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PERSPECTIVE

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Harnessing nanotechnology to expand the toolbox of chemical biology

Ryan M. Williams[1,2,7], Shi Chen[3,4,7], Rachel E. Langenbacher[2,5], Thomas V. Galassi[2,5], Jackson D. Harvey[2,5], Prakrit V. Jena[2], Januka Budhathoki-Uprety[6]✉, Minkui Luo[3,5]✉ and Daniel A. Heller[2,5]✉

Although nanotechnology often addresses biomedical needs, nanoscale tools can also facilitate broad biological discovery. Nanoscale delivery, imaging, biosensing, and bioreactor technologies may address unmet questions at the interface between chemistry and biology. Currently, many chemical biologists do not include nanomaterials in their toolbox, and few investigators develop nanomaterials in the context of chemical tools to answer biological questions. We reason that the two fields are ripe with opportunity for greater synergy. Nanotechnologies can expand the utility of chemical tools in the hands of chemical biologists, for example, through controlled delivery of reactive and/or toxic compounds or signal-binding events of small molecules in living systems. Conversely, chemical biologists can work with nanotechnologists to address challenging biological questions that are inaccessible to both communities. This Perspective aims to introduce the chemical biology community to nanotechnologies that may expand their methodologies while inspiring nanotechnologists to address questions relevant to chemical biology.

Chemical biologists leverage chemical tools to interrogate, manipulate, and perturb systems for biological discovery. Whereas chemical biology primarily relies on chemical principles to address biological questions, nanotechnology focuses on the manipulation of nanoscale synthetic materials, often in biological systems[1]. Nanomaterials are unique due to their tunable and distinct physical, chemical, and biological properties compared to bulk materials (Fig. 1). Because of their complementary goals and toolkits, chemical biology and nanotechnology can potentially be explored in a collaborative, synergistic manner. Synthetic nanomaterials can interact with biological processes on cell surfaces, inside living cells, and even within specific intracellular compartments. The size, physicochemical control, and resulting biological interactions of nanomaterials have facilitated abundant investigative directions, including chemical-, cell-, and mechanobiology, for studying a wide range of biomolecules and cellular processes. Multiple broad classes of nanomaterials, from inorganic to biodegradable polymers, have been developed for molecular delivery, enzymatic catalysis, molecular imaging, and molecular sensing. In addition, a number of nanomaterial applications have reached the clinic[2]. The unique features of nanomaterials can be of great interest to chemical biologists, although most of these technologies have yet to be extensively implemented for biological discovery. We envision the expansion of nanomaterial utility as, or together with, chemical tools for broad biological applications (i.e., nanochemical biology). There are also existing challenges in chemical biology that nanotechnologists can collaboratively address, as described herein.

Nanocarriers of bioactive chemicals

While chemical biologists have developed highly potent modulators (agonists, antagonists, degraders, etc.) of biomolecules, many of these may not exhibit ideal functionality in vivo due to limited solubility, stability, biocompatibility, poor pharmacokinetics, and/or off-target activity[3]. Nanotechnologists have developed tools to deal with the delivery and pharmacokinetic issues of otherwise poorly behaved molecules via encapsulating active cargos and targeting specific types of tissues, cells, or organelles. Specifically, nanomaterials are developed to deliver these chemicals via encapsulation and subsequent controlled release within subcellular organelles or specific organs and tissues in live animals (Fig. 1, red quadrant).

Although these benefits of nanomaterials have typically been applied for medical needs, the principles are expected to be readily transferred to the delivery of bioactive chemicals to interrogate biological systems. In addition, pharmacokinetic properties of delivered molecules, such as their half-life, can be enhanced by nanocarrier loading. This strategy allows substantial accumulation and functional modifications of encapsulated molecules at nanoparticle-targeted sites compared to free diffusion[4]. For general consideration of chemical biologists, three important aspects of formulating nanocarriers are to encapsulate bioactive molecules, to target them to desired intra/extracellular loci, and to control their temporal and spatial release.

A wide range of materials have been explored as nanocarriers of bioactive cargos (Table 1), including lipids, polymers, metals, and other inorganic materials. Liposomes, highly organized hollow lipid bilayer nanoparticles, were first described in 1965 and are currently used in the clinic to encapsulate doxorubicin (Doxil), daunorubicin (DaunoXome)[2,5], and other drugs. Their amphiphilic structure makes liposomes suitable for delivery of various compounds. Less-ordered lipoplexes such as Lipofectamine may form a complex with nucleic acids to result in nanoparticles for transfecting cells in culture. Other materials include biodegradable polymers

[1]Department of Biomedical Engineering, The City College of New York, New York, NY, USA. [2]Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [3]Chemical Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [4]Tri-Institutional PhD Program in Chemical Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [5]Department of Pharmacology, Weill Cornell Medical College, Cornell University, New York, NY, USA. [6]Department of Textile Engineering, Chemistry and Science, North Carolina State University, Raleigh, NC, USA. [7]These authors contributed equally: Ryan M. Williams, Shi Chen. ✉e-mail: jbudhat@ncsu.edu; luom@mskcc.org; hellerd@mskcc.org

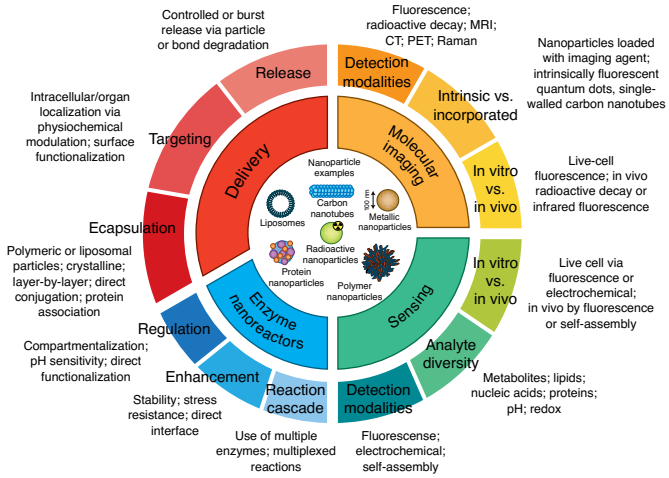

Fig. 1 | Major classes of potential contributions of nanotechnology to chemical biology. Potential contributions of nanotechnology to chemical biology are classified into four modules: nanocarriers of bioactive chemicals or delivery (upper-left red quadrant), enzymatic nanoreactors (bottom-left blue quadrant), nanoparticle-based molecular imaging (upper-right yellow quadrant), and nanoscale sensors (bottom-right green quadrant).

such as poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL), which form nanoparticles with a hydrophobic core[2,5]. Solid inorganic nanoparticles, often composed of gold, iron oxide, various metals, or carbon nanotubes, can deliver surface-adsorbed or covalently conjugated small molecules[6,7]. Nanoparticles formulated through protein–drug interactions, such as albumin-bound paclitaxel (Abraxane), have also resulted in clinical success[2,8]. The choice of nanocarrier material is important for chemical biologists in considering the cargo type, efficiency of loading and release, organelle and tissue targeting, and other biological interactions.

There are multiple strategies available for the association of pharmacologically active molecules with nanoparticle carriers. Liposomal formulations can be assembled with hydrophilic or hydrophobic small molecules loaded into the aqueous core or the lipid bilayer, respectively. Crystalline formulations represent another strategy[5,9]. Hydrophobic drugs are often encapsulated within a hydrophobic polymeric matrix via co-precipitation[10], allowing controlled release. Alternatively, chemical conjugation methods often involve a labile linker, such as a pH-responsive hydrazine–carbonyl condensation bond[11]. Solid or hydrogel matrix nanoparticle systems may also be conjugated with drugs via dithiol linkages to facilitate release[12]. Layer-by-layer assembly[13] is another strategy for loading cargoes into a polymeric nanoparticle. Porous metal–organic frameworks are also loaded via adsorption and trapping of drug molecules[14].

Nanoparticles often enter cells via endocytosis, sequestering particles in late endosomes and lysosomes. The physicochemical properties of nanocarriers can be modified to control their localization within specific subcellular compartments, allowing delivery of active cargoes to specific organelles, which can be useful with non-specific inhibitors. For example, to facilitate the escape of nanoparticles from late endosomes and lysosomes into the cytosol, particles may be engineered to swell, fuse with the endosome membrane, destabilize the membrane, or cause an increase in osmotic pressure[15]. Targeting of nanomaterials to the nucleus has been achieved via the attachment of natural or synthetic nuclear localization signals[16]. Localization to the endoplasmic reticulum and Golgi apparatus can occur by receptor-mediated retrograde trafficking, whereas mitochondrial localization has been achieved by cationic surface functionalization of nanoparticles[17,18]. In addition, methods such as electroporation, mechanical cellular manipulation via microfluidics, and direct injection enable insertion of nanoparticles into the cytoplasm[19]. Although intracellular uptake via endocytosis is the rule rather than the exception, alternative uptake may be accomplished by designing surface coatings that minimize protein adsorption, such as polyethylene glycol (PEG)-functionalized nanoparticles. While available nanomaterials can only selectively target a subset of intracellular compartments, direct collaboration may better fulfill the needs of chemical biologists to improve subcellular localization of biologically active compounds. For example, more triphenylphosphonium and polyguanidinium-containing chemical building blocks can be designed to coat nanoparticles for subcellular targeting to mitochondria and nuclei, respectively[17,18].

Table 1 | General classes of nanomaterials

Type of material	Examples	Benefits	Potential drawbacks
Lipids[2,5,46]	• Phospholipids • Synthetic lipids, many varieties • Lipid bilayers (liposomes) • Solid lipid nanoparticles • Other forms	• Biocompatible (in most cases) • Increases solubility • Well studied • Adaptable to many cargoes	• Cargo leakage • Particle stability and solubility • Reactivity of lipids • Immune response • Low drug-loading efficiency
Polymers[2,5,10,29]	• Poly-lactic-co-glycolic acid (PLGA) • Polycaprolactone (PCL) • Polymeric micelles (block copolymers)	• Biocompatible (in many cases) • Well studied • Facilitates controlled release • Increases solubility • Adaptable to many cargoes	• Some polymers have toxic degradation products • May be difficult to scale up • Potential immune response • Low drug-loading efficiency
Aggregates[2,4,8,20,21]	• Excipients include proteins (albumin) • Dyes • Polysaccharides • Other drugs	• Solubilization • Drug-loading efficiency • Generally biocompatible	• Rapid degradation • Potential for low stability
Inorganic[2,6,7,51,71]	• Quantum dots • MRI contrast agents • Carbon nanotubes	• Unique optical or magnetic properties • Do not degrade • Potential for simultaneous delivery and imaging	• Potential toxicities • Generally poor drug-loading efficiencies

In animals, nanocarriers can abrogate toxicities and side effects associated with systemic administration. Nanocarrrier-mediated delivery of chemotherapeutic agents has been shown to reduce toxicities compared to systemic administration of carrier-free therapies[20]. Even molecularly targeted small molecules, such as kinase inhibitors, exhibit suppressed toxicity profiles when encapsulated in site-targeted nanocarriers[21]. The administration of active compounds via nanoparticles for the purpose of extending chemical biology investigations in vivo is largely underutilized.

Controlling the size, shape, and surface chemistry of nanocarriers can target them to specific organs or tissues. A majority of nanoparticles exhibit localization to the liver and spleen due to their size (<200 nm) and opsonization (serum protein adsorption), resulting in primarily hepatobiliary clearance[22]. PEG and zwitterions can reduce, but often not completely abrogate, opsonization-induced liver localization[23].

The majority of nanoparticle passive targeting strategies have focused on tumor targeting. In tumor-bearing animals, nanoparticles exhibit some tumor localization via leakiness of tumor vasculature, termed the enhanced permeability and retention (EPR) effect[24]. This is often achieved by controlling particle size (keeping diameter below 200 nm (ref. [25])). There is evidence, however, that this effect may not translate well to many human cancers[26]. Additional targeting strategies have been described elsewhere[27]. Ultra-small nanoparticles (<10 nm) exhibit renal clearance from the body[28], whereas mesoscale nanoparticles (~400 nm) and quasi-one-dimensional carbon nanotubes can exhibit renal tubular localization and retention[6,29,30]. Other particles within a defined particle size range (10–80 nm) can localize to the glomeruli of the kidneys[31]. Microparticles often localize to the lungs through intravenous or inhaled administration[32,33]. Inhaled administration has also been used to target nanoparticles into the brain[34]. Despite these strategies, most particle systems still exhibit some level of liver accumulation and hepatobiliary clearance.

Nanoparticle size and shape are thus key parameters influencing their interaction with biology. Size has been controlled by speed-controlled nano-emulsion, centrifugation, and sonication methods, among others[35]. Though many nanoparticles are spherical, non-spherical particles such as fibrils, discs, and rods can exhibit altered uptake kinetics, subcellular localization, and pharmacokinetic profiles[36]. Methods to control nanoparticle shape include synthesis by deposition of materials into template molding (termed particle replication in non-wetting templates, or PRINT)[37].

A more specific targeting strategy called active targeting uses molecular recognition elements to bind to cells or tissues of interest. These targeting moieties can include antibodies, peptides, aptamers, and small molecules that are conjugated to the nanoparticle surface to enhance uptake in target tissues[38], with potential for valency effects[39]. The targeted delivery of cargoes to particular cell subsets within a target organ could enhance drug therapeutic index in vivo[40]. However, the biodistribution of particles is not often substantially altered using these methods.

Loading cargoes into nanocarriers can restrict their reactivity, metabolism, and toxicity within cells or an organism[41]. This strategy is commonly used for the administration of macromolecular cargoes to affect RNA delivery and gene editing[42,43]. However, nanoparticles can allow biologically active compounds, including inhibitors and catalysts, to be used in vivo to address biological questions without the need for substantial chemical modification.

The use of transition metals in living cells is limited. However, transition metal catalysts can be encapsulated within nanoparticles to catalyze biochemical reactions in cells (Fig. 2)[44]. A prominent example of this is polystyrene microspheres crosslinked with a bis-1,ω-acid chloride to entrap Pd^{2+}, in turn producing Pd^0 in situ. (Fig. 2b)[44]. Such Pd^0 nanoparticles can catalyze intracellular

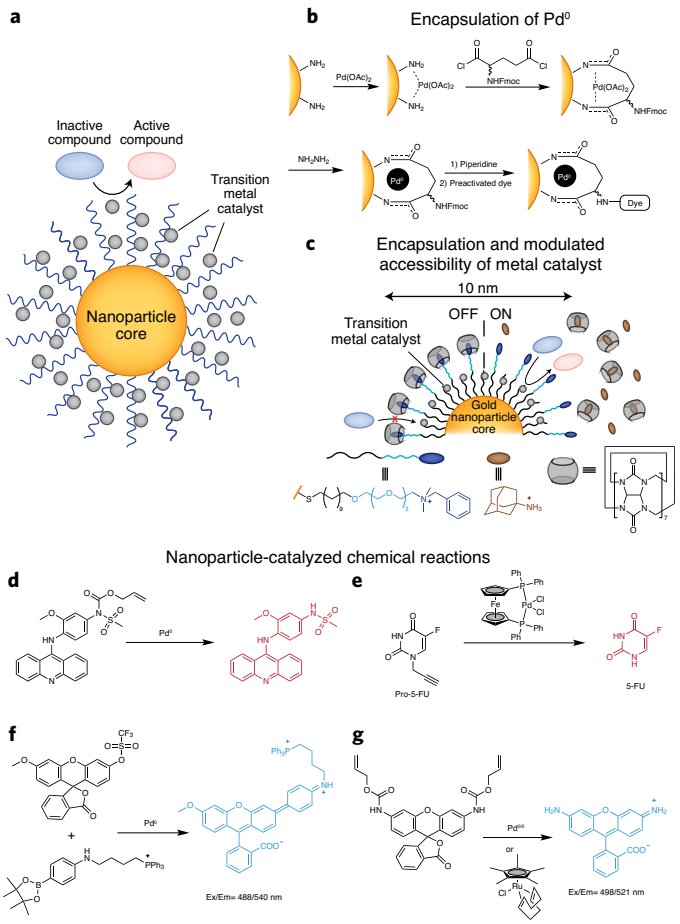

Fig. 2 | Nanoparticle-based delivery of transition-metal catalysts for intracellular chemical reactions. a, Transition-metal catalysts are immobilized onto the surface of a nanoparticle to facilitate intracellular chemical transformations. **b**, Encapsulation of the Pd^0 catalyst into a nanoparticle core via stepwise synthesis[44]. The two amines were converted into amides with glutaroyl dichloride. The coordination bonds of Pd with amine or amide are shown with the dashed lines. **c**, Encapsulation and modulated accessibility of a metal catalyst[45]. The reversible competitive host–guest interactions of cucurbit[7]uril (grey) with tertiary amine moieties surface coated on nanoparticles (dark blue) or 1-adamantylamine (brown) could modulate the accessibility of a transition-metal catalyst to inactive a substrate (light blue). **d**–**g**, Intracellular reactions catalyzed by transition-metal-encapsulated nanoparticles include activation of amsacrine (ref. [44]) (**d**), activation of 5-fluorouracil (5-FU)[45] (**e**), intracellular Suzuki–Miyaura cross-coupling to generate a mitochondria-localized fluorescent compound[44] (**f**), and activation of rhodamine 110[44,45] (**g**). Ex/Em are excitation and emission wavelengths, respectively.

Suzuki–Miyaura cross-coupling between arylboronates and aryl triflates for non-enzymatic aryl–aryl bond formation within living cells (Fig. 2f). In vitro catalytic activity of nanoparticles was demonstrated via cleavage of bis-allyloxycarbonyl rhodamine (Fig. 2g) and allylcarbamate-derivatized amsacrine (Fig. 2d). Similarly, catalytic activities of Ru^{2+} and Pd^{2+} immobilized on gold nanoparticles[45] have been demonstrated within live cells by removing the allylcarbamate of bis-*N*,*N*′-allyloxycarbonyl rhodamine (Fig. 2g) and the propargyl group of *N*1-propargyl-5-fluorouracil (Fig. 2e), respectively. This interaction can be reversible via host–guest chemistry. This exogenous regulation thus provides a modulation mechanism mimicking the allosteric properties of certain enzymes. These approaches provide examples for the exploration of metals for live-cell catalysis

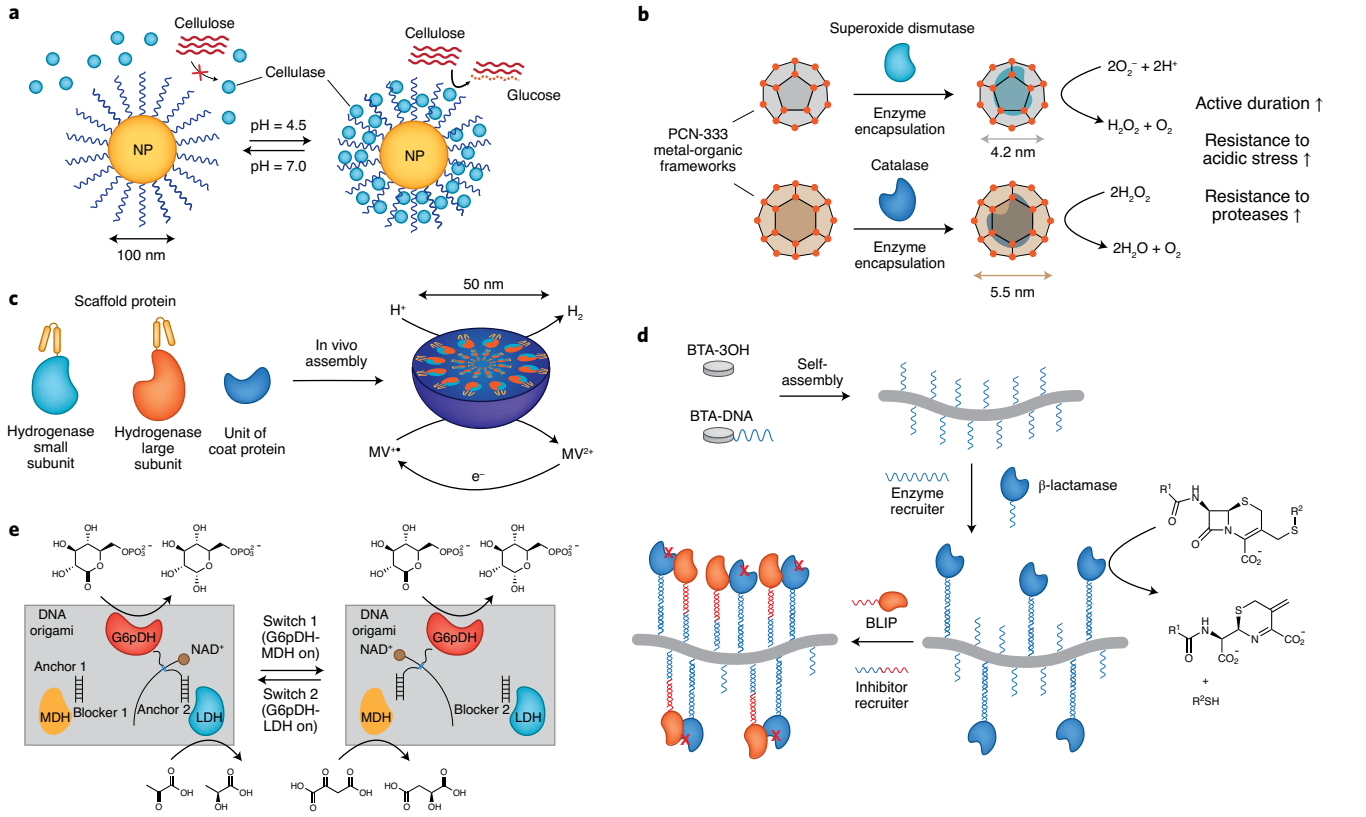

Fig. 3 | Representative examples of enzymatic nanoreactors. a, A pH-sensitive nanoreactor for interphase hydrolysis of cellulose to glycose catalyzed by cellulase[51]. There is no conversion of cellulose into glucose at pH = 7.0 (red cross). NP, nanoparticle. **b**, Encapsulation of superoxide dismutase and catalase into aluminum-based metal–organic frameworks (PCN-333) enhances the enzymes' active duration and resistance to acidic stress and proteases in the intracellular environment[52]. **c**, Co-expression of the small and large subunits of [Ni-Fe] hydrogenase EcHyd-1 with scaffold protein and coat protein in *E. coli* leads to the in vivo self-assembly of EcHyd-1 encapsulated in bacteriophage P22 capsids (right)[53]. **d**, A supramolecular self-assembling nanoreactor for DNA-mediated dynamic recruitment and regulation of β-lactamase in cells[54]. BTA, benzene-1,3,5-tricarboxamide; BLIP, β-lactamase inhibitor protein. **e**, A DNA origami-based multi-enzyme cascade nanoreactor that is switchable between two metabolic pathways[55]. LDH, lactate dehydrogenase; MDH, malate dehydrogenase; G6pDH, glucose-6-phosphate dehydrogenase.

of diverse organic reactions, potentially facilitating in situ production of bioactive molecules.

Synthetic small interfering RNA (siRNA) holds strong potential for use in studying the effects of specific pathways in biological systems, although they are particularly susceptible to nuclease degradation. Nanoformulations of siRNA and mRNA with lipids and polymers have demonstrated the ability to abrogate siRNA degradation and facilitate use in vitro or in vivo[46]. Examples include the RNA therapeutic patisiran, comprised of liposomal-formulated siRNA to treat a hereditary liver disease[43].

Nanotechnologies have also facilitated the delivery of metabolically active synthetic compounds to specific cell types for use in subsequent bioorthogonal reactions. For example, 9-azido sialic acid (9AzSia) was loaded into efficiently internalized folate-coated PEGylated liposomal nanoparticles[47]. The cargo was then available for biosynthesis of cell-surface glycans and fluorescence labeling. RGD-peptide-targeted liposomes encapsulating 9AzSia have been used to target xenografted B16–F10 cells in vivo as demonstrated by in vivo copper-free click chemistry. The same group further evaluated additional ligands to address this issue, including the antibody trastuzumab (Herceptin), an aptamer, and glycan ligands to target additional receptors[48].

Because of the diverse nanocarrier materials available, chemical biologists must choose materials for their specific needs. For a given compound, it is unlikely that a chemical biologist can exhaustively evaluate the appropriate strategy. It will thus be helpful for nanotechnologists to compare nanomaterials in parallel and provide general guidance for chemical biologists. Admittedly, cargoes harboring diverse chemical and physical properties may behave differently despite having the theoretically best available carrier. In lieu of direct collaborations, the availability of general algorithms or plug-and-play solutions would be welcomed by the chemical biology community. It would be of interest to nanotechnologists and chemical biologists to make nanocarrier materials available as kits for rapid translation of ideas to the bench.

Enzymatic nanoreactors

Nanomaterial scaffolds have been applied as reactors for enzymatic catalysis. These materials facilitate the regulation and enhancement of catalyzed reactions controlled by the nanoparticle, enabling potential reaction cascades (Fig. 1, blue quadrant). Compared to conventional homogenous assays in solution, enzyme nanoreactors provide the ability to constrain the catalysis of enzymes (Fig. 3). This technique enhances the stability and activity of enzymes, allowing enzymatic reactions to be conducted in new contexts[49]. Moreover, enzyme nanoreactors can compartmentalize multiple enzymes to control biological cascades[50].

Enzymatic stability and spatial control are notable enhancements imported by nanotechnology. A nanoscale 'enzymogel' reactor for cellulase-catalyzed hydrolysis, consisting of an inorganic

core and polymer shell, has been reported (Fig. 3a)[51]. The flexible poly(acrylic acid) shell is negatively charged and thus readily binds to positively charged enzymes such as cellulase. Cellulase loading was reversible and its mobility maintained within the shell, facilitating hydrolysis at the boundary between the shell and the cellulose substrate. Another enzymatic application of nanotechnology is the utility of metal–organic frameworks (MOFs) as an encapsulation platform for antioxidative enzymes (Fig. 3b)[52]. This work found that superoxide dismutase and catalase can be co-trapped within the MOF nanoparticle. Compared with free enzymes, encapsulation maintained their activities and chemical resistance, as demonstrated in cell culture. Another study found that enzymatic stability and protection were improved by bacteriophage-based enzyme caging (Fig. 3c)[53]. In this work, the interaction of a [Ni-Fe] hydrogenase with a scaffold protein in *Escherichia coli* allowed self-assembly into bacteriophage P22 capsids, conferring a substantial increase in activity.

Enzymatic nanoreactors can also allow temporal and multiplexed control during enzymatic reactions. A DNA-mediated self-assembled nanoreactor (Fig. 3d)[54] exhibited programmable and reversible enzyme recruitment, allowing spatiotemporal reaction control. Another DNA-mediated nanoscaffold demonstrated the ability to switch between malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) activity (Fig. 3e)[55]. The authors immobilized the enzyme glucose-6-phosphate dehydrogenase (G6pDH) coupled with a cofactor (NAD^+) on single-planar DNA-based nanoparticles. The position of the cofactor NAD^+ could be anchored in proximity with either MDH or LDH in a switchable manner and could be controlled by the DNA-based nanotechnology.

In addition to the above examples, various studies show that nanoparticles alone can mimic the activity of enzymes and catalyze diverse biochemical transformations following Michaelis–Menten kinetics. Such systems are termed 'nanozymes' and include[56] cerium oxide nanoparticles mimicking superoxide dismutase, catalase, and oxidase to scavenge reactive oxygen species generated in radiotherapy; Fe_3O_4 magnetic nanoparticles, iron chalcogenides, single-walled carbon nanotubes, and graphene oxide functioning as peroxidases; zinc-anchored gold nanoparticles mimicking the activity of phosphodiesterase; and molybdenum trioxide nanoparticles that demonstrate sulfite oxidase-like activity. Nanozymes display high catalytic efficiency, resistance to denaturation, and tunable activities derived from the redox potentials of metal ions and programmable acidity, charge, and exposed coordination sites[57]. Chemical biologists may find benefits in broadening these technologies to additional enzyme families while using similar examples to address biological questions.

Nanoparticle-based molecular imaging

Nanotechnologists have developed many biological imaging tools (Fig. 4). Such tools on the nanoscale often employ the unique physicochemical properties of nanoscale materials for a diverse array of imaging modalities for use either in vitro (including within live cells) or in vivo (Fig. 1, yellow quadrant).

Nanoscale imaging agents may be used to increase photostability, decrease systemic toxicity, and improve multiplexing in imaging studies (Fig. 5). Nanoscale probes may also enable single-molecule and multi-modality imaging capabilities[58]. These imaging agents fall into two broad categories: incorporation of small molecules such as radiotracers or fluorescent dyes into a nanostructure, and nanoscale materials that have measurable signal themselves. Though a handful of nanoimaging agents are used clinically, and several are commercially available as research tools, many nascent technologies are currently at the initial demonstration stage. As a result, there is a surfeit of underutilized molecular probe technologies that have not been examined for non-clinical, in-depth biological investigations.

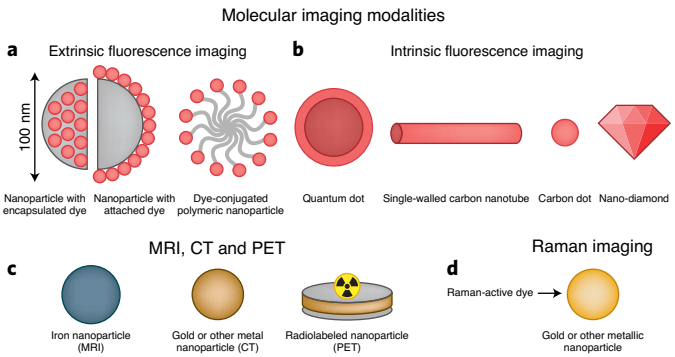

Fig. 4 | Nanotechnologies for imaging applications. Major examples include: **a**, Fluorescent dye-loaded/conjugated nanoparticles. **b**, Intrinsically fluorescent nanoparticles. **c**, Nanoparticles for several radio-imaging modalities. **d**, Dye-bound gold nanoparticles for Raman-scattering-based imaging. Scale bar is approximate.

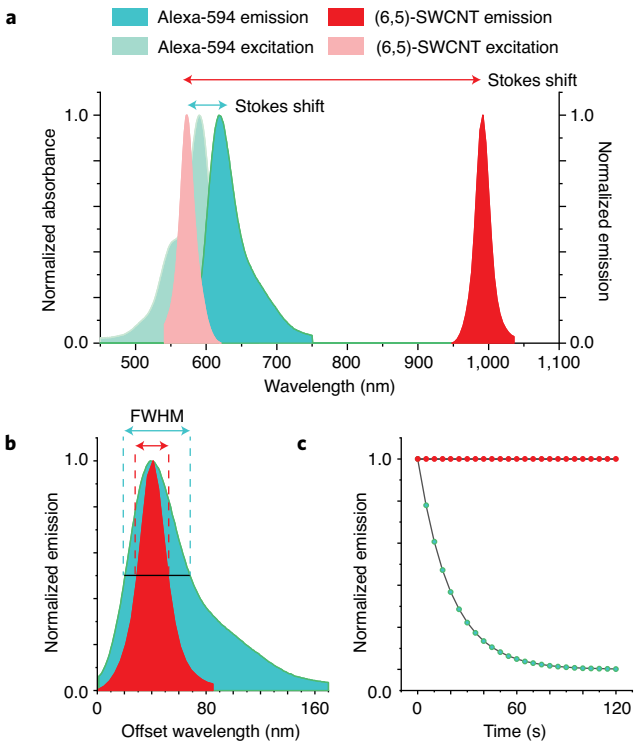

Fig. 5 | Examples of optical properties unique to certain nanomaterial imaging agents. a, A large Stokes shift compared to organic fluorophores. **b**, A narrow full-width half-maximum (FWHM) compared to organic fluorophores. **c**, Unique photostability compared to organic fluorophores. SWCNT, single-walled carbon nanotubes. The dashed lines and horizontal black lines (in **b**) represent the full-width at half of the maximum of the peak. The green outline is the emission trace.

Loading fluorophores into nanoparticles can increase signal, improve the biodistribution profile, allow for improved targeting, and protect dyes from photobleaching[59]. Due to the advantages of fluorescent nanoparticles, and the widespread use of fluorescence imaging, they are available commercially. In addition to simple fluorescence output, more complex nanostructures may be designed for subcellular targeting similar to drug delivery. One example of these is silica nanoparticles, which may be easily loaded with a wide

range of fluorophores, conjugated with modular ligands, and even co-loaded with radioimaging probes[58,59]. Sol-gels may be doped in a similar manner, and hydrophilic polymers such as cellulose, or hydrophobic polymers such as polystyrene, may be used to encapsulate hydrophilic and hydrophobic molecules, respectively[60].

Nanomaterials that function as imaging agents themselves, such as quantum dots (QDs) and carbon nanostructures, may be used for applications similar to those of fluorescent dyes and may confer additional advantages. These structures can exhibit tissue-transparent near-infrared emission, high photostability, single-molecule resolution, and narrow emission bandwidths that can confer multiplexing capabilities[61]. QDs are fluorescent particles with diameters of a few nanometers that exhibit high quantum yield and photochemical stability. The absorption and emission profiles of QDs are dependent on both composition and size[62], with typically narrow, symmetrical, and tunable bands. Single-walled carbon nanotubes (SWCNTs) also exhibit inherent, photostable, tissue-penetrant near-infrared fluorescence between 800 nm and 1,600 nm[63–67]. Carbon dots are 2–8 nm carbon structures that exhibit tunable excitation and emission in the visible-to-near-infrared range; their emission is stable, and they exhibit strong biocompatibility[68]. Fluorescence imaging via nitrogen vacancy centers of nanodiamonds is also gaining utility through surface functionalization[69], similar to other nanomaterials in this class.

Magnetic resonance imaging (MRI) is a powerful tool for noninvasive clinical diagnosis due to its soft-tissue contrast, spatial resolution, and depth of penetration[70]. Magnetic contrast agents are used to improve signal and image analysis, though conventional magnetic contrast agents can be limited by resolution. Iron oxide and lanthanide nanoparticles are used to enhance the signal-to-noise ratio (SNR) of conventional MRI[71] via shortened relaxation times (T1 and T2). Thus, it is unsurprising that these probes have found commercial and clinical use[72]. Computed tomography (CT) contrast agents are largely based on iodinated molecules that absorb X-rays. Nanoparticles composed of gold or other metals have been developed that allow greater X-ray absorption[73] compared to traditional iodinated contrast agents. Positron emission tomography (PET) imaging is often performed in addition to MRI and CT. Nanomaterials have also been synthesized that incorporate radiolabels, such as ^{89}Zr, to image tumors[74]. Several nanoparticles simultaneously incorporate multiple imaging modalities, such as fluorescence or MRI capabilities, into single particle formulations[75]. A notable example, due to its clinical translation, is a silica nanoparticle technology (C-dots) encapsulating fluorescent dyes and incorporating PET imaging agents[59].

Raman scattering is a vibrational phenomenon that imparts chemical information and can be measured via spectroscopic and imaging methods. Although relatively weak, it can be amplified by nanomaterials[76]. Benefits include high photostability and narrow spectral bands facilitating multiplexed imaging[77]. Raman probes have been used in vivo to detect and image nucleic acids, lipids, proteins, and other biomolecules of interest[78]. Metallic nanoparticles are common, as they produce relatively strong Raman signals, and surface-enhanced Raman scattering (SERS) probes are commonly used bioimaging agents.

Upconverting nanoparticles exhibit increased depth of penetration, as this property results from two low-energy excitation photons causing the emission of a single higher-energy photon from the material. These materials are of interest for use in optogenetic manipulation, in which targeted upconverting nanoparticles may add enhanced targeting and precision to optogenetic therapies, in addition to their imaging modality[79]. Additionally, they may be designed to produce signal only in the presence of a target, such as microRNA inside of a cell[79].

Nanoscale sensors

Nanoscale biosensors enable quantitative measurements of diverse analytes, often within living biological systems (Fig. 6). Similarly

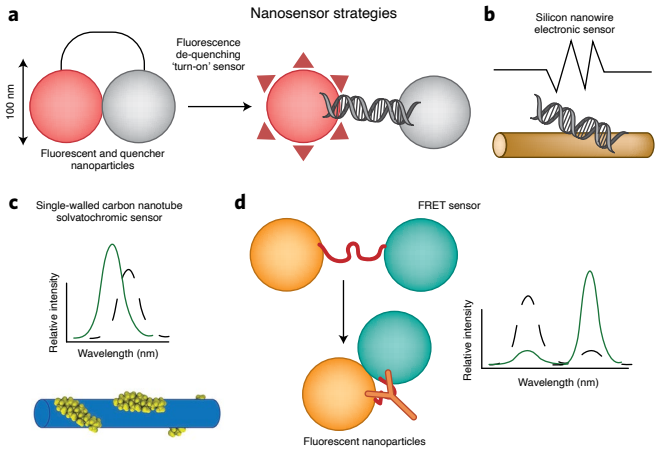

Fig. 6 | Examples of nanoscale sensor strategies. a, 'Turn-on' sensors triggered by fluorescence de-quenching. **b**, A silicon nanowire-based electronic sensor. **c**, A solvatochromic single-walled carbon nanotube-based sensor. **d**, A Förster resonance energy transfer (FRET) sensor. The graphs represent the descriptive fluorescence emission traces of the sensor from black dashed lines (before exposing analyte) to green solid line (after exposing analyte).

to molecular imaging, sensing with nanoscale materials relies on the unique physicochemical properties of the material to detect analytes through various detection modalities, both in vitro (including within living cells) and in vivo (Fig. 1, green quadrant). Analytes can include metabolites (such as lipids, metal ions, nucleic acids, and proteins) and intracellular environments (such as pH and reactive oxygen species), among others. Signal transduction can be electrical, optical, mechanical, or via other mechanisms (Fig. 6) using materials including carbon nanostructures, metals, semiconductors, and nucleic-acid-based nanostructures (Table 2). Often, new materials are studied instead of biological applications of existing materials. A strong potential exists to harness nanosensor technologies for interrogating biological systems.

Nanosensors may be conjugates or aggregates of organic dyes programmed to quench or de-quench, enabling triggered signal changes in fluorescence emission. Fluorescence restoration due to nanomaterial decoupling is a common technique demonstrated in the detection of DNA sequences and single-base mutations using self-assembled gold nanoparticle–oligonucleotide hybrids[80]. In cells, imaging of enzyme-triggered self-assembly of nanofibers from designed chemical precursors allowed the detection of inhibitors for those enzymes, potentially forming the basis of a screening assay[81]. Larger biomolecules, like microRNA, can also be detected in live cells using self-assembled nanoparticles[79]. In vivo examples include self-assembled magnetic 'nanogrenades' and peptide-functionalized gold nanoparticles that were used to detect intratumoral pH and trypsin activity, respectively[82,83].

Carbon nanostructures including carbon dots, graphene, and SWCNTs are frequently employed as sensors. In vitro, SWCNTs have been used to detect molecules such as dopamine[84,85] and protein cancer biomarkers[86], whereas thrombin has been detected with graphene-based aptasensors[87]. Notable progress has been made in developing carbon-based nanosensors to detect nucleic acids in vitro, which is reviewed elsewhere[88]. Silicon nanowires in particular can have high sensitivity due to depletion or accumulation of charge carriers, with a specific example being femtomolar-level detection of nucleic acids[89]. Carbon nanostructures have also been applied for sensing applications in living cells and in vivo, including

Table 2 | Examples of nanosensors and their characteristics

Nanoscale sensor material	Context	Common analytes	Performance
Dye-functionalized nanostructures[79–83]	• Mostly in vitro and in cells • Some in vivo examples	• Nucleic acid sequences, including single base mutations • Proteins • Enzymes	• Strong sensitivity • Signal amplification • Subject to false positives/negatives • Light penetration into biological specimens dependent on the dye
Carbon nanostructures[84,86–88,90–94,98]	• In vitro • In cells • In vivo	• Small molecules • Proteins • DNA/RNA	• Single-molecule sensitivity • Signal penetration into tissues • Multiplexing • Specialized instrumentation often needed
Silicon nanowires[89]	• Primarily in vitro	• Ions/pH • Proteins • Nucleic acids	• Very high sensitivity usually requires electrical contacts, preventing use within cells
Quantum dots/silica nanoparticles[58,59,61,62]	• In cells • In vivo	• Ions/pH	• Bright signals • Subject to potential biocompatibility or toxicity issues

a graphene-functionalized aptasensor that detects nucleolin overexpression in cancer cells[90]. Tissue-transparent SWCNT photoluminescence is exquisitely sensitive to its local environment, allowing intracellular and in vivo sensors for analytes including lipids[91,92], nucleic acids[16,93], reactive oxygen species[94], and endogenous protein cancer biomarkers[95].

While nanoscale sensors have been developed to examine important bioanalytes, few have been applied to address fundamental biological questions. Small-molecule fluorescent probes have long been used by the biological community, but certain drawbacks remain, including difficulties with producing quantitative measurements, low photostability (which often limits transient measurements), and limited in vivo use. The unique properties of nanosensors (Table 2) can potentially address these weaknesses if properly developed in collaboration with chemical biologists. Few such nanosensors are available commercially, making them relatively inaccessible to the chemical biology community. Furthermore, there are numerous bioanalytes of interest to chemical biologists, such as short-chain fatty acids that are the building blocks of reversible lysine post-translational modifications, but only a small fraction can be probed with nanosensors. Such technology gaps could be addressed more rapidly through collaborative efforts.

Nanomaterial biocompatibility

Nanomaterials interact with biological systems in novel ways that can be leveraged to develop new tools, though these interactions may also perturb the system. Nanomaterials necessarily interact with the cell during uptake, intracellular transport, and clearance. These processes are modulated by nanomaterial parameters such as size, surface chemistry, and biodegradability[37]. Depending on the mode of interaction, toxicities can occur via different molecular mechanisms, including metallic particles generating oxidative stress within the mitochondria, long multi-walled carbon nanotubes mechanically damaging the lysosomes, or QDs leaching selenium and cadmium from their cores[62]. Various strategies such as surface passivation exist to mitigate these issues and have been successfully demonstrated for the different classes of nanomaterials reviewed in this work[23] However, close collaboration between nanotechnologists and chemical biologists is thus essential to effectively leverage the capabilities of nanomaterials and ensure that deleterious interactions do not interfere with biological measurements. For example, nanomaterials consisting of iron(III) as delivery cargos[71] should be avoided when exploring biological questions involving reactive oxygen species.

Future outlook

Nanotechnologists have developed tools of potential interest for chemical, cell, and disease biology research. Biocompatible carriers have been developed to solubilize and stabilize compounds and to target locations in live cells and animals while attenuating toxicities. Nano-imaging agents are capable of high-resolution snapshots of biological processes in real time at single-molecule resolution, in live cells, and in vivo. Nanosensors can detect wide ranges of bioanalytes within live cells and in vivo, allowing researchers to better interrogate chemical or biological perturbations. Therapeutic delivery and stable signal transduction from biocompatible nanoreporters, along with high-resolution imaging modalities, can be used to gain insights into important biological processes. Although nanotechnology holds great promise for chemical biology and other fields, there are necessary advancements to be made. Areas of future progress include addressing biocompatibility and toxicity questions associated with certain materials, improving in vivo and subcellular localization strategies, increasing drug encapsulation or conjugation, and increasing physicochemical control. More broadly, those in the field of nanotechnology must improve communication with chemical biologists, make their materials available to the community, and work to address fundamental biological questions, which we hope to inspire herein. In addition, chemical biologists will benefit from quantitative guidance upon comparing and choosing nanomaterials among various choices to meet context-specific needs. Despite these areas for improvement, nanotechnology holds promise for expanding cutting-edge science and innovation at the interface of chemistry and biology.

Chemical biologists have also made substantial contributions that have advanced nanotechnology by providing access to useful molecules and molecular tools. Some notable examples include development of remarkable biorthogonal reactions such as copper-free click chemistry that allows metal-free chemical conjugation in biological systems[96], as well as Diels-Alder reactions[97]. Furthermore, material manipulation techniques, such as those for synthetic peptides and DNA, were originally developed for chemical biology. Direct research collaborations between scientists in chemical biology and nanotechnology can thus improve the engineer's toolkit as well as that of the biologist.

Many nanoscale delivery, imaging, and sensing tools have not yet been applied to address biological questions. With relatively few exceptions, outstanding tools and technologies developed in nanotechnology laboratories have remained investigative and have yet to be used to solve unmet biological problems. Many nanotechnology groups appear to focus on materials development or clinical applications. By contrast, the current generation of chemical

biologists focused on tool development carefully considers the utilities of these chemical tools in relevant biological contexts. Synergistic collaboration between nanotechnologists and chemical biologists may leverage these perspective strengths, with additional likelihood for translation into clinical technology. We envision that this Perspective may inspire not only nanotechnology experts to realize potentially promising applications in chemical biology and contribute to the existing toolbox, but also that chemical biologists may increasingly take advantage of advancements in nanotechnology.

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Author contributions

R.M.W., S.C., J.B.U., M.L., and D.A.H. conceived of the manuscript; all authors reviewed the literature and drafted the manuscript.

Competing interests

D.A.H. is a cofounder and officer with equity interest in Goldilocks Therapeutics, Inc., LipidSense, Inc., and Nirova BioSense, Inc. D.A.H. is a member of the scientific advisory boards of Concarlo Holdings, LLC and Nanorobotics, Inc. R.M.W. is a scientific advisor with equity interest in Goldilocks Therapeutics, Inc. M.L. is a member of the scientific advisory board of Epi One, Inc. P.V.J. is a cofounder and officer with equity interest in LipidSense, Inc. and an officer of Nirova BioSense, Inc.

Additional information

Correspondence should be addressed to J.B.-U., M.L. or D.A.H.