

# Multi-omics approaches to decipher the interactions of nanoparticles and biological systems

Yifan Wang<sup>1,11</sup>✉, Zhenyu Xiao<sup>2,11</sup>, Zikai Wang<sup>3,4,11</sup>, DaeYong Lee<sup>5,6,7</sup>, Yifan Ma<sup>1</sup>, Stefan Wilhelm<sup>8,9,10</sup>, Hongmei Wang<sup>2</sup>, Betty Y. S. Kim<sup>5</sup>✉ & Wen Jiang<sup>1</sup>✉

## Abstract

Understanding how engineered nanoparticles interact with biological systems is crucial for the development and implementation of nanoparticles in clinical settings. However, investigating the complexity of these interactions at the nano–bio interface requires technologies that can accurately gather biological information at the organ, tissue, cellular and subcellular levels in a high-throughput manner. In particular, imaging and multi-omics approaches provide powerful tools to study nano–bio interactions. In this Perspective, we discuss the application of transcriptomics, epigenomics, proteomics and metabolomics technologies for the investigation of nano–bio interactions, for example, to assess the biodistribution of nanoparticles in vivo, to analyse their interactions with specific cell types, and to study cellular responses to nanoparticle uptake at subcellular levels. We also examine bioinformatics and machine learning and artificial intelligence tools to assess big multi-omics data, suggesting how these might be applied to develop and optimize nanoparticles for specific applications. Finally, we highlight how multi-omics pipelines might be incorporated in the design of new nanoparticle-based treatment strategies.

## Sections

Introduction

Multi-omics technologies

Omics technologies to study nano–bio interactions

Analysing multi-omics data for nano–bio interactions

Artificial intelligence and machine learning

Designing multi-omics studies of nano–bio interactions

Outlook

Citation diversity statement

<sup>1</sup>Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. <sup>2</sup>Key Laboratory of Organ Regeneration and Reconstruction, State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China. <sup>3</sup>Shanghai Artificial Intelligence Research Institute Co. Ltd., Shanghai, China. <sup>4</sup>Xiangfu Laboratory, Jiashan, China. <sup>5</sup>Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. <sup>6</sup>Fralin Biomedical Research Institute at VTC (FBRI), Virginia Tech, Roanoke, VA, USA. <sup>7</sup>Department of Biomedical Engineering and Mechanics (BEAM), Virginia Tech, Blacksburg, VA, USA. <sup>8</sup>Stephenson School of Biomedical Engineering, University of Oklahoma, Norman, OK, USA. <sup>9</sup>Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA. <sup>10</sup>Institute for Biomedical Engineering, Science, and Technology (IBEST), University of Oklahoma, Norman, OK, USA. <sup>11</sup>These authors contributed equally: Yifan Wang, Zhenyu Xiao, Zikai Wang. ✉e-mail: [ywang45@mdanderson.org](mailto:ywang45@mdanderson.org); [bykim@mdanderson.org](mailto:bykim@mdanderson.org); [wjiang4@mdanderson.org](mailto:wjiang4@mdanderson.org)

## Key points

- Nanoparticles interact with biomolecules, cells, tissues and organs in the body at various levels.
- Imaging, genetics, epigenetics and proteomics technologies allow the precise interrogation of cellular functions and cell–cell communication and might, thus, aid in the study of nano–bio interactions.
- Biological variables, such as age and sex, impact interactions with nanoparticles and should, thus, be considered in studies investigating nano–bio interactions.
- Omics modalities might be combined to examine biological information at different levels to aid in the design of personalized nanoparticle-based therapeutic strategies.

## Introduction

Nanoparticles can be designed for various clinical applications and have been particularly explored for the delivery of drugs and vaccines<sup>1</sup>; for example, anticancer drugs can be formulated as liposomal or albumin-bound nanoparticles, outperforming some solvent-based agents in terms of safety and efficacy<sup>2</sup>, and nucleic acids, such as mRNA, can be delivered by lipid nanoparticles for vaccination. However, the clinical translation of nanoparticle-based therapeutics is often hindered by their rapid uptake and clearance by the liver and other phagocytic organs, leaving only a fraction of nanoparticles reaching the target site<sup>3,4</sup>. In addition, nanoparticles and their payloads can trigger responses in the recipient cells and induce variable biological effects. Although nanoparticle delivery<sup>3–8</sup> and cellular responses<sup>9–11</sup> have been investigated in different model systems, the precise mechanisms of how specific nanoparticles interact with the body (nano–bio interactions) are not yet fully understood. In addition, the interactions of nanoparticles with specific cells and biomolecules might be affected by biological factors, such as ageing<sup>12</sup>.

Imaging and high-throughput sequencing can be applied to investigate material–biological interactions at the nanoscale. Simultaneous profiling of the transcriptome, translome, genome, epigenome, proteome, metabolome and others, referred to as ‘multi-omics’, enables the characterization of different levels of information in the same cell at high resolution<sup>13</sup>. In addition, each of these modalities can be used separately on different tissues or cells, referred to as ‘multi-modal’. Such multi-omics and multi-modal approaches might provide powerful tools to study nano–bio interactions.

In this Perspective, we provide an overview of multi-omics and multi-modal methods that can be applied to investigate nano–bio interactions, including for the visualization of nanoparticles, to study the genetic regulation of their interactions and to identify spatial nano–bio interaction mechanisms, also examining strategies for analysing such multi-omics and multi-modal data (Fig. 1).

## Multi-omics technologies

Multi-omics studies allow the characterization of subtle heterogeneities between cells<sup>14</sup> and of the interactions between cells and nanoparticles. Imaging, genetics, epigenetics, translomics,

proteomics, metabolomics and spatial genomics have enabled a deeper understanding of gene regulation and cellular heterogeneity.

## Imaging

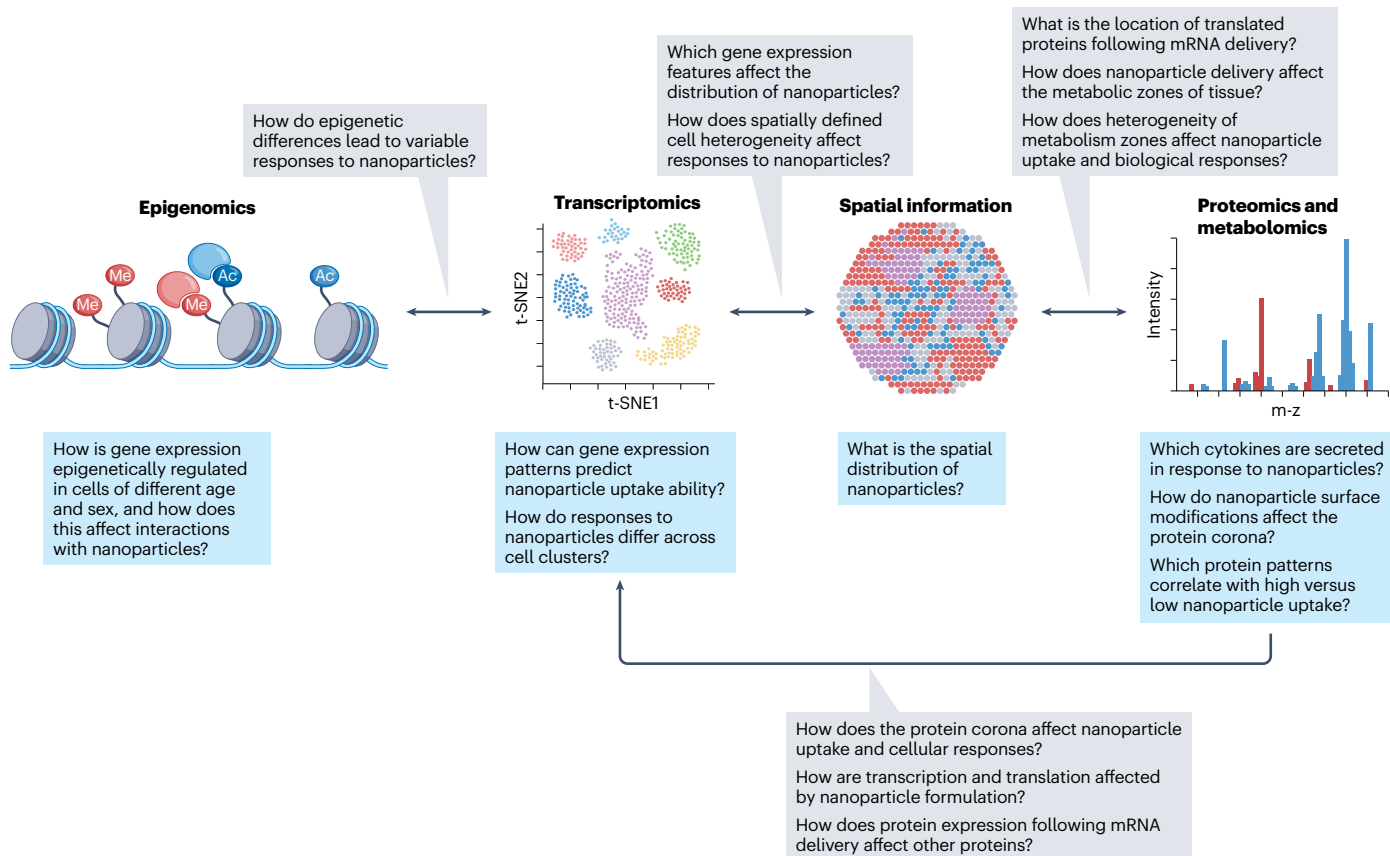
Imaging techniques can be applied to visualize the formation and organization of cells within tissue. For example, microscopy enables the imaging of tissue slides, revealing detailed information of cell types and physiological features; however, tissue slide imaging cannot provide an overall picture of the whole body. By contrast, CT and MRI allow imaging of the whole body but cannot assess cellular information. Imaging approaches that allow visualization of whole transparent mouse bodies, while providing cell-specific information, have been developed<sup>15</sup>. Nanobody-based whole-body immunolabelling approaches can penetrate deep into tissue to image intact, whole transparent mice with subcellular details. For example, nanoparticles that carry fluorescent dyes in the far-red spectrum can be delivered into transgenic reporter mice by high-pressure cardiac perfusion<sup>15</sup>. Specific cells or tissues can then be identified by fluorescence imaging in the context of the whole body, for example, to visualize all neurons in an intact mouse<sup>15</sup>. This technology provides a non-biased overview of the landscape of tissues and cells in the body, and it may be applied to study cellular changes in disease states, such as the dynamics of immune cells after stroke<sup>15</sup>. Furthermore, agents for enhanced cholesterol extraction and membrane permeabilization have been developed to allow homogeneous deep penetration of standard antibodies. This method enables whole-body multiplex imaging to assess several cell types simultaneously without requiring transgenic reporter mice. This immunolabelling platform achieves whole-body visualization of the mouse immune system and lymphatic vessels at cellular resolution<sup>16</sup>.

Imaging mass cytometry enables 3D multiplex imaging of more than 40 antibody-stained protein targets at single-cell resolution<sup>17</sup>. Correlative super-resolution microscopy integrates two or more different microscopy images of the same sample<sup>18</sup>, and super-resolution microscopy can achieve an imaging resolution of ~10 nm, thereby assessing subcellular structures and single-molecule-level reactions. Similarly, the combination of fluorescent microscopy and electron microscopy can reveal information at multiple scales or dimensions<sup>18</sup>. In addition, scanning ion-conductance microscopy can be complemented with super-resolution optical fluctuation imaging to achieve subdiffraction-resolution visualization of cytoskeleton dynamics in live cells<sup>19</sup>.

## Genetics and epigenetics

Single-cell sequencing (scRNA-seq) technologies exploit distinct barcodes to track complementary DNA (cDNA) isolated from single cells. Sequencing libraries are then built using these barcode-tagged cDNAs, which are sequenced by next-generation sequencing technologies. Thereby, hundreds of thousands of cells can be simultaneously sequenced to analyse their transcriptomes and identify cell clusters based on gene expression patterns. Compared with bulk RNA sequencing, scRNA-seq can reveal heterogeneity among cells. However, scRNA-seq cannot provide precise resolution of how transcription factors regulate genes. This can be achieved by combining scRNA-seq with single-nucleus assay for transposase-accessible chromatin with sequencing (snATAC-seq) technology.

In snATAC-seq, the Tn5 transposase carries barcodes that are inserted into open chromatin fragments to obtain sequencing results from these regions, which are typically key regulatory areas occupied by transcription factors<sup>20</sup>. Moreover, scRNA and snATAC can be combined



**Fig. 1 | Multi-omics tools to investigate nano–bio interactions.** Nano–bio interactions can be investigated at different levels using multi-omics tools. The characterization of the transcriptome, epigenome, proteome, metabolome and

spatial aspects of cells can reveal factors that regulate nano–bio interactions. t-SNE, t-distributed stochastic neighbour embedding.

to assess the same sample<sup>21</sup>, sharing a set of barcodes and enabling data integration and annotation through bioinformatics, thus improving the accuracy of identifying regulatory regions and transcription factors.

Epigenetic information can be obtained through DNA methylation profiling, relying on either 5-methylcytosine (5mC) or N6-methyladenine (6mA). In addition, transcriptome profiles can be generated alongside targeted DNA methylome profiles, for example, using single-cell methylome and transcriptome sequencing (scMT-seq) and single-cell triple-omics sequencing (scTrio-seq)<sup>22,23</sup>.

## Spatial transcriptomics

Spatial relationships between cells are crucial for regulating their functions; in particular, the cellular neighbourhood (environ) is essential for the formation of functional zones within tissues and the coordination of interactions among different cell types<sup>24</sup>. Such single-cell spatial relationships can be analysed in tissues using co-detection by indexing (CODEX) approaches, which rely on DNA-conjugated antibodies and the cyclic addition and removal of complementary fluorescently labelled DNA probes<sup>24,25</sup>. In addition, spatial transcriptomics technologies allow the quantification and localization of mRNA transcripts, while preserving their spatial context, by integrating transcriptomic information with spatial positioning<sup>26</sup>. However, compared to single-cell transcriptomics, spatial transcriptomics techniques have only limited depth of sequencing

and, thus, might not identify transcripts expressed at low levels or fail to achieve single-cell resolution. To address this shortcoming, spatial transcriptomics can be combined with scRNA-seq<sup>27</sup>; here, the spatial information can be used to classify cell types and map annotations onto the transcriptome, thereby benefitting from the deeper sequencing depth of the transcriptome for downstream analysis. Alternatively, cell types of interest can be defined based on markers on the transcriptome, and annotations can be mapped onto spatial transcriptomics to assess their spatial distribution<sup>27</sup>. In addition, scRNA-seq data can be directly integrated into spatial transcriptomics data, benefitting from the advantages of both data types<sup>28</sup>. Moreover, spatial enhanced resolution omics-sequencing (stereo-seq) can reach subcellular resolution for subcellular spatial visualization<sup>29</sup>. Stereo-seq exploits DNA nanoball-patterned arrays to provide high-resolution spatial transcriptomic analysis, by combining the large field of view and cellular resolution of DNA nanoball arrays with in situ RNA capture capabilities, making it highly sensitive and enabling the dissection of spatial cell type heterogeneity in complex tissues<sup>29,30</sup>. Combined with the algorithm probabilistic alignment of spatial transcriptomics experiments (PASTE), a method to align and integrate spatial transcriptomics data from multiple adjacent tissue slices, continuous section spatial transcriptomics may further facilitate the 3D reconstruction of virtual tissues that integrate the tissue histology with molecular and genetic information<sup>30,31</sup>.

Spatial transcriptomics can also be combined with other omics modalities to provide synergistic insights into biological processes. For example, spatial transcriptomics can be combined with mass spectrometry to simultaneously identify distinct zones of gene expression and metabolism<sup>32</sup>, or with epigenomics to identify genetic regulation networks in the context of tissue structure<sup>33</sup>.

## Proteomics and metabolomics

Cellular functions are regulated by gene transcription, but the executors of biological processes are proteins, lipids and metabolites. Importantly, the transcriptome and the activities of these biomolecules do not always correlate. In particular, transcription and protein levels might not necessarily correspond, as mRNA quantification is subject to instability and transcriptional 'bursting'<sup>34–36</sup>, that is, transcription from DNA to RNA can occur in 'bursts' or 'pulses'. In addition, the functions of proteins and lipids are further controlled by modifications, such as glycosylation and phosphorylation, which cannot be revealed by transcriptome analysis. Therefore, the characterization of biomolecules by proteomics, lipidomics and metabolomics techniques provides valuable additional information on cellular function.

The proteome refers to the collection of total protein in a cell<sup>37</sup>. Proteomics analysis usually relies on mass spectrometry to detect and identify proteins extracted from cells or tissues. However, in proteomics analysis, proteins cannot be amplified as in mRNA or DNA sequencing, making single-cell proteomics challenging owing to the small amounts of protein present in a single cell. Therefore, only abundant 'housekeeping' proteins can typically be identified in single-cell proteomics<sup>38</sup>. To address this limitation, high-throughput and high-efficiency sample preparation approaches, for example, using a nanowell chip<sup>39</sup>, and detection instruments with high sensitivity can be applied<sup>38,40</sup>. Moreover, proteomics at the tissue or organ level can include spatial context provided by mass spectrometry imaging<sup>41</sup>; here, pulsed lasers generate ionic species from the samples to obtain mass-to-charge information for peptide identification. The location of the laser pulse is known, and thus, the resulting mass spectrometry data can be associated with the tissue location on the slides. Such techniques have been used to create a map of melanoma tissue features by proteomics<sup>42</sup>, albeit with limited resolution owing to the small amounts of protein in the samples.

Alternatively, proteomics technologies not based on mass spectrometry can be applied to characterize protein patterns in cells or tissues. For example, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) uses DNA-barcoded antibodies that bind to specific proteins, thereby integrating protein and transcriptome measurements into a single-cell readout<sup>43</sup> while providing spatial contexts<sup>44</sup>. Similarly, CODEX can visualize protein expression in tissues<sup>25</sup>. These targeted proteomics methods complement mass spectrometry-based approaches to characterize specific proteins, especially those expressed at levels below the mass spectrometry detection threshold.

Mass spectrometry-based platforms can also be applied to assess metabolites. The comprehensive analysis of metabolites is defined as metabolomics<sup>45</sup>, and the analysis of lipids and lipid metabolism is referred to as lipidomics<sup>46</sup>. Here, the specific metabolites, such as lipids, fatty acids or sugar, are isolated from the cell or tissue and identified in a mass spectrometer. Metabolomics is particularly promising for biomarker discovery to characterize and predict disease progression<sup>47–49</sup>. Moreover, fuelled by instrument and bioinformatics advances, metabolomics may provide insights into the mechanisms of metabolic regulation<sup>50</sup>.

## Translatomics

Translatomics, for example, by ribosomal profiling<sup>51</sup> or full-length translating mRNA sequencing<sup>52</sup>, bridges the gap between transcription and protein expression. Here, the translation of mRNA into proteins is analysed by isolating ribosome-bound mRNA from the total mRNA of a cell<sup>53</sup>. Translatomics can be performed at single-cell resolution<sup>54</sup> and with spatial information<sup>55</sup>, and it has been explored for the analysis of the translation dynamics of tumour-infiltrated immunocytes<sup>56</sup> and embryonic development<sup>54</sup>.

## Omics technologies to study nano–bio interactions

Multi-omics technologies provide opportunities to study the interactions between cells and nanoparticles and shed light on nanoparticle uptake mechanisms, fate and spatial distribution. In addition, cellular responses to nanoparticles might be investigated at the single-cell level.

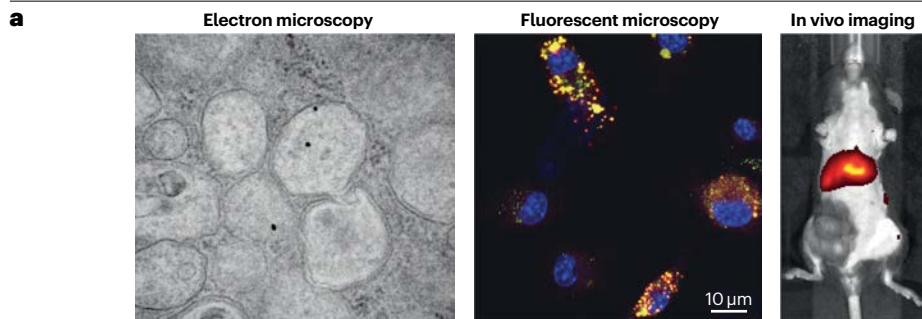
## Visualizing nanoparticles

Nanoparticles have distinct patterns of biodistribution, depending on their type<sup>57</sup>. Following intravenous administration, nanoparticles travel through blood vessels and interact with various organs and cells. The distribution of nanoparticles in the body can be affected by their physicochemical characteristics, including their size, shape and surface chemistry<sup>58</sup>. Therefore, understanding the systemic distribution of nanoparticles is crucial to the design of nanomedicines and to maximize delivery efficiency. To assess nanoparticle distribution, nanoparticles must be visualized and quantified in tissues. Electron microscopy<sup>4,8</sup> or fluorescence imaging<sup>3,59</sup> are typically used to visualize and track nanoparticles *in vivo*. However, electron microscopy can quantify only small numbers of cells, and imaging areas need to be manually chosen. Fluorescence imaging can reveal the overall distribution of nanoparticles but cannot provide resolution at the organ region or cell level (Fig. 2a). Alternatively, whole-body mouse imaging<sup>15,16</sup> can be combined with nanoparticle imaging to simultaneously visualize nanoparticles tagged with long-wavelength fluorescence labels and various cell types in the entire body. Specific tissues and cells can be selectively labelled by antibodies for imaging; for example, antibodies against CD11b can be used to label macrophages and monocytes, and antibodies against CD31 can be applied to label endothelial cells<sup>60</sup>. Simultaneous imaging of such markers and fluorescently tagged nanoparticles might allow the visualization of the systemic biodistribution of nanoparticles and investigation of their interactions with macrophages in different regions of the body, in particular, if combined with 3D imaging platforms<sup>17,61</sup>, for example, to compare the uptake of nanoparticles by liver-resident or tumour-resident macrophages.

Correlative super-resolution microscopy might be applied to visualize nanoparticle distribution at subcellular levels and nanoparticle interactions with receptors at molecular levels. Furthermore, by combining multiple imaging modalities of correlative super-resolution microscopy, different aspects of the same sample can be visualized at the nanoscale; for example, fluorescence and electron microscopy could be applied to visualize specific receptors and organelles, such as phagolysosomes<sup>62</sup>, respectively, to identify interactions of nanoparticles and their uptake in different cell types, such as tumour cells, tumour-associated macrophages and liver macrophages.

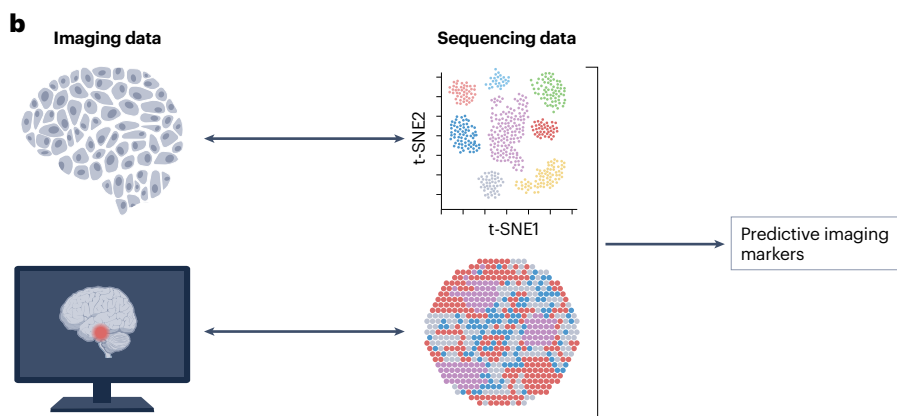
Imaging benefits from non-invasiveness and could further be combined with other multi-omics modalities, such as sequencing data, to predict nanoparticle delivery and clinical efficacy. For example, histological images of a tumour might be combined with genetic





**Fig. 2 | Imaging techniques to visualize nanoparticles.** **a**, Nanoparticles can be imaged by different microscopy techniques to localize nanoparticles and/or determine their biodistribution. '+' indicates information is available, '+++' indicates detailed information is available, and '-' indicates information is not available. **b**, Sequencing data could be combined with imaging data to predict the cellular response to nanoparticles. t-SNE, t-distributed stochastic neighbour embedding. Images in part **a** courtesy of Benjamin Schrank and Shiyang Dong.

Scope	▶		
Resolution	▶		
Systemic information	-	+	+++
Cellular information	+++	+++	-
Subcellular information	+++	+	-



information to assess nanomedicine efficacy. Similarly, MRI or CT data may be integrated with spatial transcriptomics to identify imaging markers that correlate with genetic or functional data to predict the response of an individual to a specific nanoparticle (Fig. 2b).

However, some nanoparticles, such as superparamagnetic iron oxide nanoparticles used in MRI, remain stable and can, thus, persist in tissue and be detected after labelled cells have died<sup>63</sup>. Moreover, the signal strength of nanoparticles in a cell is diluted if cells proliferate. When using fluorescent or protein labels, their stability needs to be considered to avoid missing signals from nanoparticles for which labels had been degraded<sup>64,65</sup>. Such limitations might complicate the interpretation of nanoparticle imaging results in longitudinal studies.

### Genetic regulation of nano–bio interactions

The investigation of transcriptional responses to nanoparticle uptake has typically relied on bulk RNA sequencing, which calculates the average amount of gene transcription per cell and is, thus, not suitable for heterogeneous cell populations, such as macrophages<sup>66</sup>, which

take up various amounts of nanoparticles. In addition, the amount of nanoparticles is averaged in bulk RNA sequencing, making the identification of specific cell populations with different uptake capacities impossible. Alternatively, single-cell transcription analysis can be applied to quantify the uptake of nanoparticles in individual cells by quantifying RNA or DNA barcodes on the nanoparticles<sup>67</sup>. This data can further be correlated with gene expression patterns to identify cell populations with high versus low nanoparticle uptake and signalling pathways related to uptake mechanisms<sup>67,68</sup>. For example, single-cell transcription analysis has shown that the capacity of the murine liver to take up nanoparticles, such as liposomal and polystyrene nanoparticles, varies with age owing to differences in macrophage composition and gene expression profiles<sup>68</sup>. Livers from older mice have less abundant tissue-resident macrophages and reduced expression of macrophage receptor with collagenous structure (MARCO), a scavenger receptor associated with nanoparticle uptake, corresponding to less clearance of nanoparticles, compared to livers from younger mice. Moreover, blocking of MARCO decreases the uptake of nanoparticles in the liver of

younger animals, but not in that of older animals, highlighting the need to design and apply age-appropriate nanoparticle delivery strategies<sup>68</sup>.

Sequencing techniques can also be applied to directly quantify nanoparticle uptake. For example, nanoparticles can be labelled with nucleic acids, such as RNA or DNA, to facilitate their detection in single cells by sequencing. The 'read numbers' of the specific RNA or DNA sequence then indicate the number of nanoparticles in a single cell. These data can also be correlated with gene expression patterns to assess cell populations with high and low nanoparticle uptake and identify pathways related to differences in uptake. For example, cellular heterogeneity with regard to lipid nanoparticle delivery to the liver in mice<sup>67</sup> has been studied by labelling lipid nanoparticles that carry mRNA encoding a glycosylphosphatidylinositol-anchored camelid single-variable domain on a heavy-chain antibody (aVHH) with DNA barcodes to identify recipient cell populations with variable target gene transcription and protein production at single-cell resolution. The gene expression profiles in each cell cluster are first analysed in cells without aVHH expression to assess background genes unrelated to lipid nanoparticle delivery. After subtracting the background genes, further analysis of gene expression profiles in cells with aVHH expression illustrates subtle heterogeneities in endothelial cells and Kupffer cell populations with differences in lipid nanoparticle uptake<sup>67</sup>. In addition, lipid nanoparticles show varying delivery efficiency into different cell types, depending on their formulation. The protein levels of aVHH can be further analysed through reading the DNA barcodes linked to anti-aVHH antibodies. The comparison of relative aVHH protein levels to lipid nanoparticle levels shows that lipid nanoparticle formulations may affect mRNA expression efficiency in the cell<sup>67</sup>. Furthermore, translational analysis could be applied to interrogate the factors that influence protein translation.

Different types of nanoparticles, including liposomes, polylactide-co-glycolide and polystyrene nanoparticles, have been tested with hundreds of DNA-barcoded cell lines to identify factors involved in nanoparticle uptake<sup>69</sup>. Interestingly, both the core materials and surface modifications of nanoparticles can influence their uptake by cells. Importantly, gene expression analysis allowed the identification of solute carrier family 46 member 3 (SLC46A3) as a predictive marker for lipid-based nanoparticle uptake<sup>69</sup>. Although such screening approaches may enable the identification of regulators of nanoparticle uptake, empty barcoded nanoparticles have mainly been tested thus far. However, drugs loaded within nanoparticles, including toxic chemotherapy agents, might also impact nano–bio interactions and nanoparticle uptake. In addition, proteins expressed following mRNA delivery may influence the expression of other proteins or alter the cellular response to subsequently delivered nanoparticles. Therefore, FDA-approved nanomedicines, such as liposomal doxorubicin or nanoparticle albumin-bound paclitaxel, should be barcoded to study factors associated with intra-organ distribution and cellular uptake.

Moreover, the genetic mechanisms and biological variations that regulate nanoparticle uptake should be explored. Several biological and physiological factors, including age<sup>68,70</sup> and sex<sup>70–72</sup>, impact nano–bio interactions in vivo, contributing to disparities in nanomedicine efficacy<sup>12,73</sup> and underscoring the need to account for these factors to improve the efficiency of nanoparticle delivery<sup>68,72</sup>. However, such personalized nanomedicine design would require a more comprehensive understanding of the regulatory mechanisms that drive such differences. Gene expression is regulated by numerous factors, including variant splicing of post-transcription mRNAs<sup>74</sup> and epigenetic modifications of DNA and chromatin, that influence the accessibility of

gene promoters to transcriptional factors<sup>75</sup>. In particular, epigenetics might be a key regulator of gene expression differences associated with sex<sup>76,77</sup> and ageing<sup>78</sup>.

Studies investigating epigenetics in the context of nano–bio interactions have, thus far, mainly focused on how nanomaterials influence the epigenetic regulation of cells<sup>79,80</sup> and on the use of nanoparticles to deliver drugs for epigenetic modulation<sup>81</sup>. However, how epigenetics might regulate nanoparticle clearance and cellular uptake remains unknown. Given the role of epigenetic regulation in ageing<sup>78</sup>, it might also affect the ability of cells to interact with nanoparticles. For example, the assay for transposase-accessible chromatin with sequencing (ATAC-seq) could be applied at the single-cell level and coupled with single-cell multi-omics techniques to screen for factors and pathways involved in nanoparticle uptake. Epigenomics and transcriptomics data could reveal regulation of biological function at different levels to identify differences in nanoparticle uptake by different cell types.

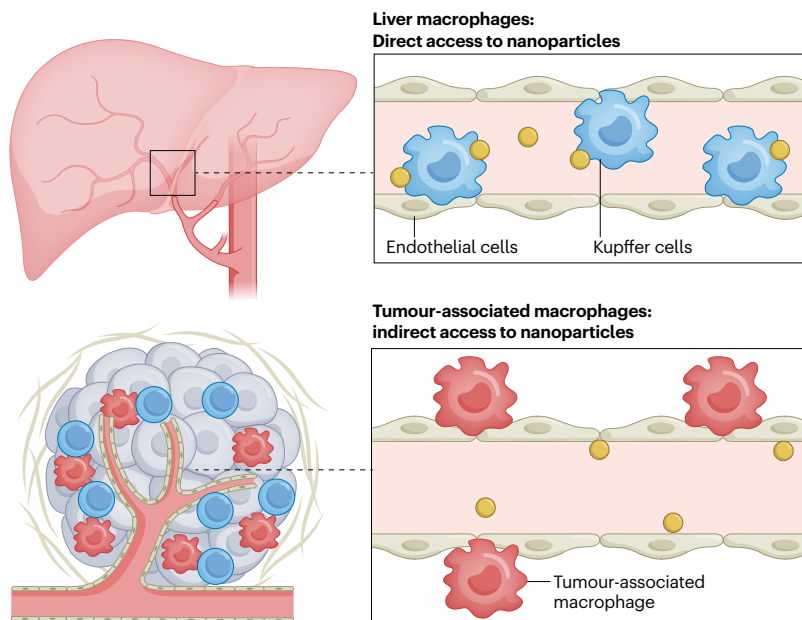
However, the analysis and interpretation of single-cell transcriptomics data<sup>82</sup> remain challenging owing to noise caused by low sample amount, doublets and batch effects, and the presence of transient cell states and cell heterogeneity<sup>83</sup>, in particular, considering the complex cellular responses to nanoparticles. For example, cells might induce transcriptive regulation of genes resulting in changes in gene expression profiles depending on the level of nanoparticle uptake. Therefore, it is difficult to untangle whether gene expression patterns cause efficient nanoparticle uptake or whether nanoparticle uptake induces these gene expression patterns. Thus, single-cell transcriptomics data should be validated by functional assays using genetic knockout, genetic knockdown or antibody blockade.

## Spatial context of nano–bio interactions

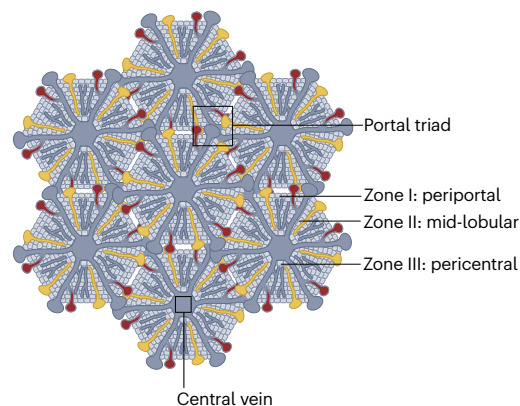
The spatial relationship between nanoparticles and cells is crucial in nanoparticle uptake because the accessibility of cells to nanoparticles is defined by their spatial location. Nanoparticles are typically unevenly distributed in organs and tumours owing to anatomical features, such as the vasculature<sup>60</sup>, and the access characteristics of different cell populations. For example, liver macrophages have direct access to nanoparticles in the blood, whereas tumour-associated macrophages are separated from nanoparticles by several biological barriers<sup>64,84</sup>. This difference in physical accessibility contributes to differences in nanoparticle uptake by macrophages (Fig. 3a). In addition, different functional zones within an organ can create diverse nano–bio interaction patterns. For example, liver lobules have periportal, mid-lobular and pericentral zones, according to their vicinity to portal vessels and central veins (Fig. 3b). Each of these liver zones is characterized by distinct cell types, metabolic characteristics and drug uptake functions<sup>85,86</sup>. Spatial RNA sequencing can be applied to identify spatially separated, distinct subtypes of liver macrophages that might affect the uptake and clearance of nanoparticles by the liver<sup>87</sup>.

Organ and tumour heterogeneity also creates spatially distinct zones of cell subtypes. For example, the presence of tumour-associated macrophages, which have spatially defined subtypes with distinct roles in tumour progression<sup>88</sup>, in areas with high cell turnover can be associated with good prognosis, whereas the presence of tumour-associated macrophages in necrotic regions predicts poor outcome<sup>88</sup>. In addition, tumours have diverse metabolic zones. Hypoxia caused by rapid tumour growth and poorly organized blood vessels might promote the metabolic programming of cells, which could impact their interactions with nanoparticles. For example, a spatially segregated group of hypoxic tumour-associated macrophages has been identified by

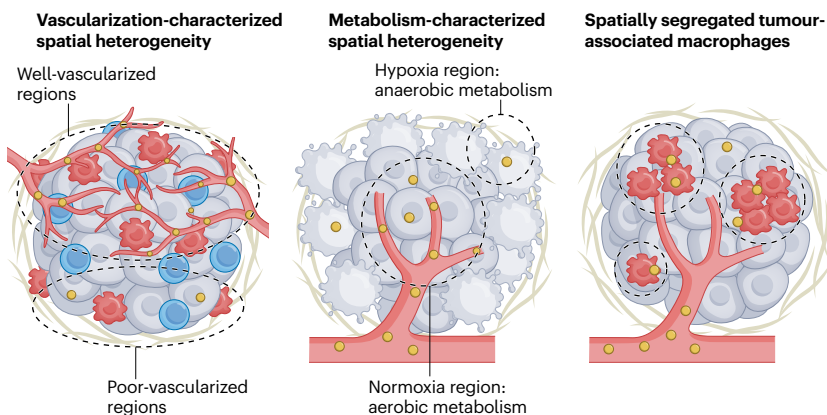
## a Direct versus indirect access to nanoparticles



## b Liver zonation

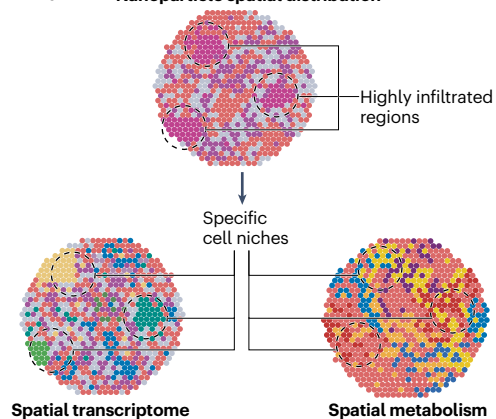


## c Spatially defined heterogeneous zones and cells in tumours



**Fig. 3 | Spatial aspects of nanoparticle biodistribution.** **a**, Macrophages can have direct or indirect access to nanoparticles. Liver macrophages (Kupffer cells) remain inside blood vessels and, thus, have direct access to circulating nanoparticles. Tumour-associated macrophages are localized outside blood vessels and, thus, have indirect access to circulating nanoparticles. **b**, The liver has different zones defined by their vicinity to the portal or central veins.

## d Nanoparticle spatial distribution



Each zone has a different cell composition, impacting nano–bio interactions. **c**, Tumours are heterogeneous tissues with regard to their microenvironment, immune cell composition, vascularization and metabolism, influencing the spatial distribution of nanoparticles and their interactions with cells. **d**, Nanoparticle distribution patterns might be assessed by spatially resolved transcriptomics and metabolomics.

spatial transcriptomics. These macrophages have the distinct function of modulating blood vessels in brain tumours<sup>89</sup> and are localized around peri-necrotic areas, where they secrete adrenomedullin, thereby destabilizing blood vessels. Blockade of adrenomedullin decreases the leakiness of blood vessels in these tumour regions, thereby increasing the delivery of anti-tumour agents. The presence of such spatially defined cell subtypes in tumours may also affect the distribution of nanoparticles and their subsequent interactions

with cells (Fig. 3c). However, how these cells interact with nanoparticles remains unknown.

Spatial scRNA-seq can be applied to assess information on the location of nanoparticles and cells in conjunction with the single-cell transcriptome<sup>26</sup>. The spatial distribution of nanoparticles can be determined by imaging their fluorescent labels or by sequencing RNA or DNA labels to identify the number of nanoparticles in distinct areas. Information on transcription or metabolism, obtained from sequencing, can

# Perspective

then be combined with spatial data to investigate the histologic, genetic and metabolic differences between these areas (Fig. 3d). For example, macrophages with high or low nanoparticle uptake could be compared in terms of gene expression profiles and their microenvironment.

## Interactions of nanoparticles with proteins

Proteomics can be applied to characterize the protein corona of nanoparticles, that is, the biomolecules on nanoparticles adsorbed from blood. The protein corona impacts the interactions between nanoparticles and cells and can be affected by nanoparticle surface modifications<sup>90,91</sup>. Genomics and proteomics analysis can be combined to assess the effects of the protein corona on nanoparticle uptake<sup>92</sup>. For example, by analysing the protein corona using mass spectrometry and by screening receptors on cancer cells through genetic knockout experiments, serum proteins and cell receptors that might be responsible for nanoparticle uptake in cancer cells could be identified. In particular, the interaction between gold apolipoprotein B-100 (ApoB; absorbed on gold nanoparticles) and low-density lipoprotein receptors on cells might mediate nanoparticle uptake<sup>92</sup>.

Mass spectrometry can also be used to directly quantify cellular uptake of nanoparticles. For example, inductively coupled plasma mass spectrometry (ICP-MS) can be applied to detect the uptake of titanium dioxide and silver nanoparticles<sup>93</sup>. Similarly, the uptake of gold nanoparticles by HeLa cells can be quantified by droplet chip ICP-MS<sup>94</sup>. The quantification of nanoparticles by mass spectrometry can also achieve single-cell resolution to obtain absolute nanoparticle numbers per cell and percentage of cells with nanoparticle uptake, as well as to distinguish between internalized versus surface-bound nanoparticles<sup>95</sup>. However, assessing the relationship between host cell proteomics and nanoparticle uptake remains challenging, in part because of the small amounts of protein present in single cells. Technical advances in single-cell proteomics, such as optimized sample preparation and delivery methods<sup>38,96</sup>, might overcome protein concentration issues, and nanoparticles can further be labelled to make them detectable by mass spectrometry to compare the proteomics of cells with high versus low nanoparticle uptake; for example, alkanethiol monolayers can be added to gold nanoparticles to serve as mass barcodes<sup>97</sup>.

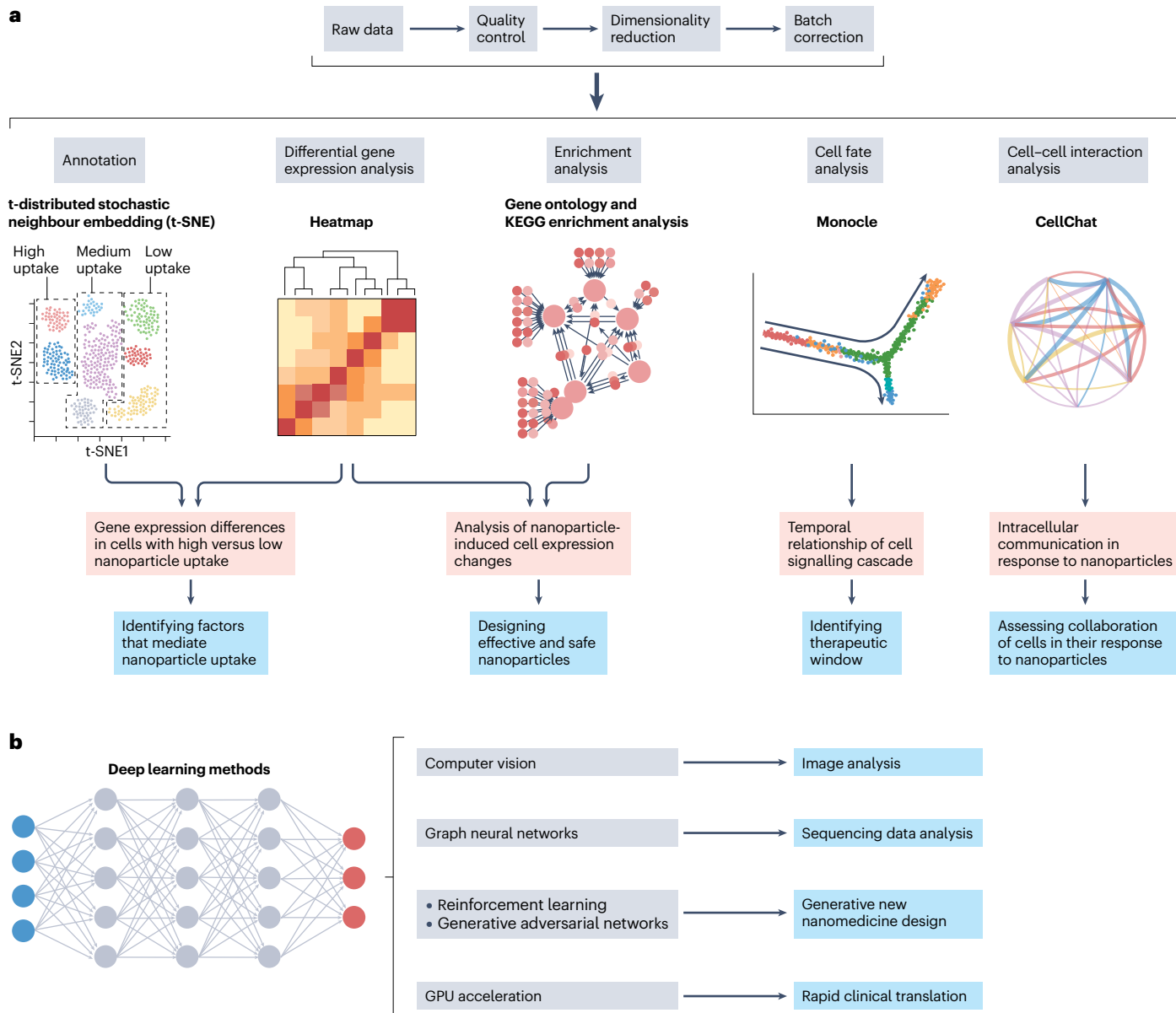
**Table 1 | Bioinformatics tools for multi-omics analysis**

Analysis	Purpose	Tools
Raw data processing	Align raw data to reference genome	Cellranger, UMI-tools zUMIs, Trim galore, STAR, featureCounts, Trimmomatic, RSEM, kallisto
Quality control	Remove low-quality cells	Seurat, Scanpy
Doublet detection	Remove doublets	DoubletFinder, scDblFinder, Scrublet, DoubletDetection, DoubletDecon
Data normalization	Adjust raw counts in the dataset for variable sampling effects by scaling the observable variance to a specified range	sctransform, scran deconvolution, CPM, logCPM <sup>144</sup> , TMM, DESeq <sup>145</sup> , quantile, Linnorm
Integration of multiple samples	Remove batch effect	CCA, R PCA, Harmony, MNN, fastMNN, BBKNN, Scanorama, limma, ComBat
Feature selection	Exclude uninformative genes that might not represent meaningful biological variation across samples	scLVM, BASiCS, M3Drop
ScaleData	Convert normalized gene expression to Z-scores	Seurat, Scanpy
Dimensionality reduction	Reduce data complexity and perform visualization	PCA, UMAP, t-SNE, scvis
Clustering	Identify cellular structure in the dataset	SC3, ZIFA, Destiny, SNN-Cliq, RaceID, SCUBA, BackSPIN, PAGODA, CIDR, pcaReduce, Seurat, Scanpy, TSCAN
Cell type annotation	Assess cellular identity of each cell	SingleR, SCSA, CellTypist, Cell-Blast, Tosica, scLearn, scID, scVI, MarkerCount, MARS
Differential gene expression analysis	Define differently expressed genes	Seurat, Scanpy, MAST, edgeR, DESeq2, t-Test, scDE, Pseudobulk
Enrichment analysis	Determine enriched pathways in a cell type-specific manner	GO, KEGG, GSEA, ssGSEA, GSVA, Reactome
Cell fate analysis	Track cell type development	Monocle, Slingshot, PAGA, DPT, Cellrank, SLICER, MST, SCUBA, TSCAN, MFA, FateID, URD, scvelo
Cell-cell communication analysis	Reveal interactions between cell types	CellChat, CellPhoneDB, NicheNet, scTensor, CellTalkDB
Spatial transcriptomics analysis	Process spatial transcriptome data	Seurat, Scanpy, Steropy
Single-nucleus ATAC-seq data analysis	Process single-nucleus ATAC-seq data	Muon, snapATAC, pyCisTopic, Signac, ArchR

ATAC-seq, transposase-accessible chromatin with sequencing.



# Perspective



**Fig. 4 | Bioinformatics and artificial intelligence strategies for analysing multi-omics data of nano-bio interactions.** **a**, The analysis of sequencing data requires the processing of raw data and its mapping to the genome. Downstream analyses include cell clustering annotation, differential gene expression analysis,

enrichment analysis, cell fate analysis and cell-cell interaction analysis. **b**, Artificial intelligence can be applied to investigate the complex data generated in multi-omics analyses. GPU, graphics processing unit; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Proteomics might further enable the study of cell responses to nanoparticle uptake. For example, proteomics might be applied to evaluate the levels of protein expression, following mRNA delivery by lipid nanoparticles, and investigate influences on other proteins in the cells. In addition, the production of inflammatory proteins and cytokines in response to nanoparticles could be assessed by proteomics<sup>98,99</sup>. Proteomics can further be combined with other omics data, such as transcriptomics, to assess regulatory networks following nanoparticle uptake and effects of post-translational

modifications, such as phosphorylation, ubiquitination and SUMOylation.

However, mass spectrometry-based proteomics analysis can have limited reproducibility in certain applications, probably owing to variations in sample preparation protocols, low stability of serum proteins, technical variability between instruments, and the sensitivity of mass spectrometers<sup>100–102</sup>. For example, proteomics analysis of protein corona formation on polystyrene nanoparticles has led to different outcomes<sup>100</sup>. Therefore, detailed protocols and parameters of sample

## Box 1 | Experimental design considerations for multi-omics studies of nano–bio interactions

### Nanoparticle injection route

The injection route is an important consideration for nanoparticle screening. In vitro injection into cell lines facilitates homogeneous incubation. In vivo, direct intra-organ injection (for example, intratumoural injection) may cause inhomogeneous distribution of nanoparticles. Therefore, intra-organ injection requires prior testing by experiments, such as immunofluorescent staining on tissue slides, to optimize timing, dosage and injection speed before proceeding with spatial sequencing to ensure that the sequenced sample is homogeneous and free of bias. Intravenous injection might enable targeted delivery to organs but is limited by liver clearance. Therefore, pilot experiments should be performed to ensure that there is a sufficient number of detectable nanoparticles at the target site before sequencing. Regardless of the injection route, spatial transcriptome analysis is typically performed in small, manually selected areas, in contrast to single-cell sequencing, for which cells are typically sorted by flow cytometry. Thus, immunofluorescence analysis of selected areas should be performed in addition to sequencing to ensure that the area and data are unbiased and representative.

### Nanoparticle dose and concentration

Low concentrations of nanoparticles might lead to weak signals. Conversely, high concentrations of nanoparticles might cause tissue and cellular toxicity and signal saturation. For example, the dosage threshold to saturate liver uptake of intravenously injected polyethylene glycol-conjugated gold nanoparticles is 1 trillion nanoparticles in mice<sup>155</sup>. Doses above this threshold exceed the clearance capacity of liver macrophages and, thus, substantially increase their tumour delivery efficiency<sup>155</sup>. Therefore, different dosages should be tested to generate a dose–response curve and identify the optimal dose for multi-omics assays.

### Assay rigour and robustness

Nanoparticles are typically detected through specific labels, which must remain functional during organ or cell harvest and throughout the sample preparation process. Therefore, at least three different sequences of DNA, RNA and peptide barcodes should be used for the same nanoparticle. Moreover, the homogeneous distribution of barcodes increases confidence in the robustness of the assay.

preparation and instrument setup should be reported to improve the reproducibility of mass spectrometry-based proteomics.

### Effects of nanoparticles on the metabolism

Metabolomics analysis can provide useful insights into nano–bio interactions, for example, by evaluating the toxicity of nanoparticles<sup>103–105</sup>. Small-molecule metabolites can be assessed at the single-cell level, coupled with genetic and spatial information, to characterize different regions of cellular metabolism<sup>32</sup>. Such approaches could also be applied to explore the relationship between specific metabolism zones and nanoparticle uptake, as well as metabolic responses to nanoparticles. In addition, lipids are major components of both cell membranes and lipid-based nanoparticles, and they can influence how nanoparticles interact with other molecules and cells. For example, cholesterol affects the composition of the protein corona on silica nanoparticles and subsequently influences their uptake and inflammatory responses in macrophages both in vitro and in vivo<sup>106</sup>. Therefore, lipidomics analysis would allow the investigation of how lipids might influence the pharmacokinetics and biological fate of nanoparticles and how lipid nanoparticles may affect lipid metabolism networks in cells.

### Analysing multi-omics data for nano–bio interactions

Complex multi-omics data can be analysed by bioinformatics approaches. Various algorithms can be applied to analyse individual analytes in single cells<sup>107–109</sup> and to integrate measurements across multiple modalities<sup>14,110,111</sup>, each capturing different aspects of cellular identity to assess differentiation trajectories, cell–cell interactions, and microenvironmental and spatial organization (Table 1). Such algorithms could also be adapted to investigate nano–bio interactions (Fig. 4a).

### Differential and enrichment analysis

Differential analysis identifies genes that significantly vary between different clusters, and enrichment analysis assesses the functions of these genes. The investigation of nano–bio interactions might also benefit from differential and enrichment analysis, for example, to compare cells with different nanoparticle uptake abilities within the same gene expression profile cluster. For example, differential analysis of single-cell RNA sequencing data has been applied to identify different subtypes of endothelial cells and their transcription features that dictate lipid nanoparticle delivery efficiency in mice<sup>67</sup>. Furthermore, differential analysis following bulk RNA sequencing could reveal different gene expression patterns in cells relating to high versus low nanoparticle uptake<sup>112</sup>. However, such approaches require statistical methods to include nanoparticle uptake as a variable in addition to gene expression. Differential analysis could also be used to analyse the influence of nanoparticle size, charge or loaded drug on gene expression<sup>9</sup> by identifying cell clusters and pathways that are affected by nanoparticles. Such data could inform nanoparticle designs that avoid or target specific cells to increase delivery efficiency or decrease toxicity.

### Cell fate analysis

Cell fate analysis, or pseudotime analysis, can be applied to infer cell differentiation sequences by using data at one time point to calculate the developmental and differentiation order of cells, that is, the temporal aspect of gene expression regulation. Cell fate analysis could also shed light on nano–bio interactions, which typically involve several sequential signalling cascades<sup>1</sup>. Instead of capturing a few pre-selected time points, cell fate analysis could estimate the sequence of activation of different cell pathways following contact with nanoparticles. Temporal knowledge of nano–bio interactions may help identify therapeutic windows to optimize the timing and dosing of nanoparticles.

## Cell communication analysis

Analyses of cell–cell communication may enable an understanding of how different cell types respond to nanoparticles and how nanoparticles impact their communication. Nanoparticles can travel through different cells in tissues and get transported in and out of organs<sup>4,8,113</sup>. Although the interactions between nanoparticles and cells have been widely explored<sup>114</sup> and strategies have been developed to achieve more efficient drug delivery by exploiting nanoparticle–cell communication<sup>115,116</sup>, how different types of cells communicate with each other to collaborate on nanoparticle transport has not yet been well studied, and how the spatial organization of cells influences communication and the fate of nanoparticles is not known. Bioinformatics-based communication analysis in single-cell sequencing might help explore such communication. By depicting cell–cell communication in the context of nanoparticle interactions and spatial considerations, certain intracellular communication pathways could be blocked to modulate nanoparticle delivery or fate. Similarly, nanoparticles could be designed to regulate cell communication.

## Artificial intelligence and machine learning

Artificial intelligence (AI) may greatly aid in the analysis of complex and big data sets, typical for multi-omics analyses, and may prove especially powerful if additional variables, such as nanoparticles, are added to the picture. Therefore, AI is being explored for the investigation of nano–bio interactions based on various data types.

## Computer vision for image analysis

Imaging of nanoparticles in the context of differently labelled cells generates complex data sets that might involve not only nanoparticles and cells but also other elements, such as blood vessels and macrophages, making manual quantification difficult and time consuming and data interpretation challenging. AI can be trained to process and recognize imaging information, transform it into data points, and use algorithms to calculate underlying patterns. For example, computer

vision, that is, machine learning combined with image segmentation techniques to allow the interpretation of visual information<sup>117</sup>, was applied to quantify nanoparticle delivery and biodistribution in mice<sup>118</sup> by analysing data from more than 30 xenograft and orthotopic tumour models to quantitatively assess the effects of different tumour vascular permeabilities on nanoparticle delivery. Similarly, machine learning can be applied to analyse microscopic images and quantify green fluorescent protein (GFP) signals in individual cells following the delivery of GFP mRNA by lipid nanoparticles<sup>119</sup>. Furthermore, cell morphology information and GFP expression data were used to train an AI model to predict GFP expression before mRNA delivery<sup>119</sup>.

## Big data analysis

Applying multi-omics data for the study of nano–bio interactions requires sequencing or mass spectrometry data of the cell to be organized and correlated in relation to nanoparticle uptake or distribution. Deep learning models, trained on multi-omics data, can be used to identify drug action targets or to distinguish cell types at the level of the individual cell<sup>120</sup>. For example, DeepLinc<sup>121</sup>, which uses deep learning analysis, allows the de novo reconstruction of cell interaction networks from single-cell spatial transcriptomics data, and it has been applied to establish models that correlate cellular molecular features with physiological and pathological states<sup>121</sup>. Moreover, graph neural networks (GNNs), deep learning models that can process graph-structured data, are useful to capture and learn from relational information within biological systems, thereby allowing the modelling and analysis of complex biological networks, such as protein–protein interaction prediction, drug discovery, gene expression analysis and biological network analysis<sup>122</sup>. For example, GNNs can be applied to predict the interactions between biomolecules, such as the affinity of protein–ligand interactions from the structure of protein–ligand complexes<sup>123</sup>, and nanoscale interactions in proteins and nanoparticles<sup>124</sup>. GNNs have also been used to train a model with thousands of chemically diverse lipids to establish a platform for lipid nanoparticle design<sup>125</sup>.

**Table 2 | Nanomaterial labelling strategies for multi-omics studies**

Application	Label	Nanoparticle type	Labelling strategies	Refs.
Imaging	Fluorescence label	Protein-based nanoparticles	Carbodiimide reaction or isocyanate reaction	59,68,118
		Gold nanoparticles	Thiol–gold interaction	113
		Lipid nanoparticles	Electrostatic interactions or carbodiimide reaction	119
		Poly (lactic-co-glycolic acid) nanoparticle	Physical loading through hydrophobic interactions	69
		Polystyrene nanoparticle	Physical loading through hydrophobic interactions	69
	Label-free	Gold nanoparticles	Polymerization of acryloyl-modified proteins	67
Sequencing	RNA	Lipid nanoparticles	Electrostatic interactions	10,67,146
		Silica nanoparticles	Carbodiimide reaction	147
	DNA	Lipid nanoparticles	Electrostatic interactions	148,149
		Gold nanoparticles	Thiol–gold interaction	150,151
		Silica nanoparticle	Streptavidin–biotin interaction	152
Mass spectrometry	Organic molecules	Gold or other metal nanoparticles	Physical interactions	92
			Thiol–gold interaction with different ligands	95,97,153
	Heavy metal	Gold or other metal nanoparticles	Physical mixture with isotopes of metal ions	93
	Label-free	Superparamagnetic iron oxide nanoparticles	Magnetic enrichment	154

Furthermore, GNNs can be trained on multi-omics data; for example, the universal graph neural network scMoGNN creates models of different modalities of single cells separately, constructing networks based on single-cell sequencing data and incorporating biological knowledge from different omics analyses as additional structural information into the graph network<sup>126</sup>, thereby capturing higher-order structural relationships between cells and modalities. Thus, GNNs might also enable the construction of a network of cells, features, environments and nanoparticle characteristics. It might be possible to further integrate cellular response data, such as gene transcription or protein expression data from multi-omics studies, with protein and lipid libraries, thereby enabling the design of nanoparticles that achieve specific cell responses.

## Predicting and optimizing nanoparticle fate

Similar to how generative AI models can be applied for designing new proteins<sup>127</sup>, reinforcement learning, supervised learning and generative adversarial network (GAN) models can be explored for the design of nanoparticles<sup>128</sup>. Such models can be further combined with clinical metadata, such as disease stage, age and sex, to personalize nanoparticle-based treatments. Reinforcement learning and GANs both enable the optimization of specific properties of proteins, molecules and antibodies<sup>129</sup>. Reinforcement learning is a machine learning approach focused on decision-making and learning from interactions with an environment, and it has been particularly explored in drug discovery to optimize the selection of potential drug candidates through chemical screening<sup>130,131</sup>. Reinforcement learning can also be applied to predict the cytotoxicity of inorganic nanoparticles by training an AI model with toxicity data of metal and metal-oxide nanoparticles from more than 100 published papers and repositories<sup>132</sup>. In addition,

reinforcement learning enables the top-down design of protein-based nanomaterials with desired systemic properties using Monte Carlo tree search, which helps the system explore different possibilities for how protein parts might fit together<sup>128</sup>. Supervised learning enables the algorithm to learn from labelled data input and output to find patterns and predict future output based on new data. This approach has been used to predict the tumour accumulation of nanoparticles by learning the relationship between biological factors and nanoparticle delivery<sup>133,134</sup>.

GANs are deep learning models consisting of a generator and a discriminator. GANs can be used to generate synthetic data for training models, simulate biological processes and produce realistic medical images for diagnostic purposes<sup>135,136</sup>. Similarly, supervised learning has been applied to analyse mass spectrometry data of the protein corona at different time points following nanoparticle injection into mice. The protein corona composition and organ accumulation data were then used to train a model that predicts nanoparticle delivery efficiency based on corona proteins<sup>137</sup>.

## Accelerating clinical translation

Multi-omics and clinical data can be integrated to predict disease outcome and assist personalized clinical decision-making<sup>138,139</sup>. Multi-omics and clinical data could also help to select an optimized personalized nanoparticle-based drug delivery platform. However, multi-omics data analysis is typically time consuming and requires substantial computational resources, which may cause delay in clinical decision-making. The capability of AI to rapidly process complex data<sup>140</sup> makes it a promising tool for solving this problem. In particular, high-performance heterogeneous graphics processing units and central processing unit computing clusters can accelerate the processing of multi-omics data<sup>141,142</sup>. For example, gene sequencing analysis requires loading of

## Box 2 | Consideration of biological variations

### Sexual dimorphism

Sex is a crucial factor in nano-bio interactions<sup>143</sup>. In particular, nanoparticle uptake<sup>71</sup>, toxicity<sup>156</sup> and immune responses<sup>157</sup> differ between sexes, partly owing to genetic and epigenetic differences that contribute to sex-specific biomolecules, immunity and disease environments<sup>143</sup>. In addition, hormone levels, pregnancy, menopause and serum protein composition might impact nano-bio interactions<sup>143</sup>. For example, the nanoparticle uptake ability of human amniotic stem cells from female donors is greater than the nanoparticle uptake ability of those from male donors<sup>71</sup>. Interestingly, the opposite trend can be observed in primary salivary gland-derived fibroblasts<sup>71</sup>. Therefore, sex should be considered in preclinical and clinical study designs of nano-bio interactions.

### Age

Systemic, cellular, genetic and epigenetic changes during ageing might impact responses to nanoparticles and should, thus, be considered in experimental design<sup>12</sup>. For example, the pharmacokinetics of nanoparticles might differ between young and ageing individuals<sup>70,158</sup>. Similarly, a population shift in liver macrophages can change nanoparticle clearance in aged mice<sup>68</sup>. Changes in macrophage receptor expression during ageing might also alter the effectiveness of nanoparticle delivery. For example, liver

clearance of nanoparticles can decline with age, thereby increasing the efficiency of tumour delivery and improving anti-tumoural responses in older animals<sup>68</sup>, owing to a decrease in the expression of the scavenger receptor macrophage receptor with collagenous structure (MARCO) in young liver macrophages, compared to aged macrophages, as shown by single-cell transcriptome analysis. Accordingly, blockade of MARCO increases tumour delivery of nanoparticles only in young mice but not in aged mice. Therefore, age-related changes should be considered in the design of experiments, in particular, if nanoparticles are designed for diseases that are common in aged individuals, such as cancer.

### Animal strains

Different strains of the same animal species might differ in their vasculature<sup>159</sup>, behaviour, such as anxiety<sup>160</sup>, and metabolism<sup>161</sup>. On the cellular level, animals of different strains may use different receptors for the same function. For example, alveolar macrophages of C57BL/6 and BALB/c mice express different scavenger receptors, which are responsible for silica uptake<sup>162</sup>. Therefore, it should be determined whether observations are strain-specific or whether they apply to the entire species. Ideally, at least two different animal strains should be tested, in addition to validation studies in human samples or genetic or proteomic databases.



the entire genome data into memory at one time. The correspondingly long analysis time may lead to delays in clinical decision-making. Using graphics processing unit acceleration and deep learning models for the integration of multi-omics data<sup>140</sup>, AI can substantially shorten the time needed from sequencing completion to data output (Fig. 4b).

## Designing multi-omics studies of nano–bio interactions

Multi-omics studies of nano–bio interactions must be designed in a way to ensure reproducibility and robustness (Box 1). Nanoparticles detected and quantified by imaging, sequencing or mass spectrometry experiments must be robustly and correctly labelled to ensure their reliable and robust detection (Table 2). Therefore, distinct labelling strategies are needed, tailored to the type of assay and nanoparticle. For example, barcoding of nanoparticles by DNA, RNA, peptide or isotope tags allows detection and quantification by single-cell sequencing and/or mass spectrometry. In addition, distinct experimental design needs to be considered to ensure reliable outcomes and reduce bias (Box 2).

## Outlook

A thorough understanding of the mechanisms underlying the interactions between nanoparticles and biological systems will greatly aid in the design of nanomedicines, for example, by improving the delivery of nanoparticles to specific organs or cell types or by modulating interactions with the immune system. Multi-omics analysis might enable the multidimensional assessment of nano–bio interactions in a physiologically relevant context, leveraging and combining different types of data and information. However, determining the in vivo biodistribution of nanoparticles remains challenging, as imaging techniques to visualize nanoparticles in the body remain limited in terms of dimension and depth. Moreover, technical issues regarding nanoparticle imaging labels with various stability can complicate the interpretation of imaging results. This limitation may not have a simple solution and might require validation by multiple experiments. Nanoparticles can be labelled with nucleic acids or peptide barcodes to allow their detection for sequencing and mass spectrometry experiments, and cellular uptake and responses can be quantified at single-cell resolution. Furthermore, imaging tools can be combined with multi-omics technologies to investigate the in vivo biodistribution of nanoparticles, for example, by combining immune-labelled whole-body imaging with 3D tissue imaging. In addition, correlative super resolution microscopy can assess the interactions of nanoparticles with cells and biomolecules at a sub-cellular level. However, depicting spatial and temporal nanoparticle trajectories remains challenging.

Importantly, variations in sex, age and other factors of cells and animal models used in nano–bio interaction studies have resulted in conflicting results<sup>68,143</sup> (Box 2). Therefore, such factors need to be considered and reported. Importantly, genetic and epigenetic heterogeneity further contribute to variable cellular responses to nanoparticles and should, thus, be accounted for in the investigation of nano–bio interactions by applying multi-omics technologies that can reveal genetic and epigenetic information at the single-cell level. Moreover, the spatial relationship between cells and nanoparticles and how spatial heterogeneity of cells contributes to variations in their interactions with nanoparticles should be explored.

The cellular responses to nanoparticles can be characterized by transcriptomics, proteomics and metabolomics analysis to identify

differences between heterogeneous cell populations of variable uptake ability or cellular response to nanoparticles and probably to also identify new regulators of cellular responses. Importantly, barcoding and sequencing of nanoparticles might shed light on the relationship between nanoparticle design and its efficacy, for example, through simultaneous multi-omics analysis of different formulations of nanoparticles in one animal model<sup>67</sup>. The mechanistic study of genetic and epigenetic regulation of nano–bio interactions might further aid in the design of personalized or recipient-tailored nanoparticles. For example, how nanoparticle design might affect mRNA delivery and protein expression can be characterized by transcriptomics and proteomics, respectively. The information obtained from different omics experiments can also be cross-compared, with the help of AI and bioinformatics tools, to reveal regulatory mechanisms and fill knowledge gaps in nano–bio interactions.

## Citation diversity statement

We acknowledge that papers authored by scholars from historically excluded groups are systematically under-cited. Here, we have made every attempt to reference relevant papers in a manner that is equitable in terms of racial, ethnic, gender and geographical representation.

Published online: 18 December 2024

## References

1. Mitchell, M. J. et al. Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discov.* **20**, 101–124 (2021).
2. Yardley, D. A. nab-Paclitaxel mechanisms of action and delivery. *J. Control. Rel.* **170**, 365–372 (2013).
3. Wilhelm, S. et al. Analysis of nanoparticle delivery to tumours. *Nat. Rev. Mater.* <https://doi.org/10.1038/natrevmats.2016.14> (2016).
4. Sindhwani, S. et al. The entry of nanoparticles into solid tumours. *Nat. Mater.* **19**, 566 (2020).
5. Blanco, E., Shen, H. & Ferrari, M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* **33**, 941–951 (2015).
6. Ouyang, B. et al. Impact of tumor barriers on nanoparticle delivery to macrophages. *Mol. Pharm.* **19**, 1917–1925 (2022).
7. Jiang, W. et al. Designing nanomedicine for immuno-oncology. *Nat. Biomed. Eng.* **1**, 0029 (2017).
8. Nguyen, L. N. M. et al. The exit of nanoparticles from solid tumours. *Nat. Mater.* **22**, 1261–1272 (2023).
9. Jiang, W., Kim, B. Y., Rutka, J. T. & Chan, W. C. Nanoparticle-mediated cellular response is size-dependent. *Nat. Nanotechnol.* **3**, 145–150 (2008).
10. Hatit, M. Z. C. et al. Species-dependent in vivo mRNA delivery and cellular responses to nanoparticles. *Nat. Nanotechnol.* **17**, 310–318 (2022).
11. Horie, M., Kato, H., Fujita, K., Endoh, S. & Iwahashi, H. In vitro evaluation of cellular response induced by manufactured nanoparticles. *Chem. Res. Toxicol.* **25**, 605–619 (2012).
12. Jiang, W., Wang, Y., Wargo, J. A., Lang, F. F. & Kim, B. Y. S. Considerations for designing preclinical cancer immune nanomedicine studies. *Nat. Nanotechnol.* **16**, 6–15 (2021).
13. Hasin, Y., Seldin, M. & Lusis, A. Multi-omics approaches to disease. *Genome Biol.* **18**, 83 (2017).
14. Baysoy, A., Bai, Z., Satija, R. & Fan, R. The technological landscape and applications of single-cell multi-omics. *Nat. Rev. Mol. Cell Biol.* **24**, 695–713 (2023).
15. Cai, R. et al. Panoptic imaging of transparent mice reveals whole-body neuronal projections and skull-meninges connections. *Nat. Neurosci.* **22**, 317–327 (2019).
16. Mai, H. et al. Whole-body cellular mapping in mouse using standard IgG antibodies. *Nat. Biotechnol.* **42**, 617–627 (2023).
17. Kuett, L. et al. Three-dimensional imaging mass cytometry for highly multiplexed molecular and cellular mapping of tissues and the tumor microenvironment. *Nat. Cancer* **3**, 122–133 (2022).
18. Hauser, M. et al. Correlative super-resolution microscopy: new dimensions and new opportunities. *Chem. Rev.* **117**, 7428–7456 (2017).
19. Navikas, V. et al. Correlative 3D microscopy of single cells using super-resolution and scanning ion-conductance microscopy. *Nat. Commun.* **12**, 4565 (2021).
20. Grandi, F. C., Modi, H., Kampman, L. & Corces, M. R. Chromatin accessibility profiling by ATAC-seq. *Nat. Protoc.* **17**, 1518–1552 (2022).
21. Wang, M. et al. Single-nucleus multi-omic profiling of human placental syncytiotrophoblasts identifies cellular trajectories during pregnancy. *Nat. Genet.* **56**, 294–305 (2024).
22. Hu, Y. et al. Simultaneous profiling of transcriptome and DNA methylome from a single cell. *Genome Biol.* **17**, 88 (2016).

23. Hou, Y. et al. Single-cell triple omics sequencing reveals genetic, epigenetic, and transcriptomic heterogeneity in hepatocellular carcinomas. *Cell Res.* **26**, 304–319 (2016).
24. Goltsev, Y. et al. Deep profiling of mouse splenic architecture with CODEX multiplexed imaging. *Cell* **174**, 968–981.e15 (2018).
25. Black, S. et al. CODEX multiplexed tissue imaging with DNA-conjugated antibodies. *Nat. Protoc.* **16**, 3802–3835 (2021).
26. Rao, A., Barkley, D., Franca, G. S. & Yanai, I. Exploring tissue architecture using spatial transcriptomics. *Nature* **596**, 211–220 (2021).
27. Stuart, T. et al. Comprehensive integration of single-cell data. *Cell* **177**, 1888–1902.e21 (2019).
28. Dong, K. & Zhang, S. Deciphering spatial domains from spatially resolved transcriptomics with an adaptive graph attention auto-encoder. *Nat. Commun.* **13**, 1739 (2022).
29. Chen, A. et al. Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays. *Cell* **185**, 1777–1792.e21 (2022).
30. Xiao, Z. et al. 3D reconstruction of a gastrulating human embryo. *Cell* **187**, 2855–2874.e19 (2024).
31. Simeth, J. et al. Virtual tissue expression analysis. Preprint at *bioRxiv* <https://doi.org/10.1101/2023.11.16.567357> (2023).
32. Vicari, M. et al. Spatial multimodal analysis of transcriptomes and metabolomes in tissues. *Nat. Biotechnol.* **42**, 1046–1050 (2023).
33. Zhang, D. et al. Spatial epigenome-transcriptome co-profiling of mammalian tissues. *Nature* **616**, 113–122 (2023).
34. Zhang, B. et al. Proteogenomic characterization of human colon and rectal cancer. *Nature* **513**, 382–387 (2014).
35. Larsson, A. J. M. et al. Genomic encoding of transcriptional burst kinetics. *Nature* **565**, 251–254 (2019).
36. Mund, A., Brunner, A. D. & Mann, M. Unbiased spatial proteomics with single-cell resolution in tissues. *Mol. Cell* **82**, 2335–2349 (2022).
37. Graves, P. R. & Haystead, T. A. Molecular biologist's guide to proteomics. *Microbiol. Mol. Biol. Rev.* **66**, 39–63 (2002).
38. Bennett, H. M., Stephenson, W., Rose, C. M. & Darmanis, S. Single-cell proteomics enabled by next-generation sequencing or mass spectrometry. *Nat. Methods* **20**, 363–374 (2023).
39. Woo, J. et al. High-throughput and high-efficiency sample preparation for single-cell proteomics using a nested nanowell chip. *Nat. Commun.* **12**, 6246 (2021).
40. Ahmad, R. & Budnik, B. A review of the current state of single-cell proteomics and future perspective. *Anal. Bioanal. Chem.* **415**, 6889–6899 (2023).
41. Norris, J. L. & Caprioli, R. M. Analysis of tissue specimens by matrix-assisted laser desorption/ionization imaging mass spectrometry in biological and clinical research. *Chem. Rev.* **113**, 2309–2342 (2013).
42. Mund, A. et al. Deep visual proteomics defines single-cell identity and heterogeneity. *Nat. Biotechnol.* **40**, 1231–1240 (2022).
43. Stoekius, M. et al. Simultaneous epitope and transcriptome measurement in single cells. *Nat. Methods* **14**, 865–868 (2017).
44. Liu, Y. et al. High-plex protein and whole transcriptome co-mapping at cellular resolution with spatial CITE-seq. *Nat. Biotechnol.* **41**, 1405–1409 (2023).
45. Clish, C. B. Metabolomics: an emerging but powerful tool for precision medicine. *Cold Spring Harb. Mol. Case Stud.* **1**, a000588 (2015).
46. Yang, K. & Han, X. Lipidomics: techniques, applications, and outcomes related to biomedical sciences. *Trends Biochem. Sci.* **41**, 954–969 (2016).
47. Mapstone, M. et al. Plasma phospholipids identify antecedent memory impairment in older adults. *Nat. Med.* **20**, 415–418 (2014).
48. Wang, T. J. et al. Metabolite profiles and the risk of developing diabetes. *Nat. Med.* **17**, 448–453 (2011).
49. Mayers, J. R. et al. Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. *Nat. Med.* **20**, 1193–1198 (2014).
50. Johnson, C. H., Ivanisevic, J. & Siuzdak, G. Metabolomics: beyond biomarkers and towards mechanisms. *Nat. Rev. Mol. Cell Biol.* **17**, 451–459 (2016).
51. Ingolia, N. T., Brar, G. A., Rouskin, S., McGeachy, A. M. & Weissman, J. S. The ribosome profiling strategy for monitoring translation in vivo by deep sequencing of ribosome-protected mRNA fragments. *Nat. Protoc.* **7**, 1534–1550 (2012).
52. Wang, T. et al. Translating mRNAs strongly correlate to proteins in a multivariate manner and their translation ratios are phenotype specific. *Nucleic Acids Res.* **41**, 4743–4754 (2013).
53. Zhao, J., Qin, B., Nikolay, R., Spahn, C. M. T. & Zhang, G. Translatomics: the global view of translation. *Int. J. Mol. Sci.* **20**, e20010212 (2019).
54. Ozadam, H. et al. Single-cell quantification of ribosome occupancy in early mouse development. *Nature* **618**, 1057–1064 (2023).
55. Zeng, H. et al. Spatially resolved single-cell translatomics at molecular resolution. *Science* **380**, eadd3067 (2023).
56. Guo, Y., Yan, S. & Zhang, W. Translatomics to explore dynamic differences in immunocytes in the tumor microenvironment. *Mol. Ther. Nucleic Acids* **34**, 102037 (2023).
57. Li, S. D. & Huang, L. Pharmacokinetics and biodistribution of nanoparticles. *Mol. Pharm.* **5**, 496–504 (2008).
58. Kulkarni, S. A. & Feng, S. S. Effects of particle size and surface modification on cellular uptake and biodistribution of polymeric nanoparticles for drug delivery. *Pharm. Res.* **30**, 2512–2522 (2013).
59. Li, R. et al. Therapeutically reprogrammed nutrient signalling enhances nanoparticulate albumin bound drug uptake and efficacy in KRAS-mutant cancer. *Nat. Nanotechnol.* **16**, 830–839 (2021).
60. Chen, Y. et al. Therapeutic remodeling of the tumor microenvironment enhances nanoparticle delivery. *Adv. Sci.* **6**, 1802070 (2019).
61. Sheth, V. et al. Quantifying intracellular nanoparticle distributions with three-dimensional super-resolution microscopy. *ACS Nano* **17**, 8376–8392 (2023).
62. Andrian, T. et al. Super-resolution correlative light-electron microscopy using a click-chemistry approach for studying intracellular trafficking. *Methods Cell Biol.* **162**, 303–331 (2021).
63. Samrot, A. V., Sahithya, C. S., Selvarani, J. A., Purayil, S. K. & Ponnaiah, P. A review on synthesis, characterization and potential biological applications of superparamagnetic iron oxide nanoparticles. *Curr. Res. Green Sustain. Chem.* **4**, 100042 (2021).
64. Stepanenko, O. V. et al. Comparative studies on the structure and stability of fluorescent proteins EGFP, zFP506, mRFP1, “dimer2”, and DsRed1. *Biochemistry* **43**, 14913–14923 (2004).
65. Campbell, B. C., Paez-Segala, M. G., Looger, L. L., Petsko, G. A. & Liu, C. F. Chemically stable fluorescent proteins for advanced microscopy. *Nat. Methods* **19**, 1612–1621 (2022).
66. Gordon, S. & Taylor, P. R. Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* **5**, 953–964 (2005).
67. Dobrowolski, C. et al. Nanoparticle single-cell multiomic readouts reveal that cell heterogeneity influences lipid nanoparticle-mediated messenger RNA delivery. *Nat. Nanotechnol.* **17**, 871–879 (2022).
- This article reports DNA-barcoded nanoparticles to screen for factors related to lipid nanoparticle delivery of mRNA at single-cell resolution.**
68. Wang, Y. et al. Age-associated disparity in phagocytic clearance affects the efficacy of cancer nanotherapeutics. *Nat. Nanotechnol.* **19**, 255–263 (2024).
- This article shows that liver clearance of nanoparticles declines with age owing to a change in liver macrophage population during ageing.**
69. Boehnke, N. et al. Massively parallel pooled screening reveals genomic determinants of nanoparticle delivery. *Science* **377**, eabm5551 (2022).
- This article reports the screening of a library of different nanoparticles and barcoded cell lines, identifying the core materials and surface modifications of nanoparticles influencing their cellular uptake.**
70. La-Beck, N. M. et al. Factors affecting the pharmacokinetics of pegylated liposomal doxorubicin in patients. *Cancer Chemother. Pharmacol.* **69**, 43–50 (2012).
71. Serpooshan, V. et al. Effect of cell sex on uptake of nanoparticles: the overlooked factor at the nanobio interface. *ACS Nano* **12**, 2253–2266 (2018).
72. Mahmoudi, N. et al. Sex-specific nanomedicine- and biomaterials-based therapies of chronic wounds. *Nat. Rev. Bioeng.* **2**, 447–449 (2024).
- This article discusses sex differences in chronic wound healing and the design of sex-specific biomaterials for chronic wound treatment.**
73. He, Y., Wang, Y., Wang, L., Jiang, W. & Wilhelm, S. Understanding nanoparticle-liver interactions in nanomedicine. *Expert Opin. Drug Deliv.* **21**, 829–843 (2024).
74. Tabula Sapiens, C. et al. The Tabula Sapiens: a multiple-organ, single-cell transcriptomic atlas of humans. *Science* **376**, eabl4896 (2022).
75. Saeed, S. et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* **345**, 1251086 (2014).
76. Hall, E. et al. Sex differences in the genome-wide DNA methylation pattern and impact on gene expression, microRNA levels and insulin secretion in human pancreatic islets. *Genome Biol.* **15**, 522 (2014).
77. Kundakovic, M. & Tickerhoof, M. Epigenetic mechanisms underlying sex differences in the brain and behavior. *Trends Neurosci.* **47**, 18–35 (2024).
78. Pal, S. & Tyler, J. K. Epigenetics and aging. *Sci. Adv.* **2**, e1600584 (2016).
79. Ng, C. T. et al. The induction of epigenetic regulation of PROS1 gene in lung fibroblasts by gold nanoparticles and implications for potential lung injury. *Biomaterials* **32**, 7609–7615 (2011).
80. Zhao, X., Toyooka, T. & Ibuki, Y. Silver nanoparticle-induced phosphorylation of histone H3 at serine 10 is due to dynamic changes in actin filaments and the activation of Aurora kinases. *Toxicol. Lett.* **276**, 39–47 (2017).
81. Sanei, M., Amirheidari, B. & Satarzadeh, N. Mutuality of epigenetic and nanoparticles: two sides of a coin. *Heliyon* **10**, e23679 (2024).
82. Lahnemann, D. et al. Eleven grand challenges in single-cell data science. *Genome Biol.* **21**, 31 (2020).
83. Kiselev, V. Y., Andrews, T. S. & Hemberg, M. Challenges in unsupervised clustering of single-cell RNA-seq data. *Nat. Rev. Genet.* **20**, 273–282 (2019).
84. Wang, L. et al. Exploring and analyzing the systemic delivery barriers for nanoparticles. *Adv. Funct. Mater.* **34**, 2308446 (2023).
85. Manco, R. & Itzkovitz, S. Liver zonation. *J. Hepatol.* **74**, 466–468 (2021).
86. Kietzmann, T. Metabolic zonation of the liver: the oxygen gradient revisited. *Redox Biol.* **11**, 622–630 (2017).
87. Guillems, M. et al. Spatial proteogenomics reveals distinct and evolutionarily conserved hepatic macrophage niches. *Cell* **185**, 379–396.e38 (2022).
- This article identifies spatially resolved niches of different liver macrophage populations.**
88. Matusiak, M. et al. Spatially segregated macrophage populations predict distinct outcomes in colon cancer. *Cancer Discov.* **14**, 1418–1439 (2024).
- This article reports that tumour-associated macrophages at different spatial locations have distinct functions, which can be related to disease outcomes.**

89. Wang, W. et al. Identification of hypoxic macrophages in glioblastoma with therapeutic potential for vasculature normalization. *Cancer Cell* **42**, 815–832.e12 (2024).
90. Mahmoudi, M., Landry, M. P., Moore, A. & Coreas, R. The protein corona from nanomedicine to environmental science. *Nat. Rev. Mater.* **8**, 422–438 (2023).
91. Lee, D., Huntoon, K., Lux, J., Kim, B. Y. S. & Jiang, W. Engineering nanomaterial physical characteristics for cancer immunotherapy. *Nat. Rev. Bioeng.* **1**, 499–517 (2023).  
**This article discusses how the physical characteristics of nanomaterials affect immunological outcomes in cells.**
92. Ngo, W. et al. Identifying cell receptors for the nanoparticle protein corona using genome screens. *Nat. Chem. Biol.* **18**, 1023–1031 (2022).
93. Hsiao, I. L. et al. Quantification and visualization of cellular uptake of TiO<sub>2</sub> and Ag nanoparticles: comparison of different ICP-MS techniques. *J. Nanobiotechnol.* **14**, 50 (2016).
94. Wang, H. et al. Study on uptake of gold nanoparticles by single cells using droplet microfluidic chip-inductively coupled plasma mass spectrometry. *Talanta* **200**, 398–407 (2019).
95. Donahue, N. D. et al. Absolute quantification of nanoparticle interactions with individual human B cells by single cell mass spectrometry. *Nano Lett.* **22**, 4192–4199 (2022).
96. Gatto, L. et al. Initial recommendations for performing, benchmarking and reporting single-cell proteomics experiments. *Nat. Methods* **20**, 375–386 (2023).
97. Zhu, Z. J., Ghosh, P. S., Miranda, O. R., Vachet, R. W. & Rotello, V. M. Multiplexed screening of cellular uptake of gold nanoparticles using laser desorption/ionization mass spectrometry. *J. Am. Chem. Soc.* **130**, 14139–14143 (2008).
98. Gioria, S. et al. Proteomics study of silver nanoparticles on Caco-2 cells. *Toxicol. In Vitro* **50**, 347–372 (2018).
99. Verano-Braga, T. et al. Insights into the cellular response triggered by silver nanoparticles using quantitative proteomics. *ACS Nano* **8**, 2161–2175 (2014).
100. Ashkarran, A. A. et al. Measurements of heterogeneity in proteomics analysis of the nanoparticle protein corona across core facilities. *Nat. Commun.* **13**, 6610 (2022).
101. Ostroff, R. et al. The stability of the circulating human proteome to variations in sample collection and handling procedures measured with an aptamer-based proteomics array. *J. Proteom.* **73**, 649–666 (2010).
102. Faserl, K., Chetwynd, A. J., Lynch, I., Thorn, J. A. & Lindner, H. H. Corona isolation method matters: capillary electrophoresis mass spectrometry based comparison of protein corona compositions following on-particle versus in-solution or in-gel digestion. *Nanomaterials* <https://doi.org/10.3390/nano9060898> (2019).
103. Hornik, Š. et al. Effects of workers exposure to nanoparticles studied by NMR metabolomics. *Appl. Sci.* **11**, 6601 (2021).
104. He, X. et al. Metabolomics of V<sub>2</sub>O<sub>5</sub> nanoparticles and V<sub>2</sub>O<sub>5</sub> nanofibers in human airway epithelial BEAS-2B cells. *Toxicol. Appl. Pharmacol.* **459**, 116327 (2023).
105. Bannusch, A. et al. Metabolomics profiling to investigate nanomaterial toxicity in vitro and in vivo. *Nanotoxicology* **14**, 807–826 (2020).
106. Tang, H. et al. Cholesterol modulates the physiological response to nanoparticles by changing the composition of protein corona. *Nat. Nanotechnol.* **18**, 1067–1077 (2023).
107. Korsunsky, I. et al. Fast, sensitive and accurate integration of single-cell data with Harmony. *Nat. Methods* **16**, 1289–1296 (2019).
108. Welch, J. D. et al. Single-cell multi-omic integration compares and contrasts features of brain cell identity. *Cell* **177**, 1873–1887.e17 (2019).
109. Cao, Z. J. & Gao, G. Multi-omics single-cell data integration and regulatory inference with graph-linked embedding. *Nat. Biotechnol.* **40**, 1458–1466 (2022).
110. Vandereyken, K., Sifrim, A., Thienpont, B. & Voet, T. Methods and applications for single-cell and spatial multi-omics. *Nat. Rev. Genet.* **24**, 494–515 (2023).
111. Athieniti, E. & Spyrou, G. M. A guide to multi-omics data collection and integration for translational medicine. *Comput. Struct. Biotechnol. J.* **21**, 134–149 (2023).
112. Aberg, C., Piattelli, V., Montizaan, D. & Salvati, A. Sources of variability in nanoparticle uptake by cells. *Nanoscale* **13**, 17530–17546 (2021).
113. Lin, Z. P. et al. Macrophages actively transport nanoparticles in tumors after extravasation. *ACS Nano* **16**, 6080–6092 (2022).
114. Behzadi, S. et al. Cellular uptake of nanoparticles: journey inside the cell. *Chem. Soc. Rev.* **46**, 4218–4244 (2017).
115. Zhang, F. et al. Multistage signal-interactive nanoparticles improve tumor targeting through efficient nanoparticle-cell communications. *Cell Rep.* **35**, 109131 (2021).
116. Ultimo, A. et al. Nanoparticle-cell-nanoparticle communication by stigmery to enhance poly(I:C) induced apoptosis in cancer cells. *Chem. Commun.* **56**, 7273–7276 (2020).
117. Chai, J., Zeng, H., Li, A. & Ngai, E. W. T. Deep learning in computer vision: a critical review of emerging techniques and application scenarios. *Mach. Learn. Appl.* **6**, 100134 (2021).
118. Zhu, M. et al. Machine-learning-assisted single-vessel analysis of nanoparticle permeability in tumour vasculatures. *Nat. Nanotechnol.* **18**, 657–666 (2023).  
**This article reports the use of computer vision and machine learning to quantify and analyse the tumour vasculature and delivery of nanoparticles.**
119. Harrison, P. J. et al. Deep-learning models for lipid nanoparticle-based drug delivery. *Nanomedicine* **16**, 1097–1110 (2021).
120. Kang, M., Ko, E. & Mersha, T. B. A roadmap for multi-omics data integration using deep learning. *Brief. Bioinform.* **23**, bbab454 (2022).
121. Li, R. & Yang, X. De novo reconstruction of cell interaction landscapes from single-cell spatial transcriptome data with DeepLinc. *Genome Biol.* **23**, 124 (2022).
122. Zhang, Z. et al. Graph neural network approaches for drug-target interactions. *Curr. Opin. Struct. Biol.* **73**, 102327 (2022).
123. Mastropietro, A., Pasculli, G. & Bajorath, J. Learning characteristics of graph neural networks predicting protein-ligand affinities. *Nat. Mach. Intell.* **5**, 1427–1436 (2023).
124. Saldinger, J. C., Raymond, M., Elvati, P. & Violi, A. Domain-agnostic predictions of nanoscale interactions in proteins and nanoparticles. *Nat. Comput. Sci.* **3**, 393–402 (2023).
125. Xu, Y. et al. AGILE platform: a deep learning powered approach to accelerate LNP development for mRNA delivery. *Nat. Commun.* **15**, 6305 (2024).
126. Wen, H. et al. Graph neural networks for multimodal single-cell data integration. In *Proc. 28th ACM SIGKDD Conference on Knowledge Discovery and Data Mining* 4153–4163 (2022).
127. Watson, J. L. et al. De novo design of protein structure and function with RFdiffusion. *Nature* **620**, 1089–1100 (2023).
128. Lutz, I. D. et al. Top-down design of protein architectures with reinforcement learning. *Science* **380**, 266–273 (2023).  
**This article reports the use of reinforced learning to design new complex protein nanomaterials with desired properties.**
129. Repecka, D. et al. Expanding functional protein sequence spaces using generative adversarial networks. *Nat. Mach. Intell.* **3**, 324–333 (2021).
130. Korshunova, M. et al. Generative and reinforcement learning approaches for the automated de novo design of bioactive compounds. *Commun. Chem.* **5**, 129 (2022).
131. Popova, M., Isayev, O. & Tropsha, A. Deep reinforcement learning for de novo drug design. *Sci. Adv.* **4**, eaap7885 (2018).
132. Jyakhwo, S., Serov, N., Dmitrenko, A. & Vinogradov, V. V. Machine learning reinforced genetic algorithm for massive targeted discovery of selectively cytotoxic inorganic nanoparticles. *Small* **20**, e2305375 (2024).
133. May, J. N. et al. Histopathological biomarkers for predicting the tumour accumulation of nanomedicines. *Nat. Biomed. Eng.* <https://doi.org/10.1038/s41551-024-01197-4> (2024).  
**This article reports the use of supervised machine learning to train an AI model that uses biological factors to predict the tumour delivery of nanoparticles.**
134. Wang, Y., Schrank, B. R., Jiang, W. & Kim, B. Y. S. Learning what keeps nanomedicines in tumours. *Nat. Biomed. Eng.* <https://doi.org/10.1038/s41551-024-01251-1> (2024).
135. Arora, A. & Arora, A. Generative adversarial networks and synthetic patient data: current challenges and future perspectives. *Future Healthc. J.* **9**, 190–193 (2022).
136. Kuo, N. I. et al. Generating synthetic clinical data that capture class imbalanced distributions with generative adversarial networks: example using antiretroviral therapy for HIV. *J. Biomed. Inf.* **144**, 104436 (2023).
137. Lazarovits, J. et al. Supervised learning and mass spectrometry predicts the in vivo fate of nanomaterials. *ACS Nano* **13**, 8023–8034 (2019).
138. Wang, C., Lue, W., Kaalia, R., Kumar, P. & Rajapakse, J. C. Network-based integration of multi-omics data for clinical outcome prediction in neuroblastoma. *Sci. Rep.* **12**, 15425 (2022).
139. Higdon, R. et al. The promise of multi-omics and clinical data integration to identify and target personalized healthcare approaches in autism spectrum disorders. *OMICS* **19**, 197–208 (2015).
140. Shen, W. X. et al. AggMapNet: enhanced and explainable low-sample omics deep learning with feature-aggregated multi-channel networks. *Nucleic Acids Res.* **50**, e45 (2022).
141. Koppad, S., B. A., Gkoutos, G. V. & Acharjee, A. Cloud computing enabled big multi-omics data analytics. *Bioinform. Biol. Insights* **15**, 1179322211035921 (2021).
142. Chen, X., Wang, C., Tang, S., Yu, C. & Zou, Q. CMSA: a heterogeneous CPU/GPU computing system for multiple similar RNA/DNA sequence alignment. *BMC Bioinform.* **18**, 315 (2017).
143. Hajjipour, M. J. et al. Sex as an important factor in nanomedicine. *Nat. Commun.* **12**, 2984 (2021).
144. Liu, Y., Zhou, J. & White, K. P. RNA-seq differential expression studies: more sequence or more replication. *Bioinformatics* **30**, 301–304 (2014).
145. Risso, D., Ngai, J., Speed, T. P. & Dudoit, S. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nat. Biotechnol.* **32**, 896–902 (2014).
146. Dahlman, J. E. et al. Barcoded nanoparticles for high throughput in vivo discovery of targeted therapeutics. *Proc. Natl Acad. Sci. USA* **114**, 2060–2065 (2017).
147. Wei, W., Lu, H., Dai, W., Zheng, X. & Dong, H. Multiplexed organelles portrait barcodes for subcellular microRNA array detection in living cells. *ACS Nano* **16**, 20329–20339 (2022).
148. Rhym, L. H., Manan, R. S., Koller, A., Stephanie, G. & Anderson, D. G. Peptide-encoding mRNA barcodes for the high-throughput in vivo screening of libraries of lipid nanoparticles for mRNA delivery. *Nat. Biomed. Eng.* **7**, 901–910 (2023).
149. Xue, L. et al. High-throughput barcoding of nanoparticles identifies cationic, degradable lipid-like materials for mRNA delivery to the lungs in female preclinical models. *Nat. Commun.* **15**, 1884 (2024).
150. Victorioso, A., Saha, S., Pandey, R. & Soleymani, L. Enhancing the sensitivity of photoelectrochemical DNA biosensing using plasmonic DNA barcodes and differential signal readout. *Angew. Chem. Int. Ed.* **60**, 7316–7322 (2021).
151. Edwardson, T. G., Lau, K. L., Bousmail, D., Serpell, C. J. & Sleiman, H. F. Transfer of molecular recognition information from DNA nanostructures to gold nanoparticles. *Nat. Chem.* **8**, 162–170 (2016).
152. Zhao, J. et al. Core-shell silica nanoparticle-based barcodes combined with a hybridization chain reaction for multiplex quantitative detection of bacterial drug-resistance genes. *ACS Appl. Nano Mater.* **6**, 23114–23121 (2023).
153. Yang, Y. S. et al. High-throughput quantitation of inorganic nanoparticle biodistribution at the single-cell level using mass cytometry. *Nat. Commun.* **8**, 14069 (2017).



154. Hofmann, D. et al. Mass spectrometry and imaging analysis of nanoparticle-containing vesicles provide a mechanistic insight into cellular trafficking. *ACS Nano* **8**, 10077–10088 (2014).
155. Ouyang, B. et al. The dose threshold for nanoparticle tumour delivery. *Nat. Mater.* **19**, 1362–1371 (2020).
156. Chen, J. et al. Sex differences in the toxicity of polyethylene glycol-coated gold nanoparticles in mice. *Int. J. Nanomed.* **8**, 2409–2419 (2013).
157. Han, H. Y. et al. Amorphous silica nanoparticle-induced pulmonary inflammatory response depends on particle size and is sex-specific in rats. *Toxicol. Appl. Pharmacol.* **390**, 114890 (2020).
158. Zamboni, W. C. et al. Bidirectional pharmacodynamic interaction between pegylated liposomal CKD-602 (S-CKD602) and monocytes in patients with refractory solid tumors. *J. Liposome Res.* **21**, 158–165 (2011).
159. Steppan, J. et al. Commonly used mouse strains have distinct vascular properties. *Hypertens. Res.* **43**, 1175–1181 (2020).
160. Corder, K. M., Hoffman, J. M., Sogorovic, A. & Austad, S. N. Behavioral comparison of the C57BL/6 inbred mouse strain and their CB6F1 siblings. *Behav. Process.* **207**, 104836 (2023).
161. Montgomery, M. K. et al. Mouse strain-dependent variation in obesity and glucose homeostasis in response to high-fat feeding. *Diabetologia* **56**, 1129–1139 (2013).
162. Hamilton, R. F. Jr, Thakur, S. A., Mayfair, J. K. & Holian, A. MARCO mediates silica uptake and toxicity in alveolar macrophages from C57BL/6 mice. *J. Biol. Chem.* **281**, 34218–34226 (2006).

## Acknowledgements

The authors thank C. F. Wogan of the Division of Radiation Oncology at MD Anderson Cancer Center for editing this manuscript. The authors thank S. Dong and B. R. Schrank of the Department of Radiation Oncology at MD Anderson Cancer Center for preparing some images in the figures. The authors thank L. Nie of the Department of Experimental Radiation Oncology at MD Anderson Cancer Center for the suggestions and discussions in proteomics. Y.W. is supported by Susan G. Komen Career Transition Award (CTA241186937). This work is supported in part by National Institutes of Health grant P30CA016672.

## Author contributions

W.J., B.Y.S.K. and Y.W. conceived the project and were responsible for all phases of the preparation of the manuscript. Y.W., Z.X., Z.W., D.L., S.W., Y.M., H.W., W.J. and B.Y.S.K. wrote the manuscript. Y.W., Z.X. and Z.W. designed the figures. D.L., Z.X. and Y.W. designed the tables. All authors searched for literature and edited the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

**Peer review information** *Nature Reviews Bioengineering* thanks Horacio Cabral, Rong Fan and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

## Related links

**ArchR**: <https://github.com/GreenleafLab/ArchR>  
**BackSPIN**: <https://github.com/linnarsson-lab/BackSPIN>  
**BASICS**: <https://github.com/catavallejos/BASICS>  
**BBKNN**: <https://github.com/Teichlab/bbknn>  
**CCA**: <https://github.com/CCA-Public/cca-tools>  
**Cell-Blast**: [https://github.com/gao-lab/Cell\\_BLAST](https://github.com/gao-lab/Cell_BLAST)  
**CellChat**: <https://github.com/scqjin/CellChat>  
**CellPhoneDB**: <https://github.com/Teichlab/cellphonedb>  
**Cellranger**: <https://github.com/10XGenomics/cellranger>  
**Cellrank**: [https://github.com/theislab/cellrank\\_reproducibility](https://github.com/theislab/cellrank_reproducibility)  
**CellTalkDB**: <http://tcm.zju.edu.cn/celltalkdb>  
**CellTypist**: <https://github.com/Teichlab/celltypist#interactive-tutorials>  
**CIDR**: <https://github.com/VCCRI/CIDR>  
**ComBat**: <https://github.com/zhangyuqing/ComBat-seq>  
**CPM**: <https://github.com/amitfrish/scBio>  
**DEseq2**: <http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html>  
**Destiny**: <https://github.com/theislab/destiny>  
**DoubletDecon**: <https://github.com/EDePasquale/DoubletDecon>  
**DoubletDetection**: <https://github.com/xnba1984/Doublet-Detection-Benchmark>  
**DoubletFinder**: <https://github.com/chris-mcginnis-ucsf/DoubletFinder>

**DPT**: <https://github.com/theislab/destiny/blob/devel/man/DPT.Rd>  
**edger**: <https://bioconductor.org/packages/release/bioc/html/edgeR.html>  
**fastMNN**: [https://github.com/satijalab/seurat-wrappers/blob/master/docs/fast\\_mnn.md](https://github.com/satijalab/seurat-wrappers/blob/master/docs/fast_mnn.md)  
**FateID**: <https://github.com/dgrun/FateID>  
**featureCounts**: [https://github.com/bpucker/RNA-Seq\\_analysis](https://github.com/bpucker/RNA-Seq_analysis)  
**GO**: <http://www.geneontology.org>  
**GSEA**: <https://github.com/zqfang/GSEAPy>  
**GSVA**: <http://www.bioconductor.org/packages/release/bioc/html/GSVA.html>  
**Harmony**: <https://github.com/immunogenomics/harmony>  
**kallisto**: [https://static-content.springer.com/esm/art%3A10.1038%2Fnb.3519/MediaObjects/41587\\_2016\\_BFnb3519\\_MOESM11\\_ESM.zip](https://static-content.springer.com/esm/art%3A10.1038%2Fnb.3519/MediaObjects/41587_2016_BFnb3519_MOESM11_ESM.zip)  
**KEGG**: <http://www.genome.ad.jp/kegg/>  
**limma**: <https://github.com/gangwug/limma>  
**Linnorm**: <https://www.bioconductor.org/packages/release/bioc/html/Linnorm.html>  
**M3Drop**: <https://github.com/tallulandrews/M3Drop>  
**MarkerCount**: <https://github.com/combio-dku/MarkerCount/tree/master>  
**MARS**: <https://github.com/snap-stanford/mars>  
**MAST**: <https://github.com/RGLab/MAST>  
**MFA**: <https://www.jstatsoft.org/article/view/v025i01>  
**MNN**: <https://github.com/MarionLab/MNN2017/>  
**Monocle2**: <https://github.com/cole-trapnell-lab/monocle-release>  
**MST**: <https://github.com/caiyuanhao1998/MST>  
**Muon**: <https://github.com/scverse/muon.git>  
**NicheNet**: <https://github.com/saeyslab/nichenetr>  
**PAGA**: <https://github.com/theislab/paga>  
**PAGODA**: <https://github.com/kharchenkolab/pagoda2>  
**PCA**: <https://github.com/topics/principal-component-analysis-pca>  
**pcaReduce**: <https://github.com/JustinaZ/pcaReduce>  
**Pseudobulk**: <https://github.com/vertesy/pseudoBulk>  
**pyCisTopic**: <https://github.com/aertslab/pycisTopic>  
**quantile**: <https://github.com/yromano/cqr>  
**RaceID**: <https://github.com/dgrun/RaceID>  
**Reactome**: <https://benchmark.dynverse.org>  
**Rpca**: [https://github.com/carpenter-singh-lab/2023\\_Arevalo\\_BatchCorrection](https://github.com/carpenter-singh-lab/2023_Arevalo_BatchCorrection)  
**RSEM**: <https://deweylab.github.io/RSEM/>  
**SC3**: <https://github.com/hemberg-lab/SC3>  
**Scanorama**: <https://github.com/brianhie/scanorama>  
**Scanpy**: <https://github.com/theislab/scanpy>  
**scDblFinder**: <https://github.com/plger/scDblFinder>  
**scDE**: <http://pklab.med.harvard.edu/scde/>  
**scID**: <https://batadalab.github.io/scID/>  
**scLearn**: <https://github.com/bm2-lab/scLearn>  
**scLVM**: <https://github.com/PMBio/scLVM>  
**scran deconvolution**: <https://github.com/MarionLab/scran>  
**Scrublet**: <https://github.com/AllonKleinLab/scrublet>  
**SCSA**: <https://github.com/bioinfo-ibms-pumc/SCSA>  
**scTensor**: <https://github.com/rikenbit/scTensor>  
**sctransform**: <http://github.com/ChristophH/sctransform>  
**SCUBA**: <https://doi.org/10.5281/zenodo.4533424>  
**scvelo**: <https://github.com/theislab/scvelo>  
**scVI**: <https://github.com/YosefLab/scVI>  
**scvis**: <https://bitbucket.org/jerry00/scvis-dev>  
**Seurat**: <https://github.com/satijalab/seurat>  
**Signac**: <https://github.com/timoast/signac>  
**SingleR**: <https://github.com/dviraran/SingleR>  
**SLICER**: <https://github.com/jw156605/SLICER>  
**Slingshot**: <https://github.com/kstreet13/slingshot>  
**snapATAC**: <https://github.com/kaizhang/SnapATAC2>  
**SNN-Cliq**: <https://github.com/BIOINSu/SNN-Cliq>  
**ssGSEA**: <https://github.com/broadinstitute/ssGSEA2.0>  
**STAR**: <https://github.com/alexdobin/STAR>  
**Steropy**: <https://github.com/STOmics/steropy>  
**TMM**: <https://github.com/pfreese/tmm>  
**Tosica**: <https://github.com/JackieHanLab/TOSICA>  
**Trim galore**: [https://github.com/miomugi/trim\\_galore](https://github.com/miomugi/trim_galore)  
**Trimomatic**: <https://github.com/timflutre/trimomatic>  
**TSCAN**: <https://github.com/zji90/TSCAN>  
**t-SNE**: <https://github.com/berenslab/rna-seq-tsne>  
**t-Test**: <https://github.com/AndreasMadsen/ttest>  
**UMAP**: [https://github.com/cole-trapnell-lab/yeast\\_umap](https://github.com/cole-trapnell-lab/yeast_umap)  
**UMI-tools zUMIs**: <https://github.com/sdparekh/zUMIs>  
**URD**: <https://github.com/farrelja/URD>  
**ZIFA**: <https://github.com/epierson9/ZIFA>

© Springer Nature Limited 2024