Assembly of Superparamagnetic Iron Oxide Nanoparticles on DNA Nanostructures

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Superparamagnetic Iron Oxide Nanoparticles (SPIONs) are one of two types[1] of contrast agents for magnetic resonance imaging (MRI) that are approved for clinical use. Their use for cancer treatment by magnetic hyperthermia is also being investigated in clinical trials. Both of these applications rely on having the correct size and shape of the magnetic material. These parameters affect the resonance frequency and relaxation times in MRI[2] and heat transfer in hyperthermia.

In this work we present a method to position SPIONs on DNA nanostructures, enabling the production of constructs with precisely engineered magnetic properties from simple components. We also investigate a novel strategy to purify the constructs with high yield. We decorate a 30 nm-long DNA nanotube with one 20 nm SPION on each end by biotin-streptavidin binding. Fully assembled structures, "dumbbells" (figure 1), are separated from unreacted components by centrifugation – a technique that is compatible with the high salt concentrations required to prevent SPION aggregation. Centrifugation separates objects according to their sedimentation coefficient, which depends on the ratio of their mass, m, and hydrodynamic radius, R_H.[3]

We find that dumbbells connected by DNA nanotubes made from single-stranded tiles centrifuge at approximately the same rate as individual SPIONs: the variability in SPION mass ($\pm$ 30 %), together with the increase in hydrodynamic radius ($\approx$ 1.38 × for a simple nanoparticle dimer)[4] makes the ranges of m/R_H similar for both single particles and dumbbells. To overcome this obstacle and achieve a clean separation we increase the hydrodynamic radius of the dumbbells significantly by the addition of 7000 bases of ssDNA.[5] The additional ssDNA is incorporated by using the DNA origami technique to fold the $\approx$ 1000 basepair nanotube and leaving the remaining $\approx$ 7000 bases of the scaffold strand unfolded (figure 1a). This ssDNA "drag tag" contains two restriction enzyme recognition sites near the nanotube which can be used to remove the drag tag after centrifugation.

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[1] The other being complexes of Gd(III)
[2] Zabow, G., et al. (2008). "Micro-engineered local field control for high-sensitivity multispectral MRI." Nature **453**(7198): 1058-1063
[3] Ko, S. H., et al. (2014). "High-speed, high-purity separation of gold nanoparticle-DNA origami constructs using centrifugation." Soft Matter **10**(37): 7370-7378.
[4] On-Line Biophysics Textbook (2000), Todd M. Schuster, editor, Separations and Hydrodynamics.
[5] Assuming a random coil conformation, a 7000 base sequence of ssDNA has a hydrodynamic radius of $\approx$ 80 nm.

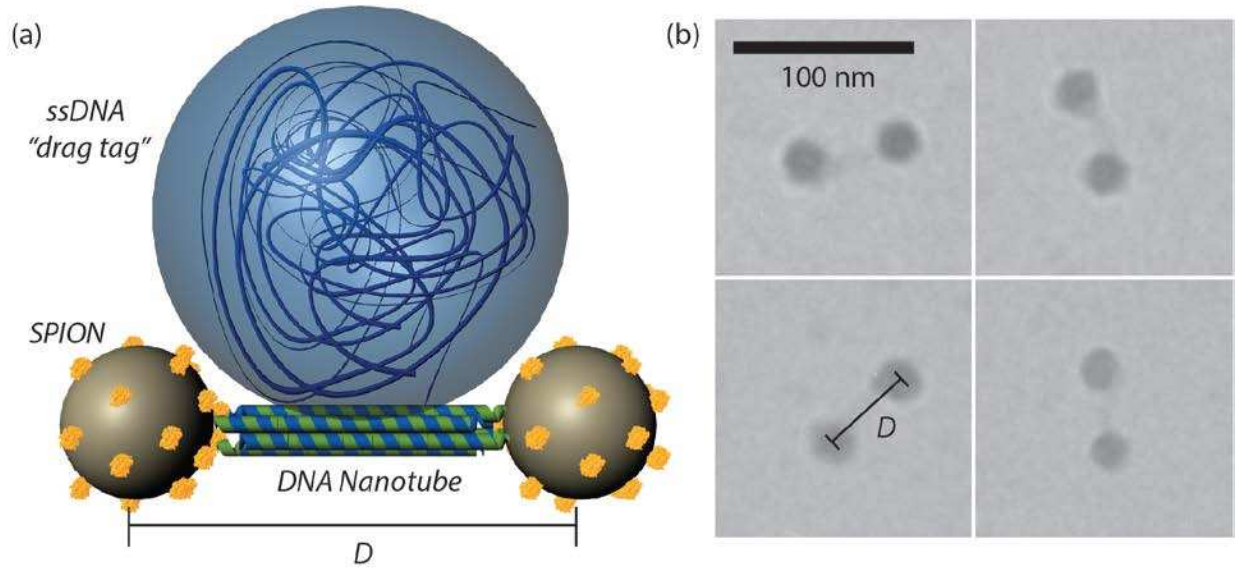

Figure 1: Schematic drawing of fully assembled dumbbells (a). TEM images of dumbbells, stained with uranyl acetate (b). The center to center distance between SPIONs, D = (54 ± 5.5) nm, (mean ± one standard deviation), consistent with our design, is indicated in the bottom left image.