Live imaging of vitamin B_{12} dynamics by genetically encoded FRET-based biosensor

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Vitamin B12 is a co-factor of various enzymes and involved in the metabolism of the cell. During infancy, low level of vitamin B12 is associated with negative consequences on the developing brain. Deficiency of vitamin B12 causes various neurological abnormalities and pernicious anemia. Different methods such as, isotopic labeling MS, NMR have been used for measuring the concentration of metabolites, however, these methods require the disruption and fractionation of tissues which suffers from contamination. Currently, no method is available for real-time monitoring of vitamin B12, non-invasively. Genetically encoded Fluorescence Resonance Energy Transfer-based biosensors have been evolved as an ideal mean to determine the metabolite concentration in live cells. Here, we report the designing of FRET-based biosensor for direct visualization of changes in vitamin B12 concentration in intact living cells. Initially, a construct was designed by using the gene fragments of vitamin B12 binding protein (BtuF), cyan variants (CFP) and yellow variants (YFP) of Green Fluorescent Protein. This construct was then shuttled in different expression vectors. The constructed FRET sensor was termed as SenVitAL (**Sen**sor for **Vit**amin **A**nemia **L**inked) which is found to be specific for vitamin B12. This biosensor is stable to pH changes, and can measure the vitamin B12 from micro-molar to mili-molar levels. In both *in vitro* and *in vivo* measurements, FRET ratio increases after the addition of vitamin B12. Furthermore the results show that the sensor can measure the vitamin B12 concentration in the cytosol of yeast and mammalian cells. Thus, the SenVitAL can serve as novel indicator to investigate the vitamin B12 import and metabolism in pro- and eukaryotic cells.