

Plasmon-Enhanced Fluorescence Detection of Blood Metabolites in Biosensors

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1 INTRODUCTION

Quick and precise detection of blood metabolites is a key parameter in monitoring and treating critically sick patients. Each analysis requires a certain amount of blood from the patient, and especially for babies in neonatal care repeated sampling can be life threatening. Therefore it is crucial to minimize the required amount of blood for analysis, which demands highly sensitive detectors. Optical detectors based on fluorescence possess high intrinsic sensitivity which by plasmonic signal enhancement can be increased even further.

Surface-enhanced fluorescence is a new technique utilizing the effect that certain metals can have on fluorophores. An excited state fluorophore in proximity of a such metal surface can interact with free electrons in the metal surface. A metal-induced emission pathway is introduced for the decay from the excited state to the ground state, which leads to increases in the quantum yield as well as decreases in excited state lifetimes. This means that the fluorescence signal can be significantly increased. [1, 2]

Blood L-lactate is a clinically valuable diagnostic indicator and there is considerable medical interest in measurements of blood lactate. Elevated concentrations of blood lactate are indicators of a considerable number of medical conditions. As examples, serum lactate levels are predictive of survival in children after open heart surgery [3] and mortality in ventilated infants [4] and may be preferable to pH for evaluating fetal intrapartum asphyxia [5]. In adults elevated blood lactate can predict multiple organ failure and death in patients with septic shock [6] and the function known to accompany decreased tissue oxygenation, hypovolemic, left ventricular failure, and drug toxicity [7]. Measurement of blood lactate is also valuable for monitoring the results of exercise and athletic performance [8]. D-Lactate is not produced by humans, and is not found in the blood except in the presence of unusual intestinal bacteria. L-lactate determinations are typically performed by enzymatic oxidation to pyruvate by lactate dehydrogenase or lactate oxidase, followed by detection of NADH or H_2O_2 , respectively [4, 9–11].

2 EXPERIMENTAL MOTIVATION

In the present work we report the possibility of monitoring lactate oxidase activity by spectral changes due to the presence or absence of L-lactate due to different oxidation states in the flavinmononucleotide (FMN) cofactor. The investigation encompass emission properties including lifetimes and spectral resolution of the free-space - as well as plasmon-enhanced emission performed with a streak camera with picosecond resolution. Studies of the excited state absorption using the pump-probe will also be reported. The improved understanding of the kinetics of the excited state is essential for establishing a comprehensive spectroscopic knowledge about the function of lactate oxidase, which is essential for optimising biosensor applications.

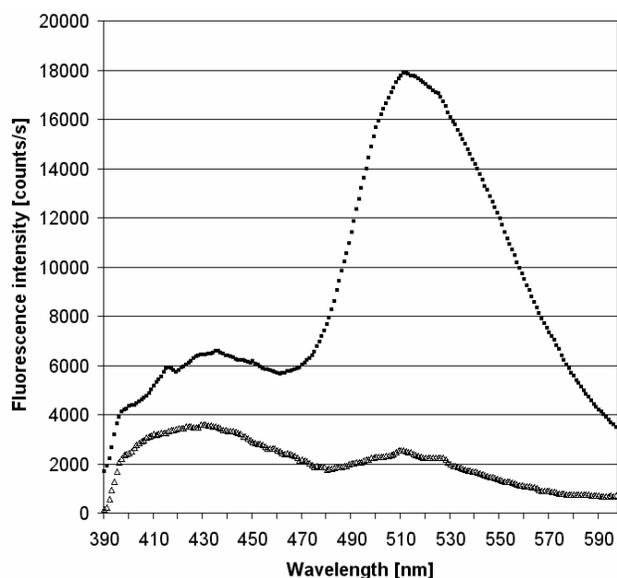


Figure 1: Fluorescence spectra of lactate oxidase (Δ) and lactate oxidase in the presence of L-lactate (\blacksquare). FMN in the active site is excited at 345 nm and emission was collected between 390 nm and 600 nm. The peak around 429 nm originates from the oxidized FMN and the peak around 512 nm comes from the protonated semiquinone FMN. It can clearly be seen that the intensity of the protonated semiquinone FMN is increased dramatically after L-lactate is added.

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