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Electronic Supplementary Information

for

Stable dispersions of azide functionalized ferromagnetic metal nanoparticles

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Materials and general methods

Dispersions of nanoparticles were produced with the aid of an ultrasonic bath (Bandelin Sonorex). The nanoparticles were analyzed by Fourier transform infrared spectroscopy (FT-IR) 5wt% nanoparticles in KBr with a Tensor 27 Spectrometer (Bruker Optics) equipped with a diffuse reflectance accessory (DiffuseIR, Pike Technologies) and elemental microanalysis (ELEMENTAR, Elementar Analysensysteme). Transmission electron microscopy (TEM) was measured with a CM12 (Philips, operated at 120 kV) and scanning transmission electron microscopy (STEM) with a NovaNanoSEM 450 (FEI, operated at 30 kV). For both, the particles were deposited on a carbon/copper grid. The nanomaterial was further characterized by magnetic hysteresis susceptibility as a powder in a gelatin capsule (vibrating sample magnetometer, VSM, Princeton Measurements Corporation, model 3900). The particle size distribution and the sedimentation velocity were measured by a dispersion analyzer (LUMiSizer 610/611, 200 rpm for 31 min 40 s then 4000 rpm for 11 h 42 min, light source: pulsed blue LED, 470 nm, detector: CCD-Line, 2048 elements, statistics: SEPView, L.U.M GmbH). The particle size distribution was further characterized by measuring the diameter of at least 169 particles per sample in STEM images. The electrophoretic mobility of the system was measured by Laser Doppler Effect using a Zetasizer Nano instrument (Malvern, UK). Particle size was measured by dynamic light scattering at a fixed angle of $\theta = 173°$ using a Zetasizer Nano instrument (Malvern, UK) equipped with laser beam of wavelength $\lambda_0 = 633$ nm, and PMMA cuvettes (Ratiolab GmbH, Dreieich, Germany). The Absorbance of nitrophenol for the biofouling test was measured with a microtiter plate reader (Infinite f200 Tecan) in a transparent flat bottom 96-well plate (TPP) at 25°C. The used centrifuge was obtained from VWR, Hitachi Koki Co, Ltd.

Chemicals 4-(2-aminoethyl)aniline (Aldrich, 97%), sodium nitrite ($NaNO_2$, Fluka, 99%), 2-bromo-2-methylpropionyl bromide (ABCR, 97%), 3-(2-Methylprop-2-enoyloxy)propane-1-sulfonic acid potassium salt (SPM, ABCR) glycidyl methacrylate (GMA, ABCR, 95%), copper(II)bromide ($CuBr_2$, Fluka, 99%), 2,2′-bipyridine (bipy, ABCR, 99%), sodium azide (NaN_3, Sigma, 99.5%), L-ascorbic acid (Sigma-Aldrich, 99%), biotin-PEG$_4$-alkyne (Click Chemistry Tools Bioconjugate technology company), β-glucosidase (from almonds, Sigma), 4-nitrophenyl-β-D-glucopyranoside (Sigma), $NaHCO_3$ (Fluka) were used as obtained if not stated otherwise.

Silica particles (Sicastar plain, micromod, 50 nm in diameter, 25 mg/mL in water, amorphous, ρ=2.0 g/cm^3), dextran-coated iron oxide nanoparticles (Fe_3O_4-dextran, nanomag-D-spio plain, micromod, 50 nm in diameter, 25 mg/mL in water, ρ=1.4 g/cm^3, saturation magnetization >69 emu/g iron, iron concentration: 2.4 mg/mL), polystyrene particles (PS, micromere plain, 50 nm in diameter, 10 mg/mL in water, ρ=1.03 g/cm^3, contain traces of negatively charged sulfate groups.), polyethyleneglycol (PEG)-coated iron oxide nanoparticles (Fe_3O_4-PEG, Ocean NanoTech, 30 nm in diameter, 1 mg/mL Fe in 0.01M borate solution (pH=5.0), ρ=5.2 g/cm^3 (density of iron oxide core), zeta potential = -10 mV to 0 mV) were used as references.

All solvents stated as dry were purified by a solvent drying system from LC Technology Solutions SP-105 under N_2 atmosphere (H_2O content < 10 ppm, determined by Karl-Fischer titration).

Synthesis of C/Co@pSPM-clicked biotin (8)

Production of C/Co-nanoparticles. The carbon coated cobalt nanoparticles were produced in a one step process by reducing flame spray pyrolysis under an oxygen-free atmosphere as described by Grass et al.[1] Elemental microanalysis: [C]: 2.83%, [H]: 0.00%, [N]: 0.01%, [S]: 0.00%.

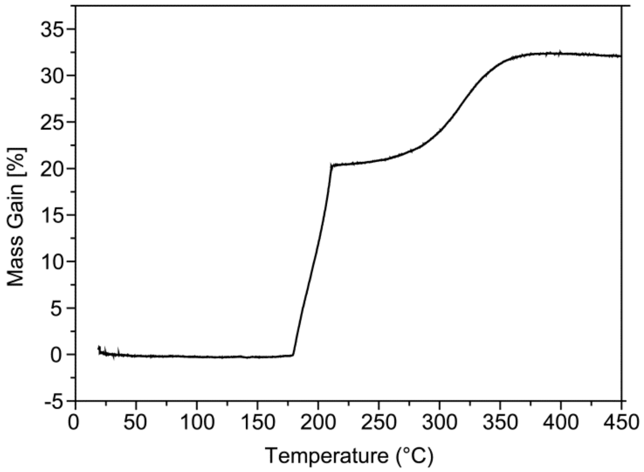

Fig. S1: Powder mass gain of carbon coated cobalt nanoparticles (C/Co) upon oxidation measured by thermogravimetry in air.

Phenethylamine functionalized C/Co-nanoparticles. The procedure was adapted from literature.[2] Carbon coated cobalt nanoparticles (C/Co, 5 g) were dispersed in water (100 mL) for 20 minutes by using a temperature controlled ultrasonic bath at 10 °C. To an ice-cooled solution of 4-(2-aminoethyl)aniline (1.03 mL, 8 mmol) in water (20 mL), concentrated hydrochloric acid (2 mL) was slowly added. This solution was then added to the dispersed carbon coated cobalt nanoparticles while sonication. An ice-cooled solution of $NaNO_2$ (820 mg, 12 mmol) in water (10 mL) was then added dropwise and gas elution was observed. The reaction mixture was sonicated for another hour before the carbon coated cobalt nanoparticles were separated with the aid of a 1.2 T neodymium-based permanent magnet (N 42, Q-40-40-20-N, Webcraft). After magnetic decantation, the particles were washed with water, ethanol, ethyl acetate and acetone (3 x 40 mL each). After each washing procedure (sonication for 3 minutes in solvent) the particles were recovered by the external magnet and the washing solvent was drained. The particles were dried in a furnace at 50 °C in *vacuo*. Spectroscopic properties were similar to published ones. IR (ν in cm^{-1}): 1665, 1503, 1370, 1019, 828. Elemental microanalysis: [C]: 4.42%, [H]: 0.31%, [N]: 0.32%, [S]: 0.03%.

C/Co@initiator (1). The procedure was adapted from literature.[2] Phenethylamine modified C/Co (2 g) were dispersed in dry THF (40 mL) in an ultrasonic bath under N_2 atmosphere. The reaction mixture was then cooled to 0 °C and under vigorously stirring, triethylamine (0.4 mL, 5.5 mmol) was added, followed by drop wise addition of 2-bromo-2-methylpropionyl bromide (0.4 mL, 3.2 mmol). The reaction mixture was stirred for 18 h while allowing the reaction mixture to slowly warm to room temperature. The nanoparticles were separated by magnetic decantation, washed and dried as mentioned before. Spectroscopic properties were the same as published. IR (ν in cm^{-1}): 1665, 1529, 1503, 1188, 1112. Elemental microanalysis: [C]: 4.75%, [H]: 0.29%, [N]: 0.27%, [S]: 0%, [Br]: 0.56%.

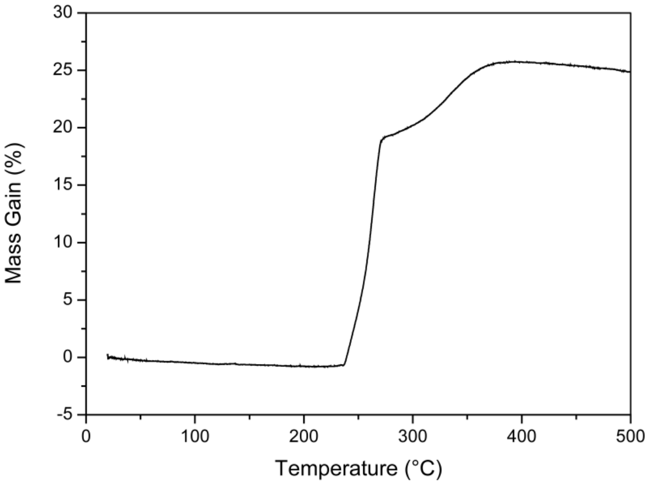

Fig. S2: Powder mass gain of C/Co@initiator (**1**) upon oxidation measured by thermogravimetry in air.

Synthesis of C/Co@pSPM (3). All reaction steps were performed under a protective nitrogen atmosphere. The monomer solution was prepared by dissolving 3-(2-Methylprop-2-enoyloxy)propane-1-sulfonic acid potassium salt (SPM, **2**) (8.6 g, 34.9 mmol) in MeOH/H_2O (2:1, 12 mL) and consecutive degassing by nitrogen bubbling for 30 minutes. $CuBr_2$ (10 mg, 0.045 mmol), 2,2'-bipyridine (54 mg, 0.35 mmol) and L-ascorbic acid (60 mg, 0.34 mmol) were added to the solution, and it was degassed for further 5 minutes. C/Co@initiator (**1**) (500 mg) were placed in a Schlenk flask and degassed (3 × high vacuum pump / N_2 refill cycles). The flask was put under vacuum and the monomer solution was added by syringe. The reaction mixture was exposed to sonication for 10 minutes to obtain a homogeneous dispersion. It was then stirred for 18 hours at room temperature. The flask was filled with nitrogen before L-ascorbic acid (120 mg, 0.68 mmol) and NaN_3 (50 mg, 0.77 mmol) were added. It was degassed for 5 minutes and a nitrogen-filled balloon was connected to the flask. The reaction solution was heated to 40 °C and stirred for 18 hours. The poly-SPM functionalized nanoparticles C/Co@pSPM (**3**) were magnetically separated. After magnetic decantation, the particles were washed three times with water. Acetone (twice the volume of the washing water) was used to destabilize the particles. It was further washed with ethanol, ethyl acetate and acetone, twice each. After each washing procedure (sonication for 3 minutes in solvent) the particles were recovered by the external magnet and the washing solvent was drained. The nanoparticles were dried in a vacuum oven at 50 °C. IR (ν in cm^{-1}): 1724, 1656, 1478, 1446, 1393, 1360, 1197, 1047, 1012, 969, 877, 795, 741, 613, 531, 442. Elemental microanalysis: [C]: 20.52%, [H]: 2.95%, [N]: 0.09%, [S]: 7.00%.

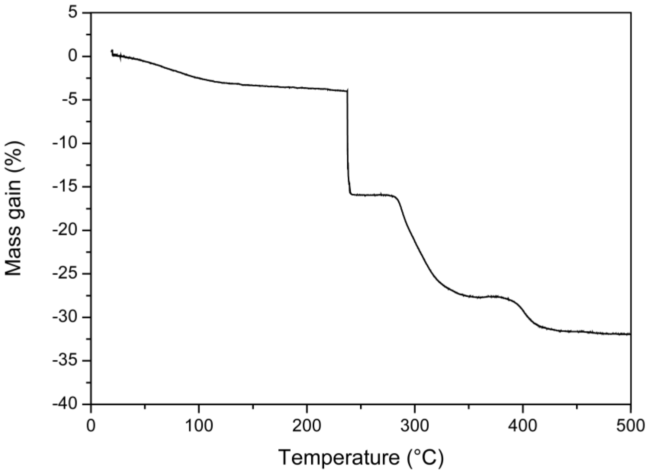

Fig. S3: Powder mass loss of C/Co@pSPM (**3**) upon combustion of the organic part of the particles measured by thermogravimetry in air.

Synthesis of C/Co@pSPM-b-pGMA (5). All reaction steps were performed under a protective nitrogen atmosphere. Glycidyl methacrylate (GMA, **4**) (0.098 mL, 0.74 mmol) was dissolved in MeOH/ H_2O (3:2, 5 mL) and was degassed for 15 minutes. $CuBr_2$ (4 mg, 0.018 mmol), 2,2'-bipyridine (20.8 mg, 0.13 mmol) and L-ascorbic acid (24 mg, 0.14 mmol) were added to the solution and it was degassed for further 5 minutes. C/Co@pSPM nanoparticles (**3**) (200 mg, 0.021 mmol Br) were placed in a Schlenk flask and degassed (3 × high vacuum pump / N_2 refill cycles). The monomer solution was added by syringe and a nitrogen-filled balloon was connected to the flask. It was stirred for 18 hours at room temperature. C/Co@pSPM-b-pGMA nanoparticles (**5**) were magnetically separated, washed and dried as described before. IR (ν in cm^{-1}): 1724, 1610, 1478, 1446, 1360, 1193, 1161, 1047, 908, 862, 777, 741, 556, 613, 531, 453. Elemental microanalysis: [C]: 21.88%, [H]: 2.78%, [N]: 1.10%, [S]: 4.05%.

Synthesis of C/Co@pSPM-N_3 (6). All reaction steps were performed under a protective nitrogen atmosphere. MeOH/H_2O (3:2, 5 mL) was degassed for 20 minutes. $CuBr_2$ (4 mg, 0.02 mmol), 2,2'-bipyridine (20.8 mg, 0.133 mmol) and L-ascorbic acid (24 mg, 0.136 mmol) were added to the solution and it was degassed for further 5 minutes. C/Co@pSPM-b-pGMA nanoparticles (**5**) (100 mg, 0.053 mmol GMA) were placed in a Schlenk flask and degassed (3 × high vacuum pump / N_2 refill cycles). The catalyst solution was added by syringe and NaN_3 (26 mg, 0.400 mmol) was added. It was degassed for further 5 minutes and then stirred at 40 °C for 2 d. C/Co@pSPM-N_3 nanoparticles (**6**) were magnetically separated, washed and dried as described before. IR (ν in cm^{-1}): 2108, 1724, 1610, 1478, 1446, 1393, 1360, 1197, 1047, 1012, 866, 777, 737, 613, 531, 442. Elemental microanalysis: [C]: 16.19%, [H]: 1.94%, [N]: 1.02%, [S]: 2.67%.

Synthesis of C/Co@pSPM-clicked (8) with R = biotin-PEG$_4$. All reaction steps were performed under a protective nitrogen atmosphere. $CuBr_2$ (3.75 mg, 0.017 mmol), 2,2'-bipyridine (19.48 mg, 0.13 mmol) and L-ascorbic acid (22.45 mg, 0.13 mmol) were added to degassed water (0.5 mL). It was degassed for further 5 minutes. Degassed DMF (0.5 mL) was added to C/Co@pSPM-N_3 nanoparticles (**6**) (30 mg, 0.011 mmol GMA) in a degassed Schlenk flask (3 × high vacuum pump / N_2 refill cycles). The catalyst solution was added to the particles, it was again degassed for 2 minutes and biotin-PEG$_4$-alkyne (19.2 mg, 0.042 mmol) was added. It was stirred at room temperature for 2 days. C/Co@pSPM-clicked nanoparticles (**8**) were magnetically separated, washed and dried as described before. IR (ν in cm^{-1}): 2108, 1724, 1674, 1620, 1478, 1446, 1353, 1314, 1233, 1165, 1040, 773, 734, 656, 610, 531, 489. Elemental microanalysis: not analyzed.

Washing procedure of nanoparticles

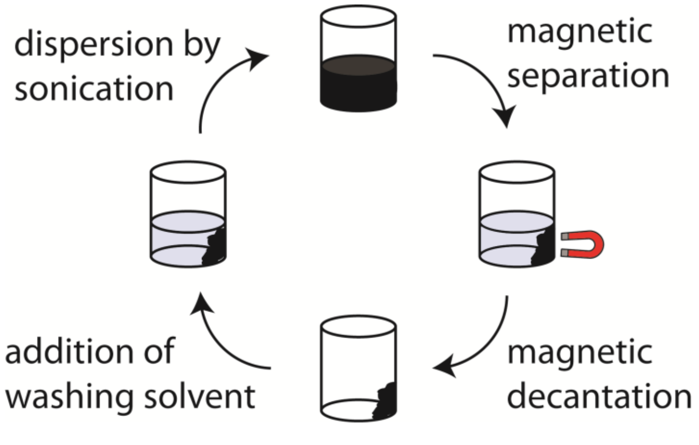

Fig. S4: General washing procedure of magnetic nanoparticles. The nanoparticles were separated from the reaction mixture by magnetic separation by holding a permanent magnet directly on the reaction vessel wall. The supernatant was then drained (magnetic decantation) and washing solvent was added. The particles were redispersed by sonication for at least 3 minutes. The particles were washed with water, ethanol, ethyl acetate and acetone 3 times. Particles containing poly(SPM) needed to be destabilized after the washing steps with water to achieve a faster magnetic separation. This destabilization was achieved by addition of acetone (twice the volume of the used washing water).

Discussion of synthesis route

In a first attempt it was tried to install the azide functionality directly onto C/Co@pSPM (**3**) by end group modification of the bromine in a one pot synthesis[3] under the same (and slightly modified conditions, concerning the amount of added NaN_3) as shown for the formation of C/Co@pSPM (**3**) in scheme 1. This attempt was unsuccessful since no azide peak was observable in the FT-IR spectra after these reactions. After changing the strategy to the shown synthesis route of scheme 1 (formation of a block-copolymer with GMA and post-modification by azide), it was observed that it wasn't possible to achieve the amount of polymerized SPM without the usage of NaN_3. The reason for this is not yet fully understood and current investigations should clarify whether this arrives from the salt concentration in the reaction or has other reasons.

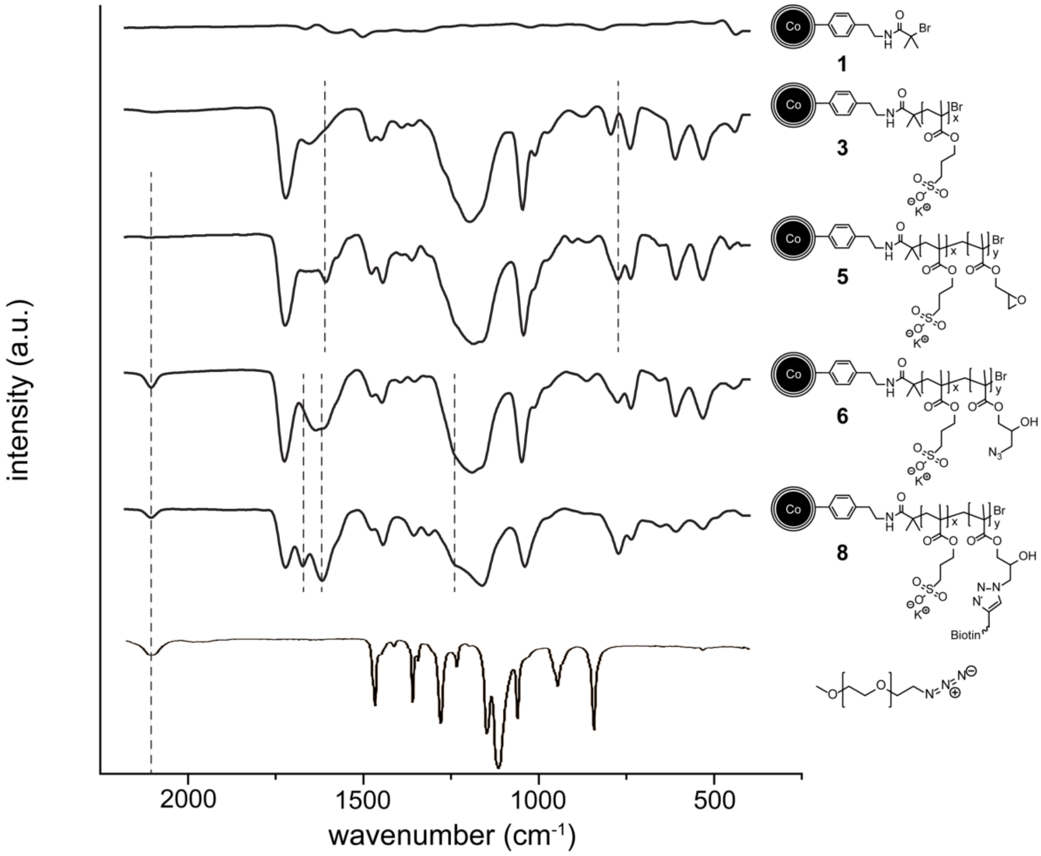

Fig. S5: Fourier transform infrared spectroscopy (FT-IR) spectra for structure confirmation of C/Co@initiator (**1**), C/Co@pSPM (**3**), C/Co@pSPM-b-pGMA (**5**), C/Co@pSPM-N_3 (**6**), C/Co@pSPM-clicked (**8**) and as a reference material azide terminated polyethyleneglycol[4]. Important changes in spectra of different functionalized nanoparticles are denoted by a dotted line. The newly appearing peaks in the spectrum of C/Co@pSPM-clicked (**8**) correspond to the amid C=O stretching vibration (1674), the urea C=O stretching vibration (1620) and the C-O-C stretching vibration (1233).

Table S1: Elemental microanalysis results for carbon, nitrogen, sulfur and bromine content of C/Co, phenethylamine functionalized C/Co-nanoparticles, C/Co@initiator (**1**), C/Co@pSPM (**3**), C/Co@pSPM-b-pGMA (**5**) and C/Co@pSPM-N_3 (**6**).

Compound	C (wt%)	N (wt%)	S (wt%)	Br (wt%)	Loading (mmol/g)	units / starter
C/Co	2.83	0	0	n.a	-	
phenethylamine C/Co	4.42	0.32	0.03	n.a	0.17[a] / 0.23[b]	
1	4.75	0.27	0	0.56	0.07[a] / 0.07[c]	
3	20.52	0.09	7.00	n.a	1.88[a] / 2.18[d]	27[a] / 31[c]
5	21.88	1.10	4.05	n.a	0.16[a]	2[a]
6	16.19	1.02	2.67	n.a	-	-

[a]: calculated with respect to increase in carbon content, [b]: calculated with respect to increase in nitrogen content, [c]: calculated with respect to increase in bromine content, [d]: calculated with respect to increase in sulfur content, n.a.: not analyzed.

In the elemental microanalysis results a carbon and sulfur loss from C/Co@pSPM-b-pGMA (**5**) to C/Co@pSPM-N_3 (**6**) is observable. It is not yet possible to explain the reason for this change. There are several possibilities: It may be hypothesized that in the formation of C/Co@pSPM (**3**) at 40 °C some part of the SPM monomer or oligomers could be non-covalently adsorbed in the polymer. This additional material contributes to a high carbon and sulfur content (see Table S1). It is attached so strongly that they are not completely washed away by the here-applied washing procedure, nor during formation of C/Co@pSPM-b-pGMA (**5**) at room temperature but appears to be released as soon as the reaction temperature in the formation of C/Co@pSPM-N_3 (**6**) is again risen to 40 °C. Full experimental confirmation of this hypothesis is currently ongoing. Explanation of the increase in nitrogen content in C/Co@pSPM-b-pGMA (**5**) is also object of further investigations. First control experiments showed that direct polymerization of GMA onto C/Co@initiator (**1**) did not show this nitrogen increase. This leads to the suggestion that the increased nitrogen content may arise from adsorption of the here used nitrogen rich ligand on the particles. One possibility is that 2,2-bipyridine complexated copper coordinates to sulfonate groups of C/Co@pSPM-b-pGMA (**5**). This suggestion is supported by the observation that the usage of another nitrogen containing ligand (N,N,N′,N″,N″-Pentamethyldiethylenetriamine, PMDETA) also leads to a nitrogen increase in the product. If the hypotheses are true needs to be established in ongoing studies.

STEM pictures for average particle size determination

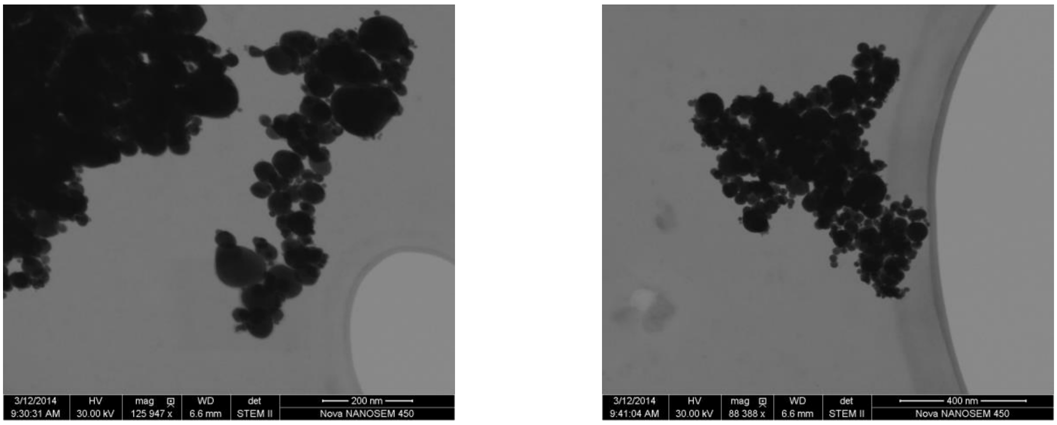

Fig. S6: Scanning transmission electron microscopy (STEM) pictures of carbon coated cobalt nanoparticles (C/Co) for average particle size determination. In total 169 particle diameters were measured to yield an average particle size of 31 ± 20 nm. The smallest particle was measured to be 7 nm and the biggest 114 nm.

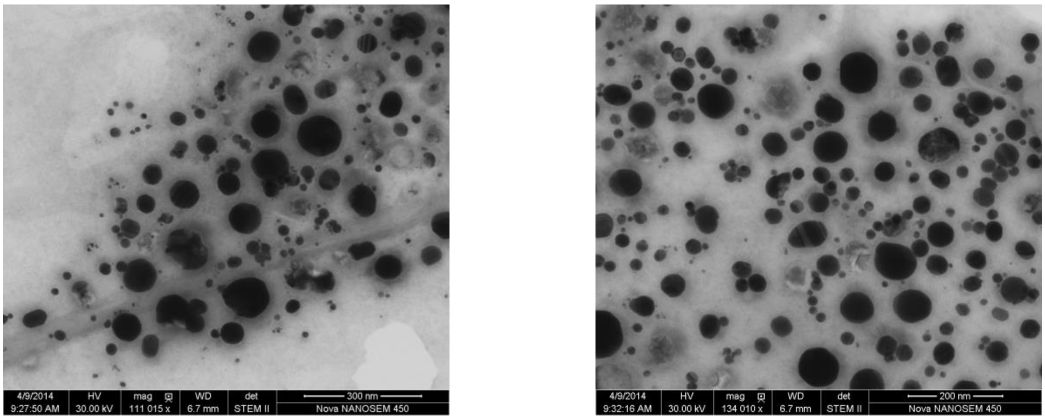

Fig. S7: Scanning transmission electron microscopy (STEM) pictures of C/Co@pSPM (**3**) for average particle size determination. In total 448 particle diameters were measured to yield an average particle size of 25 ± 18 nm. The smallest particle was measured to be 5 nm and the biggest 113 nm.

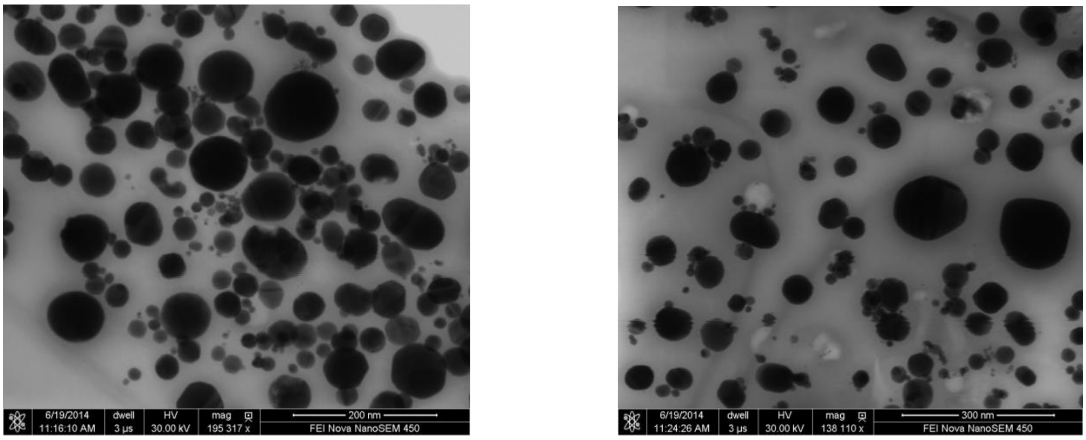

Fig. S8: Scanning transmission electron microscopy (STEM) pictures of C/Co@pSPM-clicked (**8**) for average particles size determination. In total 396 particle diameters were measured to yield an average particle size of 27 ± 19 nm. The smallest particle was measured to be 4 nm and the biggest 137 nm.

The functionalized particles are clearly separated from each other (Fig. S7 and Fig. S8) while the non-modified nanoparticles stick together and form aggregates (Fig. S6). This suggests, as more in-depth studies by Zeltner et al. showed, that the covalently attached polymer brushes indeed grew from the surface since control experiments in their work showed that physisorption alone (no covalent link) resulted in polymer loss by desorption and subsequent particle destabilization and precipitation.[2]

Determination of the amount of superparamagnetic particles

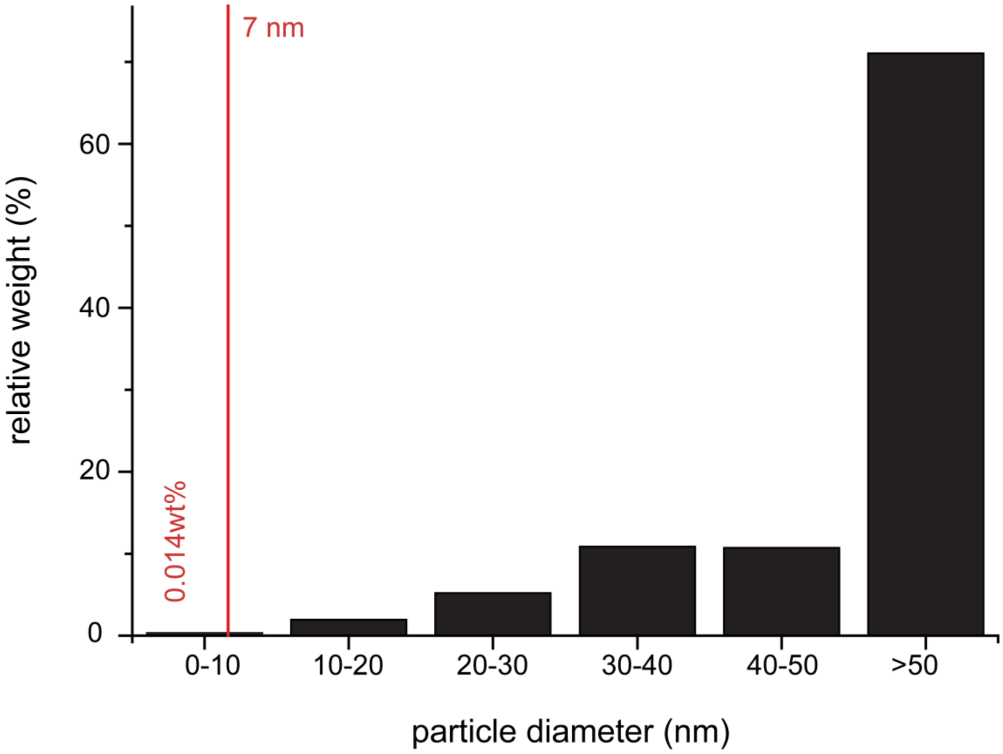

Fig. S9: Normalized size distribution of C/Co@pSPM-clicked (**8**) measured by Scanning transmission electron micrographs (STEM) shown in Fig. S8. In total 396 particle diameters were measured. The critical diameter below which the particles get superparamagnetic has been calculated to 7 nm for face-centered cubic (fcc) cobalt.[5] The amount of C/Co@pSPM-clicked (**8**) which is superparamagnetic is very small (0.014wt%).

Results of particle size distribution measurements by analytical centrifugation mesurements (LUMiSizer):

Table S2: Summerized results of sedimentation analysis by analytical centrifugation for C/Co@pSPM (**3**) and C/Co@pSPM-N_3 (**6**) in different media (H_2O, phosphate buffered saline (PBS), albumin solution (50 mg/mL in PBS, denoted as PBS&Albumin) and Dulbecco's modified Eagle's medium (DMEM)).

species	Medium	10% ≤ (nm)	16% ≤ (nm)	50% ≤ (nm)	84% ≤ (nm)	90% ≤ (nm)	Harmonic mean (nm)	Std. dev. (nm)
3	**H_2O**	20	23	32	51	57	**32**	25
	PBS	22	23	33	52	61	**33**	107
	PBS & Albumin	19	21	32	50	56	**31**	136
	DMEM	18	20	30	43	52	**28**	19
6	**H_2O**	24	27	36	50	56	**35**	17
	PBS	20	21	38	58	68	**34**	95
	PBS & Albumin	24	25	35	52	63	**35**	113
	DMEM	24	26	35	56	65	**36**	66

Investigations concerning dispersion stability in water for C/Co@pSPM with different SPM polymer chain length

Table S3: Elemental microanalysis results for carbon and sulfur content of C/Co@pSPM (**3**) with different polymer chain length (1,4,6 and 13 units of SPM per ATRP starter).

Compound	ΔC (wt%)[a]	ΔS (wt%)[a]	Loading (mmol/g)	units / starter
C/Co@pSPM-1 unit	0.63	n.a.	0.07[b]	1[b]
C/Co@pSPM-4 units	1.69	0.42	0.20[b] / 0.13[c]	4[b] / 3[c]
C/Co@pSPM-6 units	2.63	1.38	0.31[b] / 0.43[c]	6[b] / 9[c]
C/Co@pSPM-13 units	7.59	3.55	0.90[b] / 1.11[c]	13[b] / 17[c]

[a]: with respect to the used C/Co@initiator (**1**) [b]: calculated with respect to increase in carbon content, [c]: calculated with respect to increase in sulfur content, n.a.: not analyzed.

Table S4: Summerized results of sedimentation analysis by analytical centrifugation for C/Co@pSPM (**3**) with different polymer chain length in water. The particles possessing a polymer chain length of 1 and 4 SPM units are not enough stabilized and form aggregates. Furthermore, the time of magnetic separation of a dispersion of 1 mg/mL particles in water was measured (see also Fig. S10)

SPM units/starter	10% ≤ (nm)	16% ≤ (nm)	50% ≤ (nm)	84% ≤ (nm)	90% ≤ (nm)	Harmonic mean (nm)	Std. dev. (nm)	Time of magnetic separation
1	589	653	981	2584	3047	**914**	1034	25s
4	578	613	808	1011	1418	**779**	316	34s
6	21	23	35	55	63	**34**	131	>20min
13	19	20	25	42	54	**27**	24	>2 h

Fig. S10: Photographs of magnetic separation of 1 mg / mL C/Co@pSPM with different polymer length (from left to right: 1, 4, 6, 13 SPM units per ATRP starter) in water.

Saturation magnetization measurements and calculations

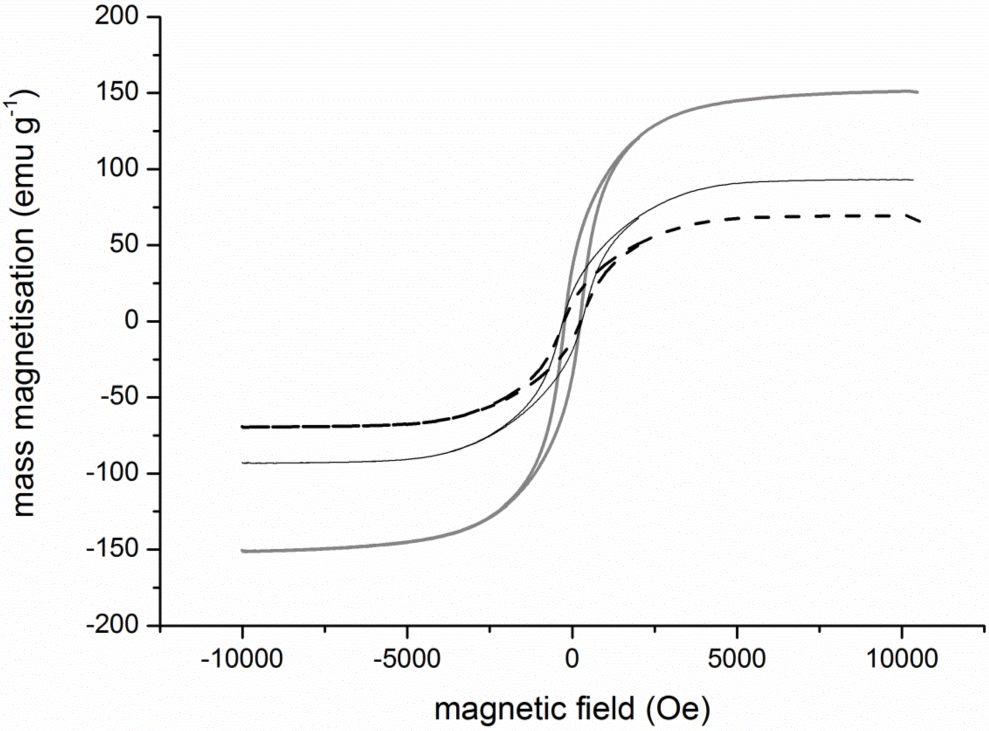

Fig. S11: Magnetic hysteresis susceptibility (VSM) of C/Co (grey), C/Co@pSPM (**3**) (dashed) and C/Co@pSPM-N$_3$ (**6**) (solid).

Total magnetization of coated particles

$$M_{tot} = M_{particle-core} \cdot \omega_{particle-core} + M_{polymer} \cdot \omega_{polymer} \tag{S(1)}$$

Where M_{tot}: total magnetization of particle core and polymer, $M_{particle-core}$: magnetization of particle core, $\omega_{particle-core}$: mass fraction of particle core, $M_{polymer}$: magnetization of polymer, $\omega_{polymer}$: mass fraction of particle core.

Since $M_{polymer}$ is nearly zero, the above formula reduces to a linear correlation, where the overall magnetization of the material is a direct result of its mass content of particle cores.

Table S5: Summerized results of sedimentation analysis by analytical centrifugation of C/Co@pSPM-N_3 (**6**) of supernatant after magnetic separation for different amounts of time (no magnetic separation, magnetic separation for 5 minutes and 10 minutes). The results show that bigger particles are separated faster than smaller ones what corresponds to published results.[6]

	10% ≤ (nm)	16% ≤ (nm)	50% ≤ (nm)	84% ≤ (nm)	90% ≤ (nm)	Harmonic mean (nm)	Std. dev. (nm)
-	24	26	38	59	67	38	20
5 min	19	20	35	51	56	31	20
10 min	13	14	18	34	36	19	113

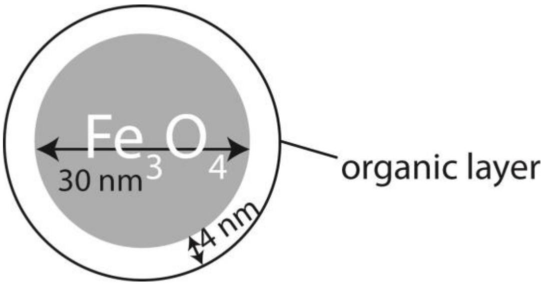

Fig. S12: Schematic representation of commercial Fe_3O_4-COOH showing the iron oxide core and the organic layer. Information given in the figure is taken from the datasheet[7] of the supplier.

In order to roughly estimate the saturation magnetization per gram material of the Fe_3O_4-COOH particles[7] the volume of the iron oxide core as well as of the organic layer was calculated. The relative weight of the organic layer was determined with the density of iron oxide (5.2 g/ cm^3) and the organic layer (1 g/cm^3) to 19.85 wt% of the iron oxide core. The iron concentration of the sample is given from the datasheet of the particles (5 mg/mL)[7]. The iron content of iron oxide is 72.4%. These calculations result in a particle weight of 8.28 mg particles per 5 mg of iron. The saturation magnetization of Fe_3O_4-dextran is given as 69 emu/g iron and is also used for Fe_3O_4-COOH. The saturation magnetization calculated as described above is: 42 emu/g material.

Biofouling test: calculations, procedure and results

The specific surface area (total surface area of a material per unit of mass) was calculated for all particles according to the formulas shown below. In the anti-fouling test the amount of particles which yield the same surface area was used.

$$A_s = \frac{4\pi r^2 \cdot n}{\rho \cdot V_{tot}}$$

$$n = \frac{V_{tot}}{V_{part}} \quad V_{part} = \frac{4}{3}\pi r^3 \rightarrow n = \frac{V_{tot}}{\frac{4}{3}\pi r^3}$$

$$A_s = \frac{3}{r \cdot \rho} \tag{S2}$$

Where A_s: specific surface area (m^2/g), r: radius of one particle (m), n: number of particles, ρ: density (g/m^3), V_{tot}: total volume (m^3), V_{part}: volume of one particle (m^3).

Diameters and densities were taken from the product specification datasheet for silica nanoparticles, dextran-coated iron oxide nanoparticles and polystyrene nanoparticles.[8] For PEG-coated iron oxide nanoparticles and for all cobalt particles diameters and densities were taken from the metal core (*i.e.* iron oxide ρ = 5.2 g/cm^3, cobalt ρ = 8.9 g/cm^3, d = 30 nm). The specific surface area of C/Co has been measured to be 15 m^2/g in average.[1]

The procedure was adapted from literature.[9] 1 mg of C/Co, C/Co@pSPM (**3**), C/Co@pSPM-N$_3$ (**6**) and C/Co@pSPM-clicked (**8**) were each put in a 2 mL Eppendorf tube. The other particles used were preserved in solution. The calculated amount of particles (for silica particle-solution: 10 µL, polysterene particle-solution: 12.9 µL, dextran-coated iron oxide nanoparticle-solution: 7 µL and for PEG-coated iron oxide nanoparticle-solution: 390 µL) were centrifuged from the particle-solution and the supernatant was removed. Enzyme-solution (0.5 mg enzyme powder/mL, 1 mL) was then added to all of the particles and dispersed by sonication for 5 minutes. The enzyme β-glucosidase was adsorbed on the particles by shaking for 1 h at 900 rpm. The particles were removed by centrifugation (15 min, maximum speed) and 48 µL of the residual solution was added to a 4-nitrophenyl β-D-glucopyranoside solution (11 mg, 0.037 mmol) in PBS buffer (1.646 mL, Gibco, adjusted to pH 6.5 with 0.1 M HCl). For 2 minutes, samples were taken every 30 seconds. 242 µL of the sample solution were immediately added to a stopping NaHCO$_3$ buffer solution (62 µL, 0.1 M, pH=11) and transferred to the microplate for absorbance measurement at 405 nm.

With the known real enzyme concentration in the enzyme powder (0.16 mg enzyme in 0.5 mg enzyme powder, determined by a commercially available BCA Protein Assay Kit, Thermo Scientific) the amount of adsorbed enzyme on the particles surface was determined (see Fig. S13).

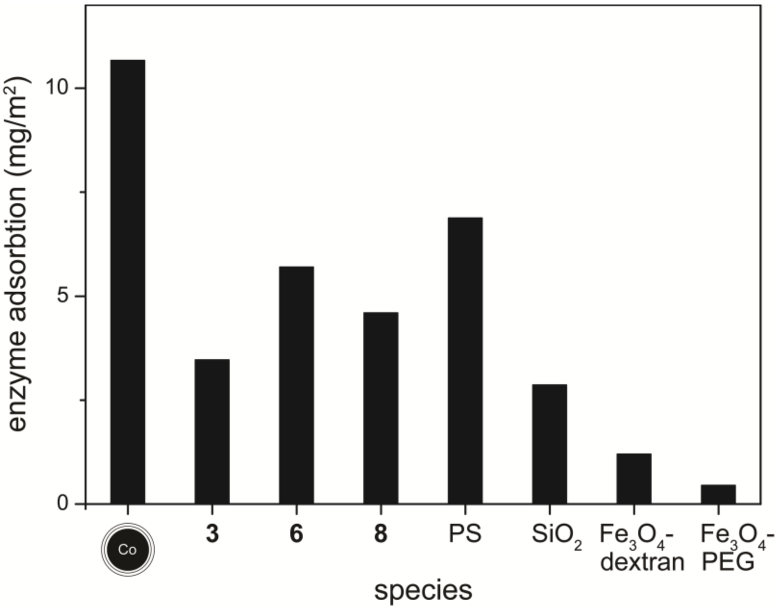

Fig. S13: Measured unspecific binding of β-glucosidase with non-functionalized C/Co nanoparticles, C/Co@pSPM (**3**), C/Co@pSPM-N$_3$ (**6**), C/Co@pSPM-clicked (**8**), polystyrene nanoparticles, silica nanoparticles, dextran-coated iron oxide nanoparticles and polyethyleneglycol (PEG)-coated iron oxide nanoparticles.

Dynamic light scattering measurements

Table S6: Particle size of C/Co@initiator (**1**), C/Co@pSPM (**3**) and C/Co@pSPM-N_3 (**6**) as a function of time measured by dynamic light scattering (DLS).

Time (minutes)	C/Co@initiator (**1**)	C/Co@pSPM (**3**)		C/Co@pSPM-N_3 (**6**)	
		Absolut diameter (nm)	Δ(nm)	Absolut diameter (nm)	Δ(nm)
0	2522	260.4		272.9	
6		264.1	3.7	274.4	1.5
12		262.8	-1.3	275.1	0.7
18		260.9	-1.9	275.7	0.6
24		263.1	2.2	277.4	1.7

Due to the high polydispersity of the used particles, DLS is well suited for qualitative assessment of the aggregation state of the particle but the actual size number determined by DLS is not accurate.[10] As shown in Table S6 there is no cluster formation over time for at least 24 minutes. The non-stabilized C/Co@initiator (**1**) form immediately clusters which are an order of magnitude bigger than the polymer-stabilized C/Co@pSPM (**3**) and C/Co@pSPM-N3 (**6**). Sedimentation analysis by analytical centrifugation and STEM/TEM clearly show a particle diameter of around 30 nm.

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