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Multiscale and multidisciplinary approach to understanding nanoparticle transport in plants

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Recent advances in nanoparticle (NP) technology have revealed potential to improve efficiencies in agriculture and plant biotechnology — specifically through controlled nutrient delivery, pathogen mitigation, and genetic engineering. However, most NP-based biotechnology applications have focused on demonstrative experimental studies, with few rigorous mechanistic explanations of how NPs translocate within plant structures. This article highlights advances in understanding NP transport in plants, categorized into NP movement across three length scales each distinguished by a different transport barrier: (i) the macroscale where different plant organs present unique obstacles to continuum transport, (ii) the microscale where the cell wall and dynamic size exclusion controls transport, and (iii) the molecular scale where cell and organelle membranes provide a hydrophobic barrier. To fully understand transport in plants and realize the benefits of responsible NP-based agri-technologies, researchers must combine knowledge from several disciplines and apply a multiscale approach to bridge knowledge about NP transport in plants across these length scales.

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Introduction

Plants are the core of our global food supply and natural greenhouse gas mitigation. Since the global population surpassed 1 billion in the early 1800s, plant engineering efforts including the agricultural revolution, plant domestication via breeding, plant genetic modification, and synthetic nutrient delivery have largely enabled our rapidly growing global population through to its current ~7.8 billion. Such technological advances are possible due to exogenous tools which, when inside plant tissues, act across numerous length and timescales. Future advances in plant engineering motivate a quantitative understanding of exogenous material transport in plants which will recursively improve their optimization for efficient and environmentally safe agri-technology.

Plants, as most biological organisms, do not indiscriminately uptake exogenous molecules [1]. Thus, abiotic carriers are often used to deliver bioengineering tools, where the carrier must enable transport through the various organs and barriers found at different length scales within a plant structure (Figure 1). The four major modes of abiotic carrier movement are: (i) uptake: transport from outside the plant to the interstitial space within tissue, (ii) translocation: transport throughout the interstitial tissue without entering cells, (iii) internalization: transport from outside a cell to inside a cell, and (iv) accumulation: the arrested transport inside or outside a cell. Three main transport regimes can be identified which hold different fundamental transport barriers: *macroscale* transport considers movement through plant organ structures such as the leaf cuticle and plant vasculature, *microscale* transport considers passage through the cell wall, and *molecular* transport considers crossing the cell and organelle lipid membranes. Transport through these scales is often accomplished with nanoscale abiotic carriers whose size (<100 nm) makes them amenable to transport in plants and whose tunable surface chemistries enable facile attachment of diverse payloads. Despite the great potential and recent progress in nanoparticle (NP) use for nutrient delivery [2–5], pathogen mitigation [2–4,6], and genetic engineering [3,7,8], NP use in plant systems has largely outpaced understanding of how their physical and chemical characteristics influence their uptake, translocation, internalization, and accumulation. These discrepancies have been illustrated in several previous reviews [9–14]. In this review, we posit that these discrepancies are, in part, a product of unintended siloing of NP research to individual length and time scales. Fundamental work on transport of endogenous fluids and metabolites in plants has yielded models of tissue growth and vascular fluid flow but have yet to incorporate NP dynamics. Because of newfound interest in NP-based abiotic carrier use in plants, we require new theories to

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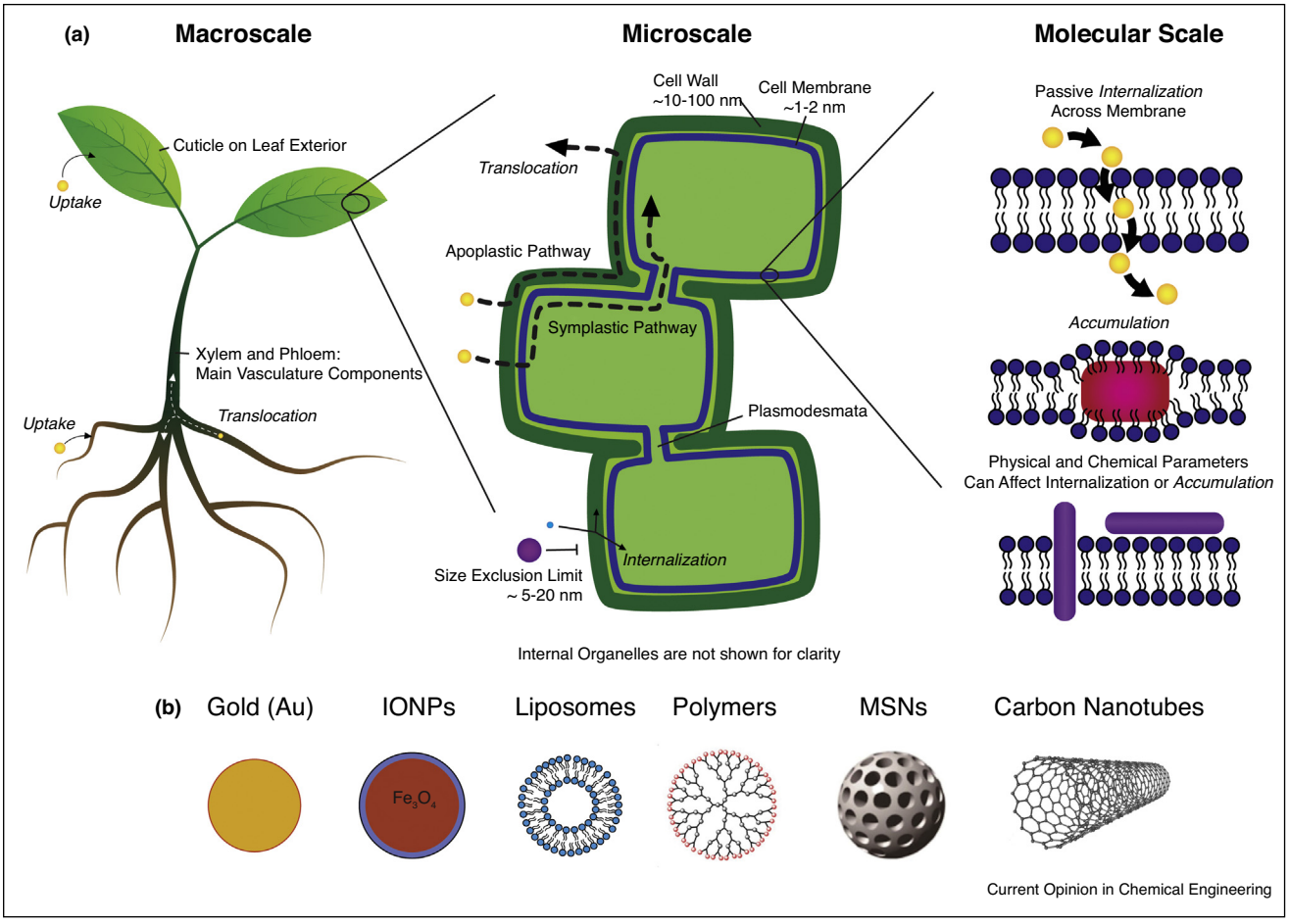
Figure 1



Nanoparticle Transport in Plants Warrants a Multiscale Approach.
a) Fundamental modes of transport include uptake, translocation, internalization, and accumulation. Understanding how nanoparticles are transported via these routes requires analysis on three scales: the macro-, micro-, and molecular scales. At the macroscale, recent work has been made to study uptake in the cuticle and root systems and translocation via the vasculature – specifically the xylem and phloem. At the microscale, recent work has compared translocation through the apoplastic pathway and internalization followed by translocation through the symplastic transport pathways. Coupled to this work are detailed studies of the dynamic cell wall size exclusion limit. At the microscale, passive internalization and accumulation within cell and organelle membranes are main foci of research.
b) Nanoparticles are defined as particles with one dimension smaller than 100 nm. They are interesting to plant engineering applications as many are smaller than the proposed cellular size exclusion limit, are easily synthesizable, and have easily functionalized surfaces. Some examples of common nanoparticles studied in the plant biotechnology and agriculture include gold nanoparticles, iron oxide nanoparticles (IONPs), liposomes, polymers, mesoporous silica nanoparticles (MSNs), and carbon nanotubes.

understand their transport and fully realize their utility for agri-technology. A mechanistic understanding of NP transport in plants, bridging macroscales, microscales, and molecular scales as reviewed herein, will improve intentional NP applications in plant biotechnology, and possibly stymie unintentional environmental consequences of mainstream NP use.

Macroscale, microscale, and molecular scale transport

Macroscale

Every plant organ has a distinct physiology which defines bulk NP uptake, translocation, and accumulation behavior. Current research at the macroscale is focused on quantifying the downstream effects of NP interactions with different plant organs, specifically in the context of uptake, translocation, and accumulation [15,16], resulting from both intentional and unintentional NP exposure [13]. The field of plant biomechanics has developed fundamental models for fluid and nutrient transport in these organs [17,18], but has yet to incorporate NPs to analogous theoretical frameworks. Much of the work done in recent years towards understanding NP macroscale transport has focused on understanding and overcoming three major barriers: (i) uptake past the leaf cuticle, (ii) translocation via endogenous vasculature

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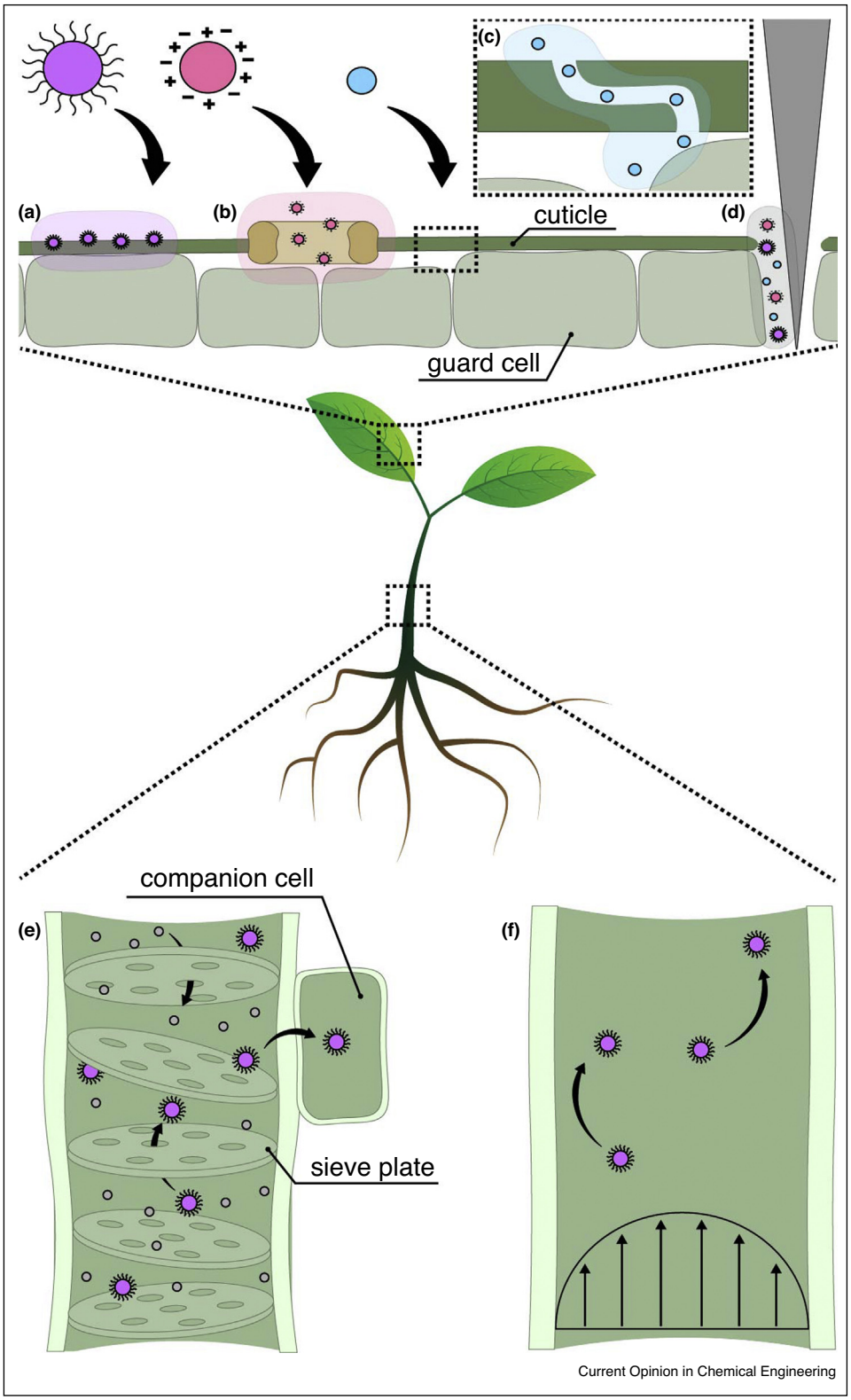
Figure 2



Methods of Nanoparticle Transport at the Macroscale through the Cuticle and Vasculature.
The cuticle (a) is a waxy coating of polymers that covers the exterior of many plant species, and functions to prevent excess transpiration of water. Pore openings in the cuticle (stomata) (b) open and close to control temperature and gas exchange. Current research suggests the cuticle presents a significant barrier for NPs introduced via foliar spray, with most types of NPs are uptaken through stomatal pathways (b) rather than

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structures, and (iii) accumulation in areas of cell division and growth.

The cuticle, a thin layer of lipid and hydrocarbon polymers covering most of the leaf exterior, is highly hydrophobic and presents an immediate barrier for NP entry into waxy plants [16,19,20] (Figure 2a–d). The primary approach for initiating NP exposure at the cuticle is to use foliar (spray-on) application of NPs. This simple technique enables study of uptake pathways into leaf tissue depending on NP size, hydrophobicity, and charge [11,16,21]. Surfactants have been long used to increase retention of applied chemicals on leaves [22] via reduction of surface tension at the leaf surface, and have also demonstrated increased NP uptake efficiency past the cuticle [23].

Alternatively, researchers have developed mechanical techniques to bypass the cuticle altogether for delivery of biological cargoes to plants. Historical procedures such as microinjection of solutions directly into cells [24] have not gained traction owing to the low-throughput and laborious nature of the technique, whereby syringeless infiltration has instead been widely adopted. This technique involves mechanically scratching the leaf to remove or weaken the cuticle before pressure-driven flooding of the leaf interstitium with an aqueous NP solution [25–27]. What remains elusive from these studies, however, is a quantitative analysis of NP transport resulting from mechanical introduction of NP into plant tissues as a function of NP physical parameters, and their effects on plant health.

Existing literature suggests cell age may affect NP transport and accumulation, although results vary based on NP core material, NP surface functionalization, and the plant organ under investigation. Within a single plant, old and young leaves can exhibit different levels of NP accumulation which depend on NP surface chemistry, implying that the interaction strength between the NP surface functional groups and plant cells can influence accumulation [16]. Alternatively, via cells that are quickly growing and dividing (such as root tips), NPs may find a lower barrier to entry relative to mature tissues [28]. New cell walls undergoing division are usually thinner and can have a larger size exclusion limit due to constant breaking and rebuilding [29], compared to their mature tissue counterparts. The thermodynamics of plant tissue growth continues to yield interesting conclusions about the dynamic nature of the cell wall [30,31], mechanistic insights that may be useful for future studies to quantify NP transport in plants.

The plant vasculature comprises xylem and phloem which are long tube-like structures that carry water and other nutrients between the roots and leaves, a distance which can be only a few centimeters for new sprouts to tens of meters for adult trees [32] (Figure 2e–f). The ability of NPs to translocate via the plant vasculature has been demonstrated experimentally [33,34], whereby current research is exploring the importance of particle charge on translocation and accumulation [19] and the effect of other NP characteristics on pathway preference for phloem or xylem channels [16,28,35–37]. A challenge of these transport studies is time resolution, where endogenous transport of water and sap can occur on timescales of minutes to hours [38,39]. However, most experiments allow days between NP infiltration and analysis of NP accumulation, suggesting experimental studies of NP transport in plants may not temporally capture the relevant kinetics of NP macroscale transport leading to accumulation. Therefore, there is significant need to pair mechanical transport theory with comprehensive studies of NP macroscale behavior, via more highly resolved temporal experiments.

Microscale

Setting aside physiological distinctions between different plant organs, much can be learned from studying NP interactions at the cellular level. One of the defining features of plant biology is the cell wall which is continuous throughout the plant tissue (Figure 3a,b). The cell wall serves as a structural support, a size exclusion filter, and a continuous transport pathway outside of cells [32]. At this microscopic length scale, we discuss recent experimental and theoretical work on translocation through and internalization across plant cell walls.

The continuous cell wall matrix, the apoplast, has been hypothesized as a pathway of NP translocation which is smaller (<5 nm) than the cell wall size exclusion limit of ~5−20 nm [11] (Figure 3a). Experiments on a variety of

passively diffusing through the cuticle (c) and into the cell mesophyll. Efficiency of traversing through either pathway is dependent on NP functionalization, charge, and size. Mechanical scratching (d) or piercing prior to pressure-driven infiltration are two methods of bypassing the cuticle.

The phloem (e) and xylem (f) are tube-like structures which connect plant roots and leaves and are central to the plant's vasculature structure. Water travels upwards through xylem channels which can range in diameter from <1 mm in young plants to as much as 2 cm in mature plants. Fluid flow in the xylem has been considered analogous to Hagen-Poiseuille pipe flow. Phloem tubes are the main transporters of nutrients including sugar, minerals, salts, amino acids, and other necessary compounds, connecting sources (i.e. photosynthetic organs where sugars and amino acids are produced) to sinks (i.e. fruiting bodies, developing tissue) throughout the plant. In phloem tubes, flow is analogous to laminar pipe flow. However, due to the higher concentrations of solutes, osmotic pressure plays a greater role than laminar flow in directing transport relative to in the xylem. The structure of phloem is more complex than that of xylem, including sieve plates and plasmodesmata openings to permit radial nutrient transport.

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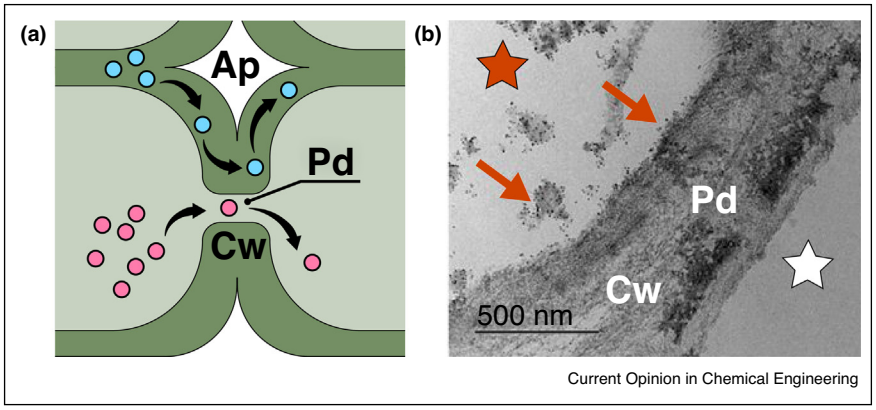
Figure 3



Cell Wall Structure, Strength and Translocation Pathways.
a) The plant cell wall (Cw) provides structural support for the cell and filters exogenous materials, presenting a unique barrier to NP transport not present in most animal cells. Comprised of a polymeric matrix of lignin, cellulose, hemicellulose and pectin, the cell wall separates individual cells from one another save for plasmodesmata (Pd) which are thin channels connecting adjacent cells. Blue circles represent NPs that were hypothetically delivered to the plant extracellular space and travel via the apoplastic pathway outside of the cell cytoplasm. Pink circles represent NPs that were hypothetically internalized by a cell and follow the symplastic pathway through cell cytoplasm and plasmodesmata. It is hypothesized that NPs below the cell size exclusion limit (SEL), are likely to transport via the apoplastic pathway by diffusing through the cell wall matrix. To transport via the symplastic pathway, NPs must be able to cross the cell membrane, which current research suggests is unlikely.
b) Electron micrograph image from Milewska *et al*. showing that neutral gold nanoparticles, after syringe injection into a root cell cytoplasm, were found to be stopped by the cell wall and do not translocate via either symplastic or apoplastic pathways. Red arrows indicate the presence of AuNPs.

plant species have confirmed accumulation of certain NPs in the apoplast [40,41], however, this does not confirm NP ability to transport *primarily* through this media. In fact, some results imply that plants actively adapt to restrict fluid flow in the apoplast upon introduction of NP [42]. Aside from NP size, NP charge may also be significant for determining NP diffusivity through the cell wall [40] (Figure 3b). Current research also suggests that NP stiffness and aspect ratio may be important factors for transport across the cell wall of non-spherical nanomaterials [43]. Overall, there is no consensus as to whether NPs translocate predominately via the apoplast or symplast and no mechanism to quantify how NP physiochemical properties affect their interactions with the cell wall. Furthermore, the results cited above must be understood in the context of their infiltration method. Physiological and chemical equilibrium changes inside tissue (including changed RNA expression) resulting from pressure-driven injection inevitably impact how NPs, water, and other nutrients are transported both spatially and temporally. More comprehensive studies of dynamic permeability and mechanisms of cell wall size exclusion may enable scientists to target NPs to cells of a specific age, location, or chemical makeup, and understand how plants respond to foreign NPs in their environments.

Cellular internalization is first limited by the cell wall size exclusion limit and then by the cell membrane. Considering the first barrier, it is commonly accepted that macromolecules and nanostructures >5−20 nm do not cross the cell wall while water, minerals, smaller macromolecules, and small ions can diffuse freely across. However, it is well known that the cell wall is heterogeneous in composition and thickness with a 'functional porosity' based on cell age, polymeric composition, hydration level, and environmental interactions [44–46]. Therefore, cell wall properties of composition, strength, and size exclusion limit are inherently-coupled [47,48] (Figure 3c). Connecting plant biomechanics to NP dynamics suggest that during pressure-driven infiltration, NP internalization is enabled by forced convection through the cell wall filter, thereby circumventing the apoplast versus symplast pathway debate. Alternatively, cell wall strength and tension depend on the local water potential [49,50]. Thus, NP internalization could be enabled by changes to the local chemical potential and surface tension at wetted interfaces, which depend on the chemical composition of the NP infiltration buffer, and can be influenced by surfactants that lower interface surface tension and cell wall integrity. There is rich literature in the plant biomechanics and biochemistry space to understand microscale cell wall structure and dynamics, though more research is needed to incorporate NP interactions, and the effects of their physiochemical properties, on NP uptake and translocation at the microscale.

Molecular scale

Research on molecular scale NP transport in plants includes both experimental studies and theoretical models of NP behavior in simplified systems (e.g. a single

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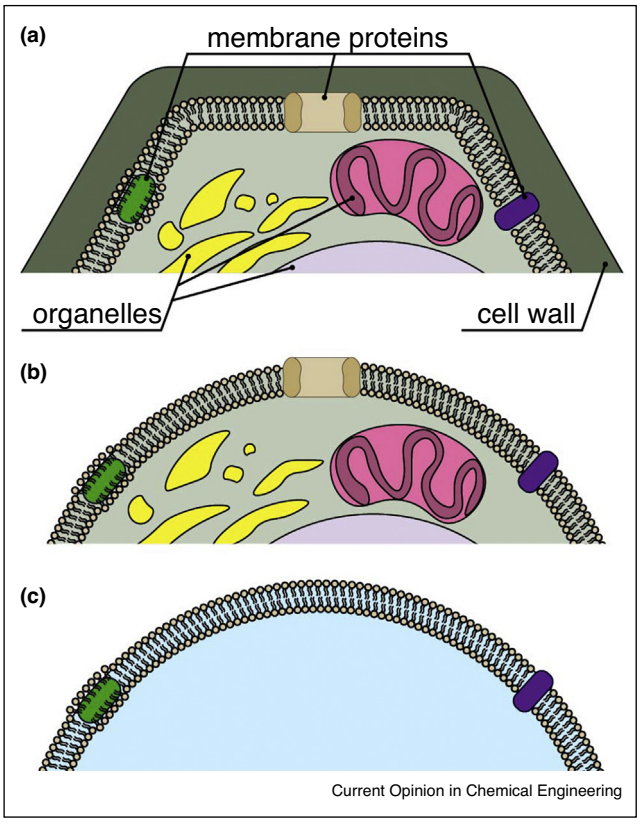
organelle, a synthetic lipid vesicle) with the primary barrier studied being the cell membrane. Cell and organelle membranes are composed primarily of amphiphilic molecules called phospholipids which form a bilayer ~1 nm thick. The outer regions of the membrane are hydrophilic while the interior is strongly hydrophobic (Figure 4). In the context of NP internalization across the lipid bilayer, continuum mechanics are no longer representative of the transport dynamics [51]. Therefore, researchers have turned to atomistic and coarse-grained molecular dynamics simulations to model interactions between NPs and and individual phospholipid head and tail groups in the cell membrane. These simulations can elucidate free energy interaction profiles and local diffusion coefficients to explain how NPs impregnate and cross the cell membrane [52,53]. Complementary efforts include *in vitro* experiments in which protoplasts (plant cells whose cell walls have been enzymatically degraded) and artificial cells such as giant unilamellar vesicles (GUVs) are exposed to NPs and uptake properties are measured. The membrane composition of protoplasts is the same as that of the whole plant cell, whereas GUV membrane composition is tunable and can be made to model a specific phenomenon but will not be fully representative of plant cell membranes (Figure 4).

The classic Meyer–Overton diffusion model and membrane pore formation model both simplify the cell membrane as a single homogeneous hydrophobic slab [51,53,54]. Other multi-step diffusive models have included NP adsorption to the membrane exterior, translocation, and desorption to the membrane interior which take slightly better account of the complexities of the lipid bilayer [55]. NP charge, functional groups, shape, and approach orientation for non-spherical NPs (such as carbon nanotubes), and other physical parameters which have been considered important for internalization have been explored in computational studies of NP — membrane interactions [52,55–57]. The development of these models and the mechanistic discussions they promote are critical to advancing knowledge of how NP characteristics influence transport across molecular scale biological barriers.

Experiments probing NP-cell membrane interactions using protoplasts and GUVs further support the significance of NP surface chemistry for internalization [58,59]. One study has shown that hollow carbon nanotubes embed themselves perpendicular to GUV membranes enabling increased water flux [60]. Similar experiments in protoplasts also show changes to total water flux along with changes in expression of water-transporting membrane proteins [61]. One of the few molecular-scale NP internalization mechanisms developed posits that interactions between oxidized groups on multi-walled carbon nanotubes and hydrophilic phospholipid head groups can bring the hydrophobic surface in proximity to hydrophobic phospholipid tails, inducing strong Van der Waals interactions [61–63]. The net effect is a local deformation of the membrane followed by NP internalization driven by further thermal fluctuations. Conclusions raised about NP internalization into protoplasts and

Figure 4



Single Cell Experimentation.
To better understand the mechanisms of NP internalization at the micro- and molecular scale, researchers have turned to single-cell experimental techniques using (a) whole cells (either as part of a whole plant or in suspension cell lines), (b) protoplasts, or (c) artificial cells such as giant unilamellar vesicles (GUVs).
For intact plant cells, the cell wall provides structural support and physical protection of the cell membrane. The cell membrane, comprised of a highly hydrophobic lipid bilayer with embedded proteins (green, purple, brown features), in conjunction with the cell wall is the primary mode of communication between a cell and the external environment.
Protoplasts refer to whole cells that have had their cell wall enzymatically degraded. They retain a similar lipid composition in their cell wall and many of the same cytoplasmic and membrane proteins. However, ligands which connect the cell wall to the cell membrane are severed. Furthermore, in the absence of structural support from the cell wall, protoplasts take on a spherical shape. Protoplasts are useful to study NP interactions directly with the cell membranes and internal organelles.
GUVs are generated *in vitro* from a solution of lipids. They are the simplest form of a cell membrane and therefore can be used to study passive uptake processes. Membrane proteins can also be introduced to GUVs in order to study specific active processes or functions.

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GUVs are informative to model NP transport across lipid membranes, but alone cannot inform mechanisms for NP internalization into regular plant cells. Nevertheless, continued joint efforts with *in vitro* experiments and molecular simulations can assist in laying the foundation for rational engineering of NP transport into plants.

Conclusion and unanswered questions

There is significant work to be done to elucidate mechanisms of NP transport in plants both to improve the intentional use of NP-based agri-technologies and to predict and mitigate unintended environmental consequences of NP exposure. Interdisciplinary research is playing a key role in these explorations. While we maintain that a comprehensive mechanistic study of NP transport in plants is necessary, we would like to highlight three unanswered questions which exemplify the challenges of bridging length scales along with disciplines.

First, what are the mechanisms tied to NP accumulation in plant organs and on a subcellular level? Accumulation requires understanding (at the macroscale) of fluid flow and stagnation points within the vasculature, (at the microscale) of the anisotropic porosity along the continuous cell wall matrix, and (at the molecular scale) of the potential for NP solvation and 'capture' within and through the cell membrane.

Second, what results, if any, are generalizable when performing experiments with protoplasts or GUVs? New research findings reinforce the importance of the cell wall and intercellular osmotic balance on cell membrane composition [64] and function [65], and thus likely also on NP transport. Protoplast research is invaluable to informing computational work on the molecular scale, but it is equally important to recognize the limitations of this technique for explaining uptake pathways at the macroscales and microscales.

Third, when and how is the choice of NP administration technique deterministic to NP uptake pathway? Just as physicians prescribe medication with a method of administration, plant scientists and engineers optimize their experiments based on plant biology, engineering intention, and the identity of the delivered agent. In addition to theories posited earlier, shear stress from syringeless injection and vacuum infiltration could create cell wall damage opening new interstitial passages, altering local osmotic balances which cause cells to swell or shrink, and/or triggering chemical signaling pathways that impact the cell membrane's endogenous uptake machinery.

Perhaps the biggest unanswered question concerning the transport of NP in plants is how to bridge these multiscale gaps in our understanding of abiotic particle transport. We strongly advocate for coupling of multidisciplinary knowledge and incorporation of mathematical and theoretical models in tandem to NP-based transport experiments, and NP-based agri-technology development. A combination of molecular biology, mathematics, and engineering will be essential to realizing both the full potential of plant-NP research and the environmentally conscious implementation of this nascent field.

Conflict of interest statement

Nothing declared.

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