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SUPPLEMENTARY INFORMATION

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On the issue of transparency and reproducibility in nanomedicine

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Supplementary Information
On the issue of transparency and reproducibility in nanomedicine

Bridging the *in vitro* to *in vivo* void with the chick embryo model

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This commentary speaks to the scientific, ethical, and economic responsibility of journals to establish guidelines and practices to accelerate nanosystem translation by standardizing characterization methods to enable establishment of fundamental structure/activity relationships and allow valid comparisons between studies. Here we are largely in agreement with the MIRABEL guidelines but would advocate for broadening the drug definition and characterization to include alternate therapeutic cargos (e.g. plasmid, protein) and require author certification of reproducibility of nanosytem synthesis and stability. For nanosystems intended for future clinical use, we recommend conducting additional studies required for regulatory agency approval. Examples of such studies include nanosystem stability in physiologically relevant media overtime, degradation and clearance in vivo, and toxicity of degradation by-products.

We argue that in vivo stability is paramount to the successful implementation of nanosystems in applications such as drug delivery. Notably in spite of many successful *in vitro* studies, nanoparticle delivery systems have not achieved their anticipated potential in vivo or in the clinic. This deficiency has been documented in a recent meta-analysis of 117 studies of nanoparticle delivery to solid tumors conducted from 2005-2015, where it was concluded that a median of 0.7% of the injected dose reached the tumor and that this value had not improved over the course of ten years[1]. Presumably lack of delivery to the tumor is a consequence of rapid uptake by the Mononuclear Phagocyte System (MPS), non-specific binding and renal clearance, which are all instability mechanisms that to date have been evaluated almost exclusively in costly, time consuming rodent models.

We propose consideration/utilization of the *ex ovo* chick chorioallanotic membrane (CAM) model as a rapid, accessible, and low cost means of evaluating nanoparticle stability and qualifying nanoparticles for in vivo use[2]. The CAM is highly vascularized and mimics the diverging/converging vasculature of the liver, spleen, and lungs, MPS organs that serve as nanoparticle traps. Intravital imaging of fluorescently-labeled nanoparticles injected into the CAM vasculature enables immediate assessment of circulation stability whilst elucidating issues with non-specific binding, aggregation, and uptake by phagocytic cells. In a 2013 study, CAM imaging revealed dramatically different circulation behaviors of colloidally stable cationic particles with identical size, shape, and zeta-potential differing only by distribution/exposure of charge[3]. Whereas these nanoparticle types (patchy charge versus uniform charge) would be deemed identical based on accepted characterization measures (DLS, TEM and zeta-potential) and therefore to have similar behavior in vivo, CAM imaging showed nanoparticles with patchy charge were immediately sequestered by non-

specific binding and white blood cells, whereas uniformly charged nanoparticles remained in circulation. This behavior was verified within a rodent model via SPECT imaging, where uniformly charged particles had a half-life double that of patchy particles[4]. The CAM model can also be applied to nanoparticle-tumor interaction studies[5]. The lack of cell-mediated adaptive immunity permits tumor cell engraftment into the CAM, allowing direct visualization of targeted binding and cargo delivery at the individual cell level[6]. Due to its highly angiogenic nature, the CAM is able to rapidly neo-vascularize tumors or re-anastamose to pre-existing tumor vasculature, thus permitting a real world oncologic assessment of nanoparticles.

While rodent model data will likely always be necessary for new investigational drugs, the CAM model confidently serves as an inexpensive and time efficient intermediary system in which to engineer and qualify nanoparticle systems for subsequent mammalian in vivo use, reducing the number of mammalian animals utilized. We believe the CAM system will help bridge the *in vitro* to *in vivo* void.

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The growing interdisciplinary field of nano(bio)medicine offers new opportunities, strategies and avenues for the discovery of new paradigms for healthcare and for extending conventional therapeutic and diagnostic techniques using novel tools and insights. A significant number of products have already progressed to routine clinical use with a solid pipeline of preclinical and clinical development. The advantages of these developments encompass the full spectrum of healthcare needs, but some notable diseases receive a much larger focus than others do.

As with all scientific disciplines, reporting of experimental procedures and analytical/characterisation, as well as data interpretation is critical to evidence-based dissemination. The rationale for defined standards, practices and protocols within the full spectrum of nanotechnology-related research fields has been debated for many years and several learned societies[1] and international institutions[2] have contributed. Regulatory bodies such as the US Food & Drug Administration (FDA) have issued a number of guidance documents[3] and have stated that they have "…long encountered the combination of promise, risk, and uncertainty that accompanies emerging technologies. Nanotechnology is not unique in this regard.", and that the "…FDA does not categorically judge all products containing nanomaterials or otherwise involving the application of nanotechnology as intrinsically benign or harmful. FDA will regulate nanotechnology products under existing statutory authorities, in accordance with the specific legal standards applicable to each type of product under its jurisdiction." [3] As such, the need for robust assessment is clear, but the case for special attention is not supported.

The reporting of science within the peer-reviewed literature must adhere to the highest possible standards, but the need for sub-field-specific checklists is unclear, as is the need to specifically highlight nano(bio)medicine which itself is a sub-field of pharmaceutics, cell biology and drug delivery. Best practice is already present across these disciplines and new approaches to redefine standards established over decades of high quality research either generate unnecessary duplication or create a parallel framework unfamiliar to experts within each field. Just as FDA sees no need to create nano-specific regulation, the scientific community should be capable of identifying poor science through existing peer-review processes. Nanotechnology has suffered from scientists allowing over-exaggeration and the propagation of unscientific mythology such as nanobots. It is the responsibility of peer-review to ensure that hype does not creep into scientific literature and that evidence-based conclusion is driven by accurate, reproducible and appropriate experimentation. The over-prescription of minimum reporting standards does also pose a considerable risk of stifling innovation. Resources are invariably limited and groundbreaking science grows from establishing early proof-of-concept. Abiding to a rigorous and standardised analytical and characterisation regime may lead to new phenomena being overlooked.

Below are a number of recommendations that are already in alignment with existing best-practice and these should continue to be adhered to within nano(bio)medicine and other fields:

a) utilisation of at least two characterisation techniques. It is widely understood that no single technique can fully characterise a disperse nanoparticle sample and data from two techniques facilitates understanding and interpretation;

b) repetition of characterisation when storing samples for long periods. Nanomaterial characteristics are known to change during storage;

c) inclusion of more than two nanomaterial comparators. Many publications include limited diversity of nanomaterial size, shape, drug loading or surface chemistry/charge, and conclusions about broad nano-specific benefits is questionable from such limited studies;
d) treatment of tissues and cells using standard incubation techniques should adhere to best practise and lack of nanoparticle interactions with plastics used as consumables should be confirmed;
d) capturing observable safety concerns throughout research and development will facilitate identification of nano-specific toxicities. Often, only cytotoxicity studies that are of limited value in assessing human safety are conducted;
e) reduction in animal use for publishing purposes as some ex vivo and in vitro assays may be sufficiently informative. It is widely understood that extrapolation from rodents to humans is highly complex and many studies have no real ambition to translate to human trials.
Nanotechnology and its use in medical interventions offers great potential for future healthcare. The accurate, reproducible, and ethical reporting of fundamental science is critical. The responsibility for credibility of publications lies squarely and correctly with scientists contributing from multiple disciplines, the community in its rigour during peer-review and journal editors in their lack of acceptance of hype and claims that are not evidence-based. Check lists and new principles that attempt to drive appropriate reporting within nano(bio)medicine should not be needed if the discipline-specific best practices are individually deployed by all that contribute to the growth and success of this important field.

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Comment on Faria’s perspective published by Nature Nanotechnology

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Engineering nanomaterials are rapidly developed for drug delivery, diagnostics, cancer therapies, tissue regenerations and so on. The initiative of Minimum Information Reporting in Bio-Nano Experimental Literature (MIRIBEL), proposed by Matthew Faria et al. recently in Nat. Nanotechnol. is timely and important for the nanobiotechnology and nanomedicine community. The use of reporting standards - research guidelines, international standards and checklists is aim to ensure the accuracy, reliability, reproducibility and comparison of experimental data. Actually, in the current

high-quality publications, most information have been provided abundantly. However, the reliability and reproducibility are still highly concerned for thorough safety assessment on nanotechology as well as clinical translation of nanomedicine.

Along with the rapid development and exploration of “nano” filed in decades, people have deeper understanding and provide more comprehensive description of the nano-bio interactions for nanomedicine. Different from conventional small or biological molecules, the examinations of these nano-bio interactions are still extremely challenging. Nanoparticles, possessing instinctive physicochemical properties, may exhibit distinctive biological effects with biomacromolecules, organelles, or even small living organisms. Therefore, the procedures of compound synthesis, sample preparation, and bio-interaction measurement may afford plenty of details and variables, which determining the fate of their therapeutic efficacies. Here the discussion of checklist does provide us a good chance to self-inspection where we are and what we have so far.

However, it must be admitted that universal standardization for nanomaterials is still unrealistic and at the immature stage. Some problems, though quite important, are still a big subject in nanotoxicity field. Strict mandatory requirements may slow down everyone's work efficiency. At the same time, we must also realize that as an interdisciplinary subject, nanomaterials cover a wide range of materials with different material characteristics. Among them, many newly emerging materials are lack of sufficient understanding, also requiring different standardization tools. Therefore, on the basis of the work by Matthew Faria et al., we would like to emphasize the following two points:

(1) It should be important to classify nanomaterials by their intrinsic physicochemical properties, e.g. metallic inorganic nanoparticles, non-metallic inorganic nanoparticles, polymer nanoparticles, peptide nanoparticles, DNA nanoparticles, cell membrane derivatives, etc. Therewith, different kinds of materials are going to have different level of scrutiny accordingly, basing on their specific aspects.

(2) It is also necessary to divide the check list into compulsory reporting summaries and suggested self-checking points according to the maturity of material developments in the field. For those issues could be defined with wide-accepted parameters, we are happy to implement the mandatory checklist. However, for those problems newly emerged or too complex to conclude, we think suggested self-checking points could be more helpful that enforce reliable developments in this field.

Last but not least, we also want to suggest some other important issues may significantly affect the conclusions on nano-bio experiments and should be seriously considered, apart from the proposed standards:

(A) For Material Characterization

1. More accurate composition information of nanoparticles should be provided: (i) For those nanomaterials from commercialized incorporations or donations, their sources should be strictly reported including the company names, catalog numbers, donor information, related literature reports, etc.; (ii) A full scan of all the elements by ICP-MS or XPS is helpful to identify possible impurities of inorganic nanoparticles during their synthesis; (iii) In terms of the critical role in nano-bio effects, surface reactivity, especially for oxidation capability should be considered and added into the standards of material characterization. (iv) Instead of showing only one or two particles EM images, more representative EM images should be provided, together with other techniques to judge the dispersity of nanoparticles in fluids.

2. Particle dissolution in biological contexts is another important characterization criteria for nanomaterials, therefore, we suggest to provide “Methods of processing the samples in nano-bio characterization”: (i) The exposure details of nanomaterials to bio-systems or biological samples,

subjected to certain processing procedures in biological environment, should be necessary to identify with their suspension procedure of nanoparticles in biological media, including sonication information, dispersion sequence, etc.; (ii) When reporting the value of hydrodynamic size and the zeta potential of nanoparticles in the liquid, it is also important to give the concentration of nanoparticles during measurements, which could be critical for those light scattering-based measurements. Reporting this information in the literature will also be helpful for reviewers and readers to judge the reliability of related data and compare the new data with other ones.

(B) For Cell Characterization

3. During cell culture procedure, several issues should be underlined: (i) Serious cell contaminations (e.g. mycoplasma infections) should be avoided and detected, which may be ignored by some chemistry or engineering labs; (ii) For toxicity or viability test in cells, we may recommend to using complementary assays for substance (MTT, CCK-8, MTS, etc) and energy (ATP) metabolism to obtain because some nanoparticles have been shown to interfere in dehydrogenase-based assays.

4. During cell imaging experiments, the following points should be important: (i) It is necessary to clarify whether the cells are live or fixed and how the cells are fixed when preparing cell samples for imaging (fluorescence imaging or TEM imaging); (ii) To differentiate the signals of labeled nanoparticles from the autofluorescence of cells, TEM imaging coupled with EDX detection is a more convincing approach to support the microcopy results.

(C) For Animal Experiments

5. In animal experiments, the total numbers of tested animals as well as the numbers of animals for statistical analysis should be reported.

6. In terms of the future perspective, although we have focused on nano-bio interactions for 15 year, there are substantial debatable results on the safety of some large-scale produced nanoparticles, e.g. SiO2, TiO2, Fe2O3, carbon nanotubes, graphene oxides, etc. It's time to call for an international collaboration of different nano-bio labs to acquire some reproducible conclusions on these materials to facilitate their commercial applications.

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After peer-reviewing multiple manuscripts notably lacking adequate characterization of nanomaterial formulations and missing critical experimental information about cells, animal models and employed laboratory procedures, our general response to the minimum information reporting in bio-nano experimental literature (MIRIBEL) concept is overall supportive. It is indeed an absolutely valuable suggestion to improve the way studies are described in manuscripts, not to the least to enable follow-up investigations and to ensure the reproducibility of reported findings. However, a close look at the MIRIBEL framework raises several concerns pivoting around an evident misalignment between the intent and the expectations. The main intention is to standardize a list of experimental readouts/parameters reported in each study referencing nanomaterials (e.g., size, shape, dimensions, synthesis process, composition, zeta potential, density in cell culture, drug loading and release, targeting, labeling among the few for nanomaterials). The expectation is to advance the field via improved reproducibility and facilitated exchange of key scientific information, thus promoting meta-analysis and in silico models, as well as allowing a more systematic comparison. The major mismatch between the intention and expectation is that the MIRIBEL proposes what to do, but evidently offers little suggestion as to how. Moreover, it implies freedom of variability in ways the studies are conducted, and reagents, models and particles are characterized. It is due to this lack of the standardized "recipe" on how to carry studies and evaluate applicable reagents, particles, and materials, that makes it difficult to expect that the intended outcomes will be confidently achieved.

Investigational New Drug enabling preclinical and clinical studies are generally conducted using a framework of Good Laboratory Practices (GLP). GLP studies are better and frequently much more extensively documented than an average basic research study, owing to rigorous requirements to both the details of these studies and the statutory demands to supporting infrastructure (independent quality control and quality assurance) that are mandatory to follow to control these studies. GLP requirements also include validation of each experimental procedure. Such validation relies on multiple parameters including but not limited to inter- and intra-assay variability, robustness, ruggedness, inter-analyst variability. As such, GLP studies take longer to design, schedule, and complete and they are unavoidably more expensive than their comparable non-GLP counterparts. Following the GLP requirements warrants the results reproducibility, as long as there is no change in the source of reagent or qualification/training of staff conducting such studies. If any change occurs, the GLP requires re-validation or cross-validation. In general, academic labs have neither infrastructure nor budgets to support the GLP studies. Switching academic labs to GLP is an unrealistic project that may lead to decreased operational efficiencies and impede the basic investigators' capability in fulfilling their main mission, which is teaching and training of a young generation of scientists.

To conclude, implementing MIRIBEL may standardize the way the manuscripts are written and the study data are presented (which is an indisputable bonus!), but it will not necessarily improve data reproducibility and other expected benefits of its implementation. Therefore, it appears that improving the approaches used for teaching next generation of scientists, emphasizing the importance of being thorough, including proper control(s) and baseline(s) which may be unique to every single study, and verifying the results by multiple methods whenever practical and whenever such methods are available, is a more reasonable – albeit more demanding and time-consuming – strategy. Much the same way each nanoparticle is unique, it will require its own unique set of well-designed controls and characterization toolkit. The journals would contribute to the improvement in reproducibility and other expected outcomes by training their editors to review each manuscript in

more methodical and coherent way, select proper peer-reviewers and publish only mature studies which were scrupulously designed, conducted, analyzed, and described while including proper controls and experimental power for sound statistical evaluation assuring reproducibility. Journals like Nature often fall in a trap of "scientific sensation" and prefer a study with unusual, provocative, or at time outright speculative results to a thoroughly done study investigating a "mundane" but interesting biological problem. Revising journals' publication policies is, therefore, another way to improve the field. Last but certainly not least, allowing more space for materials and methods would indubitably be helpful. Currently, the authors are frequently forced to shrink the experimental part of their manuscripts due to space limitations.

EUNCL/REFINE and NCI-NCL joint contribution of the Nanomedicine Characterisation Infrastructures to the MIRIBEL dialogue: enabling translational readiness

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The European Nanomedicine Characterisation Infrastructure (EUNCL) and the REFINE consortium are echoing not only the needs for minimum information reporting in bio-nano experimental literature (MIRIBEL), but would like to expand the dialogue to include minimum pre-clinical characterization requirements for translational readiness.
The EUNCL/REFINE joint effort, funded by EC-H2020, is aimed at developing a Regulatory Science Framework for Nanomedicine. Recognising the value of the MIRIBEL reporting suggestions, we emphasize that this list (as captured in Faria et al., Table 1) should be viewed as an overall recommendation rather than an absolute standard, as experience dictates that each particle is unique and may have different testing requirements. EUCNL/REFINE, jointly with the National Cancer Institute's Nanotechnology Characterization Lab (NCI-NCL), are bridging the gap between publication and translation by identifying common pitfalls in nanomedicine development, defining quality attributes for pre-clinical assessment, sharing lessons learned[1-5] and standard operating procedures (available online: http://www.euncl.eu/about-us/assay-cascade/ and https://ncl.cancer.gov/resources/assay-cascade-protocols).

The developmental path to translation can be both expensive and complex [1-5]. It is the view of EUNCL/REFINE and NCI-NCL that the evaluation of nanomedicine formulations should have a series of pass/fail analyses early in the process [6]. These suggestions arose from our combined experiences, which have seen scores of products undergo a full re-think of the synthetic process and/or composition of the product. Built on our lessons learned, aspects such as sterility and endotoxin contamination, physicochemical characterisation, batch to batch variability and stability in clinically relevant biological media[4] are most informative with regards to the overall properties and performance of the product. Furthermore, the adoption of multiparametric investigations of the immuno- and cytotoxicity responses using semi- or fully automated in vitro methodologies or representative 3D models can uncover potential show-stopper toxicities earlier in the process, allowing for refinement of the formulation at an earlier stage[7,8]. Only after these quality attributes have been assessed–using less costly techniques–should the in vivo efficacy and safety commence[9]. As noted with for the MIRIBEL reporting suggestions, these too, should be viewed as a best-practice guide for development and characterization. As with any "rule", there will always be exceptions and assays prioritization that are defined on a case by case basis.

Success, whether following the MIRIBEL reporting list or the characterization recommendations described by EUNCL/REFINE and NCI-NCL, is best achieved through a rigorous approach that is well-defined, thorough, and where applicable, makes use of properly validated assays and experimental standard operative procedures with defined quality acceptance criteria. Importantly, we support the adoption of orthogonal methodologies[4] which can be highly informative with regards to the overall properties and performance of the product. Failure to adopt these processes often leads to confounding results, lack of reproducibility, and ultimately, lost time and money. Supporting these minimum reporting and characterization recommendations will greatly advance a field that, at present, is still highly curtailed by the lack of comprehensive characterization data in literature. Thus, the extended Nanomedicine Characterisation Infrastructures are encouraging development and implementation of this process to help drive innovation in nanomedicine.

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Purposeful testing

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Faria et al. (Nat Nanotechnol. 13, 777-785, 2018) have put forward a proposal for minimal reporting requirements for publications in the bio-nano field aka the “minimal information reporting in bio-nano experimental literature” standard. We agree and we offered a similar suggestion in a recent commentary (Fadeel. Nanomedicine (Lond). 10, 1039-41, 2015). We referred to this as a “minimum information about a nanotoxicology experiment” (MIANE) standard, in line with existing reporting standards in the omics/systems biology domain. We think that checklists that take into account both characterization of the test material and of the test system are useful tools for authors and reviewers, and may also prove useful for funding agencies. After all, it is common sense that one should know the test material as well as the model system. The EU-funded project BIORIMA (biomaterial risk management) with more than 40 partner institutes (www.biorima.eu) aims to provide a risk management framework for nanobiomaterials (NBMs), i.e., engineered nanomaterials that are produced for biomedical applications such as advanced therapy medicinal products (ATMP) and/or medical devices (MD). One important aim is to develop and validate test methods reflective of the eventual deployment of NBMs as part of such applications. Currently, the failure to predict efficacy and toxicity of such devices at the preclinical stage can lead to serious delays in the development of new drugs, exposure of subjects to inefficient substances, and even unwanted side effects, as well as the initiation of expensive and unsuccessful clinical programs. From this perspective, having a mandatory reporting checklist is helpful because it satisfies the need for

transparency and reproducibility of the experimental results for regulatory purposes. We noted that Faria et al. suggest that standardized reporting of toxicity or viability studies should be demanded "in culture", but we maintain that biocompatibility studies ought to be documented in a standardized manner in vitro as well as in vivo; furthermore, a checklist for minimal reporting requirements also needs to take into account ecotoxicity testing (Malysheva et al. Nat Nanotechnol. 10, 835-844, 2015). We would like to add that one should know and describe the application of the nanomaterial that is subjected to biological testing as this information will undoubtedly inform the choice of test methods/systems. Again, this is common sense: the evaluation of nanomaterials and the study of bio-nano interactions needs to be tailored to their intended use. Surely, nanomaterials are not manufactured solely for the purpose of generating publications? Finally, the true value of minimal reporting requirements lies in their adoption by the scientific community. For comparison, the ARRIVE guidelines for reporting animal research comprises of a checklist of 20 items. The MIRIBEL proposal lists 27 items (of which the ARRIVE guidelines represent only one item under the category of biological characterization) subdivided into 34 questions. We fully agree that the principles of quality, i.e., ensuring that the results published are robust, reliable, and reproducible, and of openness and transparency (Nosek et al. Science. 348, 1422-1425, 2015) are of fundamental importance in the field of bio-nano research as in all fields of science, but one has to place a reasonable burden on scientists in order to achieve compliance.

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In a time when the credibility and reproducibility of science is questioned, the only way forward is full transparency of scientific methodology and results. We therefore welcome the MIRIBEL proposal made by Faria et al. and agree that editors and scientific journals must play a key role as the "gatekeepers of research standardization"[1,2]. The adoption of a minimum information standard enables reviewers, editors, risk assessors as well as fellow scientists to fully understand and critically assess the reliability of a study.

The MIRIBEL reporting standard is very comprehensive, and all the components to be reported will certainly add value for comparison of studies. It is, however, difficult to evaluate whether all MIRIBEL reporting components are really equally important to consider in all areas of bio-nano research. Consequently, one could fear that attempts of fulfil all components of MIRIBEL might hamper or delay publication of perfectly valid studies due to the lack of scientific, technical and/practical ability to meet one or more of the MIRIBEL components, which may be of less relevant for interpreting and understanding the results of a given experiment.

MIRIBEL aims to apply to all areas of bio-nano research covering everything from cell culture studies to *in vivo* experiments. On one hand, having one minimum reporting standard for bio-nano research that can be adopted by all editors and journals can be considered beneficial. However, designing a fully comprehensive checklist that takes into account all the experimental details in the diverse areas of bio-nano research is extremely challenging and might be very burdensome on researchers in the field. There is an inevitable trade-off between having a fully comprehensive checklist that cover all areas and one that is less ambitious and only cover specific areas. For instance, ecotoxicologists might refrain from using the MIRIBEL checklist since too many components may not be relevant or applicable in their research field. Having more than one reporting standard might make more sense and may facilitate continuous updates when e.g. demands for characterization or environmental realism of ecotoxicological studies increase[3]. If the idea of universal adoption of one reporting standard is upheld, deviations should be acceptable by reviewers, editors and scientific journals as long as they are thoroughly explained (e.g. in the supporting information) and taken into consideration by editors and reviewers as well as readers, should the paper eventually be published.

A critical point missing in the checklist of the MIRIBEL reporting standard for bio–nano research is information about methods applied to determine each component e.g. size, shape and dimensions. For instance, a vast number of methods can be applied to determine e.g. sizes, shapes and dimensions of nanoparticles. Each method has pros and cons and data the same parameter is not always comparable between methods. This needs to be made clear in the checklist and taken into account when evaluating the reported information and whether a reporting requirement has been met or not. Reporting the behavior of the test material during a given bio-nano research study (agglomeration, aggregation, dissolution, etc.) also seems not to be part of MIRIBEL, but it is well known to be crucial for interpreting test results of (eco)toxicological studies[4]. Here it would be beneficial if MIRIBEL was aligned with efforts within e.g. the Organisation for Economic Co-operation and Development and the European Chemical Agency to develop technical guidance on reporting and characterization parameters for effects studies of nanomaterials and how to practically report on many of MIRIBEL components[5, 6].

In the field of nano-ecotoxicology the number of publications has increased rapidly[7] but the relevance and reliability of the reported findings has been questioned[8]. This is partly due to the difficulties in controlling exposure during testing and insufficient reporting of testing conditions/observations, which makes it very difficult to reveal whether actual nanoparticle-specific effects occur. More emphasis is required on analytical-chemical information to verify exposure, e.g. concentrations and transformations of the nanomaterial during testing. Ideally, a series of control experiments (e.g. negative and positive controls, solvent controls, and for metals, ionic and bulk controls) have to be performed to elucidate the "nano-effect"[4]. This emphasizes the importance of introducing credibility evaluation as well as reporting standards that are applicable also to the field of nano-ecotoxicology. If the idea of one common reporting standard is upheld, the NanoCRED[3] reporting checklist (available via at www.scirap.org) may assist MIRIBEL to better encompass essential details for ecotoxicity tests with engineered nanoparticles. NanoCRED is an adjustment of the "Criteria for Reporting and Evaluating Ecotoxicity Data" (CRED) framework[9] that takes specific requirements for testing of nanomaterials into account[3]. If it is instead deemed more appropriate to develop aligned - but area-specific reporting standards - , we see the MIRIBEL (and ARRIVE[12]) guidelines as a good starting point for *in vivo* and *in vitro* studies. We suggest using NanoCRED reporting recommendations in the future work of developing checklists for ecotoxicology studies.

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How to enhance reproducibility of a certain work has long been an issue, typically for those emerging fields, including nanobiotechnology and nanomedicine. Considering the designs and characteristics of nanoparticles have grown to be extremely diverse and complicated over time, as well as the uncertainty of the *in vivo* models they are applied to, the implementation of a reporting checklist would no doubt address many reproducibility issues and enhance transparency and integrity of the research. A detailed checklist, including concrete information of chemicals, agents, instruments and animal studies is highly valuable to establish a standard among the field to facilitate comparison and expedite potential translation.

However, it should be clarified that setting a comprehensive checklist does not mean building up a "one-size-fits-all" standard. Admittedly, regarding translation, the nanomedicine filed is still at an early stage of development. We need groundbreaking fundamental discovery and related techniques in this field to clearly demonstrate their clinical potential. In other word, at this stage with very limited clinical applications in nanobiotechnology and nanomedicine, promoting new discovery and innovation to address the key challenges has a higher priority compared to setting a standard protocol or enhancing quality control. In the past a couple of years, many dynamic progresses in this field have been made, from looking into interaction details of nanoparticles and physiological environments to developing new drug delivery routes targeting diverse indications. Many specific characterization methods have therefore been developed associated with those innovations, which may need a unique checklist highlighting the major merits of a certain work. Editors and reviewers may work together to facilitate formation of such "personalized" checklist upon potential discussion with authors.

On the other hand, for a general checklist itself, further improvements could be taken into accounts. Right now, the checklist gives a comprehensive outline emphasizing on the exact details of the study showing how to repeat a method in an efficient way. In addition to pointing out the right path, it could be more important to "precisely" show readers which steps affect most on the results and which has minor influence that can be substituted by other routes. By pointing out what alternative methods they try and why those do not work out offers equally valuable experimental information on future project design and optimization.

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Data reporting in the nano-bio community has been a concern for many over the past few years. Implementation of a checklist across all the platforms for dissemination would help address this problem.

Articles are routinely published with a focus on the biological translation without proper consideration of the chemistry or physicochemical properties and how these may impact product. We believe total synthetic pathways should be included in the supplementary information. Could it be possible to use past papers from a concurrent study in the supplementary info? Using a specialised rights agreement? This would make the often-tenuous trial for chemical data more efficient and transparent.

Size data in particular is a real concern. Faria *et al.* mentions the need to understand the difference between wet and dry size measurement. It is our opinion that all papers should show data on both. Often microscopy images fail to define coatings boundaries with sizes being underestimated, whereas, light scattering often over estimates. Reporting both datasets will further the global understanding giving clarity on biological response to shape outcomes. It also needs to be understood, that addition of even one drug/ligand/protein onto the surface of particles can result in a completely different size and zeta potential which may completely alter biological properties. It is paramount that final nanomedicine product be well characterised in order to elucidate linkage between size / surface characteristics and biological response.

Experience has shown that common preclinical *in vitro* assays used for translation of small drug compounds into pharmaceutical products are not fit for purpose for nanomedicine development - particularly for cytotoxicity assessment[1,2]. Do these assays really mimic *in vivo* cytotoxicity? Probably not, especially when using larger or highly cationic nanoparticles where gravity/charge may lead to their increased surface contact with the cell membrane in monolayered cultures[1]. Additionally, a wealth of data suggests that coloured particles (particularly inorganic nanoparticles) interfere with the absorbance, fluorescence and luminescence-based viability assays[2]. This can lead to false positives. This phenomenon has been shown in numerous studies yet reports using these assays without validation are still being published. Where cell viability is the determinant factor on whether to progress nanotherapies further into *in vivo* trials, reliable scrutiny is required to minimise the use of animals in research in line with the NC3Rs. Our recommendation particularly surrounding this problem (not only limited to metallic nanoparticles) would be to include more than one assay to ensure consistency and accuracy of results. Often the only reliable assay for this is cell counting.

Our belief is that the only way for nanotechnology to move is to a more transparent collaborative effort showing entire datasets, complete thorough and well-designed studies and implementation of a checklist is possibly the first step to ensure the longevity of the field.

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Parallel to your Editorial "Reopening the dialogue" (Nature Nanotech. 13, 765; 2018), we generally agree with the "MIRIBEL" (Minimum Information Reporting in Bio-Nano Experimental Literature) principles proposed by Caruso et al. for publishing accounts of bio-nano research (Nature Nanotech. 13, 777; 2018). In recent years, research in nanotechnologies at the interdisciplinary of bio-nano field is growing rapidly. Establishing a study and reporting standard will enhance the quality and integrity of the published research, promote reuse and improvement of the results, and enable the comparison across various nanomaterials. But we do not think setting a standard like "MIRIBEL" will necessarily result in more clinical translation of bio-nano research. We see NANO as a Technology for solving problems in biomedical research, but not "doing NANO for the sake of NANO". From this aspect, we think problem-driven nanobiomedicine design is a more important prerequisite in bio-nano research for improving the success rate of clinical translation. Only a design aiming at solving a clinical problem, in combination with MIRIBEL, will possibly result in clinical translation. To be practical, extensive interdisciplinary communication is a must in the future nanobiomedicine development. Involvement of clinicians into the peer-review process may help to improve the quality of a bio-nano paper.

The action-network of nanomaterials

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Faria et al. recently suggested in this journal a 'minimum information standard' for experimental literature investigating bio-nano interactions [1]. In response to the editor of Nature Nanotechnology

reopening the dialogue on this subject [2], we propose here an 'action-network' integrating short-range, remote, and coupling actions of nanomaterials to advance comparable physicochemical property characterization of bio-nano interfaces.

The initial goal of all biomaterials science was to "*achieve a suitable combination of physical properties to match those of the replaced tissue with a minimal toxic response to the host*", leading to a large group of 'nearly inert' materials composing the bulk of the more than 2 million medical devices implanted in the United States annually during the 1960s and 1970s [3]. It is now well established that biologically active materials stimulating a programmed response from the living systems will advance their potential for improved diagnosis or therapy. As a result, by taking advantage of advanced nanotechnologies, a new generation of biomaterials is designed to elicit or monitor specific cell functions at nanoscale levels with rapid development of nanobiomedicine targeting the safe use of various nanostructured biomaterials and the accompanying 'reproducibility crisis' in this area [4]. Setting standard minimum requirements for experimental reports on bio-nano interfaces would be beneficial to improve the quality and reuse of produced data [5]; hence, a new 'minimum information standard' for the bio-nano community was proposed by Faria et al. [1] and highlighted in a recent editorial of Nature Nanotechnology [2].

The 'minimum information' concept for the characterization of nanomaterials has long been extensively discussed, and a number of distinct proposals were made by different scientists and organizations of various research interests [1, 6-32]. Except for a consensus on the potential role in improving reporting quality, none of the previous proposals has been universally recognized, and the "reproducibility crisis" remains to be solved [33].

The main challenge in establishing a 'reporting standard' and guaranteeing reproducibility in the study of nanoscale biomaterials is that the biological responses to a certain material are simultaneously related to multiple physicochemical characteristics, which interact and interplay with each other, and that time-dependent transformations must be considered for specific cases of bio-nano interactions. Before interacting with a cell, a stand-alone silver nanoparticle tends to be twinned (a typical example is shown in the lower left insert in **Figure S1**) and bound by various low energy facets (such as the {111} facets) during synthesis [34], will be stabilized by various functional groups to prevent suspension destabilization if used in liquid environments [35], then likely capped by various protein coronas in different physiological environments [36], and may be personalized by additive or nonadditive interparticle interactions [37], transforming the pristine particle parameters themselves (substructure, size, shape, surface charge, dissolution kinetics, etc.) in addition to their range and degree of contribution to the interactions with cells. Moreover, the envisaged minimum information depends on and differs with respect to the intrinsic nature and intended application of the nanomaterial. For instance, the minimal information of a polymer nanoparticle for drug delivery likely includes zeta potential, size and size distribution, amount of drug loaded, etc., which deviate significantly from that of a two-dimensional nanomaterial for osteosynthesis where nano-roughness determination should be predominant, or at least equally important to zeta potential, especially when considering its biological functionality [38].

After studying 28 previous publications on the 'minimum characterization' issue [1, 6-32], we suggest that the nanoscience and nanotechnology communities implement the following two steps to improve the reproducibility, comparability, and reusability of the vast pool of bio-nano data. First, elaborating the 'indications for use' of the designed nanomaterials in their publications. In line with the coupling of new technologies (including nanotechnology), the term 'biomaterial' has been redefined by Williams as follows: "*A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine*.'' [39]. Accordingly, nano-safety, like that of biocompatibility [40], represents an issue of a specific system, not an intrinsic property of the nanomaterial itself. That is, if the application (or experimental) environment does not meet the 'indications for use' of a certain nanomaterial, the bio-nano results may be diverse or useless. This is not the problem of the material and its design but

a consequence of inappropriate use. By addressing the indications for use, the delivery methods, dosages, cell lines, and additional microenvironments of the materials' intended applications associated with experimental assays can be refined, which is the fundamental basis for comparison and reuse of the results by different groups. Secondly, categorizing the material properties reported into different action ranges. Every engineered nanomaterial is essentially a hybrid system that stimulates specific biological systems by using a set of interplaying physicochemical parameters with respective acting ranges; elucidating these interconnected characteristics will be helpful for characterization-minimization and is fundamental to uncovering the mechanisms of bio-nano interactions more accurately. Nanomaterials themselves are complex systems that may comprise twin boundaries, core-shell structures, pores, alloys, etc.; even a standalone inorganic particle can be transformed into a core-shell structure (particle@corona) before interacting with a living cell. Therefore, the response of a living system cannot be assigned to several dependently prescribed parameters; instead, it should be related to multiple interdependent characteristics. Here, we propose to categorize the associated material parameters into three groups, i.e., short-range actions, remote actions, and coupling actions, constituting an 'action-network' of the nanomaterial system (**Figure 1**) [41]. In this network, a short-range action passively affects the concerned cells (or biological systems) merely when they come in contact with the nanomaterial; a remote action can actively reach possible biological systems even when distant from the material; and the coupling actions relate to those interactions among the sub-systems in a nanomaterial system, which may boost or undermine the biological performance of the material studied. This 'action-network' classification is helpful for defining a minimum set of parameters and customizing the associated metrics and characterization assays contributing to reproducibility. Taken determining the interacting mechanisms between silver nanoparticles and bacterial cells for example, if the silver particles are utilized immobilized on a dental material, such as titanium-based materials [42-47], the action-network may consist of particle dimensions (size/size distribution), substructures (crystal structure/crystallinity, particle-substrate interface), substrate surface potential, wettability, constituent leaching, particle spacing, and dose (in particle number per substrate area); if the intended use of the silver particles is in suspensions for oral sprays, the action-network may be composed of particle dimensions (shape, size/size distribution), substructures (crystal structure/ crystallinity, defects, twins), surface charge, self-diffusion, dispersity, and dose (in particle number per millilitre). More importantly, the zone of inhibition assay would be improper to evaluate the antibacterial efficacy of the formerly mentioned use because the mobility of the nanoparticles was constrained on the substrate surface.

This 'action-network' approach would be a suitable mind map to visualize those interacting, interplaying, and transforming characteristics involved in the definition and description of a specific bio-nano interface , producing, as a result, a primary piece of 'minimum information' for the nanomaterial designed for a certain application. Reporting and collecting a large volume of such basic pieces has the potential to advance knowledge and understanding of bio-nano interactions and their diverse applications within nanobiomedicine.

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Competing Interests

The authors declare no competing interests.

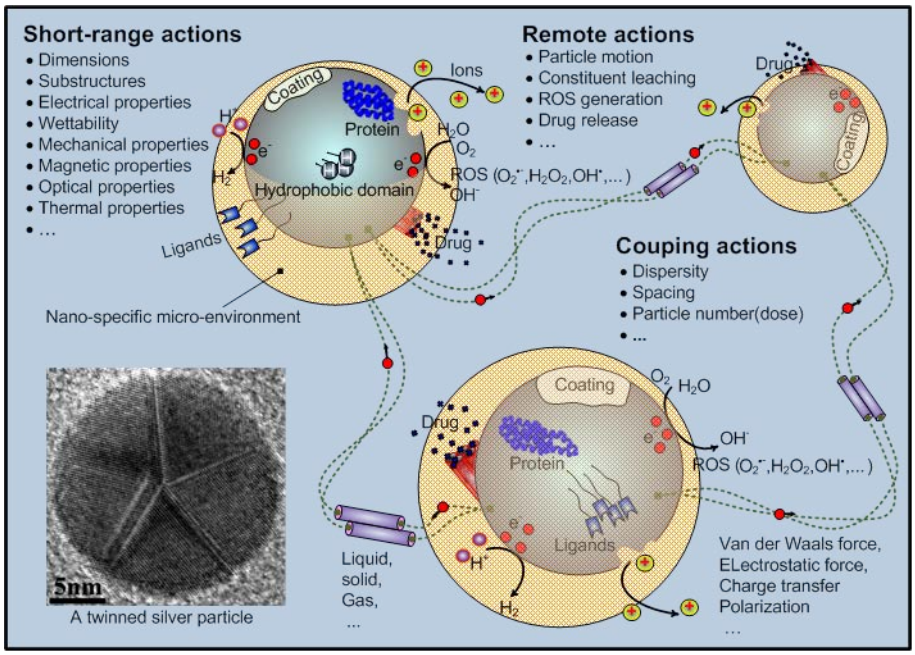

Figure 1 The action-network of nanomaterials. Every 'spherical zone' represents a nanoscale sub-system of the nanomaterials system 1541. The 'action-network' categorizes the physiochemical properties of the nanomaterials system into three groups, i.e., short-range actions (such as dimensions, substructures, electrical properties, wettability, mechanical properties, magnetic properties, optical properties, thermal properties, etc.), the remote actions (particle motion, constituent leaching, reactive oxygen species generation (ROS, generated by the nanomaterial system itself during interaction with the surrounding medium), drug release, etc.), and the coupling actions (such as dispersity, spacing, particle number/dose, etc.). Dimensions include shape, size/size distribution, surface area, etc. Substructures refer to crystal structure/crystallinity, defects, roughness, pores, surface morphology, adsorption (corona), functional groups, sub-interfaces (twins, particle-substrate interfaces), coatings, compositions (impurities), etc. Electrical properties involve particle surface charge, substrate surface potential, conductivity, bandgap, etc. Wettability can be classified as substrate wettability and particle wettability. Mechanical properties refer to rigidity, elasticity, etc. The coupling actions controlled by dispersity, spacing, and particle number (dose) are in direct correlation with the interparticle forces (such as van der Waals forces, electrostatic forces, charge transfer, polarization) and are matrix-dependent (in liquid, solid, or gas). Composition (impurity) is assigned to in the 'substructures' category because it is a basic parameter for nanomaterials design and is highly involved in substructure formation, though diversely involved in various materials characteristics. The mechanical properties, magnetic properties, optical properties, and thermal properties are case-dependent. The lower left insert displays a typical example of the twin substructure in silver nanoparticles.

Minimal reporting list for bio-nanomedicine research: go big or go home?

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The unique properties of engineered nanomaterials and their potential applications for biomedical research have resulted in significant interest and investment from both academic and industries to develop bio-nanomedicine with desirable effects in patients. The research and development process of bio-nanomedicine follows the paths of traditional drug discoveries, where the most promising candidates are identified through a rigorous preclinical testing and validation and move forward through the research pipeline.[1] However, despite the enormous literature published in the past decade, bio-nanomedicines have largely failed to justify further investigations beyond the preclinical stages due to issues relating to reproducibility or insufficient robustness of the experimental findings. As a result, increased concerns have been raised regarding the variability in nanomaterial characterizations, inconsistent experimental conditions, and lack of sufficient reporting of study protocols that jeopardize the credibility of the field.[2,3]

In light of these concerns, a growing effort has been to develop a minimal reporting list of experimental conditions in bio-nanomedicine literature aimed to minimize variability and improve reproducibility. It is worth noting that similar effort has already been ongoing in life sciences, and multiple journals have adopted standard reporting for biomedical experimentation (eg. STAR Methods)[4] or reporting checklists[5] to address the issues of transparency and reproducibility that span all biomedical research. While more stringent reporting requirements and availability of source data may improve transparency, identifying attributable causes that undermine the ability to duplicate similar findings under the same experimental conditions are necessary to improve reproducibility. Aside from rare occurrences of fraudulent fabrication of data, more often, irreproducible experimental results arise from the possibility that original findings were discovered by chance and the precise experimental conditions under which they occurred were not properly defined or cannot be replicated. In a strict sense, reproducing experimental results would require knowing and controlling for potentially infinite number of experimental variables including those that might not have been known before hand yet may influence experimental outcomes. Establishing minimal reporting list and data sharing in bio-nanomedicine to certain extent helps to mitigate this problem and provides an important first step.

A more pressing issue however, is that a large portion of the experimental findings reported in bio-nanomedicine literature tends to hold true only for a narrowly defined set of experimental conditions. For example, the inability for researcher A to replicate the experimental outcomes conducted by researcher B in a different facility does not necessarily suggest that the original work is flawed, but rather an indication of limited robustness of the study results. A robust result is more indicative of how likely the bio-nanomedicine will behave under real-world conditions, where a significant degree of heterogeneity exists (Figure 1). One way to increase robustness is to ensure that the reported findings hold true under a variety of conditions through repeated testing. In this sense, the investigators should report the range of experimental conditions under which their studies were conducted and describe the potential heterogeneity in outcomes encountered.

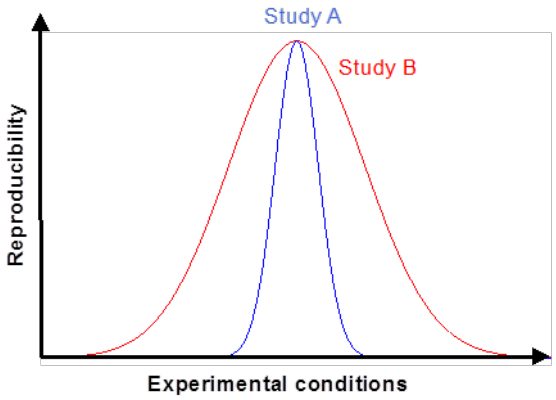

Figure 1: While both study A and B were technically reproducible, study B produced more robust results than study A.

Going back to the minimal experimental reporting checklist for bio-nanomedicine research, we applaud the authors for pushing for an experimental and reporting standards in the field, which are desperately needed. Equally important however, is such standards should place a strong emphasis on the ranges of the variables listed in addition to mean values, thus allowing better determination of the robustness of the experimental results. Furthermore, taking into considerations the potential heterogeneities that exist in the laboratory as well as in the real world, investigators should be encouraged to report outlier results and not be punished for being internally inconsistent. At minimum, justification for omitting particular sets of experimental results (in vitro and in vivo) should be provided, and ideally, with the outliers included, to minimize the risk of "cherry-picking". The minimal experimental methodology reporting checklist outlined by Faria et al. is a step in the right direction.2 The next phase would likely require the establishment of standardized parameters for reporting experimental outcomes. Collectively, these efforts will raise the bar with respect to the burden of proof to support the claims made in bio-nanomedicine research, and will be integral to our effort to advance the field.

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Reporting standards: a welcome addition to the bio-nano community to improve reproducibility and enable predictive modelling

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Minimum reporting standards are a common occurrence across a diverse range of scientific disciplines. Their introduction to the bio-nano community is a welcome addition. Continued growth

and development of the bio-nano field requires an emphasis on characterization of nanomaterial transformation in the biological and environmental milieu to connect molecular level modifications to organismal, community, and ecosystem insights. With a community of invested researchers and high quality, open data, will reconcile variability in bio-nano literature and improve our fundamental understanding of the bio-nano interface.

The importance of minimum reporting standards for nano-bio experimental literature cannot be overstated. With the increased prevalence of data-mining and meta-analyses, researchers are not just data producers, but are also data consumers. Data science methods, however, rely upon quality and breadth of publicly accessible databases. Although there are repositories for nanomaterial data, most are small, disconnected, and underutilized.[1] In a first step toward increasing data quality and enabling data comparisons, Faria et al proposed Minimum Information Reporting in Bio-Nano Experimental Literature (MIRIBEL).[2] These flexible guidelines join a growing tradition of minimal reporting standards across fields.[3–7] If adopted by the community, funding agencies, and journals, MIRIBEL could improve data quality, sharing, and communication within the bio-nano community. With open, quality databases, the depth and breadth of analyses of published data will naturally improve, catalyzing our ability to address the grand challenges of nano-bio, such as prediction of nanotoxicity, development of targeted nano-drugs, and forecasting the environmental fate of nanomaterials utilized in a myriad of consumer, industrial and engineering applications of nanotechnologies.

By making the standards a living document, MIRIBEL encourages community involvement and discussion, an aspect that may boost uptake by avoiding the impression of forcing standards upon the community. If a critical mass of researchers takes part in the design and refinement of the reporting standards, including tweaking or expanding them for specific sub-domains within this enormously diverse field, then this invested community can catalyze broad implementation. Because the guidelines span the entire broad field of nano-bio, MIRIBEL could ideally become an overarching set of principles from which more specific reporting checklists or best practice guidelines could derive, including for example, consideration of nanomaterials transformations. Perhaps then, journals and research funders can move away from the often-informal guidelines toward the use of MIRIBEL.

Addressing the challenge of interdisciplinarity

When comparing the nano-bio field to others with minimum reporting standards, nano-bio research is perhaps the most inherently interdisciplinary research area and spans the length-scales to include molecular, cellular, organismal, and even mesocosm scaled studies.8 With the ideal of connecting atomic level modifications of nanomaterials to biological (organism, community, and ecosystem) response, the field requires input from a vast array of datasets collected by researchers with diverse expertise. The heavy incorporation of existing guidelines into this first iteration of MIRIBEL emphasizes the importance of working with established expertise leveraged from intersecting research communities. The call for community involvement is also key to ensure the guidelines properly represent each layer in the length-scales and each newly evolving approach. Currently, most researchers are more traditionally trained in only one or two of these fields, and as such the reporting standards should also inform quality in experimental design across disciplines and not only serve for data reporting. Through community involvement, we can help ensure that MIRIBEL not only covers the breadth of the field, but also the depth of each sub-field by providing the details

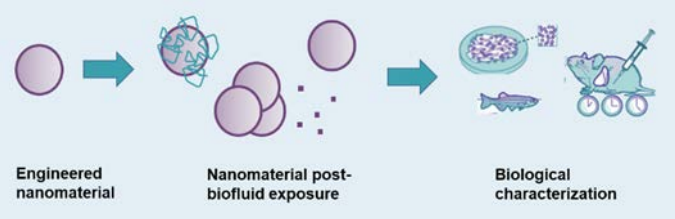

Figure 1 The biological response to a nanomaterial not only relies upon the properties of nanomaterial as synthesized, but is also dependent upon modifications incurred during dispersion and after biofluid exposure. Adapted from Faria et al.[2]

necessary to ensure proper implementation of challenging techniques by transdisciplinary researchers.

Here-in, we aim to provide the first addition or sub-field specific expansion to MIRIBEL by highlighting the need for an emphasis on nanomaterial transformations in biological and environmental milieu, and to make the case for others in the community to add details relevant to their sub-fields (such as proteomic identification of corona constituents used as an example herein) in order to guide our community of transdisciplinary researchers through some technically challenging areas in the field.

Nanomaterials transform in the biological milieu

The MIRIBEL standards, in their current form, include three components: material characterization, biological characterization, and details of experimental protocols. Indeed, these three components cover the experimental stages largely undertaken by the biomedical nanoscience community. Yet, they broadly overlook one major insight from the field thus far: Nanomaterials undergo major transformations in biological fluids and complex environmental matrices (Figure 1). These transformations can include, amongst others, dissolution, agglomeration, or alteration of the nanomaterial surface through interaction with biomolecules. The MIRIBEL guidelines do include a reminder to perform characterizations "in a fluid mimicking biological conditions"; however, this is arguably an understatement of the importance of performing materials characterization on both the nanomaterials as engineered and on nanomaterials after exposure to biological fluids. Some journals, such as ES: Nano, include a fourth component in their brief nanomaterials characterization checklist for authors, asking "Is the material altered during handling and in reaction media?".

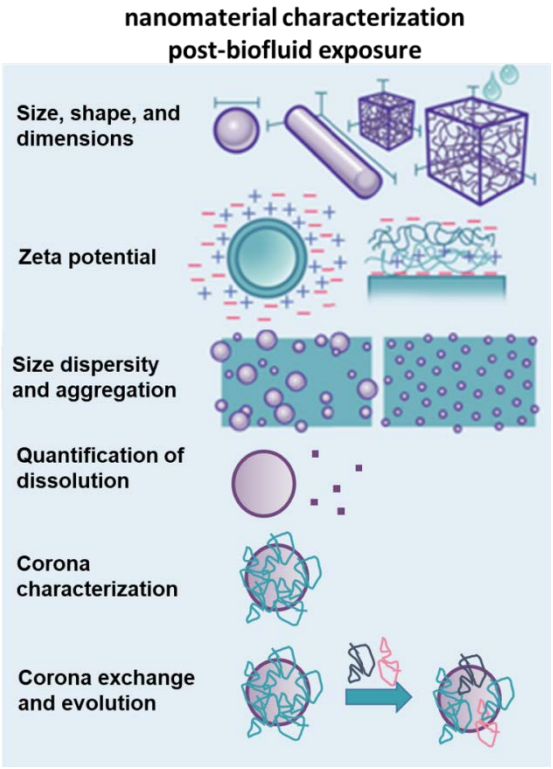

Figure 2 Outline of nanomaterial characterization post-biological exposure.

The transformations of nanomaterials can range from atomic level modifications at the surface to alterations of the nanomaterial such that it is no longer on the nanometer scale. For example, the relative instability of nanomaterials leads to agglomeration, or changes to dispersity in biofluids and, sometimes, even in high salt buffers. Indeed, transformations can occur during storage[9], leading to a call for information on nanomaterials provenance.[10] In addition, silver and metal oxide nanomaterials are often toxic, in large part, because of oxidative dissolution and release of metal cations that can occur during aging or as catalyzed by biomolecules. Alternatively, ligand exchange or loss of capping agents can alter surface chemistry, or biochemical reactions can result in changes in nanomaterial morphology. Lastly, due to the high concentrations of biomolecules and dissolved organic matter in biofluids and natural waters, these large macromolecules coat nanomaterials to form a corona. Depending on the macromolecules present, the coating is referred to as the protein corona, biocorona, or eco-corona. This new molecular coating alters the charge, chemistry, biochemical surface and can attenuate desired biological activities[11–13] of nanomaterials. Given the importance and complexity of nanomaterial transformations, we propose the addition of a fourth component to the MIRIBEL standards: a material characterization post-biofluid exposure (Figure 2)

that includes, where relevant, the constituents of the acquired biomolecule corona, and the dynamic exchange of biomolecules in the corona when entering new environments.[14,15]

Importantly, the MIRIBEL standards already include the majority of techniques required for nanomaterial characterization post-biofluid exposure, including size, shape, dimensions, and dispersity. Thus, the inclusion of post-biofluid exposure in the guidelines requires, in large part, an emphasis on the importance of nanomaterial characterization at multiple stages in the experimental process. Although it is unreasonable to expect that every study includes a full characterization of the nanomaterials post-biological exposure, it is vital to highlight the importance of such efforts and to acknowledge that the pristine characterization results may not apply following exposure to biological matrices.

By including characterization of nanomaterial transformations, nano-bio researchers can better address the 'reproducibility crisis'[16–18] by closing the loop in characterization and correlating biological responses to nanomaterials with the characteristics of the actually exposed entities. For example, this additional characterization could help identify discrepancies in biological response to nanomaterials that may be due to exposure conditions (e.g. different biological media can alter particle transformations). Studies of post-biological exposure characterization, when combined with biological response studies performed under similar conditions, can connect the molecular level insights to cellular or organismal response mechanisms. When available, characterization of post-biofluid exposed nanomaterials can improve nanotoxicity models, drug targeting, and ecological fate forecasts.[19]

A case for focused experimental design guidelines in emerging areas

The MIRIBEL standards cleanly and concisely integrate materials and biological characterization. Importantly, MIRIBEL collates established guidelines for key methods, building on existing expertise. Yet, many of these guidelines are written by, and for, experts in their respective fields. The transdisciplinary researchers within the bio-nano community may have proficiency in nanoscience, chemistry, or biology, but rarely have extensive training in all. As we argue, the Minimum Information about Nanomaterial Biocorona Experiments (MINBE)[20], technologies are emerging for more complete and biologically meaningful descriptions of nanomaterials, but few research groups at this point have the necessary capabilities. Emerging and evolving approaches are essential, however, to tackle the central, yet incredibly complex challenges of targeting and drug delivery in nanomedicine, as well as informing guidance on nanomaterial disposal and safe by design for nanomaterials applied in environmental applications.

Increasingly, researchers access some of the more costly technologies via centers with the technical expertise to support molecular level characterizations of the nanomaterials or biological response. For example, there is already a growing body of literature using omics techniques for this purpose. Here, genomics and proteomics centers can provide data on transcriptomic response to nanomaterials or identification of protein corona constituents. In these cases, minimum reporting standards already exist, in the form of Minimum Information About Microarray Experiments (MIAME)[6] and Minimum Information About Proteomics Experiments (MIAPE)[4] that can be added to MIRIBEL.

Given the challenges inherent in these techniques, however, coupled with the above-mentioned transformations of nanomaterials in biological or ecological milieu, we argue that a specific checklist for experimental design may ease communication with omics centers and translate to higher quality datasets. Since these omics techniques are growing in availability and use, the stage is set for inclusion of these approaches, in particular, into MIRIBEL. By providing resources for researchers early in their experimental design process, chances are higher that they'll participate in the curation and implementation of MIRIBEL at later stages as well.

A case study: growth and challenges in protein corona characterization

Of the emerging technologies, protein corona studies are among the most mature. A decade after the term was coined, protein coronas have demonstrated importance in both biomedical and environmental nanoscience. Corona studies have been cited as the next great challenge in nanoEHS,[21] biological response to nanomaterials,8 and nanomedicine,[22] although there is a growing concern that translating information on the corona constituents into predictions of biological response are not progressing as rapidly as had been hoped. Given the subfield's maturity within the spectrum of emerging approaches, corona characterization can serve as a case study for providing more explicit experimental design guidelines as a means to overcoming the criticism and addressing the scientific grand challenges.

Corona characterization methods that provide the identities of bound proteins include LC-MS/MS[23–25] and GC-MS.26 Given the cost and expertise required, identification of protein corona populations using these techniques is most often performed in collaboration with a proteomics center. Although MAIPE standards exist for proteomics, they are minimum reporting standards written for experts and are not meant to guide experimental design or to ease communication with centers performing analysis. Without such guides, datasets can be compromised or misinterpreted. For example, when cleaning protein samples before injection into the LC-MS/MS care must be taken to ensure that the cleaning steps do not inadvertently alter the protein population. Some solvent precipitation methods commonly recommended to clean up blood plasma protein corona samples prior to LC-MS/MS injection could lead to unintentional selectively eliminating all human serum albumin from blood plasma.[27] Although elimination of one protein from the sample may appear minor, human serum albumin is the most abundant protein in blood and has been reported as one of the four most abundant proteins bound into all studied nanomaterials coronas from 25 out of 26 studies[28]. If unknowingly removed from a sample, the reported corona composition would be significantly altered. To avoid simple, yet impactful, errors, MIRIBEL can play a central role in communicating best practice experimental design and provide checklists for communication with proteomics centers, in addition to the existing reporting guidelines.

Such experimental design guidelines could be quite timely, especially for the protein corona community. As a living document, MIRIBEL-extensions such as MINBE can serve as a central resource to the field after methodology is established, but before broad implementation across an array of samples. In cases like this, MIRIBEL has the opportunity to create sub-communities and increase user ownership of the standards to not only improve data reporting, but to also influence experimental design. In combination, data quality will improve, and a nucleus of invested researchers will form to uphold the principles of MIRIBEL.

Summary

The potential and challenge of working at the nano-bio interface lies largely in its interdisciplinarity. Despite this, it is a hugely exciting prospect for the future of nanoscience research, whereby the generation of vast quantities of data on bio-nano interactions will pave a path for nanomedicines and the development of safe by design nanomaterials.

The implementation of MIRIBEL, alongside future additions for specific sub-areas, is vital to the successful application of nanomaterials to generate novel, highly targeted and efficacious nanomedicines. It is imperative that, as a community, we strive to generate the highest quality and most reproducible data possible as it is only through this that nanomedicines will become cost effective and widely adopted. Regulatory bodies such as the FDA, and the biopharmaceutical industry, rely on high quality fundamental science data prior to pre-clinical trials and development of pharmaceuticals. Failure to provide this at a fundamental science levels run the risk of nanoscience not living up to its promise, an issue that has seen other fields such as genomics and metabolomics falter.

Furthermore, it is vital that as a community we take lessons from previous examples of new, novel chemicals being released into the environment before a comprehensive understanding of their effects were understood, such as poly aromatic hydrocarbons, endocrine disrupting chemicals, chlorofluorocarbons and other persistent organic pollutants.8 MIRIBEL represents a significant step towards avoiding these previous oversights by providing guidelines and checklists to thoroughly characterise environmental bio-nano interactions. Once nanomaterials transformations and acquisition of a biomolecule corona (or eco-corona) are incorporated into these guidelines the biological and environmental fate and risks from nanomaterials and nanomedicines can be more systematically assessed, allowing for a safe, productive and thriving nanomaterials field.

It will only be with the successful use and onward development of MIRIBEL standards that the promised advances from nanomaterials will be realized in a timely and cost-effective manner while maintaining the support of the public.[29] As such we echo the authors of MIRIBEL in rallying the community to adopt these guidelines for ongoing and future nanomaterials research, whilst continuing to evolve them as a dynamic document to capture more specialized and new technologies/methods for nanomaterial characterisation.

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Emphasis on Fundamentals, Not a "Checklist"

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The proposed nanomaterial checklist by Faria and colleagues[1] is the result of a burgeoning discourse on the lack of reporting standards in nanomaterial characterization and poor description of bio-nano experimental conditions. This is not surprising; bionanotechnology and nanomedicine are complex interdisciplinary themes where inadequate knowledge of biological techniques and protocols (and their limitations when applied to nanotechnology) by physical scientists and some biologists' lack of familiarity with nanomaterials properties and characterization technologies has contributed to a problematic and arguably downward spiral.[2] The frustration is further amplified in scenarios where researchers choose an unproven preconceived model of "proof-of-concept", yet pay little attention to systems biology and translational steps.[2,3] For instance, many nanomaterials (e.g., carbon nanotubes, graphene oxide, various metallic nanoparticles) do not have the necessary biocompatible characteristics and pharmaceutical/regulatory attributes for medicines development, yet we are inundated with their presumed therapeutic properties.

Notwithstanding, the experienced bio-nano researcher is well aware of heterogeneity surrounding nanomaterial production and challenges surrounding their characterisation and hence, stochastic biological performance. Since, this is science of diverse complexity, standardising methodology and reporting will be a daunting task. Additionally, inception of data repositories will fuel frustration. Equivocal standardisation may slowdown innovation, especially where nanoparticles act as functional tools for fundamental studies in biology. On the hander hand, there are numerous publications from the "drug delivery" community that go well beyond the proposed "minimum reporting standard" that thoroughly report on nanomaterials characteristics and experimental conditions. Some of these studies have further assessed biological performance through systems approaches and identified attributes that have allowed for better production of viable, reproducible, affordable and clinically acceptable formulations. The pharmaceutical industry has further highlighted challenges in production, characterisation and regulatory tasks surrounding the so-called "nano-similars".[4] We must openly acknowledge and embrace the experience and wealth of knowledge present within this community and implement this into the broader bio-nano arena. The proposed mandatory checklist and a nanomaterial repository for data organisation falls short of a working conceptual framework and will be too restrictive and at extreme may violate an authors' right to proprietary information. Focusing on strategies that could better train interdisciplinary scientists in biological and analytical techniques, including validation approaches to methodology optimisation is a more important solution.

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We fully endorse the importance of providing appropriate material characterization, biological characterization, and experimental protocol details in reporting on biological behavior, safety, and therapeutic use of engineered nanomaterials at the nano-bio interface, as relayed in previous editorials and perspectives, including the Perspective, "Minimum information reporting in bio–nano experimental literature"[1-5]. We are not convinced, however, that the call for standardization would be straightforward to implement as lists of "minimal information" to be provided, as called out by the proposed Minimum Information Reporting in Bio-Nano Experimental Literature (MIRIBEL)

standard. The reason for caution is the diversity of nanomaterial applications that needs to be appropriately evaluated in terms of the claims being made and the use context. While it is valuable to establish guidelines, the use of mandatory lists could create significant problems if applied uncritically or rigidly to the evaluation of manuscripts making diverse claims.

There have been multiple attempts in studying the applications and implications of nanotechnology, including for areas such as nanomedicine and nanotechnology environmental health and safety (nano EHS), to require minimum characterization standards as a result of the literature showing inconsistent, poorly reproducible, or even contradictory physicochemical properties for the same set of materials[1-5]. While no consensus could be reached on what constitutes a minimal set of characterization criteria, increased awareness and expectations of reviewers and journals of the necessity to include physicochemical characterization data appropriate to what is being claimed, has resulted in steady improvement in data quality and usefulness. While we still have a long way to go and should continue to emphasize the importance of characterization appropriate to the level of discovery or use potential, mandatory lists of "representative units" could have a significant downside in terms of fostering new innovation. For example, while several of the characterization criteria in the MIRIBEL checklist refer to intrinsic or as-synthesized materials properties (e.g., size, shape, dimensions, zeta potential), the acquisition of "extrinsic" material properties in biological media or physiological environments received minimal coverage, in spite of the dynamic properties that may be acquired in a biological environment (e.g., a protein corona, colloidal stability, hydrodynamic diameter, charge, dissolution properties). In addition, recent advances in nanosafety or nano EHS research (that we prefer to use instead of the term "nanotoxicology") show that even after considering a wide range of intrinsic and extrinsic properties, structure-activity relationships may emerge that stretch beyond the traditional properties criteria[6]. These properties could include, for example, the possibility to generate biological hazard as a result of the overlap of metal oxide nanoparticle band gap with cellular redox potential, the lipid peroxidizing effects of carbon radicals present on the graphene oxide planar surface, or complexation of structural membrane or protein phosphates by rare earth oxides leading to lysosomal damage[7-9]. Moreover, for therapeutic nanoparticles, there are several important characteristics beyond drug loading and release, targeting, and labeling as outlined in the MIRIBEL recommendations. Missing properties include consideration of colloidal stability, drug leakage, pharmacokinetics, surface PEGylation, or modifications that may alter vascular access at the tumor site, including addressing "permeabilization" that goes beyond a simplified view of an enhanced permeability and retention (EPR) effect[10]. While the listed "biological reporting standards" are relevant to biological experimentation, these are standard good laboratory practice (GLP) criteria integral to all tissue culture studies that explore exposure to chemicals, drugs, or foreign materials. It is questionable, however, whether each of the listed biological criteria should be independently reported, because that involves GLP standard operating procedures, which should then also include criteria such as instrument calibration, storage conditions, etc. While the necessary level of detail should be reported in experimental protocols, the list appearing in Table 1 can only serve as a broad guideline for the sophistication, diversity, and complexity of nanomaterial use for nanomedicine applications.

All considered, the valuable discussion about minimal reporting information by the Perspective in Nature Nanotechnology is a timely and appropriate reminder to all scientists working in this area to adhere to appropriate reporting standards. However, in our opinion, the Perspective should be used along with other reminders and editorials as a guide, rather than being implemented as a mandatory list for the reasons discussed.

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MIRIBEL: Moving Forward with Nanomedicines

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The Perspective by Crampin, Caruso and colleagues (Nature Nanotechnology, 13, 777–785, 2018) was presented as a living document of 'minimum information standard' for future experimental literature investigating bio–nano interactions. The intention of the authors is that it can be rethought and adjusted periodically. It is with great excitement that we receive the MIRIBEL proposal, as it addresses important topics of current and intense discussion within our forum. In general, the Mandatory Reporting Checklist has the potential to contribute to the establishment of comprehensive measures, promising faster and effective translation of nanomaterials into the clinics. Still, we would like to point out some additional key points to the ongoing discussion around the reporting guidance. First, information regarding the radical change of the selection of production methodologies and materials employed in early stage of drug product development should be included in order to facilitate the establishment of robust and reproducible industrial processes. In the Perspective it is not given the sufficient emphasis to the characterization of the starting materials and their quality grade. Ideally, recommendations to work with GMP materials and detailed quality characterization sheet should be proposed. Also, advances in the purification process of nanoformulations after production would be recommended.
Additionaly, the document should anticipate future guidance on the most relevant critical quality attributes for scale-up methodologies and translation into a clinical set. MIRIBEL should forsee the

participation in regulatory and pharmacopoeial initiatives towards the standard quality control attributes of nanomedicines. Coordination with regulatory bodies and industrial stakeholders is essential.
Without calling into question the credit and the value of this pioneering initiative, we identify further potential specific amendments to the document. First, the retrospective application of MIRIBEL ('reusability character') may be debatable, as only data released following the now proposed checklist must be used. This is not clear in the current version. We would also suggest that the document could be upgraded in order to better address subsequent in vivo work, namely the selection of animal models/species that could aid on the correct understading of biodistribution and bioaccumulation of nanosystems. Of course, this major change may not be straightforward and is largely dependent on specific clinical applications, which could even justify complementary guidance.
Finally, standardization of the terminology used in MIRIBEL would also be welcomed. Helpful efforts towards this objective have been undertaken in the recent "Definitions in Biomaterials" Conference held under the auspices of the International Union of Societies for Biomaterials Science and Engineering. International experts (re)examined existing scientific terms and formulated new definitions in the broad field of biomaterials, which will be released soon as a consensus compendium in order to uniform the scientific concepts on a partially overlapped field of science as bio-nano interfaces1. Additional insights on the classification of materials would reinforce the scope of these standards.

†The Nanomedicine and Nanoscale Delivery Focus Group of the Controlled Release Society (NND-FG-CRS, https://www.controlledreleasesociety.org/focus-groups/nanomedicine-and-nanoscale-delivery-nnd) aggregates a community of over 200 members from academic, industrial and regulatory settings interested in fostering an integrative and progressive discussion on the development of nanomedicines.

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Standardization: Only in an ideal world?

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The characterization of nanomaterials in biological contexts is strongly tied to nanometrology and bioanalytics and there is an implicit consensus within the community that understanding the interactions driving the biological response to nanomaterials is one of the major challenges today. The introduction of an analytically complex and ever-changing biological environment shifts the problem into multi/interdisciplinary territories, which are unfamiliar to the individual classical disciplines, such as biology, chemistry and physics. Consequently, there exists a knowledge gap

between each specialisation, which leads to an insufficient appreciation of key experimental parameters. One possible remedy involves the standardisation and clarification of protocols for designing and conducting investigations into performance and stability of nanomaterials used for medicine. Should reporting sets of parameters and protocols be made mandatory? In an ideal world, this would provide a powerful basis for enabling the refinement of nanometrological protocols; however, the complexity of the field and the large number of (convoluted) variables and parameters renders this task non-trivial. Moreover, there are several experimental challenges associated with resolution, sample size/volume and complex environments that must be considered in order to contextualise experiments and nanomaterial behaviour.

Reproducibility is a key concept to ensure consistency across studies. Accordingly, it is equally important to be able to estimate and report on possible systematic errors (accuracy) and random errors (precision). The propagation of bias and uncertainty through an analytical hierarchy may have profound ramifications in terms of the validity of the claims and conclusions drawn. Therefore, the details and justification of data evaluation must aim beyond reporting only mean, standard deviation, sample size and statistical significance. The application of fundamental inferential statistical principles provides a means of approaching experimental design and evaluation. For instance, the sample size should be chosen to reflect the tolerable error and uncertainty required for sufficient confidence in generated conclusions. In order to "compare data and ensure that published results are reliable and reproducible" (Nature Nanotechnology 13 (2018) 777–785) the required dataset must include not only the data summarized and presented in the paper itself, but also the raw data (e.g. the independent repeats of representative experiments). Data sharing is a much discussed (Nature 534 (2016) 684–687) and sometimes controversial issue and many challenges have to be overcome before it can be fully implemented. However, we are convinced that it is an important step in the right direction in the bio-nano field.

We also believe that multi- and interdisciplinary approaches are arguably basic requirements for *many* bio-nano studies. Not only should researchers be closely familiar with the related experimental techniques, but also with their limitations and pitfalls when it comes to measurements and data interpretation. As a community, we currently lack objective and accessible descriptions and assessments of analytical techniques and an understanding of their applicability outside of their original field of application. Finally, complementary knowledge and expertise should be reflected not only in the research teams, but also in the selection of reviewers with overlapping specialties; although their individual expertise may be narrower than that of the entire study, are together able to adjudicate the work.

Emphasizing cell cultures: not new but relevant

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The most important terms when reproducibility and reliability in the bio-nano-interface community are discussed are material properties, biological fluid characterization, interference testing for

toxicity assays and dosimetry. Undoubtedly, these are key parameters to assess efficacy and safety of nanomaterials for various applications. Recently, the MIRIBEL perspective stated, for the first time in relation to nanomaterials, that studies have to report specific components regarding biological *i.e.* cell characterization along with other material and experimental details. Due to inherent biological variability, which plays role also in presumably highly consistent cell cultures, a minimum of cell culturing details needs to be reported. This includes, *e.g.* seeding density including number of cells at the start of and during the experiment, cell line authentication, justification of the model and evidence that cell cultures are free of mycoplasma besides characterisation of biological fluids, especially about serum composition and content. Such information is absolutely relevant also regarding the use of *in vitro* assays for safe-by-design strategies for nanomaterial development but is missing in most of the publications up to date.

Guidance for good cell practice for cell culture techniques exists since decades, however, since we work in a very interdisciplinary field this kind of experiments are often performed by non-experts in this field. This is not only reflected by the frequently missing details on cell culture procedures in the published papers but also by ignoring characterization of appropriate cell differentiation such as *e.g.* cell morphology, expression of specific cell markers or cell polarisation. It is, however, absolutely mandatory to provide evidence that the cell culture experiments have been performed under appropriate conditions ensuring the published data is robust, reliable and reproducible and allowing a correct interpretation of the results. Unfortunately, the characterization point has not been covered by the MIRIBEL perspective and should be included as another key component when reporting bio-nano research outcomes. All the cell characterisation components must undoubtedly be included in the reporting standard, yet this category does not - apart from material dosimetry - differ much from the general guidelines on good cell practice. In our opinion, the nano-bio interface research community should focus on specific issues that arise when nanomaterial-cell interactions are tested using conventional in vitro assays. This is addressed in the experimental details section of the MIRIBEL perspective, but could be expanded. For example, the issue of nanomaterial interference does not only occur with optical pathways, but could also react with assay reagents.

To conclude, the “minimum reporting standard” initiative for studying bio-nano interactions is a step in the right direction. It is an important note that these standards are a living document. At this stage, our role as researchers in this field is to discuss and report these issues, and that journals, editors and reviewers implement such a checklist. On the other side one has to be realistic – which laboratory and which student can perform all the characterization components listed in Table 1 within a reasonable time? Will everybody in the world be able to apply all techniques to fulfil the mandatory checklist and if not do we discriminate researchers in poorer countries? Will we lose creativity when only standardized methods have to be used? The discussion has just started and has to be continued.

Translation is key

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Recently, Caruso *et al.* highlighted in *Nature Nanotechnology* the variability of reported experimental details in the bio-nanotechnology literature as "significant barriers to progress in this multidisciplinary area"[1]. To overcome these barriers, authors proposed implementation of standardized minimum information reporting guidelines[1]. We welcome this initiative and believe that official adoption of continuously refined reporting guidelines may be an important starting point for improving reproducibility of scientific discoveries in nanomedicine. Such practice, if systematically and consistently implemented, could ultimately help in facilitating the true goal of nanomedicine research, that is, to successfully translate this technology into the clinic.

With clinical translation as a key objective, results obtained at the preclinical stage must be reliable and reproducible. The importance of reproducibility cannot be overemphasized as it is at the core of the 'scientific method'[2]. Researchers should apply this method to establish scientific truth by rigorously evaluating the validity of hypotheses. Such practice requires researchers to have access to details about experimental techniques and analysis methods for a given published study. However, consistent reporting of this information is currently lacking in the nanomedicine literature, which can negatively affect the potential to reproduce published results and could compromise downstream meta-analyses or modelling studies.

In silico modelling and meta-analyses are powerful methods with increasing importance for nanomedicine. For example, meta-analyses of published studies can provide new insights into cellular toxicity of engineered nanomaterials *in vitro* and *in vivo*[3]. A recent meta-analysis by one of us focused on quantitative evaluation of nanoparticle delivery efficiency to solid tumours in preclinical animal models[4]. As the majority of published studies that were surveyed for this meta-analysis did not provide sufficient data, original authors were contacted directly to obtain the required information. Importantly, the bulk of requested information concerned nanoparticle characterization details, including physicochemical properties (*e.g.*, size, shape, and surface modification), details about nanoparticle formulation (*e.g.*, drug loading capacity), and the administered nanoparticle dose. Based on this experience with meta-analyses in nanomedicine, we can see immediate benefits of the proposed reporting guideline information. It would be meaningful to make this standardized information available to researchers in different ways, for example: (i) by publishing it alongside the corresponding paper in a standardized checklist format; and (ii) *via* curated online repositories, ideally as open-access resources. Applying meta-analyses and *in silico* modelling on these data may enable researchers to better understand and predict complex nanotechnology-biology interactions. Such insights could guide the engineering of next generation nanomedicines, for example in clinical cancer management.

In cancer nanomedicine, successful translation of nanoparticles into clinically applied agents remains a major challenge despite promising preclinical results[4–6]. In addition to the complexity of the disease and human physiology, factors contributing to this gap include insufficient nanoparticle

characterization, lack of reporting standards[1], and the use of cancer mouse models that do not faithfully recapitulate clinical cancers[7]. Many studies evaluate nanoparticle specificity and efficacy in solid tumour models exhibiting characteristics that facilitate successful preclinical outcomes (*e.g.*, rapid growth with leaky vasculature[8], localized primary tumour models that lack formation of metastases, and sensitivity to tested nanotherapeutics[7]). However, the use of such models can introduce significant bias toward false-positive results that may not translate into the clinic[7]. To improve translatability, increased clinical faithfulness in the design and execution of *in vitro* and *in vivo* models is required. This includes: (i) rigorous cell line authentication; (ii) mycoplasma testing; (iii) the integration of nanomedicine testing strategies that reflect clinical cancers more accurately[9]; and (iv) the incorporation of relevant appropriate tumour location[10], tumour microenvironment, and tumour biology[11].

With the implementation of standardized reporting guidelines, the community has an opportunity to make a push for verification and justification of selected biological models in published studies. Currently used cancer mouse models include human tumour-derived cell lines with various locations of implantation[12], patient-derived xenografts (PDX)[13], syngeneic mouse models[14], and genetically engineered mouse tumour models (GEM)[15]. However, no one single mouse model is representative of all different forms of a single tumour type; just as one human tumour cannot represent another human tumour of the same subtype. Therefore, the goal of mouse model selection in nanomedicine should focus on the most clinically faithful and relevant biological models to increase reliability of preclinical results for downstream clinical translation[16–18]. For a published study, the justification for the selected model should be clearly stated in the corresponding reporting document.

In summary, implementation of standardized official reporting guidelines as proposed by Caruso *et al.* could have lasting impact on bio-nanotechnology research with improved reproducibility and reliability, more efficient data mining, and ultimately increased clinical translatability of nanomedicines.

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Expanding MIRIBEL to biogenic nanoparticles

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The MIRIBEL (Minimum Information Reporting in Bio-Nano Experimental Literature) standard is a response to the lack of reproducibility and consensus across studies reporting interactions between nanoparticles and biological systems. In particular, MIRIBEL provides a useful checklist for reporting

nanoparticle properties, characteristics of the biological system, and protocols[1]. However, the present version of MIRIBEL is primarily focused on synthetic nanoparticles without highlighting specific considerations related to the use of biogenic nanoparticles (BiNPs), such as lipoproteins[2] and extracellular vesicles (EVs)[3]. Lipoproteins and EVs are promising diagnostic, therapeutic, and drug delivery agents, as they are representative of the secreting-cell pathophysiological status, remain intact in the blood circulation, and can display endogenous targeting properties. It is important to emphasize certain modifications and additional considerations that are necessary to standardize reporting for characterization and manipulation of BiNPs, which in many ways differ from synthetic nanoparticles.

Among these considerations include:

- Characterization of the biological source material in regard to percentage of viable cells, mass of tissue, or volume of the biological fluid.
- Description of the method used to separate BiNPs from other components in the biological source material, specifying details in regard to equipment, separation conditions, performed steps, and storage conditions.
- Characterization of isolated BiNPs in regard to sterility, particle number, and biomolecular content (e.g. protein/lipid amount).
- Evaluation of the purity of the BiNP sample.
- Evaluation of how biological properties of BiNPs change when performing separation steps, drug loading, targeting, and labeling, as the purpose of utilizing biogenic nanoparticles is usually to exploit endogenous properties.

Moreover, based on the type of biogenic nanoparticle, specific guidelines should be taken into consideration. For example, the International Society for EVs (ISEV) recently published a position paper on the minimal information for studies of EVs (MISEV2018), co-authored by more than 350 experts in the field[4]. In addition, the repository EV-TRACK (Transparent Reporting and Centralizing Knowledge in EVs), database is an open-source knowledgebase containing methodological parameters of EV-related publications[5]. EV-TRACK data mining results in a single metric that reflects the completeness of provided information required to interpret and reproduce experiments. An important aspect of promoting reproducibility, quantitative comparisons, and meta-analyses in nanomedicine is to uphold a dialogue between the synthetic nanoparticle and BiNP communities.

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Standardization meets innovation

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Discussion surrounding the standardization of information reporting in nanomedicine is one of great importance[1]. As the field matures, there is an increasing need to compare results across works, and this can be significantly hindered by inadequate data reporting[2]. To address this point, Faria *et al.* recently published a set of guidelines specifically for minimum information reporting in bio–nano experimental literature (MIRIBEL)[3]. In our estimation, the proposed requirements are practical and would not significantly raise the barrier to publication, as they would require at most a few additional measurements, or simply increased diligence during experimental design, data collection, analysis, and reporting. Their implementation would likely improve the quality of nanomedicine research in much the same way that similar guidelines have benefitted other fields[4].

Within the field of bio–nano research, there are two main types of participants. First are the engineers, material scientists, and those from related fields who are responsible for developing new nanoplatforms and nanotechnologies. The discovery and development process generally necessitate comprehensive materials characterization, and researchers working along these lines would benefit the most from the biological portion of MIRIBEL. The second population, which has been steadily growing over time, includes clinicians and scientists from other fields who wish to use predeveloped technologies as a tool, whether it be for medical treatment, fundamental studies, or other applications. These researchers may prefer to take a "black box" approach, using nanoplatforms without a deeper understanding of their underlying properties, and how exactly these may affect biological interactions or therapeutic performance. Having a set of requirements in place to ensure the reporting of basic material properties, such as size, zeta potential, dispersity, and drug loading, would ensure that the results generated can be understood within the broader context of nanomedicine. As MIRIBEL encompasses the essential aspects on both sides of bio–nano research, it would help to bridge the gap between these two populations of researchers.

Beyond MIRIBEL, it is our belief that there are certain dangers in excessive standardization, particularly in the nanosciences where a fundamental driver of research is continued innovation[5]. A main function of publication is the widespread dissemination of novel ideas, and it is important that the imposition of publishing standards does not impede the flow of such information. Due to the fast-paced evolution of biomedical nanotechnology, it also remains to be seen if the data generated can meaningfully be subjected to meta-analyses. This could be compounded by the fact that, while data reporting should definitely improve, the quality of the underlying data generated by individual research laboratories may not. Should there be a need for increased standardization in the future, it would likely occur in specific subfields where the associated technology is relatively mature, the goals and metrics are clearly defined, and oversight is possible. Despite these points, we believe that instating a set of essential guidelines will have an overall positive effect on bio–nano research. Of all the potential benefits, improved data reproducibility may be the most readily apparent, and this would be a welcome development for everyone.

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Small but not trivial…

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A recent perspective in Nature Nanotechnology proposed a "minimum information standard" to the bio-nano interaction community with a goal of enhancing the quality and reuse of published data[1]. This applaudable effort is echoed by the latest FDA draft guidance[2], which also emphasizes the importance of identifying quality attributes of nanomaterials and comprehensive understandings of bio-nano interactions. Thus, reopening the dialogue[3] and setting up such a standard will not just allow the community to more effectively communicate the discoveries but also help expedite the clinical translation of nanomaterials in the long run. Compared to what were discussed in the previous dialogues[4], much more parameters were suggested in MIRIBEL (Minimum Information Reporting in Bio–Nano Experimental Literature) in this prospective[1]. The additional parameters reflect what factors we currently believe are important to the bio-nano interactions. With the advance of our fundamental understandings of this field, we believe that MIRIBEL will continue evolving.

As a research group working on understandings of *in vivo* transport and nano-bio interactions of ultrasmall renal clearable nanoparticles[5], we would like to emphasize that seemingly small changes in the nanomaterials and biological systems could result in significant differences in bio-nano interactions. For instance, we recently observed that 7-atom differences in the particle size in the sub-nm regime could result in distinct the interactions of nanoparticles with the glomeruli[**6**]. Since *in vivo* nano-bio interactions are also tightly coupled with nanoparticle transport, anesthesia conditions and heart rates could potentially cause big variations in the studies involved in the blood flow[7]. A slight difference in the kidney injury stages can also result in the distinct nanoparticle transport and interactions *in vivo*[7]. These observations pass an important message to us that biological systems could interact with nanomaterials in a way much more sensitive than what we thought. Thus, those small differences/changes in both nanomaterials and biological systems might not be trivial and should not be overlooked in reporting the discoveries. Therefore, in addition to MIRIBEL, we should always encourage the community to more precisely and quantitatively report nanomaterials, physiological conditions and disease stages. Such the joint efforts of the community will inevitably accelerate both our fundamental understandings of bio-nano interactions and the clinical translation of nanomedicines.

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Checklist is a symptom of peer review problems

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A mandatory reporting checklist for nanobiomedicine studies (Perspective, *Nature Nanotech.***13,** 777–785; 2018) is a useful advance in this maturing field but will not substantially improve research quality without synchronous improvements in the review process.

The proposed MIRIBEL guidelines and corresponding checklist are a reasonable, conservative approach to add a minimal level of uniformity to nanobiomedicine research reports. The three-component checklist—material characterization, biological characterization, and experimental details—offers both flexibility and a clear framework for integrating specific guidelines and benchmarks from the wide range of disciplines that fall under the nano-bio umbrella. Where previous guidelines have failed due to their overly specific technical recommendations, MIRIBEL recognizes the breadth of the field and we believe a mandatory checklist in this format will not unduly burden researchers.

In our opinion, MIRIBEL contains no exceptional guideline and few that are unique to nanobiomedicine. Most of the guidelines are elementary considerations for doing good materials science and biomedical research. This makes for an appropriate checklist but spotlights shortcomings in the review process. As a community, we should be concerned that the basics are being omitted frequently enough that a mandatory checklist is under consideration. While we anticipate the checklist will improve data reporting, it will have no impact on research quality if the contents are not critically reviewed by referees and editors. Is that possible in the current state of peer review? If referees and editors are not currently demanding a rationale for the choice of biological model or study design, why should we assume that if this information is provided it will be rigorously reviewed? At best, a mandatory checklist may improve research quality by ensuring the

referees have a minimal set of information available to evaluate the study claims during the first round of revisions.

We should be realistic also of the impact a mandatory checklist will have on facilitating systematic comparisons across the literature. Unlike clinical studies, preclinical studies are fundamentally not suited to meta-analyses as they are exploratory rather than confirmatory, are designed to minimize heterogeneity, and generally have low statistical power. Integrating data from across preclinical studies to generate in silico models is also a biased approach due to unreporting of negative data. A mandatory checklist should, therefore, be implemented with the primary purpose of improving data reporting and be evaluated on those terms. The MIRIBEL checklist is a good starting point.