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Innovation Advancement & Commercialization

Platform method for enabling detection in complex samples

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Reference: TFA method

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Reference

Anal. Chem., 2015 (Link)
Anal. Methods, 2015 (Link)

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Overview

Homogeneous protein assays involve simple "mix-and-read" steps, making them attractive for rapid, point of care settings. However, these assays suffer from high background in complex samples, making sensitive detection difficult. A method has been developed that removes background for these assays, allowing fast and sensitive detection in human serum and plasma. Most existing assays can easily be adapted to take advantage of this method. This method could potentially be used wherever mix-and-read assays are used, including healthcare, R&D, Quality Assurance labs, food safety, etc.

Advantages

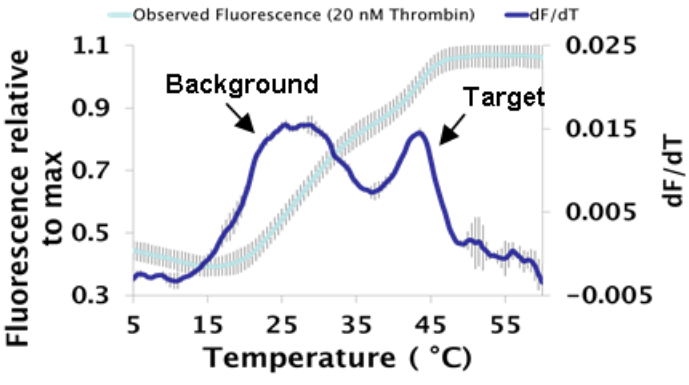
- Removes background — eliminates autofluoresence and possibly other noise
- Increases sensitivity — lowers detection limits; may enable detection with new sample types
- Cross-functional — can be used with many mix-and-read detection platforms
- Multiplexing compatible and functional in picoliter reaction volumes

Description

Homogenous protein assays are simple, rapid, quantifiable, and sensitive but suffer from high background in biological samples. This is particularly true of fluorescent assays using biological samples such as serum or plasma. Thermofluorimetric Analysis (TFA) effectively eliminates this problem, helping existing protein assays approach zero background with minimal signal loss. TFA has been demonstrated using fluorescent nucleic acid-based homogeneous protein detection assays but is compatible with most high-throughput and multiplexable systems. It can be performed using standard instrumentation, such as a qPCR machine, or used with other devices. TFA works similarly to qPCR melting curves but detects compounds other than nucleic acids. The method is based on the stability provided by the protein-probe interaction(s) instead of the nucleic acid interactions of qPCR melting curves. As proof of concept, TFA was used to improve thrombin detection 17-fold in a proximity FRET assay, lowering detection limits to a single attomole of protein in a 100 picoliter reaction. TFA was also used with an electrochemical assay to enable insulin detection in undiluted human serum where this was previously not possible. TFA could be used with currently sold homogeneous assays to enhance performance and sensitivity or be used with custom applications to enable protein detection in biological samples.

Status

- Top 10 finalist for the SLAS 2015 Innovation Award
- Compatible with detection in human serum, plasma, or other complex samples
- Demonstrated with qPCR machines; development of optimized instrumentation is underway
- Subject of PCT application PCT/US2015/059020



Separation of background from target signal. **Light blue line** shows the observed fluorescence of a nucleic acid-based proximity assay detecting 20 nM of Thrombin after subjection to a thermal gradient. **Dark blue line** shows separation of background from target signal after both thermal scan and derivation is taken. Back-ground and Target signal are labeled.