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Tesis:	" FLUORESCENCE QUENCHING-BASED IMMUNOSENSING PLATFORM FOR THE DETECTION OF CLINICALLY RELEVANT PROTEINS"

Resumen:

Immunoassays are nowadays a crucial tool for diagnostics, drug development, and environmental monitoring. However, most immunoassay methods involve cumbersome procedures such as washing, blocking, or separation steps, and in general, require many bioreagents for their operation. In this thesis, "a single-step immunosensing platform based on the fluorescence quenching capability of graphene oxide (GO) and the versatile format offered by the famous 96 microwell plates was developed". This approach was used for the detection of clinically relevant proteins such as Human immunoglobulin G (H-IgG), Prostate Specific Antigen (PSA), and Heat Shock Protein 72 (Hsp72). Our immunosensing method exploits a single antibody conjugated with a fluorophore (F-Ab).

The fluorescence intensity of F-Ab (a donor) can be strongly quenched when F-Ab is incubated within GO-covered microwell surfaces (an acceptor) via non-radiative energy transfer. However, when the analyte is added, the formation of immunocomplexes involves non-covalent intermolecular interactions such as hydrogen bonding, electrostatic interactions, hydrophobic, and Van der Waals forces. We hypothesize that such intermolecular interactions hinder the affinity between immunocomplexes and GO-covered microwell surfaces, and as a consequence, in the proposed biosensing platform, the aforementioned non-radiative energy transfer is not observable upon immunocomplexes formation. Moreover, we proved that the studied immunosensing platform can be successfully employed to monitor the association process occurring between proteins, and therefore, determine binding kinetic constants whose values obtained match with those values reported in the literature and by the respective suppliers. All in all, we developed a single-step immunosensing platform where the biodetection can be monitored in real-time, avoiding cumbersome procedures like washing, separation, or blocking steps, and it is a relatively cost-effective system as the assay is around 0.47 USD per test at the laboratory scale.

Furthermore, our outstanding immunosensing platform is a potential method for the clinical diagnosis of prostate cancer and acute kidney injury, as demonstrated here via the analysis of real samples. In addition, as the association process is monitored, this technology can be used for the determination of the antibody affinity, or in biochemistry studies where the understanding of structure/function relationships are in function of protein-protein interactions, or even in the validation of potential biomarkers where binding kinetic parameters are relevant.