

Title	抗原の蛍光レシオ検出が可能な遺伝的にコードされた新規抗体バイオセンサーの開発
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## ABSTRACT

Fluorescence biosensor is an indispensable method for tracking of small biomolecules or biological processes not only *in vitro* but also in living cells. Recently, Quenchbody, a novel fluorescence biosensor consists of an N-terminal fluorescently labeled antibody single-chain variable domain (scFv) has been reported. This biosensor allowed detection of antigen based on antigen-dependent removal of quenching effect on the labeled fluorophore. However, fluorescence intensity of single labeled Quenchbody depends on not only concentration of antigen but also amount of the biosensor in measuring sample. In addition, Quenchbody requires the incorporation of fluorophore-labeled nonnatural amino acid in a cell-free translation system, thus, limit its application in live-cell imaging. In this study, a new strategy for construction of antibody-based fluorescence biosensor in combination of Förster (or fluorescence) resonance energy transfer (FRET) and fluorescence quenching mechanisms was introduced to overcome the limitations of Quenchbody. First, fluorescence biosensors for detection of phosphotyrosine-containing peptides were developed by incorporation fluorophore-labeled nonnatural amino acid into the N-terminus of anti-phosphotyrosine scFv. This biosensor showed antigen-dependent fluorescence increase upon addition of phosphotyrosine-containing peptides. Fusion of fluorescent protein (FP) to the labeled scFv generated double labeled biosensors which allowed FRET between FP and labeled fluorophore and detection of antigen based on antigen-dependent enhancement of fluorescence ratio of fluorophore/FP. Next, genetically-encoded antibody-based fluorescence biosensors were constructed by substituting fluorophore-labeled nonnatural amino acid by protein-tag and its fluorescent ligands. The obtained biosensors exhibited fluorescence enhancement in the presence of antigens. In addition, type of fluorophore, linker length between fluorophore–ligand and orientation of protein-tag to scFv largely affected fluorescence enhancement. Fusion of FP to protein-tag-scFv resulted in double labeled biosensors which showed FRET between FP and labeled fluorophore as well as antigen-dependent enhancement of the fluorophore, allowing fluorescent ratiometric detection of antigen. Finally, an application of the novel genetically-encoded antibody-based ratiometric fluorescent biosensor was demonstrated by expression of the biosensor on the surface of mammalian cells for detection of extracellular antigen. The advantage of the present strategy over conventional strategy for FRET-based biosensor construction is that no conformational change of backbone protein upon binding to analyte is required. Therefore, it is potentially applicable for various antigen-antibody pairs in not only diagnostic analysis but also live-cell imaging.

**Key words:** single-chain antibody, nonnatural amino acid, fluorescence biosensor, protein-tag, live-cell imaging.