Abstract

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Title: Construction of Genetically Encoded FRET-Based Nanosensors for Monitoring of Heavy Metal Ions in Living Cells

Keywords: FRET, Fluorescent protein, Genetically encoded sensors, Metal ions toxicity, Non-invasive, Live cell imaging

We report the development of a nanosensor for the flux analysis of silver and manganese ions, which is increasingly emerging as an environmental toxicant associated with several human health risks. FRET-based nanosensors exploit obligatory conformational changes in a polypeptide or ligand-binding proteins upon binding to the analyte-of-interest (metals in this case) and the phenomenon of FRET of fluorescent protein pair. The novelty of this nanosensors is that it can be introduced in any cell type and real-time monitoring of the level of silver and manganese ion and it can be carried out non-invasively in any type of living cell. The study was conducted at stages including the designing and construction of genetically encoded nanosensors, expression and purification of the developed nanosensors, *in vitro* characterization (fluorescence emission spectra, buffer and pH stability, metal specificity, affinity and generation of affinity mutants) of the nanosensors and *in vivo* characterization of the developed metal specific nanosensors in bacteria, yeast and mammalian cells. These tools are highly specific to respective metal ions and stable at physiological pH ranges. The developed sensors are named as SenSil (sensor for silver) and SenMn (sensor for Manganese). We have validated the results in three systems bacteria, yeast and animal cells; and also both *in vitro* and *in vivo* studies carried out found it highly responsive towards the respective metal ions. Our results suggest that genetically encoded recombinant fluorescent sensors will be versatile tools for monitoring the flux of silver and manganese ions inside the cells without any disruption.