Fe(III)-MimochromeVI*a for the development of sensitive lateral flow immunoassays

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Lateral flow immunoassays (LFiAs) are point-of-care tests commonly used around the world thanks to their reliability, sensitivity, and specificity. When the output of the assay is a colorimetric signal, gold nanoparticles (AuNPs) are often used because of their high surface-to-volume ratio and naked-eye visible red color (when 20 nm in diameter, as is most commonly used).[1] Despite many advantages, the LFiAs lack the sensitivity required to detect many desirable biomarkers, which currently have to be detected with laboratory assays. Recent studies have therefore focused on developing signal enhancement strategies, including the use of enzyme catalyzed reactions.[2]

Herein, the artificial miniaturized peroxidase Fe(III)-MimochromeVI*a (FeMC6*a) is exploited as a strategy to obtain catalytic signal amplification in sandwich immunoassays on lateral flow strips. The here developed Human-IgG assay foresees the use of AuNPs decorated with both FeMC6*a and anti-Human-IgG as a detection antibody. After AuNPs focusing over the test line, subsequent addition and catalytic oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) by FeMC6*a induces an increase of the test line color (Figure 1).

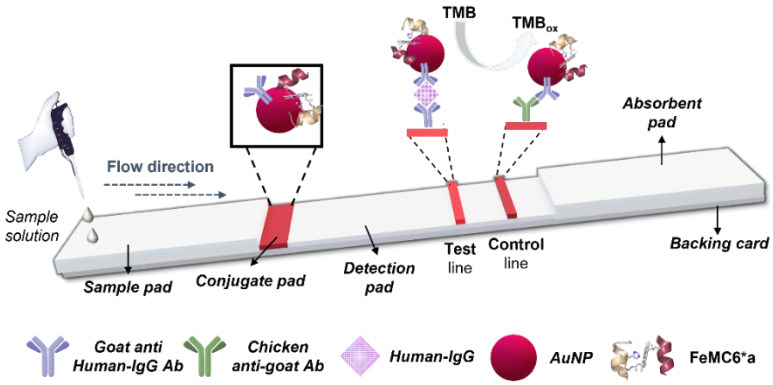

Figure 1. Schematic representation (not to scale) of FeMC6*a –assisted lateral flow two-step immunoassay for Human-IgG detection.

Our results show that FeMC6*a acts as an efficient catalyst on paper, increasing the sensitivity of LFiA up to 4 times with respect to a conventional LFiA. Furthermore, FeMC6*a achieved lower limits of detection that were found in control experiments horseradish peroxidase, its natural counterpart. This study represents a significant proof-of-concept for the development of more sensitive LFiAs, for different analytes, based on properly designed artificial metalloenzymes.

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2. Calabria, D., Calabretta, M. M., Zangheri, M., Marchegiani, E., Trozzi, I., Guardigli, M., Michelini, E., Di Nardo, F., Anfossi, L., Baggiani, C., Mirasoli, M. *Sensors* **2021**, *21* (10), 3358-3377.