# Supplementary Information

## Sophorolipids-functionalized iron oxide nanoparticles

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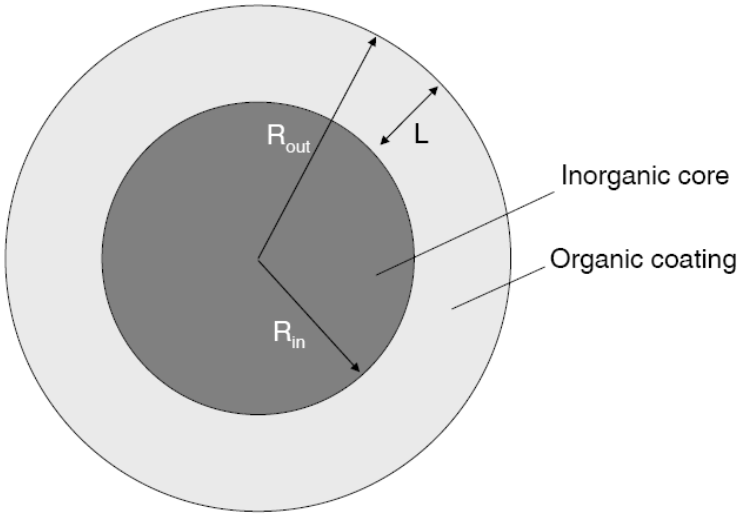
### Synthesis of acidic sophorolipids

A fed-batch fermentation was run in a Biostat® B culture vessel (Sartorius-BBI Systems) with a maximum working volume of 1.5 L. Temperature (30 °C), pH (3.5), stirring rate (800 rpm) and airflow rate (1 vvm) were controlled by the Biostat® B control unit. 100 mL of an overnight grown shake flask culture was used to inoculate the fermentor. For maintaining a stable pH, appropriate amounts of 5 M NaOH were used. There was no correction for a too alkaline pH and fermentation started at pH 5.8 and was allowed to drop spontaneously till 3.5. Later unalterable incensement was seen as the end of the fermentation process. Feeding of rapeseed oil (Sigma) was started 48 hours after inoculation, and was adjusted to the consumption rate. Additional glucose (50 g/L) was added 150 hours after inoculation. Sophorolipids were extracted by the following procedure: 3 volumes of ethanol were added to the fermentation medium and yeast cells were removed by centrifugation. The water-ethanol mixture of the supernatants was removed under reduced pressure in a rotavapor. 2 volumes of ethanol were added to dissolve the sophorolipids and the residual hydrophobic carbon source. The mixture was passed over a Whatman filter to remove the water soluble components and ethanol was evaporated under reduced pressure in a rotavapor. Solid substances were dissolved in water (pH 6.5) and residual oil and fatty acid were extracted by an equal volume of hexane. The sophorolipid mixture (about 80 % C18:1) is comparable to what it was reported by other authors.[1] The obtained sophorolipid mixture containing acidic and lactonic forms and di-, mono- and un-acetylated molecules, was hydrolysed with 5 M NaOH solution and extracted with pentanol according to procedure described in Ref. [2], giving rise to the un-acetylated acidic COOH form (17-L-([2'-O-$\beta$-D-glucopyranosyl-$\beta$-D-glucopyranosyl]-oxy)-octadecenoic acid). An average downfield 5 ppm shift from 172 ppm to 177 ppm in the $^{13}$C NMR spectra of these compounds confirms the occurrence of the free COOH group. The final compound is mainly (> 90%) constituted by acidic sophorolipids, as also verified by $^{1}$H NMR (results not shown).

### Core-shell model for the calculation of the area per molecule

The density of complexing compounds on the surface of small-scale spherical objects can be expected to vary as a function of the curvature radius of the object itself, because the

exposed surface area varies with the radius. In a trivial core-shell model, where the core represents a dense spherical particle and the shell identifies the external organic layer, the variation of the area per adsorbed molecule as a function of the core radius can be estimated on the basis of the intrinsic mass density of the organic compound, if one assumes that density is independent of the particle radius, hence curvature effects and effect of external physico-chemical parameter on the local molecular packing (a more critical discussion of this model is given in the main text).




**Scheme 1 – Core-shell model of a coated mineral nanoparticle**

For a single coated nanoparticle, as shown in Scheme 1, one can identify two radii, $R_{in}$ and $R_{out}$, where $R_{in}$ is the core radius of the bare, non-functionalized, particle, and $R_{out}$ is the radius of the bare particle plus the organic layer of size $L$, so that,

$$R_{out} = R_{in} + L$$

The volume corresponding to the organic layer alone, $V_o$, is

$$V_o = V_{out} - V_{in}$$

where, for a spherical geometry

$$V_{out} = (4/3)*\pi*R_{out}^3$$

$$V_{in} = (4/3)*\pi*R_{in}^3$$

To calculate the theoretical surface area per molecule, $s_c/N$, the calculated nanoparticle surface is

$$s_c = 4*\pi*R_{in}^2$$

and one can use the density, $\rho$, and molar mass, $M$, of the organic compound

$$N = V_o*(\rho/M)*N_a$$

where $N_a$ is the number of Avogadro. Rearrangements of these equations provide the calculated surface per molecule, $s_c/N$, given in Eq.1

$$\frac{s_c}{N}(R_{in}) = \frac{4\pi R_{in}^2}{\frac{4}{3}\pi\left[(R_{in}+L)^3 - R_{in}^3\right]\left(\frac{\rho}{M}\right)N_a} = \frac{MR_{in}^2}{\left[R_{in}^2L + R_{in}L^2 + \frac{1}{3}L^3\right]\rho N_a} \qquad \text{Eq.1}$$

In a real system constituted of functionalized nanoparticles, the surface area can be estimated through experimental techniques. The corresponding to the nanoparticles alone, $S_m$ (the subscript m stands for "measured") is the product of $s_c$ with the total number of nanoparticles, $N_p$

$$S_m = s_c*N_p$$

If the crystallographic structure (hence, the density, $\rho_{np}$) and the geometry of the nanoparticles are known from XRD and TEM, one can also estimate the total amount of inorganic, $m$, and organic, $m_o$, matter from TGA. In this case, $N_p$ is given by the ratio of the overall nanoparticle volume, $V_{np}$, to the volume of a single nanoparticle,

$$N_p = V_{np}/V_{in} = (m/\rho_{np})/V_{in}$$

As for the total number of surface molecules, $N$, it can calculated from $m_o$ and its molar mass

$$N = (m_o/M)*N_a$$

Rearrangements of terms above are given in Eq.2 for the estimation of the measured surface per nanoparticle:

$$\frac{S_m}{N}(R_{in}) = \frac{\frac{s_c}{V_{in}}\frac{m}{\rho_{np}}}{\frac{m_0N_a}{M}} = \frac{3mM}{R_{in}\rho_{np}m_0N_a} \qquad \text{Eq.2}$$

For sake of clarity, please note that in this work the following equivalence holds: $R_{in} \equiv d_{TEM}/2$, where $d_{TEM}$ refers to the average nanoparticle diameter measured by TEM.

**$^{57}$Fe Mössbauer spectroscopy**

Additional information on the physico-chemical and magnetic nature of samples can obtained by $^{57}$Fe Mössbauer spectroscopy. The room temperature Mössbauer spectrum of sample 2S-80C where the SL were added only after the precipitation of the iron oxide particles (Figuire

S1a, top), exhibits a relatively broad and asymmetric magnetic sextet centred at $\delta = 0.34(1)$ mm/s which can be fitted assuming a distribution of hyperfine magnetic fields (Figure S1b) and a very narrow quadrupole shift $\varepsilon = 0.01(1)$ mm/s. The strongest contribution to the hyperfine field distribution is centred at a field of about 46 T. These values agree well with the presence of high spin trivalent iron in multiple octahedral and tetrahedral sites in maghemite ($\gamma$-$Fe_2O_3$),[3,4] which is derived from magnetite upon spontaneous oxidation of $Fe^{2+}$ sites. The relatively low value of the maximum hyperfine field compared to literature values of well-crystallized maghemite together with the asymmetric shape of the Mössbauer profiles testify either from the finely divided nature of the maghemite particles which start to undergo superparamagnetic relaxation at room temperature, or for the presence of defects in the crystalline structure of the solid. The very slight difference in intensity observed between the corresponding lines of the spectrum at high and low velocity might be due to the presence of small amounts of magnetite in this sample.[3]

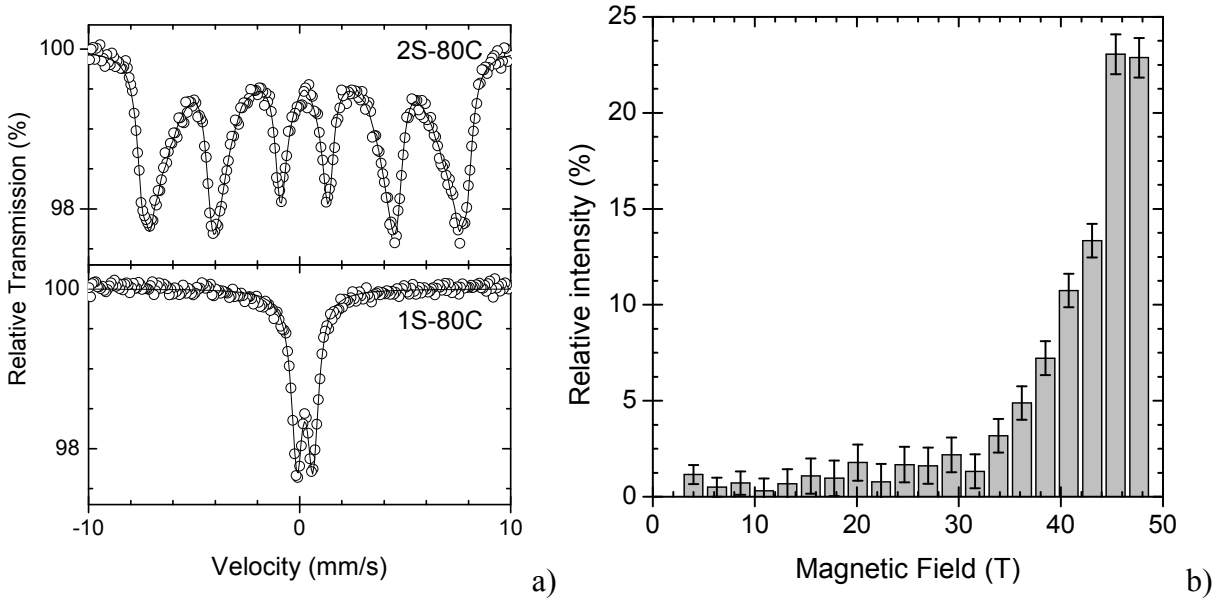



**Figure S1 – a) Room temperature $^{57}$Fe Mössbauer spectra of the sophorolipid-containing iron oxide nanoparticles obtained at T= 80°C in the two-step (2S-80C) and one-step (1S-80C) synthesis processes; b) Hyperfine magnetic field distribution obtained from the fit of the spectrum of sample 2S-80C.**

On the other hand, the room temperature Mössbauer spectrum of sample 1S-80C, where the oxide particles were precipitated in the presence of SL (Figure S1a, bottom) presents a relatively broad quadrupole doublet with an isomer shift $\delta = 0.36(1)$ mm/s and a quadrupole splitting $\Delta = 0{,}76(1)$ mm/s. Such parameters are typical of high spin trivalent iron, and agree well with the presence of superparamagnetic particles of ferrihydrite in this sample.

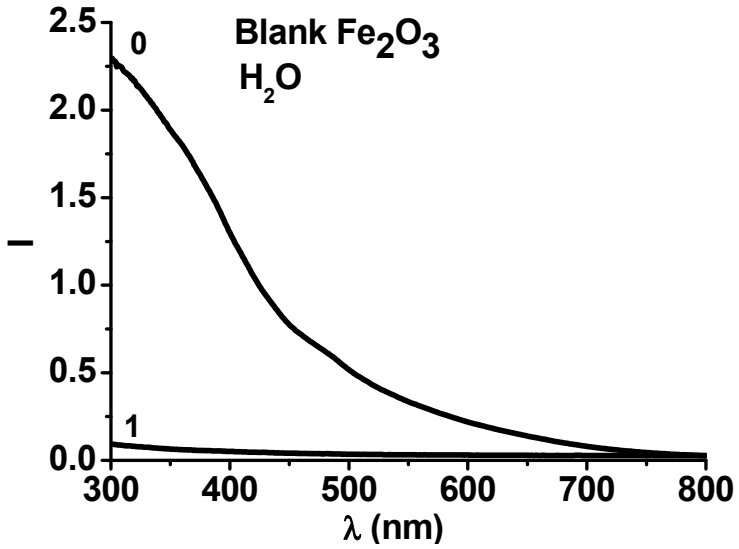



**Figure S2 – UV-vis spectra recorded on the as-prepared (0-label) and one-time filtered (1-label) blank $Fe_2O_3$ nanoparticles solution in water. Filter pore size is 0.20 μm.**

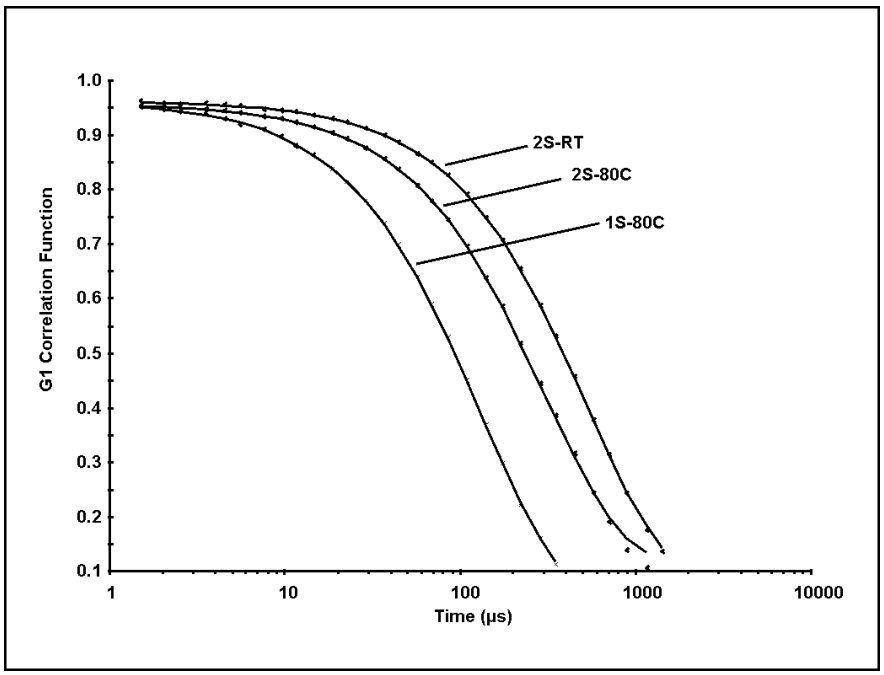



**Figure S3 – Typical correlograms obtained after DLS experiments performed on the sophorolipid-functionalized iron oxide nanoparticles synthesized at in two-steps (2S) at T= 80°C (80C) and room temperature (RT). For comparison, the correlogram of the 1S-80C sample is also reported. Each curve is averaged over three different experiments. The longer the persistency in time of the correlation function, the larger the size of the scattering objects. Fits have been done using the cumulant method.**

**TGA analysis for sophorolipids-functionalized iron oxide nanoparticles and additional information to evaluate the area per molecule.**

Eq. 2 has been used to estimate the area per molecule. The mass loss, $m_o$, due to the organic compound, here sophorolipids, has been determined from TGA curves (Figure S4) between about 250°C and 600°C. The systematic mass loss at 200°C is attributed to strongly bonded water molecules.[5]

Additional pieces of information needed to calculate the area per molecule according to Eq.2 are the mass densities, $\rho_{np}$, which for ferrihydrite and maghemite are 3.8 g/cm$^3$ and 4.9 g/cm$^3$ and the average radii of the inorganic core, $R_{in}$, measured by TEM (Figure 2 and Table 2). For 1S-80C and 2S-80C that is 1.4 nm and 4.3 nm.

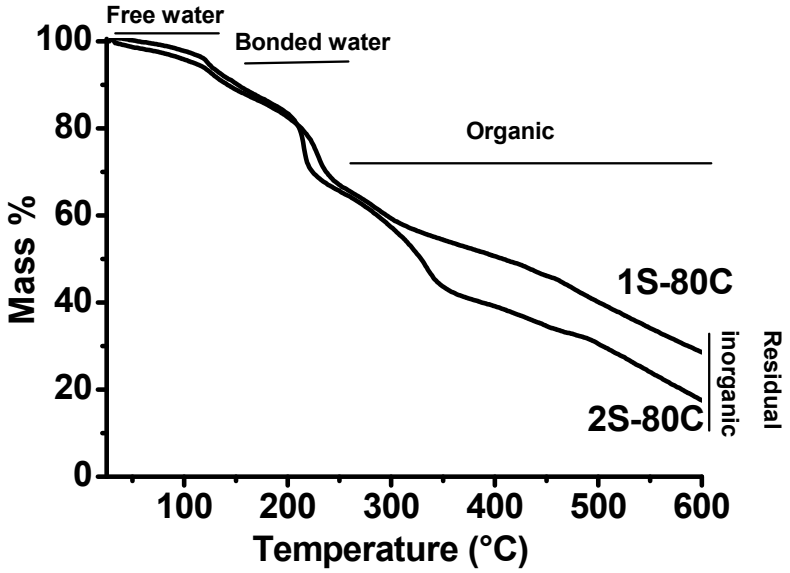



**Figure S4 – Typical TGA curves recorded on the one-step (1S) and two-step (2S) sophorolipid-functinalized iron oxide nanoparticles at T= 80C.**

[1] Davila, A.-M.; Marchal, R.; Vandecasteele J.-P. *J. Ind. Microbiol.* **1994**, *13*, 249-257
[2] U. Rau, R. Heckmann, V. Wray, S. Lang, *Biotechnol. Lett*., **1999**, 21, 973-977
[3] G. M. da Costa, E. De Grave, P. M. A. de Bakker, R. E. Vandenberghe, *J. Solid State Chem*., **1994**, 113, 405-412
[4] R. M. Cornell, U. Schwertmann, *The Iron Oxides*, Ed VCH Weinheim, 1996
[5] a) S. Yu, G. Moog Chow, *J. Mater. Chem*., **2004**, 2781-2786; b) G. E. Kellum, R. C. Smith, *Anal. Chem*., **1967**, 39, 341-345)