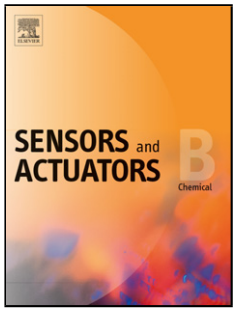

Title: Protein-induced fluorescence enhancement as aptamer sensing mechanism for thrombin detection

Authors: Saurabh Umrao, Vasundhara Jain, Anusha, Banani Chakraborty, Rahul Roy

PII: S0925-4005(18)30735-4
DOI: https://doi.org/10.1016/j.snb.2018.04.039
Reference: SNB 24514

To appear in: *Sensors and Actuators B*

Received date: 9-1-2018
Revised date: 5-4-2018
Accepted date: 7-4-2018

Please cite this article as: Saurabh Umrao, Vasundhara Jain, Anusha, Banani Chakraborty, Rahul Roy, Protein-induced fluorescence enhancement as aptamer sensing mechanism for thrombin detection, Sensors and Actuators B: Chemical https://doi.org/10.1016/j.snb.2018.04.039

Protein-induced fluorescence enhancement as aptamer sensing mechanism for thrombin detection

Saurabh Umrao[1], Vasundhara Jain[1,#], Anusha[1], Banani Chakraborty[1], Rahul Roy[1,2,3,*]

[1]Department of Chemical Engineering, [2]Molecular Biophysics Unit and [3]Center for Biosystems Science and Engineering, Indian Institute of Science, Bangalore, India 560012

Current Address: [#]Department of Biotechnology, Indian Institute of Technology, Roorkee, India 247667

[*]Corresponding author

E-mail: rahulroy@iisc.ac.in

Highlights

- A rapid and simple 'one-pot' homogenous fluorescence enhancement assay is used to detect thrombin protein.
- Applicability of protein-induced fluorescence enhancement (PIFE) for thrombin sensing is demonstrated without loss of sensitivity with respect to FRET.
- PIFE based thrombin detection displayed excellent linearity in the range of 0.25 pM-25 nM with a detection limit of 8.9 pM.
- Developed aptasensor displays high specificity and function in the presence of 'natural' backgrounds and various salt conditions.
- The proposed approach can be employed as a screening platform for DNA aptamers based protein sensing.

Abstract

Aptamer-based protein sensing can be implemented using a variety of optical and non-optical detection methods. In this report, we present protein-induced fluorescence enhancement (PIFE) based detection of DNA aptamer binding to thrombin. We demonstrate that PIFE reports on direct binding of thrombin to the aptamer strand carrying the fluorophore and hence is unaffected by salt based stabilization of the aptamer conformations as observed in fluorescence resonance energy transfer (FRET) assays. PIFE based thrombin detection displayed excellent linearity in the range of 0.25 pM-25 nM with a detection limit of 8.9 pM. In an alternate scheme, PIFE was demonstrated by placing the fluorophore on a connector strand thus bypassing the requirement to label the aptamer directly. This strategy allowed us to examine thrombin binding by variously modified thrombin aptamers and can serve as a platform for aptamer screening. We