

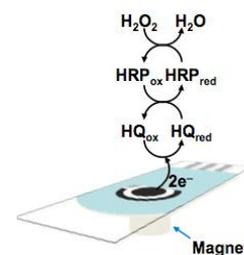
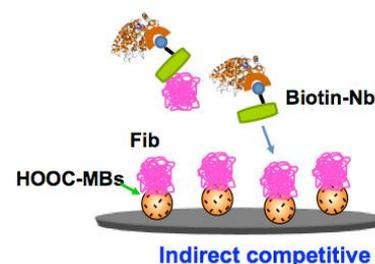
Nanobody-based electrochemical immunosensor for fibrinogen quantification in plasma

The research Group lead by Dr. Luis Ángel Fernández at The National Center for Biotechnology (CNB-CSIC), in collaboration with scientists from Complutense University of Madrid, has developed a fibrinogen immunosensing device based on the use of magnetic beads. The free fibrinogen in solution and immobilized fibrinogen compete for binding to a fixed amount of the specific biotinylated nanobody. Labeling of captured biotinylated nanobody was made with streptavidin-HRP. The magnetic beads are then captured by a magnet on the surface of screen-printed carbon electrodes. Amperometric detection is accomplished at -0.20V by measuring the catalytic current arising upon addition of H_2O_2 and using hydroquinone as redox mediator in solution. Industrial partners interested in a product license are being sought.

An offer for product license

Abnormal high fibrinogen levels in plasma are associated with cardiovascular diseases, whereas abnormal low concentrations are associated with risk of bleeding

Nanobodies are single-domain antibody fragments formed by the variable domain of heavy-chain-only antibodies found in camelids, also known as VHH. These single domain antibodies constitute the smallest domains of natural antibodies having full antigen-binding capacity (molecular weight ranging between 12 and 14 KDa) and have demonstrated to be an advantageous alternative to monoclonal antibodies. Fibrinogen is a 340 KDa plasmatic glycoprotein produced in the liver. It is involved in the final step of blood coagulation. Abnormal fibrinogen concentration in blood has been reported to be associated with cardiovascular diseases, venous thrombosis or myocardial infarction. The reference levels of fibrinogen in plasma are between 1.5 and 4.5 mg ml^{-1} . Lower protein concentration indicates risk of bleeding, whereas higher concentrations reveal an important risk for ischemic vascular or coronary accidents. Due to its clinical importance, fast, reliable and reproducible methods for the accurate determination are in great demand.



Biotinylated nanobody is captured on magnetic beads. They are then captured by a magnet on the surface of electrodes. Amperometric detection is accomplished by measuring the current upon addition of H_2O_2 .

Main innovations and advantages

- The assay can be carrying out in diluted plasma samples in a total analysis time of 90 minutes and has a detection limit of $0.044 \mu\text{g ml}^{-1}$ of fibrinogen. It only needs $0.01 \mu\text{l}$ of plasma.
- The biosensor exhibits excellent analytical performance in terms of sensitivity, selectivity, wide range of quantifiable antigen concentrations and inter-assay reproducibility.
- It has been successfully employed for the analysis of a commercial plasma sample with certified fibrinogen content.

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