

# Minimum Information Reporting in Bio–Nano Experimental Literature

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## **ABSTRACT**

Studying the interactions between nanoengineered materials and biological systems plays a vital role in the development of biological applications of nanotechnology and the improvement of our fundamental understanding of the bio–nano interface. A significant barrier to progress in this multidisciplinary area is the variability of published literature with regards to characterisations performed and experimental details reported. Herein, we suggest a “minimum information standard” for experimental literature investigating bio–nano interactions. This standard consists of specific components to be reported, divided into three categories: material characterisation, biological characterisation, and details of experimental protocols. Our intention is for these proposed standards to improve reproducibility, increase quantitative comparison of bio-nano materials, and facilitate meta analyses and *in silico* modelling.

Advances in nanoengineering have led to strong interest in how nanomaterials interact with biological systems. Research ranges from understanding fundamental biological interactions<sup>1</sup>, to developing nanomaterials for specific applications<sup>2</sup>, to identifying unintended toxicity that can be a by-product of nanoengineering<sup>3</sup>. There is considerable evidence that nano- and micro-scale materials have unique biological interactions when compared with molecules or bulk materials<sup>4</sup>. However, a major impediment to characterising, understanding, and ultimately controlling bio–nano interactions is a lack of standardisation in this area of research<sup>5,6</sup>. Standardisation enables comparison of different materials, establishment of performance benchmarks, and evaluation of engineering design choices.

Standardisation of nanomaterials is a substantial issue entwined with commercial, academic, and societal concerns. Entire texts have been written on the subject<sup>7</sup>, and the International Standards Organization has an active technical committee (ISO/TC 229) devoted to developing new standards for nanotechnology. There exist initiatives in nanotechnology focusing on material classification and characterisation<sup>8,9</sup>, in addition to well-developed efforts and evolving consensus on safety evaluation, from both health and environmental perspectives<sup>10,11</sup>. There has also been a call for standardisation within the field of nanotoxicology, the importance of which was highlighted in a previous editorial<sup>12</sup>, namely: “few studies offer consistent results that are of value, and it is difficult to compare studies because they are often carried out using poorly characterised nanomaterials and arbitrary experimental conditions.” Recommendations from the community to address these issues include promoting the use of multiple characterisation techniques<sup>13</sup>, articulating focused research questions<sup>14</sup>, and performing material characterisation appropriate to the particular type of investigation<sup>15</sup>. Despite these laudable efforts, broader uptake in the field of bio–nano research has been limited.

The use of reporting standards—research guidelines and checklists—has received broad support in the scientific community<sup>16</sup> and has been shown to improve the quality of reported research<sup>17</sup>. We believe it is time for the bio–nano science community to adopt a “reporting standard” to enhance the quality and reuse of published research. To this end, we propose MIRIBEL (Minimum Information Reporting in Bio–Nano Experimental Literature) for published accounts of bio–nano research. MIRIBEL continues a tradition of minimum information reporting standards in the biological<sup>18–20</sup> and chemical fields<sup>21</sup> and consists of specific *components* that are categorised into three *sections*: material characterisation; biological characterisation; and details of experimental protocols. We suggest that each component should be provided when reporting experiments investigating bio–nano interactions. However, some components are only relevant when reporting particular types of experiments (e.g. in culture or *in vivo*) or when using particular types of materials (e.g. those designed for drug loading or targeting). For each component of the MIRIBEL standard, we include a justification for its inclusion. **Figure 1** consists of a summary of these components, our guiding principles, and potential benefits. **Table 1** lists each component, along with a representative unit of measure. We provide a more extensive companion checklist, which includes further details on the reporting components, in the Supplementary Information. This checklist is designed to be used while preparing a manuscript to ensure that each component is considered as appropriate. (This checklist is also available via a repository (<http://doi.org/10.17605/OSF.IO/SMVTF>), allowing reuse by any researcher, journal, or publisher—which we encourage.) We conclude with general guidelines, thoughts on how application of this standard can strengthen the field, and an outlook on what can be undertaken to further improve standardised reporting in bio–nano research.

Four principles have guided our development of MIRIBEL. First, **reusability**: researchers should be able to compare new data with previously published results in a reliable

and meaningful way. Furthermore, published results should be easy to subject to further analysis, whether that be meta-analysis or *in silico* modelling. A recent meta-analysis of delivery to tumours required extensive contact with original authors<sup>22</sup>. If surveying the field requires the effort of reaching out to authors, a central reason for publishing has not been met. Secondly, **quantification**: nanomedicine is becoming an increasingly mature field, with more than 50 formulations approved for clinical use and dozens more in clinical trials<sup>23</sup>. To continue to advance the state of knowledge, both translational research and fundamental investigation need to move towards quantitative, benchmarked assessment and away from qualitative and inconclusive results. We should quantify how well our formulations are working, compare new materials with existing ones, and establish baselines for performance. Thirdly, **practicality**: the standard we propose is limited to parameters and measurements that are accessible to the majority of research teams working in the bio–nano field. We avoid components that require highly specialist equipment or experience only available to a small subset of researchers. And fourthly, **quality**: ensuring that the results published are robust, reliable, and reproducible.

**Table 1.** Summary of MIRIBEL reporting standard for bio–nano research\*

Component	Representative units	Report when...		
		in culture	<i>in vivo</i>	Experiment-dependent
<b>Material characterisation</b>				
• Synthesis and composition	-	✓	✓	
• Size, shape, and dimensions	nm	✓	✓	
• Size dispersity and aggregation	dispersity index	✓	✓	
• Zeta potential	mV	✓	✓	
• Density	kg/m <sup>3</sup>	✓		
• Drug loading and release	% by mass			✓
• Targeting	ligands/particle			✓
• Labelling	a.u./particle	✓	✓	
• Quantification of varied properties (e.g. rigidity)	-			✓
<b>Biological characterisation</b>				
• Cell seeding details	-	✓		
• Cell characterisation	ATCC designation	✓		
- Cell line authentication	-	✓		
- Passage number	-	✓		
- Mycoplasma testing	-	✓		
• Cell/tissue background signal	a.u./cell	✓	✓	
• Toxicity/viability studies	-	✓		
• Justification of biological model	-	✓	✓	
• Biological fluid characterisation	quantification of proteins, cells present			✓
• ARRIVE guidelines	-		✓	
<b>Experimental protocol details</b>				
• Culture dimensions	mm <sup>2</sup> , ml	✓		
• Administered dose	particles/ml	✓	✓	
- Method of administration	-	✓	✓	
• Imaging details	-	✓	✓	
• Delivered dose	% injected dose/g		✓	
- Tissue mass	g		✓	
• Signal of cells with nanomaterial	a.u./timepoint	✓		
• Details of data analysis	-	✓	✓	

\*For each component, we give a representative example of the units it may be reported in. We also indicate, via a checkmark, under which conditions the components should be reported. ARRIVE refers to the ARRIVE guidelines<sup>24</sup>, ATCC refers to American Type Culture Collection, and a.u. refers to arbitrary units.

## DEFINING THE SCOPE OF A MINIMUM INFORMATION STANDARD

The standard we suggest here refers *only* to the information that we believe should be included as part of published research on bio–nano interactions. We do not refer to *how* this information should be presented (e.g. in tabular form, a database<sup>9,25</sup>, and/or in one of the proposed nano-data formats<sup>26</sup>). Although we applaud these efforts, a consensus on *what* information is presented, independently of *how* and *where*, would advance the field. Additionally, there are multiple techniques for determining many of the components discussed herein. While we mention some common methods of characterisation, our intention is not to mandate specific protocols or debate their merits. Specific experimental protocols have been suggested previously, for instance in the synthesis and characterisation of different types of inorganic colloidal nanomaterials<sup>27</sup> and in pre-clinical cancer nanomedicine evaluation<sup>28</sup>. Furthermore, as much as possible, we do not seek to add to the experimental or characterisation workload typically required in this field. Though many of these components are routinely determined or calculated, they are rarely reported in totality. Finally, our intention is not to criticise existing work or suggest a specific direction for future research. The absence of standards and consistency in experimental reporting is a systemic problem across the field, and our own work is no exception.

The classification of materials as “nanoengineered” or “nanomaterials” depends on still-debated definitions of these terms. One option is to consider a material as nanoengineered when it has at least one functional dimension on the nanoscale (i.e. from 1 to 100 nm). However, guidance from the US Food and Drug Administration (FDA) states that “materials or end products may also exhibit similar properties or phenomena attributable to a dimension(s) outside the nanoscale range of approximately 1 nm to 100 nm”<sup>29</sup>. Size-specific biological interactions are unlikely to fall strictly along metric barriers, and, in this manuscript, we use the terms “nanoengineered”, “nanomaterial”, and “nanoparticle” interchangeably and broadly



to refer to the entire class of these materials. In other words, we support an inclusive definition of what constitutes a nanomaterial, and suggest these standards for bio–nano research using materials that fall within this broad definition.

## **MATERIAL CHARACTERISATION**

Despite intense research scrutiny, there are still many unknowns about how nanomaterial properties influence biological responses. The importance of a property is likely heavily dependent on what biological experiment is performed. However, there are several material properties that are known to have effects across a wide range of biological systems.

**Synthesis and composition.** The composition of a nanomaterial plays a vital role in determining its biological interactions<sup>30</sup>. Thus, including details of composition is essential. However, the extent of material characterisation that can be performed is highly dependent on the material under investigation. Requiring specific details of composition will undoubtedly be too restrictive for some materials, and not specific enough for better studied constituents. Therefore, one way to ensure adequate details of composition are included for a newly published nanomaterial is to provide high-quality, reproducible steps for synthesis. Curated examples of best practices for reporting synthesis and method details have recently been documented<sup>31</sup>. Additionally, any step of synthesis known to be particularly challenging or sensitive should be noted. Any methods of purification used should also be specified, as the presence of precursor residues in nanoparticle mixtures may alter biological responses.

**Size, shape, and dimensions.** The cellular pathways capable of internalising an engineered material and the efficiency of these pathways are constrained by that material's size and shape<sup>32</sup>. Additionally, size affects which biomolecules are adsorbed onto the material<sup>33</sup> and their conformation<sup>34</sup>. *In vivo*, size and shape affect organ distribution<sup>35</sup>. The body contains both physical filters<sup>36</sup> and cells that select for materials in particular size ranges<sup>37</sup>. For spherical

particles, characterising diameter is sufficient. For particles of other shapes (e.g. rods), measurements for every dimension should be provided. Consideration should be given to how the size of a nanomaterial changes upon interaction with a biological system, for example, due to dynamic adsorption of biomolecules onto the particle surface<sup>38</sup>. Additionally, unlike inorganic metal nanoparticles, which generally have the same size in the “wet” and “dry” states, organic nanomaterials can undergo substantial changes in size when comparing their dry to liquid state (e.g. using electron microscopy versus dynamic light scattering measurements). The protocol used to measure size and the “type” of size measured (e.g. geometric or hydrodynamic) should be provided<sup>39</sup>.

**Size dispersity and aggregation.** Minimising size dispersity and aggregation are central challenges in the preparation of colloidal dispersions of nanomaterials<sup>40</sup>. Reporting the average size is insufficient to evaluate biological responses: consider the difference between particles with a uniform distribution in size from 10 to 990 nm versus those that range from 495 to 505 nm. Both systems have an average size of 500 nm, but are likely to exhibit very different biological interactions. Thus, including characterisation information on the size dispersity of engineered particles is vital. Details on how size dispersity was assessed, including concentration of the material investigated, protocol used, and any preparatory steps such as filtering, should be included. The fluid used to assess size dispersity should also be detailed, as biologically relevant media can induce agglomeration. If interpreting light scattering data, whether number- or volume-based distributions are used should be specified.

**Zeta potential.** Variation in cellular response due to surface charge is a well-documented phenomenon<sup>30</sup>. Additionally, surface charge affects biodistribution<sup>41</sup>, influences which biomolecules adsorb onto a particle<sup>42</sup>, and is a critical determinant of colloidal stability. Surface charge cannot easily be experimentally determined, though its sign and magnitude may be inferred from surface potential. Practically, instead of the potential at the surface, the

potential at a certain distance from the nanoparticle surface, related to the electrostatic screening length, is determined<sup>43</sup>. This “zeta potential” (the electrokinetic potential of a colloidal suspension) should be provided for newly reported materials. Because the zeta potential depends on the local environment, details of the fluid (or, ideally, fluids) used to characterise zeta potential, including pH and background electrolyte concentration, should be included.

**Density (in culture).** Nanomaterial density (mass/volume) alters settling behaviours and can have a large effect on the cellular dose of engineered nanomaterials during cell culture experimentation<sup>44,45</sup>. Downstream biological effects are dependent on dose, making density an important parameter to report in conventional cell culture experiments. While precise characterisation of density may not always be possible, newly developed techniques have improved our ability to estimate density for complex materials<sup>46</sup>. Estimations or bounded ranges may also be used.

**Drug loading and release.** Many modern nanomaterial systems are designed to carry a drug<sup>47,48</sup>. If the particle presented is a carrier system, the amount of drug that can or has been loaded should be quantified. For formulations in which a drug is not “loaded”, but an inherent constituent, the amount of active ingredient should be reported. This can be reported either as percentage by mass, or as concentration of drug ‘per particle’. If making claims about drug release or stability as part of a particle formulation, these claims should be quantified. Drug release from nanomaterials—both intentional and unintentional—deserves careful consideration.

**Targeting.** Another area of considerable research interest is the design of “targeted” materials intended to demonstrate affinity for specific tissues or cells<sup>49,50</sup>. One strategy to accomplish this is through conjugation of targeting ligands to the surface of a particle, the

effectiveness of which is dependent on both the amount of ligand<sup>51</sup> and the method of attachment<sup>52</sup>. Other strategies for targeting rely on the physicochemical properties of the material<sup>30</sup>. If a carrier is designed for targeting through the addition of a ligand, the amount of ligand bound to the carrier should be reported. This is especially topical given recent questions about whether nanomedicine has a “delivery problem”<sup>53</sup>, if “targeting should be our target”<sup>54</sup>, and how the performance of targeted nanomaterials compares to clinically used targeted therapies<sup>55</sup>. The functional optimisation of targeting is complex and not well understood—a recent analysis found that only 3.5% of proteins attached to a particle’s surface had appropriate orientation for receptor recognition<sup>56</sup>, and increasing antibody concentration on a particle’s surface can reduce targeting<sup>52</sup>. This suggests that the poor results observed during 2005–2015<sup>22</sup> in tumour targeting may be due to *operational* rather than *fundamental* issues. Encouragingly, recent reports have described methods for determining receptor-binding behaviour at the molecular level on nanoparticles<sup>56</sup>. As these methods mature, more complete and biologically meaningful descriptions of nanomaterials (e.g. in terms of number or density of biologically functional ligands available on particle surfaces) will become easier to achieve. However, these emerging technologies are still new and outside the capability of many research groups. Thus, at present when a carrier designed for targeting is reported, some (semi-)quantitative assessment that the system demonstrates affinity and specificity for its target should be included. As these molecular-level assays become widespread, we believe that they will (and should) become part of future reporting standards.

**Labelling.** Nanomaterials are frequently labelled to track their biological interactions. Choices include fluorescent probes<sup>57</sup>, radiolabeling<sup>58</sup>, MRI contrast agents<sup>59</sup>, or a combination of these approaches<sup>60</sup>. In some materials, the nanomaterial itself serves as the “label”<sup>61</sup>. Regardless of the labelling strategy, the labelling intensity, per particle, should be reported. Appropriate controls should be included to demonstrate if and how labelling intensity changes

during an experiment. This can occur due to label removal from a carrier, as well as environment-related alterations to intensity measurements (e.g. fluorescence can be affected by pH and biomolecules). Additionally, consideration should be given as to whether the label itself alters the biological response to a nanomaterial. Reporting labelling intensity in arbitrary units is appropriate if the same instrument is used to measure both the nanomaterial in isolation and the biological experiment, or if standards are used for normalisation between different instruments. Otherwise, particle labelling intensity should be reported in an absolute unit (e.g. molecules of equivalent soluble fluorochrome<sup>62</sup> or atoms present). The intensity per particle should be measured as close as possible to the experimental measurements (e.g. taking flow cytometry as an example, bare particles in solution and cells incubated with particles should be measured in the same run), or an estimate or measurement of drift should be provided.

**Quantification of independently varied properties.** The characterisation requirements we have outlined above are envisioned as a minimum information standard. That is, while we believe that providing the recommended information is necessary, it may be insufficient for certain investigations. There are many other physicochemical properties of interest that have been demonstrated to influence biological responses, and exploration of such properties represents a rich space of research. Whenever possible, researchers should *quantify* and report any varied properties. For instance, if investigating the influence of particle rigidity on cellular response<sup>63</sup>, the rigidity for each particle system should be measured (e.g. by colloidal-probe atomic force microscopy<sup>64</sup>) and reported.

**General guidelines.** Some general guidelines apply across these material characterisation reporting components. First, bio–nano interactions are generally assessed in a liquid medium. To the degree possible, material characterisation should be performed in a medium relevant to the fluid used in the subsequent bio–nano experiment. For example, a particle that is monodisperse in water may be highly aggregated when exposed to serum and

can therefore display substantial differences in behaviour between the two settings<sup>40</sup>. In some cases, the “characterisation fluid” and “experimental fluid” can be the same (e.g. culture media). In other cases, a medium that mimics the biological fluid may need to be prepared (e.g. by adjusting pH or salt concentrations). Details of how this fluid is prepared, and relevant parameters for characterisation, should be included. Secondly, detailing how a parameter was assessed (i.e. what technique, equipment, and protocol were used) is essential. Thirdly, nearly all material characterisation parameters occur across a distribution, and thus including a distribution of the measured property is more informative than providing a single average value. Ideally, the raw measurement data should also be provided. Fourthly, characterising a property using two (or more) independent methods is of significantly more value<sup>13</sup>. Finally, given concerns about batch-to-batch variability, it is important to detail whether characterisation variance was determined through multiple independent measurements on the same batch of material or whether multiple batches were prepared. For some parameters, some degree of estimation may be necessary, or it may only be possible to provide upper and lower bounds, and in such cases, the estimation method should be included.

## **BIOLOGICAL CHARACTERISATION**

Cell culture studies form the bedrock of bio–nano research. They inform conclusions about new materials and provide data about whether it is ethically and financially justifiable to pursue resource-demanding *in vivo* studies. We wish to draw attention to two biological concepts that may be underappreciated in bio–nano research. The first is the tremendous degree of variability inherent in biology. Researchers should demonstrate that observations reported are due to bio–nano interactions dictated by the key properties of the tested material rather than normal biological variance. The second is the disparity between the dose that is administered (the amount of material added to the system) and the dose that reaches the site of action (in culture, the “cellular dose<sup>65</sup>”). This disparity is explicitly recognised during *in vivo* studies (e.g. by

measuring organ-level distribution); however, it is frequently ignored during *in vitro* experiments<sup>66</sup>. Owing to the wide range of physicochemical properties of nanomaterials, the difference between administered and cellular dose can vary by multiple orders of magnitude when comparing different systems. This is a problem unique to nano- and micro-sized materials: small molecules diffuse sufficiently to remain well-mixed in solution, whereas bulk materials are unmoving or would quickly settle. In culture, it is important to provide enough information to distinguish between administered and cellular dose. Without this, it is extremely challenging to compare and combine results from different studies.

**Cell seeding details.** The number of cells present during an in-culture experiment is an essential parameter for determining cellular dose. Thus, the number of cells seeded, percentage confluency at the start of an experiment, and time between seeding and experimentation should be reported for cell culture experiments. For experiments involving multiple cell types (e.g. co-culture, tumour spheroids), the number of non-target (e.g. healthy) cells and the number of target (e.g. diseased) cells should be reported. Ideally, however, the number of cells for each cell type present should be reported. Additionally, incubator conditions should be reported, including temperature, humidity, and CO<sub>2</sub> percentage. Finally, details of whether replicates were performed in parallel, or as independent incubation experiments, should be provided.

**Cell characterisation (in culture).** Two central challenges for cell culture experimentation are choosing a cell line to represent the biology of interest and ensuring that the desired cell line is actually used. Cell line authentication<sup>67</sup> is vital and should be performed regularly, either by a trusted supplier or the individual laboratory. Additionally, passage number and evidence that a culture is free of mycoplasma should be provided as both parameters substantially alter the behaviour of cells in culture<sup>68</sup>. If an experiment is conducted with a “standard” cell line (e.g. one for which American Type Culture Collection guidelines exist), the name and reference of the cell line should be provided. For primary cells, known

details of donors should be provided, including number of donors, species, age, and sex. Finally, cell cycle effects should be considered, as they can have a significant effect on cellular response<sup>69</sup>.

**Cell/tissue background signal (in culture).** The background signal in the absence of particles, using whichever signalling modality to detect particles, should be quantified and reported. This is an important, however, often neglected control that is essential for quantitative analysis across a variety of modalities (e.g. flow cytometry, microscopy, or magnetic resonance imaging).

**Toxicity or viability studies (in culture).** Many nanomaterials are designed as carrier systems for bioactive molecules, drugs, or diagnostic agents. Verification that the carrier material itself does not significantly alter cell viability of the cell line of interest should be provided. Measurements of toxicity are equally important for materials that are designed to induce cell death. Additionally, it is important to provide confirmation that the protocol used (e.g. light exposure, dyes used) is not the source of observed viability changes.

**Justification of biological model.** The choice of animal or cell model is a key factor in biological experimentation often reflecting a delicate balance between relevance, cost, availability, and laboratory experience. Researchers should bear in mind that a model should be chosen to clearly present a biological scenario for addressing the intended research question rather than to “produce the most exciting data”<sup>70</sup>. Considering this, the choice of model should be justified.

**Biological fluid characterisation.** The biomolecules that adhere to a nanomaterial upon contact with a biological fluid substantially alter key particle properties<sup>33,42</sup>. Thus, there has been substantial research into the formation of “protein coronas” around particles, and how these coronas influence biological response<sup>71</sup>. For culture media, type and percentage of serum



added, if any, should be included. Information on media and serum should include ordering and batch details, species of origin, and details of additives (e.g. antibiotics) or stabilising agents. When investigating the effects of biological fluids, it is essential to subject the fluid to the same degree of characterisation that the nanomaterial/fluid is subjected to. For instance, if a nanoparticle is incubated with serum and relative concentrations of protein adsorbed onto the particle are reported, relative concentrations of proteins in bare serum should also be reported. Alternatively, if a fluid has established reference values for protein composition, cell presence, etc., these references can be specified. Unless the characterisation of biological fluids is conducted, it is difficult (if not impossible) to determine whether differences in nanomaterial response are due to their properties or to biological variation.

**ARRIVE guidelines (*in vivo*).** For *in vivo* experiments, the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines<sup>24</sup> should be followed. ARRIVE is a 20-point checklist for reporting *in vivo* experiments, and includes details about species, strain, housing, and ethical approval.

## **EXPERIMENTAL PROTOCOL DETAILS**

**Culture dimension (in culture).** The dimensions of the cell culture used are a determining factor of the effective cellular dose<sup>44,45</sup>. If using a standard 2D culture plate, providing details of the type of plate (e.g. 24-well and supplier) and volume of media added is sufficient. For more complex systems (e.g. 3D spheroid models), full dimensions (e.g. shape, height, width, depth) of the culture should be provided. If a non-standard orientation (e.g.; cells facing downward into media solution) or flow conditions is used, full details of how the condition is achieved should be provided.

**Administered dose.** The amount of material added during an experiment is an obvious component that needs to be accurately reported. However, the choice of units for this parameter

is less obvious. For cell culture experiments, mass, volume, particle number, and surface area are all common choices for measuring “nano-dosage”<sup>72</sup>. We recommend providing sufficient characterisation information so that interested researchers can calculate all four of these dosage metrics, and we include details of required information in the checklist (Supplementary Information). In cases where the dose is administered in a complex way (e.g. fluidic particle–cell experiments), the method of administration should be clear. For instance, in the case of fluidic incubation, whether the dose was from a singular reservoir, kept constant, or recycled should be noted, as should flow rate. In the case of an *in vivo* experiment, administration details should include vehicle of administration, injection/administration location, total volume and concentration administered, and details about multiple infusions (i.e. time points or rate of administration). Additionally, methods used to normalise dosage (e.g. to body weight) should be reported, as should the concentrations before and after normalisation.

**Imaging details.** For experiments involving imaging, details of how the imaging was conducted should be provided. *In vivo*, details of any shielding that was performed (e.g. using aluminium foil to obscure injection point) should be included. Additionally, if a contrast agent was used, details of its administration (e.g. concentration, method) should be provided. In culture, any image processing that is not applied uniformly to the entire image (e.g. to highlight specific regions or features) should be explained, details of thresholding should be provided, and an image intensity scale should be included.

**Delivered dose (*in vivo*).** The amount of material that reaches the site or organ of interest is an essential reporting piece of information. As in culture systems, there are multiple choices for the dose metric used, including percentage of injected dose delivered (%ID) and percentage of injected dose delivered per gram of organ (%ID/g). Because of the diversity of bio–nano research, we recommend that sufficient information is provided to calculate the most common *in vivo* dosage metrics. Specifically, we recommend that the delivered dose is reported

as the percentage of injected dose delivered per gram of tissue (%ID/g). Normalising in this fashion allows for more direct comparison of data from different organ systems and animals. Additionally, data on weight of organs or tissues measured (and used during normalisation), and information on mass of material injected should be included to allow other researchers to calculate alternative metrics (e.g.; %ID, mg/kg). If possible, in the cases of a nanocarrier, distinguishing between carrier delivery and cargo/drug delivery is desirable. Finally, consideration of the pharmacokinetics of the material under investigation is an important part of quantifying efficacy of potential nanomedicines.

**Signal of cells with nanomaterial (in culture).** In culture, particle–cell association is commonly assessed using the signal label intensity, percentage cellular association, or by estimating particles associated per cell. Percentage cell association or cell percentage is a metric that has long been used with high-throughput flow cytometry data, and it is useful for distinguishing between heterogeneous populations of cells (e.g. in cell biology and immunology studies). However, percentage cellular association can be an unreliable metric for comparative interpretation of results in bio–nano studies. This is especially true when the signal from a single particle is less than the cell autofluorescence, or when comparing particles with different levels of fluorescence. Thus, if an in-culture experiment involves only a single cell type, we recommend that the number of particles associated per cell is estimated or that the signal intensity of cells with labelled particles is provided. For instance, if flow cytometry is used to measure cell–particle association, the mean fluorescence intensity of the channel the particle fluoresces in should be reported as a histogram or distribution of values. Researchers should also bear in mind that association is distinct from internalisation, and a number of techniques have been developed to distinguish internalisation from association or surface binding<sup>73</sup>.

**Details of data analysis.** Details of statistical and data analysis performed should be provided. Because data without uncertainty estimation has questionable value<sup>74</sup>, the number of independent experiments ( $n$ ), and details of how uncertainty is expressed (e.g. standard deviation, standard error, or confidence intervals) should be provided. Details of outlier removal and significance tests, if any, should also be provided, including any parameterisation of these methods. For data where relatively complex non-linear regression methods are used (e.g. fitting scattering data to structure), the method should be fully described, and code used for the analysis should be accessible, for instance, using open access tools<sup>75</sup>.

**General guidelines.** Whenever possible, we recommend that raw data are provided for published bio–nano experimental reports, either as part of the article submission or in a trusted data repository<sup>76</sup>. When raw data cannot be provided, the distributions of measured parameters are significantly more valuable than an average value. Additionally, we recommend that prior to *in vivo* work, the *in culture* and material characterisation components discussed herein should be assessed. For example, changes in hydrodynamic size and aggregation behaviour in blood can compromise an *in vivo* experiment.

## SUMMARY AND OUTLOOK

We present a “minimum reporting standard” for studies investigating bio–nano interactions. However, we emphasise that proposing a standard, or agreeing that standardisation is important, is insufficient to improve the field. To be useful, any standard must be widely adopted by the community, which undoubtedly requires further discussion and input. We hope that our proposal will galvanise this discussion. Furthermore, editors and journals play a key role as the “gatekeepers of standardisation”<sup>5</sup>, crucial for providing momentum to the adoption of guidelines and enforcing community-agreed standards to increase robustness, reproducibility and usefulness of published research<sup>16</sup>. To assist with this, we provide a

checklist of the MIRIBEL standard (see Supplementary Information), similar to requirements that have been implemented by leading journals for life sciences<sup>77</sup>, lasing experiments<sup>78</sup>, or suggested standards for journals such as the Transparency and Openness Promotion Guidelines<sup>79</sup>.

The growth of bio–nano experimentation and the need for standardisation echoes progress in other fields<sup>18,21</sup>. In some cases, experimental and reporting standards have been instrumental in the development of entirely new areas of research. For instance, many modelling approaches in modern systems biology would not be possible without standardisation of genomic experiments<sup>19</sup>. We expect a similar necessity for computational modelling and analysis in bio–nano research given the complex interplay of chemical and biological processes involved. A growing number of bio–nano computational research studies<sup>44,45</sup> underscore interest in this area. However, characterisation and standardisation are strict requirements for computational modelling to become widespread<sup>80</sup>.

Finally, we intend for these standards to be a living document, to be revisited and amended periodically by the community. We expect such refinements to be driven both by improvements in technology, as well as growing knowledge of the bio–nano interface. We strongly encourage those interested in standards development to contact us and join the discussion.

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## **Competing interests statement**

The authors declare no competing interests.

## **Additional Information**

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## **Figure Captions**

### **Figure 1. Summary of MIRIBEL components, guiding principles, and potential benefits.**

The development of MIRIBEL was guided by principles of reusability, quantification, practicality, and quality. If combined with journal and community adoption, MIRIBEL can lead to improved outcomes in the field, including data exchange and communication, reproducibility, deeper analysis of published data, and systematic comparison between approaches and materials.

## **Table Captions**

### **Table 1. Summary of MIRIBEL reporting standard for bio–nano research**

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