

Synthesis of nanoparticles

Monodisperse iron-oxide nanoparticles were synthesized by reduction of iron pentacarbonyl in the presence of oleic acid and oleylamine by which the Iron oxide composition and size are controllable. (see C. H. Yu, N. Caiulo, C. C. H. Lo, K. Tam, S. C. Tsang, *Adv. Mater.* **18**, 2312 (2006).

Synthesis of silica coated iron oxide nanoparticles and super-crystals

In order to encapsulate Iron oxide nanoparticles in silica, 1 mmol Igepal CO-520 polyoxyethylene(5) nonylphenyl ether was added into the 9 mL cyclohexane followed by ultrasonic treatment (Decon) for 20 minutes. 1 mL of iron oxide solution prepared by re-dispersion of the iron oxide solid in cyclohexane (1mg/ml) was added into the Igepal solution. After stirring the mixture for 4 h, 80 μ L of ammonia solution (35%) was added. Finally, 60 μ L tetraethyl orthosilicate was added and the mixture was allowed to age for 48 h to encourage hydrolysis and condensation of the silica precursor. The resulting silica coated iron oxide nanoparticles were collected by adding ethanol solution for washing a couple of times and then evaporating very slowly to grow super crystals at room temperature.

Protein labelling

Cytochrome B5 and Cytochrome C were purchased from Abcam (UK). The CytB5 was designed to be labelled by Alexa Fluor 568 for a red label, whereas CytC by Alexa Fluor 488 for green. The corresponding protein labeling kits came from Invitrogen. Protein samples (0.5 ml, 1 mg/ml) were dialyzed into degassed PBS buffer (Sigma) and 50 μ l 1M bicarbonate sodium were added to increase pH up to \sim 8.3. One aliquot of the provided fluorophores were dissolved with each pretreated protein solutions at low temperature, followed by vigorous stirring at room temperature for one hour. The excessive fluorophores were thereafter removed by applying onto HiTrap G25 Sephadex column equipped Äkta FPLC. UV-Visible spectra of final products were recorded for quantitative characterization, and the labeling efficiency was estimated to be 30 % for 44 μ M CytB5-AF568 and 91 % for 21 μ M CytC-AF488, respectively.

The fluorescence experiments

Cyt C and B5 (purchase from Abcam plc.) was used without further purification. 250 μ l (21 μ M) CytC with labelling Alexa Fluor 488 (green dye) and 195 μ l (44 μ M) CytB5 with labelling Alex Fluor 568 (red dye) were mixed with 13.88 mg nanocrystals and under rotary shaking machine for 3 hours. The samples were washed with 500 μ L by centrifuge and external magnetic field, respectively. Then other two time washings were required with total amount of 1,500 μ l PBS buffer. Samples solids were collected to determine by monitor of Confocal Microscopy as follows:

1. 200 μ l MQ water was added into each sample
2. Sonicate each sample on ice for 170s by using the Ultrasonic Homogenizer (FISHER sonic dismembrator Model 300)
3. 10 μ l of each sample was mounded on a slide and the images were captured by using the laser scan confocal microscope (Olympus, FV10-ASW; excitation at 495nm for cytoC and 575nm for cytoB5)
4. For FRET experiments, only laser excitation at 495nm was used.

Magnetic kinetic absorption

A standard 20 μ l Cyt C labelling green dye solution with concentration of 21 μ M was prepared by dissolved a given amount of 500 μ l PBS (pH=7.0). A 3.0 mg magnetic nanocrystal was immersed with gently under rotary shaking machine for 3 h in order to reach equilibrium. External magnetic field was applied to induce of cytochrom C and nanocrystals. After that, sample was collected for investigations of concentration calibration and magnetic kinetic absorption using fluorescence spectroscopy.

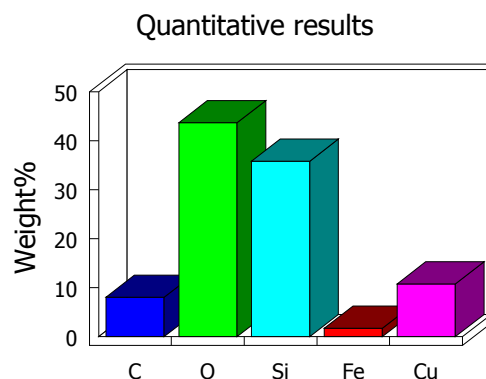
Supporting Information:

Silica coated iron oxide nanoparticles

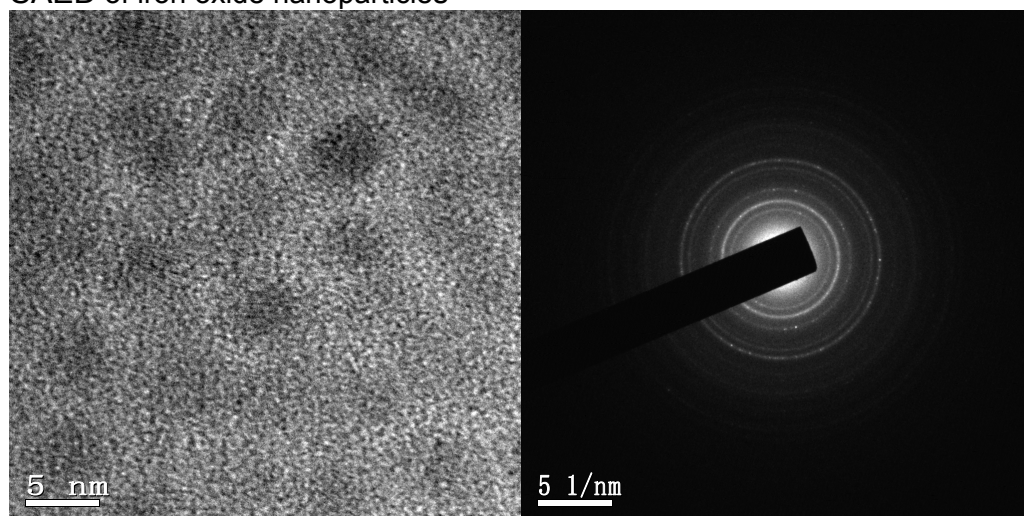
Spectrum processing:
No peaks omitted

Quantitation method : Cliff Lorimer thin ratio section.
Processing option : All elements analyzed (Normalised)
Number of iterations = 1

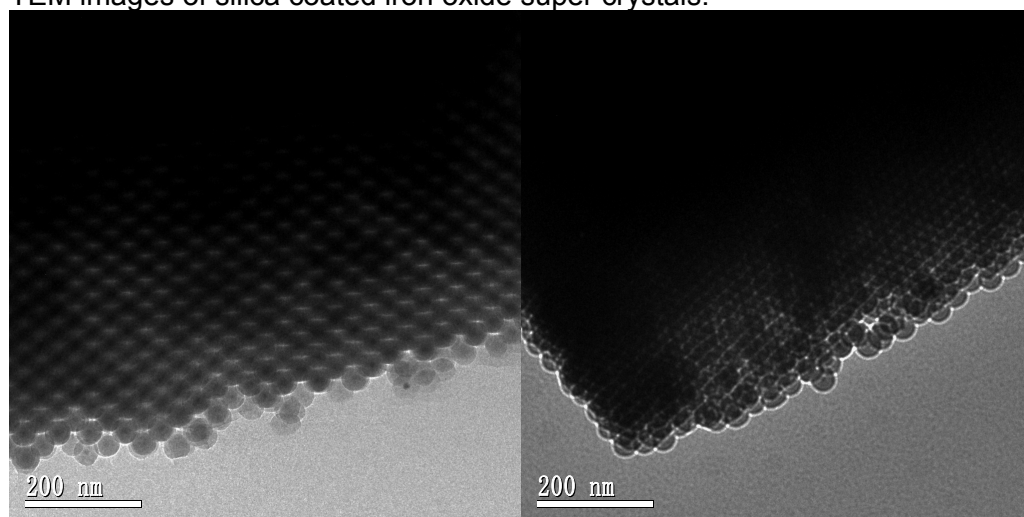
Element	Peak Area	Area Sigma	k factor	Abs Corn.	Weight%
C K	269	41	2.208	1.000	8.00
O K	1791	76	1.810	1.000	43.71
Si K	2657	85	1.000	1.000	35.83
Fe K	110	21	1.170	1.000	1.73
Cu K	583	41	1.366	1.000	10.73
Totals					100.00



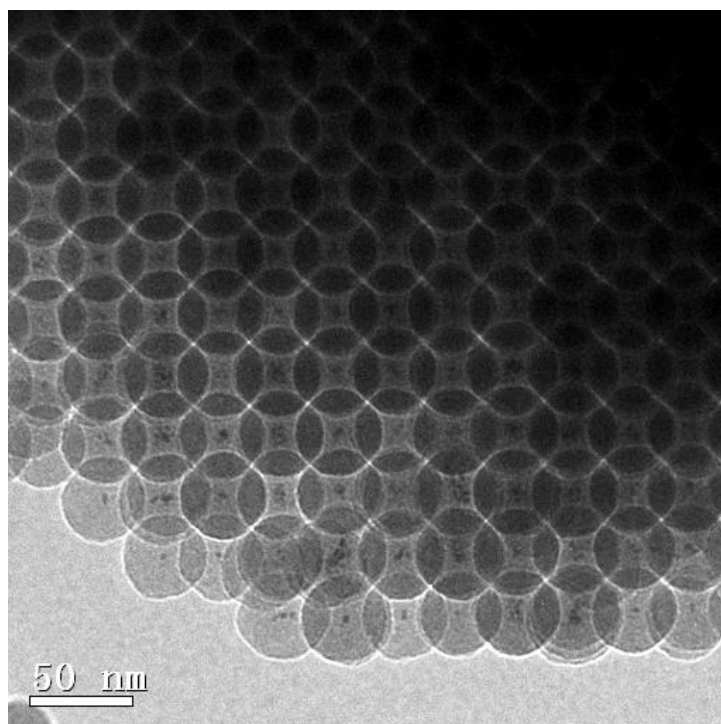
SAED of iron oxide nanoparticles



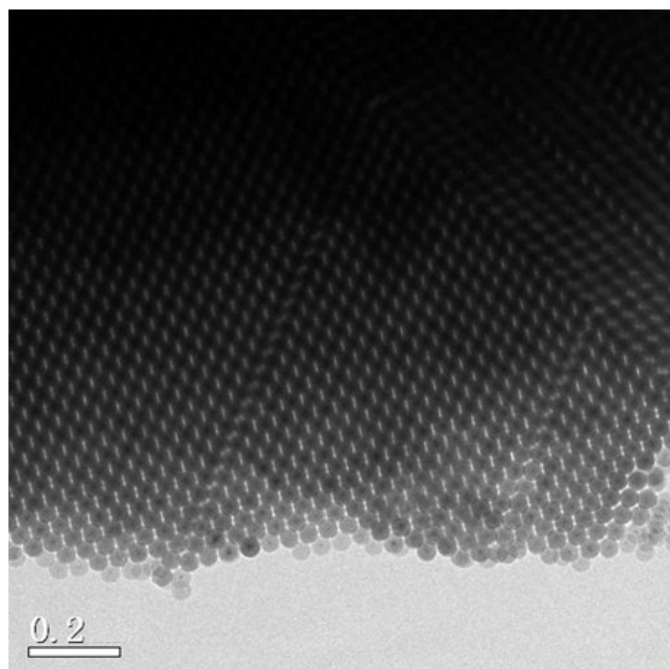
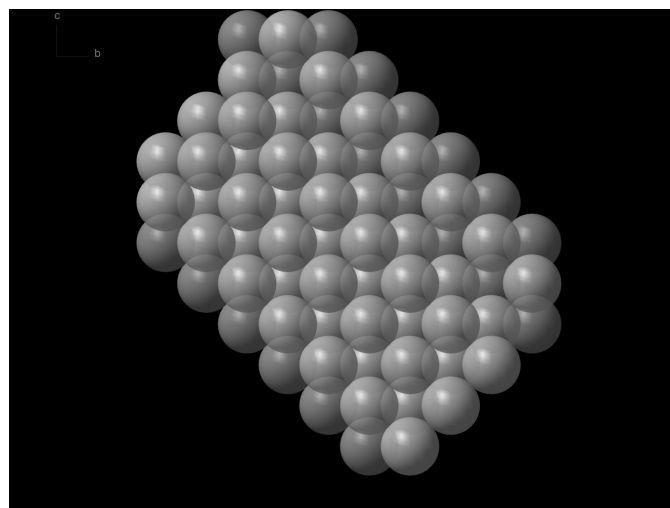
TEM images of silica coated iron oxide super crystals:



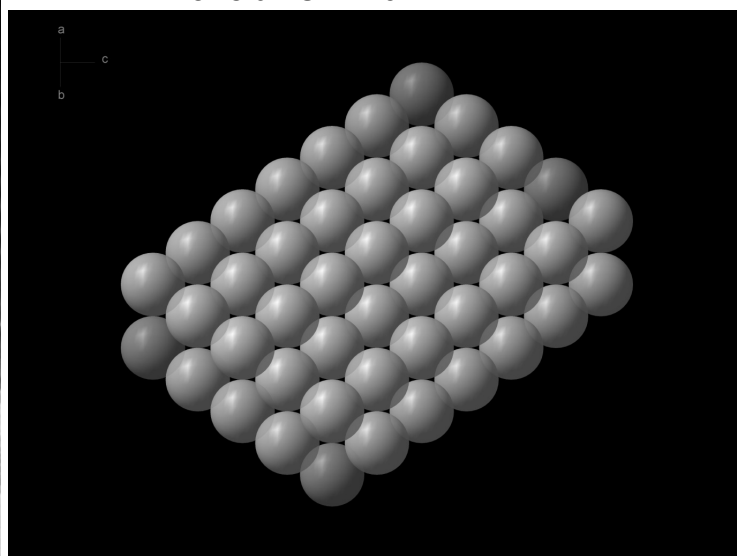
TEM images and simulated patterns of super crystals:

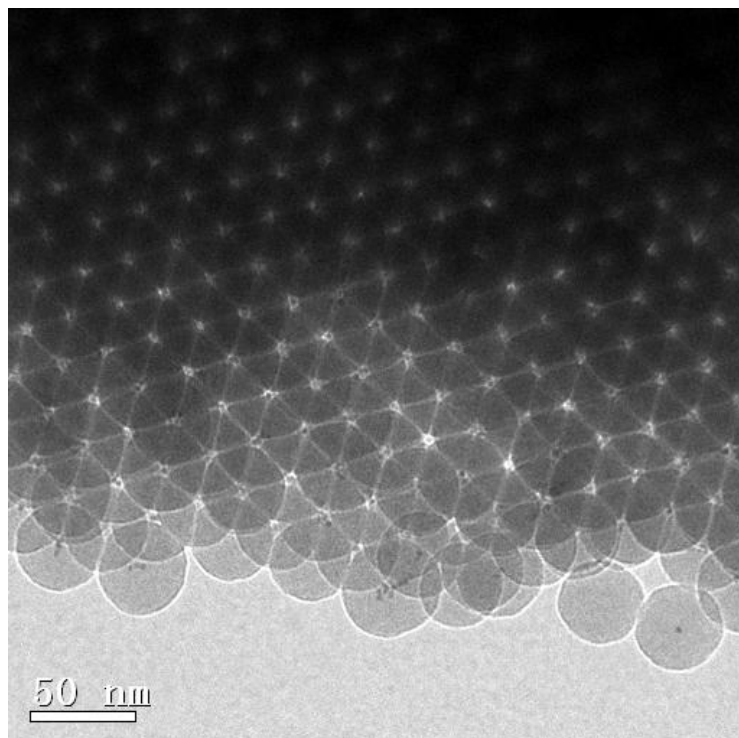


Zone axis: $\langle 100 \rangle$

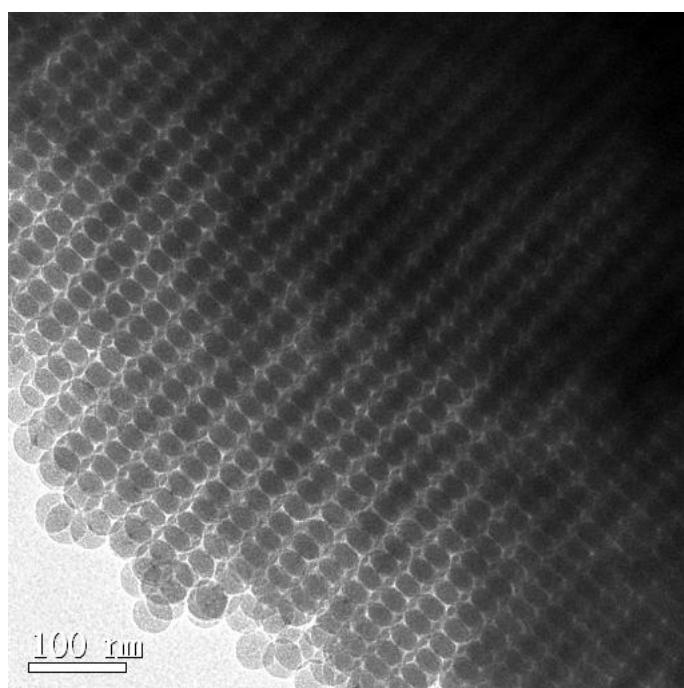
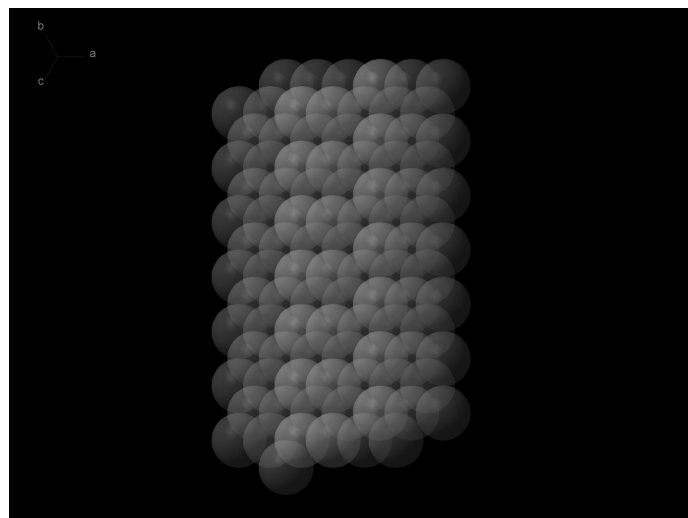


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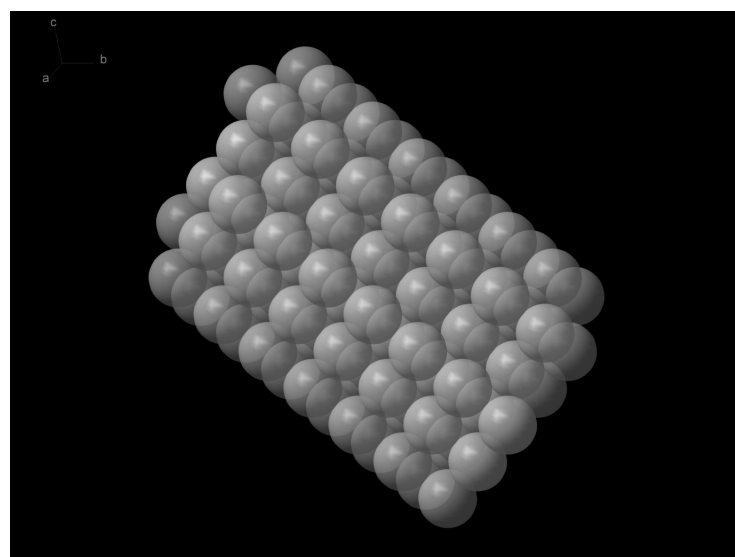




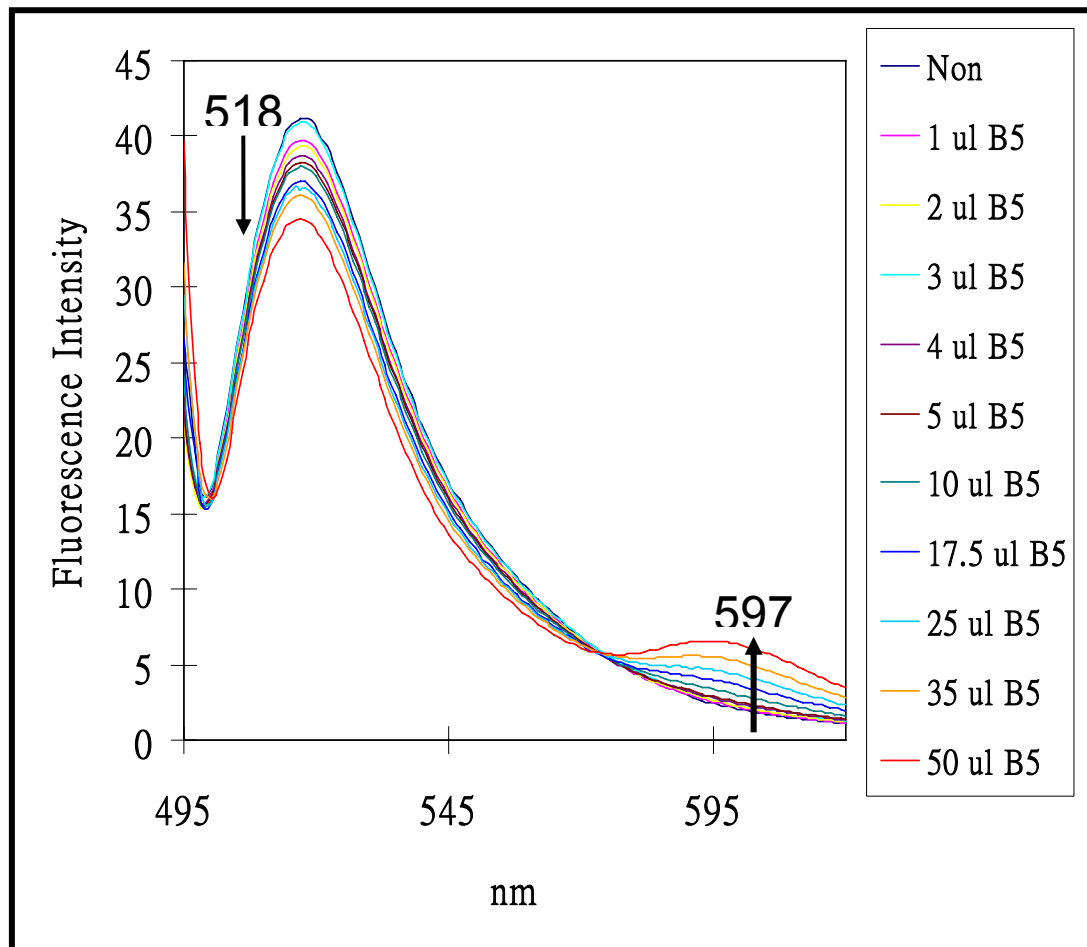
Zone axis: <111>



Zone axis: <211>

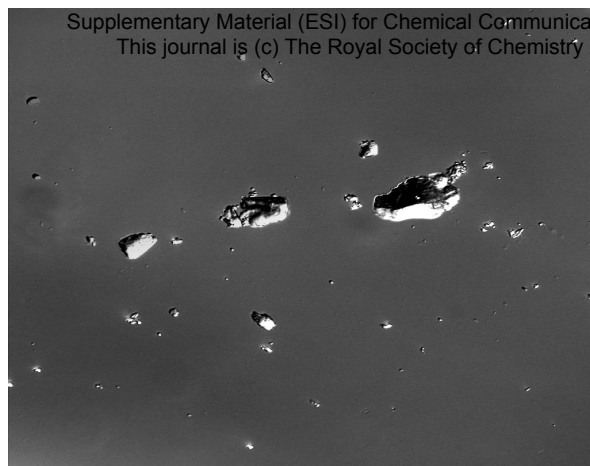
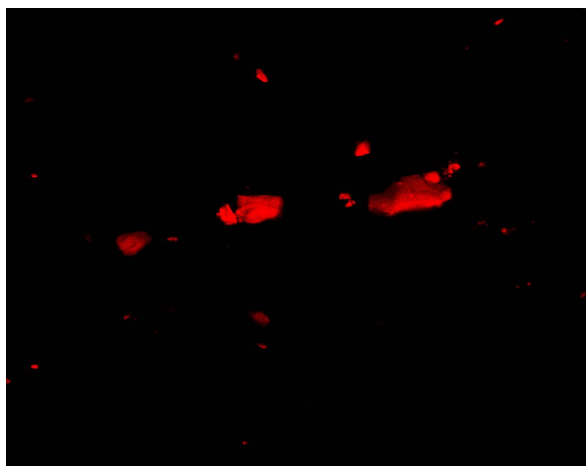


Mixed free Cytochrome C (20 μ l) with free Cytochrome B5 in 500 μ L PBS solution and excited the solution mixture at 495nm for Förster resonance energy transfer (FRET)

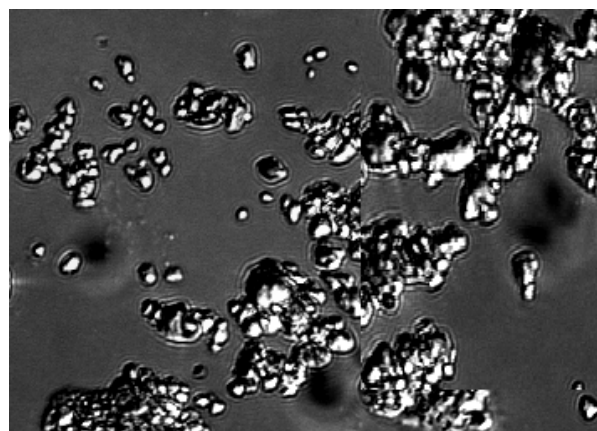
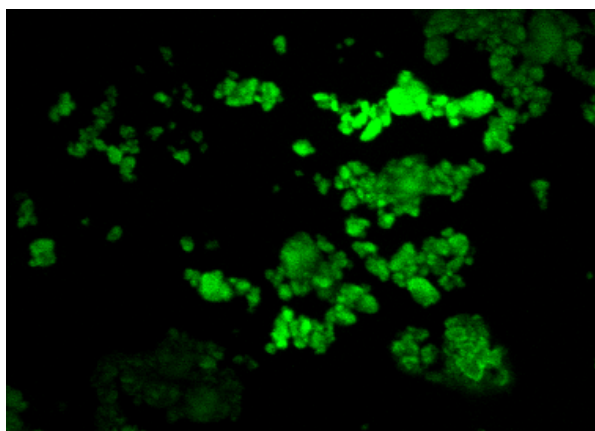


Cyt B5-Af568(red)
Emission:597 nm
Excitation/Absorbance:575 nm

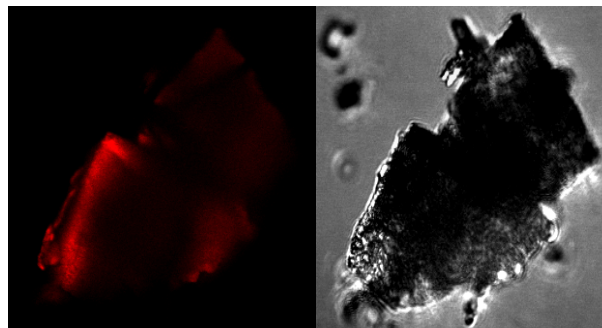
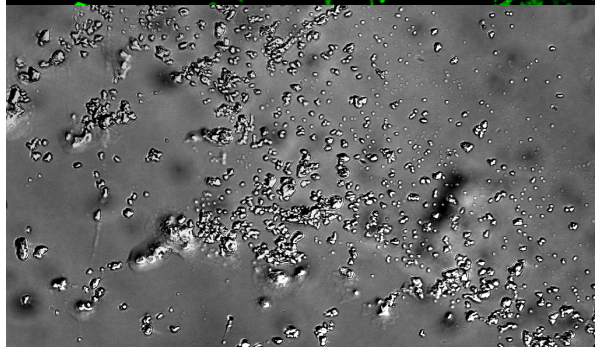
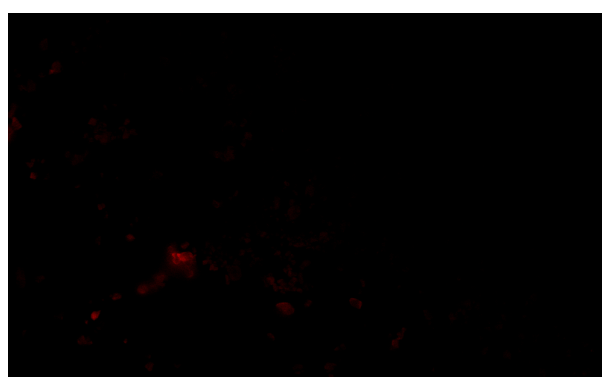
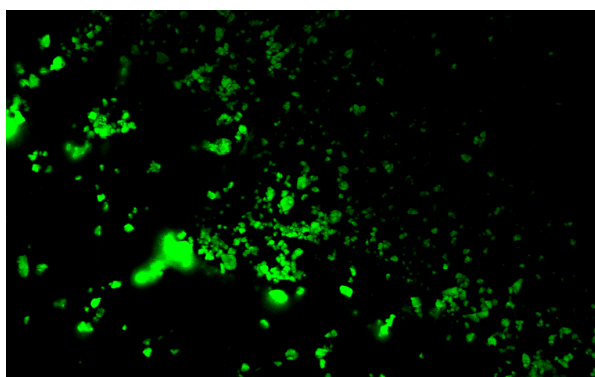
Cyt C-AF488(Green)
Emission:518 nm
Excitation/Absorbance:495 nm



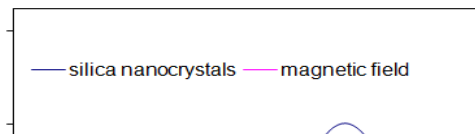
(L) red fluorescence emission with laser excitation at 575nm; **(R)** corresponding optical view to(L)

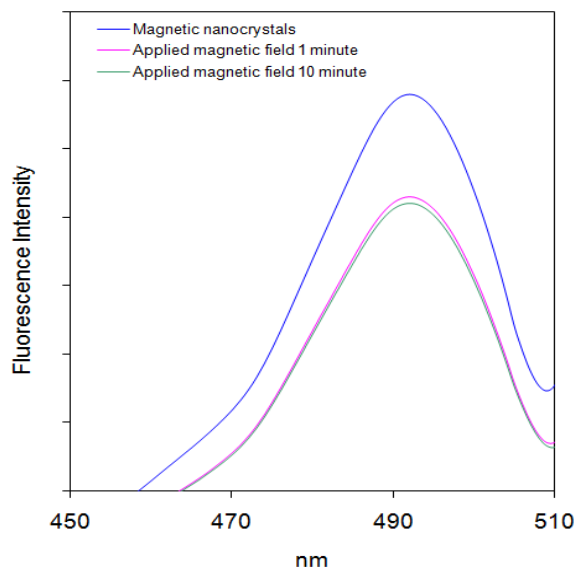


(L) green fluorescence emission with laser excitation at 495nm; **(R)** corresponding optical view to (L)



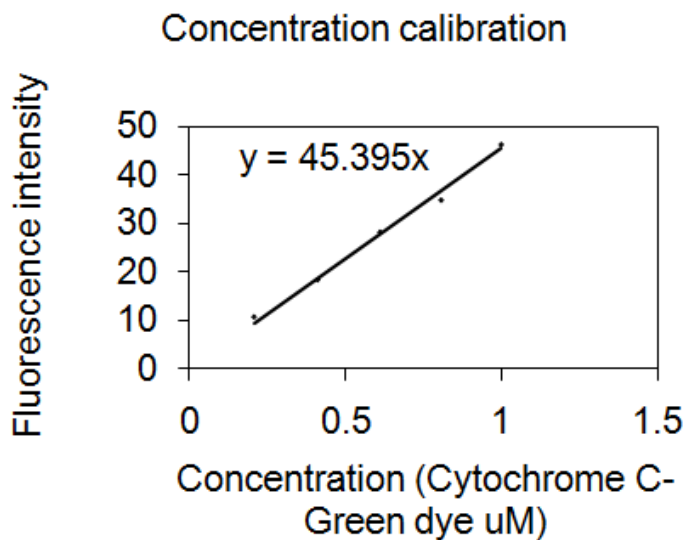
(top L) green fluorescence emission with laser excitation at 495nm; **(top R)** induced red light emission from crystals edges due to FRET; **(bottom L)** optical view; **(bottom R)** enlarged images of top R and bottom L, respectively.



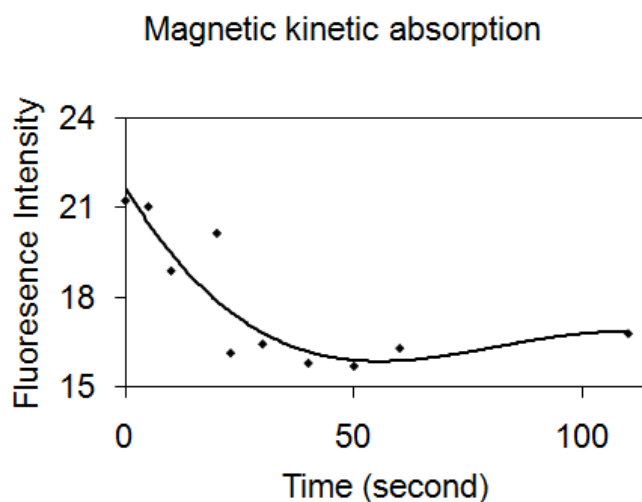


(A)

(B)



(C)



(D)

Fluorescence spectra of before and after applying external saturated magnetic field: (A) 0.81 μM Cytochrome C labeled with green dye at 500 μL PBS immersed with 3.0 mg magnetic super crystals; (B) 0.61 μM Cytochrome C labeled with green dye at 500 μL PBS immersed with 3.0 mg silica nanocrystals (Control experiment without magnetic core); (C) Fluorescence Intensities of Cytochrome C labeled with green dye of known concentrations for response calibration; (D) A studying of magnetic kinetic absorption at different times before equilibrium.