

## Fiber Optic SPR biosensor for progesterone detection in raw milk as a tool to improve reproduction

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In the last 15 years, the improved milk yields of dairy cows have been accompanied by a decreased fertility which is visible in the increased calving interval. Accurate estrous detection is crucial in reproductive management. The currently used methods of heat detection such as visual observation or activity sensors are not adequate due to a higher rate of false negatives. Detection of progesterone, a key hormone in the regulation of the estrous cycle, has the advantage of determining heat in combination with the ability of early diagnosis of pregnancy or abnormalities in estrous cycle.

In this work, a biosensor that quantifies progesterone in milk is developed. The bioassay is based on a fiber optic surface plasmon resonance (FO-SPR) sensor that was previously developed in our group as a cost-effective alternative for the expensive and more complex SPR systems used in research centers. Therein, the platform was evaluated for the detection of proteins and detection of single nucleotide polymorphisms (SNPs) using antibody- and DNA-based bioassays, respectively. The FO-SPR biosensor can perform fast, specific and sensitive detections.

For the detection of progesterone a competitive immunoassay is developed, which is needed for detection of low molecular weight molecules since the sensitivity of SPR sensors is determined by changes in weight near the sensor surface. In the competitive assay, a detection antibody is bound either to a progesterone molecule from a sample or its derivative that is immobilized on the sensor surface (Figure 1A). To improve the limit of detection, the signal of the bound detection antibody is amplified by use of gold nanoparticles (Au NPs) functionalized with a secondary antibody. This competitive bioassay showed a detection limit of 0.23 ng/ml in half diluted raw milk (Figure 1B) and the obtained dynamic range is compatible with the expected concentration range of 0.5-10 ng/ml in undiluted raw milk.

Future work will focus on reducing the assay time and increasing the reusability of the sensor. The final goal is to integrate this sensor with a sampling device in order to make the interaction with a milking robot and allow automated at-line measurements in a dairy farm.

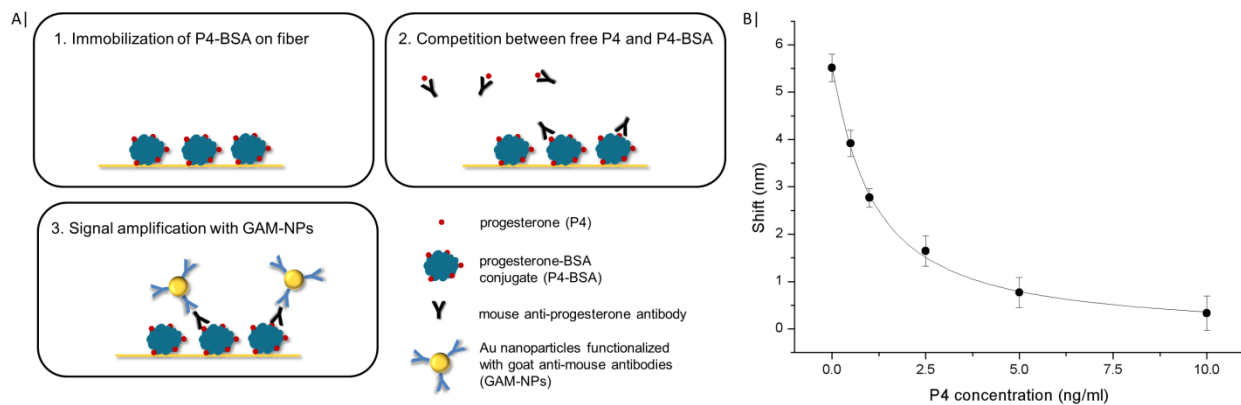


Figure 1: A | Scheme of the competitive assay for the detection of the small molecule progesterone. B | Calibration curve of progesterone detection in half diluted raw milk.