Reviewers' Comments:

Reviewer #1 (Remarks to the Author)

In the article air-stable superparamagnetic metals entrapped in a graphene oxide matrix - ultrasmall nanomagnets with peculiar morphologies and
potential applications in MRI the authors describe the synthesis of air-stable superparamagnetic nanometals (Fe, Co, Ni) trapped between thermally reduced graphene oxide (TRGO) nanosheets and suggest a potential application of these structures in MRI . Overall the article is well written and the methodology is sound but the idea by itself is not novel. Similar studies with Mn intercalated graphene towards use as MRI contrast agent have been published before and the only novelty in this study is the method of synthesis(Physicochemical Characterization, and Relaxometry Studies of Micro-Graphite Oxide, Graphene Nanoplatelets, and Nanoribbons. Paratala BS, Jacobson BD, Kanakia S, Francis LD, Sitharaman B (2012) . PLoS ONE 7(6): e38185. doi: 10.1371/journal.pone.0038185). I have the following comments for the authors to consider
1. The novelty of this study does not come out by reading the manuscript. I suggest a detailed comparison of previously published similar studies to emphasize how this study is different and what novelty it brings to the table in terms of applications.
2. Several studies have shown that MTT assay is not a good way to evaluate cytotoxicity when working with carbon based structures and can produce false positive results. I would suggest using a different assay.
3. The cytotoxicity assay was done only till 250 microg/ml of the nanocomposites but in actual animal testing 10 mg/kg (of Fe) was used. It is very difficult to relate the two studies in terms of doses tested and the rationale. What is also important to consider here is that animals get exposed to several fold higher concentrations of nanoparticles at the site of injection compared to the steady state concentration in blood. So if 250 microg/ml is the maximum dose that the authors want to use they should test atleast 5-10 fold higher doses for toxicity.
4.It is unclear why the authors choose to use 9.4T and 4.7T MRI and not more clinically used magnetic fields.
5. Comparison with more clinically used MRI contrast agents will be more helpful for assessment of efficacy in this case. From what i understand Feraspin is more tailored towards preclinical imaging.

Reviewer #2 (Remarks to the Author)

This manuscript describes the synthesis and characterization of superparamagnetic nanoparticles consisting of Fe, Co, and Ni coated in thermally reduced graphene oxide (TRGO). The manuscript is a little sloppy, and would definitely benefit from another round of polishing. The method described produces very interesting nanostructures with unusual magnetic properties. Using TRGO to protect zero valent metal nanoparticles is a really good idea, and the authors show that it works well.

The methods described by the authors are of interest to both researchers in magnetic nanostructures as well as to the wider nanoparticle community. For the most part, the particles are well characterized, and the conclusions are supported by the data. The authors completely ignore the impact of the unusual nanoring structures their particles inhabit, and this is a potentially important issue that should be addressed.

There are a number of open issues that should be addressed prior to publication.

Questions to the authors:
1. What is the impact of the ring structures on magnetic properties? The particles are close enough that they should interact magnetically with each other. Some of the unusual properties may in fact

be due to the ring structures rather than to the makeup of the particles alone. The authors should comment on this (and at least attempt to discuss the possible effects-modeling would be useful here, since the particles are unstable out of the matrix). There is a bunch of stuff out on flux closure states in magnetic rings that could be relevant.

2. What is the material inside the rings? The structures look more like disks with rims rather than rings in the case of Fe(10), since there is a lot of higher contrast material in the center. Is it iron oxide?

3. Why do the ZFC curves for the Fe particles look so odd? Peak is very small for all samples shown.

4. Have you tried another iron salt precursor? What happens to the nitrate?

Minor issues:
1. In the abstract, the authors say the provide a method for Fe, Co, and Ni, and then go on and talk solely about iron oxide hybrids without indicating a change in subject. This is confusing to the reader. In fact, I would put the universality at the end of the abstract, since the Ni and Co particles are never really discussed other than to say it is possible.

2. The statement that iron oxide nanoparticles have revolutionized biomedical diagnosis and therapy is completely over the top and factually incorrect. Most approved iron oxide contrast agents have been withdrawn from the market (the exception is used for gastrointestinal imaging only). All other uses for iron oxide nanoparticles are research only or in trial.

3. I would drop catalysis as a possible application (there already are plenty), given that the primary benefit of the TRGO coating is that it protects the nanoparticles from chemical modification! Without reactant access to the nanoparticle surface, why would these make good catalysts?

4. On page 12, the authors state that the hybrid material reaches magnetic saturation at 1 T, and states that this is a much weaker field than for iron oxide nanoparticles. This is very odd, since there are many reports of superparamagnetic iron oxide nanoparticles that are saturated at 1 T. See K. Korpany at al Mat Chem Phys, 138, 29 (2013) for an example, but there are many others. Magnetic saturation for 5 nm superparamagnetic iron oxide nanoparticles at 1 T is not that remarkable, and does not support the conclusions drawn from this observation.

5. Why show gold in the SI and not Co? Co magnetic properties should be shown if the authors want to claim that have superparamagnetic Co particles.

Reviewer #3 (Remarks to the Author)

This manuscript reports superparamagnetic metals which are trapped by a graphene oxide matrix (Fe/TRGO). In this report, the physicochemical properties of Fe/TRGO, particularly about magnetic properties are well explained by using detail characterization methods. Overall, the manuscript is also well organized. However, as considering originality and quality of data, unfortunately this reviewer thinks that this paper is not sufficient to be accepted. This reviewer rejects this paper. There are some reasons below about this decision.

This reviewer finds some similar concepts or composites using both iron oxide and graphene from other journals. For example, 'Nano Research, Vol. 5, Issue 3, pp 199-212, 2012', 'Advanced Materials, Vol. 23, Issue 46, pp 5574-5580, 2011', 'Scientific Reports, Vol. 5, pp 9298, 2015', and so on.

Besides, these authors insist on a higher r2 relaxivity than commercial one, but recent several papers about MR T2 contrast agents suggest much higher r2 values. So this reviewer thinks that the value reported in this manuscript is not really surprising.

In order to increase the stability in the body, they suggest a PEGylation method. However, in the extended data Fig 7, even with PEGylation, the colloidal condition does not look very good. So for a longer time, they might check the stability of Fe/TRGO. This reviewer suggests the measurement of hydrodynamic sizes about Fe/TRGO over time using DLS. This reviewer also thinks that this composite cannot be tested for in vivo experiments without PEGylation. As we can see large particles by eyes in the extended data Fig 7, Fe/TRGOs can be several micrometers in diameter.

In Figure 1, there are some iron oxide nanoparticles which are black in the figure, but each size is different. That means their properties will not be uniform in MRI. Many recent papers about iron oxide NPs demonstrate uniform diameters because the size distribution is critical in the MR contrast effects as well as bio-distribution.

In Figure 4, this Fe/TRGOs are accumulated in spleen, liver, kidney, and so on. There are no detail data about bio-distribution. If they inject pure iron oxide NPs, they will get similar results. So this reviewer suggests that they need to measure the distribution of Fe/TRGOs using ICP-MS. If they can compare the distribution with commercial ones, it will be better. The circulation half-life of Fe/TRGOs in the blood will be also a critical factor as a potential MR contrast agent.

Authors' replies to the Reviewer's remarks and questions

Reviewer #1

Reviewer's Comment: In the article air-stable superparamagnetic metals entrapped in a graphene oxide matrix - ultrasmall nanomagnets with peculiar morphologies and potential applications in MRI the authors describe the synthesis of air-stable superparamagnetic nanometals (Fe, Co, Ni) trapped between thermally reduced graphene oxide (TRGO) nanosheets and suggest a potential application of these structures in MRI. **Overall, the article is well written and the methodology is sound but the idea by itself is not novel.** Similar studies with Mn intercalated graphene towards use as MRI contrast agent have been published before and the only novelty in this study is the method of synthesis (Physicochemical Characterization, and Relaxometry Studies of Micro-Graphite Oxide, Graphene Nanoplatelets, and Nanoribbons. Paratala BS, Jacobson BD, Kanakia S, Francis LD, Sitharaman B (2012). PLoS ONE 7(6): e38185. doi: 10.1371/journal.pone.0038185). I have the following comments for the authors to consider.

Reply to the Reviewer's Comment: Firstly, we would like to thank the Reviewer for thorough assessment of the manuscript. However, we convincingly feel that our work brings novelty in terms of a completely new approach toward chemical application of one of the currently most studied materials – graphene oxide – and, at the same time, on the first experimental evidence of air-stable superparamagnetic metals with a potential exploitation in a broad portfolio of applications, not only in MRI. As an example demonstrating the lack of the novelty of our work, the Reviewer mentioned the paper by B. S. Paratala et al. (PLoS ONE 7, e38185 (2012). However, this paper reports on intercalation of manganese ions (Mn^{2+}) between the graphene sheets and there is no correlation with zero-valent metal entrapment. Moreover, Mn^{2+} ions do not show superparamagnetic behavior (and, hence, much weaker magnetic response compared to our hybrid system) and the r_2/r_1 relaxivity ratio (~10) is much lower than that observed for our nanocomposite system (728). As highlighted in the Introduction of our study, there was only one previously reported preparation of Co/CoO core-shell systems that exhibit superpramagnetic behavior at elevated temperatures (Skumryev et al., Nature 423, 850-853 (2003); however, to the best of our knowledge, there is not experimental work describing the universal approach towards air-stable ultrasmall zero-valent metal nanoparticles (Co, Fe, Ni) keeping their superparamagnetic properties at room temperature.

Reviewer's Point #1: The novelty of this study does not come out by reading the manuscript. I suggest a detailed comparison of previously published similar studies to emphasize how this study is different and what novelty it brings to the table in terms of applications.

Reply to the Reviewer's Point #1: As stated above, the key novelties of the work involve (i) the use of graphene oxide as a universal chemical trap enabling zero-valent metal nanoparticle stabilization (by interactions with oxygen-containing functional groups in the thermally reduced graphene oxide structure), (ii) the first synthesis of an air- and solution-stable superparamagnetic α-Fe particles with sizes below 5 nm, (iii) the possibility to control the morphology/assembly of entrapped metal nanoparticles depending on the concentrations used and (iv) the experimental demonstration that the Fe/TRGO system has an interesting potential in MRI applications. In the revised manuscript, this unambiguous novelty of the work is newly stressed in the partially rewritten Introduction and Conclusionsections. As far as comparison with similar studies is concerned, there are three relevant works to mention, i.e., the work by Skumryev et al. (Skumryev et al., Nature 423, 850-853 (2003)), the work by Bodker et al. (Phys. Rev. Lett. 72, 282 (1994)), and the work by Margeat et al. (Beilstein J. Nanotechnol. 1, 108 (2010)). In the study by Skumryev et al., the Co/CoO core/shell system was synthesized where CoO is used as a stabilizing agent; this core/shell system is superparamagnetic at room temperature, however, the presence of CoO degrades the magnetic features of the Co nanoparticles due to the exchange bias phenomenon.

The study by Bodker et al. describes a system and synthetic approach that is similar to ours in that it uses the same iron precursor (iron nitrate) and is based on impregnating the iron salt into carbon black and then subjecting the resulting system to thermal treatment under a reducing atmosphere. Importantly, the iron nanoparticles are prepared *in situ* in Mössbauer cells, enabling both sample preparation at high temperatures and sample analysis at low temperatures without any need to handle the sample in the air. Regrettably, there is no discussion regarding the stability of such nanoparticles in the air and it is very unlikely that such nanoparticles will be air-stable. Moreover, the particles' sizes and morphological features (which would typically be determined by microscopy) cannot be investigated due to their *in situ* preparation. Finally, while the synthetic approach is very interesting, it has some unaddressed limitations relating to the synthesis of larger amounts of air-stable material for practical applications. Moreover, there is no relation to the entrapment role of carbon black matrix, which is the key novelty of the manuscript exploiting the functional groups of graphene oxide for covalent stabilization of superparamgnetic metals.
The study by Margeat et al. uses a completely different but very elegant organometallic method to prepare stabilized iron nanoparticles that are embedded in a polymer matrix. The particles' size is deduced from magnetization measurements. Importantly, low temperature Mössbauer data (5 K) reveal that the resulting system is magnetically disordered with a broad distribution of magnetic hyperfine fields and two chemical fractions. The dominant fraction, which has an isomer shift of 0.35 mm/s, probably corresponds to iron(III) oxide because this isomer shift is far from that expected for single-phase α-Fe (0.10 mm/s at 5 K, see the data in our work). Moreover, the authors admit the probable dominant formation of iron(III) oxide phase in their earlier work (Lacroix et al., *J. Appl. Phys.* 103, 07D521 (2008)).
All these three papers are mentioned in the manuscript and discussed with respect to the results of our work (see Pages 11-12). Thus, we still believe that our work has unambiguous conceptual novelty in the development of the universal approach for stabilization of ultrasmall (otherwise highly reactive) zero-valent metals (Fe, Co, Ni) keeping their air-stability and superparamagnetic behavior. Importantly, the work opens the doors for a new chemical role of one of the most student current materials, graphene oxide, providing the biocompatible matrix for the covalent entrapment of nanometals.

Reviewer's Point #2: Several studies have shown that MTT assay is not a good way to evaluate cytotoxicity when working with carbon based structures and can produce false positive results. I would suggest using a different assay.

Reply to the Reviewer's Point #2: We agree with the Reviewer that there exists a variety of cytotoxicity tests; nevertheless, the MTT is still commonly used even for carbon based nanomaterials also in the recently published papers (see, e.g., R. Surudzic et al., J. Indust. Eng. Chem. 34, 250-257 (2016); J. Tian, J. et al., Biosens. Bioelectron. 80, 519-524 (2016); H. Li et al., Microchim. Acta 183, 821-826 (2016); M. Fan et al., Green Chem. 18, 1731-1737 (2016); W. B. Hu et al., ACS Nano 5, 3693-3700 (2011); Y. B. Zhang et al., ACS Nano 4, 3181–3186 (2010); A. R. K. Sasikala et al., Sci. Rep. 6, 20543 (2016)). Moreover, we performed in parallel microscopic analysis of cells labeled with the same concentrations of Fe(10)/TRGO/PEG system as used in the MTT assay to control the MTT results. We did not observe any significant changes in the cell morphology, proliferation, or deadheration of cells during the 24 hours compared to control cells (see newly added Extended Data Fig. 8).

Reviewer's Point #3: The cytotoxicity assay was done only till 250 microg/ml of the nanocomposites but in actual animal testing 10 mg/kg (of Fe) was used. It is very difficult to relate the two studies in terms of doses tested and the rationale. What is also important to consider here is that animals get exposed to several fold higher concentrations of nanoparticles at the site of injection compared to the steady state concentration in blood. So if 250 microg/ml is the maximum dose that the authors want to use they should test at least 5-10 fold higher doses for toxicity.

Reply to the Reviewer's Point #3: The Reviewer is correct that it is essential to determine the effective dose of GO-based magnetic nanocomposites to meet MRI contrast demand in vivo. In order to relate these two exposures in vivo and in vitro, the dosages of FeTRGO/PEG were converted to iron concentration according to the iron loading efficiencies which was 10 wt.%. The mouse was treated with the dosage magnitude of 10 mg Fe per kg of body weight (BW) by intravenous injection based on the references for similar works for MRI contrast purpose and our practical exploration (A. J. Cole et al., Biomaterials 32, 2183-2193 (2011)). Following our test, the whole blood of ICR mouse (BW of 35-38 g) has about 3 milliliters. From this calculation, the Fe concentration in blood is about 120 ug Fe per mL at that moment of injecting. The dosage range of 5 – 250 ug of FeTRGO per mL used for cytotoxicity assay is thus lower (4.5 times) compared to in vivo test. Nevertheless, the course (trend) of viability remains the same as control cells even at 250 ug/ml. We would like to mention here that the toxicity of graphene and graphene oxide depends, besides the particle size, particulate state, and oxygen content/surface charge, mainly on the exposure environment (i.e., whether or not aggregation occurs) and mode of interaction with cells (i.e., suspension versus adherent cell types) and not so much on their concentration. Still, we would like to stress that the key message and novelty of the manuscript lies in the covalent entrapment of SP nanometals with GO matrix. The MRI study is presented just as introductory example that such a methodology would be exoplited for more detailed MRI studies of entrapped nanometals opening the space for further optimization.

Reviewer's Point #4: It is unclear why the authors choose to use 9.4 T and 4.7 T MRI and not more clinically used magnetic fields.

Reply to the Reviewer's Point #4: Within this work, we used a very new pre-clinical 9.4 T MR scanner equipped for mouse/rat imaging; the samples were tested in terms of relaxivity before in vivo MRI. In order to compare relaxation values with commercial SPIO contrast agents and with other new contrast agents, we used the 4.7 T MRI scanner for which the relaxivity values are known in literature.

Reviewer's Point #5: Comparison with more clinically used MRI contrast agents will be more helpful for assessment of efficacy in this case. From what I understand Feraspin is more tailored towards preclinical imaging.

Reply to the Reviewer's Point #5: Nowadays, there is a problem to order and buy superparamagnetic iron oxide based commercial contrast agents. Most of them have been taken off the market. FeraSpin XS, S, M, L, XL, and XXL are agents of high relaxivity indicated for use in MRI of small animals, for example, mice. We chose the Feraspin XXL since it features iron oxide nanoparticles with the largest size (60-70 nm) and the largest r_2/r_1 value in the family of FeraSpin-type contrast agents. Thus, we could compare the relaxivity values with our system most appropriately.

Reviewer #2

Reviewer's Comment: This manuscript describes the synthesis and characterization of superparamagnetic nanoparticles consisting of Fe, Co, and Ni coated in thermally reduced graphene oxide (TRGO). The manuscript is a little sloppy, and would definitely benefit from another round of polishing. **The method described produces very interesting nanostructures with unusual magnetic properties. Using TRGO to protect zero valent metal nanoparticles is a really good idea, and the authors show that it works well.**
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There are a number of open issues that should be addressed prior to publication.

Reviewer's Point #1: What is the impact of the ring structures on magnetic properties? The particles are close enough that they should interact magnetically with each other. Some of the unusual properties may in fact be due to the ring structures rather than to the makeup of the particles alone. The authors should comment on this (and at least attempt to discuss the possible effects-modeling would be useful here, since the particles are unstable out of the matrix). There is a bunch of stuff out on flux closure states in magnetic rings that could be relevant.

Reply to the Reviewer's Point #1: The analysis of the magnetization data suggests that the ring structure does not play any crucial role on the magnetic properties of Fe/TRGO nanocomposite. The magnetic features are solely governed by the nanosized character of zero-valent iron particles encouraging evolution of superparamagnetic behavior in a broad temperature interval down to 26 K (in the case of Fe(10)/TRGO sample). Despite the fact that the nanoparticles are very close to each other, the interactions between them seems to be not so pronounced as documented by the symmetric character of the hysteresis loop at 5 K. In other words, magnetic interparticle interactions of both dipole-dipole and exchange type are negligible in the system as no exchange bias is observed. In this respect, the flux closure states are not supposed to evolve in our case.

Reviewer's Point #2: What is the material inside the rings? The structures look more like disks with rims rather than rings in the case of Fe(10), since there is a lot of higher contrast material in the center. Is it iron oxide?

Reply to the Reviewer's Point #2: We hypothesize that the rings are hollow inside. This is well documented by chemical mapping using HRTEM (see Figure 1c), when no iron or oxygen is detected inside the rings. The higher contrast in the center of the rings originates from TRGO sheets that overall in a high number.

Reviewer's Point #3: Why do the ZFC curves for the Fe particles look so odd? Peak is very small for all samples shown.

Reply to the Reviewer's Point #3: The ZFC/FC magnetization data show the contributions from both the superparamagnetic iron nanoparticles and the larger iron nanoparticles with ferromagnetic response that are, despite the washing process, present on the surface of the TRGO network (see Extended Data Fig. 1). As these iron nanoparticles show very strong ferromagnetic behavior in the whole temperature range, even their tiny amount in the sample may drown out the magnetic signal originating from superparamagnetic iron nanoparticles. Their contribution may be even stronger below the blocking temperature of the superparamagnetic iron nanoparticles when a significant drop in the magnetization of superparamagnetic iron nanoparticles is expected.

Reviewer's Point #4: Have you tried another iron salt precursor? What happens to the nitrate?

Reply to the Reviewer's Point #4: No other iron salt precursor was tried for preparation of zero-valent iron nanoparticles entrapped inside the thermally reduced graphene oxide (TRGO) matrix. Nitrates of Fe, Co, and Ni were chosen due to nitrogen atmosphere used during the synthesis of the nanocomposites. It is speculated that the nitrate then leaves the sample in gaseous forms; a hydrogen flow of 1000 mL min^{-1} was used to remove the gaseous by-products of the reaction process (see details in the Experimental section of the Supplementary Online Material file).

Reviewer's Point #5: In the abstract, the authors say they provide a method for Fe, Co, and Ni, and then go on and talk solely about iron oxide hybrids without indicating a change in subject. This is confusing to the reader. In fact, I would put the universality at the end of the abstract, since the Ni and Co particles are never really discussed other than to say it is possible.

Reply to the Reviewer's Point #5: We agree with the Reviewer. Following the suggestion of the Reviewer, the universality of the presented method for preparation of nanometals is now highlighted at the end of the abstract in order not to confuse the reader by changing the subject. The abstract now reads as:

"Superparamagnetism is a remarkable nano-scale phenomenon caused by quantum effects in magnetic nanomaterials. Computational studies have suggested that zero-valent nanometals with diameters below 5 nm may be superior alternatives to superparamagnetic metal oxides, having greater superspin magnitudes and lower levels of magnetic disorder. However, their synthesis has been hindered by their chemical instability. Here we present a method for preparing air-stable superparamagnetic iron nanoparticles trapped between thermally reduced graphene oxide (TRGO) nanosheets and exhibiting ring-like or core-shell morphologies controllable by concentration of iron precursor. These hybrids showed superparamagnetism at room temperature. They retained partial superparamagnetism even at 5 K, which has never previously been observed, and their corrected saturation magnetization of 185 Am^2/kg is among the highest values reported for iron-based superparamagnetic systems. A polymer-modified Fe/TRGO hybrid exhibited excellent chemical and colloidal stability with no in vitro toxicity at concentrations up to 250 μg/mL. Moreover, magnetic resonance imaging studies showed that the Fe/TRGO hybrid possessed very high r_2 relaxivity and experiments on a healthy mouse demonstrated its pertinence in in vivo applications. The synthetic concept was generalized by preparation of Co, Ni, and Au nanoparticles stabilized in the TRGO matrix, verifying the universality of the developed procedure."

Reviewer's Point #6: The statement that iron oxide nanoparticles have revolutionized biomedical diagnosis and therapy is completely over the top and factually incorrect. Most approved iron oxide contrast agents have been withdrawn from the market (the exception is used for gastrointestinal imaging only). All other uses for iron oxide nanoparticles are research only or in trial.

Reply to the Reviewer's Point #6: We agree with the Reviewer. In the original version of the manuscript, we meant to stress that magnetic nanoparticles, entering into the various fields of biomedicine, encouraged improvement of existing diagnostic and therapeutic methods and promoted development of novel diagnostic/therapeutic approaches with a high potentiality to find applications in future. In many studies and review papers (as already cited in the manuscript, see references Nos. 12-17), iron oxide nanoparticles are viewed as the most promising candidates for diagnostic/therapeutic purposes due to not only suitable magnetic properties but also biochemical features such as low level of toxicity (e.g., much lower compared to that of gadolinium-based compounds used in MRI), biocompatibility (Fe-metabolism in humans), and biodegradability. We agree that currently, iron-oxide-based nanoparticles are heavily tested for various biomedical

applications in studies of purely research character; however, as discussed in details in our recent review paper published in Chemical Reviews (for more details, see K. Ulbrich et al., Chem. Rev. 2016, in print, doi: 10.1021/acs.chemrev.5b00589), some iron oxide nanosystems have been successfully approved for clinical use and are routinely used in the clinics (see Figure R1 below). This implies that iron oxide nanoparticles still play a significant role in biomedicine and in future, they may effectively substitute other materials/compounds with lower performance and side effects of unpleasant nature. In order to satisfy the comment of the Reviewer, we rephrase the problematic sentence which now reads as

"Most importantly, the introduction of SP nanoparticles (especially those based on iron oxides) in various biomedical fields has been proposed to improve efficiency of diagnostic and therapeutic methods minimizing the side effects due to controlled/well-conditioned presence in a living organism and targeted treatment. They have been found effective as contrast agents in magnetic resonance imaging, magnetic carriers for targeted drug delivery, and heating elements for the induction of magnetically-assisted hyperthermia[12–17]; some of iron-oxide-based nanosystems have been already clinically approved and are used in clinics in MRI and treatment (e.g., Lumirem®, GastromMARK®, Sienna+®, Feraheme®)[18]."

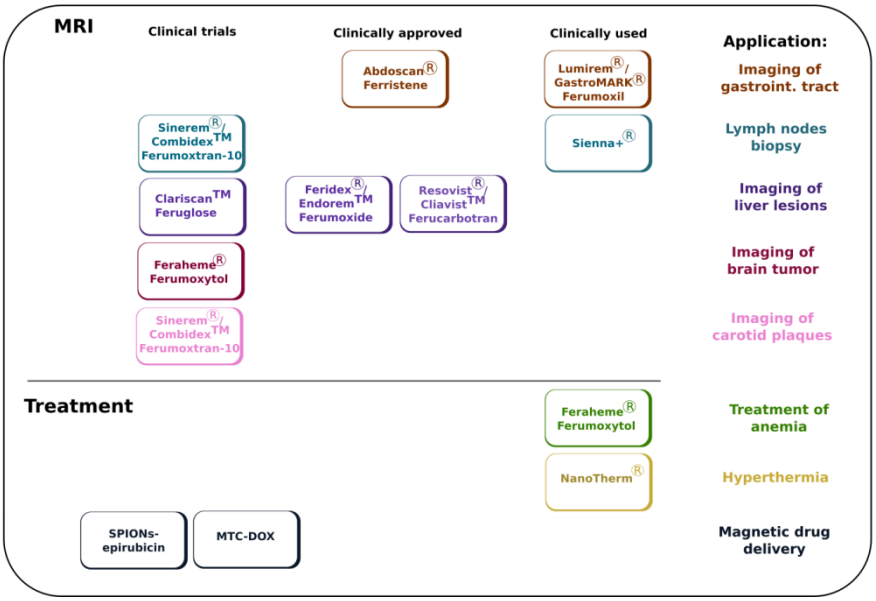

Figure R1. Overview of SPIONs that are currently undergoing clinical trials, have been approved for clinical use, and are routinely used in the clinic. Adopted from our recently published work in Chemical Reviews: K. Ulbrich, K. Holá, V. Šubr, A. Bakandritsos, J. Tuček, and R. Zbořil, Targeted Drug Delivery with Polymers and Magnetic Nanoparticles: Covalent and Noncovalent Approaches, Release Control, and Clinical Studies. Chem. Rev. 2016, in print, doi: 10.1021/acs.chemrev.5b00589).

Reviewer's Point #7: I would drop catalysis as a possible application (there already are plenty), given that the primary benefit of the TRGO coating is that it protects the nanoparticles from chemical modification! Without reactant access to the nanoparticle surface, why would these make good catalysts?

Reply to the Reviewer's Point #7: We completely agree with the Reviewer; the potential application of the developed hybrid in catalysis was removed from the revised version of the manuscript.

Reviewer's Point #8: On page 12, the authors state that the hybrid material reaches magnetic saturation at 1 T, and states that this is a much weaker field than for iron oxide nanoparticles. This is very odd, since there are many reports of superparamagnetic iron oxide nanoparticles that are

saturated at 1 T. See K. Korpany at al Mat Chem Phys, 138, 29 (2013) for an example, but there are many others. Magnetic saturation for 5 nm superparamagnetic iron oxide nanoparticles at 1 T is not that remarkable, and does not support the conclusions drawn from this observation.

Reply to the Reviewer's Point #8: It is known that the magnetic properties of nanoparticles are strongly governed by finite-size and surface effects. On lowering the nanoparticle size, these effects become more and more significant; they manifest themselves by evolution of new types of magnetic anisotropies such as local (defect-induced) anisotropy and surface magnetic anisotropy. They contribute to the total magnetic anisotropy of the nanoparticle causing development of extra easy axes of magnetization along which the atomic magnetic moments inside the nanoparticle can preferentially lie. As a result, in the absence of the external magnetic field, the alignment of atomic magnetic moments inside the nanoparticle is not uniform (i.e., the ordering deviates from perfectly ferromagnetic, antiferromagnetic and/or ferrimagnetic arrangement). In other words, inside the nanoparticle, regions with variation in spin directions emerge when atomic magnetic moments located around the defects and/or in the surface layers of the nanoparticle show a strong tendency to align in a non-uniform manner encouraged by the local and/or surface magnetic anisotropy (for details, see excellent reviews by X. Batlle et al., J. Phys. D: Appl. Phys. 35, R15-R42 (2002) and J. L. Dormann et al., Adv. Chem. Phys. 98, 283–494 (1997) as already cited in the manuscript). In order to achieve a perfect alignment of all the atomic magnetic moments inside the nanoparticle, an external magnetic field must overcome the effects of these size-evolved magnetic anisotropies. There are many examples in the literature showing that nanoparticles of oxides of transition metals with sizes of around or less than 5 nm are reluctant to orient to the direction of the external magnetic field showing spin canting phenomenon (see, e.g., E. Tronc et al., J. Magn. Magn. Mater. 221, 63-79 (2003); E. Kachkachi et al., Eur. Phys. J. B 14, 681-689 (2000); S. Brice-Profeta et al., J. Magn. Magn. Mater. 288, 354-365 (2005); H. Yu et al., Nano Lett. 5, 379-382 (2005); K. J. Woo et al., Chem. Mater 16, 2814-2818 (2004)). Thus, the magnetic field much higher than 5 T is needed to establish arrangement of all the atomic magnetic moments inside the nanoparticle into the field direction. Moreover, such ultrafine magnetic nanoparticles of transition metal oxide tend to agglomerate to reduce high surface energy. This results in evolution of interparticle magnetic interactions of both dipole-dipole and exchange type significantly modifying the magnetic anisotropy profile of an individual nanoparticle. This intensifies a magnetic disorder inside the nanoparticle when stronger external magnetic fields are then required to achieve the magnetic saturation of the nanoparticle assembly. In the case of zero-valent iron nanoparticles trapped in the TRGO matrix, the local and/or surface magnetic anisotropies are not so pronounced upon a decrease in size. In order words, for iron nanoparticles with sizes of around 3 nm, the contributions of the local and surface magnetic anisotropies are considered to be small to the overall magnetic anisotropy of the nanoparticle which is dominantly driven by the magnetocrystalline anisotropy term. Thus, in order to make it clearer to the Reviewer and the reader, the problematic sentence was rephrased as follows:

"The saturation tendency of iron nanoparticles under weak applied magnetic fields is surprising in contrast to the behavior of metal oxide (iron oxide) nanoparticles with similar average particle sizes, which generally do not exhibit complete magnetic saturation even under applied fields of up to 5 T (i.e. the applied field is not strong enough to overcome the effects of the iron oxide nanoparticles' local and surface magnetic anisotropy, which imprints specific and more energetically favorable orientations on the magnetic moments of the atoms around the defects and in the surface layers of the nanoparticles)."

Reviewer's Point #9: Why show gold in the SI and not Co? Co magnetic properties should be shown if the authors want to claim that have superparamagnetic Co particles.

Reply to the Reviewer's Point #9: As suggested by the Reviewer, we moved the HRTEM/TEM images of the Au/TRGO nanocomposite to the main manuscript (Fig. 5 in the revised version of the

manuscript). Newly, we added the magnetic properties of the Co/TRGO nanocomposite system to demonstrate its superparamagnetic behavior (with a blocking temperature at 22 K) as analogously observed for the Fe/TRGO and Ni/TRGO hybrids (see newly added Extended Data Fig. 10 of the revised version).

Reviewer #3

Reviewer's Comment: This manuscript reports superparamagnetic metals which are trapped by a graphene oxide matrix (Fe/TRGO). In this report, the physicochemical properties of Fe/TRGO, particularly about magnetic properties are well explained by using detail characterization methods. Overall, the manuscript is also well organized. However, as considering originality and quality of data, unfortunately this reviewer thinks that this paper is not sufficient to be accepted. This reviewer rejects this paper. There are some reasons below about this decision.

Reviewer's Point #1: This reviewer finds some similar concepts or composites using both iron oxide and graphene from other journals. For example, 'Nano Research, Vol. 5, Issue 3, pp 199-212, 2012', 'Advanced Materials, Vol. 23, Issue 46, pp 5574-5580, 2011', 'Scientific Reports, Vol. 5, pp 9298, 2015', and so on.

Reply to the Reviewer's Point #1: At this point, we must disagree with the Reviewer that our work lacks the novelty. The papers highlighted by the Reviewer all report on synthesis, physicochemical characterization, and applications of nanocmposites based on graphene and IRON OXIDES not ZERO-VALENT IRON! Just the stabilization of zero-valent superparamagnetic metals to be air stable and thus applicable in various technologies is viewed as a long-term challenge in the community, while there are hundreds of studies on superparamagnetic metal oxides and their composites out there. Thus, our work clearly justify the novelty requirement, i.e., use of graphene oxide as a universal chemical trap enabling metal nanoparticle stabilization (by interactions with oxygen-containing functional groups in the thermally reduced graphene oxide structure, (ii) the first synthesis of an air- and solution-stable superparamagnetic α-Fe system, (iii) free external surface of TRGO network with large surface area offers a secondary functionalization with biomolecules and/or drugs, and (iv) the experimental demonstration that the Fe/TRGO system has an interesting potential in MRI applications. Please, see also the Reply #1 to the comment of Reviewer #1.

Reviewer's Point #2: Besides, these authors insist on a higher r_2 relaxivity than commercial one, but recent several papers about MR T2 contrast agents suggest much higher r_2 values. So this reviewer thinks that the value reported in this manuscript is not really surprising.

Reply to the Reviewer's Point #2: We agree with the Reviewer that there are several systems developed and described in the literature for which higher r_2 values were reported. Here, we would like to stress that our aim was not really to "compete" with other superparamagnetic agents with higher r_2 values that have been reported in the literature (see, e.g., *Adv. Mater.* **21**, 2133 (2009); *Nat. Medicine* **13**, 95 (2007); *Nat. Mater.* **5**, 971 (2006); these works are already cited in the manuscript). We compared the Fe/TRGO composite's contrast properties to those of commercial agents to illustrate its potential range of applications. The composite actually exhibited superior contrast properties in some respects, notably with respect to the r_2/r_1 ratio. While our system exhibits r_2 values that are superior or comparable to those of commercial agents and some other iron oxide or alloy-based systems that have been reported in the literature (e.g., Jun et al., *J. Am. Chem. Soc.* **127**, 5732 (2005); Jun et al., *Acc. Chem. Res.* **41**, 179 (2008); Xu et al., *Angew. Chem. Int. Ed.* **47**, 173 (2008); Choi et al., *J. Am. Chem. Soc.* **128**, 15982 (2006)), we certainly do not wish to present it as having "record" r_2 values, and this is not a primary goal of the manuscript. Thus, the respective part of the Result and Discussion section was rephrased accordingly.

Reviewer's Point #3: In order to increase the stability in the body, they suggest a PEGylation method. However, in the extended data Fig 7, even with PEGylation, the colloidal condition does not look very good. So for a longer time, they might check the stability of Fe/TRGO. This reviewer suggests the measurement of hydrodynamic sizes about Fe/TRGO over time using DLS. This reviewer also thinks

that this composite cannot be tested for in vivo experiments without PEGylation. As we can see large particles by eyes in the extended data Fig 7, Fe/TRGOs can be several micrometers in diameter.

Reply to the Reviewer's Point #3: We do not think that DLS is an appropriate method for measuring of hydrodynamic sizes in graphene based nanomaterials in general. Since graphene sheets are two dimensional particles they are expected to present two different diffusion coefficients in solution. At the same time, DLS is a technique which produces the diameter of a hypothetical spherical particle which would have the same diffusion coefficient as the actual particles under measurement. For this reason we considered that obtaining a size for through DLS would be quite arbitrary. Thus, mostly HRTEM/TEM and/or AFM measurements are used in literature. Nevertheless, we checked the hydrodynamic size of the sample before in vivo MRI by optical microscopy where it is clearly seen that in the sample after PEGylation, sonication and size fractionation by weak centrifugation, there are particles of sizes of less than 1 μm, stable in PBS, which can be used for safety intravenous applications (according to the veterinary rules, see newly added Extended Data Fig. S10).

Reviewer's Point #4: In Figure 1, there are some iron oxide nanoparticles which are black in the figure, but each size is different. That means their properties will not be uniform in MRI. Many recent papers about iron oxide NPs demonstrate uniform diameters because the size distribution is critical in the MR contrast effects as well as bio-distribution.

Reply to the Reviewer's Point #4: Here, we would like to stress that no iron oxide nanoparticles appear in our system! This is misunderstanding from the side of the Reviewer. All the chemical analyses performed by various techniques employed in your study demonstrate that only zero-valent iron nanoparticles are present in the hybrids; they are stabilized by the oxygen functional groups of the thermally reduced graphene oxide! The size distribution of the zero-valent iron nanoparticles, derived from the statistical analysis of the representative HRTEM/TEM images, is narrow; the sizes of the zero-valent iron nanoparticles varies from 2 to 6 nm. Thus, it is speculated that such small differences in the size will not affect the MR contrast. The biodistribution is governed by the size of the hybrid.

Reviewer's Point #5: In Figure 4, this Fe/TRGOs are accumulated in spleen, liver, kidney, and so on. There are no detail data about bio-distribution. If they inject pure iron oxide NPs, they will get similar results. So this reviewer suggests that they need to measure the distribution of Fe/TRGOs using ICP-MS. If they can compare the distribution with commercial ones, it will be better. The circulation half-life of Fe/TRGOs in the blood will be also a critical factor as a potential MR contrast agent.

Reply to the Reviewer's Point #5: In this study, in vivo MRI was performed only as a pilot experiment to support the in vitro results and to show potential applicability of the newly developed material (hybrid), which has never been tested before in MRI (please see also our reply to Reviewer 1, Point 3). It is desirable to optimize the system for large in vivo study with more mice to evaluate biodistribution, pharmacokinetics and long term toxicity of our system. We did not want to work with living animals at this point of study. Nevertheless, we checked ROIs (region of interest) in macrophage rich organs such as liver, spleen and kidney before and after application of the Fe/TRGO nanocomposite and the signal intensity was significantly lower in that regions as it is demonstrated in Figure 4.
Here, we would like to stress that the key advantage of the Fe/TRGO system over, e.g., GO-iron oxide systems lies in the firm embedding of superparamagnetic iron nanoparticles in a graphene oxide matrix that prevents their release to organism/environment or aggregation. Such release/aggregation cannot easily be prevented when dealing with iron oxide nanoparticles that are non-covalently assembled onto a GO surface (Tucek et al., *ACS Nano* **8**, 7571 (2014)). Moreover, if the nanoFe-based system is used for MR imaging at the same concentration as an iron-oxide-based contrast agent (e.g., in the same GO matrix), the Fe/TRGO system can be expected to yield a much

more pronounced negative contrast due to its much higher saturation magnetization, which means it has a higher r_2 relaxivity.

Reviewer #1 (Remarks to the Author)

The authors have answered most of the questions satisfactorily. However, there are two key justifications that are still flawed
1. Along with the other factors mentioned, the toxicity of graphene and graphene oxide does depend on its concentration as shown in numerous previous studies and it is wrong to say it does not.
2. I still feel the authors the article would significantly gain from a comparison with previously clinically approved contrast agents as comparison with agents meant only for pre clinical imaging lowers the overall impact of the manuscript although it does not hamper the validity of the manuscript.

Reviewer #2 (Remarks to the Author)

The authors have satisfied my concerns with the manuscript.

Reviewer #3 (Remarks to the Author)

First of all, I would like to thank their answer and detail explanation. It was more helpful to understand their work. However, unfortunately, this reviewer still thinks that this manuscript is not enough to be published in Nat Comm.

If they only emphasize the originality in terms of the synthesis method of air-stable superparamagnetic nanometals trapped between TRGO nano sheets, this manuscript will be more appropriate to some journals oriented in pure chemistry.

If they consider their originality with in-vitro and in-vivo applications, clearly, these results are not enough to explain advantages and universality of their nanometals. They need to demonstrate results about in-vitro/vivo stability, in-vitro/vivo toxicity, in-vivo bio-distribution, in-vivo circulation half-life, and so on.

Reviewer #1

Reviewer's Comment: The authors have answered most of the questions satisfactorily. However, there are two key justifications that are still flawed.

Reviewer's Point #1: Along with the other factors mentioned, the toxicity of graphene and graphene oxide does depend on its concentration as shown in numerous previous studies and it is wrong to say it does not.

Reply to the Reviewer's Point #1: We completely agree with the Reviewer. In the revised version of the manuscript, we now stress that among other factors, the toxicity of graphene and graphene oxide depends on the concentration (see Page 6 and Page 15).

Reviewer's Point #2: I still feel the authors the article would significantly gain from a comparison with previously clinically approved contrast agents as comparison with agents meant only for pre clinical imaging lowers the overall impact of the manuscript although it does not hamper the validity of the manuscript.

Reply to the Reviewer's Point #2: The comparison with previously clinically approved contrast agents was given in the previous version of the manuscript in the Supplementary Online Material (see Supplementary Table S3). In the revised version of the manuscript, we newly compare relaxivity values of the developed hybrid with those based on superparamagnetic iron oxide (SPIO) nanoparticles (e.g., Lumirem®, GastromMARK®, Sienna+®, Feraheme®, Resovist®, Feridex®) citing appropriate references (see yellow text in Page 16). It turns out that the relaxivity of the Fe/TRGO hybrid shows a higher value compared to that of SPIO-based contrast agents (in T_2-weighted images), placing the nanocomposite among potential alternatives for MRI T_2 contrast agents. However, we stress that although the relaxivity values are very promising, the MRI results are preliminary and a further detailed study is needed to assess the bio-related features of the hybrid in a more complex way.

Reviewer #2

Reviewer's Comment: The authors have satisfied my concerns with the manuscript.

Reply to the Reviewer's Comment: We thank the Reviewer for his/her appreciation of our work during the review process.

Reviewer #3

Reviewer's Comment: First of all, I would like to thank their answer and detail explanation. It was more helpful to understand their work. However, unfortunately, this reviewer still thinks that this manuscript is not enough to be published in Nat Comm.

Reviewer's Point #1: If they only emphasize the originality in terms of the synthesis method of air-stable superparamagnetic nanometals trapped between TRGO nano sheets, this manuscript will be more appropriate to some journals oriented in pure chemistry.

Reply to the Reviewer's Point #1: We still believe that this work would open the doors for the use of the same approach for the entrapment of other ultrasmall metals with a broad portfolio of applications. Moreover, the manuscript covers the multidisciplinary topic involving

nanotechnologies, materials chemistry, magnetism, solid/state physics and biomedicine. Thus, we are strongly convinced that the manuscript is suitable for a broad readership of the Nature Communications journal.

Reviewer's Point #2: If they consider their originality with in-vitro and in-vivo applications, clearly, these results are not enough to explain advantages and universality of their nanometals. They need to demonstrate results about in-vitro/vivo stability, in-vitro/vivo toxicity, in-vivo bio-distribution, in-vivo circulation half-life, and so on.

Reply to the Reviewer's Point #2: The key message of the manuscript is to announce the experimental approach providing an effective strategy for the preparation of air/stable superparamagnetic metals, which were only predicted by theory before. MRI data/results, as already stated as a Reply to the Editorial Comment, are preliminary and are discussed mainly to demonstrate the superior magnetic properties of superparamagnetic nanometals and their potential applicability. Definitely, we do not aim to compete with well-established contrast agents (typically SPIO-based) studied and developed for tens of years. However, despite this "proof of concept" study, we definitely proved the non-toxicity of the product in in-vitro and in-vivo stages in diagnostically relevant concentrations. We newly state that in order to apply the developed hybrid in the MRI diagnostics, a more detailed work is required to address both advantages and potential drawbacks related with its in vivo applications.