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Conjugated Polymer Nanoparticles for Fluorescent Labeling of Live Cells and Delivery of Biological Molecules into Plant Cells

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Conjugated Polymer Nanoparticles are intrinsically fluorescent carbon based structures whose engineered dimensions fall below 100 nm. These nanoparticles possess advantageous photophysical properties such as high fluorescent quantum yield, large excitation coefficient and efficient signal transduction which make them use full in fluorescent Microscopy. Amphipathic structure and flexibility in further chemical modification make them permeable through biological membranes and potential use as a carrier of biological molecules. In this study CPNs were assessed for their ability to fluorescent labeling of Tobacco BY2 cells and protoplasts by co culturing in the culture medium. More than 50% fluorescent positive protoplasts were observed under B2A excitation filter after 2 hour incubation with 10 μM CPNs. The green fluorescent signal was stable for 2 days without any noticeable reduction in intensity. CPNs uptake of protoplasts was further confirmed by analyzing intracellular fluorescence and side scattering properties of protoplasts using Flowcytometry. Toxicity effects of CPNs were evaluated in terms of cell viability in protoplasts incubated with 5 to 500 μM CPNs. Protoplasts did not show significant reduction in cell viability with CPNs concentrations below 50 μM after 24 hours of incubation. Confocal analysis of BY2 cells treated with similar concentrations of CPNs demonstrated that cell wall provides a barrier for CPNs uptake by BY-2 cells.

One of the major goals of this research is to analyze the ability of CPNs to use as a transfection agent to deliver small RNAs in to plant cells. To check the hypothesis protoplasts were incubated with commercially available siGLO small RNAs mixed with CPNs in different ratios of concentrations. CPNs were able to carry those small RNAs into protoplasts after 24 hours of incubation.

In order to check whether CPNs can be used as a tool to measure dimension requirements in plasmodesmata transport of molecules in intact leaf cells, CPNs were delivered into *Nicotiana benthamiana* leaf epidermal cells using particle mediated transfer technology. Epifluorescence microscopy was used to monitor their spread immediately upon delivery. This study showed the potential use of CPNs in studying of cell to cell trafficking of molecules in plant cells.

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