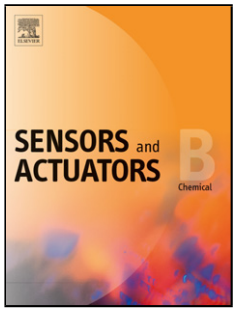

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PII: S0925-4005(17)31818-X
DOI: https://doi.org/10.1016/j.snb.2017.09.152
Reference: SNB 23243

To appear in: *Sensors and Actuators B*

Received date: 7-2-2017
Revised date: 20-9-2017
Accepted date: 21-9-2017

Please cite this article as: Paola Carullo, Marco Chino, Giovanni Paolo Cetrangolo, Sara Terreri, Angela Lombardi, Giuseppe Manco, Amelia Cimmino, Ferdinando Febbraio, Direct detection of organophosphate compounds in water by a fluorescence-based biosensing device, Sensors and Actuators B: Chemical https://doi.org/10.1016/j.snb.2017.09.152

Direct detection of organophosphate compounds in water by a fluorescence-based biosensing device

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Highlights

- A new mutant of Esterase 2 from *A. acidocaldarius* was designed and synthesized to be used as bioreceptor in a fluorescence-based biosensing device for the direct detection of organophosphate compounds.
- A mechanism of fluorescence quenching of IAEDANS-Esterase 2 complex in the presence of an organophosphate inhibitor, such as paraoxon, was described.
- A cell-flow system based on the fluorescence quenching of IAEDANS-Esterase 2 complex for a selective and sensitive detection of paraoxon in solution was developed.

Abstract: The main drawbacks in the use of acetylcholinesterase-based biosensors are their susceptibility to inhibition by too many chemicals, their limited time-stability, and the constant need for a supply of substrates for the measurements. In order to offset these deficiencies, we have addressed our studies towards the thermophilic esterase 2 from *A. acidocaldarious,* which shows a high specificity and affinity towards organophosphates and a high resistance under raw operative conditions. In particular, we have investigated the possibility of measuring the binding of organophosphates to the protein by using a fluorescent probe covalently linked near the active site. We have produced a mutant where the serine 35, a residue located at the entrance of the alcohol binding site, has been replaced by a cysteine residue. The addition of 1,5-IAEDANS as a fluorescent probe to the thiol group of the mutant-protein did not affect the capability of the enzyme to bind the paraoxon and its stability or instability over time. We have set up a continuous flow system based on a re-circulating solution of the probe-enzyme complex through a fluorimetric flow cell inside a spectrofluorimeter. The addition of paraoxon aliquots has been detected in real-time by measuring the fluorescence quenching of the probe-enzyme complex. The fluorescence signals, as well as the enzyme activity, were not affected by dilution and organic solvent addition. These results support the development of biosensing devices for the continuous monitoring of organophosphate compounds.