Genetically encoded FRET-based optical probe for quantitative analysis of mercury in living cells

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Metals are ubiquitous, have persistent and non-degradable nature. Biological systems require metal as co-factors for biochemical reactions both at the cellular and molecular level. Localizing and monitoring the distribution of metal ions and measuring their concentration in cellular and sub-cellular compartments is imperative in maintaining metal homeostasis. Mercury is a non-essential heavy metal present in the body. Its relative higher level can be toxic and prove fatal to the human health causing cancers and tumors [1]. This is highly reactive and extremely volatile environmental and occupational pollutant negatively affecting the lungs, kidneys and nervous, digestive, neuromuscular and immune systems [1,2]. Organomercurials such as methyl mercury affects the nervous system causing insomnia, memory loss, tremors and neurological dysfunction [3]. Methods available so far to study their level are not able to address the sensitivity, selectivity, are toxic and have limited spatial and temporal resolution. Subsequently, higher resolution can be achieved by using a genetically encoded Mercury FRET Sensor (MerFS) that uses *Mer*P as a receptor domain sandwiched between a donor fluorophore enhanced cyan fluorescent protein (ECFP) and an acceptor fluorophore venus at N- and C- terminus of the binding domain respectively. On binding of Hg^{2+}, *Mer*P undergoes conformational changes and produce a fluorescent response through attached fluoprophores (FPs). The receptor domain is responsible for recognition of mercury ions and produces a responsive fluorescence resonance energy transfer (FRET) signal. Mercury exposure introduces free radicals in the body which may lead to oxidative damage and affects metabolic functions. The purified MerFS was found to be highly specific towards mercury ions. A mutant MerFS-29 is the most efficient nanosensor that showed a maximal ratio change of 0.59 upon addition of mercury with an apparent affinity (K_d) of 29.09 µM, thus providing a linear detection range for mercury quantification between 11.80 µM and 58.88 µM. In case of *E. coli* cells, an increase in the FRET ratio was obtained with MerFS-29 after addition of mercury in a time-dependent manner under *in vivo* conditions. MerFS-29 was also targeted to yeast and mammalian human embryonic kidney (HEK)-293T cells which allowed dynamic *in vivo* measurement of mercury concentration, thus proving its potential in eukaryotic system. This genetically encoded tool can prove to be useful in detecting the physiological concentration of mercury ions in living cells and understanding of metal homeostasis. It also allows the real time monitoring and *in situ* detection of the metal ions to help regulate the intracellular concentration of mercury ions.

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