

Homogeneous High-sensitivity CRP assay on MagArray Biosensors

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Abstract

Background: MagArray platform is based on the detection of magnetic particles as labels in bioassays. In contrast to systems based on optical signals, magnetic signals are not affected by the common optical interference in complex matrices. In addition, since the biosensors are designed to detect magnetic particles only when particles are bound or captured to the sensor surface, this proximity detection mechanism allows the possibility of homogeneous immunoassays. We report here that we have developed a one-pot homogeneous assay for CRP with high sensitivity. We wish to demonstrate MagArray platform is well suited for homogeneous assays that require both simplicity and sensitivity.

Objective: A feasibility study of fast detection of CRP in serum samples in a homogeneous magnetic immunoassay on magnetic biosensors.

Methods: Antibody pairs for CRP assay were screened and selected on MagArray platform, and the detection antibody is conjugated to magnetic particles for both capping CRP and generating signals. The assay consists of two simple steps of mixing the magnetic particles with serum sample and addition of the mixture to magnetic sensors. Signals can be read in as short as 2 min for a result, and more accurate results can be obtained after 5 min. The whole process requires no shaking and rinsing or other separation steps. Standard curves of CRP in both pure buffer and sera were established and compared.

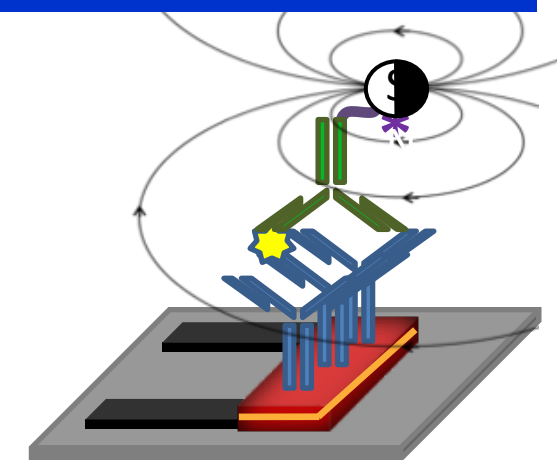
Results: The detection sensitivity of CRP in serum on MagArray platform is less than 1 mg/L for a homogeneous assay. The CVs for lower concentrations are less than 10% and less than 5% for medium and high concentrations. No prozone effect was observed for CRP concentrations of up to 2000 mg/L. Our preliminary tests showed no interference effects ($\leq 10\%$) from lipids, HAMA and Rheumatoid Factor.

Conclusions: MagArray platform provides a unique opportunity of detecting proteins in a simple and homogeneous fashion. Since this assay only involves mixing and adding reagents to the biosensors, the complexity of the assay format is greatly reduced. The detection of magnetic signals from magnetic particles in proximity is a key to applying MagArray platform for a homogeneous immunoassay.

Introduction

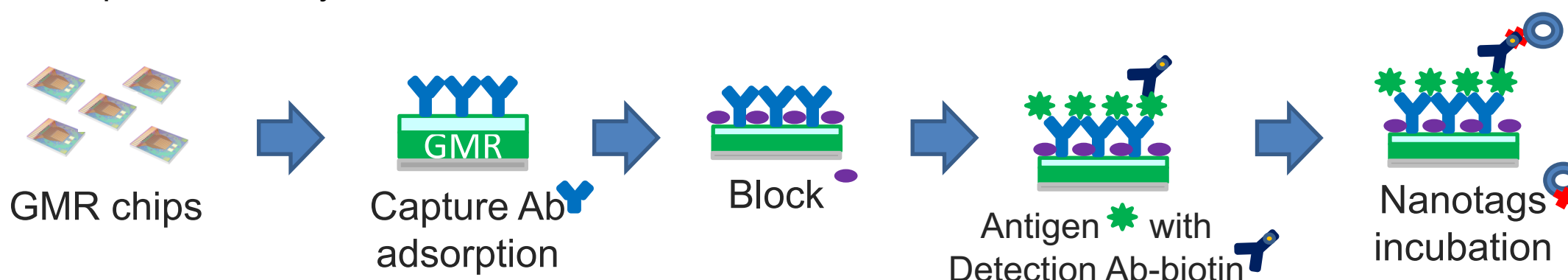
1. Principle of Operation: Case of a sandwich immunoassay

- 1) Spot a unique capture antibody over each sensor
- 2) Incubate with fluid of Interest : blood, plasma, serum, urine etc.
- 3) Add biotinylated detection antibodies
- 4) Add nanotags and detect magnetic signals - but only from surface-bound particles!



Method and Assay Format

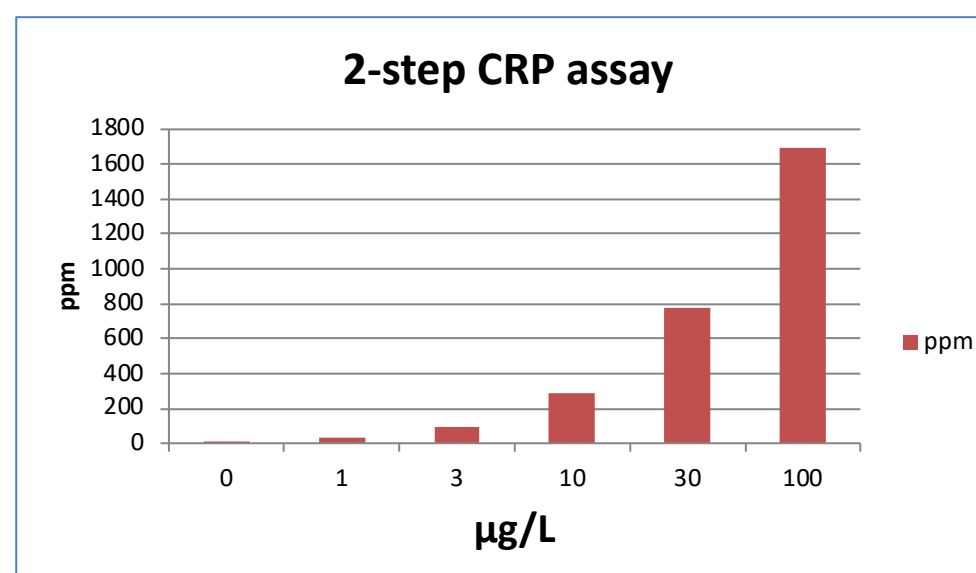
Sample measurements: detection antibodies were mixed with samples and the mixtures were added to the reaction wells on the chips and shaken for 8 min. Magnetic particles were then added to the chips for signal generation. The measurements were run for 15min in order to study the signal-time relationship. The whole assay involved no rinsing procedures and took less than 15 min in optimized assays.



Results

2-step CRP assay:

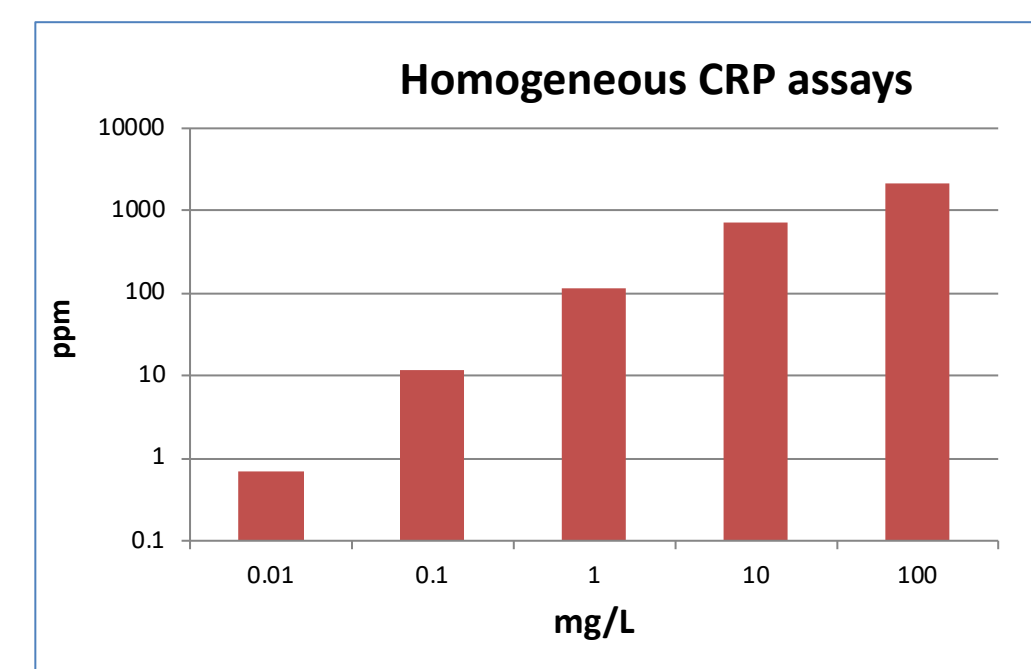
- detection antibodies were mixed with samples
- 5 min reaction
- magnetic nanotags added for signal generation and recording
- Assuming a 1:100 dilution of samples, sensitivity around 0.1 mg/L
- 20 min assay with washing step



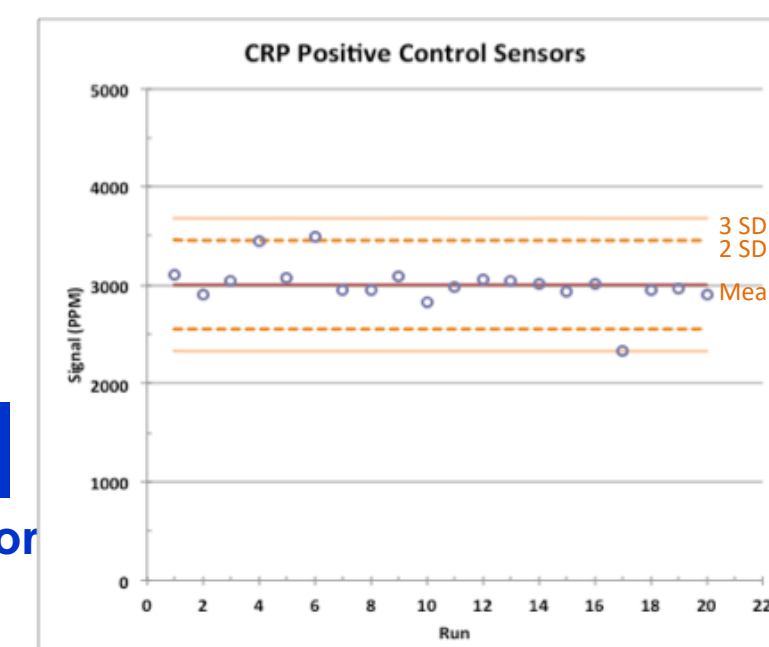
Homogeneous CRP assay:

- detection antibody coated with magnetic nanotags
- The nanotags mixed together with the sample and reacted with coated GMR chips
- Signals generation and recording
- Assuming a 1:10 dilution of samples, sensitivity around 1mg/L

- 10 min assay with no washing



Built-in positive control sensors show good reproducibility



Conclusions

MagArray biosensor is a simple and versatile platform for either wash-free or regular assay for sensitive protein detection.