Development of Antibodies That Show Antigen-dependent Fluorescence Enhancement

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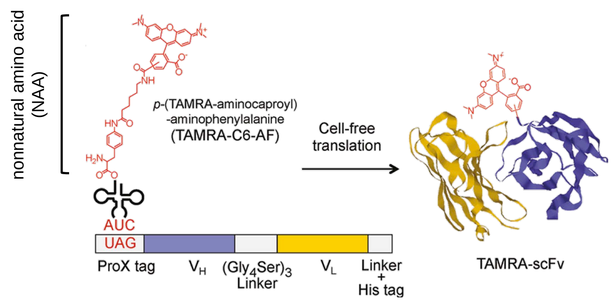
Abstract

Protein-based fluorescent biosensor is an undoubtedly powerful tool for detection and visualization of target biomolecules. Recently, it was reported that single-chain variable fragment (scFv) antibody-based fluorescent biosensor, namely Quenchbody showed a fluorescence intensity change in an antigen concentration-dependent manner. Because structurally conserved protein (*i.e.*, antibody) was employed as the scaffold of biosensor, measurements of various antigens were achieved by preparing fluorescent amino acid-incorporated scFv. However, time-consuming gene manipulation and usage of less productive cell-free translation system is required for genetic incorporation of fluorescent amino acid into scFv. Thus, alternative approach for preparation of antibody-based fluorescent biosensor is highly demanded.

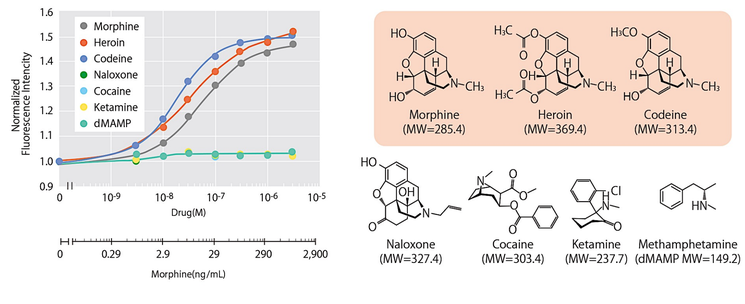
We here report a novel design and synthesis of the antibody-based fluorescent biosensor. Previous study proved that fluorescent amino acid at the N-terminal side of scFv was quenched by conserved tryptophans, but the binding of its antigen triggered a structural rearrangement of scFv followed by a cancellation of the fluorescence quenching. Accordingly, it is considered that N-terminal-selective fluorescent labeling will be useful for preparation of antibody-based biosensor. As a proof of concept, immunoglobulin (IgG) monoclonal antibody-based fluorescent biosensor was semi-synthesized by reductive alkylation of N-terminal *α*-amino groups of IgG with dye-aldehyde in a weak acidic buffer. The N-terminal-selective conjugation of fluorescent dye was evaluated by tandem mass spectrometric analysis of peptide fragments. Also, antigen-dependent fluorescence intensity change of the labeled IgG was confirmed by fluorescence spectral measurement. Fluorescent detection of several peptides and biomolecules was successfully achieved by using the IgG-based biosensor. Because a variety of different IgG are commercially available, wide applications of the IgG-based biosensor will be developed.

Research background

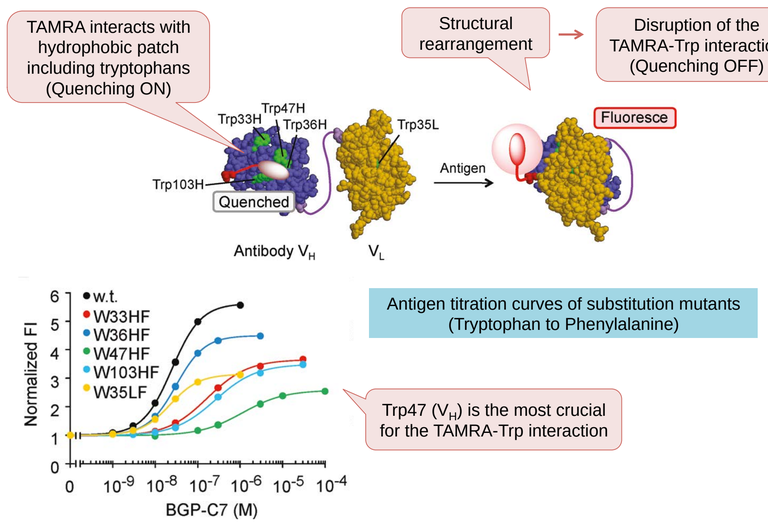
1) Incorporation of NAA into N-terminus of scFv by expanded genetic code system

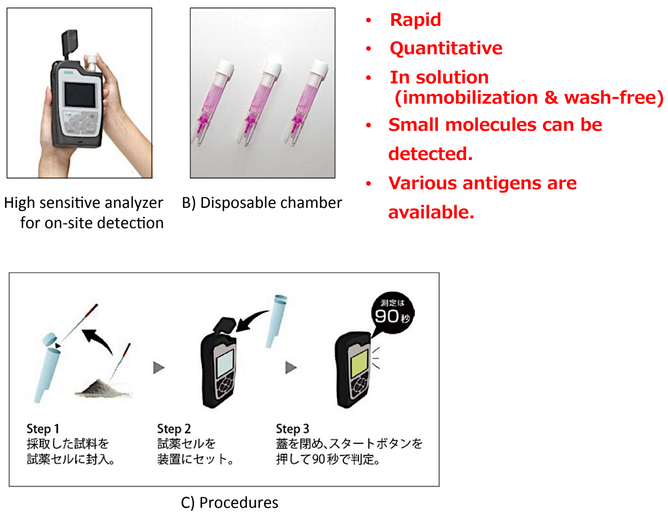


2) Fluorescence detection of controlled drugs using the fluorescent-labeled scFv



3) TAMRA on the N-terminal domain is quenched by intrinsic conserved Trp residues



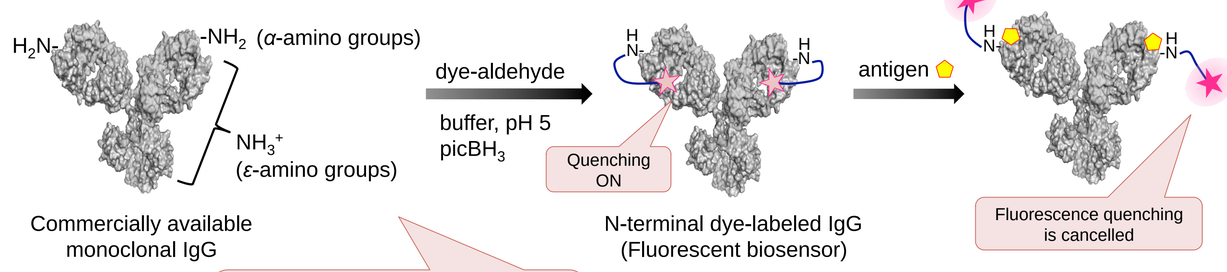


Reference

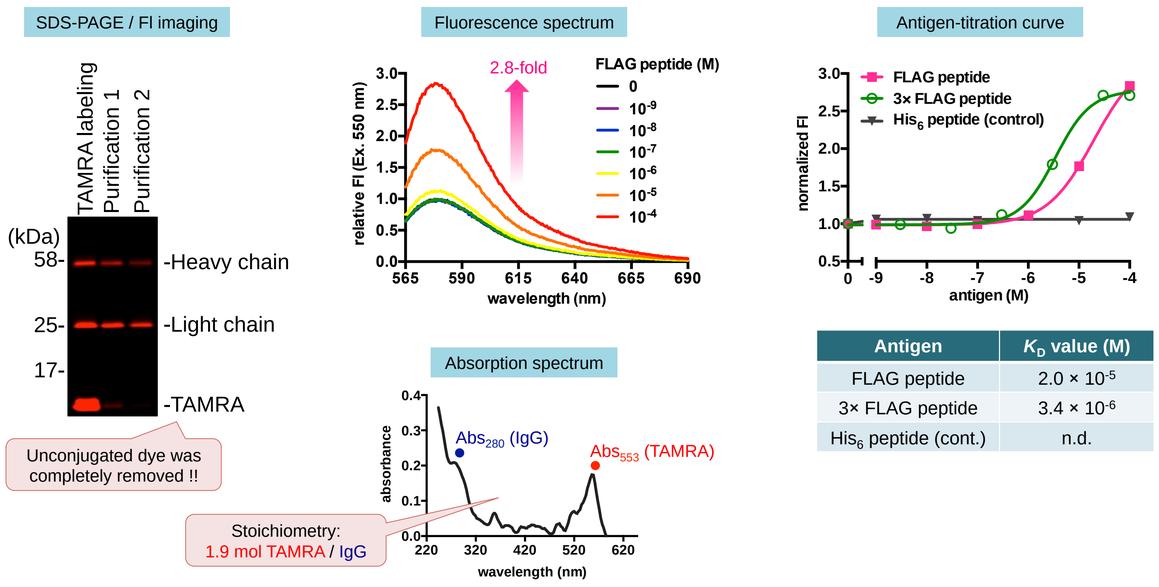
1) Ryoji Abe *et al.*, *J. Am. Chem. Soc.* **113**, 17386 (2011)

Results

1) N-terminal *α*-amino group-selective fluorescent-labeling

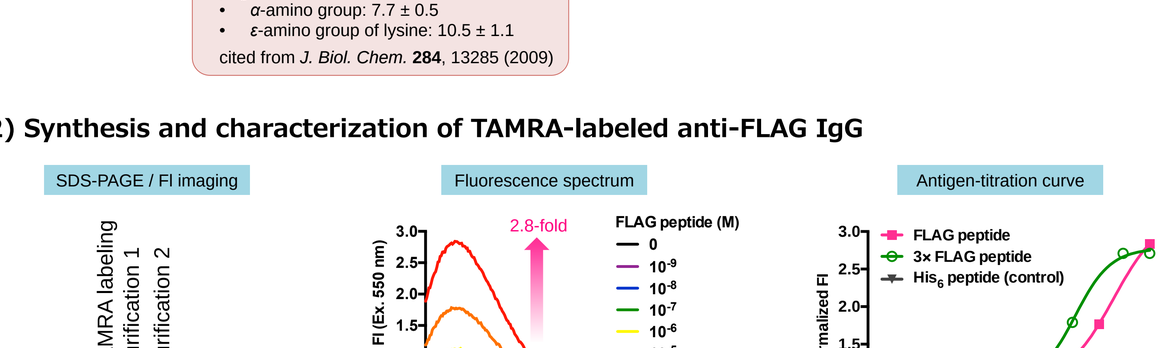


2) Synthesis and characterization of TAMRA-labeled anti-FLAG IgG

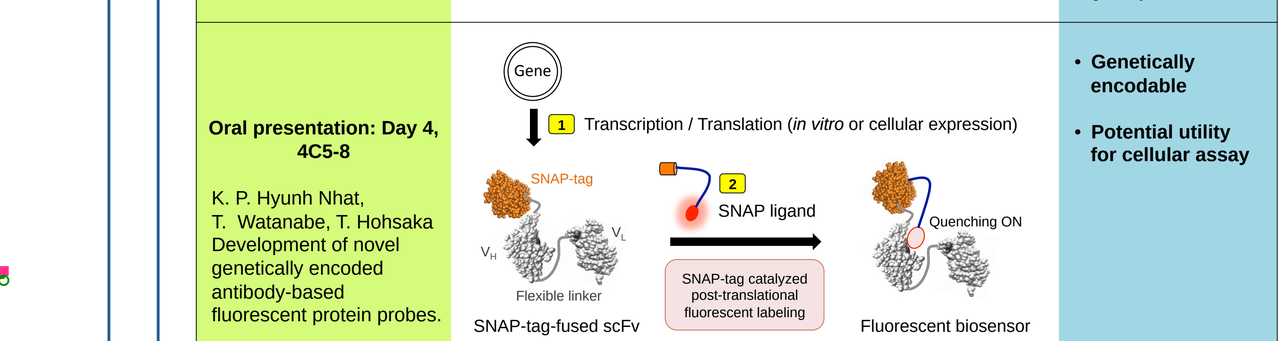


Antigen	K_D value (M)
FLAG peptide	2.0×10^{-5}
3× FLAG peptide	3.4×10^{-6}
His_6 peptide (cont.)	n.d.

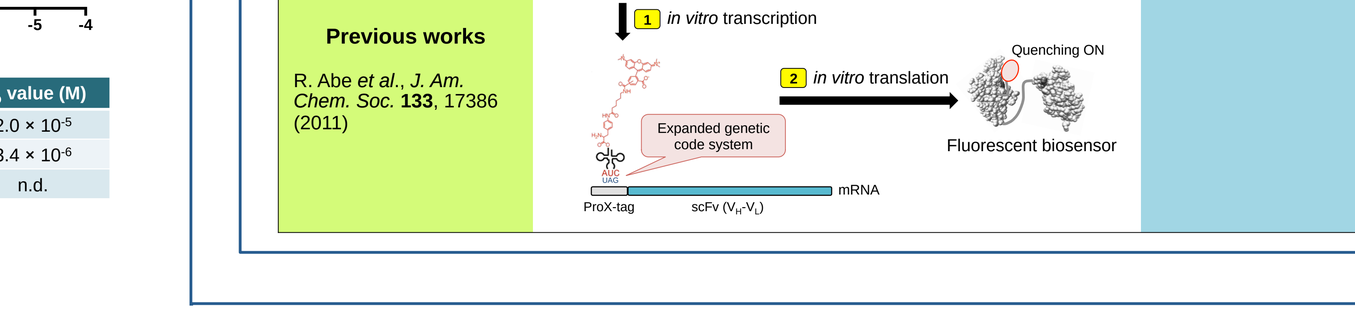
3) Optimization of fluorescent dye and alkyl linker length



4) MS/MS analysis of the trypsinized IgG (anti-c-Myc 9E10 monoclone)



5) TAMRA-labeled IgG antibodies might expand various applications



Positioning of this study

Schematic drawing of synthesis of antibody-based biosensors: strategies for incorporation of fluorescent dye	Advantages
This work **Oral presentation: Day 4, 4C5-10**	• **Simple method** • **Diversity of biosensors:** commercially available IgGs can be used. • **Cost effective ¥:** good yield.
Oral presentation: Day 4, 4C5-8 K. P. Hyunh Nhat, T. Watanabe, T. Hohsaka Development of novel genetically encoded antibody-based fluorescent protein probes.	• **Genetically encodable** • **Potential utility for cellular assay**
Previous works R. Abe *et al.*, *J. Am. Chem. Soc.* **133**, 17386 (2011)	• **Excellent site specificity**

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