Monolithic Dual Antibody Clamp (MDAC): A High Avidity FRET Construct for Instant ELISA

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Protein detection is of great importance to clinical diagnostics as it is one of the predominant methods of detecting and monitoring disease states. Protein quantitation in blood samples is typically achieved through ELISA assays. Due to the sample preparation and assay protocols, these assays are performed in off-site or centralized analytical laboratories by specialized technicians – this involves lengthy turnaround times that can impact patient outcomes. A generalized point-of-care analytical platform that addresses the need for sensitive, rapid, and sample-prep-free protein quantitation that can be carried out in the clinical setting would drastically reduce sample-to-answer times and improve clinical outcomes.

In this work, we propose a new approach to rapid clinical protein quantification, termed Instant ELISA (fig. 1a), which leverages the generality of immunosorbent techniques in an assay free of sample handling or processing steps. This is achieved with a novel affinity reagent termed monolithic dual antibody 'clamp' (MDAC). The construct comprises two fluorophore-labeled monoclonal antibodies linked by a DNA scaffold. When the antibodies bind and 'clamp down' on a single target protein, their proximity leads to a FRET signal change. Excitation and probing of the MDAC is achieved by surface coupling with a tapered fiber optic: an evanescent excitation field probes the binding state of MDAC constructs and the FRET emission response couples back into the fiber for optical detection. The fiber optic tip is directly immersed in a blood draw and quantitation is achieved within minutes. The fiber can be easily disposed and replaced for subsequent measurements.

We demonstrated clinical utility of Instant ELISA in the context of toxicity mitigation in CAR T-cell therapy, where elevated levels monocyte chemoattractant protein-1 (MCP-1) have been shown to be predictive[1] of life-threatening complications such as Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Instant ELISA was able to quantify physiologically relevant target concentrations spiked in both buffer (fig. 1b) and whole blood (fig. 1c) in less than 15 minutes.

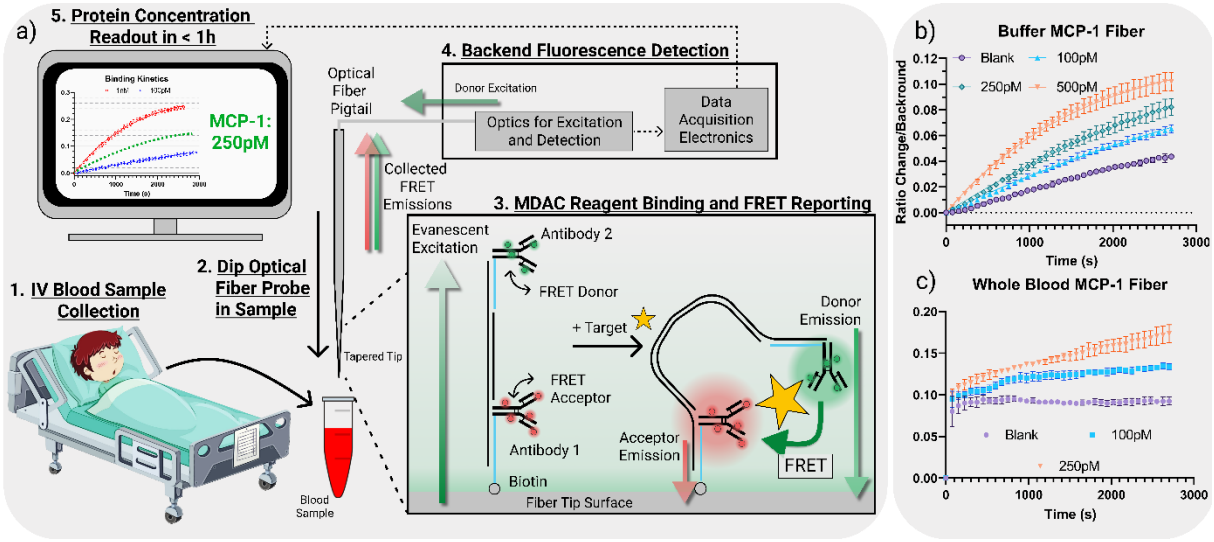

Fig. 1. a) Instant ELISA system leverages the MDAC reagent immobilized on an optical fiber tip to rapidly quantify concentrations of cytokines directly in a blood sample with no sample preparation. b) Binding kinetics of MDAC on optical fiber for MCP-1 target spiked in buffer. c) Quantification of MCP-1 target in whole chicken blood spiked with target.

[1] Hay, K. A., *et al.*; Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor–modified T-cell therapy. Blood. 2017; 130 (21): 2295–2306