

## Engineering of Reagentless Fluorescence Biosensors Based on Single-Chain Antibody Fragments

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**Abstract :** Fluorescence-based immunodiagnosics are an emerging field in biosensor development and exhibit several advantages over traditional detection methods. While various affinity biosensors have been developed to generate a fluorescence signal upon sensing varying concentrations of analytes, reagentless, reversible, and continuous monitoring of complex biological samples remains challenging. Here, we aimed to genetically engineer biosensors based on single-chain antibody fragments (scFv) that are site-specifically labeled with environmentally sensitive fluorescent unnatural amino acids (UAA). A rational design approach resulted in quantifiable analyte-dependent changes in peak fluorescence emission wavelength and enabled antigen detection in vitro. Incorporation of a polarity indicator within the topological neighborhood of the antigen-binding interface generated a titratable wavelength blueshift with nanomolar detection limits. In order to ensure continuous analyte monitoring, scFv candidates with fast binding and dissociation kinetics were selected from a genetic library employing a high-throughput phage display and affinity screening approach. Initial rankings were further refined towards rapid dissociation kinetics using bio-layer interferometry (BLI) and surface plasmon resonance (SPR). The most promising candidates were expressed, purified to homogeneity, and tested for their potential to detect biomarkers in a continuous microfluidic-based assay. Variations of dissociation kinetics within an order of magnitude were achieved without compromising the specificity of the antibody fragments. This approach is generally applicable to numerous antibody/antigen combinations and currently awaits integration in a wide range of assay platforms for one-step protein quantification.

**Keywords :** antibody engineering, biosensor, phage display, unnatural amino acids

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