Superparamagnetic iron oxide nanoparticles with photoswitchable fluorescence†

Kyung Min Yeo, Chun Ji Gao, Kwang-Hyun Ahn* and In Su Lee*

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The incorporation of superparamagnetic iron oxide nanoparticles with sulfur-oxidized diarylethene molecules resulted in a novel multifunctional nanosystem, in which the fluorescent performance and flocculation and dispersion are reversibly switched by light irradiation and external magnetic field, respectively.

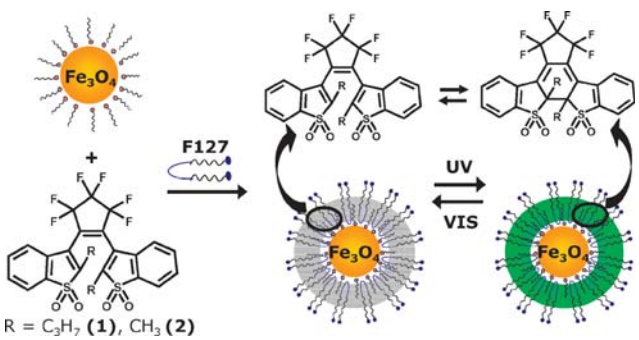
Multifunctional nanoparticles have attracted increasing attention as advanced agents for biomedical applications. In recent years, several kinds of nanoparticles combining magnetic and fluorescent properties have been fabricated through the incorporation of superparamagnetic nanoparticles with fluorescent organic dyes or quantum dots.[1,2] The combination of magnetism and fluorescence in a single nanoparticle provides unique applicability which cannot be achieved with conventional materials. For example, the motion of such fluorescent magnetic nanoparticles can be induced by the application of an external magnetic field and monitored in real time through fluorescence measurements. Furthermore, they have the potential to be used as multimodality imaging probes that enable simultaneous diagnoses using magnetic resonance and optical imaging techniques. Very recently, molecules whose fluorescence can be reversibly photoswitched were employed to achieve cellular labeling, allowing for much improved performance in super-resolution microscopy.[3] Also, several types of nanoparticles having photoswitchable fluorescence were synthesized and successfully demonstrated as a new class of probes able to distinguish the sites of interest from false positive signals.[4]

In this context, the combination of magnetic nanoparticles and photoswitchable fluorescence can provide useful tools for imaging and tracking complicated biological systems. In our previous work, we developed highly fluorescent materials with oxidized diarylethene molecules whose fluorescence is reversibly modulated by alternating UV and visible lights.[5] Very recently, the groups of Irie and Feringa reported the photoisomerization of diarylethene derivatives at the surface of metallic nanoparticles.[6,7] Based on these developments, we envisioned that the incorporation of oxidized diarylethene molecules into superparamagnetic nanoparticles would allow for the production of a multifunctional nanosystem, in which the fluorescence and the flocculation and dispersion can be reversibly switched.

Herein, we report the synthesis of sulfur-oxidized diarylethene encapsulated superparamagnetic iron oxide nanoparticles and their fluorescent performances and flocculation that are reversibly switched by light illumination and external magnetic field. To the best of our knowledge, this is the first report on nanoparticles combining superparamagnetism and photoswitchable fluorescence.[8]

The 7 nm sized iron oxide nanoparticles stabilized by oleic acid were prepared by the previously reported procedure.[9] The incorporation of fluorescent molecules was conducted by mixing iron oxide nanoparticles, the sulfur-oxidized diarylethene, and Pluronic copolymer (F127, PEO_{99}-PPO_{67}-PEO_{99}) in CH_3Cl solution. Two types of sulfur-oxidized diarylethene molecules, 1,2-bis(2-propyl-1-benzothiophene-1,1-dioxide-2-yl) perfluorocyclopentene (**1**) (Scheme 1) and 1,2-bis(2-methyl-1-benzothiophene-1,1-dioxide-2-yl)perfluorocyclopentene (**2**), were used for this work. Those compounds are known to undergo photocyclization under UV irradiation in ethyl acetate, resulting in the highly fluorescent closed-ring isomer, which reverts to the non-fluorescent open-ring isomer upon visible light irradiation.[6,7] The addition of water to the nanoparticle mixture and filtering off the floating matter generated a transparent dark brown suspension of well dispersed iron oxide nanoparticles encapsulated by **1** and **2** (**MNP-1** and **MNP-2**), respectively. The purification of the resulting nanoparticles was accomplished by repeated ultracentrifugation and dispersion in water.

Transmission electron microscopy (TEM) analysis showed that there were no discernable changes in the shape, size, or size distribution of the nanocrystals during the encapsulation



Scheme 1 Preparation and photoisomerization of **MNP-1**.

Department of Chemistry & Advanced Material Sciences, College of Environment and Applied Chemistry, Kyung Hee University, Gyeonggi-do, 449-701, Korea. E-mail: khahn@khu.ac.kr; insulee97@khu.ac.kr; Fax: 82-31-202-7337; Tel: 82-31-201-3823

† Electronic supplementary information (ESI) available: Experimental procedures for the syntheses and spectroscopic analysis, absorption spectra of **MNP-1** and **1**, fluorescence spectra of **1**, modulation of the fluorescence of **1**, temperature and field dependence of magnetization of the nanoparticles, and the cytotoxicity of **MNP-1**. See DOI: 10.1039/b807462c

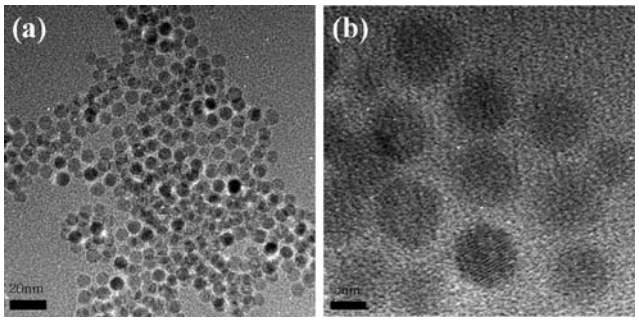


Fig. 1 (a) TEM and (b) high resolution TEM (HRTEM) images of **MNP-1**.

reaction (Fig. 1(a) and (b)). **MNP-1** exhibited good colloidal stability, such that no aggregation was detected after standing for more than one month at ambient temperature. The number of **1** molecules encapsulating each nanoparticle, determined by ICP AES for Fe and elemental analysis for S, was found to be around 200.[10] The incorporation of the molecules of **1** can be understood by their partition into the hydrophobic shells around the nanoparticle surface.[11] F127 copolymer used in this work has hydrophobic segments of polypropylene oxide (PPO) sandwiched between hydrophilic polyethylene oxide (PEO) segment. For the surface coating, the PPO segment is expected to anchor at the interface of the oleic acid shell around iron oxide while the PEO segments extend into the aqueous phase. The molecules of **1** are expected to be encapsulated within the hydrophobic shell constructed between oleic acid and the PPO segment of F127 through hydrophobic interactions. The incorporation of **2** did not result in the particles having sufficient colloidal stability in water, most likely due to the less hydrophobic nature of **2** compared with **1**. The encapsulation within the hydrophobic shell of the nanoparticles was anticipated to protect the **1** molecule from water molecules and help to maintain their fluorescence and switching properties even in aqueous suspension.

The optical and switching properties of **MNP-1** were investigated by illumination with UV and visible light. The UV illumination at 312 nm induced a dramatic enhancement in the fluorescence of the **MNP-1** suspension. The emission spectra of **MNP-1**, monitored with 410 nm excitation light, showed a gradual increase in intensity during UV light irradiation (Fig. 2(a)). The fluorescence profile of **MNP-1**, having two peaks at around 460 and 490 nm, was quite similar to that observed from the closed-ring isomer of **1** in ethyl acetate, indicating the induction of the cyclization of the encapsulated **1** molecules by UV light (ESI†). The formation of the closed-ring isomer of the encapsulated **1** was also evidenced by the new absorption maximum at 410 nm in the absorption difference profile, derived by subtracting the absorption of **MNP-1** before UV irradiation (ESI†). The fluorescence quantum yield of 0.12 for the closed-ring isomer of **MNP-1** was slightly lower than the value of 0.14 for the molecules in ethyl acetate solution. Nevertheless, the photocyclization process of the **1** molecules was influenced and slowed down by their encapsulation. The aqueous suspension of **MNP-1** was found to reach the photo-stationary state in 30 min, while only 3 min was needed for the molecules in ethyl acetate solution. Upon its

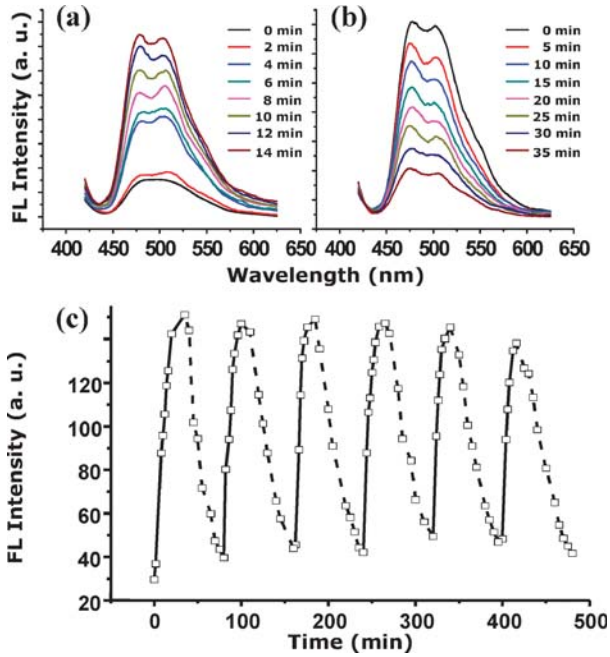
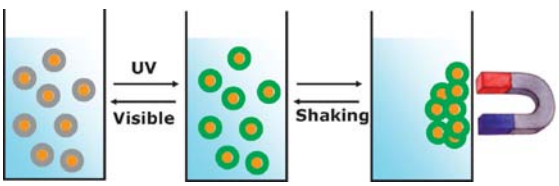


Fig. 2 Fluorescence spectra changes of an aqueous suspension of **MNP-1** with UV irradiation (a) at 315 nm (b) and subsequent illumination of visible light at 420 nm. (c) Modulation of the fluorescence signal of **MNP-1** in aqueous suspension, monitored at 505 nm with excitation at 410 nm, upon alternative illumination with UV at 312 nm (solid line) and visible light at 420 nm (dotted line).

subsequent illumination with visible light at 420 nm, the fluorescence of the suspension was decreased to its original intensity, most likely due to the reversion of the closed-isomer of **1** into the open-ring isomer (Fig. 2(b)). In order to evaluate the reversibility of the switching process, the fluorescence modulation of **MNP-1** was monitored at 505 nm in aqueous suspension with alternating cycles of UV (312 nm) and visible light (420 nm). As shown in Fig. 2(c), the fluorescence of **MNP-1** was reversibly modulated, at least for six cycles, without any apparent fatigue effects or photo-bleaching. Such a reversible fluorescence modulation (Scheme 2) even under aqueous media demonstrates the potential of **MNP-1** to be used in on-off switching probes for biomedical applications. **MNP-1** showed reversible magnetization and demagnetization during the measurement of field dependent magnetization at 300 K, indicating the superparamagnetic property (ESI†). When a small magnet was placed on the side of the vessel, the nanoparticles moved along the magnetic gradient and almost all of them were concentrated close to the magnet within 12 h.[12] The concentrated nanoparticles were easily caused to drift apart into the dispersion by shaking or



Scheme 2 The on–off fluorescence switching and reversible flocculation and dispersion of **MNP-1**.

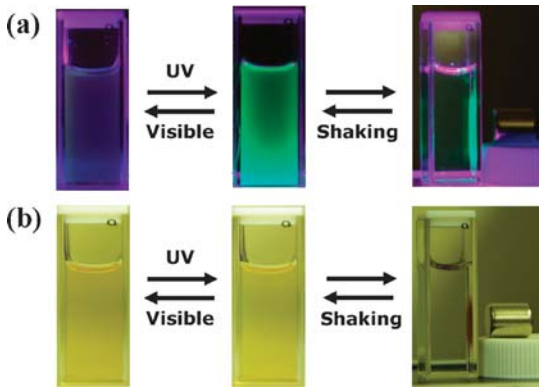


Fig. 3 Photographs of the aqueous suspension of **MNP-1**, taken (a) under 365 nm excitation light and (b) natural light, after illuminating with UV at 312 nm for 20 min (middle), placing a small magnet on a side of vessel for 12 h (right), shaking and subsequently illuminating with visible light at 420 nm for 40 min.

vortexing. Fig. 3 depicts the reversible flocculation and dispersion of **MNP-1** controlled by the application of an external magnetic field and their on-off fluorescence switching induced by alternating illumination of UV and visible light, demonstrating their unique combination of superparamagnetism and photoswichable fluorescence. The *in vitro* cytotoxicity of **MNP-1** was evaluated on Raw 264.7 lines by using MTT assay method. **MNP-1** did not show any significant toxicity to cells at experimental conditions up to 10 μM of Fe concentration measured by ICP AES, indicating their suitability for biomedical applications including cellular labeling (ESI†).

In summary, we synthesized a novel bifunctional nanosystem through the encapsulation of sulfur-oxidized diarylethene molecules onto iron oxide nanoparticles. Also, we demonstrated the reversible and controllable switching of its fluorescence and flocculation. We believe that the unique approach described in this work can provide useful tools for imaging and tracking complicated biological systems.

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