Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants – Critical review

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Abstract

Uptake, transport and toxicity of engineered nanomaterials (ENMs) into plant cells are complex processes that are currently still not well understood. Parts of this problem are the multifaceted plant anatomy, and analytical challenges to visualize and quantify ENMs in plants. We critically reviewed the currently known ENM uptake, translocation, and accumulation processes in plants. A vast number of studies showed uptake, clogging, or translocation in the apoplast of plants, most notably of nanoparticles with diameters much larger than the commonly assumed size exclusion limit of the cell walls of $C245–20$ nm. Plants that tended to translocate less ENMs were those with low transpiration, drought-tolerance, tough cell wall architecture, and tall growth. In the absence of toxicity, accumulation was often linearly proportional to exposure concentration. Further important factors strongly affecting ENM internalization are the cell wall composition, mucilage, symbiotic microorganisms (mycorrhiza), the absence of a cuticle (submerged plants) and stomata aperture. Mostly unexplored are the roles of root hairs, leaf repellency, pit membrane porosity, xylem segmentation, wounding, lateral roots, nodes, the Casparian band, hydathodes, lenticels and trichomes. The next steps towards a realistic risk assessment of nanoparticles in plants are to measure ENM uptake rates, the size exclusion limit of the apoplast and to unravel plant physiological features favoring uptake.

Abbreviations: (C)FPE: (clathrin-mediated) fluid-phase endocytosis; EDS: energy dispersive X-ray spectroscopy; ENMs: engineered nanomaterials; MSNs: mesoporous silica nanoparticles; NPs: nanoparticles; nZVI: zero-valent nano iron; QDs: quantum dots; (S)TEM: (scanning) transmission electron microscopy; SWCNTs/MWCNTs: single-walled/multi-walled carbon nanotubes; SEL: size exclusion limit; SPIONs: super paramagnetic iron oxide nanoparticles; $\mu$-XANES synonym $\mu$-NEXAFS: $\mu$-X-ray absorption near edge structure; $\mu$-XRF: $\mu$-X-ray fluorescence spectroscopy;

Keywords

Engineered nanomaterials, excretion, internalization, nanoparticles, plant physiology

Introduction

Naturally occurring nanoparticles (NPs), in enormous quantities and varieties, are distributed throughout the atmosphere, oceans, soil systems, terrestrial freshwater systems and in/on most living organisms (Colvin, 2003; Hochella et al., 2008; Wiesner et al., 2009). Concern over the possible environmental and toxicological impacts of increasingly manufactured and used engineered nanomaterials (ENMs) has stimulated research on the potential for plant (cell) uptake, accumulation and transformation of ENMs – Materials that several decades ago might have been simply described as dissolved by virtue of their ability to pass through a 0.45 micron membrane (Dietz & Herth, 2011; Scheringer, 2008; USEPA, 1996; von Moos et al., 2014). Plant–ENM interactions, in the process, have led plant biologists to rethink the role of (nano)particles as sorbents and/or sources of heavy metals, pesticides and essential nutrients to plants and phytoplankton (Baken et al., 2014; Santner et al., 2012; Schwab et al., 2013; Van Moorleghem et al., 2013). Two decades ago, research on the uptake of ENMs by plants was motivated primarily by interest in their capability as vehicles for gene transfer (Fisk & Dandekar, 1993; Torney et al., 2007; Wang et al., 1988). Over the last decade (Figure 1), scientists have begun to consider plant–ENM bioaccumulation and transformation as processes affecting the environmental fate of ENMs. Their ecotoxicity, and their behavior in ecosystems include propagation within food webs (Al-Salim et al., 2011; Klaine et al., 2008; Navarro et al., 2008; Zhu et al., 2008). The frequency of publications addressing nanoparticle uptake by plants jumped over the last 5 years to $\approx$40 peer reviewed original publications per year in 2013 (Figure 1).

Both terrestrial and aquatic plants, including phytoplankton, are expected to be exposed to ENMs from ENM-containing...
sewage sludges (predicted annual input \( \sim 1.01–2380\mu g \) ENMs kg\(^{-1}\) year\(^{-1}\) sludge treated soil, where land application is allowed), supposedly quickly increasing levels of novel nanoformulations of pesticides or fertilizers (Gardea-Torresdey et al., 2014), wastewater treatment effluent (0.001–15 ng L\(^{-1}\) year\(^{-1}\)) and atmospheric sources from factory or laboratory exhaust including contaminated rainfall (\(<<1\mu g \text{ m}^{-3}\) year\(^{-1}\); Gottschalk et al., 2009; Hendren et al., 2011). In soil, the exposure concentrations are predicted to be five orders of magnitude higher than those in water, and aerial exposure is expected to be much lower. Still, determining which compartment is relevant for plant NP uptake is challenging due to the different mobility of the NPs, e.g. in the absence or presence of natural organic matter (Ek et al., 2004; Zhao et al., 2012b). In addition, the ability of different plants to take up NPs is currently unpredictable (Dietz & Herth, 2011).

In the water compartment, at least for types of ENMs well-dispersible in water (Wilkinson et al., 1997), NP-plant accumulation is well documented (Colman et al., 2014; Glenn et al., 2012). Depending on the background particles, ENMs agglomerate more or less rapidly (Therezien et al., 2014). Agglomerated ENMs can again re-suspend due to many natural processes, e.g. turbulence in rivers (Gottschalk & Nowack, 2011), or suspension by natural organic matter (Hyung et al., 2006; Schwzyzer et al., 2012, 2013). Submerged hydrophytes, as compared to terrestrial plants, must exhibit much faster mass transfer rates to acquire enough CO\(_2\) and other dissolved gases from the water (Sculthorpe, 1967). These mass transfer rates are enabled by an underdeveloped or absent epidermis and/or protective lipophilic cuticle (Sculthorpe, 1967). Consequently, well-dispersed ENMs can interact directly with the cellulose cell wall. This can facilitate uptake.

In the terrestrial compartment, ENM accumulation in plants is favored due to exposure concentrations in the \( \mu g \text{-mg kg}^{-1}\) range (Gardea-Torresdey et al., 2014; Gottschalk & Nowack, 2011). The mobility of the NPs, and uptake into plants was found to be low (Gardea-Torresdey et al., 2003). Eight different ENMs in a system resembling a sandy groundwater aquifer were transported maximally 14 m (organic NPs), and 2.4 m, respectively (inorganic NPs; Lecoanet et al., 2004). Nanoparticle mobility in soil was found to be even lower (Darlington et al., 2009). Keeping in mind that under environmental conditions, biovoids, mucilage and exudates from roots, hyphae and bacteria significantly alter soil porosity and transport (Oades, 1993; Zhao et al., 2012b), this low mobility of ENMs shows that (a) NPs can accumulate in the first few meters or centimeters of the soil, in close interaction with the rhizosphere (Gardea-Torresdey et al., 2014; Hawes et al., 2000; McNear, 2013; Walker et al., 2003) (i.e. the root system environment where mucilaginous and exudates act) and (b) the key process keeping plant–ENM accumulation low in soil is slow desorption kinetics or sequestration (Avanasi et al., 2014; Wang et al., 2013b; Zhao et al., 2012b; Zhao et al., 2014).

To date, ~240 peer-reviewed original publications have addressed ENM–plant uptake (Figure 1). Recent reviews have addressed toxic and stimulating NP effects, summarized NP concentrations found in plants, and discussed how plants are exposed to NPs in soil, water and air (Gardea-Torresdey et al., 2003; Handy et al., 2008a; Husen & Siddiqi, 2014; Jackson et al., 2013; Klaine et al., 2008; Ma et al., 2010a; Miralles et al., 2012a; Navarro et al., 2008; von Moos et al., 2014). Central, not recently systematically addressed topics are the actual barriers, pathways and transport processes that NPs face in plants. What kind of chemical and physiological barriers of plant cells do NPs have to cross before they reach the cytoplasm? How (fast) do – particularly non-dissolving – nanoparticles translocate within plants? Which anatomical features of plants favor uptake? Where in plants will NPs accumulate? And do plants, at all, excrete insoluble NPs again? Dietz and Herth provided an initial overview for several hypothetic pathways for NPs into plants in 2011. Recent studies have begun to reveal which of these processes are significant. New or scarcely described sites of uptake, transport, accumulation and excretion have been studied, such as plant-associated microorganisms (mycorrhiza), mucilage, the cuticle, non-endocytic uptake across cell membrane, necrotic tissue, root hairs and trichomes. Other topics continue to cause confusion, such as the actual size and morphology of “pores across the cell wall”, and several other types of “pores” or “channels”. The goal of this review is therefore to (a) provide an update on pathways for NPs into plants; (b) systematically describe, for the first time, the micro-architecture of these and all other major pathways for NPs in plants, including uptake, translocation and excretion, with special attention to less explored, but potentially relevant pathways (termed here “loopholes”); (c) mathematically describe uptake processes based on the gained knowledge on NP transport within plants; and (d) identify priorities for research on NP–plant interactions.

**Cell-level nanoparticle interactions**

Plants and phytoplankton exhibit specific barriers against intruders and ENMs (Figure 2; Fahn, 1982; McNear, 2013). The thickness and architecture of the barriers varies with species, tissues and environmental conditions, e.g. in drought-tolerant plants, the cell wall, cuticle and the Caspian strip are tighter to minimize water loss (Fahn, 1982), and the xylem (refer the “Vasculature” section) is strongly sectored (Choat et al., 2008). The plant itself, by depositing cell materials, can create additional barriers. For instance, cell wall thickenings such as papilla, or callose, suberin and lignin deposits represent plant responses to the mechanical intrusion of pathogens (Figure 2A; Basavaraju et al., 2009; Carpita & Gibeaut, 1993; Voigt, 2014). The following subsections describe all cell-level pathways and barriers for NPs in detail.

**Symbiotic bacteria and mycorrhizae**

Nearly all pathogenic and symbiotic relationships in roots are initiated in the extending cell wall region of elongation...
CuO and nano-ZnO exposed mung bean (Vicia faba L., 2013) significantly affected the levels of bioavailable metal nutrients in the root bacterium Pseudomonas chlororaphis (Belimov & Dietz, 2000) colonized by rhizobia; Guo & Chi, 2014), a key ingredient in many livestock diets. Glycine max (soybean), Phaseolus vulgaris (bean), and Trifolium repens (clover) are N-fixing bacteria (e.g. Rhizobium leguminosarum). They erode few μm sized holes in the root hair cell wall (Mateos et al., 2001) to induce bacteria-containing nodules (McNear, 2013). These nodules can alleviate heavy metal accumulation in symbiotic legume hosts (soybean, cadmium 22% reduced as compared to control without nodules). A possible mechanism is that the nodules supply the plant with Fe for defense reactions (Guo & Chi, 2014). In non-legumes, the presence of the same or comparable bacteria can lead to an increase of heavy metal uptake (ryegrass, Lolium multiflorum, increase of cadmium 12% (Guo & Chi, 2014); barley, Hordeum vulgare, increase of 14% (Belimov & Dietz, 2000)), probably because such bacteria perforate the cell wall, but cannot establish a symbiosis. Data on the effect of rhizobia to NP uptake are almost non-existent.

Ubiquitous mycorrhizal fungi can form symbiotic partnerships with all higher plants (McNear, 2013; Raven, 2003) except for ferns and a few exotic plant divisions (Figure 3). Fungi symbionts play a major role in soil mineral weathering (Bonneville et al., 2009; Oades, 1993), since they increase the effective plant root surface by up to 10-fold (McNear, 2013). These factors combine to enhance plant uptake of water, nutrients (Duran et al., 2013;
Figure 3. Key uptake or accumulation pathways possible for nanoparticles (NPs) into the root in the presence of the two major types of mycorrhiza, illustrated on a dicot root. Both the endo- and the ecto-mycorrhizal fungi (ENF and ECF, respectively) infuse the intercellular space of the plant. Endomycorrhiza: the hyphae infiltrate the intracellular space, which facilitates apoplastic solute transport (red solid line). Cell walls of specific cells in the root cortex are penetrated, resulting in hyphae filled cells in direct contact with the cell membrane (arbuscules). This enhances the symplastic solute transport (red dotted line) via arbuscules. Ectomycorrhiza: the ENMs form a mat of hyphae (fungal mantle, blue area outside root) on the root, penetrate the extracellular space (Hartig net, blue area between cells), but do not penetrate the cell walls. Only near plasmodesmata in the cell walls, the hyphae are in direct contact with the cell membrane. Vesicles: Swellings of inter- or intra-cellular hyphae for storage. Hyphopodia: fungi attachments to the root surface to apply mechanic pressure. Two NP pathways are shown on each side to illustrate where the NPs enter the symplast for the first time. Image adapted from Di Laurenzio et al. (1996) and McNear (2013).

As a whole, symbiotic microorganisms consistently enhanced accumulation of heavy metals (Cd, Cr, Zn), non-metals (P, Se) or ENMs (QDs) for true grasses (Poaceae; Belimov & Dietz, 2000; Duran et al., 2013; Guo & Chi, 2014; Whiteside et al., 2009) and reduced heavy metal (Cd, Zn, Ni), nano-Ag, and nano-FeO uptake for legumes (Feng et al., 2013; Guo & Chi, 2014). This is especially remarkable given that the monocot family of the true grasses was – with few exceptions (Koelmel et al., 2013; Lin & Xing, 2007) – normally more resistant to heavy metal (oxide) and lipid-based NP uptake and toxicity than dicots (Judy et al., 2012; Ma et al., 2010b; Nadiminti et al., 2013; Wang et al., 1988). Only in protoplasts (plant cells with the cell wall removed) both legume and true grass cells behaved similarly (Shen et al., 2010). Symbionts must partially dissolve or perforate the cuticle and cell walls (Mateos et al., 2001; McNear, 2013). The robust micro-architecture of the cell wall in true grasses (refer the ‘The cell wall and pectins’ section) (Carpita & Gibeaut, 1993; Ma & Yamaji, 2006) may be a disadvantage when it comes to establishing beneficial interactions with microorganisms.

Mucilage and exudates

Mucilage and exudates (M/E) are excreted into the rhizosphere via the root cap (border cells) and root hairs of most plants (Driouich et al., 2013; Hawes et al., 2000; McNear, 2013; Walker et al., 2003; Zhang et al., 2011), thereby consuming 5–21% of all photosynthetically fixed carbon (Walker et al., 2003). Further sites of M/E production that can interact with NPs are seed cell envelopes (Yang et al., 2012), and the cell surface or debris of some phytoplankton species (Figures 2 and 5; Baldi et al., 1997; Bar-Ze ev et al., 2015; Tufekci et al., 2010; Vymazal, 1995). Insoluble, high molecular weight mucilage (Driouich et al., 2013; McNear, 2013; Walker et al., 2003) and soluble, low molecular weight exudates (McNear, 2013; Walker et al., 2003) are highly responsive sensing, protecting and sometimes, lytic agents on the outermost cell surface. They therefore form the first barrier for NPs (Figure 2A and Supplementary Information (SI) for details on M/E composition and function). Mucilage and exudates actively protect the plant cells against environmental stress such as mechanical friction and toxic metals. They also increase bioavailability of nutrients, and interact with symbiotic microorganisms (Driouich et al., 2013; Horst et al., 1982; Hawes et al., 2000; McKenzie et al., 2013; McNear, 2013; Vervaeke et al., 2004). As discussed in detail below in this section, the mainly protective function of the M/E extends to (nano)particles depending on NP solubility, size and surface coating. Nanoparticles accumulate and dynamically interact with M/E of the outer mucilaginous plant surface (Burkhardt et al., 2012; Cherchi et al., 2011; Driouich et al., 2013; Ma et al., 2011; Yang et al., 2012; Zhang et al., 2011, 2012b), and of phytoplankton (Miao et al., 2009; Nielsen et al., 2008; Verneuil et al., 2014). Little is known about the role of M/E in NP uptake of plant parts other than roots, except for one report of nano-Pb...
accumulated in necrotic tissue of leaves (Uzu et al., 2010), likely captured in mucilage of the lysed cells.

Nanoparticles in terrestrial plants frequently accumulated and aggregated primarily near the roots, the root tips, specifically the root cap (border cells) or M/E on the root cap and hairs (Al-Salim et al., 2011; Aubert et al., 2012; Geisler-Lee et al., 2013; Glenn et al., 2012; Kurepa et al., 2010; Lee et al., 2013; Nair et al., 2011; Navarro et al., 2012; Zhang et al., 2011, 2012b; Zhao et al., 2012b; Zhu et al., 2008). Root absorption has been shown to be reversible: The fraction of negatively, neutrally and positively charged nano-Au (charge determined by surface stabilizers) removable from the root surface (=surface-adsorbed) varied between 14 and 90% of the total root concentration across rice (Oryza sativa), perennial ryegrass (Lolium perenne L.), radish (Raphanus sativus) and pumpkin (Cucurbita maxima) (Zhu et al., 2012). For cucumber (Cucumis sativus L.), only 7.5–8.9% of supposedly positively charged nano-$^{114}\text{CeO}_2$ adhered to the root after five washes using deionized water (Zhang et al., 2011).

Nano-Ag was found accumulated in the mucilage and border cells of thale cress (Arabidopsis thaliana). The resulting brown discoloration of the root cap – typical for nano-Ag exposure – is usually visible by the naked eye (Geisler-Lee et al., 2013; Thuesombat et al., 2014). This discoloration consists of precipitates on the root cap, and root hair tips: exclusively in the NP-exposed plants, a scattering signal was found in confocal dark-field microscopy, being specific for electron dense particulate matter (Geisler-Lee et al., 2013). Nano-CeO$_2$ also accumulated in the mucilage, based on transmission electron microscopy (TEM) observations of massive numbers of NPs immobilized in gel-like electron-transparent matter on the outermost root cap layer (Zhang et al., 2011).

Mucilage and exudates of plants or microorganisms acidify the environment of the rhizosphere (Ma et al., 2011; Schaller et al., 2013), which promotes dissolution of NPs (Lv et al., 2015; Ma et al., 2011; Parsons et al., 2010; Vannini et al., 2014; Zhang et al., 2012b), even some that are normally insoluble (Battke et al., 2008; Schwabe et al., 2015; Zhang et al., 2012b). Nano-Au, for example, seems to dissolve in the rhizosphere, although it is unclear which exudates would be enough aggressive to protonate and oxidize gold (Taylor et al., 2014). And, Ce$^{3+}$ from nano-CeO$_2$ contributed significantly to the uptake of total Ce in pumpkin (Cucurbita maxima), wheat (Triticum aestivum) and sunflower (Helianthus annuus, Schwabe et al., 2015). Soluble NPs that translocate from root to shoots therefore often have a smaller diameter than before dosing in the stock suspension, and can dissolve completely before uptake in extreme cases (Badireddy et al., 2014; Lv et al., 2015; Vannini et al., 2014; Zhang et al., 2012b).

Accumulation of NPs on the root surface (mucilage) can coincide with reduced root–shoot translocation which was often associated with a positive NP surface charge (Zhu et al., 2012). For example, positively charged nano-Au consistently accumulated to a greater extent on the negatively charged root surface than was negatively charged nano-Au (Zhu et al., 2012). Negatively charged nano-Au, or QDs in turn exhibited higher translocation rates because of less association with negatively charged plant surfaces (refer the “Endocytosis” section; Wang et al., 2014; Zhu et al., 2012). Nevertheless, some NPs such as superparamagnetic iron oxide nanoparticles (SPIONs) do translocate from roots to shoots regardless of charge (Ghafariyan et al., 2013). Further, negatively charged NPs such as Alizarin Red S labeled nano-TiO$_2$ can strongly accumulate in the thick mucilage sheath of seeds (Kurepa et al., 2010; Yang et al., 2012).

Portion of the NPs coming in contact with roots traverses the mucilage, particularly if the surface charge is negative as discussed above, or if the NPs are relatively small. Large numbers of nano-Ag <$20\text{nm}$ as observed by TEM coupled with energy dispersive X-ray spectroscopy (TEM-EDS), accumulated in the cell walls of root cap cells, despite the presence of mucilage (Geisler-Lee et al., 2013). It is not known to which extent NPs in the apoplast (refer the “Apoplast versus symplastic transport” section) of root cap cells are immobilized while the root is growing, and to which extent they actively diffuse through the mucilage into the cell walls. In one case, dye-labeled <$5\text{nm}$ nano-TiO$_2$ were more concentrated in the cortex than in the root caps suggesting extensive diffusion through the mucilage (Kurepa et al., 2010). However, these results have to be interpreted with caution. The dye used to label nano-TiO$_2$ (Alizarin Red S) loses its red color at a pH <6.5, and the acidic environment of root tips can quench this color. Further observations indicating that NPs may pass mucilage size-selectively were 80 nm NPs that were, unlike 20 nm NPs in the same experiment, absent in the apoplast of root caps (Geisler-Lee et al., 2013). Similar results were obtained by Zhang et al. (2012b). Size exclusion either occurred during translocation through the mucilage, or the cell wall.

Possible NP specific cell surface and mucilage binding mechanisms – besides the weak van der Waals forces – include hydrogen bonds (Schwab et al., 2011), or the above-mentioned electrostatic attraction of positively charged NPs and negatively charged (because acidic) mucilage (Zhang et al., 2011; Zhu et al., 2012). Neutrally and negatively charged NPs accumulating on the root surface show that additional mechanisms overriding the electrostatic repulsion can apply (Zhao et al., 2012b). In this case, NPs must form stronger bonds with the plant surface such as the mentioned hydrogen bonds (Schwab et al., 2011), or covalent bonds. Another possibility for NPs to override the electrostatic repulsion is sorption hysteresis (trapping) by irreversibly diffusing into micro-pores of the mucilage polymers. “Trapping” mechanisms known for bacteria can include recognition by extracellular deoxyribonucleic acid (exDNA) (Driouich et al., 2013). To quantify such binding mechanisms, exact knowledge of both the M/E, and NP surface properties, including stabilizing agents, is essential.

Phytoplankton and NPs agglomerate extensively, which can be the result of excess mucilage production in response to NP exposure (Cherchi et al., 2011; Kadar et al., 2012; Nielsen et al., 2008; Verneuil et al., 2014). Disintegrated plankton colonies can produce gel-like transparent polysaccharide exopolymer particles (TEP) that are known to promote particle aggregate formation in salt- and freshwater environments (Bar-Zeev et al., 2015). Another possible cause of excessive mucilage production by specific dinoflagellate and diatom species is viruses (Baldi et al., 1997). Similar to root tip mucilage, phytoplankton mucilage consisted of polysaccharides and alcohols released upon cell lysis. It was resistant to mechanic stress (e.g. attack by pathogens) and chemically inert (Baldi et al., 1997; Tufekci et al., 2010). Phytoplankton mucilage can externally complex NPs, similar as it complexes toxic heavy metals: extensive accumulation of NPs was seen in polyphenolic mucilage of the brown algae Fucus vesiculosus (Nielsen et al., 2008). Likewise, Anabaena variabilis responded to prolonged nano-TiO$_2$ aggregate exposure by increasing its mucilage layer thickness (Cherchi et al., 2011). Isochrysis galbana secreted an extracellular matrix to bind zero-valent nanomaterials (nZVI) (Kadar et al., 2012). Washing experiments on nano-Ag adsorbed to the green alga Chlamydomonas reinhardtii demonstrated that up to 97.6% of the nano-Ag was bound to the outer surface of the cell (Piccioppo et al., 2012). Electron microscopy images of the same algal species confirmed similar behavior for nano-TiO$_2$: the bulk of NPs adhered to the outer algal surface (Quigg et al., 2013). Own work has shown that the phytoplankton in agglomerates with MWCNTs remains viable.
(Schwab et al., 2011), and no uptake occurred (Schwab et al., 2013).

Clearly, mucilage in direct contact with exposed water or soil accumulates large fractions of NPs, which tends to reduce root–shoot translocation and uptake into cells. If washing procedures were used, roots, shoots and phytoplankton cells all showed high extracellular, adsorbed NP concentrations. The mucilage has a rather general protective effect against NP translocation across a wide variety of terrestrial plants, and NP sizes and coatings. The protective effect appears to extend especially, but with exceptions, against small (Zhang et al., 2011), and positively charged NPs (Zhang et al., 2011; Zhu et al., 2012).

**Cuticle**

All regions of the plant exposed to air, and most of the root rhizodermis except for the developing root tips and mucilage, are covered by a lipophilic cuticle (Figures 2 and 5, exceptions are listed in the “Uptake across the cell membrane” section) (Albersheim, 2011; McNear, 2013; Schreiber, 2005). The cuticle protects the plant from uncontrolled water loss, and efficiently protects the plant against attachment of pathogens, particles and dirt (Neinhuis & Barthlott, 1997; Schreiber, 2005). The hydrophobicity and chemical inertness of the cuticle presents a repellent barrier for polar compounds either entering, or leaving the plant-unlike the sticky hydrophilic, reactive mucilage (Albersheim, 2011; Schreiber, 2005). The cuticle is the first barrier for NP uptake in all regions of the plant bare of symbionts or mucilage, i.e. most of the root periderm, and all the aerial parts (Figure 1). All kinds of NPs, such as negatively charged mesoporous silica nanoparticles (MSNs, 20 nm) (Hussain et al., 2013), or neutrally charged lipid-based liquid crystalline NPs (150-300 nm) (Nadiminti et al., 2013), were reported to accumulate above the anticlinal cell walls or in the cuticle. In one case, nano-TiO$_2$ was shown to produce holes in the cuticle (Larue et al., 2014b). More direct observations of NP uptake across the cuticle are not available.

Actual uptake of NPs through the cuticle would be striking in view of its robustness. Epicuticular waxes can additionally infiltrate the simple layer of cutin in cuticles. These waxes form crystals of a wide variety of size and shape on the cuticle surface serving to increase roughness for higher repellency (in extraordinary cases called the lotus-effect) (Albersheim, 2011; Nadiminti et al., 2013; Neinhuis & Barthlott, 1997). Being the main barrier for pesticides, the cuticle’s permeability for organic molecules has been extensively studied. Its permeability for molecules increases with the substance’s lipophility and diffusion coefficient (Schreiber, 2005; Wild et al., 2005), and can be artificially increased by classical surfactants solubilizing epicuticular waxes (Hu et al., 2010; Nadiminti et al., 2013; Schreiber, 2005). Lipophilic molecules accumulate in the cuticle above anticlinal cell walls, and then translocate along pectins (middle lamellae) through the apoplast (refer the “Apoplast versus symplastic transport” section).

Like for pesticides, the permeability of the cuticle for NPs can be enhanced by surfactants (Hu et al., 2010; Nadiminti et al., 2013). Adding a surfactant enabled uptake of quantum dots (QDs, 4–7 nm) in maize (Hu et al., 2010). Dicot (or eudicot) cuticles were more permeable than those of monocots for lipid-based nanostructured liquid crystalline NPs, which were used as surfactant replacement to alter the wax morphology. The more robust cuticle was ascribed to the greater anatomical punch strength and fracture toughness of the tested true grass species (Nadiminti et al., 2013).

For hydrophilic compounds, special polar paths in the cuticle were postulated, i.e. regions permeable to water and other polar solutes (Figure 5; Schreiber, 2005; Schreiber et al., 2006). Thinned, permeable regions of the cuticle exist in various plant tissues possessing secretory/uptake functions, such as trichomes, mucilage-covered root hairs and tips, and hydathodes (refer the “Plant loopholes” section; Fahn, 1982). Further permeable regions of either the cell wall or the cuticle are present near cell junctions, as shown using secondary ion mass spectroscopy imaging for ~1 nm [Mo$_6$Br$_{14}$]$^{2-}$ clusters (refer the “The cell wall and pectins” section; Aubert et al., 2012). By comparing the rate constants of $^{15}$N and $^{13}$C uptake, a pore size of cuticular leaf tissues (i.e. tissues bare of stomata) for polar solutes of 2.0–2.4 nm was calculated (Eichert & Goldbach, 2008). In the presence of the naturally cuticle-free stomata, the pore size jumped to >100 nm, underscoring the importance of the cuticle for repellency of polar solutes (Eichert & Goldbach, 2008). Remarkably, neither the thickness of the cuticle nor the amount of wax correlates with the water permeability (Albersheim, 2011). Instead, plants exhibiting increased cutin synthesis (wax inducer I, WIN1 overexpression) showed increased drought tolerance. This illustrates that the micro-architecture of the cuticle plays an important role in determining water (im)permeability (Albersheim, 2011), consistent with the notion of polar paths. Charged NPs (i.e. the vast majority of ENMs) might enter or be excreted through polar paths, e.g. in lettuce, fruits and berry cultivars possessing many trichomes.

As mentioned earlier, in submerged aquatic plants, the cuticular waxes on the leaves are absent, and the cuticle is much thinner to facilitate nutrient uptake into the leaves (Glenn et al., 2012; Ma & Lin, 2013). Indeed, some submerged plant and phytoplankton species easily accumulate NP concentrations in the $1\text{ng g}^{-1}$ range, corresponding to ~2 orders of magnitude accumulation as compared to the water column (Colman et al., 2014). Further research should focus on visualizing NP uptake in shoot tissues of submerged aquatic species to elucidate why and where this accumulation occurred. Unbranched roots in submerged plants often serve primarily as anchors (Fahn, 1982). Leaves of submerged plants are excellent models to quantify the permeability of the cell wall as they are directly exposed to suspended NPs (Glenn et al., 2012).

**The cell wall and pectins**

The cellulose cell wall—the NP barrier most plants share—is a fiber-reinforced composite material with a similar architecture in all plants: a cellulose–hemicellulose network resisting tension, and a coextensive pectin network to resist compression and shear forces (Figure 2; Albersheim, 2011; Vymazal, 1995). Pectic polysaccharide exudates from the cell wall form the middle lamella, which connects cells and merges with the cuticle (refer the “Cuticle” section). The cell wall is normally a few hundred microns up to several µm in fairly homogeneous thickness (Figure 2A). Exceptions are the endo- and epidermis (Figure 3; Fahn, 1982; Sattelmacher & Horst, 2007) and aged secondary cell walls. The latter can reach up to several microns in thickness in the xylem. The other extreme are developing primary cell walls of specific meristematic cells, or root hairs, which can be <100 nm thin (Albersheim, 2011; Fahn, 1982; Galway, 2006). This may explain, besides the mucilage, the accumulation of a broad range of NPs (CNTs, fullerenes, nano-TiO$_2$, nano-CeO$_2$, nano-$^{141}$CeO$_2$, nano-Ag and nano-Au) in the thin-walled root cap border cells (Geisler-Lee et al., 2013; Liu et al., 2010; Wild & Jones, 2009; Zhang et al., 2011; Zhai et al., 2014).

In the endo- and epi-dermis (Figure 3), suberin can reinforce the cell walls—and potentially block NP transport—to an extent varying by >2 orders of magnitude in different species (Fahn, 1982; Sattelmacher & Horst, 2007). Similar to suberin, elevated
As the pectins limit the porosity of the cell wall (Carpita & Gibeaut, 1993; Fahn, 1982), their decomposition is the key target of many symbionts and pathogens. Similarly, the degradation of pectins by nZVI-induced OH\(^\circ\) radicals was demonstrated (Kim et al., 2014), an effect that will have to be studied for further OH\(^\circ\) radical producing NPs. Less branched pectins (homogalacturonans) are present near adhered and separated cell walls (Willats et al., 2001), and higher permeability in these regions was indeed documented, e.g. for \(^{81}\text{Br}\) and \(^{99}\text{Mo}\) of <1nm [\(\text{MoOBr}_4\)\(^{1-}\)] clusters (Aubert et al., 2012). A different micro-architecture of the cell wall is, besides of the dissimilarities in cuticular waxes mentioned in the “Cuticle” section, another potential explanation for the different uptake and toxicity of NPs in dicots and monocots (especially true grasses; Judy et al., 2012; Ma et al., 2010b, 2013; Sun et al., 2014). Pectin polymer networks in true grasses and many other monocots (Type I walls) are more firmly cross-linked to the cellulose than those of dicots (Type II walls; Carpita & Gibeaut, 1993). Further, the Si content reinforcing the cell wall is elevated in true grasses (Ma & Yamaji, 2006; Wu et al., 2013). On a macroscopic scale, monocot leaves with parallel veins – especially the graminoid (grass) species – are generally most resistant to tearing, i.e. they have a 3.6–4.2 times higher strength per density than the leaves of other plants (Onoda et al., 2011). For all these reasons, and in view of the frequent strong NP accumulation in this plant group, NPs may penetrate the cell wall easier in dicots than in monocots.

The pore size in the pectin network (middle lamellae; Wild et al., 2005) was determined to range from ~0.6 to 4.8nm (Eichert et al., 2008). This size range and the lipophilicity favor accumulation for small neutrally or nearly neutrally charged NPs.

### Uptake across the cell membrane

Besides protoplasts, transport across the cell membrane is a central process in thin-walled plant parts such as root hairs and pollen tubes (refer the ‘‘Cuticle’’ and ‘‘The cell wall and pectins’’ sections; Galway, 2006; Šamaj, 2012). The plant cell membrane (i.e. plasmalemma, plasma membrane) is selectively permeable to allow small non-polar ions easy diffusion across the membrane (Figure 2). Larger and polar molecules including water, ions and exotic particles cannot easily diffuse through the phospholipid bilayer. Nevertheless, in Mammals, such molecules and NPs with special size, shape and surface charge, such as iron oxide NPs, functionalized CNTs, as well as cationic QDs, dendrimers, and other metallic NPs are clearly able to cross the cell membrane (Bhirde et al., 2011; Verma & Stellacci, 2010). ‘‘Endocytosis’’ and ‘‘Non-endocytic pathways’’ sections discuss the less well known uptake pathways of NPs across the plant cell membrane, which vary – similar to uptake in mammalian cells – depending on size, shape and surface charge.

Protoplasts – plant models with the cell wall enzymatically removed – are used to study plant cell membrane uptake. This plant model is used to circumvent the technical difficulties of studying membrane uptake processes in plants; the plant cell wall’s bright autofluorescence, and impermeability to fluorescent dyes used in staining procedures (Etxeberria et al., 2006; Kole et al., 2013; Torney et al., 2007).

### Endocytosis

Endocytosis is invagination of the cell membrane for the uptake of extracellular materials, including NPs (Figure 2A). Fluid-phase endocytosis (FPE) mechanisms differ for NPs of comparable size but different charge (Etxeberria et al., 2006): In NP-exposed protoplasts of Acer pseudoplatanus (sycamore maple), 40nm positively charged Texas Red labeled polystyrene nanoparticles were primarily found in the central vacuoles.
Neutrally charged polyethylene glycol coated NPs (Etxeberria et al., 2006; Moscatelli et al., 2007; Onelli et al., 2008; Šamaj, 2012). Oxidized SWCNTs, <500 nm in length, traversed Nicotiana tabacum (cultivated tobacco) plant cell membranes. Again, unspecified FPE was discussed as a possible mechanism, as nanotube uptake was significantly decreased upon treatment with known FPE inhibitors (Figure 2A-b; Liu et al., 2009). Endocytosis-like vesicles formed after prolonged exposure of Arabidopsis protoplasts to SWCNTs (Shen et al., 2010). Clathrin-independent FPE of solutes does not require binding of a specific ligand to a cell membrane localized receptor, occurs in larger vesicles and is in line with macro- or micro-pinocytosis (Šamaj, 2012). Macropinocytosis however was to date reported to occur, together with pathways like the alveolar pathway, CLIC/GEEC pathway and phagocytosis, exclusively in animal cells (2001; Šamaj, 2012, Sánchez-Serrano).

Fluid-phase endocytosis was also found in uptake experiments in protoplasts with nano-Au of opposite charge (Onelli et al., 2008). Whereas positively charged nano-Au attached to the negatively charged cell membrane and got internalized to a great extent, the negatively charged nano-Au bound weakly to the cell surface and was taken up only occasionally (unspecific FPE; Onelli et al., 2008). Again, unlike the negatively charged nano-Au, the uptake of positively charged nano-Au was partially clathrin-dependent (CFPE; Onelli et al., 2008). Similar selective endocytosis of positively charged NPs was found for MSNs (~200 nm in diameter) in protoplasts (Torney et al., 2007) and positive and negative nano-Au in pollen tubes (Moscatelli et al., 2007). This charge-related selectivity is in agreement with results in Mammals (Bhirde et al., 2011). The second important factor for endocytosis is size. On the one hand, large, non-functionalized, negatively charged MSNs (~200 nm) were not internalized in protoplasts (Torney et al., 2007). On the other hand, small non-functionalized MSNs (5–15 nm) in suspended walled cells were able to pass through the membrane via endocytosis. This demonstrates a bias of endocytosis towards small NP size (Xia et al., 2013). As a whole, these results show that positively charged NPs <120 nm can be internalized by CFPE. Less frequently, inert NPs regardless of charge and <200–500 nm are taken up by non-selective FPE.

Non-endocytic pathways

Aquaporins (water channels) are proposed NP uptake pathways (Fitzpatrick & Reid, 2009; Rico et al., 2011; Sattelmacher & Horst, 2007). These protein channels serve to non-selectively uptake water and small non-ionic solutes of generally <1 nm in diameter. Aquaporin facilitates water transport in the otherwise almost impermeable cell membrane (Fitzpatrick & Reid, 2009; Zangi & Filella, 2012), and aids the plant to switch between symplastic and apoplastic transport (Sattelmacher & Horst, 2007). The aquaporin channel diameter at the midpoint is 0.2–0.25 nm (Tyrerman et al., 2002), and single Hg^{2+} ions can block them (Sattelmacher & Horst, 2007). Uptake of insoluble NPs of >1 nm in diameter through these water channels (and similar protein pumps or channels in the cell membrane) is extremely unlikely (Hu et al., 2010; Schaller et al., 2013; Wang et al., 2011a). Indeed, no research found any protein channel including aquaporins to be the main pathway for insoluble NP uptake. Upon exposure to NPs, aquaporin was sometimes up- (Khodakovskaya et al., 2012) and sometimes down-regulated (Lu et al., 2010; Taylor et al., 2014). The change of aquaporin expression may be a response to the reduced water flow through the plant when NPs clog the apoplast (Asli & Neumann, 2009; Lu et al., 2010; Ranathunge et al., 2005).

Large MWCNTs can mechanically penetrate the cell wall and the cell membrane of wheat roots without ever being completely internalized (Figure 2A; Wild & Jones, 2009). Multi-walled CNTs <100 nm can skip endosomal processing and directly enter Madagascar rosy periwinkle (Catharanthus roseus) protoplasts (Serag et al., 2011). Surface-dependent NP uptake mechanisms have been tested on mammalian cells but not on plant cells. For example, NP surface ligands can interact with the cell membrane. A recently discovered, for plants yet unexplored mechanism based on the ligand–membrane interaction is snorkeling (Figure 2A; Van Lehn et al., 2013; Verma et al., 2008). Due to the similar thickness and lipid bilayer structure of cell membranes in animals and plant cells, snorkeling is also a potential way through the cell membrane in plants. Mechanisms such as snorkeling demonstrate that there is still plenty of room for new discoveries in plant cell membrane uptake.

**Cell-level imaging of nanoparticle interactions**

While TEM is of invaluable help to visualize NP uptake in plants, such observations of ENMs in plants without confirmed chemical composition have to be treated with extreme caution (Brandenberger et al., 2010; Lombi et al., 2011). Plants contain many electron dense NPs mistakable for the NPs under investigation (e.g. NPs from the soil or growth medium, ferritin, CaCO₃, SiO₂-nH₂O phytoliths or NP synthesis byproducts; Bauer et al., 2011; Brandenberger et al., 2010; Fahn, 1982; Mueller, 2011; Petersen et al., 2014; Richmond & Sussman, 2003; Wu et al., 2013). Transmission electron microscopy staining agents and buffers can produce similar artifacts, especially osmium tetroxide, cacodylate, lead citrate, uranyl acetate and ferri- or ferrocyanide (Ayache et al., 2010; Huang et al., 2011; Rivlin & Raymond, 1987). All these agents are prone to form electron dense precipitates of shapes and sizes similar to the investigated NPs. Finally, the investigated NPs can dissolve (Badireddy et al., 2014) and re-precipitate in the plant (Ma et al., 2011; Parsons et al., 2010; Schaller et al., 2013; Vannini et al., 2014; Zhang et al., 2012b). Chemical confirmation by micro- or preferably nano-analytical techniques can identify the NPs, e.g. using μ-X-ray fluorescence (μ-XRF), or high resolution TEM to identify the crystalline structure or distinct shape of the NPs (Gaff et al., 1964; Zhao et al., 2012a,b). The importance of this validation step is underscored by results such as those for cucumbers exposed to nano-Yb₂O₃. These NPs dissolved, and the electron dense NPs seen in the plants were in fact YbPO₄ precipitates (Zhang et al., 2012b).

**Plant loopholes**

A few important exceptions apply to the continuity of the barriers now discussed (Figure 4). Certain parts of plants such as crevices, eroded cuticles or thinned cell wall regions can facilitate NP uptake, and also allow excretion.

**Wounds**

Wounds are an infection pathway for many plant pathogens (Huang, 1986) and offer a direct route for NPs into the cytoplasm
as well (Figure 5; Dietz & Herth, 2011). Nanoparticle uptake due to wounding was reported, e.g. on cut edges of plants (Al-Salim et al., 2011), but without information on the relative contribution (e.g. by grazing or mechanical ruptures) to total plant NP uptake. Fluorescence time series of root cross-sections exposed to 4–7 nm fluorescent QDs showed, after 12 h of exposure, high uptake in one of 11 xylem vessels (Hu et al., 2010), suggesting a connection to a lesion in the root. In leaves foliarly exposed to nano-Pb, the NPs accumulated in necrotic tissue (Uzu et al., 2010). Plants recognize wounded regions within seconds to minutes (Łukaszuk & Ciereszko, 2012), then block plasmodesmata of adjacent cells by callose in less than 10 min (Egeria densa) to hours (Lee & Lu, 2011; Roberts & Oparka, 2003), and deposit lignin and suberin to fortify the cell wall within hours (Denness et al., 2011). In line with this, most NPs accumulated in the first few centimeters of a cut stem: roses (Rosa hybrida) dipped into 50 mg L$^{-1}$ nano-Ag suspensions accumulated low mg kg$^{-1}$ concentrations of Ag in leaf tissue, and ~90% of the Ag was found in the cut stem (3–5 mg kg$^{-1}$ dry weight, ~60% in the first 2 cm: Lu et al., 2010). Taking into account a typical dry weight–fresh weight conversion factor of around 10 on a leaf basis, this accumulation is consistent with the uptake observed.

Several of the above and following statements base on (confocal) fluorescence light microscopy, mainly of fluorescent QDs, and fluorescence imaging without cross-verification and images of unexposed controls is challenging to interpret. In plants and phytoplankton, many artifacts, for instance, the above-mentioned fluorescence quenching (phyto-quenching) (Al-Salim et al., 2011), were demonstrated (Al-Salim et al., 2011; Navarro et al., 2012; Shukla et al., 2005; Wang et al., 2013d). False positives also occur, e.g. due to the bright autofluorescence especially of chloroplasts and cell walls (Kole et al., 2013; Wang et al., 2014; Yamaji & Ma, 2014). Useful techniques to identify light scattering, or fluorescent NPs are, e.g. hyperspectral imaging (Badireddy et al., 2012), and emerging fluorescence techniques such as spectral unmixing (Wang et al., 2014), single-molecule microscopy, and fluorescence correlation spectroscopy (Jares-Erijman & Jovin, 2003; Michalet et al., 2005). Alternatively, QDs emitting in the near-infrared region (900–1300 nm) can be used for uptake studies, provided they are not phytotoxic (Michalet et al., 2005).

Lateral root – ruptured epidermis

Controlled forms of wounding occur when roots lose trichomes (Fahn, 1982). The young lateral root tip, in gymnosperms and angiosperms starting in the pericycle (Figure 3), penetrates the cortex and eventually rips the (previously died) root endo- and epidermis including the Casparian strip, resulting in a wound around its base (Fahn, 1982). The wound, as described above, is quickly sealed by the mucilage of ripped cells and repaired. Still, this crevice is an hotbed for certain bacteria (Huang, 1986). Nanoparticles possibly accumulate in this region as well (Dietz & Herth, 2011; Zhai et al., 2014; Zhang et al., 2012a; Zhao et al., 2012a). So far, this has been indirectly confirmed in two cases (Lv et al., 2015; Ranathunge et al., 2005). Other results showed absence of NPs near the base of emerging lateral roots (Geisler-Lee et al., 2013).

Root hairs – water and nutrient transporters

The mucilage-covered root hairs are present in the zone of root maturation above the root tip and cope with most of the mass transfer, i.e. water and nutrient uptake in roots (Figure 5; Fahn, 1982; Zhu et al., 2012). Root hairs emerge from single specialized epidermal cells (=trichoblasts) of the parent root, which are not directly connected to the vascular system (Fahn, 1982; Navarro et al., 2012). The root hairs’ cuticle and cell wall are thinner and more permeable than those of normal cells (Galway, 2006), and their cell walls are not reinforced by Si (Ma & Yamaji, 2006). In line with this,
comparisons of different plant species showed strongest nano-Au accumulation in radish (6–10 nm NPs), and Carolina mosquito fern (*Azolla caroliniana*, 4 and 18 nm NPs). This was ascribed in all cases to the root hairs of these species (Glenn et al., 2012; Zhu et al., 2012). Further, QDs (Al-Salim et al., 2011; Navarro et al., 2012) and CNTs (Canas et al., 2008; Miralles et al., 2012b) accumulated on root hairs. Frequently, less or no root hairs grew in plants exposed to NPs (Aubert et al., 2012; Dietz & Herth, 2011; Wang et al., 2011b; Yin et al., 2011). This toxic effect is likely associated with the fact that large fractions of NPs can get stuck to root hairs (Al-Salim et al., 2011; Canas et al., 2008; Miralles et al., 2012b; Navarro et al., 2012). The lost root hairs demonstrate why we also must measure NP uptake at low effect concentrations: at toxic concentrations, the uptake mechanisms of plants are different from those at low concentrations. Adverse effects or accumulation can occur at both high and low concentrations (Feng et al., 2013; Zhai et al., 2014). As the root elongates, the root hairs fall off and the trichoblasts dies (Fahn, 1982; Galway, 2006), leaving lysed cells, i.e. pathways for NPs. Another pathway is created when bacteria perforate the root hair cell walls to establish symbiotic connections (refer the ‘Symbiotic bacteria and mycorrhizae’ section; Mateos et al., 2001).

**Hydathodes – water safety valves**

Hydathodes, small cavities at the leaf tip lacking cover by a cuticle (Figure 5), allow excretion (=guttation) of excess water...
across the leaves of herbaceous plants such as *Brassica* (mustard family), *Poaceae* (true grasses), *Crassulaceae* (stonecrop family), and *Saxifragaceae* (saxifrage family; Huang, 1986). Gutttation is important for leaves as their cell walls, unlike those of roots, are not saturated with water. Non-volatile substances can therefore not be discharged to the apoplastic for excretion (Sattelmacher & Horst, 2007). Hydathodes are an entrance pathway for pathogenic bacteria. Uptake or excretion of NPs may occur across the hydathodes (Hong et al., 2014) especially after a guttation period when the water droplet is sucked back into the plant (Huang, 1986). Indeed, accumulation of insoluble NPs near hydathodes was found in cucumbers, where radioactive $^{141}$Ce, from largely insoluble nano-$^{141}$CeO$_2$, was detected in leaf tips and serrations at the end of the vascular bundle (Zhang et al., 2011). Similarly, nano-SiO$_2$ accumulated in ionic form, and was excreted as a salt near the tips of the leaves (Schaller et al., 2013).

**Stomata – gas transporters**

Stomata, most abundant on the abaxial (=lower) leaf surface, are the key sites for transpiration in the aerial parts of phototrophic terrestrial plants, and in a few submerged water plants (Figure 5; Fahn, 1982; Hirano et al., 1995; Veraverbeke et al., 2003). During the day, the plants keep these pores wide open to facilitate evaporation of water. Stomata, like lenticels, lack a cuticle to improve gas diffusion. They can exhibit an increased roughness (NP attachment) due to salt precipitates (Burkhardt et al., 2001, 2012), and an increased permeability for polar substances (Schreiber, 2005). Exclusively in the presence of stomata, the pore size of leaves of three dicotyledons, as determined by comparing uptake rates of $^{15}$N and $^{13}$C, was $>$100 nm (Eichert & Goldbach, 2008). All this underscores the role of stomata for NP deposition and uptake. Indeed, a wealth of studies documented stomata or substomatal chambers blocked by micrometer, sub-micrometer, and nano-sized particles (Eichert et al., 2008; Farmer, 1993; Hirano et al., 1995; Hussain et al., 2013; Uzu et al., 2010), and related adverse effects, particularly for mosses and lichens (Farmer, 1993; Hirano et al., 1995). Blocked stomata or guard cells can lead to reduced water transpiration, increased leaf temperature and then to reduced growth, photosynthesis and diffusive resistance (Farmer, 1993; Hirano et al., 1995).

Exclusive foliar uptake of ions or NPs can lead to accumulation of heavy metals on and in the leaves: *Lettuce (Lactuca sativa)* growing near a secondary lead smelter – with roots covered – accumulated 335 ± 50 mg kg$^{-1}$ Pb in the washed leaves (Uzu et al., 2010). When nano-CeO$_2$ was applied on cucumber leaves, ~3% of the total cerium in the plant was found in the roots, indicating leaf-root translocation of either NPs or cerium ions (Hong et al., 2014). Also, 73.0–81.0% of the totally applied nano-CeO$_2$ adhered to the outer leaf surface. Foliar application of NPs (including insoluble nano-TiO$_2$) resulted in NP translocation from stomatal cavities to plant tissues such as adjacent cells, the vasculature and roots (Larue et al., 2014a,b; Wang et al., 2013c). Notably, translocation coincided with solubility of the NPs: Leaf uptake of nano-ZnO, nano-MgO and nano-TiO$_2$ led to consistently lowest translocation to roots for the least soluble nano-TiO$_2$ (1.49–5.45% of total plant concentration in root), and highest shoot–root translocation for the most soluble nano-MgO (11.5–26.14%) (Wang et al., 2013c). Again, partially dissolving NPs cannot be ruled out as a cause for the measured concentrations. Direct (visual) observations of stomatal uptake of non-dissolving NP, and translocation from there to adjacent plant cells are rarely reported.

In the case of root NP uptake, Lu and co-workers noted that stomata closed, and transpiration slowed down when cut flower stems were dipped into NP suspensions. Consequently, NPs might as well block the stomata from inside, or clog the vasculature, leading to decreased water fluxes (Lu et al., 2010). Mysteriously, accumulation of black particles (MWCNTs?) in stomata was seen by light microscopy after root NP exposure (Smirnova et al., 2011). However, the primary effect to be anticipated is cell wall pore, channel, or vasculature clogging by NPs, since NP exposure often affects water fluxes in cut and intact plants (Asli & Neumann, 2009; Farmer, 1993; Hirano et al., 1995; Sattelmacher & Horst, 2007; Zhai et al., 2014).

Interestingly, several mosses bare of stomata such as *Hylorocium splendens* (no roots, no stomata) or *Sphagnum spp* (pseudostomata only) were more vulnerable to dust than those possessing stomata (Farmer, 1993). The presence of stomata therefore does not necessarily increase vulnerability to particles in different plant species. Consequently, NP stomatal uptake, hydathode and trichome uptake all have to be considered for the total foliar NP uptake.

**Lenticels – gas safety valves**

Lenticels, cavities not bearing a cuticle on surfaces of roots, stems and fruits of most plants (Fahn, 1982; Greve et al., 1987), act as “gas safety valves” for excess gas in stress situations when the stomata are closed and cannot volatilize water (Figure 5; Veraverbeke et al., 2003). Due to the non-existence of a cuticle, lenticels exchange gas, and to some extent, water and salt aerosols (Fahn, 1982; Greve et al., 1987; Huang, 1986). The comparable stomatal “gas valves”, which also lack a cuticle, possess a calculated pore size of $>$100 nm (refer the “Stomata – gas transporters” section; Eichert & Goldbach, 2008). No studies on the permeability of lenticels for NPs are available.

**Trichomes – defensive appendages**

Trichomes, unicellular and multicellular appendages of the epidermis (Fahn, 1982), are present on most surfaces of most plants, mainly for protective purposes (Figure 5; Wagner, 1991). There are $>$300 morphological types of trichomes (Wagner, 1991), which can be very coarsely divided into non-glandular and glandular (Fahn, 1982). Many types of trichomes, such as the peltate–shaped (non-glandular), accumulate internalized or airborne metals, thereby alleviating their toxicity (Larue et al., 2012b; Moore et al., 2012; Ruffini Castiglione & Cremonini, 2009; Sarret et al., 2013). Accumulation of NPs in non-glandular trichomes has so far not been investigated.

Glandular trichomes excrete salt solutions, sugar solutions (nectar), terpenes (resin) and gums (polysaccharides), e.g. as protection against pests, or excessive light irradiance (Fahn, 1982; Sánchez-Serrano, 2001; Wagner, 1991). Due to the secretory properties, glandular trichomes are permeable for diffusion of polar substances (polar paths, refer the “Cuticle” section; Schreiber, 2005; Schreiber et al., 2006). Glandular trichomes can accumulate large quantities (up to 10% of leaf dry weight) of metabolic substances between the pectin layer and the cuticle. These substances are exuded through pores in the cuticle if a given pressure is exceeded (Fahn, 1982; Wagner, 1991). Glandular trichomes can excrete NPs, as visualized by TEM in pumpkin (*Cucurbita pepo*), after C-coated nano-Fe$_x$O$_y$ particles were injected into the leaf pith cavity of the leaf petiole (Corredor et al., 2009). The same type of NPs also accumulated – according to black discolorations visible by light microscopy – in or on wheat trichomes after NP exposure of the roots (Cifuentes et al., 2010). Untransformed nano-TiO$_2$ ($27 ± 4$ nm) as identified by μ-X-ray absorption near edge structure (μ-XANES) was recently demonstrated to translocate from the roots of cucumber into leaf trichomes (Servin et al., 2012), and was also detected in
cucumber fruits (Servin et al., 2013). The contribution of trichomes to total NP accumulation is not known.

Although trichomes may also, due to their permeability, take up NPs (Navarro et al., 2008), no studies have verified this up to date. The role of trichomes on NP uptake likely depends on their wax content and number. On the one hand, non-waxy intact or broken trichomes are an avenue for microbial phytopathogens, e.g. in fluffy young leaves of tomato and lettuce (Huang, 1986), and this may extend to NPs. Especially leaves with few or non-waxy trichomes, such as trees and shrubs in temperate forests, and submerged plants, become hydrated easily, leading to facilitated attachment of bacteria and particles on the leaf surface (Neinhuis & Barthlott, 1997). Trichomes can also be, together with lesions (wounds), the main sites of foliar uptake of pesticides (Buick et al., 1993). On the other hand, for the majority of NPs being less lipophilic, the trichomes are likely more important for the lotus-effect. Plant surfaces covered with numerous waxy trichomes are extremely water-repellent, and therefore (nano)particle-repellent, due to their high roughness and lipophilicity (marsh and non-submerged plants) (Neinhuis & Barthlott, 1997).

Plasmodesmata

Symplastic transport (refer the “Apoplastic versus symplastic transport” section), i.e. cell-to-cell transport of cytoplasmic components including NPs, is strongly regulated by the plasmodesmata (Figure 4; Lucas & Jung-Youn, 2004; Kim, 2005; Roberts & Oparka, 2003). First direct observations by TEM of NPs or precipitates in or near plasmodesmata suggest that NPs of <15–40 nm in diameter can enter plasmodesmata (Geisler-Lee et al., 2013; Zhai et al., 2014). Due to the scarce direct observations of NPs in plasmodesmata, the role of symplastic NP transport is still a mystery. Nanoparticles are most often detected in the apoplast (refer the “Vascular and non-vascular transport processes” section) and not in the symplast. Studies proposing NP transport through plasmodesmata (Figure 4, Supplemental Table S1) often did so based on increased metal ion concentrations in different plant parts. This does not rule out the possibility of apoplastic transport (refer the “Apoplastic versus symplastic transport” section), or dissolution of the NPs, and therefore studies with more and less direct observations will be discussed below separately.

In plants, plasmodesmata are the only connections of the cytoplasm of adjacent cells, and provide the cytoplasmic pathways for communication (Figure 4). The plasmodesmata architecture and number varies depending on the species and tissue (Roberts & Oparka, 2003; Robards, 1975). Small cells can have as much as 10³–10⁶ plasmodesmata, and the density in various cell types ranges from 0.6 to 140 plasmodesmata mm⁻² as determined by TEM. Frequencies of >1 Mio mm⁻² occur (Robards, 1975).

Structure and size exclusion limit (SEL)

To understand NP transport through plasmodesmata, it is important to realize that plasmodesmata are not hollow. The basic structure of plasmodesmata is a linear or branched channel across the cell wall, lined by the cell membrane. The channel contains a strand termed desmotubule of endoplasmatic reticulum (ER) connecting the ERs of two cells (Figure 4). Most models consider the desmotubule to be hollow, but occasionally a central rod is proposed. The space in between the cell membrane and the desmotubule, in which most solutes are transported, is called cytoplasmic sleeve (Roberts & Oparka, 2003). The desmotubule and the cell membrane are connected by proteins, which subdivide the cytoplasmic sleeve into several microchannels of 3–4 nm in diameter (Lucas & Jung-Youn, 2004; Roberts & Oparka, 2003). The plasmodesmata have, due to the size of the microchannels, a basal size exclusion limit (SEL) in mature tissues of ~3–4 nm, or 580–900 Da (Dietz & Herth, 2011; Roberts & Oparka, 2003; White et al., 1994). Molecules larger than the basal SEL, such as RNA, proteins, transcription factors and plant viruses, undergo conformational changes in the plasmodesmal channel (Roberts & Oparka, 2003; White et al., 1994). Given the inability of most NPs to undergo conformational changes, this means that plasmodesmata, under normal circumstances, do not transport NPs >3–4 nm (Dietz & Herth, 2011). Only solutes smaller than the basal SEL, such as sugars, amino acids and ions, directly diffuse through the microchannels in plasmodesmata. Plasmodesmata in mature cells are ~20–40 nm in diameter in the neck region of the cytoplasmic sleeve, and 50–60 nm in diameter at the widest midpoint (Figure 4; Lin et al., 2009; Robards, 1975). Wider diameters of <200 nm were observed occasionally (Robards, 1975).

Flexible size exclusion limit

A recent change of paradigm from rigid to fluid, dynamic and flexible plasmodesmata underscores the flexibility of the SEL. The plasmodesmata aperture can be modified by calcium (Roberts & Oparka, 2003), proteins (Roberts & Oparka, 2003), viruses (Scholthof, 2005), environmental stresses (Dietz & Herth, 2011; Larue et al., 2012a; White et al., 1994) and actin (White et al., 1994). For example, deposited callose near the cell walls surrounding the plasmodesmata strongly reduces the permeability of plasmodesmata in short time (wounding, refer the “Wounds” section). Stressing plasmodesmata by plasmolysis increased their permeability for dyes (White et al., 1994). Further, all plant viruses translocate through plasmodesmata via movement proteins. These proteines dilate the plasmodesmata openings at the neck region to enable passage of viral nucleic-acid–protein complexes (Scholthof, 2005). Some viruses such as the tomato spotted wilt virus, perhaps pass as intact virions, thereby permanently modifying the plasmodesmal structure (Scholthof, 2005). By such and other mechanisms, the SEL of plasmodesmata can be expanded to ~30–40 kDa. Then, macromolecules possessing a hydrodynamic diameter of ~10 nm can transit through plasmodesmata (Dietz & Herth, 2011; Larue et al., 2012a). For NPs, the value is smaller, because unlike macromolecules, most NPs cannot undergo conformational changes (Dietz & Herth, 2011). Also, NPs themselves may dilate the SELs of plasmodesmata, either directly, or via NP-induced structural changes (Larue et al., 2012a). Nano-TiO₂ disrupted microfilaments in wheat roots, which was proposed to enable the transit of nano-TiO₂ with diameters >20 nm from cell to cell (Larue et al., 2012a).

Directly observed NP transport

Without specific upregulation, and assuming the proteins in the desmotubule are flexible, the symplastic pathway may be passable, or blocked, by NPs <20 nm, which is the distance between the desmotubule and the cell membrane (Figure 4; Robards, 1975). In agreement with this size, Geiser-Lee and co-workers, and own work showed that nano-Ag 20 + 40 nm, and nano-Au ~15 nm in diameter, respectively, or similar sized precipitates can accumulate in or near plasmodesmata (Figure 4; Geiser-Lee et al., 2013; Zhai et al., 2014). The silver containing aggregates were identified by STEM-EDS in concentrated spots. The role of trichomes on NP uptake likely depends on their role in the desmotubule (Figure 4; Dietz & Herth, 2011).
Indirectly observed NP transport

Supplemental Table S1 shows representative studies that observed, by indirect or direct evidence, NP transport through plasmodesmata. The most frequently mentioned NPs in association with the plasmodesmata pathway in plants were fullerene, nanoparticulate silver, copper oxide, (C-coated) iron oxide, cerium dioxide, titanium dioxide, zinc oxide and gold. It is important to keep in mind that nano-Ag, nano-CuO, nano-ZnO and, to a lesser extent, nano-CeO2 and C-coated nano-FeOx can partially dissolve during the experiment, which can be promoted by the acidic environment of the rhizosphere (refer the “Mucilage and exudates” section). The initial size of these particles therefore does not correlate with the size of the plasmodesmata.

Transport through plasmodesmata was proposed based on the size of the investigated NPs, (Ghafariyan et al., 2013), radial NP transport in plants (Corredor et al., 2009), root–shoot translocation (Lin et al., 2009), accumulation of the metal present in the NP in different plant parts (Nekrasova et al., 2011), NP presence in the xylem (Sun et al., 2014) and observation of electron dense particulate matter in the xylem and phloem (Huang et al., 2011). Most notably, Zhang and co-workers demonstrated root–shoot translocation of 7 and 25 nm nano-141CeO2, in cucumber plants by tracing the radioactive isotope 141Ce in the leaves. Based on the presence of 141Ce in non-vascular leaf tissues and absence in leaf veins, nano-141CeO2 may have traversed plasmodesmata when it was released into the symplast. This is supported by the fact that 141Ce was absent in the vasculature-unlike 141Ce3+ (Zhang et al., 2011). Cerium from nano-CeO2 was also found to translocate from roots to shoots of tomato plants, and to accumulate in fairly high concentrations (0.5 g kg⁻¹ tissue by ICP-MS) in tomato fruits (Wang et al., 2012a). Tomatoes are connected to the phloem only. Consequently, nano-CeO2 was able to translocate along the phloem (Wang et al., 2012a), which belongs to the symplast and thus involves transport through sieve areas and/or plasmodesmata (Figures 4 and 5).

Vasculature

In higher plants, the vasculature determines the extent of NP translocation (Figure 4). The presence of NPs in the vasculature is well documented (Al-Salim et al., 2011; Asli & Neumann, 2009; Cifuentes et al., 2010; Corredor et al., 2009; De La Torre-Roche et al., 2013; Ghafariyan et al., 2013; Hu et al., 2010; Huang et al., 2011; Larue et al., 2012a; Whiteside et al., 2009; Zhai et al., 2014). The vasculature, consisting of xylem (apoplast) and phloem (symplast), possesses different architectures in different species (Fahn, 1982). In the dead, lignified, long, cylindrical xylem conduits (vessels and tracheids), NPs follow the pathways of transpiratory water (Figure 5; Al-Salim et al., 2011; Asli & Neumann, 2009; Cifuentes et al., 2010; Corredor et al., 2009; Ghafariyan et al., 2013; Hu et al., 2010; Huang et al., 2011; Zhai et al., 2014). From the xylem, NPs can distribute within the rest of the plant and be loaded into the phloem. Nanoparticles in the xylem have often been observed (Al-Salim et al., 2011; Corredor et al., 2009; Huang et al., 2011; Hussain et al., 2013; Marchiol et al., 2014; Wang et al., 2012b). Pits equipped with pit membranes in the tracheids mainly limit NP transport in the xylem vasculature, and primary pit fields in sieve areas are the bottlenecks of the phloem sieve cells (Fahn, 1982; Raven, 2003). Due to pore sizes of >>100 nm, NP transport is limited in the macroporous perforation plates in the xylem vessel elements (=vessel members), and sieve plates in phloem sieve tube members (for a schematic overview on all vessel types; Supplemental Figure S2; Fahn, 1982; Raven, 2003; Sokolowska & Sowiński, 2013). In gymnosperms, the macroporous vessel elements and sieve tube members are absent. Instead of macroporous conduits, the pits of gymnosperms (termed torus-margo pits) possess a larger pore size (refer the “Pit membranes” section; Choat et al., 2008; Liese & Johann, 1954). Transport in the gymnosperm xylem is exclusively regulated by these larger torus-margo pits in the xylem, and primary pit fields in the phloem (difference is marked by an asterisk in Figure 5). The extent of vascular translocation of NPs can be considered as a function of water flux in the plant. The flux again strongly depends on drought tolerance and plant architecture (Birbaum et al., 2010; Choat et al., 2008; Ma et al., 2013; Raven & Handley, 1987; Rico et al., 2013; Schwabe et al., 2013; Thuesombat et al., 2014; Wang et al., 2012a, 2014). Drought-tolerant plants possess a strongly sectored xylem (Choat et al., 2008), and tighter subcellular barriers (refer the “Cell-level nanoparticle interactions” section; Fahn, 1982). In line with this, the vast majority of drought-tolerant plants was more resistant to NP uptake than less drought-tolerant plants (Birbaum et al., 2010; Ma et al., 2013; Rico et al., 2013; Schwabe et al., 2013; Thuesombat et al., 2014; Wang et al., 2012a, 2014).

Phloem NP transport was rarely investigated and is challenging to verify due to other electron dense organelles and granules present in the cytoplasm of the living cells. Initial studies demonstrated leaf-root translocation of insoluble NPs to a limited extent, and NPs were found in tomatoes and other non-vascular tissues (Larue et al., 2014b; Wang et al., 2012a; Zhang et al., 2011), indicating phloem transport (Hong et al., 2014). The mechanisms of NP transport in phloem are important for NP transport into fruits and seeds (Sokolowska & Sowiński, 2013).

In some >20 angiosperm plant families, such as lettuce, poppy or hemp, specialized cells containing latex (laticifers), may transport NPs (Hagel et al., 2008). Very little is known about the role of these veins in NP transport.

The Caspian strip

The Caspian strip, or Caspian band, is a layer of interstitial cell walls sealed by lipophilic hydrocarbons (lignin and suberin) located in between cells of the endodermis (Figures 2 and 5; Fahn, 1982; Sattelmacher & Horst, 2007). Forming a mostly water impermeable barrier in the apoplast between the cortex and the vasculature, the Caspian strip is the last barrier for apoplastically travelling NPs before uptake into the vascular system. NP accumulation along the Caspian strip has been demonstrated (Sattelmacher & Horst, 2007; Sun et al., 2014; Zhao et al., 2012a). Suberin lamellae, which can coat the cell wall surface of the endodermis facing the vasculature, and probably Si (Ma & Yamaji, 2006), further fortify the Caspian strip (Sattelmacher & Horst, 2007). Due to this barrier, most solutes crossing the endodermis are forced to pass the cytoplasm of the endodermal cells.

Numerous observations of NPs present in the vasculature demonstrate that the Caspian strip is often circumvented (Al-Salim et al., 2011; Asli & Neumann, 2009; Cifuentes et al., 2010; Corredor et al., 2009; De La Torre-Roche et al., 2013; Ghafariyan et al., 2013; Huang et al., 2011; Hu et al., 2010; Larue et al., 2012a; Whiteside et al., 2009; Zhai et al., 2014). Also, the suberin contents associated with a tighter Caspian strip, e.g. in rice and maize, are not correlated with the different NP uptake rates in these plants (Birbaum et al., 2010; Rico et al., 2013; Sattelmacher & Horst, 2007). One explanation for this is that at least some plants, to facilitate transport for nutrients, take up most nutrients in the outermost regions of the root epidermis and the outer cortex into the symplast (Sattelmacher & Horst, 2007), as illustrated in a study on uptake of <1 nm [Mo6Br14]2– clusters (Aubert et al., 2012). Further, the Caspian strip contains permeable regions in so-called passage cells in the endodermis (Fahn, 1982; Luttge, 1971;
Yamaji & Ma, 2014). Additional leaking occurs in young roots where the Casparian strip is absent (Sattelmacher & Horst, 2007; Zhang et al., 2011). These findings relativize the effectiveness of the Casparian strip as a barrier for NP uptake, and underscore the importance of other NP barriers located in the rhizosphere such as microorganisms, mucilage, the cuticle, the cell wall and root hairs.

**Pit membranes**

Many observations of root–leaf NP translocation and of NPs in the vasculature (Cifuentes et al., 2010; Corredor et al., 2009; Jansen et al., 2009; Lin et al., 2009; Sun et al., 2014; Zhai et al., 2014; Zhao et al., 2014; Zhu et al., 2008) show that NPs can translocate through pit membranes (Figure 5). Electron microscopy showed that the microfibril-layered membranes of pits of different species are variable in thickness (70–1892 nm), average pore diameter (10–225 nm, all data from angiosperms) and architecture (Jansen et al., 2009). Plants possessing wide pores in the pit membranes are conifers (up to 200 nm; Liese & Johann, 1954), ferns and vessel-less angiosperms (Choat et al., 2008). The average pit membrane porosity determined using particle suspensions was 10–200 nm for gymnosperms (conifers; Liese & Johann, 1954), and only <5 nm for angiosperms. The variability in angiosperms is high, and pit membrane porosities of 5–100 nm (max. 420 nm) have been measured (Choat et al., 2004, 2008; Jansen et al., 2009).

To move from the root to the leaves, water – and NPs moving along with water – must cross many thousands of pits before they reach the leaves (Choat et al., 2008). Macroporous pit membranes render plants vulnerable for the spread of pathogens (Choat et al., 2008; Jansen et al., 2009), and given their permeability to micro- and nanoparticles (Choat et al., 2004; Liese & Johann, 1954), the same may apply for NPs. Tall plants have to cope with high pressure differences, reflected by an highly negative hydraulic potential in the xylem and resulting in smaller pores in the pit membranes especially in the upper parts of the plants (Choat et al., 2008). Experiments on tall-growing plants like maize and poplar trees indeed showed no or very little NP translocation (Birbaum et al., 2010; Wang et al., 2014; Zhao et al., 2012b). This indicates that low pit membrane porosity (tallness), and high segmentations of the xylem (drought-tolerance) can limit the extent of NP translocation (Thuesombat et al., 2014; Wang et al., 2014), which however has to be verified under more controlled conditions.

**Nodes**

Graminaceous plants (i.e. Poaceae, true grasses) have a specialized vascular system in the nodes to strongly regulate nutrient transportation into leaves and seeds (Yamaji & Ma, 2014). Many angiosperms possess nodes, especially in the dicotyledons (Fahn, 1982). The role of nodes for NP uptake remains to be elucidated. Comparative studies of NP uptake or effects in plants with/without nodes usually report less or absent NP transport/effects in the latter (Jacob et al., 2013; Ma et al., 2013; Pokhrel & Dubey, 2013; Schwabe et al., 2013).

**Vascular and non-vascular transport processes**

**Apoplastic versus symplastic transport**

Nanoparticles crossing the cuticle and pectins, or entering through one of the pathways shown in Figures 2 and 5 eventually reach the cell wall. Depending on size and charge, the NPs can translocate from there via the apoplastic pathway, i.e. through the extracellular spaces such as cell walls and longitudinal channels in the cell walls, but also middle lamellae, intercellular spaces and the xylem as described in the previous sections and Figure 5 (Geisler-Lee et al., 2013; Sattelmacher & Horst, 2007). The apoplastic also serves as a storage to certain minerals (Si, Fe, Ca; Sattelmacher & Horst, 2007) and can accumulate NPs (Ma et al., 2011; Zhang et al., 2012b). Longitudinal channels in the cell walls of diameters of <30 nm (anticlinal cell walls), and <40 nm (epidermal cell walls) were directly observed using TEM as early as 1964 to facilitate the axial apoplastic NP transport (Chen et al., 2010; Gaff et al., 1964). The symplastic pathway (Figures 4 and 5), on the other hand, is relevant for the distribution of photosynthates produced in mature leaves across the plant, and for long distance transport in some mosses and macroalgae (Lee & Lu, 2011; Raven, 2003). Symplastic transport requires the NPs to cross the cell membrane via fluid-phase endocytic, or non-endocytic pathways (refer the “Uptake across the cell membrane” section) and move from cell to cell through plasmodesmata (refer the “Plasmodesmata” section), sieve plates or primary pit fields as mentioned above (Lee & Lu, 2011; Zangi & Filella, 2012). The apoplastic pathway, i.e. the passage outside of cell membranes, is therefore considered to be the uptake pathway of lowest resistance (Raven, 2003; Sattelmacher & Horst, 2007).

The available literature provides no definite answer whether NPs prefer transport through the apoplast or symplast. However, to date, most data support transport through the apoplast. First, despite all efforts to visualize symplastic NP translocation in plant cells, the currently largest directly observed insoluble NPs clearly blocking plasmodesmata had a diameter of only ~15 nm (Figure 4; refer the “Plasmodesmata” section; Zhai et al., 2014). Nano-TiO2 and China ink particles commonly used to block the water flux in the apoplast have a much larger diameter of ~30–50 nm (Asli & Neumann, 2009; Sattelmacher & Horst, 2007). This in agreement with the 36 nm that were recently found to be the first nano-TiO2 particle diameter, above 25 nm, not translocating in wheat (Larue et al., 2012a). These sizes are about one-third of the size of the abovementioned longitudinal channels in the cell walls (Chen et al., 2010; Gaff et al., 1964), demonstrating a permeability of these or other channels in the apoplast for NPs of <36–50 nm. Second, NPs of all kinds, hydrophilic, lipophilic, regardless of charge and even those as small as 1 nm, were mainly found in the intercellular space, cell walls or middle lamellae of roots and the xylem, i.e. in the apoplast (Aubert et al., 2012; Corredor et al., 2009; Geisler-Lee et al., 2013; Hu et al., 2010; Lee et al., 2008; Li et al., 2014; Ma et al., 2011; Sun et al., 2014; Whiteside et al., 2009; Zhai et al., 2014). In the leaves, NPs were detected both in the apoplast and symplast (Corredor et al., 2009; Gaff et al., 1964; Ma et al., 2011; Larue et al., 2014b; Li et al., 2014; Sun et al., 2014). The leaves are the sites where solutes transported via the xylem are translocated back into the symplast. In some cases, NPs were also shown to accumulate above, or in between the anticlinal cell walls of leaves (Hu et al., 2010; Hussain et al., 2013; Kole et al., 2013; Nadiminti et al., 2013). Thus, a growing body of evidence shows that NPs prefer to translocate through the apoplast. Micro- or nano-analysis should address whether these particles move or are immobile (transport or storage), and whether they consist of NPs, re-precipitated NP ions or other small particles (refer the “Microalgae and exudates” section).

**Uptake rates**

Low NP translocation in drought-tolerant plant species with low water transpiration rates, and vice versa (Birbaum et al., 2010; Ma et al., 2013; Rico et al., 2013; Schwabe et al., 2013; Thuesombat et al., 2014; Wang et al., 2012a, 2014), shows that NP uptake rates correlate with the transpiration in vascular plants. In line with this, blocking the water flux in roots using Hg2+...
reduced the uptake of QDs (Hu et al., 2010), and as outlined above, NPs were found to move along with the transpiratory water (Al-Salim et al., 2011; Asli & Neumann, 2009; Cifuentes et al., 2010; Corredor et al., 2009; Ghafooryan et al., 2013; Hu et al., 2010; Huang et al., 2011; Zhai et al., 2014). Transpiration is proportional to the mass transport of water into the plant, and roughly proportional to the area of leaves in the plants (Ma et al., 2013; Thuesombat et al., 2014; Wang et al., 2014). Uptake rates for insoluble NPs into plants are currently not directly available.

For soluble NPs such as QDs, nano-Ag, nano-ZnO, nano-SiO2, nano-Pd, nano-Rh and nano-Pt, uptake and effects have often been shown, or suspected, to be governed by the uptake of dissolved ions (refer the "Mucilage and exudates" section; Battke et al., 2008; Colman et al., 2014; Ek et al., 2004; Handy et al., 2008b; Lv et al., 2015; Petersen et al., 2014; Schaller et al., 2013; Vannini et al., 2014; Wang et al., 2013b,d), especially for nano-ZnO, which dissolves within minutes (Jiang & Hsu-Kim, 2014; Petersen et al., 2014). Removal, uptake or excretion of soluble NPs from various plant-containing systems followed first-order kinetics (Colman et al., 2014; Wang et al., 2013d). Explanations on the basic reaction kinetics are provided in the "Details on uptake/elimination rate equations" section in Supplementary Information, and in Supplemental Equations (S1)–(S3). A back-of-the-envelope calculation (uptake concentration divided by duration of exposure) of the uptake rates into roots using selected literature data resulted in values ranging from 1.00 to 0.57 mg kg\(^{-1}\) d\(^{-1}\) for nano-Ag in a size range of 20–150 nm (rice) (Thuesombat et al., 2014), to 6.0 and 164 mg kg\(^{-1}\) d\(^{-1}\) for 4 nm QD long term uptake in poplar for negatively and positively charged NPs, respectively (Wang et al., 2014). These values for soluble NPs have to be interpreted with due caution because disentangling the uptake of soluble NPs from the uptake of their ionic counterparts is extremely difficult (Colman et al., 2014; Larue et al., 2014a; Petersen et al., 2014).

Uptake rates for insoluble NPs, and into shoots, are sometimes several orders of magnitude lower, e.g. 3.8, 3.7 and 0.83 µg kg\(^{-1}\) d\(^{-1}\) for 15, 25 and 50 nm nano-Au, respectively (poplar; Zhai et al., 2014). Schwabe et al. (2013) reported 0 (zero) and 2.2 mg kg\(^{-1}\) d\(^{-1}\) for 17–100 nm nano-CeO\(_2\) in wheat and pumpkin (Cucurbita maxima), respectively. In rice, concentrations corresponding to nano-CeO\(_2\) uptake rates between 3.7 and 10 mg kg\(^{-1}\) d\(^{-1}\) were measured in an exposure concentration range of 62.5–500 mg L\(^{-1}\) (Rico et al., 2013). Nano-CeO\(_2\) uptake rates started to level off at 10 mg kg\(^{-1}\) d\(^{-1}\). Concentrations corresponding to fairly high uptake rates for 25 nm nano-CeO\(_2\) were also found for tomatoes (7.1 mg kg\(^{-1}\) d\(^{-1}\); Wang et al., 2012a). Again, these calculated uptake rates are rough estimates, and placeholders until the results of more systematic experiments are available.

Unlike the symplastic pathway, the apoplastic pathway is non-selective, because it does not require endocytosis or passage through plasmodesmata. Instead, the apoplast is continuous with the cell wall (Supplemental Figure S1) using Equation (1) applying up to an exposure concentration of 250 mg L\(^{-1}\) and resulted in a value for T × TSCF of 0.39 L kg\(^{-1}\) d\(^{-1}\) (Rico et al., 2013) showed that Equation (1) applies up to an exposure concentration of 0.039 L kg\(^{-1}\) d\(^{-1}\) (\(R^2 = 0.76\)), T was not available). However, the \(R^2\) increased to 0.99 and the value for T × TSCF changed to 0.028 L kg\(^{-1}\) d\(^{-1}\) if the regression was not forced through zero, indicating that a different process, e.g. increasing occlusion of the vascular system, occurred in parallel. At 500 mg L\(^{-1}\), severe effects were observed in the plant and the uptake rates, as expected, increased no longer linearly with the concentration. Equation (1) and first-order uptake kinetics are only valid if NPs actually translocate in the plant. If NP uptake is dominated by adsorption on the plant surface, e.g. in leaves and in drought-tolerant tall plants, the process is limited by the total plant surface area and can be expected to follow classical sorption isotherms under equilibrium conditions, and second order, or pseudo-second order reaction kinetics (Supplemental Equation (S3) and the "Details on uptake/elimination rate equations" section in Supplementary Information; Schwab et al., 2014; Zhang et al., 2012a).

Conclusions and gap analysis

Although the uptake of NPs in plants received a lot of attention in the last 5 years, there is still relatively little solid information on the pathways of NPs into plants. Nevertheless, the new results on uptake and excretion barriers, pathways and processes outlined in the previous sections show that invaluable knowledge was gained in the qualitative description of NP uptake in plants. While the following gap analysis is far from totally comprehensive, some urgent scientific challenges were included which must be addressed along with typical issues on NP uptake and translocation in plants.

Understudied or overlooked uptake processes

It is obvious from the above discussion of NP uptake processes that much research is needed on the pathways of NPs into plants. To know where in the plant NPs will accumulate, and which plant species are most exposed, it is essential to find out the major pathways NPs use to enter and leave the plants, and how these pathways differ in different plants. The size of the pathway is thereby not proportional to its importance: the tiny, invisible root hairs, for instance or mycorrhiza and rhizobia, have received almost no attention despite their pivotal role for the water and...
nutrient uptake into most plants. An important next task is therefore to scrutinize all possible pathways regardless of size or topicality for NP uptake. Further, current research indicates that the most size limiting of the subcellular barriers is the cell wall. It is important to realize that NPs pass the cell wall more easily than previously thought. Due to this, the size exclusion limits of the cell walls in roots and the apoplastic, responsible for root–shoot translocation, are to date one of the major factors of uncertainty for NP translocation. Its interspecies variability is $>2$ orders of magnitude based on the variability of deposited suberin, or the variability of the pit pore size. Tests aiming at quantifying uptake using different NPs sizes, coatings and plant species, should not only focus on quantification of NP uptake, but also on analysis of apoplastic micro-architecture, and cell wall components such as pectins, suberin, lignin and Si. Such experiments, on a broad range of species, supported by micro- or nano-analytical techniques such as XRF or TEM-EDS, will shed light on NP uptake. Knowledge gained on the NP uptake processes in plants opens new fields for the application of nanotechnology in agriculture – under reserve that the nanomaterials used are easy to biodegrade and less toxic than the conventional pesticide they replace. Nanoparticles adhering to specific plant surfaces, entering through barriers at the cellular level, or through plant loopholes, translocating to their optimal site of action and decomposing after they are no longer needed could revolutionize agriculture and replace more toxic conventional pesticides, seed coatings and fertilizers. With this and the general increase of human and environmental exposure to NPs in mind, NP uptake studies should also provide more information directly related to consumer safety: NP transport into fruits and seeds (e.g. apples, citrus fruits, wheat, rice, millet and soybean), root tubers (e.g. potatoes, cassava) and leaves (e.g. spinach, herbs, lettuce).

**Uptake and translocation rates (in soil)**

A lot of studies are currently devoted to disentangling ion uptake from soluble NP uptake. This basic information on NP uptake is needed, but not sufficient to allow for risk assessment of NPs in plants. What is also needed is the uptake kinetics that NP obey when passing the apoplastic or the symplastic pathway. Such a rate law will make the data of different studies comparable to each other. In the ‘Vascular and non-vascular transport processes’ section, we described a rate law for NPs adapted from well-established models for apoplastic transport of metal complexes and pesticides. The rate law was applicable to NP uptake kinetics of recent studies. Experimental data for different species, exposure times and concentrations will improve the robustness of this, and many other models. Such studies are particularly useful for risk assessment if they are conducted in soil and at low, environmentally relevant exposure concentrations. Nanoparticle uptake differs significantly in non-soil and soil systems, and can be higher at chronically than at acutely toxic concentrations.

**Defense mechanisms**

Given all the NPs in environment, the many exposure routes and the considerable potential of NPs to accumulate in plants, there are not very many examples of toxicity (Gardea-Torresdey et al., 2014; Jackson et al., 2013; Ma et al., 2010a; Miralles et al., 2012a). Hormesis was observed in the presence of NPs, i.e. stimulation due to activation of repair mechanisms at low doses (Calabrese, 2005). For instance, growth and transpiration increased at low doses of nano-Ag (Wang et al., 2013a). This lack of toxicity despite uptake indicates that plants have evolved defense mechanisms to handle natural occurring NPs a long time before they were exposed to engineered NPs. Research on such defense mechanisms is still in its infancy.

**Wanted reporting data**

Specifically for plant uptake studies, the usefulness and comparability of experimental results can be invaluable enhanced if along with the gained knowledge, these few more parameters are reported:

- Plant genus and species (Latin).
- Shape and primary NP size with standard deviation, optimally accompanied by a histogram and with the hydrodynamic diameter in pore water or growth medium.
- Chemical composition of the coating and the surfactant, and surface charge of the NPs, optimally measured in hydroponic suspension or pore water.
- Numerical values (averages and 95% confidence intervals, with SI units) of all measured tissue and exposure concentrations, weights of plant parts, root surfaces, transpiration or water consumption of the plant. If a soluble NP is tested: solubility of the NPs, optimally in growth medium in the presence of plants.

**Plant–soil interactions**

Not only bioaccumulation but also the strong influence of plants on the soil structure or vice versa can change the way NPs are transported. Most of the upland area of our planet is covered by vegetation, which produces natural organic matter, exudates and mineral phases of tremendous diversity. Understanding how this affects NP transport and fate is crucial for a realistic NP risk assessment. Further, NPs can facilitate the transport of nutrients (P), and organic molecules into plants or phytoplankton, or immobilize heavy metals in the roots, all interesting features with a potential to improve efficiency in agriculture, e.g. by nanofertilizers, nano-pesticides, nanoparticulate seed coatings or by new methods to reduce the heavy metal burden of edible parts in food crops.

**Validated analytics and visualization**

It is challenging to validate analytical quantification methods when certified standard reference materials are missing. Affordable international standard reference materials (soil, sediment, water, plant tissue such as flour and leaves), and isotopically labeled standards are needed at least for the most relevant insoluble ENMs such as nano-Au, nano-TiO$_2$, nano-CeO$_2$, Fullerences, CNTs and graphene. Further, validated washing procedures are needed for the individual NPs to allow reliable differentiation between adsorbed (surface bound) and absorbed (internalized) NPs in plant tissue. Finally, visualization techniques require the same rigorous quality control as classical analytical techniques. False positives or negatives can arise from techniques relying on identifying an analyte (the NP) in a complex matrix based on a single criterion (wavelength range, electron-opaqueness). Most of these problems can be solved by additional control experiments, and by identifying the investigated NPs by a second or third specific characteristic. Another option is to cross-validate the result by one of the emerging visualization techniques possessing spectral, and/or three-dimensional resolution. Characterizing NP uptake at non-toxic, environmentally relevant conditions will push the limits of these analytical tools. Doing so is essential to forecast NPs accumulation in plants, the implications for food webs and eventually, to perform realistic assessments of the risks of nanotechnology for today and the future.

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Supplementary material available online
Supplementary Table S1, Figures S1–S2 and Equations S1–S3.