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Towards Realizing Nano-enabled Precision Delivery in Plants

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Abstract

Nanocarriers that can precisely deliver active agents, nutrients, and genetic materials into plants will make crop agriculture more resilient to climate change and sustainable. Nano-agriculture is still a developing field and there are significant scientific and societal barriers to overcome, however, lessons can be learned from mammalian nanomedicine. Greater understanding of how nanocarrier properties affect their interactions with plant surfaces and biomolecules, and their ability to carry and deliver cargo to specific locations, is needed to improve efficiency and efficacy. New tools are required to rapidly assess nanocarrier-plant interactions and to explore and verify the range of viable targeting approaches in plants. Elucidating these interactions can lead to the creation of computer generated *in silico* models (digital twins) to predict the impact of different nanocarrier and plant properties, biological responses, and environmental conditions on the efficiency and efficacy of nanotechnology approaches. Developing sustainable, safe, and socially acceptable nanocarriers for precision delivery in plants will require convergence across both scientific and societal boundaries.

Introduction

Industrial fixation of $N_{2(g)}$ and massive terraforming of the Earth's land mass have enabled sufficient food production for society, but current agricultural practices are unsustainable. At the same time demand for food is increasing globally,¹ while climate related events (e.g., heat waves, drought, and salinity) are predicted to *decrease* crop yields.²⁻⁴ Agrochemical inputs are also getting more difficult and expensive to source (e.g., P and water), soils are being degraded and lost at rates higher than they are formed,⁵ and crop pests are becoming more difficult to control with a warming climate. Greenhouse gas emissions from agriculture account for 14% to 28% of global greenhouse emissions,⁴ further exacerbating climate change and the corresponding impact on crops. There is a tremendous need for disruptive technologies and alternative agricultural practices⁶ to overcome challenges to meeting future food demand and to meeting many of the seventeen Sustainable Development Goals set forth by the United Nations, including zero hunger, climate action, and preserving life in water and on land.

Plant nanobiotechnology approaches along with novel nano-enabled delivery approaches can improve the efficiency of agrochemical use, increase crop resilience, and increase yields(**Figure 1**).⁷⁻⁹ Nano-enabled carriers (nanocarriers - NC), made of active agents (AA) such as copper or zinc, and polymer, silica, and carbon nanomaterials loaded with AAs, have demonstrated the potential to improve plant resilience against disease, promote photosynthesis, and protect plants against environmental threats like heat and salinity stress.^{6,8} Published data on the impact of nanoscale micronutrients suggests increases in uptake and translocation of 20-60%, resulting in similar increases in biomass or yield⁹⁻¹¹. NC can enhance delivery of AA, genetic material, or anti-stress agents to specific biological targets, including plant leaves, roots and vascular tissues, and organelles (chloroplast and mitochondria).¹²⁻¹⁹ However, there are many challenges to making these approaches efficient.

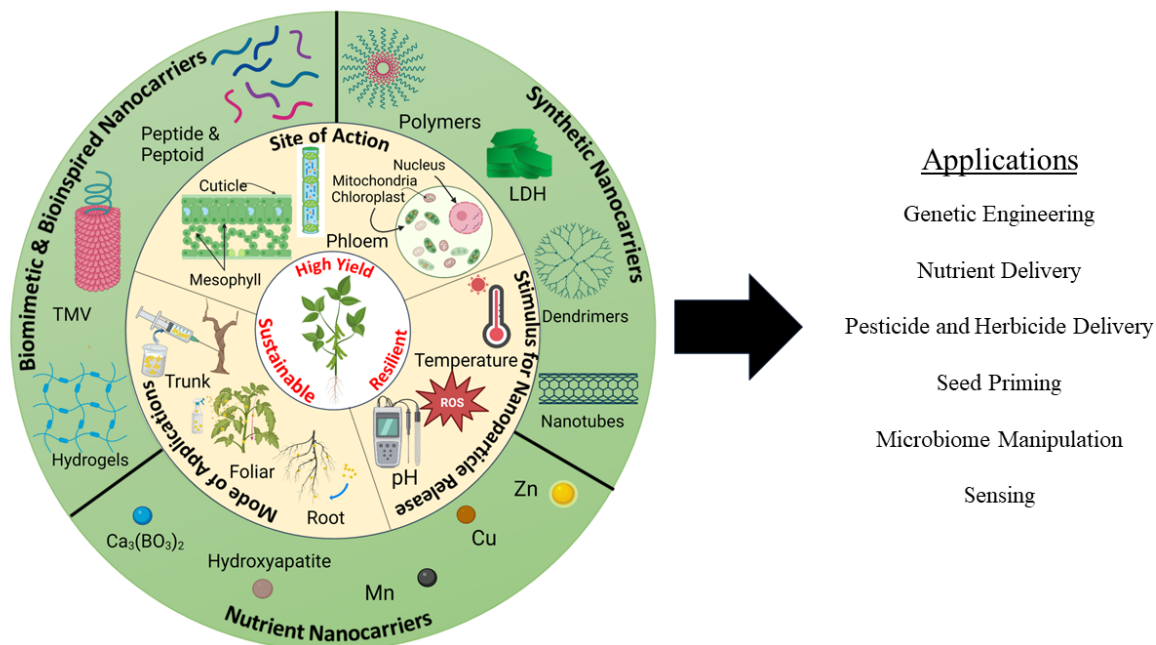


Figure 1. Many synthetic and biobased NC have been proposed to deliver active agents (AAs) into plants. NC can be applied to plants on leaves, roots, or stems and be targeted to tissues (e.g., phloem) or organelles (e.g., chloroplasts) for the release of an AA based on a desired stimulus (e.g., pH or ROS). NC have demonstrated improvements in AA delivery efficiency, enhancements to crop resilience to stress (e.g., heat or salt), and yield enhancement.

The challenges of using NCs to deliver nutrients or active agents in plants parallel those in nanomedicine, and thirty years of nano-enabled precision drug delivery in humans can inform a path forward for delivery in plants. Work in nanomedicine to develop biocompatible formulations, biotargeting principles, and controlled release mechanisms (e.g., based on pH or temperature),^{20,21} and the development of pharmacokinetic models for assessing NC-cell interactions,²² uptake, efficacy, and toxicity potential in mammalian systems can be leveraged for design of precision delivery approaches for plants. Recent studies have adapted approaches pioneered in nanomedicine to provide “targeted delivery” to specific plant organs with some success (Table 1). However, there are significant differences between precision delivery in plants compared to

precision drug delivery in mammals, leading to several key knowledge gaps that must be addressed. These important differences include the following:

- Plants have different cell barriers for nanomaterial uptake compared to mammalian cells, including cell walls, cuticle, and mucilage. The cell walls made of cellulose, hemi-cellulose, and pectin in plants are a crucial structural barrier that does not exist in mammalian systems.
- Important biological targets in plants are different than in mammalian cells, organelles such as chloroplasts and vascular tissues like phloem, and the corresponding targeting biorecognition molecules need to be identified.
- Therapeutic compounds and active agents to be delivered in plants are different than in mammalian cells, potentially requiring a new NCs.
- The massive scale of agriculture necessitates the sustainable sourcing of low-cost materials for agrochemical delivery and for scalable manufacturing approaches. This is a significant departure from nanomedicine and will require innovation in both NC design and manufacturing.

Table 1. Examples of targeted delivery of nanocarriers and chemical cargoes in plants through biorecognition.

Target	Surface moieties enabling targeting	Nanocarrier	Designed functions	Ref.
Phloem	Sucrose	Carbon dots (CD)	Promotes uptake and delivery of chemical cargo to phloem by β -cyclodextrin functionalize CDs	[18]
	Glycine methyl ester	Glycine methyl ester conjugated Polysuccinimide NC	Nanocarrier delivery of fungicide mediated by plant amino acid transporter AtLHT1	[29]
Stomata guard cells & trichomes	LM6M antibody/BSA	Gold NC	NP delivery to leaf stomata and trichomes	[27]
Nucleus	Peptide (PKKKRKVKHKHKHKHKHKHKHKHKHKH/RRRRRRRRR)	CPPs/nucleus penetrating peptides	Improved nucleus internalization of cargo	[30]
Chloroplast	Peptide (KH9-OEP34)	Organelle penetrating peptides	Improved organelle internalization of cargo	[31] [25]
	Peptide (MASSMLSSATMV)	Quantum dots (QD)	Delivery of chemical cargoes by β -cyclodextrin functionalized QDs into chloroplasts	[12]
	Peptide (MASSMLSSATMVGGGGG GKHKHKHKHKHKH)	Single-walled carbon nanotubes (SWCNT)	Delivery of plasmid DNA-coated SWCNT into chloroplast	[13]
	Peptide (MASSMLSSATMVGGC)	Carbon dots (CD)	Delivery of chemical cargoes by β -cyclodextrin functionalized CDs into chloroplast	[13]

There is tremendous opportunity for nano-enabled precision delivery, but there remain many key unanswered questions about the phenomena controlling the complex interplay between nanocarriers (NC) and the biological processes they affect. Nanocarrier-protoplast interactions have been studied²³ and approaches for targeting a NC to a specific plant tissue or organelle are emerging.^{12,15,16,18,24–31} However, the fundamental processes controlling the interactions between NC and important plant surfaces (e.g., cell walls) and milieu of organic compounds in the vasculature (xylem and phloem) that may affect their uptake and translocation, transformation, and efficiency are not known. There is currently no understanding of how plants adapt to manage nanoparticulate entities in their vasculature, or ways to harness or control NM transformations in plants for beneficial purposes. Model experimental systems for probing the underlying mechanisms of interactions of nanomaterials with plant surfaces are not available, and atomistic, coarse-grained, or continuum models to predict NC transport or targeting in plants based on plant and NC properties are needed. Model development is further challenged by a bifurcated field,

with crops falling into monocots and dicots with varying physiology. Together, this currently prevents development of robust structure-activity relationships for the design of efficient engineered NC. Achieving solutions to this wide range of challenges will require the convergence of thought, approaches, and technologies across disciplinary and societal boundaries.

This perspective discusses the critical technical challenges that must be overcome to realize high value opportunities for nano-enabled precision delivery of agrochemicals and biomolecules in plants and presents a conceptual research roadmap to overcome those challenges. This includes recommendations for coordinating research activities, and sharing tools, methods and information to advance the process of discovery and translation into practice. The societal challenges to implementing nanotechnology for crop production in a safe, sustainable, and socially responsible manner are also discussed.

Leveraging structure-property relationships of nanomaterials for efficient active agent delivery

Nanomedicine is a clinical reality, and nanoscale carriers are used for gene and drug delivery. Based on lessons learned in nanomedicine, successful nanocarriers are those that interface well with organisms, readily overcome biological barriers (i.e., extravasate from the vessels into target tissue and cells) and harness biological helpers (i.e., disease-associated protein expression or changes in pH), mitigate off-target effects, and maximize efficacy. This is achieved by selecting appropriate material compositions and properties because they govern the fate of the NC in the biological system. These same properties are also integral to AA packaging, delivery, and release. While some of the materials and chemistry concepts are transferable between these disciplines, the biology of plants and mammalian systems is distinct and the translational and commercialization space differs.

The multitude of factors influencing NC design for targeted and controlled release, size, rigidity, surface chemistry, aspect ratio, and ligand density (**Figure 2**) offer both opportunities and challenges. While this diverse design space provides a wide array of potential NCs for achieving efficient delivery in plants, the process of exploring this through trial-and-error or intuition-guided approaches is labor-intensive. Machine learning strategies, such as those used in designing high glass transition polymers,³² drugs and small molecules,³³ self-assembling peptides,³⁴ and lipid nanoparticles³⁵ can guide a more systematic exploration of the design space.³⁶ These data-driven machine learning strategies will need to work closely with experimental and campaigns and modeling to rapidly map relationships between NC structure and its function and identify the most promising unexplored NC candidates for precision delivery in plants.

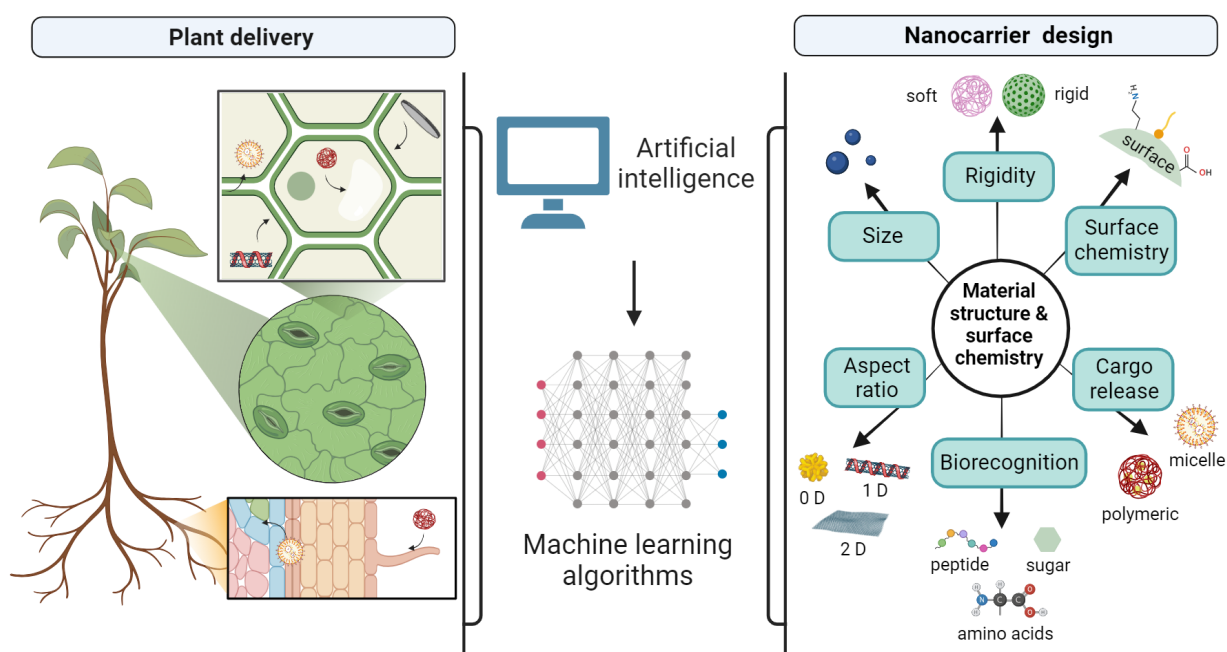


Figure 2. Design of nanocarrier properties (e.g., size, rigidity, and aspect ratio), biorecognition molecules and surface chemistries determines their uptake, translocation, and targeting in plants, as well as active agent release mechanisms. Artificial intelligence including machine learning algorithms can guide experimental research by mapping relationships between structure and function of nanocarriers for precise delivery in plants.

Material structure and surface chemistry define NC-plant interactions, uptake, and translocation. Although systematic analysis of how NC properties influence interactions with plant surfaces and milieu are limited, available examples provide insights into the engineering design space. Zeta potential magnitude determines the uptake of NC across plant cell and organelle lipid membranes with highly charged nanomaterials being able to spontaneously disrupt and penetrate lipid bilayers.^{23,37} Surface charge also dictates the translocation and distribution of nanoparticles in plant cells and organelles, where negatively charged nanoparticles preferentially translocate between organs through the vasculature whereas positively charged nanoparticles remain on the applied organs.^{15,28,38} Adhesion of particles to the cuticle on plant leaves is promoted by adding positively charged surface moieties³⁹.

Size, surface functionality, and rigidity are also important controls of the efficiency of interactions with plant surfaces and translocation of nanomaterials across cell barriers. Many size exclusion limits (SEL) have been reported, e.g., at least 50 nm for spherical gold particles through plant cell walls and cuticles of wheat,¹⁵ < 500 nm for cell membranes, and < 10 nm for symplastic transport in the plant cell via the plasmodesmata.⁴⁰ While these SEL appear to severely limit the types and dimensions of possible NC, these measurements were largely based on rigid materials. Some reports have indicated plant delivery with less rigid (low stiffness), deformable polymeric NC violates the afore-mentioned SEL cut-off.^{41,42} Relatively large (~10 nm in diameter and 200 nm in length) “soft” (i.e., less rigid) polymeric materials were taken up by leaves more readily than “hard” metal and metal oxides particles of similar size.⁴³ However, it was recently described that rigid inorganic nanoparticles require less energy to overcome the energy barrier for membrane internalization than soft organic nanoparticles.⁴⁴ As such, NM rigidity appears to be a key property affecting delivery across the plant cell wall.⁴⁵ The chemical identity of the nanoparticle surface also plays an important role controlling interactions with hydrophilic and hydrophobic components of plant membranes.^{15,26} Polyphenolic compounds coating NC surfaces promoted adhesion and retention to the waxy leaf surface due to H-bonding of the phenolic OH groups and the leaf

cuticle.⁴⁶ While biomolecule uptake in plants is well-studied, there is relatively little known about the mechanisms of uptake and translocation of nanoparticles in plants, especially for soft biomaterial interactions with plant surfaces, tissues, and organs. This knowledge is needed to engineer effective NC from sustainably sourced biomaterials.

The aspect ratio of the NC is a relatively unexplored design variable to consider. Many of the NC described in the literature are hard spheres (e.g. metals and metal oxides), but this morphology may not be optimal for attachment to plant surfaces or delivery across plant cell barriers, such as cell walls. Nanomedicine has demonstrated that low and high aspect ratio (AR > 1) materials have distinct *in vivo* trafficking and cell interactions. Work delivering high AR carbon nanotubes and polymers in plants indicates that the smallest dimension of a high AR material controls its transport^{23,37}, and that there are differences between how monocots and dicots transport high aspect ratio polymeric NC⁴³. Rods and filaments or sheets, and in general materials with AR >1 have a larger surface to volume ratio and therefore more contact sites with plant surfaces compared to spheres.⁴⁷⁻⁴⁹

Biomimicry and bio-inspired designs. Biomimicry and bio-inspired designs and coatings can be leveraged to promote uptake and translocation in plants.^{12,13,16,50} Plant viruses for example achieve long-distance movement inside the infected plant through passive transport along with the photoassimilates⁵¹. Plant viral cell-to-cell movement is often achieved through specialized movement proteins, so there is an opportunity for biomimicry to promote mobility of virus-like particles in plants. Coating NC with biorecognition motifs (e.g. peptides or sugars) can use specific cell receptors to promote uptake and translocation in plants¹⁸. For example, carbon dot based NCs coated with guiding peptides or sucrose enhance transport of chemical cargoes into chloroplasts or phloem, respectively, through biorecognition transporters on their membranes^{13,18}. Similarly, zwitterionic properties of plant viruses have been leveraged to develop plant virus-based carriers for improved nematicide delivery through soil to plant roots⁵²⁻⁵⁴.

Structure determines nanocarrier cargo and release to its target. Nanobiotechnology promised to provide controlled, stimuli-responsive, or on-demand release of AA cargoes. We can leverage slow-release approaches from the medical community, but the formulation chemistry needs to be adjusted to the vastly different time scales for agriculture (days to months) as compared to biomedical applications (minutes to hours, days, or weeks). For instance, Bioclay™ nanosheets provided the slow release of double-stranded RNA (dsRNA) in plants for several weeks and improved plant resistance to viral and insect pathogens^{47,48}. In general, high aspect ratio nanomaterials with large surface to volume ratios are better suited for the delivery of larger molecules such as plasmid DNA (pDNA)^{13,14,16,19}. However, current approaches of electrostatic binding of pDNA to nanomaterials lack sufficient control over cargo *release* dynamics needed for DNA integration into the plant genome^{14,55}. Small spherical carbon dots coated with cyclodextrins, which are used for biomedical applications, can also load and deliver a wide range of AAs from herbicides and pesticides¹³ either by slow or triggered release depending on cyclodextrin type.¹⁸

Biologically or environmentally responsive NC. Many adverse outcomes in agriculture are a result of environmental conditions, e.g., heat stress, salt stress, or water stress, that produce excess reactive oxygen species. There are also pH gradients (~5.5 - 8) across plant cells and organelles. Core-shell NC that respond to changes in, for example, pH, ROS, or temperature can deliver AAs to a specific location in the plant or in response to stress events. For example, nutrient (N, P, K, Cu)-loaded nanocapsules with a fungal enzyme-responsive core increased crop photosynthetic output of wheat and soybean⁵⁶. and a temperature responsive core-shell polymer carrying a plant hormone that is released at temperatures above 40 °C relieved heat stress in tomatoes for at least 15 days⁴³. ROS-responsive core-shell polymeric NC promoted photosynthesis under plant stress by scavenging ROS while releasing magnesium (a macronutrient that forms the central core of chlorophyll) from the NC core⁵⁷.

Multi-staged designs where the structure-function is tailored toward each plant microenvironment could be envisioned, i.e., a core-shell principle where the outermost layer is

designed to facilitate plant surface attachment or entry – upon entry, functional moieties are exposed to facilitate long-distance vs. cell to cell movement, targeting of organelles and/or pathogens of interest, with subsequent release of cargo upon interaction with target. Better understanding of the interactions of NC with plant biological barriers and their *in vivo* transformations will be critical to overcome several challenges, including the need to avoid non-target interactions and loss of payload en-route to target. However, more complex formulations are more difficult and costly to manufacture, which may be unsuitable for agronomic applications. Targeted delivery of NC loaded with AA is expected to be concentration dependent where AA efficacy increases with concentration of NC. However, targeting of NC at very high efficiency could potentially result in detrimental effects in plants. This would require determining optimal concentrations of NC that are both biocompatible and maximize the AA delivery and efficacy

Biotargeting approaches for precise delivery of nanomaterials and their cargoes

Biotargeting is a cutting-edge technique that aims to deliver nanomaterials and their cargoes to specific plant tissues, cells, or organelles, and plant pathogens^{13,16,27}. This approach can increase the delivery efficiency of AAs to more than 70%^{12,13}. Biorecognition mediated delivery relies on coating NC with biomolecules such as peptides, aptamers, signaling tags, and sugar adducts^{18,25,58} to guide the nanomaterials and their cargoes to their target via specific plant or pathogen receptors and membrane integral components⁵⁹. Developing biotargeting methods for plants requires an ability to coat nanomaterials with targeting biomolecules that can specifically bind them to desired plant cells or organelles' surfaces, as well as understanding of how the NC properties and surface chemical composition controls their interaction with the plant surfaces and milieu^{12,13}. Complete understanding of the plant membrane properties shared or differentiating target plant taxa will also be crucial for designing NC capable of biotargeting⁶⁰.

Lessons learned from nanomedicine. Biorecognition mediated delivery has been accomplished in nanomedicine by coating nanomaterials with domains with high affinity and

specificity for selected targets^{61–63}. This biorecognition approach has also been used for developing selective adsorbents, enzyme immobilization, drug delivery, and immunoengineering^{64–70}. Combining binding peptides or molecules with another functional aspect of the NC, such as gene silencing or nutrient delivery, allows for precisely targeted delivery of both the cargo and NC function. Designing biorecognition molecules for targeted delivery in plants should be analogous to drug delivery methods in humans and animals^{12,71}. For example, identifying target molecules, such as tissue-specific proteins or surface receptors, could enable the design of NC that preferentially bind to a targeted plant surface (**Figure 3**). Developing a library of plant-specific and tissue-specific targets is paramount for these efforts. Tools to design cell-penetrating biomolecules have been explored in nanomedicine but not in plants⁷². Binding studies of target moieties with protein affinity domains, nucleic acid aptamers, signaling tags, and sugar adducts help create complementary pairs to specifically bind to the target tissues^{73–75}. Tools such as phage and yeast display, systematic evolution of ligands by exponential enrichment (SELEX), and thermal shift assays can identify potential binding domains for improved specificity and performance^{73,76–78}. Adapting these tools for plants should enable the design of NC for targeted delivery in plants.

Biotargeting approaches can also be used for efficient delivery of AA to plant pathogens including fungi, bacteria, viruses, and nematodes that localize within the plant vasculature or roots. This approach has led to the development of multifunctional nanomaterials for biomedical applications that can target multiple signaling pathways in infected cells, reducing toxicity and increasing therapeutic efficacy^{79,80}. Recent studies have indicated that NC can be designed to target receptors on the surface of fungi cell wall components^{81,82} or lipopolysaccharides on bacteria, enabling precise delivery of AA^{83,84}. NC can also be designed to deliver pesticides to control plant pathogens that localize within the plant vasculature (xylem or phloem) or in the roots. For example, designing NC that interact with proteins or sugars, or specific transporters and channels present in the phloem¹⁸. Plant immune receptors have also inspired the design of NC

for targeted drug delivery⁸⁵. NC able to recognize pathogen-derived molecules could selectively target and deliver therapeutic agents to infected cells or tissues while avoiding healthy cells. The Lipid Exchange Envelope Penetration (LEEP) model has been used to describe how size and charge of nanomaterials affect their capacity to cross cell membranes and distribute within plant protoplasts and organelles^{23,37}. This modeling approach could be extended to understand how bioactive targeting moieties affect internalization of NC in plants. Overall, receptor-mediated signaling mechanisms in plants⁵⁹ offer a powerful platform for advanced plant delivery systems through NC-mediated biotargeting strategies.

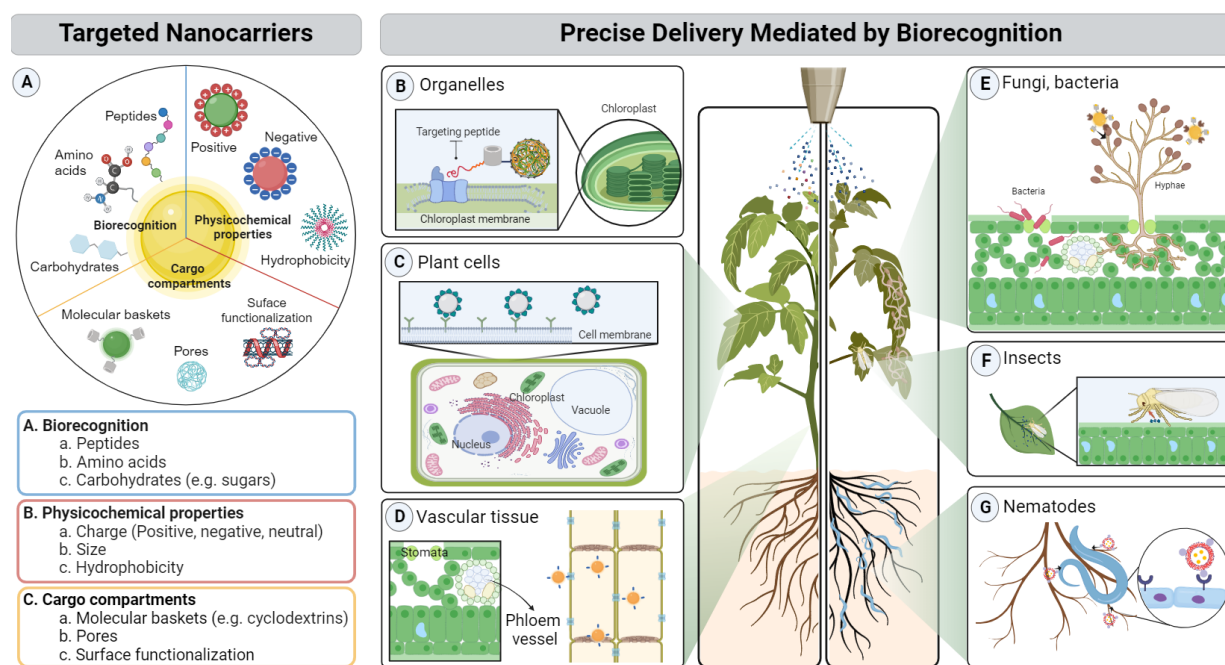


Figure 3. Precision delivery of nanocarriers with cargoes to target organelles, cells and tissues mediated by biorecognition. A) Nanocarriers with cargo compartments are designed with physicochemical properties (e.g., charge, size, and hydrophobicity), and coated with biorecognition molecules (e.g., peptides, amino acids, and carbohydrates) for precise delivery to specific organelles, cells, and tissues. The porous interior, molecular baskets, or surface functionalization of the nanocarriers enable the delivery of a wide range of AAs. Biorecognition mediated delivery of nanocarriers and their cargoes to plant B) organelles such (e.g., chloroplasts), C) cells (e.g. stomata or trichomes), and D) tissues (e.g., phloem). Nanocarriers could also be used for biorecognition mediated delivery to plant pests and pathogens, including E) fungi and bacteria, F) insects, and G) nematodes. Alternatively, nanocarriers coated with pathogen-derived molecules could selectively recognize infected cells or tissues.

Targeted delivery mediated by the plant or pathogen biorecognition molecular machinery.

Biorecognition mediated delivery of NC with chemical or DNA cargoes to specific organelles (e.g., chloroplasts and mitochondria)^{13,16} or cells (e.g., stomata)²⁷ has recently been demonstrated using guiding peptides or proteins that recognize specific receptors in the target organelle or cell membrane. Plant viruses encode unique multifunctional proteins (e.g., movement proteins - MPs) with specific domains that interact with proteins from the host cells⁸⁶. The MPs facilitate the transport of virus-like biomolecules⁵⁰ by enlarging cell-to-cell connections (plasmodesmata), then use structural proteins for targeted delivery to specific sites in plants⁸⁷. The strategies that viruses use for penetrating and translocation inside plants can potentially be used to provide precision delivery in plants to solve real-world problems. There are significant benefits of targeting AA delivery for disease control to specific organs, tissues or cells of interest where pathogens infect or colonize, including increased AA efficacy and reduced application doses of environmentally toxic pesticides. Some examples of potential targeted nanocarriers for plant pathogen control include tobacco mild green mosaic virus (TMGMV) for plant rhizosphere delivery of nematicides⁵², carbon dot nanocarriers for AA delivery to the plant vascular tissue (e.g., phloem) where microbial pathogens localize¹⁸ or targeted nanocarriers to stomatal guard cells on the leaf surface to limit bacterial or fungal pathogen entry²⁷. However, we currently have a poor fundamental understanding of the delivery mechanisms by biorecognition in plants, the physical and chemical interactions of nanocarriers with plant interfaces, and the potentially side effects of biotargeting on plant (or non-target organisms) physiological and developmental processes. High throughput assays and design tools used in nanomedicine for drug discovery will be instrumental for advancing bio-targeting in plants.

Nanocarrier transformations for targeted and controlled delivery

Experience with nanomedicine indicates that *in vivo* NC transformations, e.g., biocorona formation⁸⁸, dissolution, or enzymatic degradation⁸⁸ are critical in controlling both targeting and AA delivery efficacy. NC will interact with a plethora of biomolecules on plant surfaces (e.g., lipids in membranes and pectin/cellulose in cell walls) and in plant cytosolic, apoplastic, or vascular fluids. Yet, the range of transformations of NC (**Figure 4**) once they are on or inside of plants remains largely unexplored.

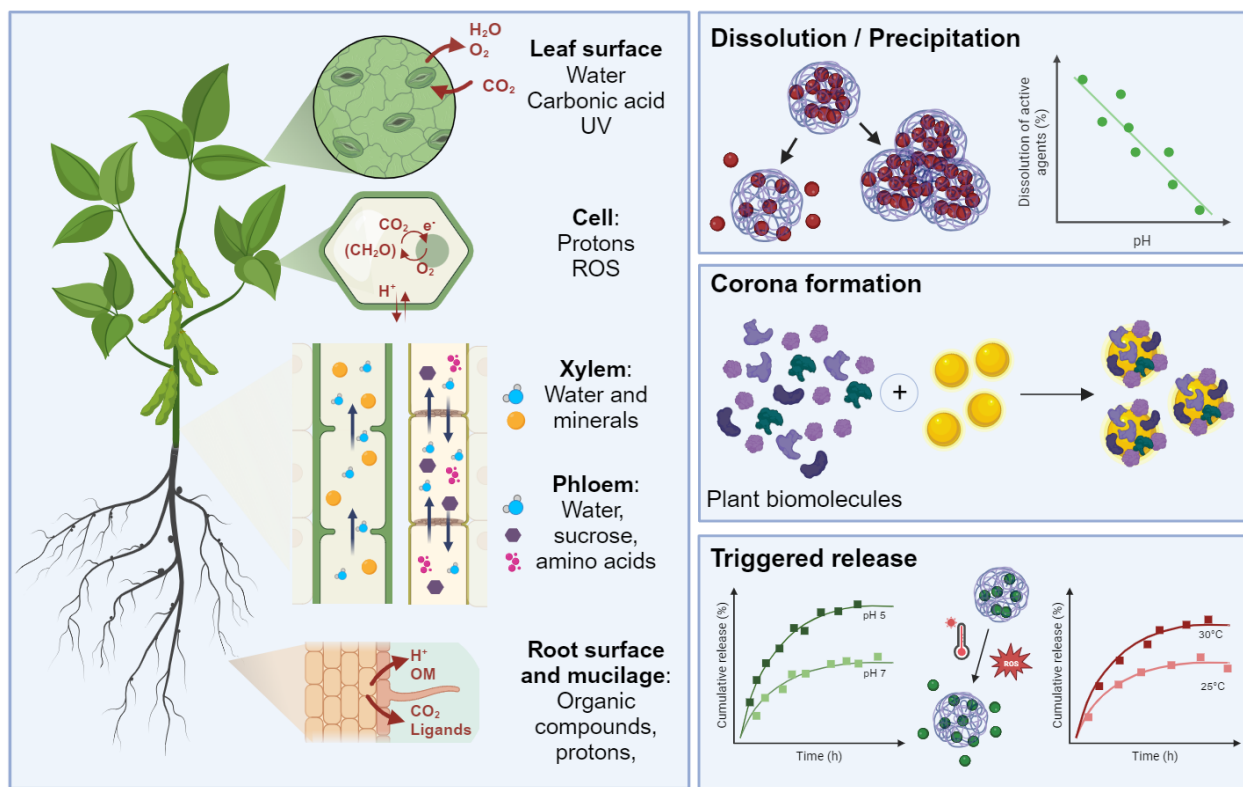


Figure 4. Nanocarrier transformations for targeted and controlled release. Nanocarriers may transform in a myriad of ways on and inside of plants. This includes transformations on the leaf and root surfaces after application, interaction with ROS in cells, and interaction with components in the vasculature (dissolved minerals, sugars, amino acids, and proteins). These interactions can lead to dissolution, corona formation, and potentially trigger the release of the AA it is carrying, either intentionally or unintentionally. These transformations will ultimately control the fate of the nanocarriers inside of the plants and efficacy of the intended treatment.

Exogenous transformations at the plant outer surfaces. The cuticle, a hydrophobic plant surface that prevents water loss from plants, is the first key biointerface encountered by foliar applied NC. Root peripheral and border cells are the first biointerface encountered by soil or hydroponically applied NC. Both barriers can promote the dissolution of kinetically unstable NC^{49,89,90}. The rhizosphere (the interface between soil and roots) has gradients of physical and chemical conditions due to root exudates (carbon-based biomolecules) that can promote NC transformations. For instance, wheat plant root exudates led to a pH increase in rhizosphere soil that modulated proton-induced dissolution of CuO NC⁹¹. Dissolution of Fe-Al/Ge nanotubes⁹² and CuO NC⁹³ by plant-derived siderophores have been reported. By tuning the rate of NC dissolution, or by engineering the materials to specifically interact with biomolecules on these interfaces can control the rate of delivery of these agents into plants⁴⁹. Overall, better understanding of the range of biomolecules in leaves, roots (e.g., root exudates), and stems that interact with and mediate the transfer or complexation of NC is needed to design efficient materials⁹⁴.

Endogenous transformations inside of plants. Uptake of NC into plant cells relies on the translocation across cell walls. The cell wall is the outermost layer of plant cells, formed by a complex mixture of cellulose fibers, pectin, and other biomolecules depending on the plant species. The underlying chemical and molecular level transformations of NC within cell walls or cell membrane components, and how they affect their subsequent translocation or interactions with plant biomolecules such as lipids, carbohydrates, or proteins are unknown. As whole plant translocation requires that the NC cross a cell wall and membrane, NC translocating through the plant vasculature have likely acquired a biocorona^{23,37,95} further altering the designed surface properties. Reports on the interactions between plant proteins and NC are limited^{96,97} and the impact of these transformations on translocation and biotargeting are unknown. Systematic studies are needed to understand how NC properties such as size, charge, chemical identity, and

hydrophobicity impact the formation of plant protein coronas and subsequent NC translocation, targeting, and efficacy.

Controlled release by NC transformations. Large swings in pH as NC translocate in plants can potentially be leveraged to elicit a transformation for targeted release of an AA. For example, apoplastic fluids can be acidic (pH of 5.5-6), while the pH in the cytosol and chloroplasts is slightly basic (pH of ~7.5-8). For example, leaf surface pH changes during formation of carbonic acids enabled the slower release of RNAi from Bioclay™ nanosheets for combating plant pathogen infections⁴⁸. While a pH-triggered release is common in biomedical applications, they have been underutilized for delivery of AAs in plants. The generation of ROS or reactions with sulfur-based analytes in the glutathione pathway can also be used for triggered and targeted AA release, but few studies have explored these approaches⁵⁷.

Challenges for delivery mediated by transformations. NC that can utilize biotransformation for controlled and targeted delivery in plants can improve efficiency and efficacy of AA and plant nutrients delivered to plants. However, there is limited understanding of the acquired coatings of biomolecules on NC and how this affects their transformation and biotargeting in plants. Categorizing the fundamental interactions of NC with the wide diversity of proteins and biomolecules in plant species will require a large set of model systems. The *in vivo* characterization of these processes is even more challenging to explore given the difficulty in extracting plant phloem and xylem in sufficient quantities for study and the lack of tools for measuring NC transformations *in vivo*. In addition, these interactions will likely be modulated by the plant life cycle as well as by responses to biotic and abiotic environmental conditions.

Creating predictive digital model plant systems

The development of a plant “*digital twin*”, *i.e.*, a virtual (computer-based) representation of a plant, including its biosurfaces, organs and vasculature is a potentially transformative tool for enabling

targeted delivery of NC in plants. “Digital twins” have been widely used in manufacturing, infrastructure management, and predictive maintenance^{98,99}, but there is no existing plant “digital twin” to virtually test the efficacy of various NC biotargeting or controlled release strategies. Creating such a model would enable rapid in silico design and testing of different NC configurations for a specific function, e.g., enhanced delivery of an AA to a target site within the plant. The concept of the plant “digital twin” is analogous to the use of computation for personalized human medicine, frequently referred to as the development of a “digital patient” e.g., computational fluid dynamics (CFD) in support of patient-specific stents or patient-specific prostheses^{100,101}. In both cases, the goal is to numerically simulate physical laws governing transport and chemical phenomena (e.g., adsorption, advection, diffusion, or transformation) with geometries and boundary conditions specific to the individual under consideration. These models are *intrinsically multi-scale*, integrating knowledge from the atomistic scale (e.g., molecular transport across cuticular membranes or within ion channels)^{102,103} to the continuum scale (e.g., pressure-driven flow within the phloem or cohesion-tension driven flow within the xylem)^{104,105}.

Key enabling computational methodologies. There are several computational frameworks that need to be developed as the backbones for a digital plant model (**Figure 5**). Each framework provides information at different length- and time-scales as nanoscale, mesoscale, continuum, and system level.

Nanoscale. Molecular dynamics (MD) and Monte Carlo (MC) simulation can be used to model nanoscale (e.g., tens of nanometers and time-scale of nanoseconds to microseconds) transport phenomena¹⁰⁶ such as transport of NCs across plant biointerfaces. Both MD and MC require molecular-scale knowledge about material compositions, microstructures, and mechanics. While these properties can be determined for the NCs using available analytical techniques, the molecular scale properties of plant surfaces such as the cuticle, cell wall,

epidermis, etc. are less certain and variable across plant species. Atom simulations based on classical molecular mechanical (MM) force fields¹⁰⁷ may potentially be used to simulate important transport phenomena or chemical transformations on the order of thousands of atoms¹⁰⁸, but the amorphous microstructure of biological materials will make it challenging. A critical step in the application of such simulations to plant surfaces will be the parameterization and validation of the force field parameters for the relevant components.

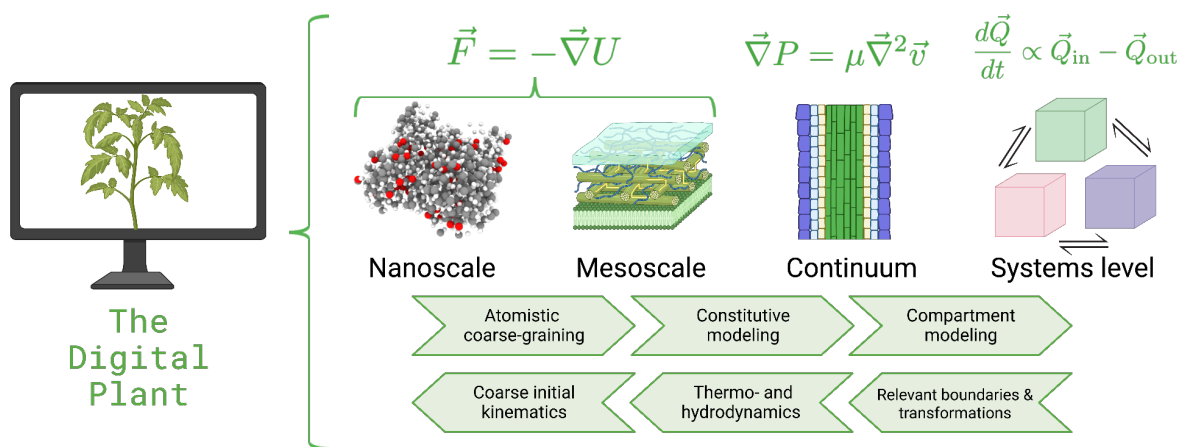


Figure 5. Development of a plant “digital twin” will enable rapid discovery of nanocarrier designs that enable efficient targeted delivery into plants. It will require the integration of models at different scales that capture the relevant biochemistry and biophysics at the nanocarrier-plant biointerface. Parameterizing those models will also require development of model plant surfaces for detailed mechanistic studies both in vitro and in vivo.

Mesoscale. Coarse-grained molecular dynamics (CGMD) simulations using the MARTINI model¹⁰⁹ have lower resolution, but are typically two to three orders of magnitude faster than corresponding all-atom simulations, making it possible to simulate substantially larger systems (e.g., a patch of cytoplasmic membrane at the length scale of ~100 nm)¹¹⁰, and for much longer time-scales than all-atom simulations. Similar models at comparable or even lower resolution¹¹¹ have been developed for plant cell walls¹¹² and used to explain their unique mechanical properties¹¹³.

Continuum. Many plant transport systems can be modeled using classical transport laws (e.g., Navier-Stokes), so there are a range of continuum computational fluid dynamics (CFD) methods that are suitable for studying transport in plants. For example, finite-element-based approaches have been used to model transpiration in stomata¹¹⁴ and flows within xylem;¹¹⁵ finite-volume-based approaches have been used to model root uptake of water and nutrients^{116,117} and hydrogen peroxide signaling within plants¹¹⁸. Flows into and within plants may exhibit phenomena that pose unusual modeling challenges, including multi-phase flows (as is the case in transpiration), free surfaces (as is the case for droplets on a leaf), or geometrically complex boundary conditions (as is the case for flows in xylem and phloem). In such cases, other CFD approaches may be fruitful, including the Lattice Boltzmann method, which has been used to model porous flow through bordered pits in xylem (a system that presents particularly complex boundary conditions)¹¹⁹.

Systems-level. A particularly useful advance for modeling targeted delivery in plants would be the development of *botanokinetic/botanodynamic* (BK/BD) models, in close analogy with pharmacokinetic/pharmacodynamic (PK/PD) models for drug delivery in humans¹²⁰. Similar to a PK/PD model, a BK/BD model would describe the movement of the NMs or NCs within and between various *compartments* of a plant (e.g., a plant's cuticular membrane, organelle like chloroplasts, or the rhizosphere). BK/BD models are a natural multi-scale framework for digital plants, as they directly interface with all of the previously discussed simulation methodologies, each of which can be cast as a specific compartment within a BK/BD model. Developing the necessary general-purpose BK/BD computational tools will enable efficient optimization and sensitivity analysis¹²¹, a necessary feature to enable rapid discovery of new NC and targeted AA delivery approaches.

Benchmark data to develop and calibrate digital plants. Modeling the behaviors of NC in plants will require the collection of benchmark quantitative data on plant surface properties to calibrate and validate those models. In particular, it will be necessary to quantify the structural composition and properties of selected surfaces, e.g., the chemical composition of plant cell wall and cuticle surfaces, organelle membranes, and endomembranes. In addition to the chemical composition and structural features of plant surfaces, it might also be necessary to include the mechanical properties of plant surfaces in plant-nanomaterial interaction models. For example, the mechanical properties of plant surfaces such as, tensile strength¹²², contact angle, porosity, and roughness may predict the movement of NC through key plant barriers. Some are relatively easy to measure using existing techniques (e.g., contact angle of a leaf cuticle), but measuring these properties *in vivo* for internal structures such as cell walls, will be challenging. In addition to the physical and chemical properties of important plant surfaces, the properties of phloem and xylem that may affect transport in the plants must also be measured. For example, the rheological properties of these fluids would be needed to accurately model sap flow transport rates as well as diffusion between cells or across plant biosurfaces¹²³. While many of these properties are available in the plant biology literature, quantitative measurements of the interactions of NC with plant biosurfaces are limited yet necessary for the development and calibration of plant “digital twins”. It is also worth emphasizing that, as is the case for all digital twins, useful digital plants would necessarily require extensive calibration and validation, at all scales studied by the model. A high near-term priority is the development of canonical experiments that will be used to validate digital plant models.

Critical challenges for the development of digital plants. The development of a viable plant “digital twin” for studying NC behaviors *in vivo* is a daunting task. This is especially true given the range of plant biological diversity. In direct analogy with “digital patient” models, in order to be useful, plant “digital twins” must also account for the biological diversity of plants. For example,

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there is an enormous range of cuticular compositions and microstructures¹²⁴ and a large diversity in plant vascular structures.¹²⁵ A fruitful starting point may be to develop representative models for broad groupings of main crop plants that have well-characterized differences in relevant anatomical features (e.g., monocots vs. dicots, C4 vs C3 photosynthesis)^{126,127}. Another critical need for building digital plant models will be the development of open-access simulation codes and datasets, including complex datasets from -omics (e.g., genomic, proteomic, metabolomic) analyses. It will be particularly important to develop consistent formats and standards for workflows, data, and metadata, which will require significant collaboration (and some amount of enforcement) within communities of researchers, publishers, funding agencies, and industrial partners. On this front, it may be particularly instructive to look to the successes of and challenges faced by analogous efforts to digitally twin materials, exemplified by the Materials Genome Initiative^{128,129}.

New data acquisition tools and methods for tracking nanomaterial mediated delivery

Development of new tools, capabilities, and model systems will be critical to development of digital plant models and realizing the benefits of nano-enabled precision delivery of agrochemicals. These include i) *in vivo* characterization and detection of NC, ii) increasing the throughput and speed of data acquisition *in vitro*, iii) development of *in vitro* model plant surfaces for study, and iv) facilitating NC biotargeting and controlled release in plants (**Figure 6**).

Tools for in vivo characterization and detection of NC. Tools for qualitative and quantitative *in vivo* characterization of NC in plants are needed for tracking their behavior in plants, developing plant “digital twins”, and for rapid development of effective NC-mediated delivery applications. Methods for imaging NC *in vivo* are available but have limitations. For example, NC can be tracked using their intrinsic fluorescence^{12,13,95}, or a labeling dye^{130–133}. NC can also be tracked using

selected elements in the NC using laser-ablation¹³⁴ or inductively coupled plasma mass spectrometry (ICP-MS)^{15,135}, synchrotron X-ray fluorescence (XRF) imaging^{15,27,28,136,137}, or electron microscopy with energy dispersive X-ray analysis¹³⁸. However, most of these tools are destructive and only provide two-dimensional information. Three-dimensional information is often needed to assess the ability of an NC to pinpoint selected targets, e.g., chloroplasts. Here, X-ray tomography, magnetic resonance imaging (MRI), and confocal microscopy tools can provide three dimensional reconstructions. They can also be used *in vivo* or even *in planta*^{12,13,139}. All of these methods may require a higher mass of NC than exists in the plant tissues so approaches to increase the sensitivity of these methods are still needed.

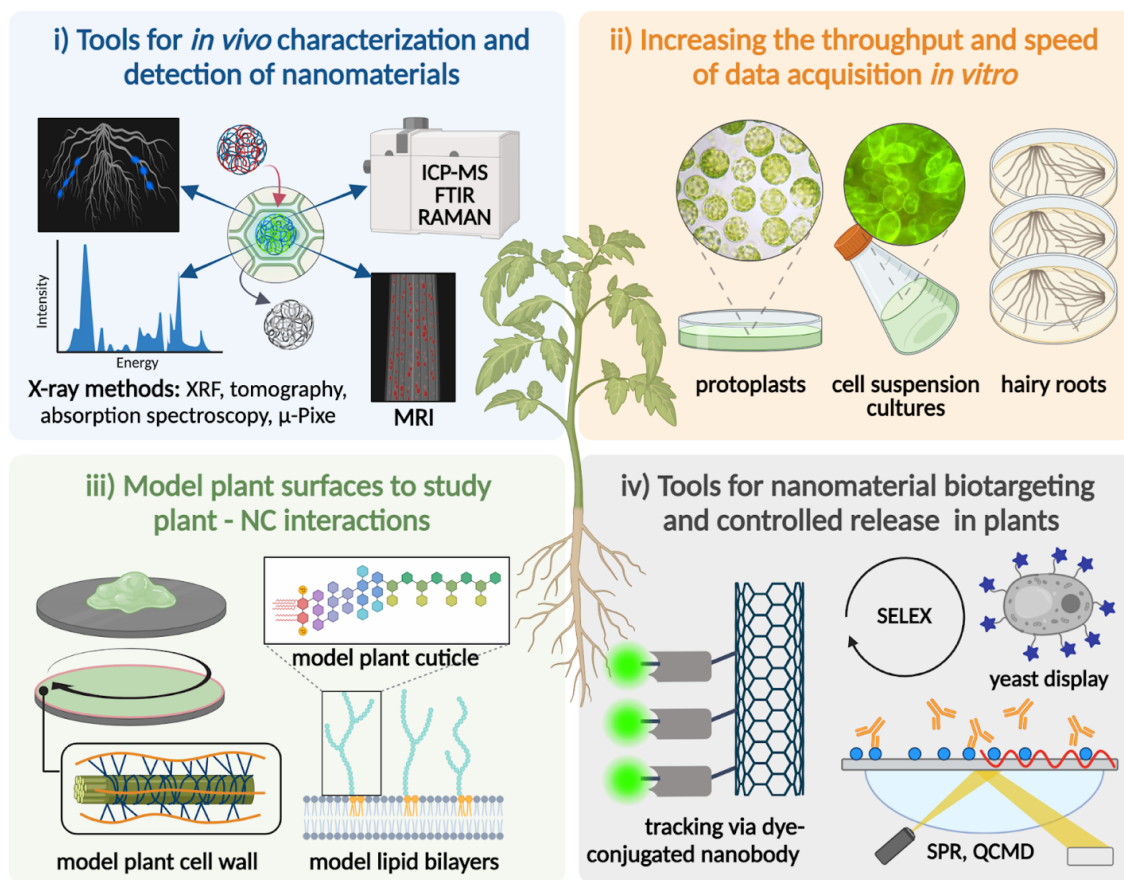


Figure 6. New tools and technologies needed to design nanomaterials and nanocarriers for precision agrochemical delivery. These include new methods for the **i)** *in vivo* characterization and detection of NC in plants, **ii)** increasing the throughput and speed of data acquisition via *in vitro* plant systems, **iii)** model plant surfaces to characterize NC-plant interactions, and **iv)** nanomaterial biotargeting in plants.

Tracking NC transformations *in vivo* is also a challenge, but some tools exist. Changes in speciation of inorganic NC can be measured using synchrotron X-ray absorption spectroscopy¹⁴⁰ or isotopic tags¹⁴¹. Techniques like FTIR, RAMAN, or Micro-Particle Induced X-ray Emission (μ -Pixe) can be used for tracking NC surface modifications and interactions with functional groups on a plant surface.¹⁴² Gaining a deeper understanding of NC transformation and persistence in plants may require extracting and isolating the NC before they are characterized using single particle ICP-MS,^{143–145} single particle ICP-TOF-MS¹⁴⁶ or 3D excitation emission matrix (3D-EEM).¹⁴⁷ These techniques also all have different limits on sensitivity, spatial resolution, and the size range that may be observed. They also require extraction from the plant which may alter their properties prior to analysis, leading to artifacts. Methods that avoid these artifacts need to be developed and validated.

Increasing the throughput and speed of data acquisition from in vitro plant cell and tissue-based systems. Characterization the distribution of delivered NC in plants is currently limited by the relatively low throughput of using whole plants. Inspired by biomedical research approaches, the study of plant-NC interactions should utilize *in vitro* plant cell and tissue model systems to significantly increase throughput and data collection. Example *in vitro* plant systems include protoplasts,¹⁴⁸ cell suspension cultures,¹⁴⁹ and hairy roots.^{150,151} Protoplasts are individual plant cells devoid of cell walls. They are isolated from plant tissues followed by either mechanical or degrading enzyme cell wall removal. From a small amount of tissue, $\sim 10^8$ cells can be quickly obtained, which enables rapid testing of how NC interact with plant cell membranes, where they localize intracellularly, their cellular toxicity, and transformations they experience in plant cells, such as protein, carbohydrate, or lipid corona formation. The limitations of protoplasts are that important cellular internalization information may be missed given the lack of cell walls, and lack of feasibility of long-term studies due to the short life span of protoplasts. Plant cell cultures are an alternative approach. They are formed by cells with walls, a key barrier for NC entry, and under optimal conditions in a liquid culture can divide and propagate indefinitely.¹⁵² Plant cell cultures

have been used to investigate carbon nanotube and quantum dot toxicity,^{153–156} and can potentially be used to elucidate interactions of NC with native plant cell walls in a high throughput manner.¹⁵⁷ Hairy roots, created through genetic transformation using *Rhizobium rhizogenes*, are plant root tissues that can be maintained in culture and mimic natural root anatomy and function.^{151,158} They are routinely used for answering fundamental plant cell and root biology questions.^{159–161} Hairy roots could provide a useful platform for assessing NC-root surface interactions or NC transport and transformation in plant tissues.

New model systems for studying plant surface-NC interactions. Experimental model plant surfaces are also needed to elucidate the mechanisms of NC-plant biosurface interactions. While not yet widely available, some examples exist. Model plant cuticle layers were designed using self-assembled monolayers (SAMs) with specific biomolecular composition to understand how cationic copper oxide nano-spikes helped adhere those NC to plant leaves.⁸⁹ Model cell walls made of cellulose, lignin, and pectin were created on quartz crystals to understand the chemical interactions between NC and cell walls. Similar approaches can be used to explore how structural defects in the cuticle or cell walls, or natural openings on the plant surface such as trichomes will affect NC uptake and translocation. Model plant lipid bilayers such as sulfoquinovosyl diacylglycerols, a novel model chloroplast membrane, can be constructed to study the association of NC with and uptake across cell membranes using QCM-D⁶⁰. Future opportunities could include generation of more representative and complex plant tissue platforms, such plant organs-on-a-chip with multiple organs - e.g. high throughput platforms with both roots and shoots enabling the study of NC uptake and translocation and delivery of agrochemical cargoes.

Tools for nanomaterial biotargeting and controlled release of cargoes in plants. Techniques widely used in the biomedical field for intra- and inter-cellular NC tracking and drug delivery discovery could be adapted for tracking NC in plant tissues and organelles. For instance,

nanobodies - smaller variants of antibodies (2-4 nm)¹⁶² - with high affinity to a NC can be conjugated with markers (e.g., fluorophores or radioactive elements) for NC visualization *in planta*. Similarly, SELEX platforms can be used to identify aptamers (short single-stranded DNA or RNA molecules) that selectively bind to a specific target.¹⁶³ Furthermore, there are existing screening tools to build targeting libraries of plant biomolecules and chemicals and their binding domains using techniques like phage/yeast display, molecular docking, or other adsorption/binding assays (SPR, QCMD) on tissue extracts, whole cells, pathogens, and bioinformatics tools.^{164,165} This would facilitate the creation of databases of potential biomolecules for biotargeting applications in plants. *In silico* approaches using artificial intelligence programs such as AlphaFold¹⁶⁶ and machine learning to predict protein structures and plant biomolecule interactions could allow virtual screens for putative binding domains and *de novo* design of NC tracers for biotargeting research. However, the accuracy of *in silico* methods still needs to be validated by experimental approaches, as these predictive algorithms have limitations (e.g., determining conformations of intrinsically disordered proteins)¹⁶⁷ and cannot yet fully replace experimental structure determination.¹⁶⁸

Reliable methods for controlled release of AA cargoes on or within plants at a specific location or time, or both sparse in the published literature. Importantly, the time scales for release of an AA or nutrients in an agricultural setting is likely to be very different that in animal drug delivery. For example, optimal delivery of N and P in crops may need to be tuned to timescales of weeks or months which has not been demonstrated by NC-mediated delivery approaches to date. Thermodynamics and DFT approaches were recently used for tuning copper oxide and phosphate NC dissolution in plants to achieve the maximum benefit from the materials⁴⁹. Direct evidence of the controlled release in plants is difficult to obtain due to the general lack of *in vivo* materials characterization tools that can operate on physiologically relevant time scales.

Approaches used in biotargeting may also prove to be useful for controlled release of cargoes by identifying plant biomolecules that trigger release at the desired location.

Sustainable development and use of nanocarriers in agriculture

Convergence across scientific and societal boundaries to foster sustainability. Improving the sustainability of agriculture is a complex socio-economic problem that requires integration of expertise from diverse academic disciplines and from critical non-academic stakeholders to create combinatorial methods and courses of inquiry.^{169,170} The merging of knowledge to spur critical innovation in nano-enabled agriculture will require methods for designing and managing convergent research approaches in large, complex scientific endeavors; examples include Interdisciplinary Studies,¹⁷¹ the Science of Team Science,¹⁷² and Integration and Implementation Sciences (I2S).¹⁷³ Authentic co-production of knowledge with diverse stakeholders across the entire innovation to implementation cycle, from the inception of formulating questions and throughout investigation and translation is critically important to its success.¹⁷⁴ Viable, sustainable solutions will only emerge if ideas, approaches, technologies, and human systems understanding across a broad range of perspectives are harnessed, combined, and communicated intentionally and inclusively to fuel critical innovations.

The products or processes designed to improve food and agricultural production systems, their development, manufacturing, production, use, and deployment also need to be sustainable. Perspectives and expertise from social sciences, humanities, and an array of non-academic partners, as well as considerations of human and environmental health, societal and ethical implications, and technological and economic considerations¹⁷⁵ must be included in formulating the research questions from inception.^{176,177} Meeting the expectation will require application of team science methods to advance transdisciplinary objectives (**Figure 7**). Intentionally inclusive approaches are also needed to develop and use nanomaterials for agricultural production, adhering to core concepts of sustainability and responsible innovation.^{178–180}

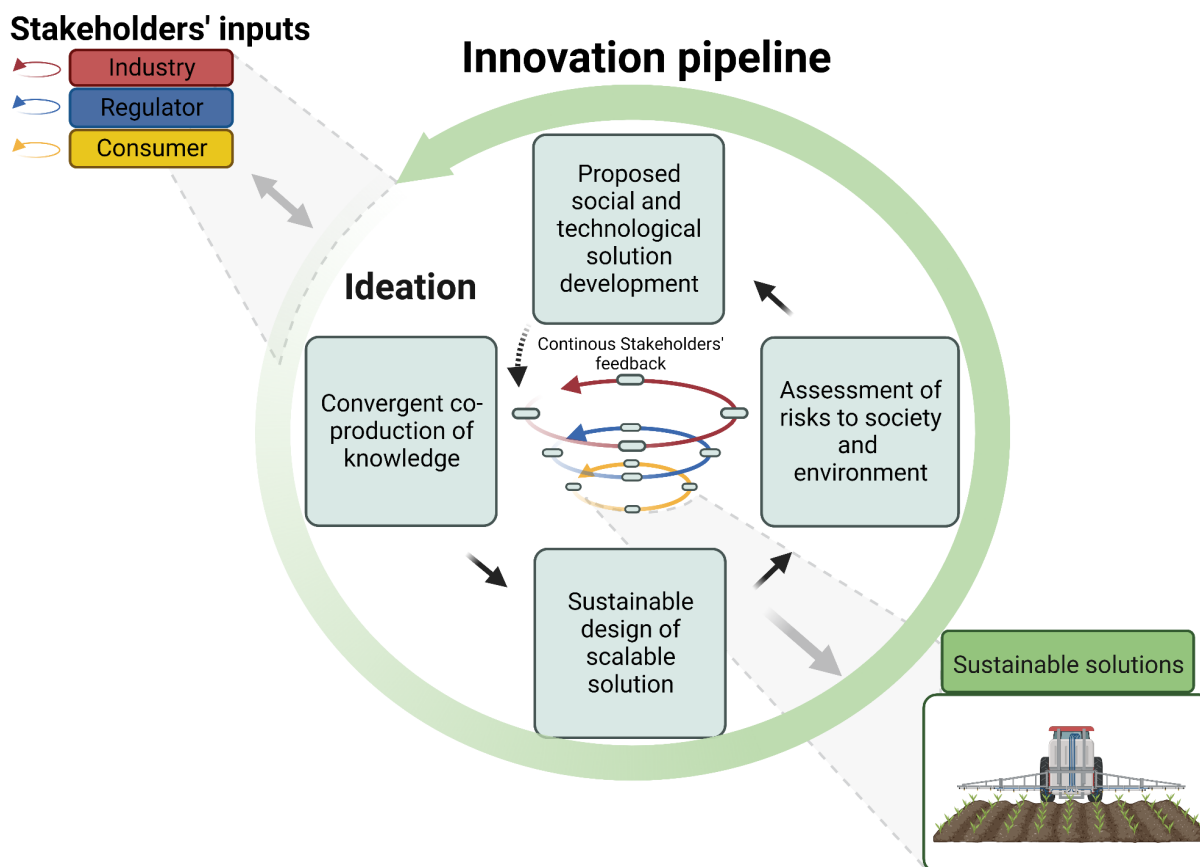


Figure 7. Integrated convergence and team science approaches with continuous feedback mechanisms are needed to structure and facilitate interdisciplinary projects to address the intersectional challenges related to sustainable and responsible nano-agricultural applications. It is crucial to develop shared mental models for both the problem and solution spaces, and converge on shared language to enable boundary-spanning integration of broad sources of knowledge.

Nanocarrier scalability and manufacture. The enormous scale of global agriculture demands that novel technologies be scalable at a magnitude similar to the oil and gas and bulk chemical industries. Process scalability is critical for commercial manufacture¹⁸¹, so barriers to scalability must be considered early in the development process. For example, microfluidics techniques used in developing nanomedicines are fundamentally limited to scale-out (i.e. many small-scale units running simultaneously) rather than scale-up, which is usually prohibitively expensive and inefficient. As a result, only a handful of nanomedicine formulations have reached

the market.¹⁸² The nanomedicine field provides useful metrics for success in agrichemical nanoformulation development and research, including AA loading, encapsulation efficiency, and process controllability. Active agent loading, i.e. the mass of AA per mass of delivery vehicle in the formulation^{183,184} must be high to achieve a desired dose of AA while minimizing excipient mass. This can also minimize the potential for unwanted environmental impacts. For example, free carbohydrates used as matrix formers may serve as a food source promoting growth of undesirable microorganisms and a detrimental effect on the leaf microbiome.¹⁸⁵ Encapsulation efficiency, i.e. the mass of AA encapsulated in a delivery vehicle per mass of AA introduced into a formulation process, should be maximized to reduce manufacturing waste and cost because the AA may be the most expensive material in a formulation and recovery and recycling of unencapsulated AA may not be possible.

Understanding risk and impacts on the environment and society. Researchers, innovators, and other stakeholders must strive to avoid mistakes of the past and avoid collateral or unintended consequences from rushed application of new technologies by better understanding potential risks of nano-enabled applications and incorporating stakeholder needs and perspectives into research and development.¹⁸⁰ For nano-enabled agriculture this includes assessing the environmental fate and potential toxicity and ecotoxicity of the NC in different environmental matrices (e.g., soil, water, sediment), assessing the effects of these materials on soil properties and function,¹⁸⁶ and researching growers' and consumers' perceptions of NC in agriculture.^{180,187,188} For example, stakeholders are more supportive of nanotechnological innovations for agricultural with greater benefit-to-risk ratios,¹⁸⁰ e.g. nano-ZiO₂ used to combat citrus greening and nano-vaccines used to decrease rates of *Salmonellosis* in egg-laying hens were viewed favorably, while the use of nanomaterials for food improvement, e.g. nano-TiO₂ to whiten infant formula, were not. This approach can avoid the reluctance of adoption of novel technologies in food and agriculture systems, such as occurred for the first generation of genetically modified organisms that were implemented without adequate inclusion of interested

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parties.¹⁸⁹ For example, the use of degradable biopolymers^{190,191} in place of non-degradable synthetic polymers used widely in agriculture for seed coatings, slow-release fertilizers, and plastic film mulching, but with the same functionality, would alleviate concerns about toxicity and persistence and promote adoption.^{192–194}

The scale of agriculture also means that materials used for agrochemical delivery must be sustainably sourced. For example, agriculture generates large amounts of ‘waste’ lignocellulose that can be used to prepare biodegradable nanomaterials,¹⁹⁵ and seafood waste can be used to derive natural, biodegradable biopolymers (e.g., chitosan) for NC use.¹⁹⁶ Carbon dot NC for agrochemicals can be manufactured from a range of agricultural wastes including plant or animal derivatives including leaves, stems, husks, urea,^{13,197,198} and silica extracted from husks can be used for seed treatments to enhance germination.^{199,200}

Regulatory landscape and challenges for nanotechnology mediate delivery approval. New technologies will undergo regulatory scrutiny, and this is especially true for NC. Formulating NC from materials that are generally regarded as safe (GRAS), such as certain biopolymers and virus-like particles, would also help in the regulatory process. While some inorganic NC have raised concerns regarding their potential toxicity to consumers²⁰¹ or to plants, biomolecule-based NC can potentially be more biocompatible and biodegradable than inorganic or synthetic counterparts. These properties will make the NC more sustainable than many currently used formulation components, and more acceptable to stakeholders.

Outlook

Nano-enabled precision delivery of active agents in plants will transform agriculture, but there are critical technical challenges that must be overcome to realize the benefits of this suite of technologies. The relationships between the structure and surface properties of nanocarriers and their interactions with plant surfaces and biomolecules, and their ability to carry and deliver the

desired AA must be elucidated to enable bio-inspired NC designs. Multifunctional NC employing biotargeting approaches adapted from nanomedicine could precisely deliver nutrients and AA to specific plant tissues, cells or organelles. This will enhance the effectiveness of nano-enabled applications like genetic modification by delivering gene-editing tools (e.g., plasmid DNA, RNA, and CRISPR/Cas9) directly to specific plant genomes, cells or tissues, or delivering AA to locations with specific plant pests while minimizing off-target effects. However, realizing these benefits will require greater understanding of the range of biotargeting tools available, their mechanisms of action and biotransformation, and the potential for any undesired detrimental impacts on plants. NC will transform inside of plants through interactions with plant biomolecules and various pH and redox gradients encountered in cells. The impact of these transformations on the efficacy or targeting, or the leveraging of these interactions for precision delivery in plants has not been widely explored or exploited. Better understanding of these transformations is needed to design reliable delivery and targeting strategies in plants. The fate of NC internalized into plant tissues also needs to be considered in their design. NC in plant residues may return to the soil or be transported offsite. While many NC that are applied to plants may transform ²⁰², the transformation products are likely to persist in plant tissues and potentially in soils unless they are biodegradable. Current tools used to assess the fate and lifecycle impacts of nanomaterials currently do not include these considerations and must be adapted for this purpose. ²⁰³

The development of new NC for nano-enabled precision delivery is hampered by the lack of high throughput screening methods and by the absence of predictive models. Similar to the “digital patient” models used to provide personalized medicine in humans, a multi-scale plant “digital twin” that captures important NC-plant surface interactions and biotargeting chemistry could be used to virtually screen for the efficacy of numerous different combinations of materials properties and biomolecular compositions to guide and accelerate NC design and development. Developing such models will require new in vivo tools to detect and characterize NC and in vitro tools to rapidly

quantify the interactions of NC with important plant surfaces. It will also require better understanding of plant physiological responses to the presence of the NC, a formidable challenge given the diversity of plant species, growth cycles, and potential environmental conditions to explore. This perspective focused on the development of digital plants from the scale of molecules to organisms. A digital plant model at this level of organization could potentially be incorporated into already existing crop or ecosystem models²⁰⁴ to simulate nanomaterial and environmental interactions at a larger scale. Overcoming these scientific challenges to develop globally sustainable nano-enabled precision delivery approaches will require convergence across both scientific and societal boundaries.

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