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Imaging of vitamin B_{12} dynamics by genetically encoded fluorescent nanosensor in living cells

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Vitamin B_{12} (cobalamin) is a co-factor of various enzymes and involved in the metabolism of every cell of human body. Deficiency of vitamin B_{12} causes various neurological abnormalities and pernicious anemia. Various methods like isotopic labeling MS and NMR have been used for measuring the level of cellular metabolites or signaling molecules, but these methods require the disruption and fractionation of tissues which suffer from contamination. Genetically encoded FRET-based sensors have been constructed to determine the metabolite concentration in live cells. Here, we report the designing of fluorescence resonance energy transfer-based nanosensors for direct visualization of changes in cobalamin concentration in intact living cells. Initially, a construct was designed by using the vitamin B_{12} binding protein (BtuF), cyan (CFP) and yellow (YFP) variants of green fluorescent protein. This construct was then shuttled in different expression vectors. This FRET sensor was named as SenVitAL (sensor for vitamin anemia linked) which is found to be very specific for vitamin B_{12}. This sensor is stable to pH changes, and measures the vitamin B_{12} in a concentration-dependent manner with an apparent affinity of (K_d) ~157 µM. In case of *E. coli*, increase in the emission intensity ratio was specifically observed after exposure to vitamin B_{12}. Both *in vitro* and *in vivo* measurements, FRET ratio rises after with the addition of vitamin B_{12}. Moreover, the results show that the SenVitAL can evaluate the vitamin B_{12} concentration in the cytosol of yeast and mammalian cells, proving its potential in eukaryotic system. Consequently, the sensor can serve as novel indicator to investigate the vitamin B_{12} flux and, would help to elucidate their complex roles in metabolism.

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