

Supporting Information for  
**Absence of Cytotoxicity towards Microglia  
of Iron Oxide ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) Nanorhombhedra**

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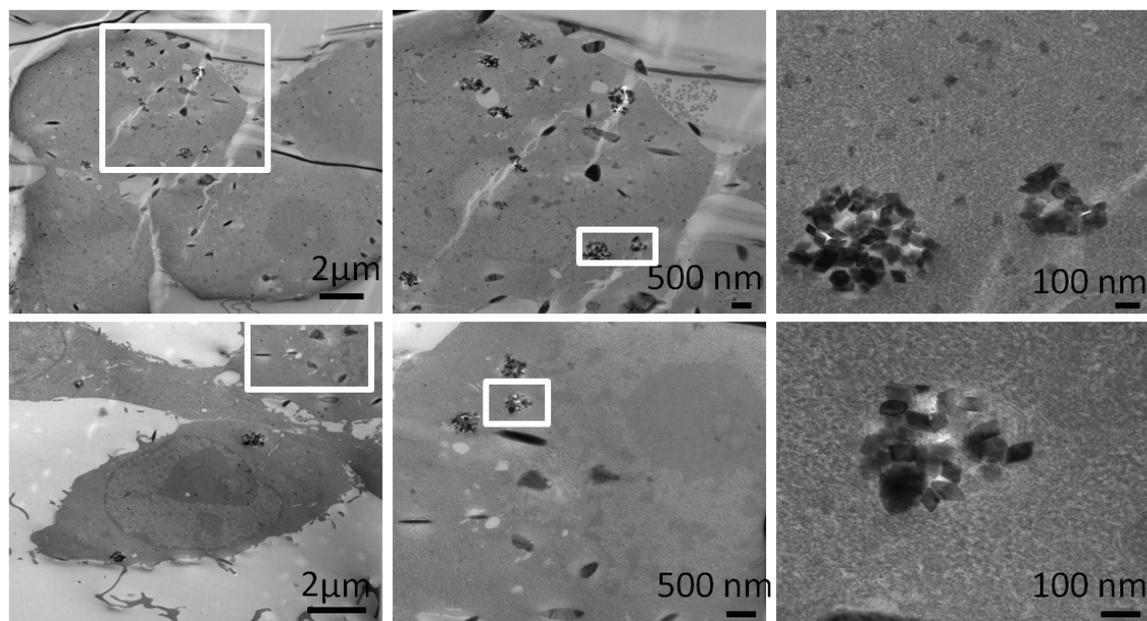
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## **Experimental Section: Additional Protocols I**

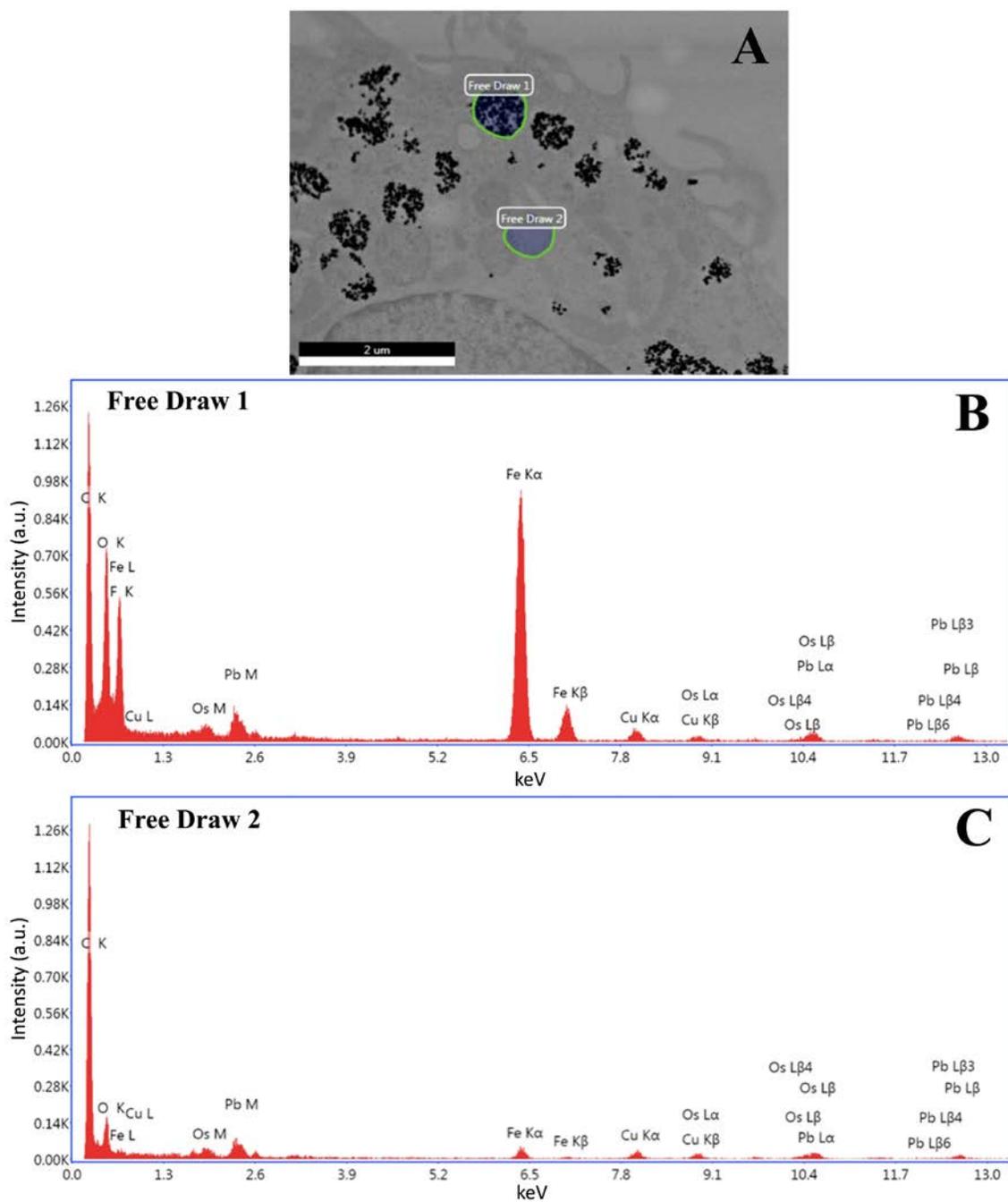
### **Stand-alone $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb Synthesis -**

For the synthesis of average-sized (i.e. ~75 nm)  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb, a reaction protocol from the prior literature was used.<sup>1</sup> Initially, a 0.04 M solution of cetyltrimethylammonium bromide (CTAB) was freshly prepared by adding in 1.458 g to 80 mL of distilled water. The solution was subsequently allowed to stir at 200 rpm for about an hour. After the CTAB was fully mixed in, a solution of 0.01 M of iron chloride (FeCl<sub>3</sub>) was prepared by adding in 0.162 g of FeCl<sub>3</sub> to the CTAB solution followed by stirring for an additional 30 minutes. The solution mixture was subsequently transferred into a 100 mL volumetric flask; distilled water was then added, and the resulting solution was thoroughly shaken and mixed. 18.4 mL of the solution was later placed in a 23 mL Teflon autoclave and oven-heated to 120°C for 12 hours.<sup>1</sup>

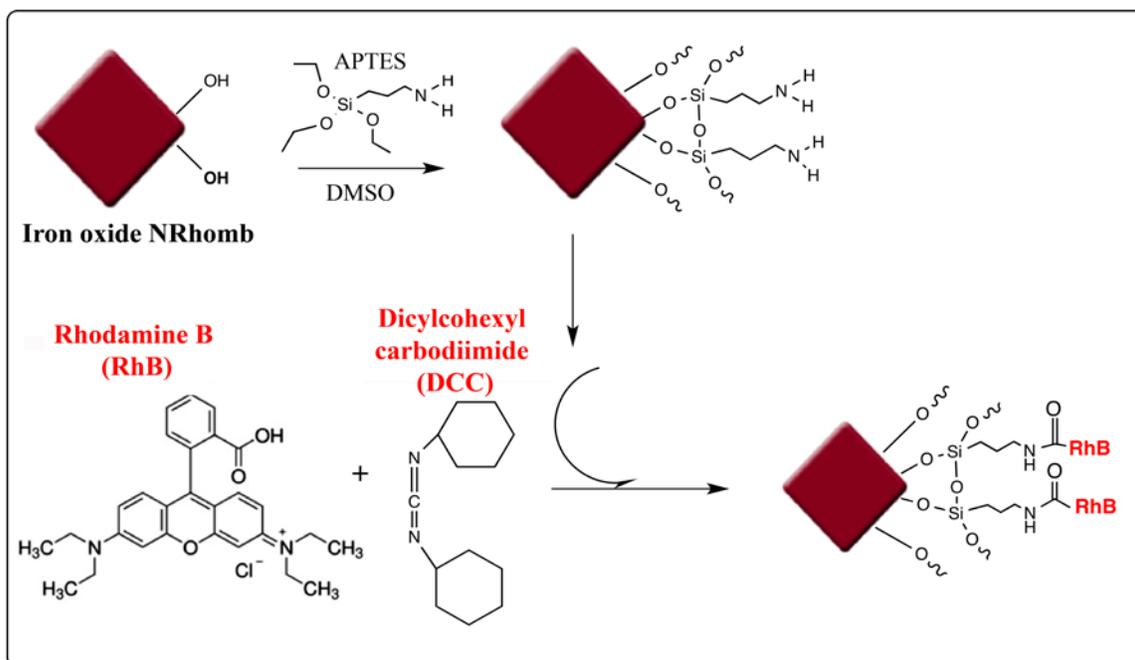
The net result of this hydrothermal process enabled the hydrolysis of the iron chloride precursor and subsequent transformation to the desired Fe<sub>2</sub>O<sub>3</sub>. A slightly modified reaction protocol was employed for the production of smaller sized (i.e. ~47 nm)  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb. Specifically, the concentrations of both CTAB (0.02 M) and FeCl<sub>3</sub> (5 mM) were reduced by half, and the procedure described above was followed.



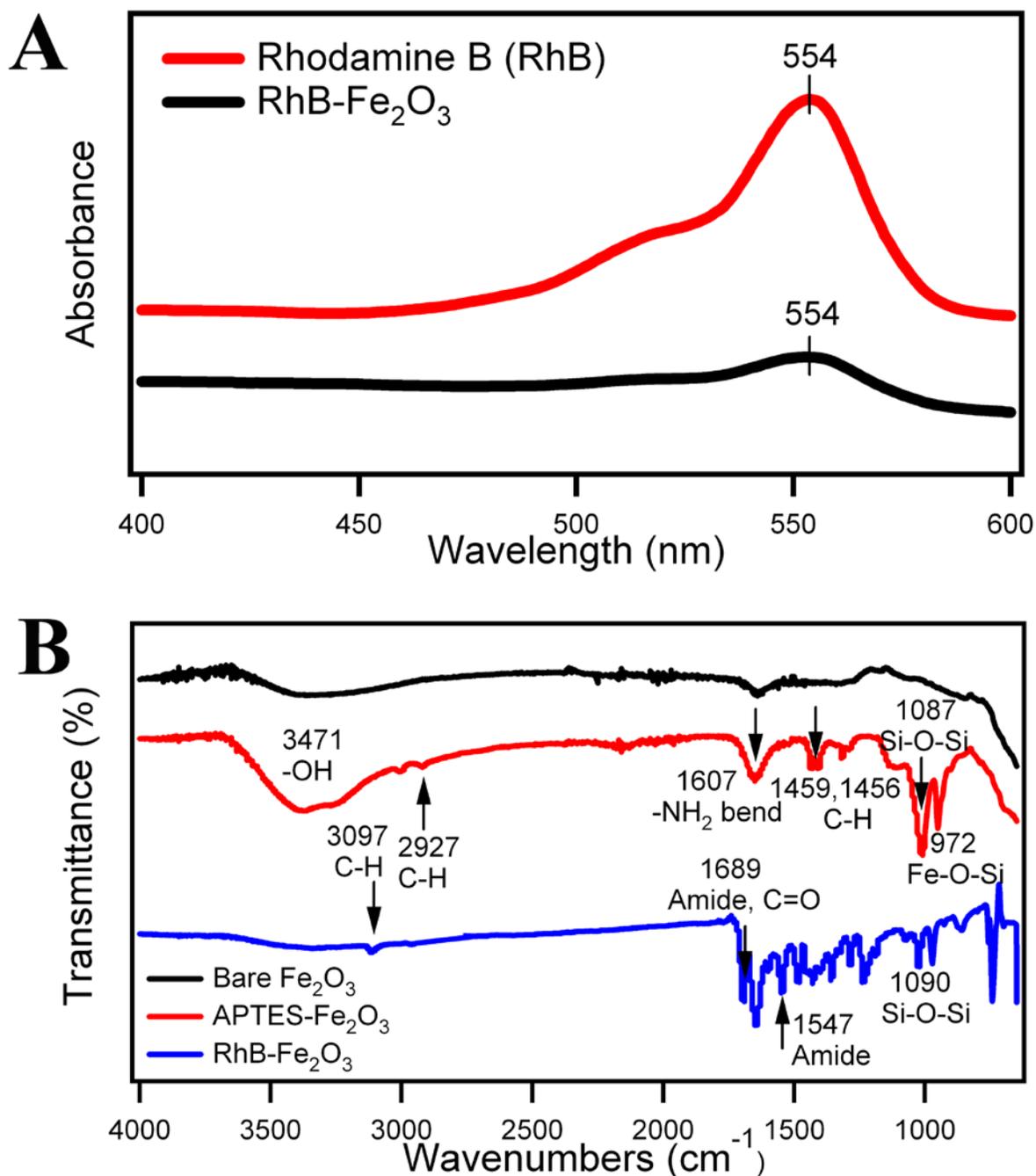
**Figure S1. Cultured N9 microglia engulf bare  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb.** Electron microscopy images of untreated cells and of cells exposed to  $\sim$ 45 nm and  $\sim$ 75 nm bare  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb. White squares surround areas chosen for magnification, which are shown in the subsequent images.



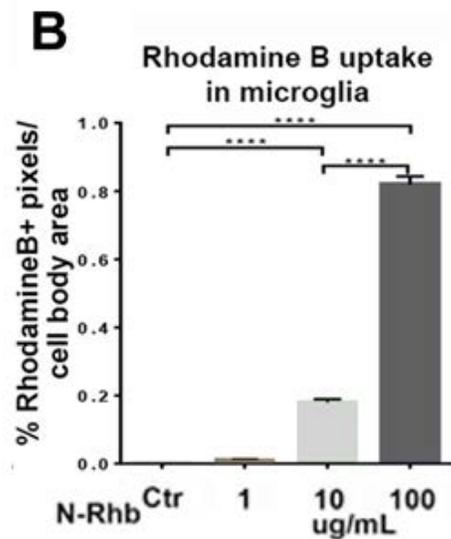
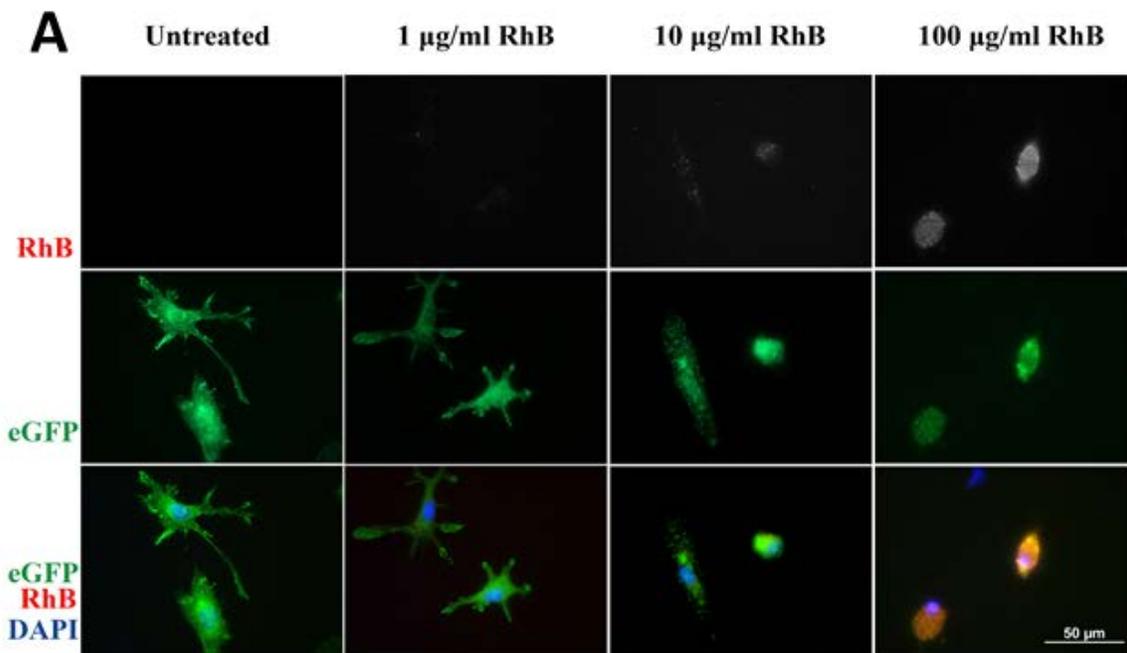
**Figure S2.** Energy-dispersive X-ray spectroscopy data on (A) Fe<sub>2</sub>O<sub>3</sub> N-Rhomb-containing microglia cells in a region incorporating Fe<sub>2</sub>O<sub>3</sub> N-Rhomb (B) and in an area without Fe<sub>2</sub>O<sub>3</sub> N-Rhomb (C).



**Figure S3.** Reaction schematic for the conjugation of RhB dye onto  $\alpha\text{-Fe}_2\text{O}_3$  N-Rhomb.



**Figure S4. Characterization of RhB-labeled N-Rhomb.** (A). UV-visible spectra of RhB organic dye (red line) and RhB-functionalized  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb (black line). (B). Infrared spectra of bare  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb (black line), APTES-functionalized Fe<sub>2</sub>O<sub>3</sub> (red line), as well as RhB-labeled  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb (blue line), respectively.



**Figure S5. Uptake of bare Rhodamine B in microglia cells. (A).** Confocal images of microglia exposed to 0 (Untreated), 1, 10, and 100  $\mu\text{g/mL}$ , respectively, of bare RhB. DAPI was used to stain cell nuclei. Scale bar = 50  $\mu\text{m}$ . **(B).** Quantification of the RhB fluorescence of microglia treated with bare RhB at varying concentrations. Data are shown as mean  $\pm$  SEM. \*\*\*\*  $p < 0.0001$ .

## **Experimental Section: Additional Protocols II.**

### **Chemical Modification of $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb - RhB -functionalized 47 nm $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> N-Rhomb**

As a first, preparative step towards coating our iron oxide nanoparticles with Rhodamine, the surfaces of our 'smaller-sized' nanostructures were first chemically modified with amine-terminated groups, derived from aminopropyl triethoxy silane (APTES) as described by a prior methodology.<sup>2</sup> Briefly, 0.1 g of Fe<sub>2</sub>O<sub>3</sub> NRhomb and 70 mL of dimethyl sulfoxide (DMSO) were added to a 150 mL Schlenk-line flask. The flask was then de-gassed with pure nitrogen (N<sub>2</sub>), stirred, and heated at 85°C for 26 hours. The product was subsequently washed in DMSO and heated at 120°C for 22 hours in order to thermally 'cure' it, a process which both stabilizes and strengthens the crosslinking APTES network onto the Fe<sub>2</sub>O<sub>3</sub> nanostructures. The product was then collected and the APTES bound Fe<sub>2</sub>O<sub>3</sub> NRhomb, with the desired pendant amine groups exposed, were used for subsequent conjugation with the Rhodamine B (RhB) dye.

Hence, to functionalize our rhombohedra with the labeled dye (Figure S3), 1 mg of RhB (95%, Aldrich) was dispersed in 10 ml DMSO and the exposed carboxyl groups of the RhB dye were activated using 35 mg of 1,3-dicyclohexyl-carbodiimide (DCC), a biochemically inspired coupling agent. Subsequently, ~1.6 mg of amine-terminated, APTES modified Fe<sub>2</sub>O<sub>3</sub> was added to the reaction mixture. The solution was allowed to stir for 24 hours at room temperature under dark conditions, thereby yielding the desired covalent amide bond linkages between the RhB molecules and the APTES-functionalized Fe<sub>2</sub>O<sub>3</sub> NRhomb. The product was subsequently washed in water for 3 times and later dispersed in a cell culture medium at a final concentration of 1 mg/mL.

## **Results – Structural Characterization.**

### **Conjugation of RhB labeled-Fe<sub>2</sub>O<sub>3</sub> N-Rhomb**

The conjugation of RhB onto Fe<sub>2</sub>O<sub>3</sub> N-Rhomb can be explained from the reaction scheme in Figure S2. It is apparent that the DCC carbodiimide coupling agent aids in activating the pendant carboxyl groups of the RhB, which can then readily attach onto the amine-terminated Fe<sub>2</sub>O<sub>3</sub> with simple stirring for 24 hours. The presence of the fluorescent dye RhB incorporated within RhB-labeled N-Rhomb Fe<sub>2</sub>O<sub>3</sub> was subsequently confirmed using UV-visible and IR spectroscopy (Figure S3), as well as with confocal microscopy.

For UV-visible data, the nanostructures gave rise to a maximum of ~565 nm, corresponding to the known absorption peak of the organic dye RhB (Figure S3A). In terms of IR analysis (Figure S3B), the RhB-conjugated Fe<sub>2</sub>O<sub>3</sub> N-Rhomb were compared with both bare Fe<sub>2</sub>O<sub>3</sub> and with APTES-functionalized Fe<sub>2</sub>O<sub>3</sub>, prior to covalent attachment. For bare Fe<sub>2</sub>O<sub>3</sub> and APTES-functionalized Fe<sub>2</sub>O<sub>3</sub>, both materials exhibited OH stretching vibrations, located at ~3471 cm<sup>-1</sup>. The amino-silanized Fe<sub>2</sub>O<sub>3</sub> spectrum gave rise to peaks, located at ~972 cm<sup>-1</sup> and ~1607 cm<sup>-1</sup>, respectively, corresponding to a Fe-O-Si band and a primary NH<sub>2</sub> stretch, respectively. As for the RhB-conjugated Fe<sub>2</sub>O<sub>3</sub> N-Rhomb (Figure S3B), stretching vibrations appeared at ~1547 cm<sup>-1</sup> and 1689 cm<sup>-1</sup>, respectively, which were associated with the expected vibrational signature of amide NH bonds. Additional peaks in the fingerprint region could be correlated with the known structure of the RhB compound.

## References

1. Z. Pu, M. Cao, J. Yang, K. Huang and C. Hu, *Nanotechnology*, 2006, **17**, 799-804.
2. L. Ye, R. Pelton and M. A. Brook, *Langmuir*, 2007, **23**, 5630-5637.