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Use of Nanoparticles to Study and Manipulate Plant cells**

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Fluorescence microscopy has developed into a key technology of the postgenomic era in biology, because it combines structural information with molecular specificity. However, the resolution of this approach is limited by bleaching and optical cross-reference of the fluorescent labels. Fluorescent semiconductor quantum dots (QDs) provide excellent bleaching stability and tunable emission spectra, and therefore would be an excellent alternative to overcome these limitations. However, to apply them to cell biology, three challenges have to be met: bioconjugation to molecular probes that confer the specificity of the label, passage through the external barriers of the cell, and suppression of toxic side effects of the nanoparticles. In plant cells that are ensheathed by a cellulosic cell wall, these challenges are especially prominent. Moreover, plants are located at the start of the food chain and thus of high relevance for the ecotoxicological assessment of nanomaterials. We have therefore explored the application of nanoparticles to plant cell biology. We have first evaluated different strategies to visualize microtubules by QDs in vitro and in cellula. By using silica-coated QDs coupled to anti-tubulin antibodies we were able to image microtubules in tobacco BY-2 cells by direct immunofluorescence making use of the superior bleaching stability of the nanoparticle label. To adapt this tool for in vivo imaging, we have successfully employed Trojan Peptoids as vehicles into living tobacco cells. We want to extend this strategy not only to use functionalized nanoparticles for life-cell imaging, but also to adapt them as tool to manipulate intracellular architecture.

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[**] *Abbreviations: BY-2 Nicotiana tabacum L. cv. Bright Yellow 2, CPP cell-permeating peptide, FITC fluorescein isothiocyanate, QDs Fluorescent semiconductor quantum dots*

Biology has entered the so-called postgenomic era, where the assignment of gene to function has become a central task. By the use of reverse genetics, expressed sequences of unknown function are analyzed with respect to phenotypes that occur upon overexpression or suppression of the corresponding gene products, and with respect to their intracellular localization. In the past, light and electron microscopy had focussed on the visualization of cellular structures. Recently, microscopy has adopted a new, enormous impact as central technique for so-called functional genomics. Here, in addition to the visualization of structures, reliable information about the molecular nature of these structures is crucial. This requirement has stimulated an impressive development of fluorescence microscopy that has evolved from a fairly exotic technique used by only a handful of specialists into a central tool of molecular biology. There are basically three motive forces that drive this development: in vivo imaging, spatial (and temporal) resolution, and molecular specificity. To achieve these goals, the technological progress on the side of microscopical devices has to be complemented by advances on the side of the fluorescent label, and it is at this point, where fluorescent semiconductor quantum dots (QDs), mostly based on CdSe have stimulated the interest of cell biologists.

In fact, QDs have already been successfully employed for biological approaches. For instance, derivatized QDs could be targeted to cancer cells[1,2] and to the surface of unicellular pathogens.[3] During in vitro studies, the potential of QDs for spatiotemporal resolution has been demonstrated by single molecule tracking.[4] The high bleaching resistance of the QDs made it possible to visualize the hand-over-hand processivity of myosin V during in vitro sliding assays[5] or to achieve routine two-color super-resolution imaging and single-molecule detection with standard fluorescence microscopes and inexpensive digital color cameras.[6] However, so far, the application of nanoparticles for cell biological questions has been rather limited. This is basically caused by three limitations that are still far from being solved:

(i) All cells are surrounded by a lipid bilayer, the plasma membrane, that is only permeable for very small molecules such as water or ethanol. In several organisms, such as bacteria, fungi, and plants, the plasma membrane is ensheathed by a so-called cell wall that is made up as a composite material, i.e. of fibers that are embedded into an amorphous matrix.
(ii) The compounds of QDs, such as cadmium are frequently highly toxic, i.e. they either elicit adaptive defence responses of the target cell or even kill the cell.
(iii) Nanoparticles by themselves do not confer specific binding to biological targets, which is a prerequisite for their use in a biological context. This means that the particles have to be coupled to biomolecules that harbor such specificities. The functionality and the specificity of these biomolecules must not be impaired by their conjugation to the (mostly much larger) nanoparticles.

We have addressed these challenges choosing plant cells for the following reasons: First, they possess a cellulosic cell wall as additional barrier outside the plasma membrane – any technique that works in plant cells can therefore easily be adapted to animal cells. Second, plant cells are endowed with a tremendous developmental flexibility that has possibly evolved in response to their sessile lifestyle (animals run away, plants adapt). Plant cells are therefore more robust in terms of stress-tolerance and are therefore expected to compensate potential stresses caused by nanoparticle uptake much better than other cell systems, e.g. the highly sensitive mammalian cells. However, the specific toxicity of a particle does not only depend on the physiology of the target cell, but also on the physicochemical properties of the particle itself. For instance, functionalized fullerene particles are less toxic to mammalian cells than pristine fullerenes[7,8] or hydrophilic particles that were less toxic in mammalian cells as compared to hydrophobic particles turned out to be more toxic, when administered to plant cells.[9] Third, plants are located at the start of the food chain – any ecotoxicological evaluation of nanomaterials must therefore take into account the mechanisms of uptake into and the accumulation in plant cells.

As model system, we have used cell cultures of tobacco (*Nicotiana tabacum* L. cv. Bright Yellow 2, ''BY-2'') that can be maintained in liquid culture. In our previous work we have shown that BY-2 cells represent a minimal morphogenetic system for plant organization. These cell cultures can divide after addition of the plant hormone auxin and develop into cell files consisting of four to ten individual cells. The divisions within a file do not occur randomly, but are coordinated. We could show that polar auxin transport mediates and synchronizes the divisions within a cell file that thus behaves as a supracellular entity. This synchrony is based upon a positive feedback between auxin, actin organization, and cell polarity.[10,11] Thus, the BY-2 culture represents a very simple multicellular plant organism, where we can study, how individual cells are coordinated into an entity – a basic question of developmental biology. Most importantly, there exists a broad panel of fluorescently tagged marker lines for BY-2, where different components of the cytoskeleton, but also a broad range of other subcellular structures are labeled by fusion with fluorescent proteins. Thus, at the present stage, BY-2 is the system par excellence to perform plant cell biology.

1. Specificity: Bioconjugation to Microtubules as Case Study

The application of QDs for cell biology is limited by their cytotoxicity, their large size, and the need to stabilize those nanoparticles colloidally in the aqueous environment typical for biological applications. Moreover, the particles have to be conjugated to a biomolecule harboring specific binding to the cellular target. We have employed two strategies to coat and bioconjugate QDs.

(i) Coating with BSA through ligand exchange.[12] This approach provides a better electrostatic colloidal stabilization of the nanoparticles in water and is more versatile with respect to active groups for the covalent coupling of proteins. Moreover, the resulting particles are smaller in diameter.

Briefly, CdSe/ZnS nanoparticles in chloroform were supplemented with methanol and sedimented by centrifugation. Subsequently, DMSO and 2-mercaptoethanol (ME) were added and the particles were heated and reprecipitated again by acetone. These hydrophilic particles were then suspended in bovine serum albumine in a Tris–glycine buffer, purified by agarose electrophoresis, dialyzed against PBS, and coupled with the target antibodies by a standard protocol based on *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC). (ii) Coating with silica shells.[12,13] The silanol groups on the surface decrease hydrophobicity and the tendency to agglomerate in aqueous environment. It is possible to introduce specific surface functionalities by modification of surface hydroxyls with amines, thiols, carboxyls, or methacrylate. Briefly, the luminescent CdSe/ZnS core-shell nanoparticles were encapsulated in silica using a water-in-oil microemulsion method. The particles in chloroform were supplemented with cyclohexane, NP-5 as surfactant, and tetraethyl orthosilicate as precursor for silica formation. By vigorous stirring a microemulsion formed, such that upon addition of an aqueous ammonia solution the encapsulation process was triggered and proceeded for 24 h at room temperature. Subsequently, the nanoparticles were precipitated from the microemulsion using acetone and washed in sequence with butanol, propanol, ethanol, and water to remove any surfactant and unreacted educts. Depending on the application, we found that both strategies have their specific advantages or disadvantages.

To date, there is no general solution for coupling biomolecules to nanoparticles under preservation of their biological activity. To assess the feasibility of the two bioconjugation strategies, we used two assays: (i) Self organization of microtubules in vitro. Microtubules assemble from tubulin dimers by self-organization after addition of GTP, magnesium ions, and warming up to 30–40 °C. The formation of microtubules can thus be used as indicator whether the bioconjugation has impaired the functionality of the coupled protein. (ii) Specificity *in cellula*. Microtubules are a central element of the cytoskeleton. In interphasic plant cells, they are organized in characteristic parallel bundles that are rapidly replaced by distinctly different microtubule structures once the cell undergoes cell division.[14] By using monoclonal antibodies against tubulin, and fluorescently labeled polyclonal secondary antibodies raised against the anti-tubulin antibody, it is possible to visualize those microtubular structures. Up to 50 secondary antibodies bind to one anti-tubulin antibody such that the signal is amplified by this so-called indirect immunofluorescence sufficiently to be visualized by fluorescence microscopy. By conjugation of the QDs to the anti-tubulin antibody it should be possible to visualize those microtubule structures directly, without the help of the secondary antibodies. If the specific microtubule structures become visible, this indicates not only that the antibody has maintained its specificity despite conjugation to the QDs, but also that the intensity of the QD-label is sufficient to be detected even without amplification by a secondary antibody.

2. Ligand Exchange

BSA-coated QDs were synthetized as described previously.[12] CdSe/ZnS or InP/ZnS nanoparticles were first rendered hydrophilic by replacing chloroform by DMSO and 2-ME and then suspended in bovine serum albumine in a Tris–glycine buffer and then purified by agarose electrophoresis, followed by dialysis. These BSA-coated particles were then coupled with purified neurotubulin using standard coupling *N*-(3-dimethylaminopropyl)-*N*′-EDC. Alternatively, the particles were conjugated to a monoclonal antibody against α-tubulin.

Microtubules were successfully self-assembled from the QD-conjugated tubulin (Fig. 1(A)), but these microtubules were accompanied by numerous agglomerations and misshaped microtubules, which was especially pronounced in the InP-conjugates. These agglomerations were probably caused by tubulin that was functionally impaired and thus did not undergo efficient assembly. We therefore designed an alternative strategy, where tubulin dimers were assembled in the presence of QD-conjugated anti-tubulin antibodies (Fig. 1(B)). In this approach, the agglomerations were reduced, and the microtubules were of normal structure (Fig. 1(C)). Although the binding of the antibody to its epitope was obviously preserved, the labeling of microtubules was not continuous, indicating that still a considerable proportion of the antibody had not been efficiently conjugated.

In the next step, the CdSe-QD-antibody conjugates were used to follow the dynamic reorganization of microtubules

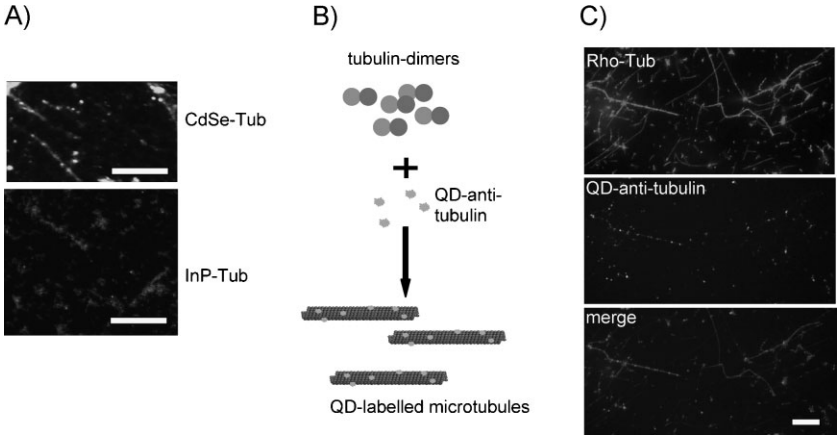

Fig. 1. Test for the functionality of QD-bioconjugates obtained by the ligand-exchange approach. (A) Microtubules assembled from either CdSe- or from InP-conjugate tubulin. (B) Alternative strategy, where the QDs are conjugated to anti-tubulin antibodies. (C) Microtubules assembled from rhodamin-labeled tubulin in the presence of CdSe-QD-anti-tubulin antibodies. Upper image: rhodamin-signal, central image: QD-anti-tubulin signal, lower image: merge of both signals showing that the microtubules are decorated with the QD-conjugated antibody. Size bars 10 μm.

through the cell cycle of a tobacco cell culture.[12] The QDs visualized fluorescent dots that decorated the various arrays of microtubules. The specificity of the antibody was maintained after conjugation with the nanocrystals, and the antibodies correctly represented the dynamics of cell-cycle-dependent microtubular reorganization. However, this approach did not yield a contiguous signal. These findings were consistent with those from the in vitro experiment. The ligand-exchange approach, while maintaining the specificity and functionality of the bioconjugated molecule suffered from a relatively high background of unconjugated biomolecules. This means that additional purification steps are required to obtain completely coupled biomolecules.

3. Silica Shells

Silica-coated CdSe-QDs were synthetized and functionalized with amino side groups as described previously,[13] and either conjugated directly to tubulin or to anti-tubulin antibodies using standard protocols either based on cyanamide or EDC, respectively. Similar to the ligand-exchange coupling, the tubulin conjugated to the silica coated QDs could be assembled into microtubules in vitro,[13] again with a certain background of agglomerations and malformed microtubules. However, when the silica-coated QDs were conjugated to anti-tubulin antibodies and tested for their performance *in cellula*, they visualized contiguous microtubules[12] in the same pattern as found for conventional indirect immunofluorescence. Thus, silica-coated QDs can be used as labels for direct immunofluorescence in plant cells. Given the fact that up to 50 secondary antibodies bind to one primary antibody in the conventional indirect immunofluorescence, one has to conclude that the fluorescence intensity produced by an individual nanocrystal-conjugated antibody is very high. The use of indirect immunofluorescence is strongly limited in mulitplexing, where several signals have to be detected simultaneously. It is extremely difficult to safeguard against illegitimate cross-reaction of the secondary fluorescent antisera. Direct immunofluorescence would be a good alternative but has been limited by low signal strength because the signal amplification due to the labeled secondary antibody is lacking. We could demonstrate in our study that this limitation can be overcome by direct coupling of QDs to highly specific antisera.

4. Toxicity: Why to Take Care of Cadmium

Nanoparticles harbor their specific properties not only by their chemical nature, but also by their small size. This stimulated a public debate on their potential toxicity. This debate suffers from the difficulty to discriminate toxicity caused by the chemical nature of the material from the structural toxicity caused by the small dimensions of nanoparticles. Triggered by a report on the phytotoxicity of phenantrene-coated aluminum nanoparticles[15] that had obtained considerable attention also in the public, concerns on the adverse effects of nanoparticles on plants were raised. However, this conclusion was later shown to be inappropriate, because it ignored the chemical toxicity of aluminum.[16] It should be kept in mind that toxicity does not necessarily require actual uptake of nanomaterials, for the toxicity of fullerenes has been proposed to be caused by membrane damage through reactive oxygen species that are generated upon dissolving weakly functionalized fullerenes in water.[7]

Most QDs are based on CdSe-semiconductor nanocrystals. Depending on the tightness of the coating around these crystals, small amounts of Cd^{2+}-ions are expected to leak out. This is not further problematic during in vitro applications as long as the concentration of Cd^{2+} is not in the range of other bivalent cations such as Mg^{2+} that are required for the assembly of microtubules. It is also not problematic for direct immunofluorescence, because here fixed and permeabilized cells are used. The situation becomes different, when the QDs are used in the context of living cells, where potential effects on signaling have to be taken into account. Cytotoxicity of CdSe QDs has been shown to depend on the tightness of the coat around the nanoparticle core,[17] but also on their stability against aggregation.[18] In fact, the primary injury caused by Cd^{2+} ions appears to be caused by binding to sulfhydryl groups in critical mitochondrial molecules. The inactivation of these thiol groups results in a permeabilization of the mitochondrial membrane, breakdown of the potential, and the generation of reactive oxygen species that are central as triggers for apoptotic cell death[19].

We therefore studied the effect of Cd^{2+} ions in BY-2 cells and observed that cadmium induced massive cell death during all stages of the cell cycle.[20] However, both the progression and the forms of the cell death differed pronouncedly, depending on the phase of the cell cycle. Whereas application of cadmium during or immediately after mitosis (so-called M and G1 phases) was not accompanied by DNA cleavage, indicating a ''trivial'' non-programmed character of the death, we observed apoptosis-like programmed cell death induced by even low doses of cadmium during the doubling of DNA or prior to the onset of mitosis (so-called S and G2 phases). The biological significance of this finding might be connected with the need to preserve genetic integrity in dividing meristematic cells (mostly in S- or G2-phase), whereas suppression of programmed cell death response in differentiated cells (mostly in G1 phase) might help to avoid death of the whole plant, and thus enables the initiation of the recovery and adaptation processes.

These findings are consistent with toxicity studies in mammalian cells, where expression profiles of QD-treated cells were compared over controls.[21] Whereas genes related to oxidative stress were upregulated, several genes involved in the control of the cell cycle and the set-up of the mitotic spindle were found to be downregulated.

Thus, even small amounts of Cd^{2+} ions leaking through the shell and are still not causing acute toxicity are biologically relevant, because they are expected to interfere with the signaling machinery that is controlling programmed cell

death in both plant[20] and animal cells.[21] There are basically two options to circumvent this undesired side effect: either using nanocrystals based on less toxic compounds such as InP or ZrS or improving the tightness of the shell. Since silica is inert in most solvents, it is a good candidate to reduce leakage of toxic ions.

5. Toward In Vivo Applications: Trojan Peptoids as Vehicles

There have been reports of spontaneous uptake of carbon nanoparticles into plants that accumulate in the vasculature.[9] However, these particles remain in the apoplast, i.e. they apparently do not cross the membrane barrier. The only exception so far seem to be carbon nanotubes that have been shown to enter into living tobacco cells by endocytosis and are then dumped to the vacuole, but apparently do not reach the cytoplasm.[9] This is confirmed by studies on membrane passage of fullerenes showing that, although pristine C_{60} particles can readily pass the membrane, functionalized fullerenes were orders of magnitude slower.[8] In fact, a recent study monitoring the spread of gold nanoparticles into estuarine ecosystems showed dramatic accumulation in clams, but only residual representation in *Spartina alterniflora* that was used as representative for higher plants in those experiments.[22]

To extend the potential of nanoparticles from in vitro and in situ applications to plant cell biology in vivo, we have explored a couple of strategies to cross cell wall and plasma membrane of plant cells that is generally impermeable for molecules exceeding 20–30 Da. This might be achieved by physical approaches such as particle bombardment, microinjection with borosilicate needles, or nanopulse electrical discharge.[23] However, these approaches are either extremely cumbersome (microinjection), will target only to few cells (particle bombardment), or induce apoptotic cell death (nanopulse electrical discharge). Therefore, chemical approaches to achieve membrane passage are highly warranted.

Permeabilization with Triton-X 100 or dimethyl sulfoxide allowed to introduce nanocrystal-antibody conjugates into tobacco cells allowing for visualization of the various plant-microtubule arrays formed during the cell cycle.[12] However, this approach is not feasible for studies in living cells, because the viability of the cells is dramatically affected, which is aggravated by the considerable turgor pressure (5–10 Bar) exerted by the expanding vacuole upon the inner face of the permeabilized membrane.

Thus, a specific requirement for membrane permeabilization is that they have to discriminate between different cellular compartments. While the plasma membrane has to be rendered permeable, the vacuolar has to remain intact. This is critical, because leakage of the vacuolar membrane will release large amounts of acidic and often toxic compounds that will kill the target cell within minutes. However, the vacuolar membrane (the tonoplast) and the plasma membrane differ in charge, such that it should be possible to design cell-permeating peptides (CPP) that bind preferentially to the plasma membrane but not to the tonoplast. CPPs have been widely used as ''Trojan Horses'' to deliver various cargoes such as peptides, nucleotides, or other bioactive molecules into mammalian cells.[24] Due to their positive charge, they are expected to bind differentially to membranes depending on their charge. In fact, it was possible to develop a permeabilization protocol using the CPP alamethicin naturally produced by the fungus *Trichoderma viride* and producing helicoidal structures[25] (Fig. 2(A)). This protocol preserved the viability of BY-2 cells that were even found to resume division after alamethicin had been removed. The non-invasive nature of the treatment was also manifest by the rich and filigrane actin cytoskeleton that could be visualized in those cells by rhodamine-conjugated phalloidin (Fig. 2(B)). A simple and reliable quantitative assay to determine the pore sizes introduced by CPPs was developed. For alamethicin, the exclusion size of the pores was estimated to range between 4 and 10 kDa (Fig. 2(C)), which is in the range of the pores that can be induced by rigidification of the membrane using aldehyde cross-linking (data not shown) and still too small for most nanoparticle-protein conjugates. However, by extending the alamethicin backbone by spacers it should become possible to increase the size exclusion limits of the pores sufficiently to allow passage of nanoparticles.

The uptake of CPPs depends basically on their short size, high content of cationic residues, and a variable spacing between the charges, whereas the conformation of their backbone seems to be of minor importance.[26] Since CPPs are rapidly degraded by proteolytic enzymes, peptide mimetica with modified backbones have been explored as alternatives.

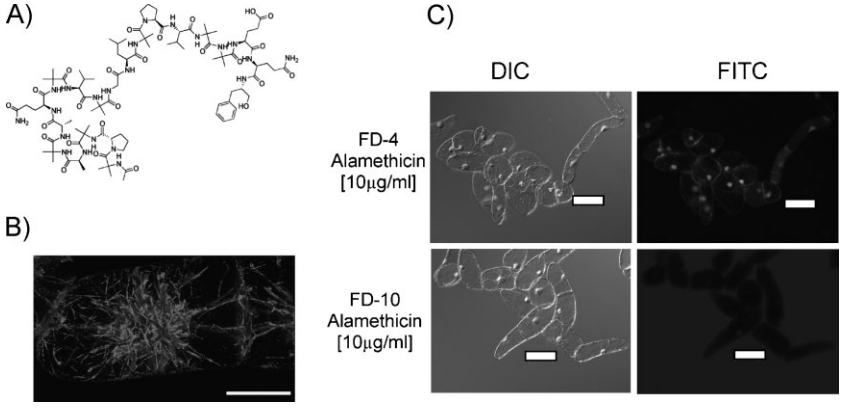

Fig. 2. Non-invasive permeabilization of the plasma membrane in BY-2 cells by alamethicin. (A) Structure of alamethicin. (B) Rich and filigrane actin network visualized by rhodamin-conjugated phalloidin in a cell that had been treated with 10 μg/ml alamethicin. Size bar 20 μm. (C) Uptake of dextranes conjugated to fluorescein isothiocyanate (FITC) into BY-2 cells upon incubation with alamethicin. Whereas a 4-kDa dextrane conjugated to (FD-4) can readily penetrate into the cell, a 10-kDa dextrane (FD-10) is excluded. Cells are shown in differential interference contrast (DIC) and in a fluorescent filter set-up detecting the FITC signal. Size bar 50 μm.

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Especially peptoids (oligo-*N*-alkylglycines) are very stable against proteases.[27] In peptoids the side chains are attached to the nitrogen atom instead of the carbon, such that the hydrogen-bonding is reduced. Therefore, in contrast to α and β-peptides that have also been used as CPP alternatives, peptoids do not aggregate. Peptoids have been used as successfully as molecular transporters for drug delivery into mammalian cells, because they are effective, water soluble, and nontoxic.[28] However, so far there has been no report on the use of Trojan Peptoids in plant cells.

We therefore investigated carrier-peptoids with or without guanidinium side-chains with regard to their uptake into plant cells, the cellular mechanism of uptake, and their intracellular localization[29].

We could show that, in contrast to polyamine peptoids (polylysine like), fluorescently labeled polyguanidine peptoids (polyarginine like) entered rapidly into tobacco BY-2 cells forming vesicular structures without affecting the viability of these cells. A quantitative comparison of this uptake with endocytosis of fluorescently labeled dextranes indicated that the main uptake of the guanidinium peptoids occurred between 30 and 60 min and thus clearly preceded endocytosis. Dual visualization with the endosomal marker FM4-64 showed that the intracellular guanidinium peptoid was distinct from endocytotic vesicles. Moreover, Wortmannin, an inhibitor of receptor-mediated endocytosis, blocked the uptake of fluorescent dextrane as test cargo, but did not impair the uptake of Trojan Peptoids.

Once the polyguanidine peptoids had entered the cell, they associated with actin filaments (Fig. 3) and microtubules. By pharmacological manipulation of the cytoskeleton we could demonstrate that this association with the cytoskeleton was necessary for uptake. Especially the actin inhibitor latrunculin B very efficiently impaired uptake and intracellular spread of the guanidium carrier.

This promising approach for membrane passage into walled, intact plant cells can now be extended by using Trojan Peptoids with larger cargoes (such as inhibitors, nanorods, proteins), but also photocleavable versions of Trojan Peptoids that will allow to unload the cargo in the cell upon irradiation.

6. Where to go

In addition to adapting QDs for plant cell biology, we pursue a second route for the application of nanoparticles. Plants lack the separation of immortal germ cells from mortal soma. All plant cells are therefore basically totipotent. In other words: plants consist of stem cells, which means that epigenetic control has a conspicuous impact. During recent years, dynamic changes of intracellular architecture have been identified as central element of plant development. This role of architectural changes for signaling has been uncovered mainly by advances in life imaging. The use of fluorescent jellyfish proteins as protein tags in combination with novel microscopical methods has allowed to follow the dynamic changes of specific proteins in the context of the living cell or even in the context of the intact organ. In order to analyze the biological function of these spatiotemporal changes, it is necessary to manipulate them. Genetical strategies such as overexpression or knock-down approaches allow to modulate the overall activity of the respective proteins. However, to understand the impact of architectural responses for signaling, it is necessary to manipulate, in addition, the spatiotemporal pattern of protein activity in subcellular dimensions.

The methodological repertory to control the spatiotemporal distribution of specific biomolecules of interest at the cellular and subcellular level is still to be extended. Especially in plant cells, where development is controlled by an innate directionality of individual cells, such a directional displacement would allow functional tests that are more stringent than mere genetic manipulation of overall protein activities. The methods to adapt QDs as a tool for plant-cell imaging should therefore be extended to other nanomaterials with specific physical properties (especially manipulation by magnetic fields). If for instance, microtubules could be rendered magnetic and aligned by a magnetic field, this would allow to control the axis of cell division (Fig. 4). In the case of actin, a similar alignment would allow to control the directionality of the ensuing organism (Fig. 4).

A second important goal of future work will be to target the Trojan carriers with their loaded cargo to specific sites of the

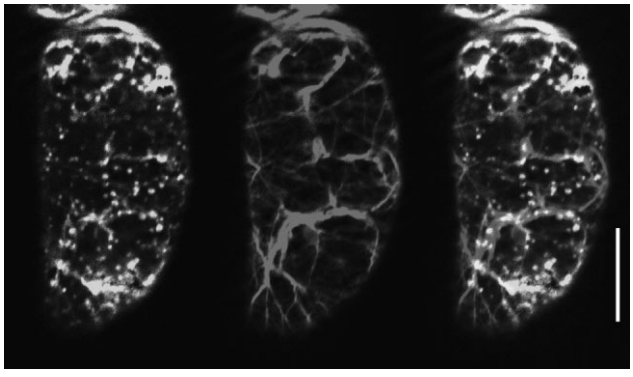

Fig. 3. Uptake of a polyguanidine Trojan Peptoid into tobacco BY-2 cells expressing the actin-binding domain of plant fimbrin in fusion with (RFP, center). The peptoid is conjugated to FITC and forms vesicular structures in the cell (left). The merge (right) shows the vesicles containing the peptoid decorate actin filaments. Size bar 20 μm.

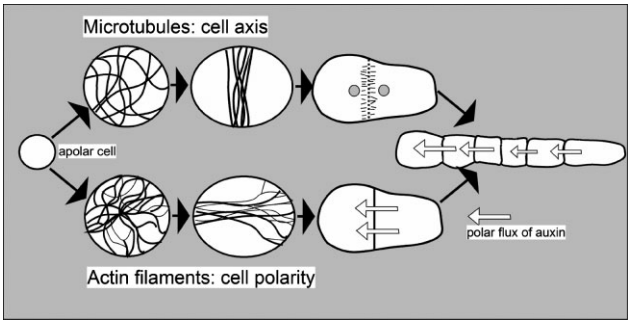

Fig. 4. Definition of axis and directionality by cytoskeletal architecture in plant cells that have been stripped of their innate structure by digestion of their cell wall. If magnetic nanoparticles would be targeted to microtubules and/to actin filaments it would be possible to control axis and directionality of the resulting cell file by external magnetic fields.

cell. This might either be achieved by modulating the charge or by fusion of specific peptides that are designed to mediate binding to the target structure of choice. The aim must be to use nanoparticles not only to watch, but also to manipulate cellular architecture.

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