Engineering of tyrosine code with unnatural amino acid for development of metal-ion chelating biosensor

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Metal ions detection is very important, hence metal ion binding sites are introduced into protein by directed evolution and structural based rational design strategies for development of an efficient protein-based biosensor. The major limitation in proteomics research is all organisms use the similar common 20 amino acids as building block for the biosynthesis of proteins. The solution to this limitation is to use non natural amino acids that possess unique side chains and would provide a powerful tool for manipulating and probing protein function in cells. Here, we replaced the tyrosine codon with 3, 4-dihydroxy L-phenylalanine (L-DOPA) into green fluorescent protein (GFPdopa) by selective pressure incorporation. The introduced new functionality allows to link metal ion binding to chromophore spectral changes in DOPA-GFP protein in the presence of Cu2+. Binding of metal ion did not affect the wavelength shift of fluorescent excitation and emission peak and resulted in higher fluorescence emission quenching compare to excitation peaks of the protein. This selectivity and reversibility is significant characters of DOPA-GFP protein will be valuable in variety of sensing applications.