PROJECT FINAL REPORT

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[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.

[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

Inhaltsverzeichnis

1. Final publishable summary report

1.1. An executive summary

Magnetite nanoparticles, especially superparamagnetic iron oxide nanoparticles (SPION), are established contrast agents for magnetic resonance imaging (MRI). Magnetosomes, magnetite nanoparticles of biological origin have been shown to have better contrast properties than current formulations, possibly because of their larger size. Here, we present an integrated study of magnetosomes and synthetic magnetite nanoparticles of varying size and therefore with different magnetic properties. We test not only the contrast properties of these particles but also their cytotoxicity and demonstrate the higher contrast of the larger particles. A theoretical model is presented that enables us to simulate the R2/R1 ratio of a contrast agent and confirm that larger particles offer higher contrast. The results from this study illustrate the possibility to obtain colloidal stability of large magnetic nanoparticles for MRI applications, and serve as an impetus for a more quantitative description of the contrast effect as a function of size.

1.2. A summary description of project context and objectives

Nanoscience and nanotechnology are currently revolutionizing sectors such as medicine, information technologies, environmental or materials sciences, and creating new opportunities for our societies. In this context, magnetic nanoparticles (MNP) are key components to the development of novel nano- and biotechnologies. Magnetosomes are unique hybrid magnetic MNP produced by magnetotactic bacteria (MB). They are employed in applications ranging from extraction of DNA to the development of immunoassays and uses in spintronics are envisaged. However, only a very limited amount of MNP (few mg per day) can be formed by MB, and the formation principles remain to be tackled.

Biomimetics, i.e. combining biological principles with chemistry, will pave the way to understand biomineralization of tailored MNP and to find out high-value high-yield synthetic routes to solve scientific and technological challenges. Specifically, we aspire at bridging the gap between different fields of science. For the first time, we blend biological and genetic approaches with chemical and physical knowledge to understand the key parameters controlling the size, shape, composition and assembly of hybrid MNP in vivo and in vitro. We combine nanoscience and nanotechnology to modify these properties and develop an ensemble of magnetic nanomaterials of higher values. This approach leads to original contributions of innovative nature based on the combined skills of the partners to manufacture and characterize the biological, chemical, structural and magnetic properties of the MNP. The industrial partner has key importance in managing and assessing the applicability of the MNP in Magnetic Resonance Imaging (MRI). Finally, our cell biologist partner tests the biocompatibility of the designed systems. In 3 years, we aimed at being able to synthesize hybrid MNP with tailored magnetic and size properties by low-cost high-yield synthesis for applications in MRI.

In addition, our main research outcomes should be recognized within the field of biomedicine and in the longer term that they should also enable the development of better contrast agent for MRI. The results obtained within the project confirmed, that contrast agents which are based on monocrystalline particles, offer very promising contrast properties compared to commercial available ones. Therefore, the dissemination of the results will open new perspectives in the field of contrast agent research and imaging.

In order to achieve this recognition, dissemination is a crucial step to reach the medical doctors that in practice use the particles as contrast agent, the pharmaceutical industry that develop and sell contrast agents but also the potential patients that need to be aware of the development in the field to potentially accept to be part in the development of therapeutic agents in the future.

The long term goals described above clearly require a long period of time before the scientific findings done in the project can be translated in the clinics. Therefore, the short term goals of the dissemination part in the consortium were focused towards the communication of our main scientific results within dedicated scientific journals as well as conferences. As there is always a lag time between the achievement of the results, their publication in scientific journals, and the feedback received by the authors from the scientific community, it might take some additional years even to fully achieve these somewhat shorter term objectives.

1.3. A description of the main S&T results/foregrounds

In the first half of the project, synthetic routes were developed to produce biological and biomimetic magnetite nanoparticles of different dimensions. In addition, analytical techniques were developed to characterize the produced materials in term of mineralogical and magnetic properties, as well as biocompatibility and effect as contrast agent for magnetic resonance imaging.

We aimed at producing nanoparticles in such quantities that a single batch of materials would be used by all consortium members for further characterization. We planned to perform an integrated analytical study to correlate the MRI outcomes with the physical properties of the nanoparticles.

In the second period, and as planned, we first modified our production strategies to ensure reproducibility of the synthetic approach (WP1). In addition, we modified our synthetic routes in such a way that we obtained nanoparticles in such quantities that a single batch of materials could be used by all consortium members for characterization.

With the nanoparticles synthesized in sufficient amount, we performed an integrated analytical study to correlate the physical properties of the nanoparticles (magnetic properties, crystallographic properties, biocompatibility) (WP2) with the MRI outcomes (WP3). Finally, we developed a theoretical model to correlate the particle properties with the contrast properties (WP4).

WP1

We produced a very large number of different samples (˜50 bacterial (LMU) and ˜80 synthetic (MPG)) of magnetite nanoparticles. Procedures of the production of both biogenic and synthetic samples were continually refined in response to the results of sample characterization, in order to achieve the highest purity magnetite and the best performance of the particles as MRI contrast agents.

Both biological and biomimetic procedure were modified to enhance the outcome of the synthesis in such a way that a single batch was sufficient to send materials to all partners of the WP2 and WP3. In addition, partners from WP2 and 3 showed that the batches were exhibiting similar properties for similar conditions thereby ensuring reproducibility and quality control.

WP2

Within the EU project, Bio2MaN4MRI, we analysed a very large number of magnetite nanoparticles (produced in WP1). Our efforts are illustrated below by selected, typical examples of 5 biogenic and 6 synthetic samples (Table 1), for which we present the results of our characterization experiments. The samples are categorized as small biogenic (SB), large biogenic (LB), small inorganic (SI) and large inorganic (LI).

1.4. Structure, size and morphology of the nanoparticles (UP and MPG)

Transmission electron microscopy (TEM) imaging is used to study the morphologies and sizes of the particles. The later is also assessed by X-ray diffraction (XRD), which in combination with selected-area electron diffraction (SAED) provide information about the crystal structure of the particles. All samples consist of nanoparticles of pure magnetite, as indicated by the observed peaks in the X-ray diffractogram (Figure 1) and by diffraction maxima measured in SAED patterns (Figure 2c and d).

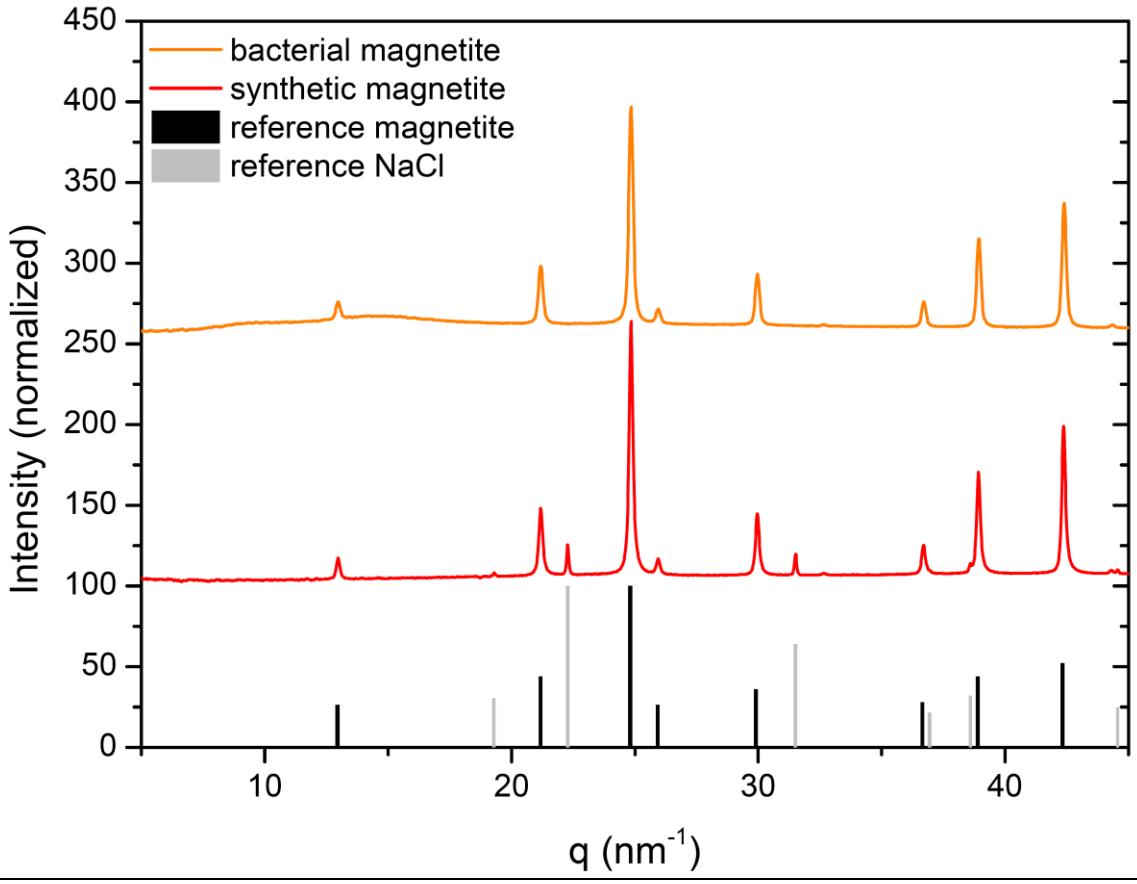


Figure 1: X-ray diagram of representative bacteria (LB3 3, orange) and synthetic (LI 2, red) samples as well as reference materials.

Synthetic samples

The sizes of the individual nanoparticles range from a few to several tens of nanometers (Table 1). The mean sizes, which are determined by TEM for the particles in the SI samples, are in good agreement with the sizes obtained using synchrotron X-ray diffraction. The size of

larger particles (LI samples) is difficult to determine from TEM micrographs because of aggregation.

Table 1: Summary of the properties of the nanoparticles. Acronyms used for a sample's names: SB stands for Small and Biological, LB for Large and Biological, SI for Small and Inorganic, LI for Large and Inorganic, s for stabilized. d_c, d_h are core and hydrodynamic diameter, respectively.

Sample description	d_c [nm]	d_h [nm]	Stabilizing agent	M_{rs}/M_s	H_{cr}/H_c	EZ4U NIH3T3 [IGC_{50}, mg/ml]	EZ4U RAW264.7 [IGC_{50}, mg/ml]	EZ4U MC3T3-E1 [IGC_{50}, mg/ml]	LDH NIH3T3 [IGC_{50}, mg/ml]	LDH RAW264.7 [IGC_{50}, mg/ml]	LDH MC3T3-E1 [IGC_{50}, mg/ml]	R1 [L/mmol*s]	R2 [L/mmol*s]	R2/R1
SB1	24	90		0.05	N/A							9.0	320.7	35.6
LB1	41	143		0.38	1.5							8.6	541.5	63.1
LB2	38	84		0.38	1.3	0.94	0.05	0.13	0.82	0.07	0.12	8.8	477.1	54.2
SB2	29	76		0.20	2.8	0.72	0.18	0.12	0.54	0.16	0.12	11.0	442.5	40.1
LB3	32			0.25	1.4	0.68	0.19	0.13	0.48	0.43	0.12			
LI1s	28	115	DOPA	0.20	1.78							12.1	330.1	27.1
LI2	36			0.22	1.57	0.34	0.29	0.65	0.27	0.37	0.53			
LI3	33			0.20	1.60	0.50	0.23	0.65	0.44	0.22	0.53			
LI4	26.5			0.21	1.65	0.31	0.48	0.49	0.22	0.65	0.20			
SI1	17			0.06	2.25	0.52	0.15	0.58	0.48	0.55	0.33			
SI1s	17	123	DOPA									14.9	244.5	16.4
SI2	18			0.09	2.46	0.53	0.24	0.45	0.49	0.55	0.20			
SI2s	18	100	DOPA	0.01	N/A							15.3	302.6	19.7
	5	63		0.01	N/A	0.16						20.0	219.3	11.0

All samples were very similar in that they contained clusters of nanoparticles in random orientations (Figure 2a), with most particles having euhedral (octahedral or cuboctahedral) shapes and typically perfect structures (Figure 2b), as suggested by high-resolution TEM images. SAED patterns obtained from both clusters of crystals (producing ring patterns, as in Figure 2c) and from individual nanoparticles were consistent with the structure of defect-free magnetite (Figure 2d).

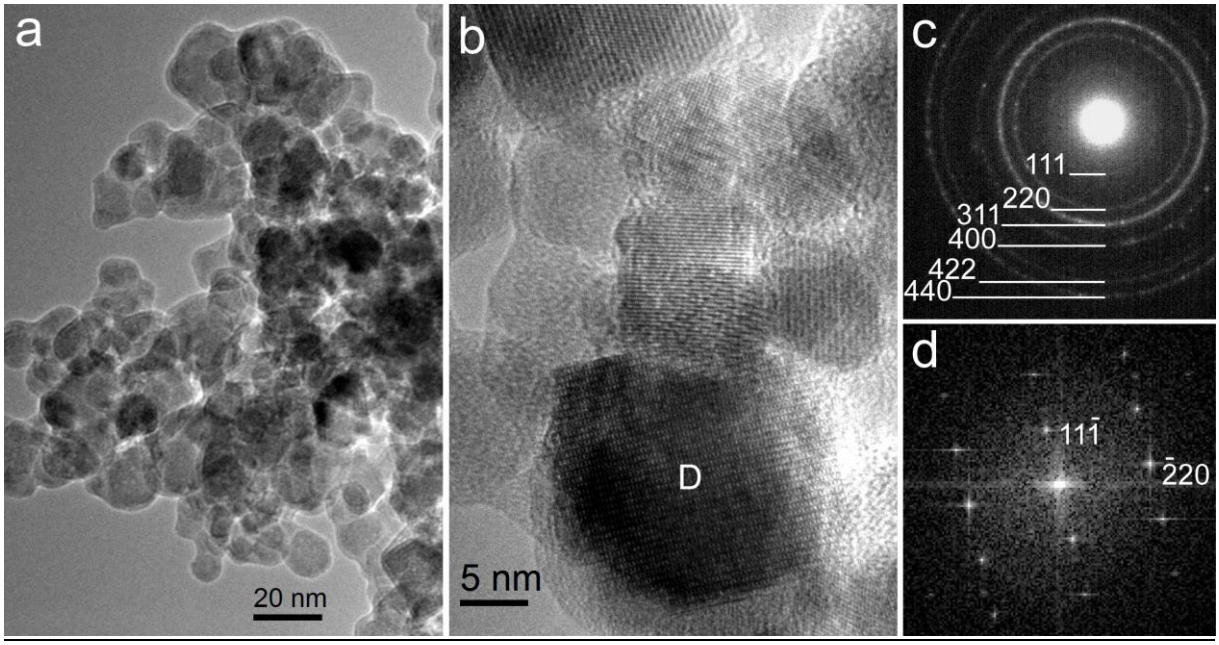


Figure 2: (a) A typical view of magnetite nanoparticles in SI1. (b) HRTEM image of several nanoparticles, showing euhedral shapes and perfect crystalline structures. (c) SAED pattern obtained from a cluster of nanoparticles, and (d) a Fourier transform of the HRTEM image of particle D in (b). Both the ring pattern in (c) and the intensity maxima in (d) can be indexed according to the structure of magnetite.

LI2 and LI3 consist of mostly small (~15 nm) magnetite nanoparticles; however, these samples also contain larger aggregates that apparently formed from randomly or similarly oriented nanocrystals.

Biological samples

In all biological samples, the magnetosomes extracted from different strains of magnetotactic bacterium *Magnetospirillum gryphiswaldense (Table S1)*, were still enveloped by the magnetosome membrane. This surface layer prevents clustering/aggregation of the particles, and results in a dispersed distribution of magnetosomes on the TEM grid. Typically, particles larger than 30 nm diameter form chains, whereas smaller particles appear randomly scattered (Figure 3).

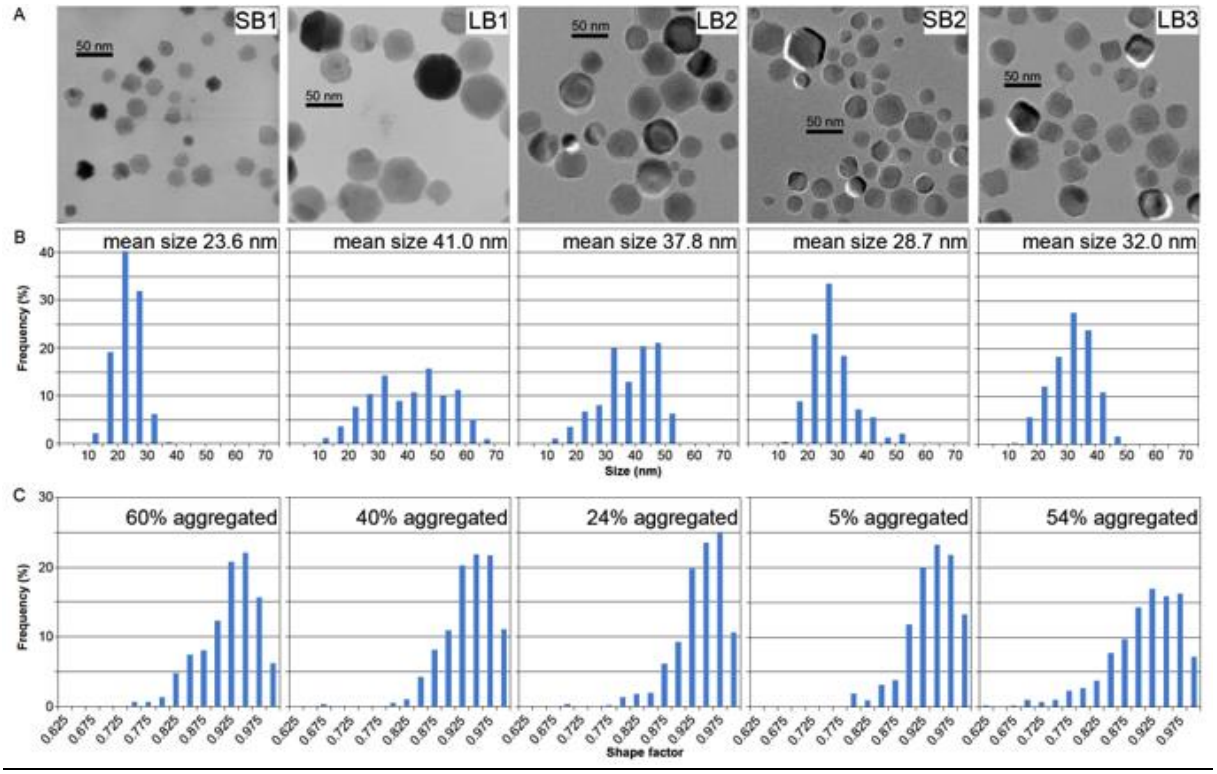


Figure 3: (a) Bright-field TEM images of typical magnetite magnetosomes from five different bacterial samples, with the sample identifiers indicated on the top (cf. Table x). (b) Particle size distributions as measured from TEM micrographs. (c) Shape factor (width/length) distributions of particles in the same samples, with the fraction of twinned or aggregated particles indicated in each panel.

Compared to magnetosomes of the wildtype particles of mutant strains (LB2) show a difference in both particle size and shape. Whereas wildtype particles produce a negatively-skewed size distribution, size distributions in mutant samples are lognormal-like (SB2), Gaussian (SB1 and LB3) or broad, double-peaked (LB1) (Figure 3b). The mean size of the distributions increases from 23.6 nm (SB1) to 41 nm (LB1). Concerning the shapes of the particles, samples LB2 and SB2 contain wild-type-like, mostly single crystalline, euhedral particles (Figure 4a).

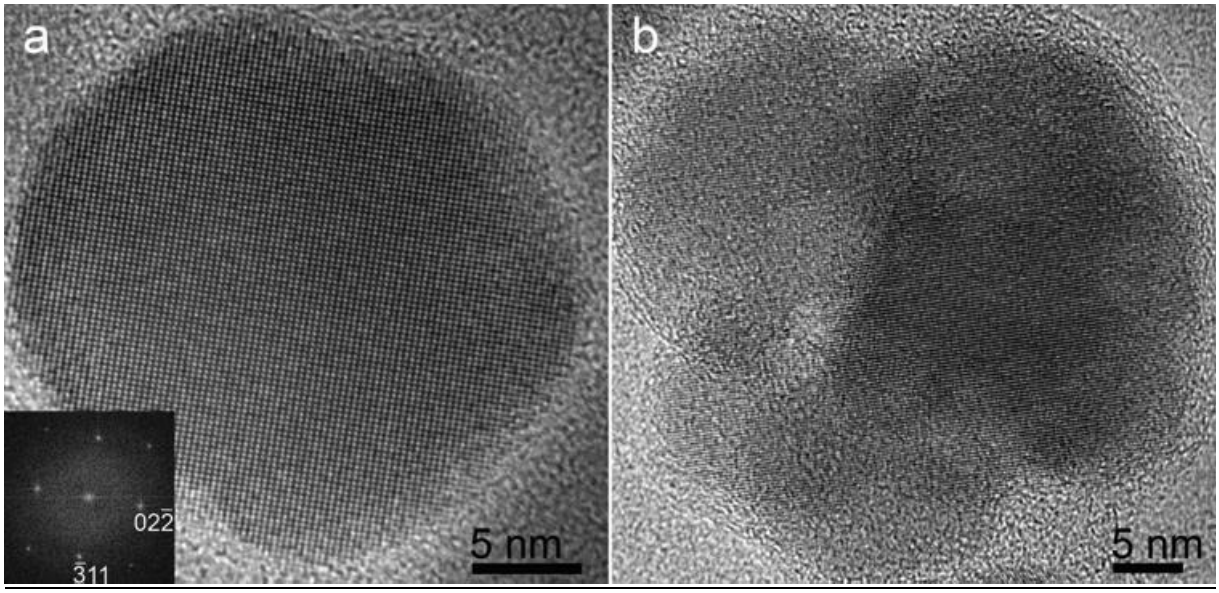


Figure 4: HRTEM images of (a) a single-crystal magnetosome with a perfect structure from SB2, with the Fourier transform of the image in the lower left, indicating that the crystal is viewed along the [223] crystallographic direction, and (b) an aggregate magnetosome that consists of at least three individual crystals, from sample LB3.

The shape factor distribution is relatively narrow in these samples and resembles the curves that are typically obtained for magnetosomes from the wildtype (**Figure 3**c). In the other samples, however, a large fraction of the magnetosomes were either twinned or multiply aggregated. This effect was most pronounced in the case of SB2, which contained relatively large crystals, most of which are composed of several crystallites (**Figure 4**b). The aggregated nature of the magnetosomes clearly affects their shape factor distribution, because samples containing a large fraction of twinned or aggregated particles produce broad shape distributions (**Figure 3**c). The TEM-based particle size and shape measurements are entirely consistent with the bulk magnetic data as shown below.

1.5. Magnetic properties (ETH)

Biological samples show a large variation in their magnetic properties (Table 1). SB1 has a closed hysteresis loop, which would be expected for true SP behavior (**Figure 5**). The hysteresis loops of the other biological samples are open and the ratios of the remanent saturation magnetization to saturation magnetization (M_{rs}/M_s) and remanent coercivity to coercivity (H_{cr}/H_c) are compatible for a mixture of SSD and SP particle sizes. The synthetic samples all show an open hysteresis loop (**Figure 5**). The magnetization ratio is variable and suggests SSD particle size with a significant SP

contribution. The approach to saturation magnetization of synthetic particles is slower (low susceptibility) and requires much higher fields to reach saturation as compared to biologic particles.

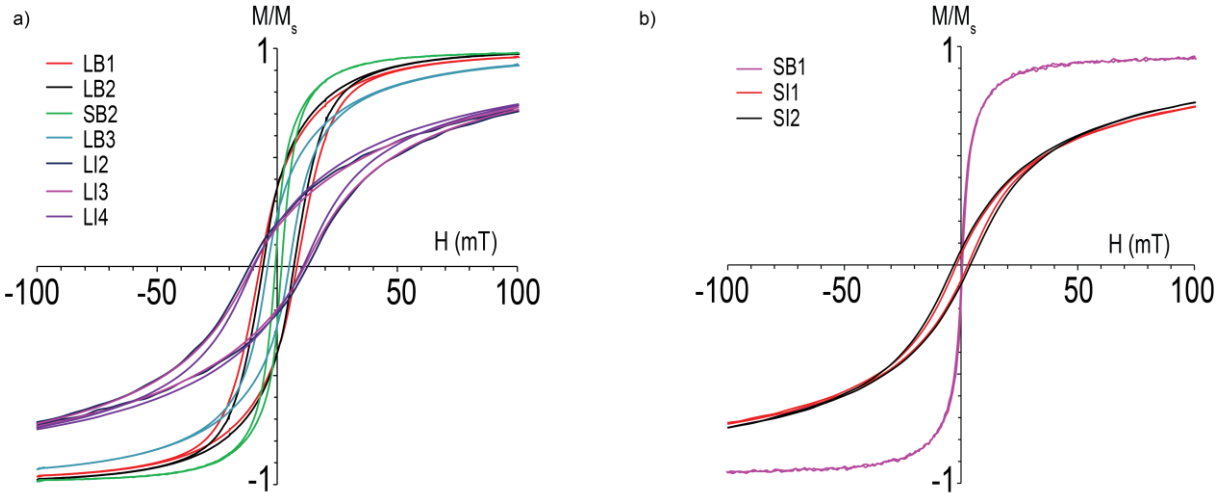


Figure 5: Normalized hysteresis loops for chemically synthetized and biologically mineralized magnetite nanoparticles with average particle size in the a) SSD and b) SP domain.

First order reversal curve (FORC) analysis is a powerful technique for characterizing ferromagnetic minerals (s.l.), their domain state (SP and SD), and the extent of interactions between particles[12]. FORC distributions located near the origin, i.e at the cross section of interaction axis (H_b) and the coercivity axis (H_a), with an upward offset signify non-interacting particles, whereas a spread along H_a signifies a distribution in particle size. A spread along H_b on the FORC diagram indicates the presence of interacting SD particles. FORC diagrams for SB1, SB2, SI1, and SI2 reveal the dominance of magnetite nanoparticles that are SP (Figure 6). The other samples contain a broader mixture of SP and SSD particle sizes.

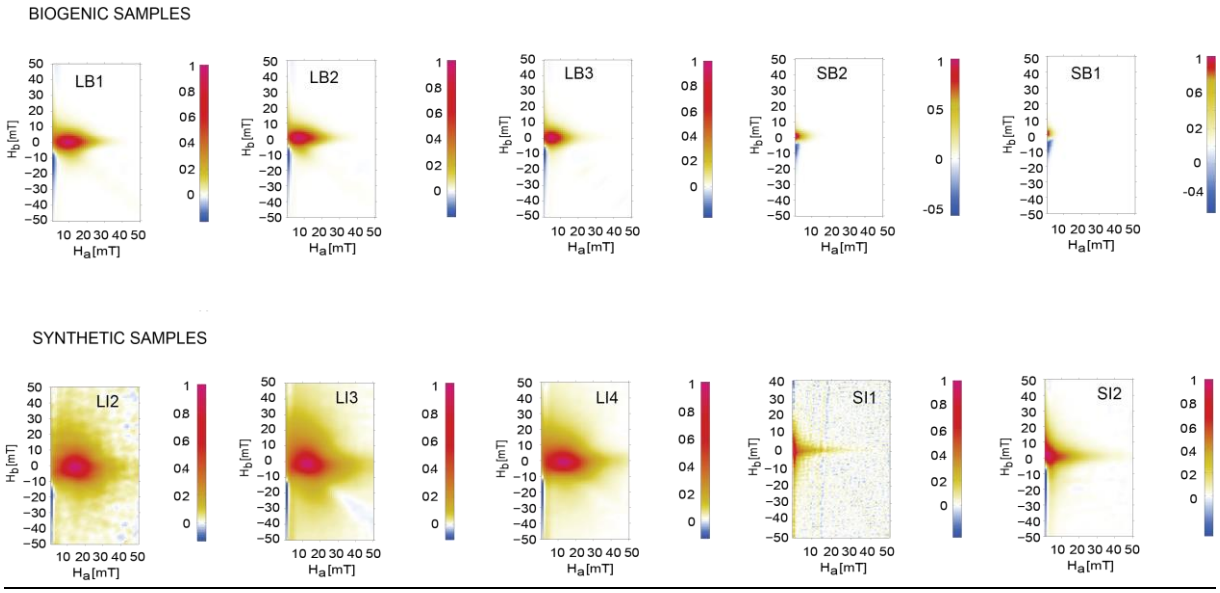


Figure 6: FORC diagrams for biogenic and synthetic magnetite nanoparticles. Smoothing factor is 2.

Decomposing the FORC measurements into the reversible and irreversible parts of the induced magnetization clearly shows the relative magnitudes of the SP and SSD contributions (Figure 7). Samples LB1, LB2, LI2, LI3, and LI4 have a larger contribution of irreversible magnetization, i.e., SD particle size. LB3 has less of a contribution from SSD particles, and samples SB2, SI1 and SI2 are largely SP. Only SB1 is almost purely SP in its magnetic behavior.

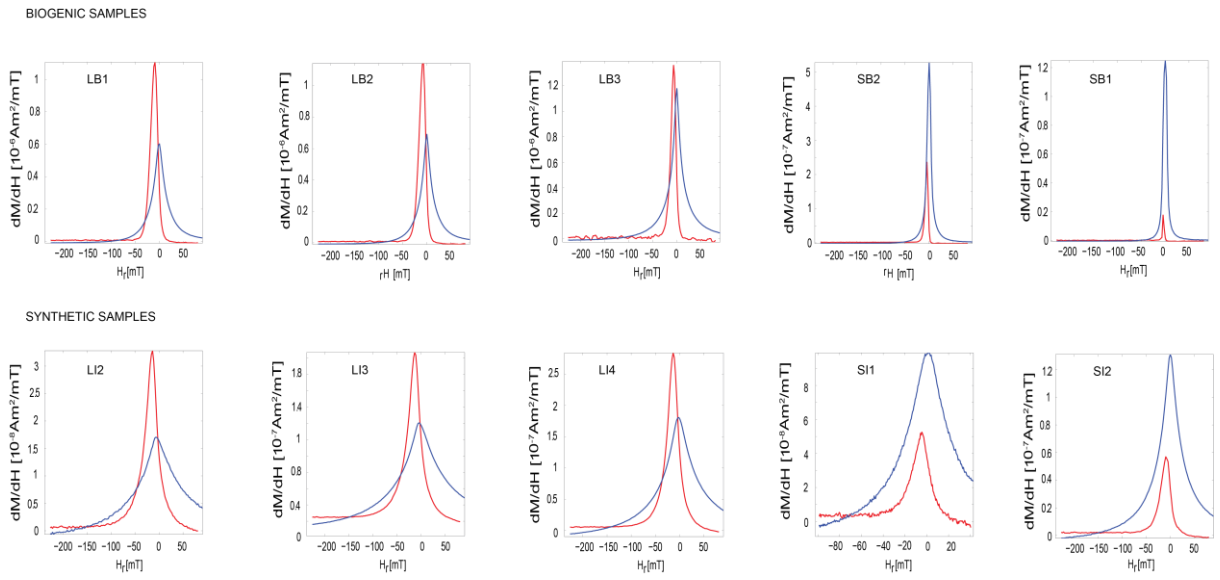


Figure 7: Derivative of magnetic moment of the reversible (blue curve) and irreversible (red curve) part of the induced magnetization versus reversal fields (Hr) for both biologic and synthetic samples From

FORC analysis one sees that the biological SSD samples exhibit a lower spread on the H_b (interaction) axis, varying between ± 15 mT, compared to the synthetic samples that range from -30 mT to +40 mT. This suggests a lower degree of interaction among magnetite particles from biologic origin as compared to chemically synthesized. There is also a larger spread in the coercivity distribution of the synthetic samples, e.g., range from 0 mT to 40 mT for SI1 with the smallest particle size distribution, compared to SB1. It should be noted that the larger degree of interactions in the synthetic samples may lead to a larger effective magnetic particle size in comparison to their physical size, i.e., aggregates of SP particles behave like SSD size. Afterward SI1 was coated with L-3,4 Dihydroxyphenylalanin (DOPA) (SI1s), which inhibited particle interaction. The FORCdistribution is concentrated at the origin, as would be expected for purely SP particles, and the magnetization is largely reversible (Supplementary Figure 2) similar to what is found for SB1. Resovist shows a largely reversible magnetization similar to what is seen in SI1s and SB1 (Supplementary Figure 3). Truncating the FORC distribution to suppress the very low coercivity, however, shows that there are particles with a higher coercivity (cf., inset Supplementary Figure 3). This higher coercivity tail is not found in SB1 and SI1s.

FORC analysis demonstrates that the magnetic properties of samples SB1, SB2, SI1 and SI2 are dominated by the SP fraction in the sample. Samples LB1, LB2, LB3, LI2, LI4 and LI4 may also have SP particles, but the SSD particles dominate. Coating the synthetic particles is successful in breaking down particle interaction, so that their magnetic properties are SP.

1.6. Biocompatibility (LBIO)

The biocompatibility of the synthetic inorganic and biological samples was tested by different assay systems: EZ4U and LDH assay, using various cell lines: MC3T3-E1 osteoblast, NIH3T3 fibroblasts and RAW264.7 macrophages (Table 1). IGC_{50} values obtained by EZ4U and LDH assays give similar results for all synthetic inorganic and biological samples within one cell line. More specifically, IGC_{50} values of the synthetic inorganic particles, which were determined in the three cell lines, range from 0.15 – 0.65 mg/ml in the case of EZ4U assay and between 0.20 – 0.65 mg/ml in the case of LDH assay. These results demonstrate that IGC_{50} values of the synthetic inorganic particles with sizes between 17 – 36 nm do not depend on size.

The biocompatibility of numerous types of artificially synthesized nanoparticles, e.g. chitosan, silica and zinc oxide, has been determined using various cell lines[14]. IGC_{50} values of 0.1 – 0.25 mg/ml were reported for iron oxide nanoparticles[15]. The IGC_{50} values from this study are not broader and

only slightly higher than values reported in these other investigations and show that cytotoxicity of our synthetic inorganic iron oxide particles is comparable to synthetic inorganic particles from e.g. chitosan or zinc oxide.

The IGC_{50} values of the biological particles show a larger variation than those of synthetic inorganic particles. They are, however, similar within one cell line. IGC_{50} values evaluated by EZ4U and LHD assay in MC3T3-E1 and RAW264.7 cells range from 0.05 – 0.19 mg/ml and 0.07 – 0.43 mg/ml, respectively. In NIH3T3 cells IGC_{50} values were higher than in the other two cell lines, which cannot be explained at present. IGC_{50} value in NIH3T3 cells determined by EZ4U and LDH assay range from 0.48 – 0.94 mg/ml. Thus, the applied genetic manipulation of the biological nanoparticles did have an effect on IGC_{50} values in the tested cell lines. In general, the inorganic particles are less toxic to the cells than the biological ones, probably due to the presence of remaining bacterial cell components on the magnetosome membrane.

This work has shown that stable magnetic nanoparticles with a limited size distribution have been produced either biologically or synthetically, with particle size distribution narrower for the biological samples. Small particles have predominantly SP behavior, whereas the magnetic properties of larger particles show a combination of SP and SSD behavior. Stabilization of the synthetic, inorganic particles prevents aggregation, so that small particles are almost exclusively SP in their magnetic properties.

WP3

1.7. Colloidal stability

The biological samples were found to be colloidally stable without further stabilization. This behavior can be attributed to the magnetosome's membrane, which is sufficient to stabilize the particles in aqueous media. The synthetic particles on the other hand, specifically those exhibiting large crystal sizes and hence high magnetic attraction forces, are prone to aggregation. Thus, stabilization is essential for these nanoparticles, because a surface modification during the synthesis step is missing.

Particles can typically be colloidally stabilized using two different approaches: the electrostatic and the steric stabilization. Iron oxide nanoparticles for biomedical applications are typically sterically stabilized by using biocompatible dispersants, such as dextran[13], polyethylene glycol (PEI) [14] or poly (vinyl alcohol)[5]. These dispersants are physically adsorbed on the particle surface and prevent the particles from aggregation due to steric repulsion. Because the non-ionic stabilization polymers do not lead to any colloidal stable dispersion, cationic polymers such as Polyethylenimine and chitosan were found to be good dispersants for the synthetic particles (data not shown). PEI and chitosan are known to offer an exceptionally strong adhesion because of additional ionic interactions with the particle surface[3,15]. Electrostatic stabilization, in turn, can be achieved by introducing an anionic/cationic charge[5] either due to anions/cations or due to monomers with a charged functional groups; i.e. citric acid[16]. The most promising results were obtained using L-3,4-Dihydroxyphenylalanin (L-DOPA), which is known to exhibit high affinity anchor groups for iron oxide particles and lead to highly stable dispersions[17].

The colloidally unstable particle dispersion was treated with an ultrasonic tip (Bandelin Sonoplus, duration = 30 s with 60% power) to reduce the size of large particle aggregates. Afterwards, the particle dispersion was mixed 1/1 (v/v) with a 10 wt% polymeric solution and a saturated solution of L-DOPA, respectively. The solution was shaken for 3h (Thermomixer comfort, Eppendorf) and finally centrifuged (Biofuge pico, Heraeus) for 5 min @ 4000 rpm. The resulting supernatant, containing the stabilized particles, was removed and analyzed in terms of particle size (DLS) and iron content.

The stabilization of the synthetic particles LI1, SI1, and SI2 was successful, and the stabilized dispersion of SI2s using L-DOPA is shown in Figure 8. The change in colloidal stability can be clearly visually observed and is also reflected by the hydrodynamic diameter, which is in the range of

100 – 125 nm after the stabilization. The stabilized particles show good colloidal stability until present, two months after treatment. Stabilization of the samples LI2, LI3 and LI4 was not successful. This can be attributed to the larger core sizes, which lead to stronger magnetic interaction (i.e., larger contribution of irreversible magnetization, cf. Figure 7), which results in irreversible aggregation.

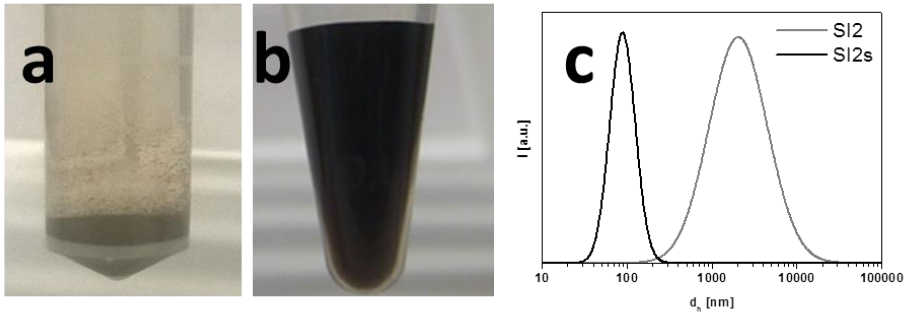


Figure 8: Sample SI2 a) before and b) after stabilization using L-DOPA. The log-normal distribution of the hydrodynamic diameter (c) of the original and stabilized particles clearly indicates the stabilization effect.

1.8. Contrast properties in MRI

In general, paramagnetic and superparamagnetic substances lead to a shortening of the relaxation times T_1 (spin – lattice relaxation time) and T_2 (spin-spin relaxation time) of the hydrogen protons in the surrounding tissue. Iron oxide nanoparticles exhibit an especially strong shortening of T_2, for which they are also called T_2-contrast agents. The higher the shortening of T_2, which is equivalent to a high R_2 relaxivity, the higher the obtained T_2 weighted contrast is. Contrast agents in general are characterized by the ratio of the relaxivity R_2/R_1. Therefore, a high T_2 weighted contrast can be obtained by combining high R_2 values with low R_1 values. In order to evaluate the findings, the contrast properties of the here investigated particles were compared with the known contrast agent Resovist®.

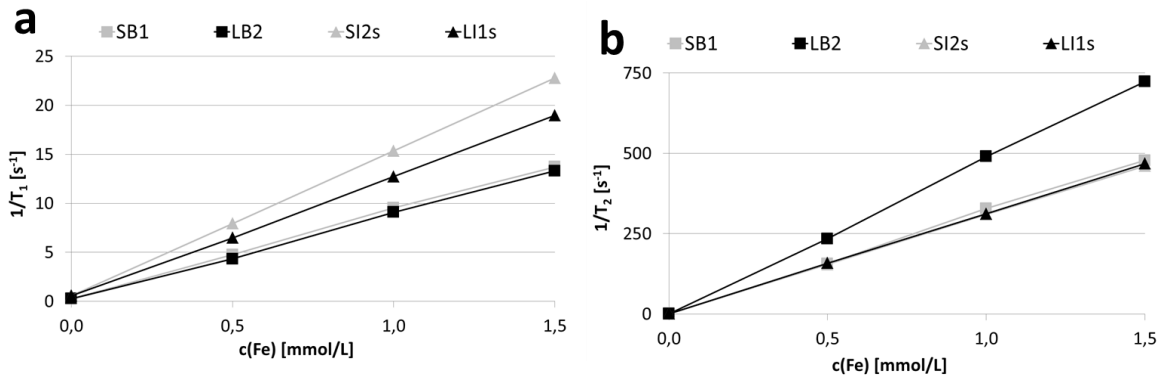


Figure 9: Concentration dependent relaxation rates (a) 1/T1 and (b) 1/T2 determined at 0.94T @ 39°C. The biological particles are represented by the cubes and synthetic particles are represented by triangles, respectively.

Figure 9 illustrates the relationship between the relaxation rates $1/T_1$ and $1/T_2$ and iron concentration. As expected, both relaxation rates are increased with increasing iron concentration. The relaxation rates of all particles show good linearity in the measured concentration range, which is an indication for non-interacting particles and colloidal stability. Colloidal stability is mandatory for the determination of the relaxivity values R_1, R_2 and $R_2/R_{1,}$.

The dependency of the relaxivity from the core diameter d_c of the different particles is shown in Figure 10. Independent of the origin of the particles, biological or synthetic, the R_2 relaxivity increases roughly linearly with the core diameter d_c. The increase of the R_2 relaxivity with the particle size can be explained by the fact, that larger particles exhibit stronger magnetic moments, which lead to a faster dephasing of the hydrogen protons and, therefore, to a stronger shortening of T_2. It is known that the T_2-effect dominates for iron oxide particles over the T_1-effect. We also observe a dependence of R_1 with a decreasing T_1 effect with increasing core diameter (decreased R_1 relaxivity). Considering both the R_1 and R_2 relaxivities, the decisive R_2/R_1 ratio increases with the core diameter.

It has to be noted that in addition to the dependence of the relaxation properties with the core diameter d_c, a strong dependence is also found with the hydrodynamic diameter d_h (data not shown). Therefore, only particles with similar hydrodynamic diameters can be compared directly (see Table 1).

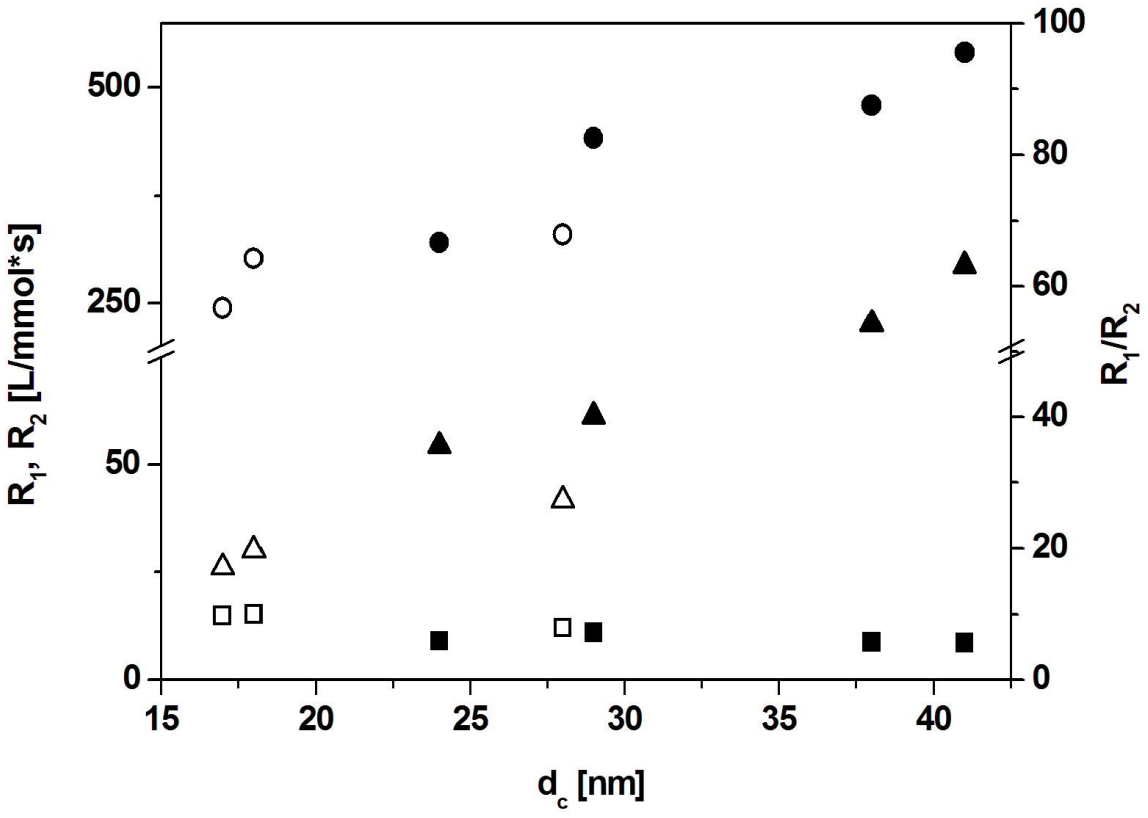


Figure 10: Size dependence (dc) of the R1 relaxivity (square), R2 relaxivity (circle) and the ratio R2/R1 (triangle). Biological particles are represented by the filled symbols and synthetic particles are represented by blank symbols, respectively.

The biological particles were measured in the original state because of their sufficient colloidal stability. Compared to Rseovist, the smallest biological particles SB1 (d_c = 24 nm) exhibit a 1.5 fold increase in R_2 and a 3.2 fold increase in R_2/R_1. In accordance with the observed size-dependence, the large biological particles LB2 (d_c = 38 nm) show an improved relaxation behavior with an 2.2 fold higher R_2 relaxivity and, due to the low R_1 relaxivity, even a 4.9 fold higher R_2/R_1 relaxivity.

The relaxation properties of the original synthetic particles could not be measured because of colloidal instability; therefore only the DOPA-stabilized particles were used for the determination of the relaxation properties. The R_2 relaxivity is increased compared to that of Resovist, but due to the overall smaller core diameters only to a limited degree. The SI2s particles with the smallest core diameter (d_c = 18 nm) show a 1.4 fold increase in R_2 and a 1.8 fold increase in R_2/R_1. The observed size dependence also applies for the biomimetic particles where the sample LI1s with a larger core

diameter (d_c = 28 nm) show enhanced relaxation effects. Accordingly, LI1s provides a 2.5 fold increase of R_2/R_1 compared to Resovist.

Both types of particles, the biological as well as the synthetic ones, provide enhanced relaxation properties compared to the known MRI contrast agent Resovist. The significant larger particle core diameter leads to an enhanced T_2-effect and therefore higher R_2relaxivity. Combined with a low R_1 relaxivity, the R_2/R_1 relaxivity values of these particles are highly improved. Due to the insufficient colloidal stability of the large synthetic particles, only the small particles were investigated. These particles offer enhanced contrast properties with respect to Resovist but reduced contrast compared to the biological magnetosomes. Since no enhanced toxicity was found for larger particles but higher toxicity for biological particles. Therefore combining size effect, colloidal stability and toxicity, improvements in the stabilization of the synthetic particles, in particular the particles with large core diameters have potential for providing particles with enhanced relaxation properties without problems associated with toxicity.

WP4

1.9. Simulation of contrast properties

In order to better understand factors that affect relaxivity in a particles system, a model has been developed to calculate changes in relaxivity by varying different physical parameters, such as the core and hydrodynamic diameters, the particle's magnetization and its anisotropy, external field strength, or the ratio between "inner" and "outer" viscosities. Expressions for the longitudinal T_1 and transversal T_2 relaxation times of protons are derived from a semi-classical model, where the protons are described in the frame of quantum mechanics, and magnetic nanoparticles are treated classically as macroscopic objects. Magnetic nanoparticles create random magnetic fields acting on the protons. Their randomness originates first from the distance between the nanoparticle and the proton, which is random due to thermal diffusion of water molecules; and second due to thermal fluctuations of the magnetic moment of the particle. As a result the longitudinal and transversal relaxation times of the proton are expressed through time correlation functions of the magnetic moment of the nanoparticle and the function j [18], describing the thermal motion of the proton in the vicinity of the nanoparticle. Time correlation functions of the magnetic moment are calculated using the "egg-yolk" model [19], which takes into account the simultaneous Brownian rotation of the particle ("egg") and the magnetic moment ("yolk"). The model accepts three non-dimensional parameters corresponding to external field strength, anisotropy of the particle and ratio of "inner" and "outer" viscosities of the particle. In the limits of a weak field and rigid dipole, the time correlation functions may be well approximated by single exponentials with the characteristic decay times, which correspond to the relations obtained in [20]. These limits, however, are outside the range of the magnetic field strength used in MRI. For an intermediate range of magnetic field strength, the time correlation functions should be approximated by a sum of exponentials as follows:

$$\overline{\mu_{z\tau}\mu_{z0}} = \mu^2\left(C_{z,0}^2 + \sum_k C_{z,k}\, e^{-\tau/\tau_{z,k}}\right)$$

$$\overline{\mu_{x\tau}\mu_{x0}} = \overline{\mu_{y\tau}\mu_{y0}} = \mu^2 \sum_k C_{xy,k}\, e^{-\tau/\tau_{xy,k}}$$

These expressions can be checked with the numerical simulation $\sum_k C_{z,k} = 1 - 2\frac{L(\xi)}{\xi} - L(\xi)^2$, where $L(\xi)$ is the Langevin function and $\xi = \frac{mH}{k_B T}$ the Langevin parameter.

As a result for the longitudinal ($T_1^{-1} = R_1 c$) and transversal ($T_2^{-1} = R_2 c$) coefficients of the relaxivity $R_{1,2}$ (c is the molar concentration of iron in the sample), we obtained [21]:

$$R_1 = A \times \left\{ 6C_{z,0}^2 j(i\omega_I \tau_D) + 6\sum_k C_{z,k} j\left(i\omega_I \tau_D + \frac{\tau_D}{\tau_{z,k}}\right) + 14\sum_k C_{xy,k} j\left(i\omega_I \tau_D + \frac{\tau_D}{\tau_{xy,k}}\right) \right\} \quad (1)$$

$$R_2 = \frac{R_1}{2} + A \times \left\{ 4C_{z,0}^2 + 4\sum_k C_{z,k} j\left(\frac{\tau_D}{\tau_{z,k}}\right) + 6\sum_k C_{xy,k} j\left(\frac{\tau_D}{\tau_{xy,k}}\right) \right\} \quad (2)$$

where

$$A = \frac{16\pi\gamma_I^2 M_s^2 \rho M V_m}{135 D R n_{Fe}}$$

$$j(z) = \mathrm{Re}\left\{ \frac{1 + \frac{1}{4} z^{1/2}}{1 + z^{1/2} + \frac{4}{9} z + \frac{1}{9} z^{3/2}} \right\}$$

and where γ_I is the proton gyromagnetic ratio, M_s the saturation magnetization of the particle, ρ the density of the particle, M the molar weight of molecules that constitute the particle, V_m the magnetic volume of the particle, D the self-diffusion coefficient of water molecules, R the radius of the particle, n_{Fe} the number of magnetic atoms in the constituting molecule, and $\tau_D = R^2/D$ is the characteristic diffusion time of the proton in the vicinity of the particle.

The model described by the relations (1) and (2) was checked with existing experimental data. The relaxivity coefficient R_2 increases linearly with size of the nanoparticle according to [22]. This result is in agreement with relation (2). It is known that the relaxivity of the nanoparticles R_1 exhibits maximum as a function of the external magnetic field strength. It flattens out with the increase of the particle size or magnetic anisotropy constant as shown in [23] by comparison of the relaxivity data for maghemite and cobalt ferrite nanoparticles. This is in agreement with the numerical simulation data, which according to relations (1), (2) predict this behavior. The verified model was applied to the experimental data.

Experimental data for various synthetic inorganic and biological samples are shown in Table 2. Numerical simulations were performed to compute relaxivities using the model and are shown in Table 3. The physical parameters of the particles, i.e., core diameters and field of saturation magnetization were chosen to reflect values obtained from the real particles. An exception was the hydrodynamic size of the particle which was kept close to the magnetic core size, with only a small (2.5 nm) non-magnetic layer. The thickness of the non-magnetic layer id based on the ability of

water molecules to enter the non-magnetic coating layer. Use of the measured hydrodynamic size leads to model relaxivity values that are in disagreement with experiment values.

Table 2 Experimental data. Relaxivities R_1,R_2 are measured at B = 0.94T, T = 39°C.

Sample	d_c [nm]	d_h [nm]	M_s [kA/m]	R_1 [L/mmol*s]	R_2 [L/mmol*s]	R_2/R_1
LI1s	28	115	357	12.1	330.1	27.3
SI1s	17	123	358	14.9	244.5	16.4
SI2s	18	100	329	15.3	302.6	19.8
SB1	24	90	-	9.0	320.7	35.6
LB1	41	143	-	8.6	541.5	63.0
LB2	38	84	-	8.8	477.1	54.2
SB2	29	76	-	11.0	442.5	40.2

Table 3 Results of numerical simulation. Common parameters: magnetite particles in water, anisotropy energy density K = 10 kJ/m^3, B = 0.94T, T = 39°C. Here ε is the ratio between "inner" and "outer" viscosities of the nanoparticle [4].

Sample	d_c [nm]	d_h [nm]	M_s [kA/m]	ε	R_1 [L/mmol*s]	R_2 [L/mmol*s]	R_2/R_1
LI1s	28	33	357	0.0031	15.7	460	29.3
SI1s	17	22	358	0.0023	17.5	160	9.1
SI2s	18	23	329	0.0022	14.9	153	10.3
SB1	24	29	300	0.0024	11.9	234	19.7
LB1	41	46	300	0.0030	8.1	723	89.3
LB2	38	43	300	0.0029	8.7	617	70.9
SB2	29	34	300	0.0026	10.8	349	32.3

To demonstrate the effect of saturation magnetization M_s on relaxation parameters various values were used in numerical simulation and the results are shown in Figure -Figure . Other parameters

were kept the same as in Table 3 and $d_h = d_c + 5$ nm. Both R_1 and R_2 increase with M_s, as is expected by relations (1) and (2) but their ratio is invariant.

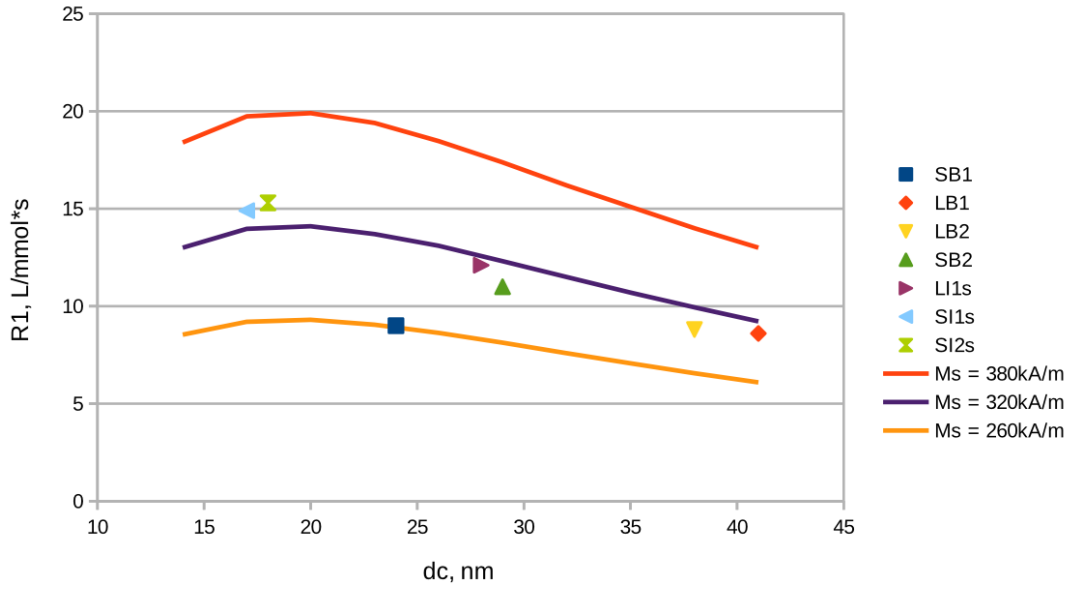


Figure 11: Comparison of R1 measured at 0.94T (markers) with those computed numerically for various values of magnetization (lines).

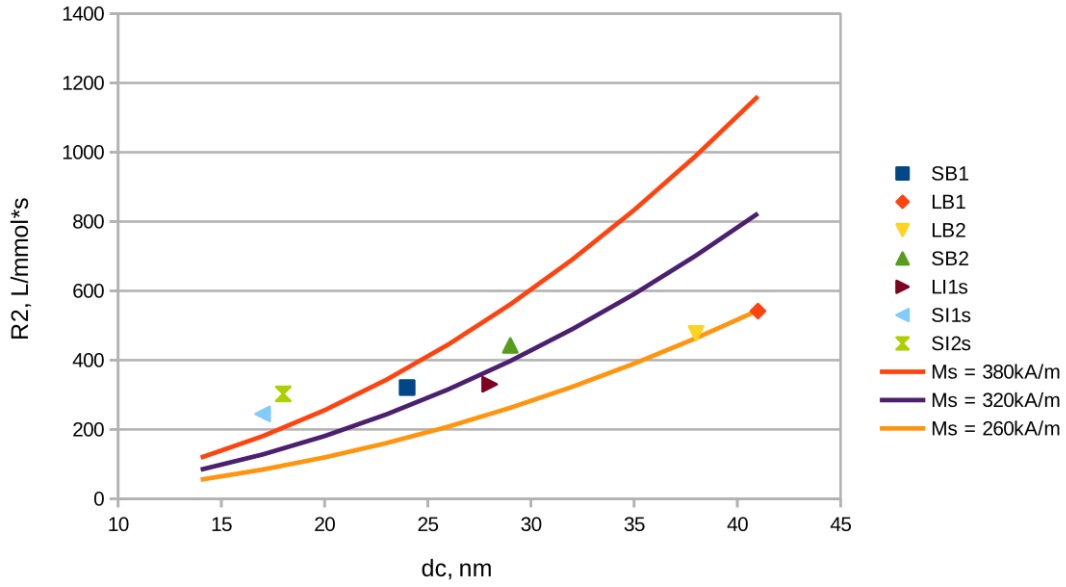


Figure 12: Comparison of R2 measured at 0.94T (markers) with those computed numerically for various values of magnetization (lines).

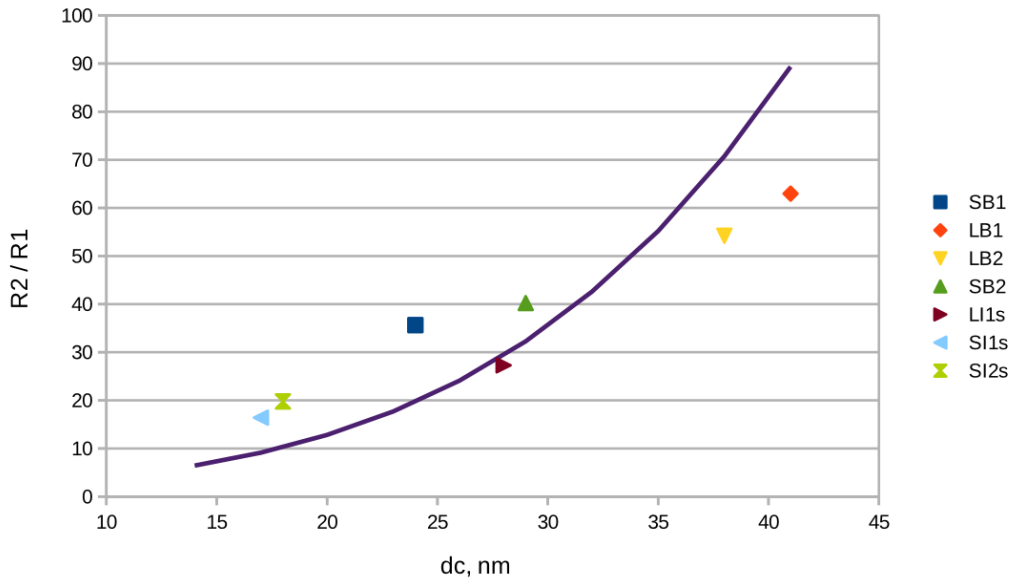


Figure 13: Comparison of R2/R1 values measured at 0.94T (markers) with those computed numerically (line).

1.10. Biological determinants for magnetosome properties

Magnetotactic bacteria (MTB) are able to form nanometer-sized particles of the magnetic iron minerals magnetite or greigite. The intracellular synthesis of these nanoparticles takes place in specific membrane-enclosed compartments called magnetosomes and requires a strict genetic control over intracellular differentiation, biomineralization, and assembly into highly ordered magnetosome chains. In *Magnetospirillum gryphiswaldense* the genetic control over magnetite biomineralization is achieved by a set of specific genes that are organized in several operons within a conserved genomic magnetosome island (MAI).

To elucidate the functions of genes encoded within the magnetosome island (MAI) of *M. gryphiswaldense* mutants with large deletions within the MAI were constructed. Whereas the majority of MAI genes have no detectable function in magnetosome formation and could be eliminated without any effect (Fig. 14 and Fig. 15, ΔA1-A5), only deletions that comprised at least one of the four major operons showed defects in magnetite biomineralization (ΔA7-A19). Single deletion of the *mms6*, *mamGFDC*, or *mamXY* operons for example affected size, morphology and organization of magnetite crystals (Fig. 14). Interestingly, even a strain with a combined deletion of these three operons still synthesized small irregular magnetic particles (ΔA13). In contrast, deletion of the 17 kb large *mamAB* operon completely abolished magnetite biomineralization.

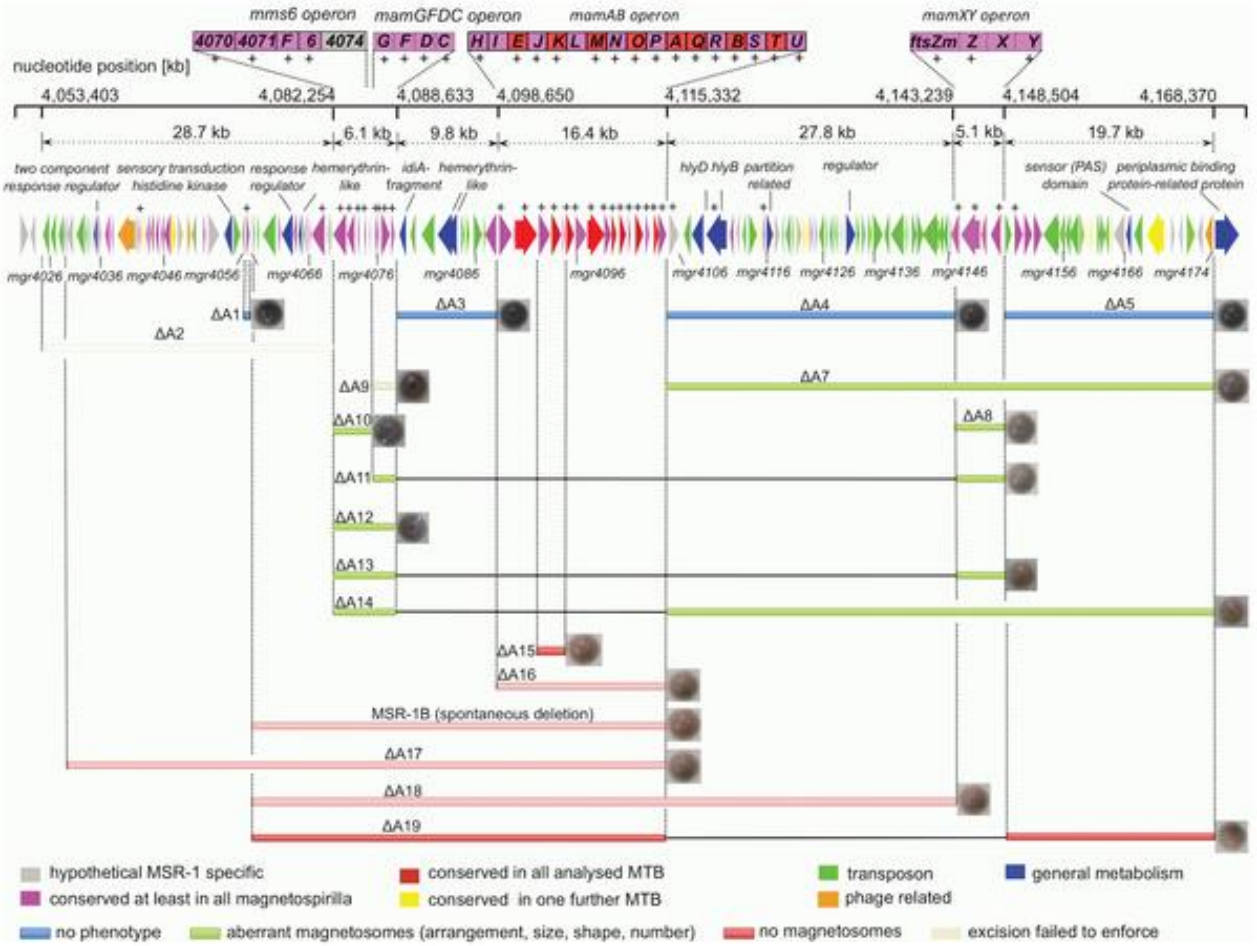


Figure 14: Molecular organization and characteristics of various deletion mutants of the *M. gryphiswaldense* MAI.

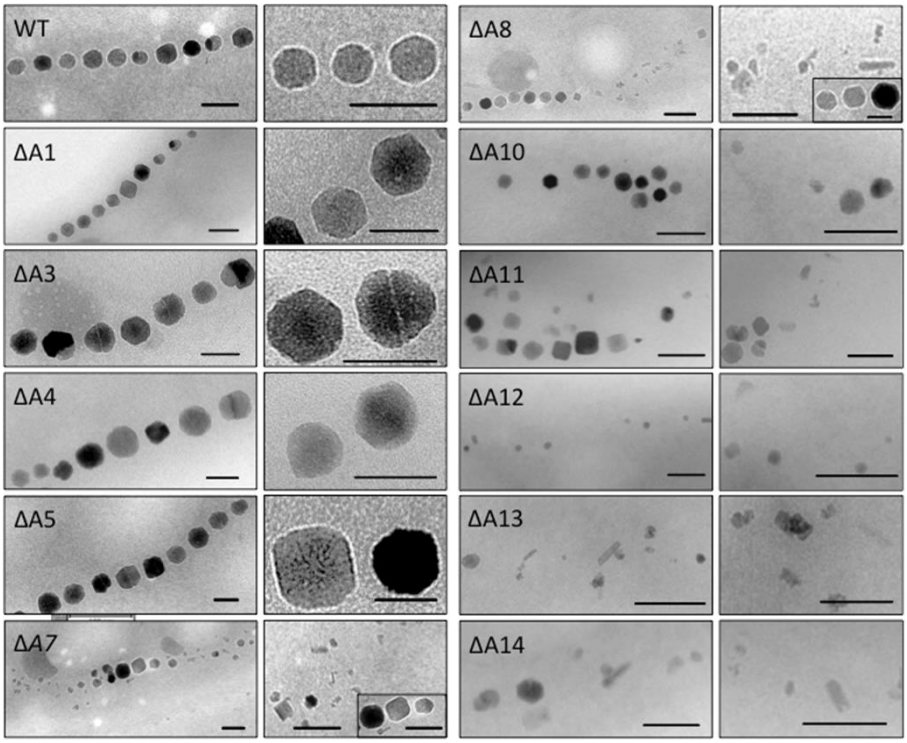


Figure 15: Summary of magnetosome morphologies observed within several deletion mutants. Scale bar for WT to ΔA5: 50 nm; ΔA7 to ΔA14: 100 nm.

This indicates that the *mamAB* operon is the only region of the MAI that is essential for magnetite biomineralization, whereas the *mamGFDC*, *mms6* and *mamXY* operons have crucial and partially overlapping functions for the formation of functional magnetosomes.

These magnetosome operon deletion analyses indicated that the proteins encoded by the *mamGFDC* and *mms6* operons are involved in the size control of magnetite biomineralization. Thus, we tested if additional copies of these operons would lead to the formation of magnetite particles with increased diameters. Therefore, a second copy of the *mamGFDC* or *mms6* magnetosome operon was inserted into the chromosome of a *M. gryphiswaldense recA* mutant strain. Our analyses showed that a *mamGFDC* duplication resulted in increased magnetosome particle sizes only, whereas a *mms6* operon duplication strain showed an increased accumulation of magnetite particles per cell and also formed particles with an increased diameter (Tab. 4). Thus, overexpression of some genetic determinants results in increased magnetosome particle sizes and numbers.

Tab.4. Comparison of the average particle size and number per cell between the *M. gryphiswaldense* wildtype and strains with *mamGFDC* or *mms6* operon duplications, respectively.

Strain	Crystal size	Increased size [%] compared to WT	Crystal number per cell	Increased number [%] compared to WT	Crystal number per cell >40 [%]
WT	35.6±13.0	0	34.3±8.4	0	20.0
ΔRecA+mamGFDC	44.9±13.5	26.1%	35.3±12.8	2.9%	41.7
ΔRecA+Mms6op.	45.7±14.2	28.4%	46.6±14.3	35.9%	83.7

WP 5

1.11. Project management during the period

WP5 contains the general coordination of the project, especially interaction with the European Commission, organization of the project meetings, the project correspondence and requests from the partners, project reporting which includes the submission of the deliverables via the participant portal and also the support to the project coordinator. In addition to this the project management is also responsible for all financial administration regarding dissemination and explanation of financial issues to all project partners and the preparation of the financial statement – Form C.

There were 3 consortium management tasks:

Task 1: Coordination of the project, EC interaction, global financial administration, progress report system, intermediate and final reports and updates of grant and consortium agreement.

Task 2: Disseminating and exploiting knowledge to all partners. Organization of meetings of the steering committee, of the advisory board and of the IPUDC will be part of the tasks:

- Designing and maintaining partner specific templates for collecting input to the required EC documents,
- Implementing and maintaining of a project-specific database for reporting and controlling, including the adaptation of the structure after changes in the work plan and the consortium,
- Drafting and maintaining the dissemination and exploitation plan following the EC's requirements,
- Preparing and post-processing of EC reviews from the consortium-side including support in the implementation of recommendations from the EC and reviewers,
- Preparing, executing and post-processing of major project meetings such as Steering Committee meetings,
- General Assemblies and meetings with the advisory board (tasks: agendas, invitations, location of meeting places, organization of rooms and equipment, preparation and distribution of materials, minutes and action lists),
- Implementing and maintaining the project infrastructure, e.g., the internal platform for information exchange and email lists,
- Handling of legal issues, IPR issues and maintenance of the consortium agreement, if obligatory
- Handling of the project correspondence and the day-to-day requests from partners and external bodies.

- Scientific coordination meetings of the scientific steering committee are not covered by the WP Management.

Task 3: Dissemination for the establishment and the maintenance of the project.

During the first and second period there were no problems at any work package or with communication with any partner, like also no changes in the project consortium.

During the both reporting periods we had six project meetings at the Max-Planck-Institute of Colloids and Interfaces in Golm:

1. Kickoff-Meeting on 10th October 2011
2. Interim Meeting on 15th May 2012
3. Review Meeting on 18th February 201
4. Interim Meeting on 19th June 2013
5. Review Meeting on 18th November 2013
6. Exploitation Strategy Seminar and Internal Project Meeting on 15th – 17th July 2014

1.12. The potential impact and the main dissemination activities

Our main goals are clearly to show that the scientific outcomes of the research done in this project are recognized within the field of biomedicine and in the longer term that they enable the development of better contrast agent for MRI. Further tests towards application in hyperthermia and magnetic particle imaging (MPI) were developed towards the end of the project as an additional part that was not in our Description of Work (DoW).
Because the here generated results are basically of scientific interest, the dissemination is focused on the scientific Imaging-community. The results obtained within the project confirmed, that contrast agents which are based on monocrystalline particles, offer very promising contrast properties compared to commercial available ones. Therefore, the dissemination of the results will open new perspectives in the field of contrast agent research and imaging.
In order for this to be achieved, the dissemination is a crucial step in order to reach the medical doctor using the particles as contrast agent, the pharmaceutical industry that develop and sell contrast agents but also the potential patients that need to be aware of the development in the field to potentially accept to be part in the development of therapeutic agents in the future.

The long term goals described above clearly require a long period of time before the scientific findings done in the project can be translated in the clinics. Therefore, the short term goals of the dissemination part in the consortium was focused towards the communication of our main scientific results within dedicated scientific journals as well as conferences (see list below). As there is always a lag time between the achievement of the results, their publication in scientific journals, and the feedback received by the authors from the scientific community, it might take some additional years even to fully achieve these somewhat shorter term objectives.

1.13. Key subjects to be disseminated

The key subjects our dissemination was centred around are:
1. The development of methods for synthesizing biological magnetite in high amount and isolating them with high purity
2. The development of methods for synthesizing synthetic magnetite nanoparticles with controllable size and colloidal stability

3. The fact that the nanoparticles we developed in the consortium are leading to contrast agent with properties much higher than current standards
4. The development of methodology and guidelines related to the use and characterization of magnetic nanoparticles (magnetic characterization, transmission electron microscopy, X-ray diffraction, biocompatibility tests)
5. The development of a code of Brownian dynamics for calculation of relaxivity induced by magnetic nanoparticles

1.14. Identification of target audience (incl. Key pen holders and stakeholders in the decision-making process)

The methods we developed will be of immediate relevance and potential use for university groups as well as R&D Scientists working on magnetic nanoparticles in general and on the development of contrast agent in particular. The target audience therefore includes:

- R&D Scientists/Physicians/Researchers in the field of e.g. nanoscience, nanotechnology and imaging
- MRI community: Clinicians, researcher and scientists with a focus on contrast agents with enhanced contrast properties
- MPI community: mainly physicist for basic research to advance the method

We are also performing networking with other project supported under FP7 as multiple members of the consortium are also present in other project. As an example, the coordinating group (MPG) is also present in the Nanoathero project, which also includes the use of magnetic nanoparticles for their use in atherosclerosis disease.

The work perform under the program presented here has already been published in several scientific journal and have in part started to be cited, showing that the dissemination work is already effective in the scientific community. In addition, some partners (e.g. ETHZ) already offer the measurements as a service to industry, which are interested in better understanding the performance of their particles. At present the ETHZ already had three industrial partners, who have profited from their analyses. Finally, our industrial partner is currently testing the particles in vivo with the potential to bring our materials to the market for small animal testing.

1.15. Message shaping, identification of channels, organizations and spokespersons

A wide range of channels was identified and the according actions were performed in order to reach the targeted groups. This included:

- Scientific posters presented at conferences and workshops such as e.g. materials research conferences in general and imaging conferences, especially MRI and MPI conferences in particular
- Project flyer presented at conferences, workshops, and open house day
- Scientific publications published in scientific journals
- Presentation of the work at a company booth at conferences
- Communication of the results to scientists in the field of MRI, MPI and preclinical imaging by attending meetings, conferences etc.
- Discussion with the scientists of our advisory board, which have established position within the biomiedical and pharmaceutical fields.

1.16. Exploitation of results

Below you find summarized the main points regarding the exploitation of the results. For the full strategical explanation, the reader is referred to the report related to the exploitation strategy seminar report. 6 main exploitable results have been identified:

ER01: **METHOD FOR SYNTHESIZING BIOLOGICAL MAGNETITE IN HIGH AMOUNT AND ISOLATING THEM WITH HIGH PURITY**

Genetically engineered bacterial strains for the isolation of biological magnetite nanoparticles of different sizes (18 to 45 nm) and narrow size distribution. Improvements of the magnetosome isolation procedure in addition to the generation of overexpression strains or increased the magnetite yields more than two-fold and decreased the processing time by several hours.

In Bio2MaN4MRI, we aimed at developing low-cost high-yield synthesis of size-controlled magnetite. Without being able to provide quantitative data due to the fact that the process was only developed at a fundamental level and no market analysis was performed, the route we developed is based on the coprecipitation technique, which yield a nearby 100 % yield in term of iron. Due to its poor solubility, the iron used in the synthesis is total converted in iron oxide. In addition, due to synthetic development described in the different reports, we could avoid the formation of

contaminating phases, therefore, the formed iron oxide is 100 % magnetite and all the iron used in the process is thus transformed to magnetite without lost. As for the cost aspect, the formation of size-controlled magnetite particles was before our endeavor only possible at high temperature (T>200°C) and high pressure (P>2bars) conditions, with the addition of organic additives, all far from green chemical conditions. Our process is performed at room temperature and atmospheric pressure without any additive needed (the additive is used for stabilization), therefore clearly reducing the cost purely associated to synthesis.

Selected additive:

Polyarginine

As presented in the final report, the synthetic set-up was modified in order to provide both, higher reproducibility of the chemical outcomes (the biomimetic magnetic nanoparticles) between different batches as well as higher quantities for a single batch in order to test all the properties of the particles originating from a unique synthesis. These modification required testing of several synthetic parameters such as reactants preparation or reaction conditions. Please find below the main modified parameters chosen in order to achieved the requested goals:

- Reproducibility:

Magnetite formation is highly dependent on oxygen concentration as magnetite is highly prone to oxidation. We have shown that other iron oxide can formed (see for example sample 179 in interim report 3). Therefore, effort was put to prepare deoxygenated solutions for the syntheses. We have gone from a nitrogen bubbling to an argon bubbling of the starting solution and from a simply closed reactor to a hydrated argon flow on the head space of the reactor to prevent oxidation. This indeed resulted in the absence of any contaminant phase in the outcome of the synthesis.

- Quantities:

Magnetite formation was typically perform in a 2 mL reactor in order to test the effect of biological additive on the properties of the reaction outcome. This is due to the fact that biological determinants cannot be obtained in large quantities and needed to be tested in concentration that were high enough to expect any effect. However, in order to test in particular the magnetic properties as well as the biocompatibility of the nanoparticles, reaction in 20 mL-scales were necessary. Simply changing the added volume of the iron and base solution or their addition rate was not an acceptable solution since

it also changed the physic-chemical conditions recorded in the reactor and thereby we could not obtained the large particles we needed anymore. We had to test a combination of different volume of the initial solution in the reactor, different iron and base concentration as well as the addition rate at which the iron solution is added in the reactor to obtain again particles with the desired dimension.

The list of tested additives includes

Peptides:

S G V Y K V A Y D W Q H

G Q S E H H M R V A S F

H M K S T V G G P D G W

G L H T S A T N L Y L H

Y S S D Q E P A R D H N

Polypeptides/polymers:

Polyarginine

Polygutamic acid

Polyethyleneimine

Proteins:

MamJ

MtxA

MamP

ER02: **METHOD FOR SYNTHESIZING SYNTHETIC MAGNETITE NANOPARTICLES WITH CONTROLLABLE SIZE AND COLLOIDAL STABILITY**

We have developed a method to synthesize magnetite nanoparticles with controllable size (10 to 50 nm). The average dimension of the crystals can be varied by varying the physicochemical conditions of the syntheses and / or the duration time. However, due to the magnetic interaction between particles of larger sizes (larger than 30 nm), the particles tend to aggregate. Therefore, the particles need to be coated for use. Several additives have been tested and we finally identified one additive that enable the formation of these large particle in a colloidally stable state in a single step rather than first synthesis and then stabilization, thereby saving time and costs.

For steric stabilization we tested different polymeric additives including charged as well as neutral ones. The neutral and biocompatible polymers dextran and hydroxyethyl starch and the negatively charged polyacrylic acid didn't lead to satisfying results. The positively charged polyethylenimine was able to stabilize the particles but in matters of toxicity aspects not being considered further.

For electrostatic stabilization we tested anions (NaOH) and cations (HNO3) which didn´t lead to success. The same behavior we found for the positively charged monomer citric acid. The most promising results we obtained using L-3,4-Dihydroxyphenylalanin (L-DOPA), which is known to exhibit high affinity anchor groups for iron oxide particles that leads to highly stable dispersions. We decided to go further with L-DOPA.

ER03: **CONTRAST AGENT WITH PROPERTIES MUCH HIGHER THAN CURRENT STANDARDS**

We have investigated various iron oxide nanoparticles of biological and synthetic origin concerning their contrast properties in MRI and, optionally, also the contrast properties in MPI and hyperthermia. We found a size dependence of both, synthetic as well as biological particles, where the larger particles exhibit improved contrast properties in MRI. After considering colloidal stability aspects, the most promising results were found for the biological magnetosomes, where the largest particles show an up to factor 5 higher R2/R1 relaxivity compared to the known MRI contrast agent Resovist ®. Additionally, the biological magnetosomes show very promising results in MPI and hyperthermia. In the case of the synthetic particles colloidal stability aspects has to be considered. Despite the promising results concerning their contrast properties, for the use as contrast agents, additional *in vivo* characterization need to be performed.

ER04: **CODE OF BROWNIAN DYNAMICS FOR CALCULATION OF RELAXIVITY INDUCED BY MAGNETIC NANOPARTICLES (THROUGH USE OF MODELS OF MAGNETIC NANOPARTICLES AS CONTRAST AGENTS (FURTHER PROJECT RESULT))**

Developed is a code to describe Brownian dynamics based on coupled motion of magnetic moment and particle is developed for calculation of MRI relaxivity induced by magnetic nanoparticles. The code vastly improves present simulation models in that it uses more sophisticated correlation functions, which are fitted with series of exponentials. Unlike other codes, it allows for the input of a large number of parameters that affect relaxivity. The simulation is designed for use in R&D, e.g., academic research at university clinics, biomedical engineering, and chemical synthesis or research in pharmaceutical companies, who are developing products for pre-clinical imaging. We will make this code available as open source software over the group website, and have started to publicize it through conference presentation and journal publications.

ER05:**METHODOLOGY AND GUIDELINES RELATED FOR USE AND CHARACTERIZATION OF MAGNETIC NANOPARTICLES**

Physical particle size in fine nanoparticle systems may not be directly correlated to the effective magnetic particle size due to particle agglomeration, interaction or surface oxidation. We have exploited a method using first-order reversal curves for a semi- quantitative discrimination between the amounts of superparamagnetism to magnetically- ordered particles.

Furthermore, we demonstrate how long-term storage environment affects the chemical composition and magnetic properties of magnetic nanoparticles. This may influence usage in applications.

ER06: **(VISUAL) TEACHING MATERIAL**

We have produced a unique collection of results that characterize morphology, magnetic proprieties, toxicity and relaxivity properties of biomimetic and biogenic magnetic nanoparticles that are invaluable in training students and researchers working in the area of synthesizing and characterizing magnetic nanoparticles. Very few studies that have produced nanoparticles for MRI provide such an in depth description of all of the above properties. We demonstrate the potential of this integrative, multi-disciplinary approach. This information will be used in lectures at the universities of those who were involved in the project. Our work will be promoted in a series of publications and book

chapters that will serve as an example of the advantage of taking a multi-disciplinary approach. Any researcher, lecturer or student will have access to any published material. This ER is available now.

DISCUSSIONS&CHARACTERISATIONS

All-over, the teamwork on the Exploitable Results (ERs), which took place in a small group, was very much committed with fruitful discussions. In particular thinking and brainstorming on which of the results of the project could be exploited, at a time when project work has almost been completed was tremendously effective. In addition to the three exploitable results (ERs) of the ESS preparation phase, further ERs were identified during the ESS and characterized.

Exploitation defined as use of the project results, by commercial means as well as by research means seemed to be most appropriate for evaluating this project and its results, bearing in mind the research focus of the project and that the majority of partners are RTDs. Applying this definition, the project comes up with altogether 6 exploitable results.

4 out of the 6 will be exploited in further academic research, as well as commercially, through means of small scale manufacturing and selling or consulting services and training, offered to academia and industry. This two-ways-exploitation is mainly done by the lead partners of the respective exploitable result.

More in detail, this means:

The partner **LMU (ER01)** and the partner **MPG (ER02)** apply their methods for synthesis of particles with improved contrast properties for further research projects in the scientific community, and at the same time provide it as lab service at small scale to demanding companies. One of their first industry side customers to buy the particles will be the partner NanoPET, who needs these particles to achieve ER03. For ER01 and ER02 scalability has been proven in the lab during the project, the next step would be to scale up to industrial requirements.

The industrial partner **NanoPET** will use the synthesised particles of ER01 and ER02 for its **ER03**, the contrast agent, which has been developed during the project mainly under the aspect of contrast characteristics. After the project, NanoPET will further develop and test the CA mainly on toxicity, biodistribution and other characteristics. Meaning that following these further development steps, this company will create a product for selling on the preclinical imaging market (mainly MPI and hyperthermia) for animals in approx. 1-2 years.

ER04, after further 1-3 months for coding by partner **UL**, this ER will result in an open source software for the simulation and calculation of MRI relaxivity induced by magnetic nanoparticles. As application in research it will allow numerical results, where currently expensive testing is applied. In addition, it has to be mentioned that a further project result is used to establish ER04: models of magnetic nanoparticles as contrast agents. As these models go into ER04 as a major contribution, they are not mentioned separately as further ER, even when this could have been done. These models will be used also apart from ER04 and will go into further scientific research activities **ER05** will enhance the lead partner's **(ETH)** research and lab services to characterise magnetic properties of nanoparticles by methods developed throughout the project adapted from the use in rock magnetism. This commercial offer is targeted to customers in academia as well as in industry. Demand comes mainly from the research community, in particular academic research. Industrial customers are companies who synthesise magnetic nanoparticles for applications in pharmacy, the sensor industry and catalytic industry

ER06 is exploited by all of the partners through means of further scientific research and teaching at universities

IPR ISSUES

With regard to ER01, ER02, and ER05, as well as specifically ER03, the exploitation claims and ownership shares of the single partners (see IPR chapter) have to be considered when it comes to revenue generating activities. The respective partners should come to an agreement on whether and how to compensate for the partners' shares on foreground by means of a written exploitation agreement.

Concerning the specific IPR situation of ER03, patenting seems to be most likely at first glance. Nonetheless the patent situation, in particular in the field of MRI is seen to be difficult, due to much publishing activity of results concerning contrast properties of similar particles. Hence patenting is considered of being not suitable, as a patent application process is expected to be not successful at the moment. Nonetheless, this view should be updated by an intensive technology and patent search as development towards the applicable product progresses. Experiments to show the difference between the novel CA and what has been published so far would help to proceed towards a patent. This is usually recommendable, as for biotech companies who would like to stay in business in the

biotech industry, patents are absolutely fundamental, not only with regard to financing, even when these patents are aggressively contested by competitors.

The market entry of this result would most probably be very successful combined with the right market entry strategy. A contrast agent for the application of preclinical imaging for animals in hyperthermia and MPI is evaluated as not existing yet. If this will still be the case after an estimated time to market of 1-2 years, a first mover advantage could be achieved. Having further convincing arguments for a successful market entry, NanoPET should consider, among others, the new H2020 SME instrument to fund and support commercialisation for ER03.

Synoptic table of Exploitable Results

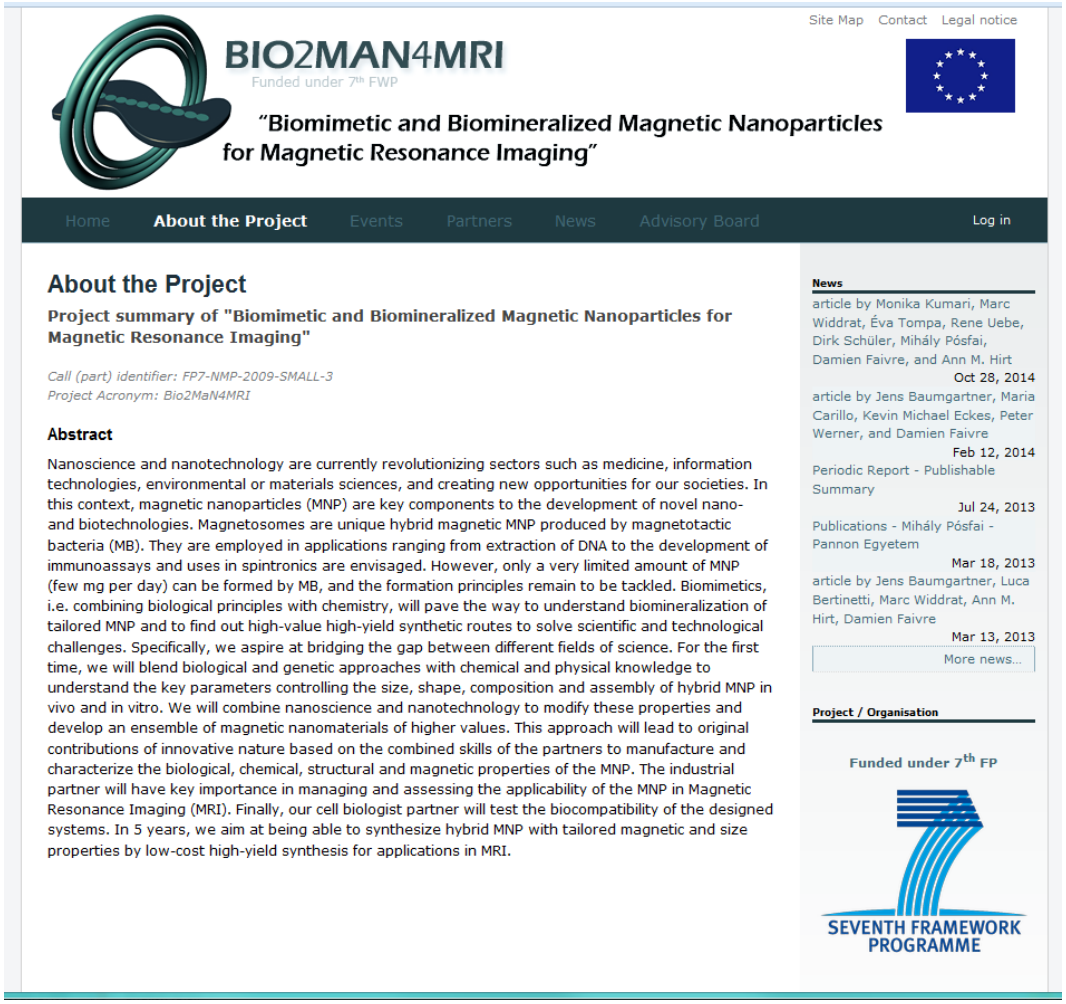
	Exploitable Result	Lead Partner	IPR owners of the ER	Partners planning to exploit	Planned additional partners	Main risks to be addressed
1	Method for synthesizing biological magnetite in high amount and isolating them with high purity	LMU	LMU MPG UP ETH UL LBIO nanopet	LMU MPG UP ETH UL LBIO nanopet	n/a	Scaling up of fermentation; Nobody buys the product. Product to be created could be too expensive.
2	Method for synthesizing synthetic magnetite nanoparticles with controllable size and colloidal stability	MPG	MPG UP ETH UL LBIO nanopet	MPG UP ETH UL LBIO nanopet	n/a	Nobody buys the product. Product to be created could be too expensive.
3	Contrast agent with properties much higher than current standards	nanoPET	nanopet MPG UP	nanopet MPG UP	n/a	Nobody buys the product. Too expensive; Result

			ETH UL LBIO LMU	ETH UL LBIO LMU		aiming at replacing existing and well entrenched technologies
4	Code of Brownian dynamics for calculation of relaxivity induced by magnetic nanoparticles (through use of models of magnetic nanoparticles as contrast agents (further project result))	UL	UL	UL	n/a	none
5	Methodology and guidelines related for use and characterization of magnetic nanoparticles	ETH	ETH MPG UP LMU	ETH MPG UP LMU	n/a	n/a
6	(Visual) Teaching Material	ETH, UP, UL	ETH MPG UP LMU UL Nanopet LBIO	ETH MPG UP LMU UL Nanopet LBIO	n/a	n/a

1.17. Project public website

One of the main tools for dissemination to the large public is also our website: http://plone.mpikg.mpg.de:9335/bio2man4mri/

The website presents the research done in the project in a general fashion to be understood by the general public. In addition, the different project partners and their roles are outlined. The website gives link towards the scientific outcomes of the project and is therefore a real platform of exchange. An example of one of the page is shown below. Furthermore, project logo, diagrams or photographs illustrating and promoting the work of the project (including videos, etc…), as well as the list of all beneficiaries with the corresponding contact names can be submitted without any restriction.



1.18. Person-months used in both reporting periods

Contract N°:	245542		Acronym:	Bio2MAN			Period 1&2	
PARTICIPANTS	UNIT (*PERSON-MONTHS or EUROS*)	TYPE of EXPENDITURE	PLANNED	ACTUAL EXPENDITURE			Pct. Spent	REMAINING RESOURCES
				Period 1	Period 2	Total	TOTAL	
			e	a1	b1	e1	a1+b1/e	e-e1
Beneficiary 1 *MPIKG*	*P-M*	Work Package 1	40,00	18,00	28,00	46,00	115%	-6,00
	P-M	Work Package 2	36,00	18,00	18,00	36,00	100%	0,00
	P-M	Work Package 3				0,00	0%	0,00
	P-M	Work Package 4				0,00	0%	0,00
	P-M	Work Package 5	18,00	9,00	9,00	18,00	100%	0,00
	P-M	*Total*	94,00	45,00	55,00	100,00	106%	-6,00
Beneficiary 2 *Pannon Egyetem*	*P-M*	Work Package 1				0,00	0%	0,00
	P-M	Work Package 2	36,00	9,50	32,40	41,90	116%	-5,90
	P-M	Work Package 3				0,00	0%	0,00
	P-M	Work Package 4				0,00	0%	0,00
	P-M	Work Package 5				0,00	0%	0,00
	P-M	*Total*	36,00	9,50	32,40	41,90	116%	-5,90
Beneficiary 3 *UL*	*P-M*	Work Package 1				0,00	0%	0,00
	P-M	Work Package 2				0,00	0%	0,00
	P-M	Work Package 3				0,00	0%	0,00
	P-M	Work Package 4	36,00	35,86	47,46	83,32	231%	-47,32
	P-M	Work Package 5				0,00	0%	0,00
	P-M	*Total*	36,00	35,86	47,46	83,32	231%	-47,32
Beneficiary 4 *nanoPET Pharma*	*P-M*	Work Package 1				0,00	0%	0,00
	P-M	Work Package 2				0,00	0%	0,00
	P-M	Work Package 3	48,00	21,90	20,90	42,80	89%	5,20
	P-M	Work Package 4				0,00	0%	0,00
	P-M	Work Package 5				0,00	0%	0,00
	P-M	*Total*	48,00	21,90	20,90	42,80	89%	5,20
Beneficiary 5 *LMU*	*P-M*	Work Package 1	40,00	12,00	16,98	28,98	72%	11,02
	P-M	Work Package 2				0,00	0%	0,00
	P-M	Work Package 3				0,00	0%	0,00
	P-M	Work Package 4	24,00	1,50	13,63	15,13	63%	8,87
	P-M	Work Package 5				0,00	0%	0,00
	P-M	*Total*	64,00	13,50	30,61	44,11	69%	19,89
Beneficiary 6 *LBIO*	*P-M*	Work Package 1				0,00	0%	0,00
	P-M	Work Package 2	36,00	15,00	18,00	33,00	92%	3,00
	P-M	Work Package 3				0,00	0%	0,00
	P-M	Work Package 4				0,00	0%	0,00
	P-M	Work Package 5				0,00	0%	0,00

	P-M	*Total*	36,00	15,00	18,00	33,00	92%	3,00
Beneficiary 7 *ETH Zürich*	*P-M*	Work Package 1				0,00	0%	0,00
	P-M	Work Package 2	36,00	18,00	18,00	36,00	100%	0,00
	P-M	Work Package 3				0,00	0%	0,00
	P-M	Work Package 4				0,00	0%	0,00
	P-M	Work Package 5				0,00	0%	0,00
	P-M	*Total*	36,00	18,00	18,00	36,00	100%	0,00
Totals	*P-M*	Work Package 1	80,00	30,00	44,98	74,98	94%	5,02
	P-M	Work Package 2	144,00	60,50	86,40	146,90	102%	-2,90
	P-M	Work Package 3	48,00	21,90	20,90	42,80	89%	5,20
	P-M	Work Package 4	60,00	37,36	61,09	98,45	164%	-38,45
	P-M	Work Package 5	18,00	9,00	9,00	18,00	100%	0,00
	P-M	*Total*	350,00	158,76	222,37	381,13	109%	-31,13

2. Use and dissemination of foreground

2.1. Introduction

2.1.1. The Plan for the Use and the Dissemination of Foreground - PUDF

The Plan for the Use and the Dissemination of Foreground summarizes the strategy and the concrete actions for the protection, exploitation and dissemination of the results generated by the project.

The current release is to be updated and completed during the project life in order to be delivered, in its final official form by the end of the project[1]. when partners are expected to report with enough details on the actual and expected "use" to be made of the foreground, i.e. on their strategy and concrete activities to disseminate and exploit the project results[2].

The PUDF it is structured in two parts: the first describing the strategy and the concrete actions for exploitation of the project's results and, the second, concerning dissemination. Information is presented by exploitable result to facilitate usability by partners.

As far as Ownership, use, dissemination and access rights the PUDF refers to what it is ruled in "Part C INTELLECTUAL PROPERTY RIGHTS, USE AND DISSEMINATION" of the Annex II - General Conditions to the FP7 Model Grant Agreement:

- The beneficiaries shall report on the expected use to be made of foreground in the plan for the use and dissemination of foreground. The information must be sufficiently detailed to permit the Commission to carry out any related audit.

- Any dissemination activity shall be reported in the plan for the use and dissemination of foreground, including sufficient details/references to enable the Commission to trace the activity. With regards to scientific publications relating to foreground published before or after the final report, such details/references and an abstract of the publication must be provided to the Commission along with an electronic copy of the published version or the final manuscript accepted for publication.
- Any dissemination action concerning foreground must include a statement acknowledging the financial support of the European Community, as well as a disclaimer specifying that it reflects only the author's view, exempting the Community from any liability. Any publicity concerning the project must also display the EU emblem.

[1] Article II.4.2.b of the standard EC Grant Agreement for FP7 projects defines the "Plan for the Use and Dissemination of the Foreground[1] (PUDF)" as one the contractual reports to be delivered by project end and as a means for the Commission to assess the success of a project.
[2] Article II.29 - FP7 Grant Agreement - Annex II – General Conditions Version 6, 24/1/2011

In the PUDF "Use" is defined[3] as the direct or indirect utilisation of foreground in further research activities other than those covered by the project, or for developing, creating and marketing a product or process, or for creating and providing a service. "Direct use" implies that partners utilise the results themselves for commercial applications (e.g. by producing and/or commercialising a new product or by integrating a new process into their manufacturing plant) and/or for further research ("further" with respect to the scope of the project in which the foreground is generated). "Indirect use" implies that partners may allow third parties to exploit the research results through a specific agreement.

It is to be noted that in H2020 exploitation and dissemination will play an important role. They are defined[4] as follows:

- ′exploitation′ means the use of results in further research activities other than those covered by the action concerned, or in developing, creating and marketing a product or process, or in creating and providing a service, or in standardisation activities;
- ′dissemination′ means the public disclosure of the results by any appropriate means (other than resulting from protecting or exploiting the results), including by scientific publications in any medium;

2.1.2. PUDF: Patents & protection

Publication and dissemination of foreground are granted with the approval of the Consortium, making sure that the period of secrecy needed for a successful patent application is respected. Any patent applications relating to foreground filed shall be reported in the plan for the use and dissemination of foreground, including sufficient details/references to enable the Commission to trace the patent (application). Any such filing arising after the final report must be notified to the Commission including the same details/references.

[3] Source: http://www.ipr-helpdesk.org/documents/ES_UseForegroundFP7_0000006654_00.xml.html

[4] Regulation of the European Parliament and of the Council laying down the rules for participation and dissemination in ″Horizon 2020 -the Framework Programme for Research and Innovation (2014-2020)″ and repealing Regulation (EC) No 1906/2006 - Draft

Contractors have to inform the Consortium and the Commission of its intention to publish on its foreground. Publication can be impeded if another contractor can show that the secrecy of the foreground is not guaranteed.

Where the foreground is capable of industrial or commercial application and its owner does not protect it, the Union may, with the consent of the beneficiary concerned, assume ownership of that foreground and adopt measures for its adequate and effective protection.

2.1.3. PUDF and Consortium agreement

The Consortium Agreement is a very important document when it comes to ownership and sharing of knowledge or project result, as it sets out or further defines how the consortium agrees on the use and dissemination of the project results.

The background[5] that is brought into the project will always remain the property of the partner involved. Those partners making available pre-existing know-how during the course of the project will specify any conditions for access thereto in the Consortium Agreement.

The Consortium agreement will dedicate one section or one appendix to define which access rights to the background may be granted. Also background to be excluded from access rights in any event will be specified in another dedicated section or appendix. All other background will be considered as unnecessary and excluded from the access rights.

In the case of the foreground, i.e. the project results and any IPR that can be attached to them, typically it is owned by the participant that carried out the work from which it resulted. Partners working in the same WP shall have Access Rights to all foreground and background needed for the execution of the WP, from all WP Partners. Participants from other WPs will enjoy the same access to foreground and background, if these form part of a deliverable or are necessary for the execution of the sub-project. Bilateral agreement between the Contractors participating in the same WP or in other WPs may be set if Contractors believe that foreground or background forms part of a deliverable of the other WPs or is necessary to

carry out activities in the other WPs. These access rights can be extended to affiliates that are participating to the project, but these rights will expire at the end of the project.

[5] "Background" is project-related information and IP rights held by participants prior to the signature

2.2. Key Exploitable Results

Synoptic table of Exploitable Results

#	Key Exploitable Result	Lead Partner
1	Method for synthesizing biological magnetite in high amount and isolating them with high purity	LMU
2	Method for synthesizing synthetic magnetite nanoparticles with controllable size and colloidal stability	MPG
3	Contrast agent with properties much higher than current standards	nanoPET
4	Code of Brownian dynamics for calculation of relaxivity induced by magnetic nanoparticles (through use of models of magnetic nanoparticles as contrast agents (further project result))	UL
5	Methodology and guidelines related for use and characterization of magnetic nanoparticles	ETH, MPG
6	(Visual) Teaching Material	ETH, UP, UL

2.2.1. Exploitable result No 1

Characterization of the result

Exploitable Result number 1:

METHOD FOR SYNTHESIZING BIOLOGICAL MAGNETITE IN HIGH AMOUNT AND ISOLATING THEM WITH HIGH PURITY

Brief Description	Genetically engineered bacterial strains for the isolation of biological magnetite nanoparticles of different sizes (18 to 45 nm) and narrow size distribution. Improvements of the magnetosome isolation procedure in addition to the generation of overexpression strains o increased the magnetite yields more than two-fold and decreased the processing time by several hours.
Innovativeness introduced compared to already existing Products/Services	The superior magnetic properties and high colloidal stability of magnetosomes provide the basis for many biotechnological or biomedical applications. Precise size control of the magnetosomes through genetically engineered mutants (30-40 samples). Scalability of the fermentation process (100 liter now and 10 liter before). Procedure to get contamination free magnetosome.
Unique Selling Proposition (competitive advantages)	➔ from here see ER n°3; method of ER n°1 will be used to achieve ER n°3

Priority map of Exploitable result

Risk matrix

	Key Exploitable Result ER 01	Degree of importance for the risk of not achieving Exploitation in industry. (1 low - 10 high)	Probability of risk happening (1 low - 10 high)	Risk Grade	Scope and type of potential intervention	Feasibility of Intervention Please rate from 1 to 10 (1 low- 10 high)	Priority Level
	Technological Risk Factors						
5	Scaling up of fermentation	*6*	*6*	**36**	Talk to experts with know-how in fermentation upscaling	*6*	**294**
6	Procedure to get contamination free magnetosome	*10*	*1*	**10**	Talk to expert with experience in sterilization procedures	*5*	**50**
	Market Risk Factors						
9	Nobody buys the product. Product to be created could be too expensive.	*7*	*7*	**49**	Rationalization of the production set-up	*8*	**392**

	Environmental/regulatory Risk Factors						
21	Influence of laws and regulations	*6*	*2*	**12**	Talk to regulation authorities.	*2*	**24**

Priority map result for Exploitable result

The Priority Map, is divided in 4 quadrants:

- the "No Action" quadrant shows risks with little influence on the exploitation of the project;
- the "Control" quadrant shows factors to be monitored periodically to exploit project results in the proper manner;
- the "Action" quadrant shows risks which require immediate interventions and implementation of contingency plans. Thanks to such interventions, these factors will move toward the "Control" quadrant;
- the "Warning" quadrant shows the most critical factors, the ones for which it is difficult to have an impact with immediate interventions. High number of factors in this quadrant could lead the project to face high risks of failing in exploiting its results.

2.2.2. Exploitable result No 2

Characterization of the result

Exploitable Result number 2:

METHOD FOR SYNTHESIZING SYNTHETIC MAGNETITE NANOPARTICLES WITH CONTROLLABLE SIZE AND COLLOIDAL STABILITY

Brief Description	We have developed a method to synthesize magnetite nanoparticles with controllable size (10 to 50 nm). The average dimension of the crystals can be varied by varying the physicochemical conditions of the syntheses and / or the duration time. However, due to the magnetic interaction between particles of larger sizes (larger than 30 nm), the particles tend to aggregate. Therefore, the particles need to be coated for use. Several additives have been tested and we finally identified one additive that enable the formation of these large particle in a colloidally stable state in a single step rather than first synthesis and then stabilization, thereby saving time and costs.
Innovativeness introduced compared to already existing Products/Services	The superior magnetic properties and high colloidal stability of magnetic nanoparticles provide the basis for many biotechnological or biomedical applications. Reach larger size than previously achieved (scientific community) Large and colloidally stable particles.
Unique Selling Proposition (competitive advantages)	➔ from here see ER n°3; method of ER n°2 will be used to achieve ER n°3

Priority map of Exploitable result

Risk matrix

	Key Exploitable Result ER02	Degree of importance for the risk of not achieving Exploitation in industry. (1 low - 10 high)	Probability of risk happening (1 low - 10 high)	Risk Grade	Scope and type of potential intervention	Feasibility of Intervention Please rate from 1 to 10 (1 low- 10 high)	Priority Level
	Technological Risk Factors						
5	Upscaling	*7*	*6*	**42**	Ask experts for advice	*5*	**240**
	Market Risk Factors						
9	Nobody buys the product. Product to be created could be too expensive.	*7*	*5*	**35**	Rationalization of the production set-up	*8*	**392**
	Environmental/regulatory Risk Factors						
21	Influence of laws and regulations	*8*	*2*	**16**	Close monitoring is necessary.	*2*	**32**

Priority map result for Exploitable result

The Priority Map, is divided in 4 quadrants:

- the "No Action" quadrant shows risks with little influence on the exploitation of the project;
- the "Control" quadrant shows factors to be monitored periodically to exploit project results in the proper manner;
- the "Action" quadrant shows risks which require immediate interventions and implementation of contingency plans. Thanks to such interventions, these factors will move toward the "Control" quadrant;
- the "Warning" quadrant shows the most critical factors, the ones for which it is difficult to have an impact with immediate interventions. High number of factors in this quadrant could lead the project to face high risks of failing in exploiting its results.

2.2.3. Exploitable result No 3

Characterization of the result

Exploitable Result number 3:

CONTRAST AGENT WITH PROPERTIES MUCH HIGHER THAN CURRENT STANDARDS

Brief Description	We have investigated various iron oxide nanoparticles of biological and synthetic origin concerning their contrast properties in MRI and, optionally, also the contrast properties in MPI and hyperthermia. We found a size dependence of both, synthetic as well as biological particles, where the larger particles exhibit improved contrast properties in MRI. After considering colloidal stability aspects, the most promising results were found for the biological magnetosomes, where the largest particles show an up to factor 5 higher R2/R1 relaxivity compared to the known MRI contrast agent Resovist ®. Additionally, the biological magnetosomes show very promising results in MPI and hyperthermia. In the case of the synthetic particles colloidal stability aspects has to be considered. Despite the promising results concerning their contrast properties, for the use as contrast agents, additional *in vivo* characterization need to be performed.
Innovativeness introduced compared to already existing Products/Services	The superior magnetic properties and high colloidal stability of magnetosomes provide the basis for many biotechnological or biomedical applications. NanoPET, the industrial partner, is planning to apply these particles to create contrast agents for preclinical imaging, depending on in vivo studies.

	Iron oxide particles which are currently used for CA´s are normally based on clustered particles with small particle core sizes. The class of iron oxide nanoparticles which addressed in this project are monocrystalline magnetic particles with large crystal sizes. Compared to clustered particles, the big advantage are the superior magnetic properties of the particles, to be used as CA´s with enhanced contrast properties. On the one hand we investigated biological magnetosomes, obtained from magnetotactic bacteria, and on the other hand we used biomimetic approaches to synthesize monocrystalline particles. A comprehensive characterization of the particles including the morphology, magnetic properties, toxicity and contrast properties will help to identify the most promising particles for use as CA´s. We will show the feasibility to use these particles as potential and innovative contrast agents in MRI, MPI and hyperthermia. Potential of a specific CA for MPI and hyperthermia in the field of preclinical imaging (especially for biological magnetosomes). <u>o No specific CA for hyperthermia and MPI on the market => Possibility of a first specific CA</u> o Superior contrast properties in MRI compared to products on the market o Higher sensitivity o Higher contrast => dose reduction =>cost reduction o Because of high contrast => possible use of the particles for molecular imaging

Unique Selling Proposition (competitive advantages)	No specific solution for MPI and hyperthermia available.

	Various iron oxide nanoparticles (material) available but only few iron oxide based CA for preclinical MRI available on the market. o Compared to existing CA: high contrast and high sensitivity in MRI o Possible CA with simultaneously excellent properties for MRI, MPI and hyperthermia (especially for the biological magnetosomes) Reducing concentration of CA means less material and consequently reduced costs.
Product/Service Market Size	Contrast agents: The global contrast media market is worth $6.2 billion in 2012, and estimated to grow at a CAGR of 6.8% to reach $8.6 billion by 2017 (Source: ASDR-107222). The current market potential of the MR contrast agent segment is between 80 – 90 million € a year in Germany.
Market Trends/Public Acceptance	Contrast agents: The global contrast agent market, though fairly mature at present (2012), still shows potential to grow over the next five years. It is propelled by the increasing demand for diagnostic imaging and image guided surgical procedures, technological advances in diagnostic imaging and the ever-increasing incidence rates of cancer and cardiac disorders. The U.S. represents the largest contrast agent market worldwide, followed by Europe and Japan.
Product/Service Positioning	o Niche market: Preclinical Imaging o imaging agents for R&D, possible for MRI, MPI and Hyperthermia (especially biological

	magnetosomes)
Legal or normative or ethical requirements (need for authorisations, compliance to standards, norms, etc.)	Contrast agents production following GLP guidlines; Ethical and self-imposed requirements: no legal requirements for the production of preclinical imaging agents but the production should be in accordance with with animal welfare and ethical guidelines which means e.g. no toxic or harmfull materials are used and formulation under physiological conditions (pH = 7.4, b = 300 mosmol/kg) In the area of preclinical imaging no approval for new CA required; For human applications Regulatory Affairs barriers.
Competitors	R&D; Pharmaceutical companies focused on the development of preclinical imaging agents, companies in the field of nanoscience (material manufacturer).
Prospects/Customers	In general R&D; e.g. preclinical imaging facilities, research institutes, academia (universities, university of applied science), physicians (researcher) o Academia : - Focus on R&D (publication); e.g. University, Medical Schools, University Clinics, Research Institutes, and national institutes (FDA; US Army) o Pharma: - R&D with focus on productlines and research; e.g. global players as known o Device manufacturer: - Focus on application regarding their

	devices
Cost of Implementation (before Exploitation)	For the use as contrast agents, additional *in vivo* characterization need to be performed. Testing. Estimated
Time to market	For preclinical imaging (animals): short, approx. 1-2 years. For application for human beings: long (due to regulatory requirements).
Foreseen Product/Service Price	cannot be estimated at this time
Status of IPR: Background (type and partner owner)	**NANOPET:** contrast agent research; biocompatible formulation; colloidal stabilisation
Status of IPR: Foreground (type and partner owner)	**NANOPET:** magnetosomes around 10 times better than standard, biomimetic nanoparticles around 3 times better than standards
Status of IPR: Exploitation Forms (type and partner owner) **e.g. direct industrial use, patenting, technology transfer, license agreement, publications, standards, etc.**	Exploitation is possible in the field of preclinical imaging. The patent situation, in particular in the field of MRI is difficult, because a lot of results concerning contrast properties of similar particles are already published. Especially the biological magnetosomes are promising in the field of MPI and Hyperthermia. **NANOPET:** manufacturing, selling, further research
Partner/s involved expectations	Own exploitation on market for preclinical imaging
Exploitation: Sources of financing foreseen after the end of the project (venture capital, loans, other grants, etc.)	Inhouse financing

Priority map of Exploitable result

Risk matrix

	Key Exploitable Result ER03	Degree of importance for the risk of not not achieving long term and successful Exploitation in industry. (1 low - 10 high)	Probability of risk happening (1 low - 10 high)	Risk Grade	Scope and type of potential intervention	Feasibility of Intervention Please rate from 1 to 10 (1 low- 10 high)	Priority Level
	Technological Risk Factors						
5	Novel product could be analysed and copied due to first market entry	*4*	*6*	**24**	License to big company. Depending on specific situation.	*4*	**96**
6	Result aiming at replacing existing and well entrenched technologies	*7*	*6*	**42**	Offer free trials together with customer sales talks.	*6*	**252**
	Market Risk Factors						
9	Nobody buys the product. Too expensive	*9*	*5*	**45**	Reassess production costs and make functional and value analysis	*8*	**360**
17	Off time supply of financial means	***6***	***2***	**12**	Search for different sources of financing for the development work (VC, loans, grants, etc.)	*5*	**60**

	Environmental/regulatory Risk Factors						
21	Influence of laws and regulations	*6*	*2*	**12**	Close monitoring is necessary. Talk to regulation bodies	*2*	**24**

Priority map result for Exploitable result

The Priority Map, is divided in 4 quadrants:

- the "No Action" quadrant shows risks with little influence on the exploitation of the project;
- the "Control" quadrant shows factors to be monitored periodically to exploit project results in the proper manner;
- the "Action" quadrant shows risks which require immediate interventions and implementation of contingency plans. Thanks to such interventions, these factors will move toward the "Control" quadrant;
- the "Warning" quadrant shows the most critical factors, the ones for which it is difficult to have an impact with immediate interventions. High number of factors in this quadrant could lead the project to face high risks of failing in exploiting its results.

2.2.4. Exploitable result No 4

Characterization of the result

Exploitable Result number 4:

CODE OF BROWNIAN DYNAMICS FOR CALCULATION OF RELAXIVITY INDUCED BY MAGNETIC NANOPARTICLES (THROUGH USE OF MODELS OF MAGNETIC NANOPARTICLES AS CONTRAST AGENTS (FURTHER PROJECT RESULT)

Brief Description	Code of Brownian dynamics based on coupled motion of magnetic moment and particle is developed for calculation of MRI relaxivity induced by magnetic nano particles.
Innovativeness introduced compared to already existing Products/Services	Magnetic moment time correlation functions are numerically calculated over a broad range of physical parameters of the particles. The correlation functions are fitted with series of exponentials. And the obtained relaxation times are used to calculate the MRI relaxivity. Previously only some asymptotic relations were available for a limited range of parameters.
Unique Selling Proposition (competitive advantages)	Open source
Product/Service Market Size	Simulation for researchers and industry in MRI
Product/Service Positioning	See above; low price or for free
Legal or normative or ethical requirements (need for authorisations, compliance to standards, norms, etc.)	none
Competitors	Unknown so far

Prospects/Customers	In general R&D; e.g. preclinical imaging facilities, research institutes, academia (universities, university of applied science),

	physicians (researcher) o Academia : - Focus on R&D (publication); e.g. University, Medical Schools, University Clinics, Research Institutes, and national institutes (FDA; US Army) o Pharma: - R&D with focus on productlines and research; e.g. global players as known
Time to market	1-3 months of coding
Foreseen Product/Service Price	cannot be estimated at this time; probably free of charge
Adequateness of Consortium Staff	Good
External Experts/Partners to be involved	none
Status of IPR: Background (type and partner owner)	**UL:** theoretical basis in physics of magnetic nanoparticles
Status of IPR: Foreground (type and partner owner)	**UL:** algorithm
Status of IPR: Exploitation Forms (type and partner owner) **e.g. direct industrial use, patenting, technology transfer, license agreement, publications, standards, etc.**	**UL:** open software tool for numerical simulation
Partner/s involved expectations	**UL:** establish open software tool for academic research community
Exploitation: Sources of financing foreseen after the end of the project (venture capital, loans, other grants, etc.)	Probably grants

Priority map of Exploitable result

Risk matrix

	Key Exploitable Result ER04	Degree of importance for the risk of not achieving long term and successful Exploitation. (1 low - 10 high)	Probability of risk happening (1 low - 10 high)	Risk Grade	Scope and type of potential intervention	Feasibility of Intervention Please rate from 1 to 10 (1 low- 10 high)	Priority Level
	Technological Risk Factors						
5	Reluctance to accept numerical results	*5*	*7*	**35**	Publish good results not available previously.	*5*	**175**
	Financial/management Risk Factors						
17	Off time supply of financial means.	***8***	***6***	**48**	Search for different sources of financing for the development work (VC, loans, grants, etc.).	*5*	**240**

Priority map result for Exploitable result

The Priority Map, is divided in 4 quadrants:

- the "No Action" quadrant shows risks with little influence on the exploitation of the project;
- the "Control" quadrant shows factors to be monitored periodically to exploit project results in the proper manner;
- the "Action" quadrant shows risks which require immediate interventions and implementation of contingency plans. Thanks to such interventions, these factors will move toward the "Control" quadrant;
- the "Warning" quadrant shows the most critical factors, the ones for which it is difficult to have an impact with immediate interventions. High number of factors in this quadrant could lead the project to face high risks of failing in exploiting its results.

2.2.5. Exploitable result No 5

Characterization of the result

Exploitable Result number 5:

METHODOLOGY AND GUIDELINES RELATED FOR USE AND CHARACTERIZATION OF MAGNETIC NANOPARTICLES

Brief Description	Physical particle size in fine nanoparticle systems may not be directly correlated to the effective magnetic particle size due to particle agglomeration, interaction or surface oxidation. We have exploited a method using first-order reversal curves for a semi-quantitative discrimination between the amount of superparamagnetism to magnetically-ordered particles. Furthermore, we demonstrate how long-term storage environment affects the chemical composition and magnetic properties of magnetic nanoparticles. This may influence usage in applications.
Innovativeness introduced compared to already existing Products/Services	• Characterizes the effective magnetic particle size, which will define the magnetic properties, which is needed in their application. • Provides a potential standardized method for characterizing the concentration of superparamagnetic versus magnetically ordered particles. • Can be applied to a broad range of magnetic compositions • Demonstrated how storage can affect

	physical properties of the magnetic nanoparticles
Product/Service Positioning	Establishes a standard method that can be used by anyone Makes recommendation for long term storage
Legal or normative or ethical requirements (need for authorisations, compliance to standards, norms, etc.)	none
Competitors	none
Prospects/Customers	Academia: groups synthesizing magnetic nanoparticles Pharma Industry R&D Industrial Engineering R&D
Cost of Implementation (before Exploitation)	none
Time to market	available now
Foreseen Product/Service Price	
Status of IPR: Background (type and partner owner)	theoretical basis and experimental technics
Status of IPR: Foreground (type and partner owner)	guidelines for particle storage; particle characterisation methods
Status of IPR: Exploitation Forms (type and partner owner) **e.g. direct industrial use, patenting, technology transfer, license agreement, publications, standards, etc.**	**MPG:** further research, publishing **UP:** further research, publishing **LMU**: further research, publishing **ETH:** further research (in particular COST Action TD1402 for magnetic hyperthermia), publishing
Exploitation: Sources of financing foreseen after the end of the project (venture capital, loans, other grants, etc.)	grants for improvement work

2.2.6. Exploitable result No 6

Characterization of the result

Exploitable Result number 6:

(VISUAL) TEACHING MATERIAL

Brief Description	We have a unique collection of particles in that have been characterized by a series of different methods. Results from characterization of magnetic nanoparticles produced under the project will be integrated into lectures at the ETH, UP and guest lectures at other universities. We have used a range of transmission electron microscopy techniques for the characterization of magnetic nanoparticle systems. Experience from this work was transpired into a graduate textbook chapter on TEM methods in studying biominerals. Principles of working of contrast agents in MRI.
Innovativeness introduced compared to already existing Products/Services	Have gathered /established an expertise that integrates different characterization which allows us to demonstrate the advantage of a mutl-disciplinary approach. The combination of TEM techniques for the study of nanoparticles allows for a simultaneous characterization of structural, morphological, compositional and magnetic properties on the nanoscale.
Prospects/Customers	Students
Cost of Implementation (before Exploitation)	none
Time to market	available now

Status of IPR: Background (type and partner owner)	knowledge
Status of IPR: Foreground (type and partner owner)	knowledge
Status of IPR: Exploitation Forms (type and partner owner) e.g. direct industrial use, patenting, technology transfer, license agreement, publications, standards, etc.	**MPG:** teaching, further research, publishing **UP:** teaching, further research, publishing **UL:** teaching, further research, publishing **LMU:** teaching, further research, publishing **ETH:** teaching, further research, publishing
Partner/s involved expectations	N/A
Exploitation: Sources of financing foreseen after the end of the project (venture capital, loans, other grants, etc.)	N/A

2.3. Priority map of Exploitable result

N/A

2.4. Ground Identification

GROUND IDENTIFICATION

Apart from the yellow boxes, you just need to put a cross "x" where appropriate

1	Result number	1
2	Result Title	**METHOD FOR SYNTHESIZING BIOLOGICAL MAGNETITE IN HIGH AMOUNT AND ISOLATING THEM WITH HIGH PURITY**
3	Result Description	Genetically engineered bacterial strains for the isolation of biological magnetite nanoparticles of different sizes (18 to 45 nm) and narrow size distribution. Improvements of the magnetosome isolation procedure in addition to the generation of overexpression strains o increased the magnetite yields more than two-fold and decreased the processing time by several hours.

		MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12		DETAILS
4	Partners willing to go to the market		X												*Accept the share of investments & risks*
5	Porte-parole partner		X												*For coordination purpose only*
6	Partners providing background knowledge willing to claim rights		X												*Only those not listed in 4*
7	Partners providing background knowledge NOT willing to claim rights														*However may request NDA on their Foreground Knowledge*

8	Partners providing foreground knowledge willing to claim rights	X	X	X	X	X	X	X						*Only those not listed in 4*
9	Partners providing foreground knowledge NOT willing to claim rights													*However may request NDA on their Foreground Knowledge*

		FREE	NEGOTIATION	NO
		In case all the partners agree to transfer the exploitation rights to the business team	*In case there is still room to clarify the IP/IPR*	*In case at least one partner do not agree to transfer the rights (VETO)*
10	Status as regard the exploitation right from other partners		X	

			MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12		DETAILS
11	Nature of activity foreseen for this result by the partner	M		X												*Manufacturing, Realisation*
		A														*Assembly*
		R	X	X	X	X	X		X							*Research*
		C		X												*Consultancy, Training*
		U														*Utilisation in other business*
		SD		X												*Selling, Distribution*
		S														*Services*

		YES	NO	
12	Consensus Rights Transfer to the group			*Put a cross for YES in case no partner did block the transfer right during the second ESS*
13	Ad hoc Partnership building			*YES in case you agree to consider going for specific partnership with the other partners as listed in 4*

14	New legal entity		*YES in case you agree to consider setting a new legal entity with the other partners as listed in 4*
15	Single engagement	X	*YES in case we got the acceptance from all partners that you can run in a free way*

GROUND IDENTIFICATION

Apart from the yellow boxes, you just need to put a cross "x" where appropriate

1	Result number	**2**
2	Result Title	**METHOD FOR SYNTHESIZING SYNTHETIC MAGNETITE NANOPARTICLES WITH CONTROLLABLE SIZE AND COLLOIDAL STABILITY**
3	Result Description	We have developed a method to synthesize magnetite nanoparticles with controllable size (10 to 50 nm). The average dimension of the crystals can be varied by varying the physicochemical conditions of the syntheses and / or the duration time. However, due to the magnetic interaction between particles of larger sizes (larger than 30 nm), the particles tend to aggregate. Therefore, the particles need to be coated for use. Several additives have been tested and we finally identified one additive that enable the formation of these large particle in a colloidally stable state in a single step rather than first synthesis and then stabilization, thereby saving time and costs.

	MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12		DETAILS
4 Partners willing to go to the market	X													*Accept the share of investments & risks*
5 Porte-parole partner	X													*For coordination purpose only*
6 Partners providing background knowledge willing to claim rights	X													*Only those not listed in 4*
7 Partners providing background knowledge														*However may request NDA on their Foreground Knowledge*

	NOT willing to claim rights													
8	Partners providing foreground knowledge willing to claim rights	X		X	X	X	X	X						*Only those not listed in 4*
9	Partners providing foreground knowledge NOT willing to claim rights													*However may request NDA on their Foreground Knowledge*

	FREE	NEGOTIATION	NO
	In case all the partners agree to transfer the exploitation rights to the business team	*In case there is still room to clarify the IP/IPR*	*In case at least one partner do not agree to transfer the rights (VETO)*
10 Status as regard the exploitation right from other partners		X	

		MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12		DETAILS
11	Nature of activity foreseen for this result by the partner														
	M	X													*Manufacturing, Realisation*
	A														*Assembly*
	R	X		X	X	X		X							*Research*
	C	X													*Consultancy, Training*
	U	X													*Utilisation in other business*
	SD	X													*Selling, Distribution*
	S														*Services*

		YES	NO	
12	Consensus Rights Transfer to the group			*Put a cross for YES in case no partner did block the transfer right during the second ESS*

13	Ad hoc Partnership building		*YES in case you agree to consider going for specific partnership with the other partners as listed in 4*
14	New legal entity		*YES in case you agree to consider setting a new legal entity with the other partners as listed in 4*
15	Single engagement	X	*YES in case we got the acceptance from all partners that you can run in a free way*

GROUND IDENTIFICATION

Apart from the yellow boxes, you just need to put a cross "x" where appropriate

1 Result number: **3**

2 Result Title: **CONTRAST AGENT WITH PROPERTIES MUCH HIGHER THAN CURRENT STANDARDS**

3 Result Description:

We have investigated various iron oxide nanoparticles of biological and synthetic origin concerning their contrast properties in MRI and, optionally, also the contrast properties in MPI and hyperthermia. We found a size dependence of both, synthetic as well as biological particles, where the larger particles exhibit improved contrast properties in MRI. After considering colloidal stability aspects, the most promising results were found for the biological magnetosomes, where the largest particles show an up to factor 5 higher R2/R1 relaxivity compared to the known MRI contrast agent Resovist ®. Additionally, the biological magnetosomes show very promising results in MPI and hyperthermia. In the case of the synthetic particles colloidal stability aspects has to be considered. Despite the promising results concerning their contrast properties, for the use as contrast agents, additional *in vivo* characterization need to be performed.

	MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12		DETAILS
4 Partners willing to go to the market			X											*Accept the share of investments & risks*
5 Porte-parole partner			X											*For coordination purpose only*
6 Partners providing background knowledge willing to claim rights			X											*Only those not listed in 4*

7	Partners providing background knowledge NOT willing to claim rights													*However may request NDA on their Foreground Knowledge*
8	Partners providing foreground knowledge willing to claim rights	X	X	X	X	X	X	X						*Only those not listed in 4*
9	Partners providing foreground knowledge NOT willing to claim rights													*However may request NDA on their Foreground Knowledge*

		FREE	NEGOTIATION	NO
		In case all the partners agree to transfer the exploitation rights to the business team	*In case there is still room to clarify the IP/IPR*	*In case at least one partner do not agree to transfer the rights (VETO)*
10	Status as regard the exploitation right from other partners		X	

		MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12		DETAILS
11	Nature of activity foreseen for this result by the partner														
	M			X											*Manufacturing, Realisation*
	A														*Assembly*
	R	X	X	X	X	X		X							*Research*
	C														*Consultancy, Training*
	U														*Utilisation in other business*
	SD			X											*Selling, Distribution*
	S			X											*Customise products*

YES NO

12	Consensus Rights Transfer to the group			*Put a cross for YES in case no partner did block the transfer right during the second ESS*
13	Ad hoc Partnership building			*YES in case you agree to consider going for specific partnership with the other partners as listed in 4*
14	New legal entity			*YES in case you agree to consider setting a new legal entity with the other partners as listed in 4*
15	Single engagement	X		*YES in case we got the acceptance from all partners that you can run in a free way*

GROUND IDENTIFICATION

Apart from the yellow boxes, you just need to put a cross "x" where appropriate

1 Result number: **4**

2 Result Title: **CODE OF BROWNIAN DYNAMICS FOR CALCULATION OF RELAXIVITY INDUCED BY MAGNETIC NANOPARTICLES (THROUGH USE OF MODELS OF MAGNETIC NANOPARTICLES AS CONTRAST AGENTS (FURTHER PROJECT RESULT))**

3 Result Description:

Code of Brownian dynamics based on coupled motion of magnetic moment and particle is developed for calculation of MRI relaxivity induced by magnetic nano particles.

	MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12	P13	DETAILS
4 Partners willing to go to the market							X							*Accept the share of investments & risks*
5 Porte-parole partner							X							*For coordination purpose only*
6 Partners providing background knowledge willing to claim rights							X							*Only those not listed in 4*
7 Partners providing background knowledge NOT willing to claim														*However may request NDA on their Foreground Knowledge*

	rights													
8	Partners providing foreground knowledge willing to claim rights							X						*Only those not listed in 4*
9	Partners providing foreground knowledge NOT willing to claim rights													*However may request NDA on their Foreground Knowledge*

		FREE	NEGOTIATION	NO
		In case all the partners agree to transfer the exploitation rights to the business team	*In case there is still room to clarify the IP/IPR*	*In case at least one partner do not agree to transfer the rights (VETO)*
10	Status as regard the exploitation right from other partners			

		MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12	P13	DETAILS
11	Nature of activity foreseen for this result by the partner														
	M							X							*Manufacturing, Realisation*
	A														*Assembly*
	R							X							*Research*
	C							X							*Consultancy, Training*
	U														*Utilisation in other business*
	SD							X							*Selling, Distribution*
	S														*Services*

		YES	NO	
	Consensus Rights			*Put a cross for YES in case no partner did block the transfer right during the second ESS*
12	Transfer to the group			
	Ad hoc Partnership			
13	building			*YES in case you agree to consider going for specific partnership with the other partners as listed in 4*
14	New legal entity			*YES in case you agree to consider setting a new legal entity with the other partners as listed in 4*
15	Single engagement			*YES in case we got the acceptance from all partners that you can run in a free way*

GROUND IDENTIFICATION

Apart from the yellow boxes, you just need to put a cross "x" where appropriate

1 Result number: 5

2 Result Title: ***METHODOLOGY AND GUIDELINES RELATED FOR USE AND CHARACTERIZATION OF MAGNETIC NANOPARTICLES***

3 Result Description:

Physical particle size in fine nanoparticle systems may not be directly correlated to the effective magnetic particle size due to particle agglomeration, interaction or surface oxidation. We have exploited a method using first-order reversal curves for a semi-quantitative discrimination between the amount of superparamagnetism to magnetically-ordered particles.
Furthermore, we demonstrate how long-term storage environment affects the chemical composition and magnetic properties of magnetic nanoparticles. This may influence usage in applications.

	MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12	P13	DETAILS
4 Partners willing to go to the market					X									*Accept the share of investments & risks*
5 Porte-parole partner					X									*For coordination purpose only*
6 Partners providing background knowledge willing to claim rights	X	X		X	X									*Only those not listed in 4*
Partners providing								96						*However may request NDA on their Foreground Knowledge*

7 background knowledge

	NOT willing to claim rights													
8	Partners providing foreground knowledge willing to claim rights	X	X		X	X								*Only those not listed in 4*
9	Partners providing foreground knowledge NOT willing to claim rights													*However may request NDA on their Foreground Knowledge*

		FREE	NEGOTIATION	NO
		In case all the partners agree to transfer the exploitation rights to the business team	*In case there is still room to clarify the IP/IPR*	*In case at least one partner do not agree to transfer the rights (VETO)*
10	Status as regard the exploitation right from other partners		X	

		MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12	P13	DETAILS
11	Nature of activity foreseen for this result by the partner														
	M														*Manufacturing, Realisation*
	A														*Assembly*
	R	X	X		X	X									*Research*
	C	X				X									*Consultancy, Training*
	U														*Utilisation in other business*
	SD														*Selling, Distribution*
	S					X									*Services, Contract Research*

		YES	NO	
	Consensus Rights			*Put a cross for YES in case no partner did block the transfer right during the second ESS*
12	Transfer to the group			
	Ad hoc Partnership			
13	building			*YES in case you agree to consider going for specific partnership with the other partners as listed in 4*
14	New legal entity			*YES in case you agree to consider setting a new legal entity with the other partners as listed in 4*
15	Single engagement	X		*YES in case we got the acceptance from all partners that you can run in a free way*

GROUND IDENTIFICATION

Apart from the yellow boxes, you just need to put a cross "x" where appropriate

1 Result number: **6**

2 Result Title: **(VISUAL) TEACHING MATERIAL**

3 Result Description:

We have a unique collection of particles in that have been characterized by a series of different methods. Results from characterization of magnetic nanoparticles produced under the project will be integrated into lectures at the ETH, UP and guest lectures at other universities.

We have used a range of transmission electron microscopy techniques for the characterization of magnetic nanoparticle systems. Experience from this work was transpired into a graduate textbook chapter on TEM methods in studying biominerals.

Principles of working of contrast agents in MRI.

	MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12	P13	DETAILS
4 Partners willing to go to the market														*Accept the share of investments & risks*
5 Porte-parole partner														*For coordination purpose only*
Partners providing								100						*Only those not listed in 4*

6 background knowledge
willing to claim rights

7	Partners providing background knowledge NOT willing to claim rights													*However may request NDA on their Foreground Knowledge*
8	Partners providing foreground knowledge willing to claim rights													*Only those not listed in 4*
9	Partners providing foreground knowledge NOT willing to claim rights													*However may request NDA on their Foreground Knowledge*

		FREE	NEGOTIATION	NO
		In case all the partners agree to transfer the exploitation rights to the business team	*In case there is still room to clarify the IP/IPR*	*In case at least one partner do not agree to transfer the rights (VETO)*
10	Status as regard the exploitation right from other partners			

		MPG	LMU	NanoPet	UP	ETH	LBIO	UL	P8	P9	P10	P11	P12	P13	DETAILS
11	Nature of activity foreseen for this result by the partner														
	M														*Manufacturing, Realisation*
	A														*Assembly*
	R														*Research*
	C	X	X	X	X	X	X	X							*Consultancy, Training*
	U														*Utilisation in other business*
	SD														*Selling, Distribution*

S														Services

		YES	NO	
	Consensus Rights			*Put a cross for YES in case no partner did block the transfer right during the second*
12	Transfer to the group			*ESS*
13	Ad hoc Partnership building			*YES in case you agree to consider going for specific partnership with the other partners as listed in 4*
14	New legal entity			*YES in case you agree to consider setting a new legal entity with the other partners as listed in 4*
15	Single engagement	X		*YES in case we got the acceptance from all partners that you can run in a free way*

2.5. IPR

Background

N°	Key Exploitable Result	Lead Partner	All partners	Background IPR in Detail
1	**Method for synthesizing biological magnetite in high amount and isolating them with high purity**	LMU	-	LMU: synthesis of magnetosomes, isolation from cells, different strains available

2	**Method for synthesizing synthetic magnetite nanoparticles with controllable size and colloidal stability**	MPG	UP, nanoPET	MPG: improve coprecipitation technique UP: controlled size by different time (UP basic synthesis)
3	**Contrast agent with properties much higher than current standards**	nanoPET	-	Nanopet: contrast agent research; biocompetible formualtion; colloidal stabilisation
4	**Code of Brownian dynamics for calculation of relaxivity induced by magnetic nanoparticles**	UL	-	theoretical basis in physics of magnetic nanoparticles

5	**Methodology and guidelines related for use and characterization of magnetic nanoparticles**	-		theoretical basis and experimental technics
6	**Teaching Material**	-		knowledge

2.6. Foreground

N°	Key Exploitable Result	Lead Partner	All partners	Foreground IPR in Detail
1	**Method for synthesizing biological magnetite in high amount and isolating them with high purity**	LMU	-	enhanced yield, enhanced isolation process, non-bacterially contaminated samples

2	**Method for synthesizing synthetic magnetite nanoparticles with controllable size and colloidal stability**	MPG	-	Imrove experimental set up, enhanced yield, enhanced colloidal stability by use of additives, enhanced monodispersity
3	**Contrast agent with properties much higher than current standards**	Nanopet	-	magnetosomes around 10 times beter than standard, biomimetic nanoparticles around 3 times better than standrds (MRI)
4	**Code of Brownian dynamics for calculation of relaxivity induced by magnetic nanoparticles**	UL		algorithm

5	**Methodology and guidelines related for use and characterization of magnetic nanoparticles**	ETH, MPG		guidelines for particle storage; particle characterisation methods
6	**Teaching Material**	All universities		knowledge

2.7. Exploitation Claims

N°	Key Exploitable Result	Lead Partner	Exploitation Claims in Detail	Partner + Share
1	**Method for synthesizing biological magnetite in high amount and isolating them with high purity**	LMU	LMU: manufacturing, selling, publishing, further research; UP: further research; ETH: further research; MPG: further research	LMU: 88% MPG: 2% UP: 2% ETH: 2% UL: 2% LBIO: 2% nanopet: 2%

2	**Method for synthesizing synthetic magnetite nanoparticles with controllable size and colloidal stability**	MPG	MPG: manufacturing, selling, publishing, further research; UP: further research; ETH: further research	MPG: 90% UP: 2% ETH: 2% UL: 2% LBIO: 2% nanopet: 2%
3	**Contrast agent with properties much higher than current standards**	Nanopet	nanopet: manufacturing, selling, publishing, further research;	nanopet: 88% MPG: 2% UP: 2% ETH: 2% UL: 2% LBIO: 2% LMU 2%
4	**Code of Brownian dynamics for calculation of relaxivity induced by magnetic nanoparticles**	UL	UL: open software tool for numerical simulation, publishing	UL: 100%

5	**Methodology and guidelines related for use and characterization of magnetic nanoparticles**	ETH, MPG	ETH: Selling LabServices to research and business; further research, publishing; MPG: further research, publishing; UP: further research, publishing; LMU: further research, publishing;	ETH: 25% MPG: 25% UP: 25% LMU: 25%
6	**Teaching Material**	All universities	ETH: teaching, further research, publishing; MPG: teaching, further research, publishing; UP: teaching, further research, publishing; LMU: teaching, further research, publishing; UL: teaching, further research, publishing;	ETH: 18% MPG: 18% UP: 18% LMU: 18% UL: 18% nanopet: 5% LBIO: 5%

2.8. Summary

Table Exploitable foreground and its use

Exploitable foreground need to consider:

- Further R&D activity and/or collaboration needs for exploitation (and which risks are implied)
- IP-related aspects
- Customer detection (focus on factors that affect purchasing decisions)
- Features of the target market (size, growth rate, share that the technology/product could reach, driving factors likely to change the market, legal, technical and commercial barriers, other technologies likely to emerge in the near future…)
- Positioning (how the company entitled to the technology exploitation is positioned or should be positioned in the market)

Description of Exploitable Foreground	Exploitable Results	Sector(s) of application	Time to Market	Patents or other IPR exploitation Forms/Claims	Owner & Other Beneficiary(s) involved
Genetically engineered bacterial strains for the isolation of biological magnetite nanoparticles of different sizes (18 to 45 nm) and narrow size distribution. Improvements of the magnetosome	Method for synthesizing biological magnetite in high amount and isolating them with high purity	Biotechnology	Already in use at small scale with the end of the project. Upscaling should be available within a couple of years if dedicated funds are available.	LMU: manufacturing, sell, further research; UP: further research; ETH: further research; MPG: further research	LMU: 88% MPG: 2% UP: 2% ETH: 2% UL: 2% LBIO: 2% nanopet: 2%

Description of Exploitable Foreground	Exploitable Results	Sector(s) of application	Time to Market	Patents or other IPR exploitation Forms/Claims	Owner & Other Beneficiary(s) involved
isolation procedure in addition to the generation of overexpression strains o increased the magnetite yields more than two-fold and decreased the processing time by several hours.					

We have developed a method to synthesize magnetite nanoparticles with controllable size (10 to 50 nm). The average dimension of the crystals can be varied by varying the physicochemical conditions of the syntheses and / or the duration time. However, due to the	Method for synthesizing synthetic magnetite nanoparticles with controllable size and colloidal stability	Bio- and nanotechnology	Already in use at small scale with the end of the project for testing their effect as contrast agent for MRI in small animals. Upscaling should be available within a couple of years if dedicated funds are available.	MPG: manufacturing, sell, further research; UP: further research; ETH: further research	MPG: 90% UP: 2% ETH: 2% UL: 2% LBIO: 2% nanopet: 2%

Description of Exploitable Foreground	Exploitable Results	Sector(s) of application	Time to Market	Patents or other IPR exploitation Forms/Claims	Owner & Other Beneficiary(s) involved
magnetic interaction between particles of larger sizes (larger than 30 nm), the particles tend to aggregate. Therefore, the particles need to be coated for use. Several additives have been tested and we finally identified one additive that enable the formation of these large particle in a colloidally stable state in a single step rather than first synthesis and then stabilization, thereby saving time and costs.					

We have investigated various iron oxide nanoparticles of biological and	Contrast agent with properties much higher than current standards	Bio-, and nanotechnologies, medical field(MPI, MRI,	For preclinical imaging (animals): short, which means approx. 1-2 years.	nanopet: manufacturing, selling, further research	nanopet: 88% MPG: 2% UP: 2% ETH: 2%

Description of Exploitable Foreground	Exploitable Results	Sector(s) of application	Time to Market	Patents or other IPR exploitation Forms/Claims	Owner & Other Beneficiary(s) involved

synthetic origin concerning their contrast properties in MRI and, optionally, also the contrast properties in MPI and hyperthermia. We found a size dependence of both, synthetic as well as biological particles, where the larger particles exhibit improved contrast properties in MRI. After considering colloidal stability aspects, the most promising results were found for the biological magnetosomes, where the largest particles show an up to factor 5 higher R2/R1 relaxivity		Hyperthermia)	For application for human beings: long (due to regulatory requirements).		UL: 2% LBIO: 2% LMU 2%

Description of Exploitable Foreground	Exploitable Results	Sector(s) of application	Time to Market	Patents or other IPR exploitation Forms/Claims	Owner & Other Beneficiary(s) involved
compared to the known MRI contrast agent Resovist ®. Additionally, the biological magnetosomes show very promising results in MPI and hyperthermia. In the case of the synthetic particles colloidal stability aspects has to be considered. Despite the promising results concerning their contrast properties, for the use as contrast agents, additional *in vivo* characterization need to be performed.					

Code of Brownian dynamics based on coupled motion of magnetic moment	Code of Brownian dynamics for calculation of relaxivity induced by magnetic nanoparticles (through use of models of	Research, medical field	1-3 months of coding	UL: open software tool for numerical simulation	UL: 100%

Description of Exploitable Foreground	Exploitable Results	Sector(s) of application	Time to Market	Patents or other IPR exploitation Forms/Claims	Owner & Other Beneficiary(s) involved
and particle is developed for calculation of MRI relaxivity induced by magnetic nano particles.	magnetic nanoparticles as contrast agents (further project result))				

Physical particle size in fine nanoparticle systems may not be directly correlated to the effective magnetic particle size due to particle agglomeration, interaction or surface oxidation. We have exploited a method using first-order reversal curves for a semi-quantitative discrimination between the amount of superparamagnetism to magnetically-	Methodology and guidelines related for use and characterization of magnetic nanoparticles	Industry and academia, production of magnetic (nano)particles	Available now	ETH: Selling LabServices to research and business; further research, publishing; MPG: further research, publishing; UP: further research, publishing; LMU: further research, publishing	ETH: 25% MPG: 25% UP: 25% LMU: 25%

Description of Exploitable Foreground	Exploitable Results	Sector(s) of application	Time to Market	Patents or other IPR exploitation Forms/Claims	Owner & Other Beneficiary(s) involved
ordered particles. Furthermore, we demonstrate how long-term storage environment affects the chemical composition and magnetic properties of magnetic nanoparticles. This may influence usage in applications.					

We have a unique collection of particles in that have been characterized by a series of different methods. Results from characterization of magnetic nanoparticles produced under the project will be integrated into	(Visual) Teaching Material	Teaching at universities and research facilities	Available now	ETH: teaching, further research, publishing; MPG: teaching, further research, publishing; UP: teaching, further research, publishing; LMU: teaching, further research, publishing; UL: teaching, further	ETH: 18% MPG: 18% UP: 18% LMU: 18% UL: 18% nanopet: 5% LBIO: 5%

Description of Exploitable Foreground	Exploitable Results	Sector(s) of application	Time to Market	Patents or other IPR exploitation Forms/Claims	Owner & Other Beneficiary(s) involved
lectures at the ETH, UP and guest lectures at other universities. We have used a range of transmission electron microscopy techniques for the characterization of magnetic nanoparticle systems. Experience from this work was transpired into a graduate textbook chapter on TEM methods in studying biominerals. Principles of working of contrast agents in MRI.				research, publishing	

2.9. List of applications for patents, trademarks, registered designs, etc.

N/A (no patents or other IPR applied for during the project lifetime)

2.10. Specific dissemination to science and academia

2.10.1. Publishable results: scientific publications

	Title	Author(s)	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Related to Ex.Res. n°
1	Keeping nanoparticles fully functional: long term storage and alteration of magnetite	Widdrat M., Kumari M., Pósfai M., M. Tompa, Hirt A. M., and Faivre D.			*ChemPlusChem*	2014	cplu.201402032	2
2	Biomimetic Magnetite Formation: From biocombinatorial approaches to mineralization effects	Baumgartner J., Carillo M. A., Eckes K., Werner P and Faivre D.,	Vol. 30 (8)		*Langmuir*		2129-2136	2
3	Formation of magnetite at low temperature: From superparamagnetic to stable single domain particles	Baumgartner J., Bertinetti L., Widdrat M., Hirt A., and Faivre D.	Vol. 8 (3)		*PLoS ONE*		e57070	2
4	Genetic dissection of the mamAB and mms6 operons reveals a gene set essential for magnetosome biogenesis in Magnetospirillum gryphiswaldense.	Lohße, A., S. Borg, O. Raschdorf, I. Kolinko, E. Tompa, M. Posfai, D. Faivre,	2014/07 Vol. 196(14)	American Society for Microbiology	*Jopurnal of Bacteriology*	2014	2658 - 2669	1

		J. Baumgartner and D. Schüler.						
5	Bacterial magnetosome biomineralization--a novel platform to study molecular mechanisms of human CDF-related Type-II diabetes.	Zeytuni N, Uebe R, Maes M, Davidov G, Baram M, Raschdorf O, Friedler A, Miller Y, Schüler D, Zarivach R.	2014/05/12 Vol. 9(5)	Public Library of Science	*PLoS ONE*	2014	e97154 pages 1 - 11	1
6	Cation diffusion facilitators transport initiation and regulation is mediated by cation induced conformational changes of the cytoplasmic domain.	Zeytuni N, Uebe R, Maes M, Davidov G, Baram M, Raschdorf O, Nadav-Tsubery M, Kolusheva S, Bitton R, Goobes G, Friedler A, Miller Y, Schüler D, Zarivach R.	2014/03/21 Vol. 9(3)	Public Library of Science	*PLoS ONE*	2014	e92141 pages 1 - 14	1

7	Biosynthesis of magnetic nanostructures in a foreign organism by transfer of bacterial magnetosome gene clusters.	Kolinko I, Lohße A, Borg S, Raschdorf O, Jogler C, Tu Q, Pósfai M, Tompa E, Plitzko JM, Brachmann A, Wanner G, Müller R, Zhang Y, Schüler D.	2014/03 Vol. 9(3)	Macmillan Publishers Limited	*Nature Nanotechnology*	2014	193 - 197	1
8	Analysis of Magnetosome Chains in Magnetotactic Bacteria by Magnetic Measurements and Automated Image Analysis of Electron Micrographs	Katzmann E, Eibauer M, Lin W, Pan Y, Plitzko JM, Schüler D.	2013/12 Vol. 9(3)	American Society for Microbiology	*Applied and Environmental Microbiology*	2013	7755 - 7762	1
9	The magnetosome proteins MamX, MamZ, and MamH are involved in redox control of magnetite biomineralization in Magnetospirillum gryphiswaldense.	Raschdorf, O., F. D. Müller, M. Pósfai, J. M. Plitzko and D. Schüler.	2013/09 Vol. 89(5)	John Wiley & Sons	*Molecular Microbiology*	2013	872 - 886	1

10	The cation diffusion facilitator proteins MamB and MamM of Magnetospirillum gryphiswaldense have distinct and complex functions, and are involved in magnetite biomineralization and magnetosome membrane assembly	Uebe, R., K. Junge, V. Henn, G. Poxleitner, E. Katzmann, J. M. Plitzko, R. Zarivach, T. Kasama, G. Wanner, M. Pósfai, L. Böttger, B. Matzanke, and D. Schüler	2011/11 Vol. 82(4)	John Wiley & Sons	*Molecular Microbiology*	2011	818 - 835	1
11	Coupled stochastic dynamics of magnetic moment and anisotropy axis of a magnetic nanoparticle	R.Taukulis, A.Cēbers	Vol. 86	Phys.Rev.E		2012	61405	4
12	Band formation by magnetotactic spirillum bacteria in oxygen concentration gradient.	K.Ērglis, D.Zhulenkovs, M.Belovs, J.Valeinis, A.Cēbers.	Vol. 48	Magnetohydrodynamics		2012	607-614	4
13	Three dimensional dynamics of a particle with a finite energy of magnetic anisotropy in a rotating magnetic field	J.Cīmurs, and A.Cēbers.	Vol. 88	Phys.Rev.E		2013	62315	4
14	Diffusion in active magnetic colloid	R.Taukulis, A.Cēbers		JMMM	http://www.sciencedirect.com/science/journal/aip/03048853	2013		4

15	Biominerals at the nanoscale: transmission electron microscopy methods for studying the special properties of biominerals. In Minerals at the Nanoscale	Pósfai, M., Kasama, T. and Dunin-Borkowski, R.	Vol. 14	European Mineralogical Union Notes in Mineralogy	*European Mineralogical Union and the Mineralogical Society of Great Britain & Ireland, London,*	2013	375-433	1
16	Phylogenetic significance of composition and crystal morphology of magnetosome minerals	Pósfai, M., Lefèvre, C.T., Trubitsyn, D., Bazylinski D.A., and Frankel, R.B.	Vol. 4		*Frontiers in Microbiology,*	2013	344	1
17	Growth defects and epitaxy in Fe3O4 and γ-Fe2O3 nanocrystals	Rečnik, A., Nyirő-Kósa, I., Dódony, I. and Pósfai, M.	Vol. 15		*CrystEngComm*	2013	7539-7547	1
18	Novel methods for the synthesis of magnetite nanoparticles with special morphologies and textured assemblages	Nyirő-Kósa, I., Rečnik, A., and Pósfai, M.			*Journal of Nanoparticle Research*	2012	1150-1159	1

19	Distinguishing magnetic particle size of iron oxide nanoparticles with FORC analysis	Monika Kumari, Marc Widdrat, Éva Tompa, Rene Uebe, Dirk Schüler, Mihály Pósfai, Damien Faivre, Ann M Hirt	Vol.116		*Journal of Applied Physics*	in review	124304	2
20	Experimental mixtures of superparamagnetic and single domain magnetite with respect to Day-Dunlop plots	Monika Kumari, Nico Senn, Rene Uebe, Éva Tompa, Wolfram Lorenz, Dirk Schüler, Mihály Pósfai, Ann M Hirt			*Geohemistry, Geophysics, Geosystems*	in preparation		1
21	Assessing the self-assembly of synthetic and biogenic magnetite nanoparticles	Monika Kumari, Rene Uebe, Marc Widdrat, Damien Faivre, Dirk Schüler, Ann M Hirt			*Geopyhsical Journal International*	in preparation		2
22	Is 135° ridge on the lower half of the first order reversal curve diagram an artifact?	Monika Kumari, Marc Widdrat, Izabela Bobowska, Damien Faivre, Ann M. Hirt			*to be decided*	in preparation		1

23	Effect of freeze-thaw cycles on the magnetosomal chains of magnetotactic bacteria	Monika Kumari, Rene Uebe, Dirk Schüler, Ann M Hirt			*to be decided*	in preparation		5
24	Magnetic Properties of Iron Oxide Nanoparticles and Methods in Their Characterization	Monika Kumari			*PhD Thesis ETH Zurich*	in preparation		5
25	Biogenic and Biomimetic Magnetite Nanoparticles for different biomedical Applications	David Heinke, Alexander Kraupner, René Uebe, Marc Widdrat, Dietmar Eberbeck, Makis Angelakeris, Nicole Gehrke, Damien Favre, Dirk Schüler, Andreas Briel			To be decided	In preparation		5

2.10.2. Communications at conferences

	Title	Author(s)	Conference	Approx. number of auditors	Place	Year	Notes
1	Functional Magnets Assemblies: Biological design and Synthetic Approach	D. Faivre	CEA Seminars	20	Saclay, France	2014	
2	Magnetite Synthesis: from the Magnetotactic Bacteria to the Beaker	D. Faivre	GDCh Chemiedozententagung	100	Paderborn, Germany)	2014	
3	Magnetosome Biomineralization and Assembly in Magnetotactic Bacteria	D. Faivre	Biomineralization seminars	20	Weizmann Institute of Science, Rehovot, Israel	2014	
4	Magnetite Formation Mechanisms in Magnetotactic Bacteria and in the Beaker	D. Faivre	Nano Center Seminars	50	Ben Gurion University of the Negev, Beer Sheva, Israel	2014	
5	The bacterial art of making and using nanomagnets	D. Faivre	Kuratorium of the Research Park Golm	30	Potsdam, Germany	2013	

6	The solid-state side of bio- inorganic chemistry: biological and biomimetic formation of magnetite	D. Faivre	Colloquium Bio-inorganic Chemistry	50	Dortmund, Germany	2013	
7	Biological and synthetic formation of iron oxides	D. Faivre	Colloquium Particle Synthesis	40	Erlangen, Germany	2013	
8	Synchrotron Radiation as a tool to analyze magnetite nanoparticles	M. Widdrat and D.Faivre	8th European NESY Winter-School & Symposium on Neutrons and Synchrotron Radiation	100	Planneralm, Austria	2013	
9	Synthetic and biological magnetite: from iron colloids to functional chains	P. Vach, J. Baumgartner, M. Widdrat, and D. Faivre	Chemiedozententagung, GDCh	50	Berlin, Germany	2013	
10	Magnetic Nanoparticles: Navigating from bacteria to robots	D. Faivre	Symposium Inorganic Chemistry	30	Ulm, Germany	2012	
11	Simple but organized: biological and biomimetic mineralization and assembly of magnetic nanoparticles	D. Faivre	ERC Grantees Conference 2012	250	Strasbourg, France	2012	

12	Functional iron oxides: from bacteria to synthetic nanoswimmers	D. Faivre		50	University Granada, Granada, Spain	2012	
13	Synthetic and biological magnetite: from iron colloids to functional chains	D. Faivre		30	Max Planck Institute for Dynamics and Self-Organizatio n, Göttingen, Germany	2012	
14	Nanoparticles of Magnetite: Formation and Applications	D. Faivre			University Granada, Granada, Spain	2012	
15	Synthesis of size-controlled stable single domain magnetite nanoparticles	M. Widdrat, J. Baumgartner, and D. Faivre	COST meeting of the CM 0902 Action	60	Bari, Italy	2012	
16	High-resolution X-ray diffraction as a tool to study bio- and biomimetic minerals: studies of inorganic magnetite and magnetosomes	D. Faivre	Ringberg Symposium 2010: Molecular Bionics – From Biomineralization to Functional Materials	50	Ringberg Castle, Germany	2010	

17	Experimental study on mixtures of superparamagnetic and single domain magnetite with respect to Day-Dunlop plots	Monik Kumari, Nico Senn, Rene Uebe, Dirk Schüler, Ann M Hirt	American Geophysical Union		San Francisco, CA, USA	2013	
18	Assessing the self-assembly of synthetic and biogenic magnetite nanoparticles	Monika Kumari, Stephan Handschin, Rene Uebe, Dirk Schüler, Ann Hirt	European Geosciences Union General Assembly		Vienna, Austria	2014	http://meetingorganizer.copernicus.org/EGU2014/EGU2014-8263.pdf
19	Assessing superparamagnetic iron oxide nanoparticles with first order reversal curves	Monika Kumari, Marc Widdrat, Éva Tompa, Rene Uebe, Alexander Krauppe5, Dirk Schüler, Mihály Pósfai, Damien Faivre, Ann M Hirt	10th International Conference on the Scientific and Clinical Applications of Magnetic Carriers		Dresden, Germanyl	2014	http://www.mfd.mw.tu-dresden.de/icmc/media/Book-of-Abstracts.pdf

20	Is 135° ridge on the lower half of the first order reversal curve diagram an artifact?	Monika Kumari, Izabela Bobowska, Damien Faivre, Ann M. Hirt	14th Castle Meeting		Évora Portugal	2014	https://www.fc.ul.pt/sites/default/files/fcul/public/14Castle_meeting/Kumari%20M%20abstract%20Castle14.pdf
21	Day-Dunlop Plots: Use in assessing mixutres of single-domain and superparmagnetic particles	Monika Kumari	Earth Planetary Magnetism Seminar ETHZ		Zurich, Switzerland	2014	
22	Experimental study on mixtures of superparamagnetic and single domain magnetite with respect to Day-Dunlop plots	Monik Kumari, Nico Senn, Rene Uebe, Dirk Schüler, Ann M Hirt	American Geophysical Union		San Francisco, CA, USA	2013	
23	Assessing the self-assembly of synthetic and biogenic magnetite nanoparticles	Monika Kumari, Stephan Handschin, Rene Uebe, Dirk Schüler, Ann Hirt	European Geosciences Union General Assembly		Vienna, Austria	2014	http://meetingorganizer.copernicus.org/EGU2014/EGU2014-8263.pdf

24	Assessing superparamagnetic iron oxide nanoparticles with first order reversal curves	Monika Kumari, Marc Widdrat, Éva Tompa, Rene Uebe, Alexander Kraupner, Dirk Schüler, Mihály Pósfai, Damien Faivre, Ann M Hirt	10th International Conference on the Scientific and Clinical Applications of Magnetic Carriers		Dresden, Germanyl	2014	http://www.mfd.mw.tu-dresden.de/icmc/media/Book-of-Abstracts.pdf
25	Is 135° ridge on the lower half of the first order reversal curve diagram an artifact?	Monika Kumari, Izabela Bobowska, Damien Faivre, Ann M. Hirt	14th Castle Meeting		Évora Portugal	2014	https://www.fc.ul.pt/sites/default/files/fcul/public/14Castle_meeting/Kumari%20M%20abstract%20Castle14.pdf
26	Day-Dunlop Plots: Use in assessing mixutres of single-domain and superparmagnetic particles	Monika Kumari	Earth Planetary Magentism Seminar ETHZ		Zurich, Switzerland	2014	

27	Bacterial magnetosomes as a new type of biogenic MPI tracer	Alexander Kraupner, David Heinke, René Uebe, Dietmar Eberbeck, Nicole Gehrke, Dirk Schüler, Andreas Briel	4th International Workshop on Magnetic Particle Imaging		Berlin, Germany	2014	http://www.iwmpi.org/fileadmin/files/2014_IWMPI/Abstracts/IWMPI2014_Book_of_Abstracts.pdf
28	BIOGENIC MAGNETITE NANOPARTICLES FOR DIFFERENT BIOMEDICAL APPLICATIONS	David Heinke, Alexander Kraupner, René Uebe, Dietmar Eberbeck, Makis Angelakeris, Nicole Gehrke, Dirk Schüler, Andreas Briel	10th International Conference on the Scientific and Clinical Applications of Magnetic Carriers		Dresden, Germany	2014	http://www.mfd.mw.tu-dresden.de/icmc/media/Book-of-Abstracts.pdf
29	Biomineralization by magnetotactic bacteria and the biomimetic synthesis of magnetic nanoparticles.	Pósfai, M., Tompa, É., Nyirő-Kósa, I., Kovács, A., Dunin-Borkowski, R.E., Tóth, B., Vonderviszt, F., Uebe, R., Schüler, D.	92nd Annual Meeting of the Deutsche Mineralogische Gesellschaft		Jena. Germany	2014	
30	Biomineralization by magnetotactic bacteria and the biomimetic synthesis of magnetic nanoparticles.	Tompa, É., Nyirő-Kósa, I., Pósfai, M., Tóth, B., Vonderviszt, F.	4th Meeting on Magnetotactic Bacteria		Rio de Janeiro, Brazil	2014	

31	Biomineralization by magnetotactic bacteria and the biomimetic synthesis of magnetic nanoparticles.	Kovács, A. Uebe, R., Schüler, D., Lefèvre, C.T., Bazylinski, D.A., Frankel, R.B., Tompa, É., Pósfai, M., Dunin-Borkowski, R.E.	4th Meeting on Magnetotactic Bacteria		Rio de Janeiro, Brazil	2014	
32	Magnetic minerals in bacteria and the biomimetic synthesis of magnetic nanoparticles	Pósfai, M	5th International Students Geological Conference		Budapest	2014	
33	Bio-assisted synthesis of magnetic filaments	Tompa, É., Tóth, B., Vonderviszt, F., Nyirő-Kósa, I., Pósfai M.	4th Central European Mineralogical Conference		Skalsky Dvur, Czech Republic,	2014	
34	Synthesis of magnetic nanotubes using modified flagellar filaments	Tompa É., Tóth B., Vonderviszt F., Nyirő-Kósa I., Pósfai M.	Technical Chemistry Meeting		Veszprém, Hungary	2014	
35	Synthesis of nanocrystalline magnetite using magnetotactic bacteria and bio-nanotechnological approaches	Tompa É., Pósfai M.	9th Winter School in Mineral Sciences		Tihany, Hungary	2014	
36	Biomineralization and biomimetic synthesis of magnetite nanoparticles	Tompa, É., Nyirő-Kósa, I., Uebe, R., Schüler, D., Pósfai, M.	Goldschmidt Conference		Florence, Italy	2013	

37	Biominerals at the nanoscale: transmission electron microscopy methods for studying the special properties of biominerals	Pósfai, M., Kasama, T., Dunin-Borkowski, R.E.	Union school on "Electron Microscopy and Nanoscale Phenomena in Minerals"		Granada, Spain	2013	
38	Structural studies of planar defects and surface layers in magnetite/maghemite magnetic nanocrystals	Nyirő-Kósa I., Rečnik, A., Dódony, I., Pósfai, M.	Annual Meeting of the Hungarian Microscopy Society		Siófok, Hungary	2013	
39	Structures and transformations of synthetic and bacterial iron monosulfides. Microscopy and Microanalysis	Pósfai, M., Csákberényi-Malasics, D., Kovács Kis, V., Rodriguez-Blanco, J.D., Benning, L.G., Rečnik, A.	Microscopy and Microanalysis		Phoenix, USA	2012	
40	Biological control over size, shape, aggregation and magnetic properties in magnetite nanoparticles	D. Schüler	Symposium "Generation of Inorganic Functional Materials-Implementation of Biomineralization Principles"		Ringberg Castle, Germany	2012	

2.11. Contribution-Benefit-Matrix

Exploitable Result 1

Partner names as numbered in the DoW	Partner MPG	Partner UP	Partner UL	Partner NANOPET	Partner LMU	Partner LBIO	Partner ETH
Partner MPG							
Partner UP							
Partner UL							
Partner NANOPET							
Partner LMU					X		
Partner LBIO							
Partner ETH							

Exploitable Result 2

Partner names as numbered in the DoW	Partner MPG	Partner UP	Partner UL	Partner NANOPET	Partner LMU	Partner LBIO	Partner ETH
Partner MPG	X						
Partner UP							
Partner UL							
Partner NANOPET							
Partner LMU							
Partner LBIO							
Partner ETH							

Exploitable Result 3

Partner names as numbered in the DoW	Partner MPG	Partner UP	Partner UL	Partner NANOPET	Partner LMU	Partner LBIO	Partner ETH
Partner MPG	X			X			
Partner UP							
Partner UL							
Partner NANOPET	X			X	X		
Partner LMU				X	X		
Partner LBIO							
Partner ETH							

Exploitable Result 4

Partner names as numbered in the DoW	Partner MPG	Partner UP	Partner UL	Partner NANOPET	Partner LMU	Partner LBIO	Partner ETH
Partner MPG							
Partner UP							
Partner UL			X				
Partner NANOPET							
Partner LMU							
Partner LBIO							
Partner ETH							

Exploitable Result 5

Partner names as numbered in the DoW	Partner MPG	Partner UP	Partner UL	Partner NANOPET	Partner LMU	Partner LBIO	Partner ETH
Partner MPG	X	X	X	X	X	X	X
Partner UP	X	X	X	X	X	X	X
Partner UL	X	X	X	X	X	X	X
Partner NANOPET	X	X	X	X	X	X	X
Partner LMU	X	X	X	X	X	X	X
Partner LBIO	X	X	X	X	X	X	X
Partner ETH	X	X	X	X	X	X	X

Exploitable Result 6

Partner names as numbered in the DoW	Partner MPG	Partner UP	Partner UL	Partner NANOPET	Partner LMU	Partner LBIO	Partner ETH
Partner MPG	X	X	X	X	X	X	X
Partner UP	X	X	X	X	X	X	X
Partner UL	X	X	X	X	X	X	X
Partner NANOPET	X	X	X	X	X	X	X
Partner LMU	X	X	X	X	X	X	X
Partner LBIO	X	X	X	X	X	X	X
Partner ETH	X	X	X	X	X	X	X

3. Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	NMP4-SL-2011-245542
Title of Project:	Biomimetic and Biomineralized Magnetic Nanoparticles for MagnetiResonance Imaging
Name and Title of Coordinator:	Dr. Damien Faivre

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***0Yes XNo***
2. Please indicate whether your project involved any of the following issues (tick box) :	***NO***
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human genetic material?	
• Did the project involve Human biological samples?	
• Did the project involve Human data collection?	
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	

• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	
• Were those animals transgenic small laboratory animals?	
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	0 Yes X No
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	2	1
Work package leaders	2 (initially NanoPET)	4 (final NanoPET)
Experienced researchers (i.e. PhD holders)	3	6
PhD Students	4	3
Other	2	1

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**7**
Of which, indicate the number of men:	3

D	Gender Aspects		
5.	**Did you carry out specific Gender Equality Actions under the project**?	X	Yes No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	○
❑	Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
X	Other:	Girls day				

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X No

E	Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

X Yes- please specify: Open day

○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

X Yes- please specify: Lecture associated materials

○ No

F	Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

X Main discipline[3]:1.2

X Associated discipline[3]:1.3

X Associated discipline[3]:1.4

G	Engaging with Civil society and policy makers		
11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	X ○	Yes No

[3] Insert number from list below (Frascati Manual).

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

- ○ No
- ○ Yes- in determining what research should be performed
- ○ Yes - in implementing the research
- X Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ Yes X No

12. Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- X Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- X Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	X
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
Education, Training, Youth		Foreign and Security Policy		Space	
Employment and Social Affairs		Fraud		Taxation	
		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- X International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**25**
To how many of these is open access[4] provided?	**19**
How many of these are published in open access journals?	
How many of these are published in open repositories?	
To how many of these is open access not provided?	
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available X no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[5]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**0**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑ Increase in employment, or	❑ In small & medium-sized enterprises
❑ Safeguard employment, or	❑ In large companies
❑ Decrease in employment,	❑ None of the above / not relevant to the project

[4] Open Access is defined as free of charge access for anyone via Internet.
[5] For instance: classification for security project.

X	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*
Difficult to estimate / not possible to quantify	*X*

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

X Yes ○ No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes X No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

❑	Press Release	❑	Coverage in specialist press
❑	Media briefing	X	Coverage in general (non-specialist) press
❑	TV coverage / report	X	Coverage in national press
X	Radio coverage / report	❑	Coverage in international press
X	Brochures /posters / flyers	X	Website for the general public / internet
❑	DVD /Film /Multimedia	X	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

X	Language of the coordinator	X	English
X	Other language(s)		