and end-to-end reactions is illustrated in Figure 5(a).

In terms of this model, the addition of the surface chemistry can be understood as a non-reversible interaction effectively compartmentalizing a single aggregate on the surface. We extend the above model by a notion of irreversibly anchoring a free aggregate to a surface

FIG. 5. (a) Schematic illustrating competition between side-to-side and end-to-end binding of chains. The model assumes that pairs of free chains can anneal in either configuration, but with different kinetics, yielding relatively faster equilibration of side-to-side binding. (b) Schematic of surface-binding as an irreversible reaction transforming free chains to surface-bound chain bundles. Exchange of unbound chains from bound partners provides a mechanism to separate aggregates and produce a high yield of individual chains.

and extend the reaction model to describe gain or loss of non-anchored chains within an anchored aggregate,

This model implicitly assumes that the surface is sufficiently large that no two aggregates anchor close enough to interact with one another. The introduction of the irreversible reaction directly implies that the steady-state of the reaction must be a transfer of all chains to an anchored, single-filament state S_1 . This anchored, single-chain state is the observed nanoparticle chain. A schematic describing this model of the influence of surface chemistry in separating side-to-side chains appears as Figure 5(b).

In summary, we have described an approach to engineer the formation of linear ferromagnetic nanoparticle structures in zero-field amenable to engineering into biomimetic systems. Unlike similar structures that are quickly displaced by solvent front evaporation, these functionalized MNPs were able to bond robustly to substrate and preserve self-assembled MNP structures that spontaneously form in colloid suspensions. In the future, the underlying chemistry could be easily modified for integration into filamentous, cytoskeleton networks for embedding into biomimetic artificial cells. We expect these results will be of value in fields ranging from MEMS-based memory devices to biophysics, and in particular, help reveal and mimic the applied physical properties of MNPs assembled intracellularly by magnetotactic bacteria.

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- ¹⁴See supplementary material at <http://dx.doi.org/10.1063/1.4742329> for detailed experimental methods.