Electronic Supplementary Information (ESI) for

Metal carbonyls supported on iron oxide nanoparticles to trigger the CO-gasotransmitter release by magnetic heating

Peter C. Kunz[a,]*, Hajo Meyer[a], Juri Barthel[b], Silvio Sollazzo[c], Annette M. Schmidt[c,]* and Christoph Janiak[a,]*

[a] Institut für Anorganische Chemie und Strukturchemie, Heinrich-Heine-Universität, Universitätsstr. 1, D-40225 Düsseldorf, Germany. Fax: (+)49 211 8112287.
E-mail: peter.kunz@uni-duesseldorf.de, hajo.meyer@uni-duesseldorf.de, janiak@uni-duesseldorf.de

[b] Ernst Ruska-Centrum für Mikroskopie und Spektroskopie mit Elektronen, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany. E-mail: ju.barthel@fz-juelich.de

[c] Institut für Physikalische Chemie, Universität zu Köln, Luxemburger Str. 116, D-50939 Köln, Germany.
E-mail: sisollazzo@aol.com, annette.schmidt@uni-koeln.de

Experimental section

All chemicals were used as received: Iron(III) chloride hexahydrate (99%, Grüssing GmbH), iron(II) chloride tetrahydrate (98%, Alfa Aesar), iron(III) nitrate (99%, UCB Chemie GmbH), ammonia (25%, VWR), hydrochloric acid (37%, VWR), nitric acid (65%, AppliChem), acetone (p.a., VWR), DL-3,4-dihydroxy-phenylalanine (99%, Sigma-Aldrich), myoglobin from equine skeletal muscle (95%, Sigma-Aldrich), dodecacarbonyl ruthenium (99%, ABCR), sodium hydroxide (99%, ABCR), ethanol (p.a., VWR), chloroform (p.a., VWR), acetone (p.a., VWR), 3-morpholino-propanesulfonic acid (99%, Fluka). Deionized water with a resistance of 0.05 µS/cm was prepared by a Synergy (Millipore) device. All reactions and workup was carried out under ambient air atmosphere unless stated otherwise.

IR-spectra where recorded on a Bruker Tensor 37 IR-spectrometer with KBr discs. IR spectroscopic measurements of the black-colored IONP-CORMs in attenuated total reflectance (ATR) mode gave only very small signal intensities. After optimization IR-spectra of IONP-CORMs were obtained as KBr discs. For this, the functionalized nanoparticles **2** and **3** were ground for prolonged time with dry KBr to achieve a fine distribution. Care was taken to keep the IONP-CORM concentration low (1-2 mg / 30-50 mg KBr) because of strong absorption bands. The integration time was set to 50 scans per wave number to obtain good quality spectra.

Powder X-ray diffractograms (PXRD) were measured on a Bruker-D2 Phaser (Cu-Kα, λ = 1.5406 nm; 14 h exposure time; detector set to 0.25 V of the lower detection limit).

Dynamic light scattering (DLS) measurements were carried out on a Malvern Zetasizer Nano S with a 4 mW He-Ne Laser (633 nm) in 173° backscattering mode. The analyte (10 mg) was dispersed in 5 mL of deionized water by sonication and transfered to a cuvette. The cuvette with the dispersion was equilibrated at 20 °C for 300 s. Each measurement lasted 600 s and a refractive index of 2.63 was used for maghemite.[1]

Inductively-coupled plasma optical emission spectra (ICP-MS) were collected at the "Laboratorium der Urkantone, by Dr. Nikos Agorastos (nikos.agorastos@laburk.ch), Föhneneichstr. 15, Postfach 363 6440 Brunnen, Switzerland" with a Perkin Elmer Elan DRC-e (Dynamic Reaction Cell environmental).

Transmission electron micrographs (TEM) were obtained with a Fei Titan 80-300 TEM at Ernst-Ruska Centrum (ER-C) of Forschungszentrum (FZ) Jülich. For the sample preparation 5 mg of the particles

---

[1] Handbook of Chemistry and Physics 78th ed., 1997/98 (1997)

were dispersed by sonication in dimethyl sulfoxide (DMSO, 2 mL). One drop of this dispersion was placed on holey thin film of amorphous carbon on a copper grid. After a contact time of 5 min the grid was floated on a water surface and held vertically to rinse of the solvent.

Syntheses

Maghemite nanoparticles (Fe_2O_3-NPs) (1):

Maghemite-NPs were synthesized by dissolving 3.26 g (16.4 mmol) of iron(II) chloride tetrahydrate and 8.69 g (32.1 mmol) of iron(III) chloride hexahydrate in 380 mL of deionized water and adding dropwise 20 mL of 25% ammonia under vigorous stirring. The black precipitate was sedimentated with a permanent magnet and the supernatant decanted and discarded. Nitric acid (60 mL, 2 mol/L) was added to the solid and stirred for 5 min. After the addition of 60 mL (0.35 mol/L) iron(III) nitrate solution the suspension was heated to reflux for 1 h. The solid was again sedimentated with a permanent magnet and washed twice with 40 mL (2 mol/L) nitric acid and twice with acetone (50 mL each). Finally the solid was dried under vacuum. Yield 2.18 g. A powder X-ray diffractogram confirmed the maghemite phase (Fig. S1).

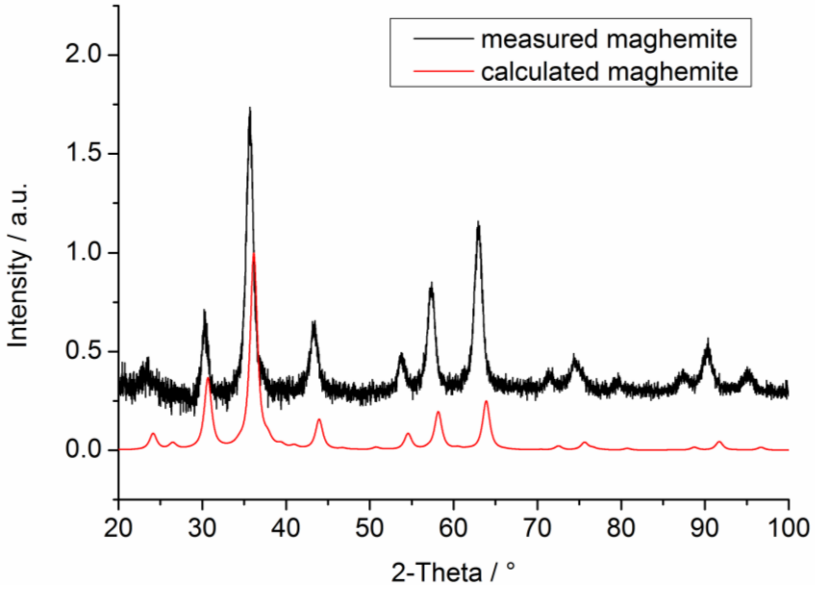
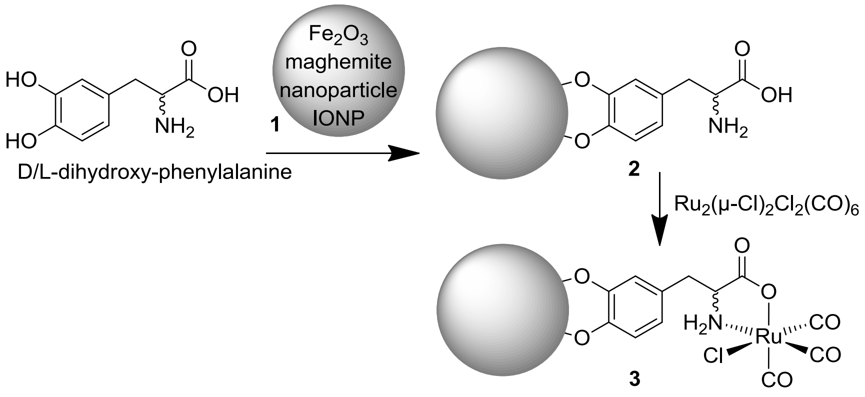


Figure S1 Measured and simulated powder X-ray diffractogram of maghemite Fe_2O_3-NPs **1**.

D/L-Dihydroxy-phenylalanine-(D/L-DOPA-)functionalized maghemite-NPs (2):



Maghemite-NPs **1** (100 mg) were dispersed in 100 mL of deionized water. D/L-3-(3,4-Dihydroxyphenyl)-alanine (D/L-DOPA) (160 mg) was dissolved in 50 mL of deionized water by sonication. Both solutions were combined and the pH adjusted to 7.4 by addition of an NaOH solution. The solid was sedimentated with a permanent magnet and washed twice with acetone (50 mL each). Yield: 190 mg. IR (KBr): $\tilde{\nu}/cm^{-1}$ = 1619 (m), 1484 (m), 1404 (w), 1384 (s), 1260 (m), 1221 (w), 1116 (w), 810 (w), 631 (s), 577 (s).

The diameter and its variance of the maghemite-NPs **1** and the D/L-DOPA-functionalized maghemite-NPs **2** were determined from DLS measurements (Fig. S2).

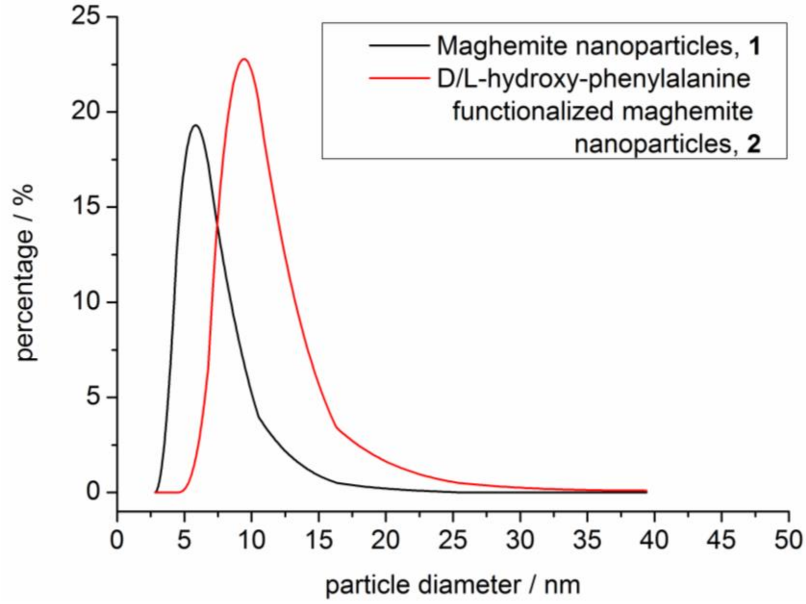


Figure S2 DLS curves of particle diameters for maghemite-NPs **1** and D/L-DOPA-functionalized maghemite-NPs **2**.

CORM-functionalized maghemite-NPs, CORM@IONP (3):

D/L-DOPA-functionalized maghemite-NPs **2** (95 mg), $[Ru_2Cl_4(CO)_6]^2$ (90 mg, 0.18 mmol) and 50 mg of sodium methanolate were dispersed/dissolved under nitrogen atmosphere in 40 mL of methanol. The suspension was heated to reflux for 2 h and the solid was sedimentated with a magnet. After washing three times with acetone (10 mL each) the solid was dried under vacuum. Yield: 80 mg. IR (KBr): $\tilde{\nu}/cm^{-1}$ = 3417 (br, s), 2961 (w), 2925 (w), 2856 (w), 2360 (w), 2238 (w), 2059 (m), 1986 (m), 1619 (s), 1482 (m), 1387 (w), 1262 (m), 1114 (w), 806 (w), 631 (s), 581 (s).

For CORM-3 a facial configuration was found for the $Ru(CO)_3$ fragment.[3] Detailed IR-spectroscopic investigations on CORM-3 showed a strong pH-dependence of the CO bands concerning their position and number (3 versus 2 bands) (s. "Fig. 9" from the Johnson publication[3] on p. 7 here in the ESI). In acidic solution the amino-carboxylato chelate ring is opened by protonation of the carboxylate group. In basic solution there is a nucleophilic attack of an OH^--group onto a CO ligands with formation of a [Ru]-C(=O)OH-ligand. Further addition of base deprotonates this ligand to $[Ru]-C(=O)O^-$. However, this pH-dependent transformation of the $Ru(CO)_3$ fragment does not coincide with a loss of CO.[3]

The maghemite-(Fe_2O_3-)NPs are most likely basic on their surface due to the pH adjustment to 7.4 by addition of an NaOH solution upon the preparation of **2** and due to the free amino acid groups. The isoelectric point of phenylalanine is 5.48.[4]

Under basic conditions with one equivalent of NaOH, CORM-3 shows only two strong bands at 2058 and 1985 cm^{-1}.[3]

The diameter and its variance of the CORM-functionalized maghemite-NPs **3** were determined from TEM pictures (Table S1, Fig. S4 and Fig. S5). The CORM-functionalized maghemite-NPs **3** could not be efficiently dispersed anymore in water for a DLS measurement.

Table S1 Particle diameter (number average) of the CORM-functionalized maghemite-NPs **3**.

number of particles with diameter measured	141
average diameter / nm	9

---

[2] A. Mantovani and S. Cenini, *Inorg. Synth.* **1976**, *16*, 51–53.

[3] T. R. Johnson, B. E. Mann, I. P. Teasdale, H. Adams, R. Foresti and C. J. Green, R. Motterlini, *Dalton Trans.* **2007**, 1500–1508.

[4] H.-D. Jakube and J. Jeschkeit, in *Peptide, Proteine,* Verlag Chemie, Weinheim, 1982, p. 40.

standard deviation σ / nm	2

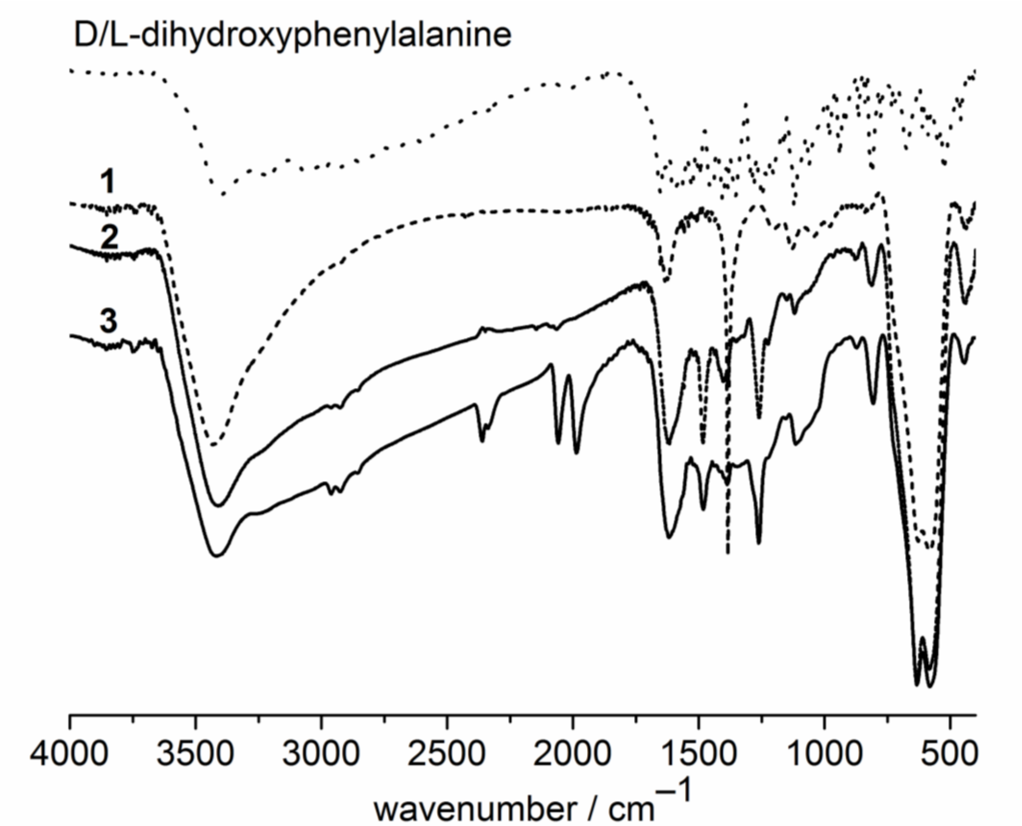


Figure S3 IR-spectra (in KBr) of D/L-dihydroxyphenylalanine, the non-functionalized (**1**) and functionalized nanoparticles (**2** and CORM@IONP, **3**).

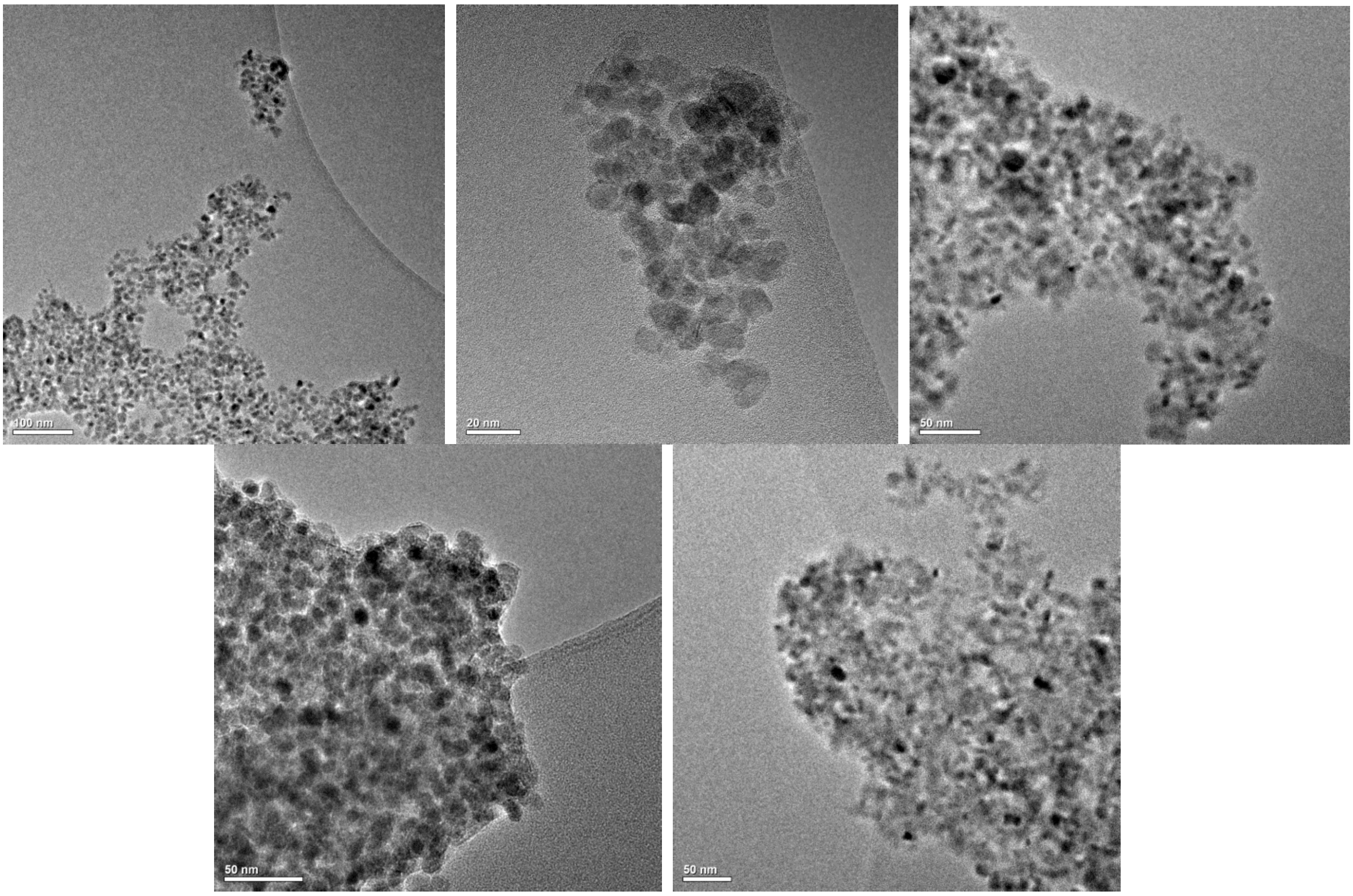


Figure S4 TEM pictures of CORM-functionalized maghemite-NPs **3** which were used for the statistical evaluation in Table S1 (Fei Titan 80-300 TEM, ER-C FZ Jülich).

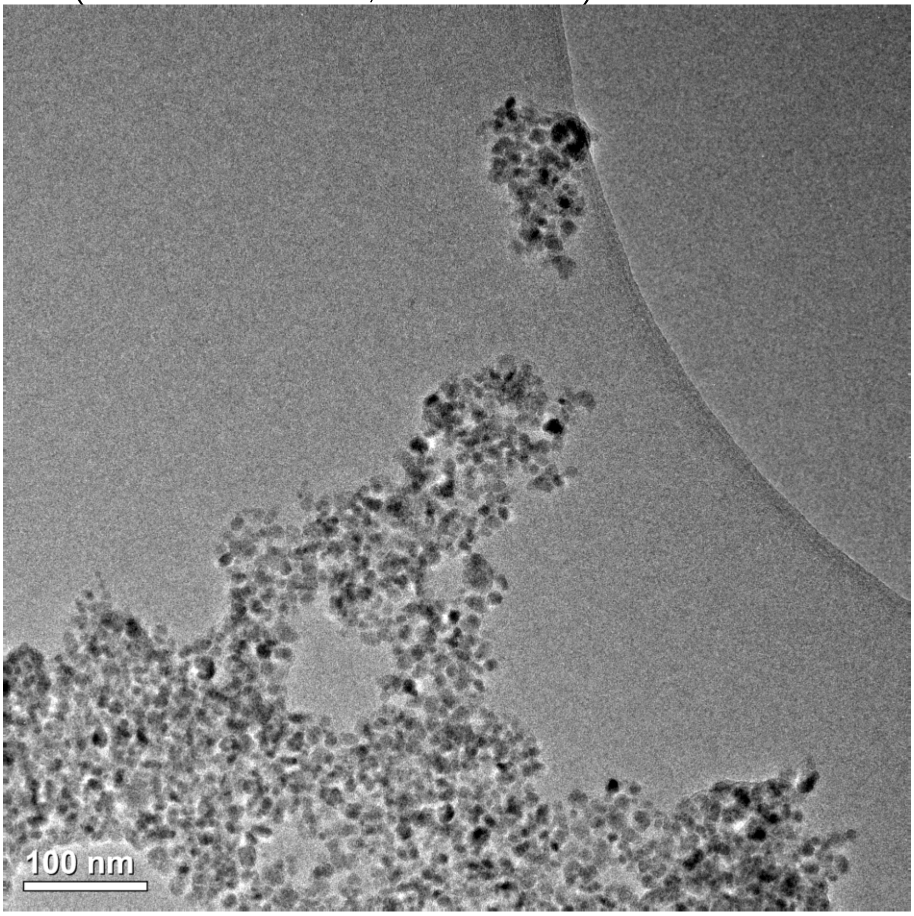


Figure S5a Enlarged TEM picture from Fig. 1a or Fig. S4 of the CORM-functionalized maghemite-NPs **3** (Fei Titan 80-300 TEM, ER-C FZ Jülich).

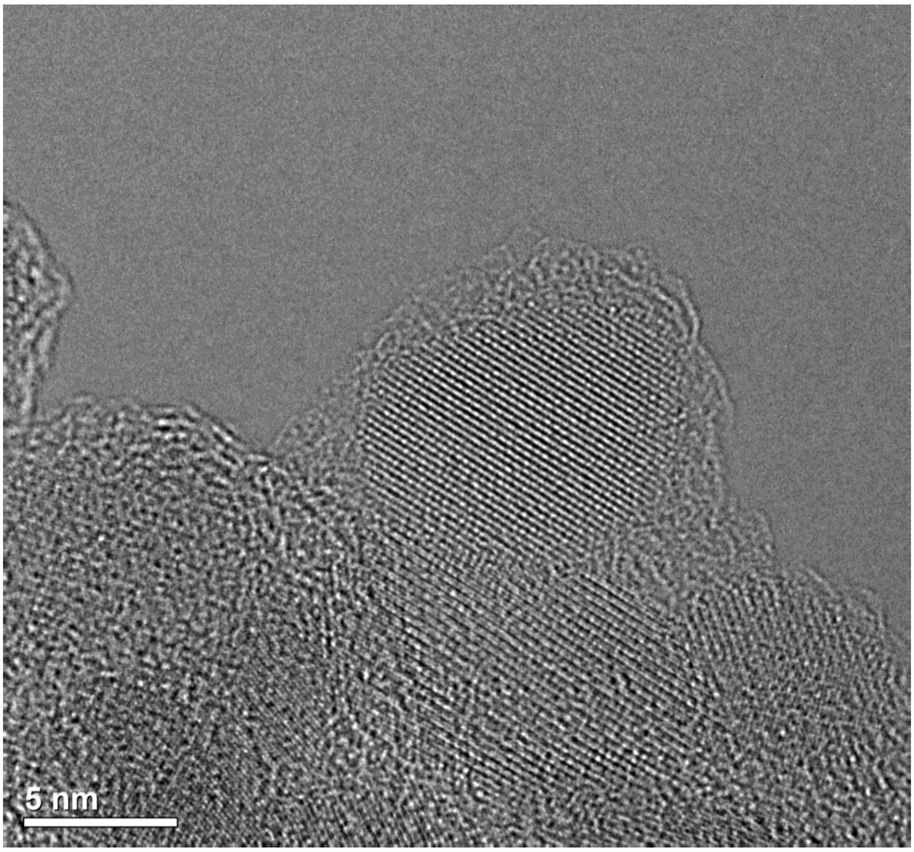
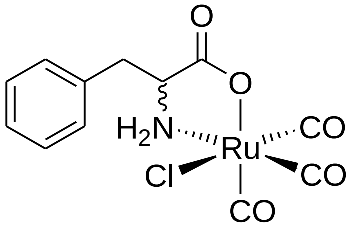


Figure S5b Enlarged TEM picture from Fig. 1b of the CORM-functionalized maghemite-NPs **3** (Fei Titan 80-300 TEM, ER-C FZ Jülich).

Tri(carbonyl)-chlorido-phenylalaninato-ruthenium(II), (4):



4

D/L-Phenylalanin (2-amino-3-phenylpropionic acid) (74 mg, 0.45 mmol), $[RuCl_2(CO)_3]_2$ 117 mg (0.23 mol) and sodium ethanolate (42 mg, 0.62 mol) were added in dry methanol (50 mL) under nitrogen atmosphere and stirred at room temperature for 18 h. The solvent was removed in vacuum. The residual solid was dispersed in 50 mL of dry tetrahydrofuran and filtered through Celite. The solution was narrowed in vacuum and the product precipitated with n-hexane as a yellow solid. Yield 90 mg (0.23 mmol, 51 %). IR (KBr): $\tilde{\nu}/cm^{-1}$ = 2137 (s), 2057 (s), 1984 (m), 1644 (s) (see Fig. S6a). MS (EI): m/z = 320 $[M - CO, Cl]^+$; ^1H NMR (500 MHz, d_8-THF) δ = 7.38 (d, *J*=7.5, 2H, 2,6-(ortho-)C_6H_5), 7.31 (t, *J*=7.5, 2H, 3,5-(meta-)C_6H_5), 7.21 (d, *J*=7.3, 1H, para-C_6H_5), 3.97 (m, 1H, CH), 3.42 (dd, 1H, diastereotopic H of CH_2), 3.04 (m, 1H, diastereotopic H of CH_2). In the NMR a yellow solid precipitates from the THF solution within 2 h and the NMR spectrum shows additional small signals at 6.42 and 5.54 which are indicative of formation of another product, perhaps from CO/THF ligand exchange).

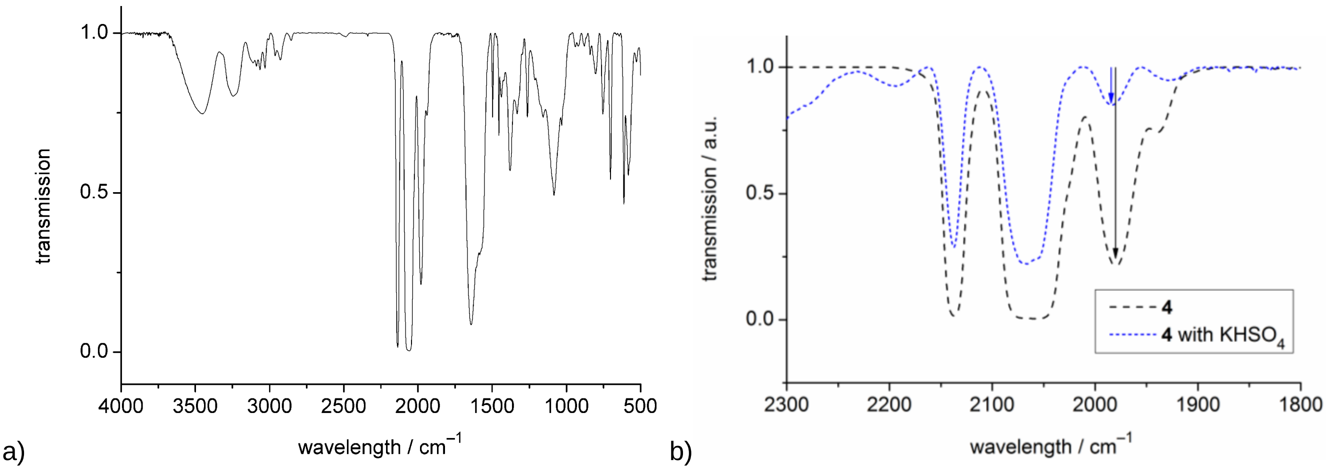
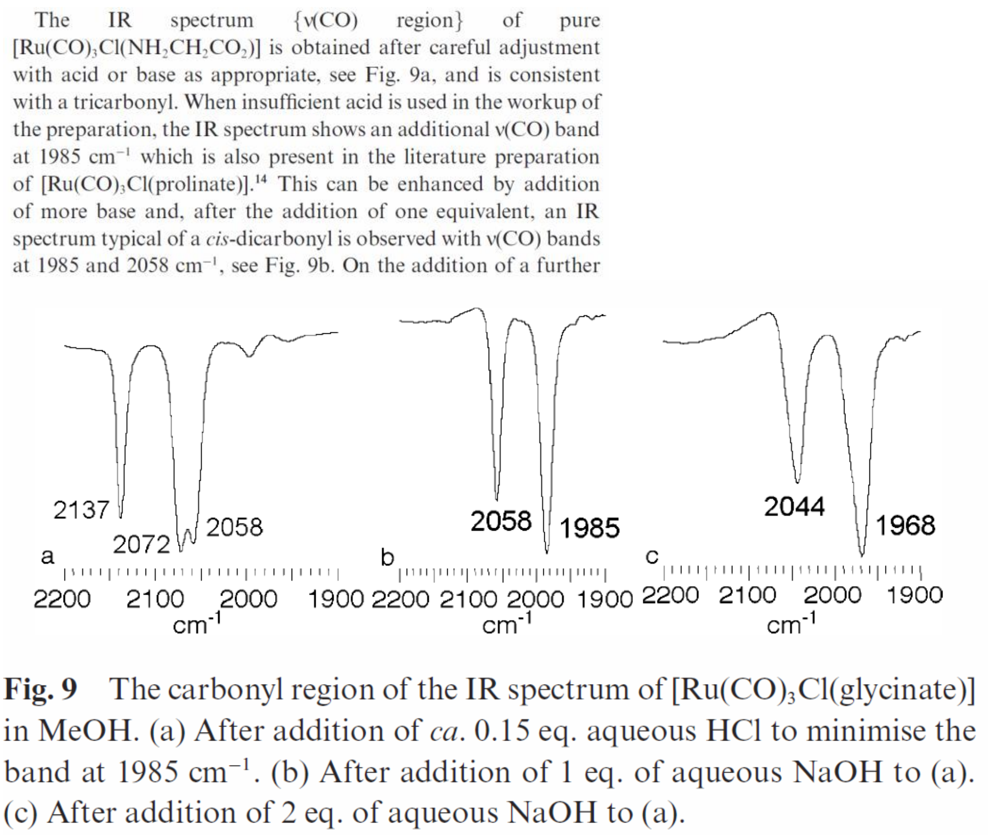


Figure S6-1 (a) IR spectrum (KBr) of the tri(carbonyl)-chlorido-phenylalaninato-ruthenium(II) complex **4** and (b) after addition of 1.5 equivalents of acid ($KHSO_4$). The spectrum in (b) agrees with the IR of CORM-3 after addition of 1.5 eq. aqueous HCl (bands at 2137, 2072 und 2058 cm^{-1}).[3] The two spectra in (b) have been normalized to 1.0.

The IR spectrum in Fig. S6-1(b) shows the minimization of the band at 1984 cm^{-1} after the addition of acid to give the IR spectrum of pure **4**, in agreement with ref. 3 (see text and "Fig. 9" from this source, given below) and consistent with a tricarbonyl fragment carrying different trans ligands. The high concentration was chosen to clearly show the effect of the added acid. The splitting of the second band into two bands at 2072 and 2058 cm^{-1} for a symmetric $[M(CO)_3L_3]$ complex is due to the unsymmetry of the $[Ru(CO)_3Cl(phenylalaninato)]$ complex **4**.

The IR spectra of the $[Ru(CO)_3Cl(amino\ acetate)]$ complexes are more complicated than for simple symmetric tricarbonyl $[M(CO)_3L_3]$ fragments.
We cite here from the paper of T. R. Johnson, B. E. Mann, I. P. Teasdale, H. Adams, R. Foresti, C. J. Green, R. Motterlini, *Dalton Trans.* **2007**, 1500–1508. ("*Metal carbonyls as pharmaceuticals? [Ru(CO)$_3$Cl(glycinate)], a CO-releasing molecule with an extensive aqueous solution chemistry*").
The following text and "Fig. 9" on the next page is copied from this paper:



The IR spectrum {ν(CO) region} of pure $[Ru(CO)_3Cl(NH_2CH_2CO_2)]$ is obtained after careful adjustment with acid or base as appropriate, see Fig. 9a, and is consistent with a tricarbonyl. When insufficient acid is used in the workup of the preparation, the IR spectrum shows an additional ν(CO) band at 1985 cm^{-1} which is also present in the literature preparation of $[Ru(CO)_3Cl(prolinate)]$.[14] This can be enhanced by addition of more base and, after the addition of one equivalent, an IR spectrum typical of a *cis*-dicarbonyl is observed with ν(CO) bands at 1985 and 2058 cm^{-1}, see Fig. 9b. On the addition of a further

Fig. 9 The carbonyl region of the IR spectrum of $[Ru(CO)_3Cl(glycinate)]$ in MeOH. (a) After addition of *ca.* 0.15 eq. aqueous HCl to minimise the band at 1985 cm^{-1}. (b) After addition of 1 eq. of aqueous NaOH to (a). (c) After addition of 2 eq. of aqueous NaOH to (a).

We have added different equivalents of base by grinding solid **4** with the chosen amount of solid NaOH and KBr in the presence of moisture.

The carbonyl region of the IR spectra of $[Ru(CO)_3Cl(phenylalaninato)]$, **4** after addition of different equivalents of NaOH is displayed in Fig. S6-2 (next page).

The reaction of **4** with 0.5, 1.0 and 1.5 molar equivalents NaOH gives two lines at ~2033 and 1952 cm^{-1} in good agreement with the two vibrations at 2058 and 1985 cm^{-1} or 2044 and 1968 cm^{-1} observed from the reaction of CORM-3 with 1 eq. or 2 eq. of aqueous NaOH, respectively (see "Fig. 9" above).

The reaction of **4** with 0.5 eq. NaOH still exhibits the original CO vibrations of unreacted **4**, detected here at 2133, ~2050 and ~1971 cm^{-1}. The latter two vibrations overlap with the new vibrations.

Determination of surface coverage:

Spherical particles of 8 nm average diameter have an average surface area of ($4\pi r^2$ =) 201 nm^2 and an average volume of ($4/3\ \pi r^3$ =) 268 nm^3.

With the density of Fe_2O_3 of 5.25 g/cm^3, the average mass of such a Fe_2O_3-particle is $1.4 \cdot 10^{-18}$ g.

The formula mass of Fe_2O_3 is 159.69 g/mol.

Hence, a Fe_2O_3-particle of 8 nm average diameter contains about $8.77 \cdot 10^{-21}$ mol.

Multiplication with $N_A = 6.022 \cdot 10^{23}\ mol^{-1}$ yields 5300 formula units or ~10600 Fe atoms per particle.

The necessary surface area of a catecholate, that is, dioxyphenylalaninato ligand was estimated as the circular area of a benzene ring (0.28 nm^2). The ligand size estimation is based on a perpendicular attachment mode of the catecholate, $C_6H_3R(o\text{-}O_2)^{2-}$ to the surface and its rotational projection. In this it will cover approximately the size of a benzene ring, C_6H_6, taking into account the van-der-Waals radii of the H-atoms.

Thus, 201 / 0.28 = 717 or ~700 dioxyphenylalaninato ligands could theoretically be coordinated to an 8 nm Fe_2O_3-particle.

ICP-MS measurements yielded a molar Ru : Fe ratio of 1 : 25. This corresponds to about 400 Ru-atoms per particle. Hence, the surface area coverage of an 8 nm Fe_2O_3-particle with CO-releasing Ru-molecules is 400 / 700 or about 57% based on the estimated number of dioxyphenyl-alaninato ligands.

More dioxyphenyl-alanine ligands than needed for the CORMs can still be present as the RuCl(CO)$_3$-fragment is coordinated to the already surface bound dioxyphenylalanine ligand in the D/L-dihydroxy-phenylalanine-(D/L-DOPA-)functionalized maghemite-NPs **2**.

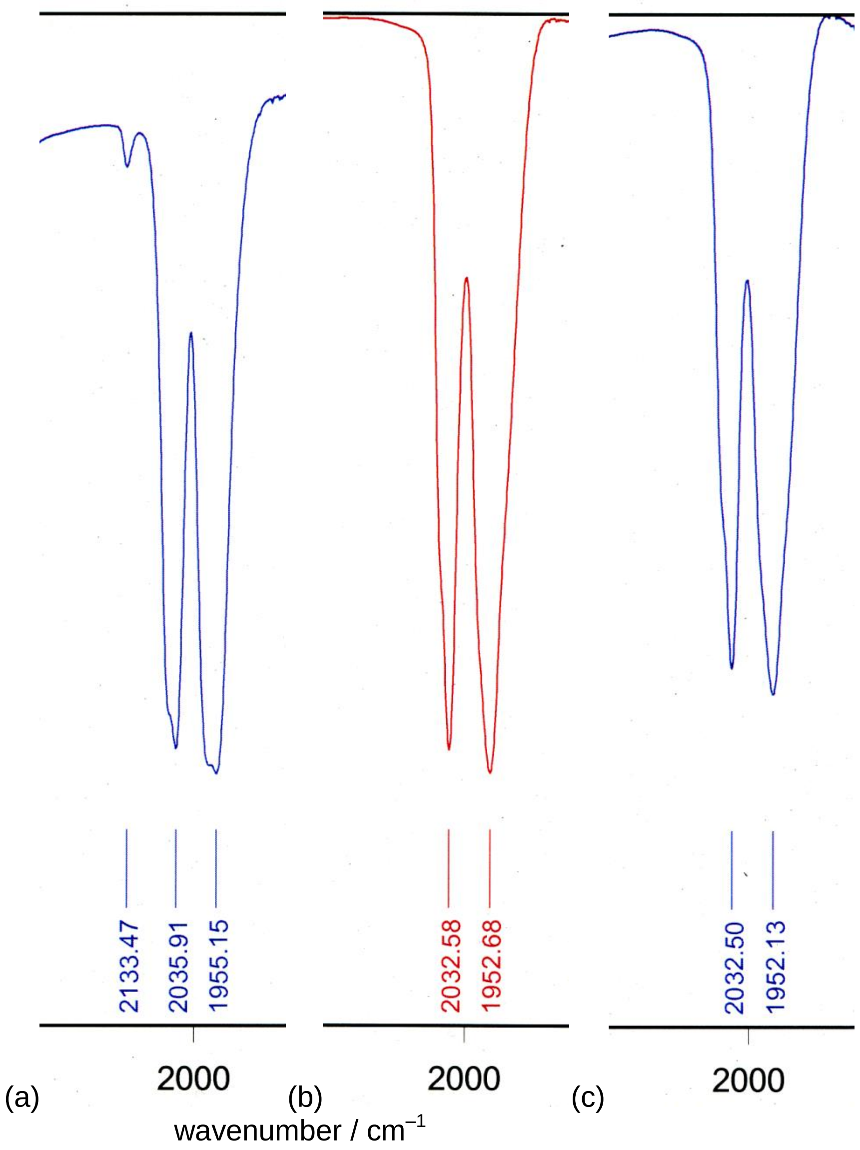


Fig. S6-2 The carbonyl region of the IR spectrum of [Ru(CO)$_3$Cl(phenylalaninato)], **4** with different NaOH equivalents: (a) **4**+0.5eq. NaOH, (b) **4**+1.0 eq. NaOH and (c) **4**+1.5eq. NaOH; all as KBr pellets.

Magnetic properties of maghemite-(Fe$_2$O$_3$-)NPs:

The magnetic properties of maghemite-NPs were determined by Vibrating Sample Magnetometry (VSM). The EV7 system of ADE Magnetics Co. consists of a 3472-70 electro magnet of GMW Magnet Systems Co. with a maximal field strength of $2.8{\cdot}10^8$ A·m^{-1} and a vibrating inducer with a frequency of 75 Hz. For each sample a hysteresis curve was measured from $-1.59{\cdot}10^6$ A·m^{-1} to $1.59{\cdot}10^6$ A·m^{-1} (Fig. S7). The saturation magnetization M_s and the starting susceptibility χ_{ini} of the maghemite-NPs were determined from this hysteresis curve. From these values and the volume-based saturation magnetization M_0 of maghemite ($3.9{\cdot}10^5$ A·m^{-1}) the volume-averaged magnetic particle diameter ($d_{v/a}$) of the maghemite-NPs can be calculated (Table S2). In addition, the saturation magnetization yields the mass fraction μ_{mag} of maghemite (Fe$_2$O$_3$) in dispersed as well as dried nanoparticles (Table S2).

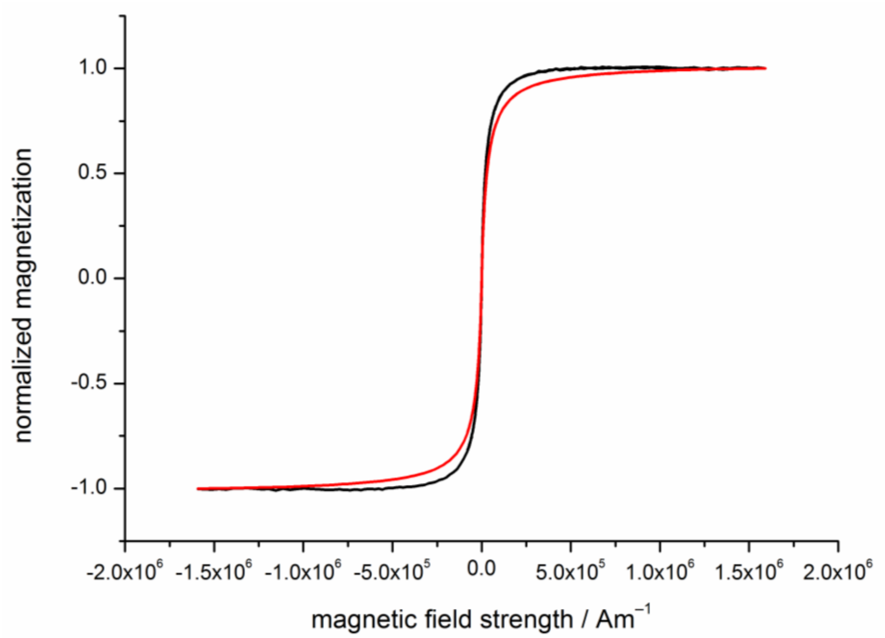


Figure S7 Hysteresis curves of maghemite-NPs **1** (normalized to the saturation magnetization); black as particle dispersion in H_2O, red as dried, solid powder.

Table S2 Magnetic properties of D/L-DOPA-functionalized maghemite-NPs **2**.

Sample	M_s [a]	χ_{ini} [a]	μ_{mag} [a]	$d_{v/a}$ [a]
Dispersion in H_2O, pH 8 [b]	50.8 $A{\cdot}m^{-1}$	0.002	0.07 % [c]	13.0 nm
Solid [d]	53.8 Am^2/kg	0.002 m^3/kg	67.31 %	11.6 nm

[a] M_s = saturation magnetization, χ_{ini} = starting susceptibility, μ_{mag} = mass fraction Fe_2O_3, $d_{v/a}$ **=** volume-averaged magnetic particle diameter.

[b] Fuid sample as particle dispersion in H_2O; therefore the units for M_s and χ_{ini} are volume-based.

[c] For the fluid sample, that is, particle dispersion in H_2O the whole dispersion was measured including the solvent. The value of μ_{mag} corresponds to the mass fraction of Fe_2O_3 in this dispersion and, therefore, is different and much smaller than the value for the (pure) solid sample of **2**.

[d] Sample as dried, solid powder with mass-based units for M_s and χ_{ini}.

Determination of CO-release with a myoglobin assay:

UV/Vis-spectra were collected on a Specord S 100 UV/Vis-spectrometer of Analytik Jena Co. This work was carried out under nitrogen or argon and with thermostated cuvettes.

The stock solution was prepared from 20 mg (1.17 µmol) myoglobin, dissolved in 13 mL 3-(N-morpholino)-propanesulfonic acid buffer (0.1 mol/L, pH 7.4) and reduced with sodium dithionite ($Na_2S_2O_4$, 100 mg, 0.575 mmol, corresponding to a concentration of 44 mmol/L).

The cuvette was charged with a counter flow of inert gas with 1.4 mL of the myoglobin assay solution and 1.5 mg of CORM-functionalized maghemite-NPs **3**.

The intensity change of the absorptions bands of myoglobin between 500-700 nm was followed as a function of time with and without alternating magnetic field (Fig. S8, S9). For the measurements in the alternating magnetic field, the thermostated cuvette was placed in the coil of the alternating current (AC) generator (Fig. S10).

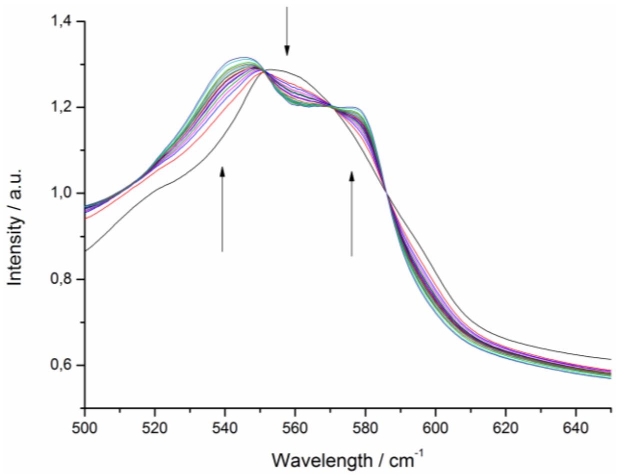
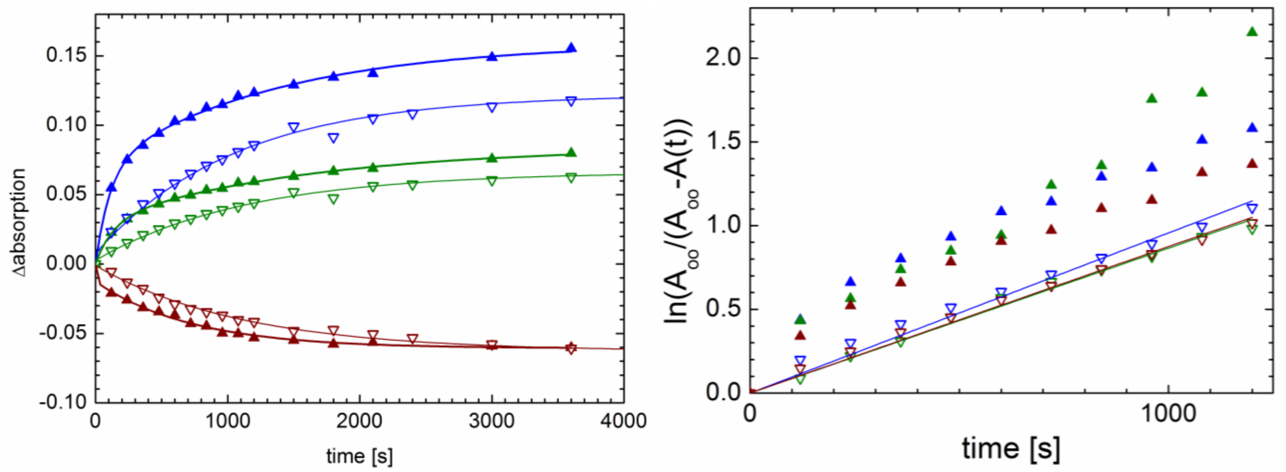


Figure S8 Typical change of the absorptions bands of myoglobin between 500-640 nm during the CO-release from **3**, here with alternating magnetic field (247 kHz, 31.7 kAm^{-1}, 39.9 mTesla) at 25 °C.
Intensity changes were followed at 541, 556 and 578 nm.



(a) (b)

Figure S9 Typical CO-release curves with a myoglobin assay from CORM@IONP, **3** at 25 °C. (Additional CO-release curves from three other experiments are shown on the next page).
In (b) a first-order rate law is assumed for the plot of $\ln(A_{oo}/(A_{oo} - A(t))$ versus time.
Empty symbols (∇): without field; filled symbols (▲): with alternating magnetic field;
blue symbols (∇,▲): change in the intensity at 541 nm;
red-brown symbols (∇,▲): change in the intensity at 556 nm;
green symbols (∇,▲): change in the intensity at 578 nm.
From Fig. 2a in the publication and Fig. S8 it is evident that the decrease in intensity of the middle band at 556 nm (red-brown symbols in Fig. 2b and Fig. S9a) is partially compensated by the increase in intensity of the overlain outer bands at 541 and 578 nm. The overlap intensifies with time as the two outer bands grow. Hence, at longer release times the middle band and the kinetic curve derived there from cannot be used for evaluation. To a smaller extend this is also the case for the two outer bands whose intensity increase is compensated by the decrease in the middle band. A deconvolution of the three bands was not possible. We therefore concentrated on the starting rate within the first 1000 s for the derivation of the kinetic data, (Fig. S9b).

Table S3 Half-lives for the CO-release from CORM@IONP, **3**.[a]

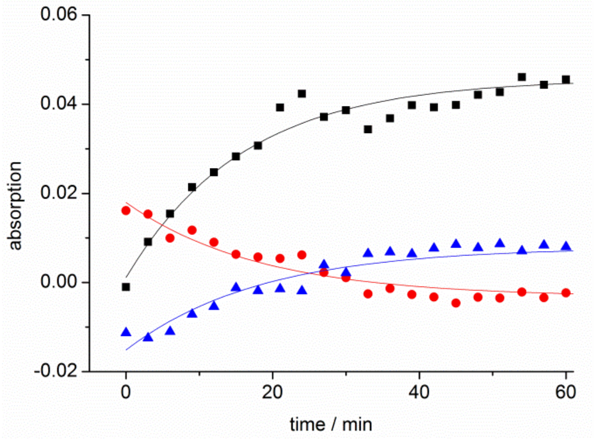
λ / nm	Half-life, $t_{½}$ / min no field, 25 °C	 alternating magnetic field, 25 °C
541	12(1)	6(1)
556	13(1)	6.7(7)
578	13(1)	8(1)

[a] The half-life for CO-release from CORM@IONP, **3** decreases with increasing temperature; at 30 °C $t_{½}$ = (11 ± 1) min and at 40 °C $t_{½}$ = (5 ± 1) min (each without magnetic field).

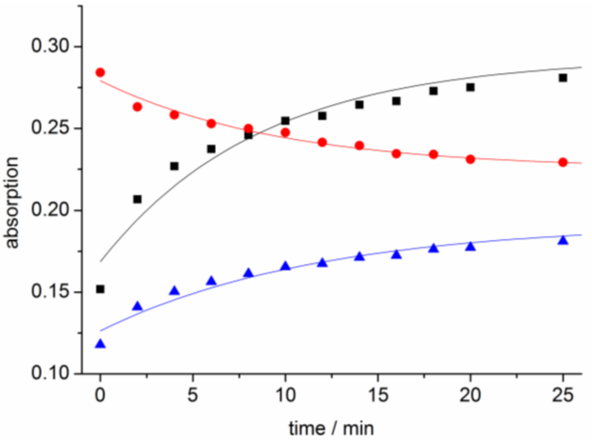
Additional CO-release curves from three other experiments:

no field, 25 °C:

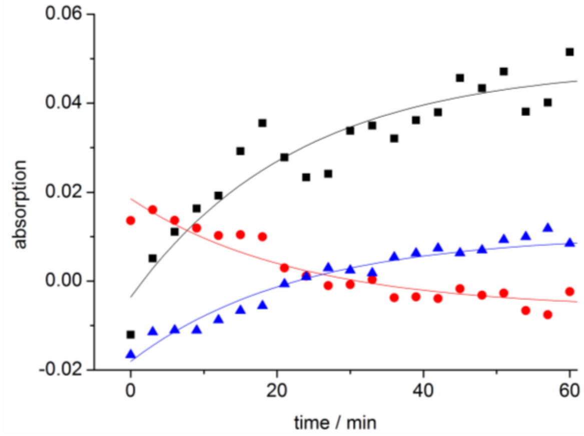
with field, 25 °C



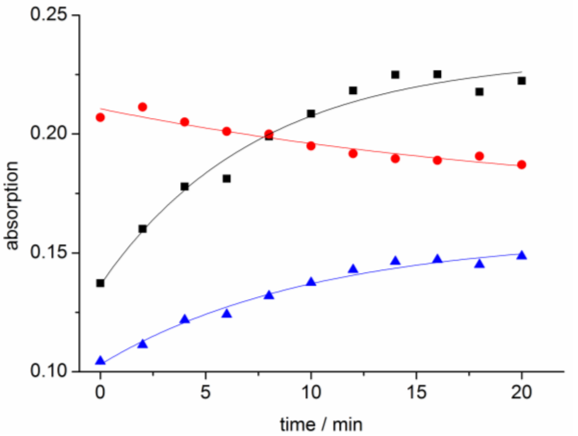
averaged half-life: $t_{1/2}$ = 12(1) min



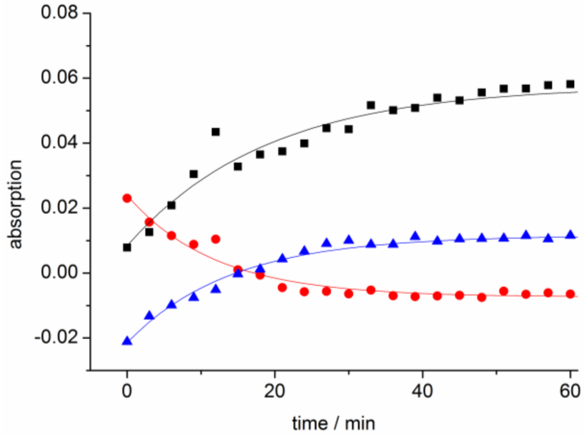
$t_{1/2}$ = 7(1) min



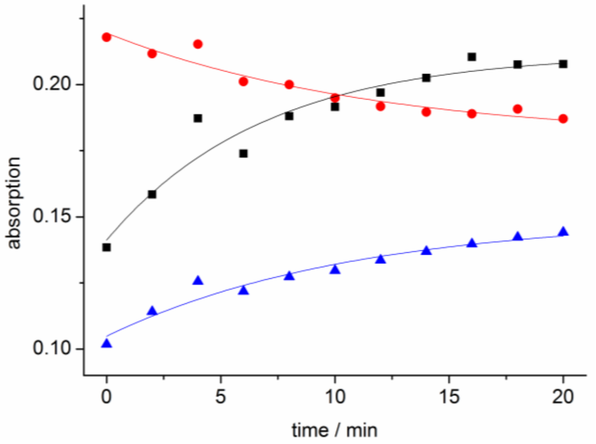
averaged half-life: $t_{1/2}$ = 16(1) min



$t_{1/2}$ = 7(2) min



averaged half-life: $t_{1/2}$ = 11(1) min



$t_{1/2}$ = 6(2) min

(Absorption graphs were normalized to the isosbestic point at 587 nm.)
Intensity changes were followed at 541, 556 and 578 nm to give the typical CO-release curves from which the half-life can be quantified.
Black symbols (■) absorption intensity at 541 nm;
red symbols (●) absorption intensity at 556 nm;
blue symbols (▲) absorption intensity at 578 nm.

Figure S10 Thermostated cuvette in the coil of the alternating current (AC) generator.

After the CO-release experiment with the myoglobin assay with and without alternating magnetic field IR-spectra in KBr were measured for CORM@IONP, **3**. Two bands remained for the CO stretching vibrations (Fig. S11). The IR spectra of reacted CORM@IONP show two bands at 2060 and 1986 cm^{-1} which are either indicative of remaining CORM@IONP, **3** or of a cis-Ru(II)$(CO)_2$ fragment bound to protein. It has been proven in a recent study that the interaction of CORM-3 with proteins results in the loss of the chloro, glycinato and one CO ligand. The rapid formation of stable adducts between the protein and the remaining *cis*-Ru(II)$(CO)_2$ fragments was confirmed by different methods, including IR-spectroscopy.[5]

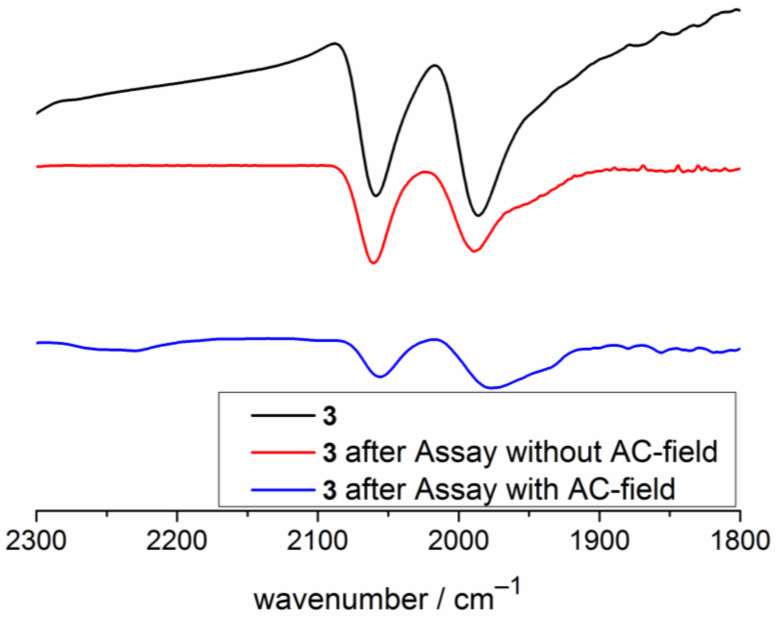


Figure S11 IR-spectra (in KBr) of the CO-stretching region of CORM@IONP, **3** and after the CO-release experiment with and without alternating magnetic field.

---

[5] T. Santos-Silva, A. Mukhopadhyay, J. D. Seixas, G. J. L. Bernardes, C. C. Romão and M. J. Romão, *J. Am. Chem. Soc.*, 2011, **133**, 1192-1195.

The half-life for the CO-release from the tri(carbonyl)-chlorido-phenylalaninato-ruthenium(II) model compound **4** was determined to (2.7±0.2) min and (3.0±0.4) min without and with alternating magnetic field, respectively (Fig. S12). These two half-lives are consistent within experimental error, hence, there is no effect of the alternating field on a $Ru(CO)_3$ fragment in a non-magnetic complex.

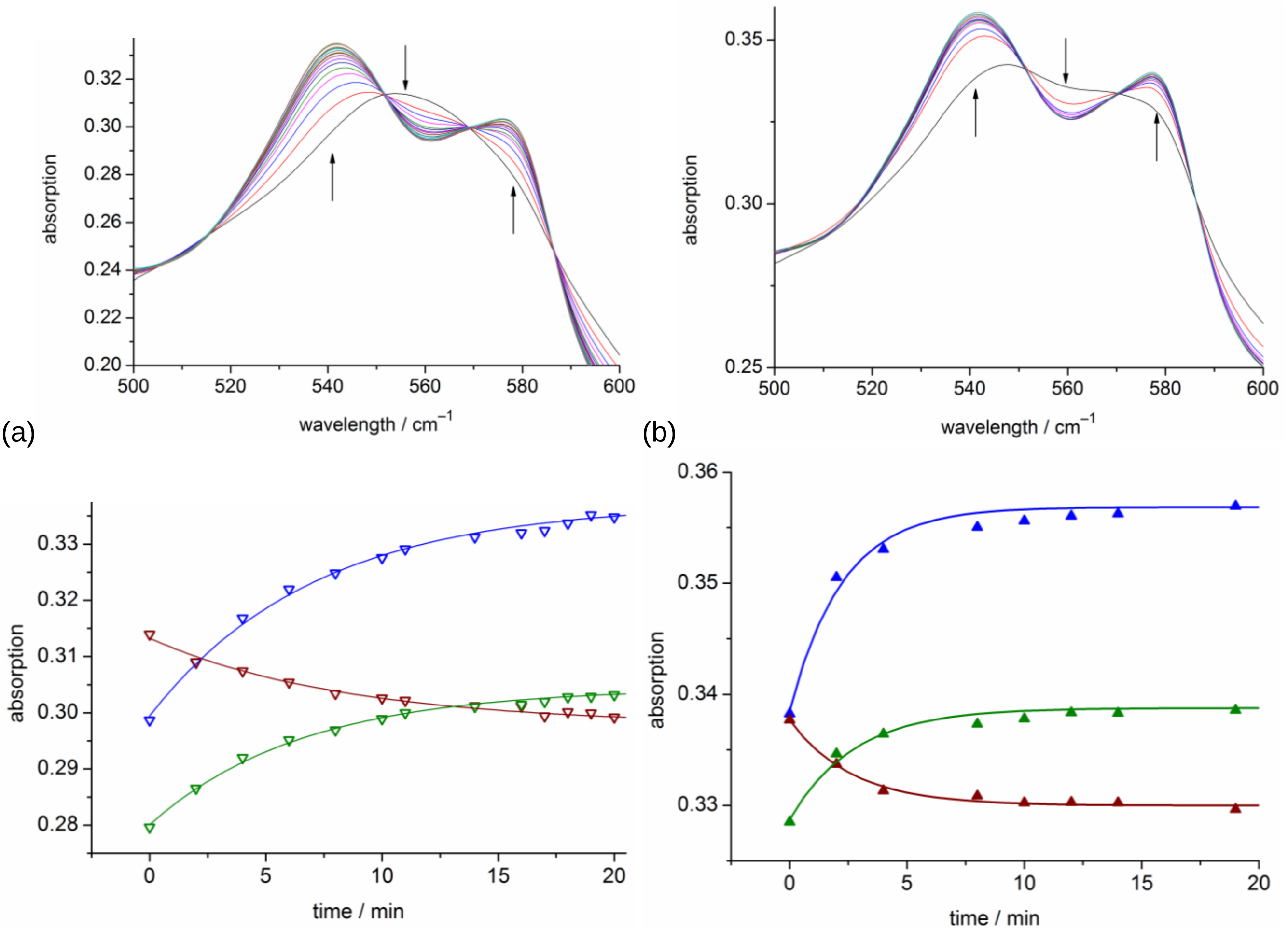


Figure S12 Typical change of the absorptions bands of myoglobin between 500-600 nm during the CO-release from **4**, (a) with no and (b) with alternating magnetic field (247 kHz, 31.7 kAm^{-1}, 39.9 mTesla) at 20 °C. Intensity changes for the kinetic evaluation were followed at 541, 556 and 578 nm (arrow positions).

The myoglobin assay is carried out with the use of sodium dithionite, $Na_2S_2O_4$ as reducing agent. To test for such an influence of dithionite, we have carried out the myoglobin assay as described above with different sodium dithionite concentrations (44 mmol/L, 88 mmol/L und 176 mmol/L). No significant deviation of the half-lives was found for the CO-release (Figure S13). Different from a recent literature report[6] we have not seen a significant influence of the amount of dithionite onto the rate for the CO-release from **3**.

We note that we have used in our study a high excess of sodium dithionite over myoglobin (Mb) with molar ratios of $Na_2S_2O_4$: Mb from 500 : 1 up to 2500 : 1 for the three dithionite concentrations. In the literature6 the molar ratio $Na_2S_2O_4$: Mb was varied from 3 : 1 to 90 : 1. In this range a significant rate enhancement for the CO-release was found at higher dithionite ratios.

---

[6] S. McLean, B. E. Mann, R. K. Poole, *Anal. Biochem.* **2012**, *427*, 36-40.

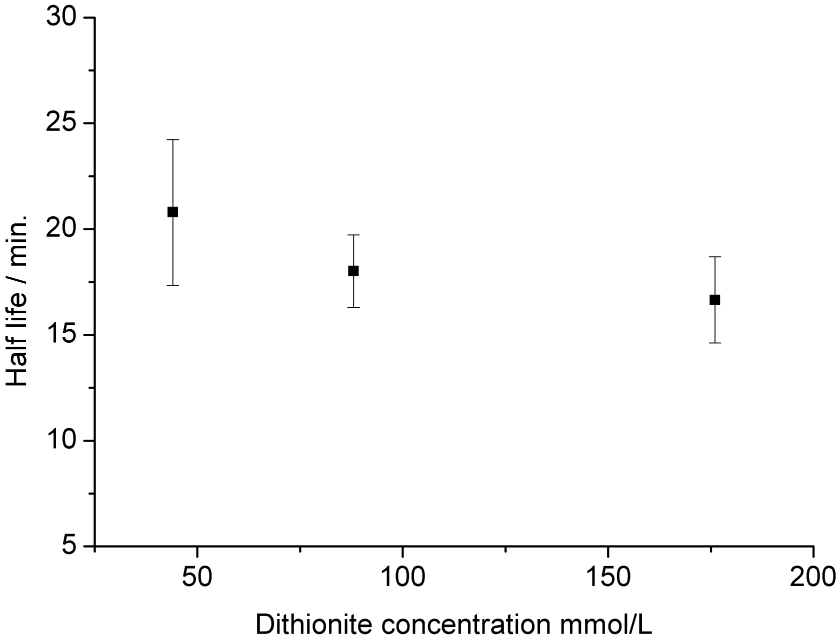


Figure S13 Half-life for the CO-release from CORM-functionalized maghemite-NPs **3** at 20 °C and different sodium dithionite concentrations (without alternating magnetic field).