Chapter 7

# Plant Nanobionics and Its Applications for Developing Plants with Improved Photosynthetic Capacity

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Additional information is available at the end of the chapter

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**Abstract**

In the present scenario, the ever-growing human population, a decreasing availability of land resources and loss of agricultural productivity are the major global concerns, and these possess a challenge for scientific community. To feed the increasing world population, an increase in the crop productivity with available land resources is one of the essential needs. Crop productivity can be increased by engineering the crop plants for tolerance against various environmental stresses and improving the yield attributes, especially photosynthetic efficiency. Nanomaterials have been developed with new functional properties like improved solar energy harvest. With these nanomaterials, nanobionic plants were developed by the facilitated kinetic trapping of nanomaterials within photosynthetic organelle, that is, chloroplast. The trapping of nanomaterials/nanotubes improved chloroplast carbon capture, that is, photosynthesis by improving chloroplast solar energy harnessing and electron transport rate. Besides improving photosynthesis, nanotubes like poly(acrylic acid) nanoceria (PAA-NC) and single-walled nanotube-nanoceria (SWNT-NC) decrease the amount of reactive oxygen species (ROS) inside extracted chloroplast and influence the sensing process in plants, and these are beneficial for a number of physiological processes. The nanobionic approach to engineer plant functions would lead to an era of plant research at the interface of nanotechnology and plant biology. In this chapter, nanobionic approach, transfer of nanomaterial to plants and their offspring and its potential applications to improve photosynthesis will be discussed.

**Keywords:** nanobionics, photosynthesis, productivity, stress, sustainability

## 1. Introduction

Nanotechnology is an emerging field of natural science dealing with materials of nano (1–100-nm) scale. NASA defined nanotechnology as 'the creation of functional materials,

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devices and systems through a control of matter on the nanometre scale and exploitation of novel phenomena and properties (physical, chemical, biological) at that length scale' [1]. The different applications of nanotechnology include the designing, characterization, production and application of structures, devices and systems. Nanomaterials (NMs) have unique properties like high surface area and improved optical property. For a chemical or a biological reaction, the rate of reaction depends on the surface area of the reactants, and due to the large surface area, nanomaterial-mediated reactions operate at a high rate. Plant biology is one of the oldest branches of science, aiming the study of different aspects of plants. The combination of plant biology and nanotechnology resulted in nanobionics which employs the nanotechnology for the improvement of plant productivity by improving plant growth, development and photosynthetic efficiency [2]. During the synthesis of materials at nanoscale, different properties of these materials get altered, and these altered properties get translated in various applications. Nanobionics is one of the important applications of nanotechnology which involves the improvement of plant or plant productivity using nanomaterials. The nanomaterial can be prepared by direct and synthetic route followed by milling, grinding, homogenization at high pressure and sonication to reduce its size at nanoscale [3, 4]. With unique physicochemical properties, that is, high surface area, high reactivity, tunable pore size, and particle morphology of nanoparticles, the nanomaterials have a large scope of novel application in the field of biotechnology and agricultural industries [5]. The nanomaterials are of different types:

*Natural nanomaterial*—Materials created independently without the involvement of human being. The natural nanomaterials are sea salt, sea spray, soil dust, volcanic dust, sulphates from biogenic gases, and so on.

*Anthropogenic (adventitious) nanomaterial*—Material created as a result of human action. The welding fume and particulates (sulphates and nitrates) resulting from the oxidation of gases [6], and soot resulting from the combustion of fossil fuels are the best example of anthropogenic nanomaterial.

*Engineered nanomaterial*—Nanomaterial designed and manufactured with human interest. The engineered nanomaterials are of organic and inorganic nature.

As the name indicates, the organic nanomaterials consist of carbon atom itself [7] and are polymeric structures with specific nano-characteristics, while inorganic nanomaterials are inorganic by nature. The engineered nanomaterials are of scientific interest because of their huge potential for different applications. The engineered nanomaterials are classified as carbon-based nanomaterials (NMs), metal-based NMs, metal oxides, dendrimers and composites [8]. The nanotubes are linear materials with nanometre size. Carbon nanotubes are long, thin cylinders of carbon molecules having good conductivity of heat, high strength and different electrical properties. The carbon nanotubes (**Figure 1**) are single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). The double-walled carbon nanotubes are known for higher thermal and chemical stability as compared to single-walled carbon nanotubes [9]. Inorganic nanomaterials are inorganic by nature and consist of metals and metalloid oxides, quantum dots (QD), dendrimers having different kinds of features such as nanofibres, nanowires and nanosheets.

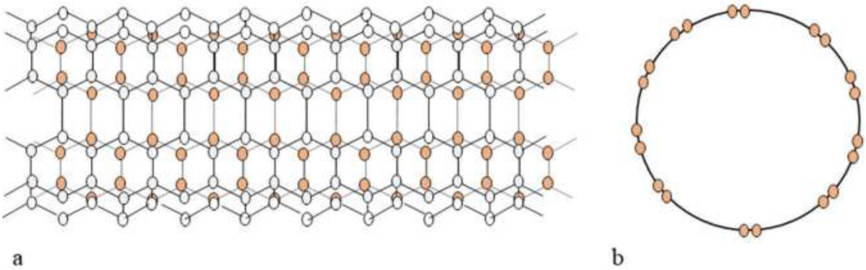


**Figure 1.** Single-walled carbon nanotube (a) and a cross section of single-walled carbon nanotube (b).

Nanobiotechnology is an emerging field of bioengineering and has enormous potential to modify or augment the plant function by employing the nanomaterial. With nanobiotechnological advancement, plants (1) are capable of imaging objects in their environment, (2) self-powering themselves as light sources, (3) with infrared communication devices and (4) having self-powered groundwater sensors developed [10]. The solar energy harnessing and biochemical sensing can be improved in plants by introducing nanomaterial in them [11], and nanobionic plants were developed for enhanced photosynthesis and biochemical sensing. Nanobionic plants can detect various chemicals present in the environment and have potential use as a plant-enabled sensor for monitoring environmental changes. The nano-encapsulated nutrients commonly referred to as nanofertilizers release the nutrients on demand basis, and thus these are beneficial for crops to regulate plant growth and enhance the target activity [12, 13]. The engineered carbon nanotubes are shown to boost seed germination, growth and development in plants [14, 15]. Comparatively, very few studies have been conducted on nanoparticles which are beneficial to plants. Nanotechnology has a great potential to develop new tools for the incorporation of nanoparticles into plants to augment the existing functions [16].

### 1.1. Entry of nanoparticles in plant cells

The characteristic feature of plant cell is its cellulosic surrounding, that is, cell wall. The plant cell wall behaves as a barrier for superficial ingression of different external agents including nanoparticles into plant cells. Cell wall possesses pores which provide sieving properties to cell walls, and this range from 5 to 20 nm [17]. Nanoparticles or aggregates of nanoparticles with a diameter less than the pore diameter of the cell wall could pass through pores and can reach the plasma membrane [18]. There is additionally a chance for the enlargement of pores or the induction of new cell wall pores upon interaction with engineered nanoparticles which in turn enhance nanoparticle uptake. Further internalization of nanoparticles or aggregates of nanoparticles occurs through endocytosis by forming a cavity-like structure surrounding the nanoparticles by a plasma membrane. Alternatively, they may cross the membrane via carrier proteins or through ion channels. In the cytoplasm, the nanoparticles may bind with different cytoplasmic organelles and interfere with the metabolic processes [19]. In leaf surface applied nanoparticles, the nanoparticles enter through the stomatal apertures or through the bases of trichomes and thereafter get translocated to tissues [20, 21, 22]. The nanoparticles penetrate the plant cell wall and enter into the space between plant cell wall and plasma membrane due to small size, capillary action and Van der Waals forces.

#### *1.1.1. Uptake and distribution of nanomaterials in plant cell*

Improvement in an agronomic attribute of plant system, that is, photosynthetic efficiency with the help of nanomaterial, needs a successful uptake and transfer of nanomaterials in plant cell. The plant cell wall has pores of an average diameter of 5–20 nm. These pores allow the passage of solutes while constraining the diffusion of massive particles and macromolecules including some enzymes [23]. Plants cell employ several strategies to avail nanomaterial (carbon nanotubes) through cell wall and cell membrane depending on the size of the nanomaterial. The entry of nanoparticle in plant cells depends on size and charge [24, 25]. The single-walled carbon nanotubes are of 1–2 nm and are smaller than cell wall pores (5 nm). These nanotubes could be perceived directly through a spontaneous leakage into the apoplast [26]. Thus, for spontaneous leakage, the single-walled carbon nanotubes must be truncated to a commensurable size [27]. The introduction of wide-diameter carbon nanotubes into walled plant cells could also occur through local hydrolysis of the cellulosic cell wall. The cellulose molecules immobilized on the surface of carbon nanotube generate local lesions in the cell wall which facilitate the uptake of carbon nanotubes [28].

The leakage of carbon nanotubes through the cell wall pores has been reported in cells of *Nicotiana tobacum* and *Catharanthus roseus* [29, 30]. The first experimental evidence for the internalization of single-walled carbon nanotubes (SWNTs) has been shown in *N. tobacum* [31]. Temperature-dependent uptake of single-walled carbon nanotubes in *N. tobacum* suggests the internalization of nanotubes through endocytosis [30]. On the other hand, it has been reported that there is no effect of temperature and light on SWNT transfer to lipid bilayer [11]. Multi-walled carbon nanotubes (MWCNTs) could also penetrate the cell membrane of plant protoplasts [30]. When MWCNTs are in close vicinity of protoplast of *C. roseus*, the nanotube aggregations increase the tonicity of cell medium and facilitate the penetration of MWCNTs. Active transport of nanoparticles has also been reported through the lipid bilayer [32].

The metal oxide nanoparticles may be transported through root to leaf or leaf to root in plants [33], and it was studied in hydroponic [34] and soil [35] culture. The negatively charged nanoceria translocates at a higher rate from root to leaf as compared to positively charged nanoceria [36]. The metal oxide nanoparticles are absorbed by root endodermis through apoplastic and symplastic routes, and these are then transported to stem, leaf, fruit and grains [37–39] through a vascular cylinder [40]. Similarly, the mono-dispersed mesoporous silica nanoparticles penetrate into the roots through symplastic/apoplastic pathways and then to the aerial parts of the plants through vascular system [41]. The uptake of metal oxide nanoparticle has been shown by seeds [42], seedlings [38] and mature tubers [43]. The metal oxide nanoparticles may enter through leaf stomata or cuticle and then to stem and root through phloem sap [44, 45]. The single-walled carbon nanotubes and nanosheets are transported into cultured plant cells by endocytosis or internalized in plant root cells via non-endocytic pathways [31, 46]. Silver nanoparticle enters in *Arabidopsis* protoplasts through mechanosensitive channels [47].

#### *1.1.2. Uptake of nanomaterial by organelles*

Different cellular organelles have been reported to uptake the nanomaterials. Serag et al. [48] reported the vacuolar uptake of SWNTs by labelling the SWNTs with fluorescein isothiocyanate (FITC). Following incubation of plant tissues with FITC-labeled SWNTs, fluorescence signals were detected in the cell vacuoles. Further measurement of diffusion coefficient (Deff) supported vacuolar accumulation. To confirm vacuolar uptake, the Deff was measured using fluorescence recovery in a photobleached area (FRAP). The Deff varied according to the size of a macromolecular complex containing fluorescent label. FRAP helped to study the fractions of molecules capable of recovering in the photobleached area and confirmed the accumulation of SW-F inside the vacuoles [48]. Further, the use of probenecid, an inhibitor of carrier-mediated transport, indicated the accumulation of SW-F in vacuoles.

SWNTs transport passively through chloroplast lipid bilayer through kinetic entrapping or by disrupting lipid bilayers [11, 49]. As SWNTs come in contact with the chloroplast's outer envelope, it wraps around the glycerolipid (forming most of the chloroplast's outer envelope). As nanotubes perforated through the envelopes, they are covered with a layer of lipids that irreversibly binds them to the interior side of the chloroplast. The formation of temporary pores has been noticed in the plasma membrane to internalize the nanoparticles like quantum dots and silica nanosphere [49, 50]. Also, the negatively or positively charged nanoparticles spontaneously penetrate lipid envelopes of the extracted chloroplasts [51].

### 1.2. Generational transmission nanomaterials

The generational transmission of nanomaterials was studied in rice [52] using a bright field microscopy. Tissue of rice plants at various developmental stages were sampled, washed, sectioned and imaged to track the transmission after 1 week of incubating in 20 mg $l^{-1}$ $C_{70}$ solution. Black aggregates were frequently observed in seeds and roots and less frequently in stems and leaves which indicated that the sequence of nanoparticle uptake was from the plant seeds and roots to stems and leaves. The appearance of black aggregates was mostly found in and near vascular system. It was suggested that the transport of $C_{70}$ occurred simultaneously with the uptake of water and nutrients in the xylem [52]. Further, to investigate generational transmission of nanomaterials, mature seeds from the control plants and $C_{70}$-treated plants were germinated and second generation was raised. In second generation, black aggregates were also spotted in the leaf tissues, however, with much less frequency [52]. The results were supported by Fourier transform (FT)-Raman and IR spectra from both first- and second-generation rice plants.

## 2. Employment of nanotechnology for the improvement of photosynthetic activity and plant productivity

Photosynthesis is the most fundamental and vital physiological process in plant kingdom. It converts the light form of energy into chemical form in chloroplasts using chlorophyll and $CO_2$ and $H_2O$ as raw materials, and stores in the bonds of sugar molecules. This form of energy is later used as the energy currency to regulate various processes. In green plants, chloroplasts are the site of synthesis for chemical energy, that is, carbon-based fuels. With the help of light energy, the captured atmospheric $CO_2$ is converted into different forms of sugars [53]. Photosynthetic apparatus utilized less than 10% of the sunlight [54], and there are possibilities to improve the solar energy conversion efficiency in photosynthetic organisms. The improvement in photosynthetic efficiency requires broadening the range of solar light absorption [55] particularly in the near-infrared spectra which are able to penetrate deeper into living organisms. With unique properties and higher stability, the nanomaterials can form chloroplast-based photocatalytic complexes having an enhanced and improved functional property under *ex vivo* and *in vivo* conditions [11]. It is clear that neither all the absorbed photons are involved in electron flow under intense light conditions nor chloroplast captures maximum solar energy under non-saturating light [56, 57]. The SWNTs have discrete optical and electronic properties and a broad range of absorption spectra (ultraviolet, visible and near-infrared). The enhancement of light reaction after the insertion of SWNTs in chloroplasts isolated from commercially available baby spinach leaves (*Spinacia oleracea* L.) has been observed [11]. Chloroplast does not have a broad range of absorption spectra and it cannot absorb spectra outside its absorption ranges of spectra. The boosted photosynthetic reactions might be attributed to electronic bandgap of semiconducting the SWNTs which converts the absorbed solar light into photosynthetic excitons [58]. Depending on their inherent light interaction capabilities, nanoparticles (NPs) interfere and alter the photosynthetic efficiency, photochemical fluorescence and quantum yield in plants. Keeping up with the importance of process, the researchers attempted either to mimic the process of photosynthesis artificially or to improve the existing efficiency in planta using nanotechnology-based inventions. The plants have been augmented to harvest more light energy by delivering carbon nanotubes into chloroplast. These carbon nanotubes serve as artificial antennae allowing chloroplast to capture wavelengths of light outside the normal range, that is, ultraviolet, green and near-infrared [11, 16]. Various reports are available on the enhancement of photosynthetic activity in plants through *in vivo* or *ex vivo* approaches. In subsequent text, a few cases will be highlighted to show the relevant progresses made by a nano-technologist for the improvement of agronomic attribute.

Plant photosystems include reaction centres (RCs) and the antenna chlorophylls; they are held in the membrane by weak intermolecular interactions. The antenna chlorophyll absorbs photons and transfers to the RCs and then electrons are transferred to the next electron acceptor. Naturally, photosynthetic machinery absorbs light within certain wavelength intervals. It has been reported that if nanoparticles conjugate with these RCs and antenna chlorophyll, there is an exciton enhancement effect [59]. Nanoparticle conjugate with light-harvesting complex absorbs a wider range of wavelength interval. Nanomaterials conjugated with a photosynthetic system strongly increase the rate of production of excited electrons due to the plasmon (metal nanoparticle having an oscillating free electron) enhancement effect. This excited electron can be used for photocurrents or chemical reactions. The association of metal nanoparticle with photosynthetic system has been reported to enhance the efficiency of photosystem. The incorporation of metal nanoparticles with light-absorbing chlorophyll molecules enhances the photon field which is referred as plasmon enhancement effect. Thus, the production of exited electrons has been reported to increase due to plasmon resonance and electron-hole separation [60]. In support of this, experimental proofs were generated for the increased rate of the formation of ATP molecules. With hybrid structure, the rate of formation of the excited electron was reported to enhance as compared to photosystem alone [61]. Artificial structures composed of a photosynthetic system and various metal nanoparticles also display strong enhancements of photosynthetic efficiency, and this cause the parallel increases in light absorption by chlorophylls and energy transfer from chlorophylls to nanoparticles [60, 62, 63].

Artificially, the quantum dots (artificial antennae absorbing light efficiently in a wide range of photon energies from solar spectrum) conjugated with a reaction centre complex of *Rhodobactor sphaeroides* purified from natural light-harvesting complexes showed an efficient transfer of excitation energy to reaction centre. The efficient energy transfer from QDs to the bacterial RC clearly offers an opportunity of the utilization of nanocrystals to enhance the photosynthetic biological functioning [59]. A silver nanowire conjugated with light-harvesting complex from the dinoflagellate *Amphidinium carterae* showed strong enhancement in fluorescence intensity of protein-bound chlorophyll molecules [64]. The increase with silver nanowire conjugate was recorded up to an average of 10-fold increase in chlorophyll fluorescence [65], and this indicates a higher rate of generation of excitations in the chlorophylls [66].

Metal nanoparticles have the ability to influence the energy conversion efficiency in photosynthetic systems. The binding to Au and Ag nanoparticles with chlorophyll molecule results in a novel hybrid system, which could produce 10 times more excited electrons due to plasmon resonance and fast electron–hole separation [60]. Electron transfer from excited fluorophore to Au or Ag nanoparticles has been reported [65, 67–69]. The concentration-dependent effects of Au nanoparticles (5–20 nm) on PSII chlorophyll, a fluorescence quenching in soybean leaves, have been observed [70]. Falco et al. [70] observed a shift in fluorescence towards a higher wavelength in Au nanoparticle-treated soybean leaves. An enhanced PSII quantum efficiency was reported in Ag nanoparticle-treated Indian mustard [71].

Giraldo et al. [11] reported 49% increase in electron transfer rate under *ex vivo* conditions (in extracted chloroplast from baby spinach leaves) after treatment with SWNTs. SWNTs also enhanced the light reaction *in vivo* in leaves of *A. thaliana*. Similarly, carbon nanotubes in spinach thylakoid improved photo-electrochemical activity under illumination [72]. Noji et al. [73] reported that nanomesoporous silica compound (SBA) conjugated with photosystem II (PSII) maintained the high and stable oxygen-evolving ability of PSII in *T. vulcanus*. The applied $TiO_2$ nanoparticles caused the transfer of charges between light-harvesting complex II (LHCII) and $TiO_2$ NPs because of their photocatalytic properties [74] which induced reduction–oxidation reaction. Ze et al. [75] reported an increased expression of LHCII b and contents of LHCII in the thylakoid membrane of *A. thaliana* after the application of $TiO_2$ nanoparticles. It was found that $TiO_2$ NPs promote the light absorption by chloroplast and regulate the distribution of light energy from PSI to PSII by increasing LHCII content, which in turn accelerate the transformation from light energy to electronic energy, water photolysis and oxygen evolution.

Nadtochenko et al. [62] observed an enhanced electron transfer efficiency in isolated photosynthetic reaction centres using alumina nanoparticles. The bread wheat (*Triticum aestivum* L.) showed an increase in grain number, biomass, stomatal density, xylem-phloem size, epidermal cells and water uptake after seed priming with MWCNT [76]. $TiO_2$ nanoparticles have been reported to protect chloroplasts from aging during long illumination regimes, promote chlorophyll formation and stimulate Rubisco activity, which in turn results in increased photosynthesis or enhanced photosynthetic carbon assimilation [71, 77, 78]. With exogenous application of $TiO_2$, Qi et al. [79] observed an improved net photosynthetic rate, water conductance and transpiration rate. Nano-anatase was reported to promote electron transport chain reaction, photoreduction activity of PSII, evolution of $O_2$ and photophosphorylation of chlorophyll under both visible and ultraviolet light [80]. A higher photosynthetic carbon reaction due to Rubisco carboxylation was observed as a result of nano-anatase-induced marker genes for Rubisco activase mRNA, enhanced protein levels and activities of Rubisco activase [81]. On the contrary, the exogenous application of $TiO_2$-anatase NPs resulted in a reduced PSII quantum yield, photochemical quenching, electron transfer rate, chlorophyll fluorescence and higher non-photochemical quenching and water loss [82]. Nano-$TiO_2$ reported to improve water absorption, seed germination, plant growth, nitrogen metabolism and photosynthesis [63, 76, 83, 84]. $TiO_2$ NPs were reported to alleviate heat stress through regulating stomatal opening [79].

Nano-$TiO_2$ (rutile) influences the photochemical reaction in spinach chloroplasts [85, 86]. The spinach treated with 0.25% nano-$TiO_2$ showed improved up-hill reaction and oxygen evolution. The noncyclic photophosphorylation activity was found to be higher than cyclic photophosphorylation in chloroplasts. This increase in photosynthesis with nano-$TiO_2$ might be associated with the activation of a photochemical reaction in spinach chloroplasts [85, 86]. Similarly, an increase in dry weight, chlorophyll formation, the ribulose bisphosphate carboxylase/oxygenase activity and the photosynthetic rate was reported in aged spinach treated with 2.5% nano-$TiO_2$ rutile [83]. The nano-anatase $TiO_2$ improved light absorbance, conversion of light energy to electron energy and ultimately to chemical energy, and this promotes carbon dioxide ($CO_2$) assimilation. Treatment of nano-anatase $TiO_2$ improved Rubisco-carboxylase activity 2.67 times in spinach as compared to control, which consecutively activates Rubisco carboxylation and eventually the rate of photosynthesis increase [87]. Pradhan et al. [88] found that Mn-NPs induced an increase in the hill reaction rate in mung bean (*Vigna radiata*).

In the recent time, NMs are used as a vital tool for improving plant growth and productivity under adverse environmental conditions, that is, salt stress. The Si nanoparticles in the soil have been shown to alleviate salt stress, enhance seed germination, improve activities of antioxidative enzymes, photosynthetic rate and leaf water content [89, 90]. Increased leaf, pod dry weight and grain yield were recorded in soya bean using nano-iron oxide [91]. The β-cyclo dextrin-coated iron nanoparticles penetrate the biological membranes of maize and increase the chlorophyll pigments (up to 38%) as compared to control [92]. The spray of citrate-coated

$Fe_2O_3$ nanoparticle spray on *Glycine max* had positive effects on root elongation and photosynthesis rate. Also, the elongation of root and an increase in seed germination were observed in *Zea mays* L. with silica ($SiO_2$) nanoparticles treatment [93]. Maize with a treatment of 1500 ppm of ZnO nanoparticulates showed the highest germination and seedling vigor index [94].

## 3. Future prospects

Nanotechnology has enormous potential to create novel and improved functional properties in photosynthetic organelles and organisms for the enhancement of solar energy harnessing. The upward translocation from root to leaf opens up greater opportunities for their use in various delivery applications. The SWNTs delivered by this spontaneous mechanism have the potential for increasing chloroplast carbon capture by promoting chloroplast solar energy harnessing and electron transport rates. It has been shown that when nanoparticles enter into plant cell, various metabolic changes occur that leads to an increase in biomass, fruit/grain yield, and so on; therefore, further mode and action can be elucidated to evaluate the possibility of their uses. The nanomaterials have the potential to be utilized for the transport of DNA and chemicals into plant cells [95, 96] which offers new opportunity to target specific gene manipulation and expression in the specific cells of the plant. With nanomaterial, the output of a crop can be increased while reducing the input through a better understanding of nanoparticle interaction with plants. The nanobionics approach to engineer plant function will lead to a new area of research at the interface of nanotechnology and plant biology.

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## Conflict of interest

The authors declare no conflict of interest.

## Abbreviations

| | |
|---|---|
| Deff | Diffusion coefficient |
| FITC | Fluorescein isothiocyanate |

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| FRAP | Fluorescence recovery in a photobleached area |
| MWNT | Multi-walled nanotubes |
| PAA-NC | Poly(acrylic acid) nanoceria |
| QD | Quantum dots |
| SWNTs | Single-walled nanotubes |
| SBA | Nanomesoporous silica compound |
| LHCII | Light-harvesting complex II |

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