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Signal Enhancement of a Maltose FRET-Sensor by Engineering GFP-linkers and the Binding Site

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Fluorescence resonance energy transfer (FRET) between cyan-fluorescent protein (cFP) and yellow-fluorescent protein (yFP) recently was used as an indicator of the ligand-mediated conformational changes, after the amino and carboxy termini of a binding protein are properly fused with two fluorescent proteins. Sugar binding proteins recognizing maltose, arabinose, allose, glucose, or ribose were introduced as a sensing element for FRET sensors of this work. Sugar-binding site locates deep within the cleft between N- and C-modules and, in the presence of sugars the protein undergoes a hinge-like conformational change, which would cause a variation in distance and/or angular orientation between FPs. N- and C-terminal linkers connecting SBP and FPs would play an important role on the FRET variation (ΔFRET). Herewith a cFP-MBP-yFP library with different linkers was prepared and screened for the enhanced ΔFRET. Two residue-linkers gave better sugar-sensitive fluorescence in general and five variants of two residue-linkers were selected from 5,000 variants. The W62L mutant, having a mutation in the binding site of MBP, was found to enhance ΔFRET over 3-fold while the dissociation constant to maltose changed from 1mM to 100mM. [Supported by Biogreen21 grant of Korea]