**Creating optical nano-biosensors from engineered antibodies for protein detection**

*Jiaul Islam,A Christian Fercher,A,B,C Martina L. Jones,B,C Ashley M. Buckle,D Christopher B. Howard,B Toby D. M. Bell,E Stephen Mahler,B,C Simon R CorrieA,B*

ADepartment of Chemical Engineering, Monash University, Clayton, Australia, ARC Centre of Excellence in Convergent BioNano Science and Technology

B Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, St Lucia, Australia

CARC Training Centre for BiopharmaceuticalInnovation, Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, St Lucia, Australia

DDept. of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, Australia5

ESchool of Chemistry, Monash University, Clayton, Victoria, Australia

Biosensors based on fluoro-labelled antibody fragments combine the recognition and transduction element into the same molecule leading to real-time result detection and reducing the need for laborious, multi-step assays (1). The key challenge is the efficient site-specific modification of antibodies with environmentally-sensitive fluorescent dyes, without affecting binding functionality. Fluorescence labelling via unnatural amino acids (UAAs) is a relatively new and highly efficient method for 100% efficient site-specific fluorescence labelling, and can be genetically incorporated into any permissible site during protein synthesis (2). Although over 100 UAAs have been incorporated into various proteins for diverse applications including antibody drug conjugates and bispecific antibody development using Fab, to date none of the UAAs has been incorporated into scFv for biosensing applications nor has this been used for detection of large biomolecules (e.g. protein).

We demonstrate that incorporation of environmentally sensitive fluorescent UAA (Anap) into a permissible site of antibody fragments (e.g. anti-EGFR scFv) can be used for detection of target binding by monitoring the wavelength and/or intensity changes in emission spectra. A mutation screen was initially performed in order to identify the Anap mutation site that yielded the largest spectral change. We found that, across two different protein/antibody case studies, that only relatively hydrophobic amino acids within the binding interface could be mutated to generate optically-reactive species, and that the affinity of the mutants was not significantly affected. Here we will present the strategy for producing and characterising these biosensors, along with our latest results on incorporation of biosensors into functional devices.

**References**

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