

# Sticky cellulose hydrogel as reusable a platform for a SERS based immunoassay

Maria João Oliveira<sup>1,2</sup>, Inês Cunha<sup>1</sup>, Miguel P. de Almeida<sup>3</sup>, Elvira Fortunato<sup>2</sup>, Rodrigo Martins<sup>2</sup>, Eulália Pereira<sup>3</sup>, Luís Pereira<sup>1</sup>, Hugh J. Byrne<sup>4</sup>, Ricardo Franco<sup>2\*</sup>, and Hugo Águas<sup>1\*</sup>

<sup>1</sup>CENIMAT-I3N, Departamento de Ciência dos Materiais, Faculdade de Ciências e Tecnologia, FCT, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; Daniela.gomes@fct.unl.pt (D.G.); emf@fct.unl.pt (E.F.); rfpm@fct.unl.pt (R.M.)

<sup>2</sup>UCIBIO, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; mj.oliveira@campus.fct.unl.pt (M.J.O.); r.palomares@fct.unl.pt (R.J.)

<sup>3</sup>REQUIMTE/LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, 4169-007 Porto, Portugal; mpda@fc.up.pt (M.P. de A.); eulalia.pereira@fc.up.pt (E.P.)

<sup>4</sup>FOCAS Research Institute, Technological University Dublin, Kevin Street, Dublin 8, Ireland. [Hugh.Byrne@tudublin.ie](mailto:Hugh.Byrne@tudublin.ie) (H.B.)

\* Correspondence: ricardo.franco@fct.unl.pt (R.F.); hma@fct.unl.pt (H.A.)

## Abstract

Immunoassays using Surface-Enhanced Raman Spectroscopy (SERS) are especially interesting on account not only of their increased sensitivity, but also of their easy translation to point-of-need formats. The basis for these assays are bioconjugates of polyclonal antibodies and anisotropic gold nanoparticles functionalised with a Raman reporter. These bioconjugates, once loaded with the antigen analyte, can react in a sandwich format with the same antibodies linked to a surface that is used for detection on a microfluidics or immunochromatographic platform. The detection success depends upon the binding event, thus an appropriate immobilisation of antibodies necessary for subsequent reaction on the selected surface is mandatory. Here, we used bioconjugates of gold nanostars functionalised with 4-mercaptobenzoic acid, and anti-horseradish peroxidase antibodies and a sticky cellulose-based membrane. All steps of bioconjugate formation were fully characterised by Ultraviolet-Visible Spectroscopy, Dynamic Light Scattering, Scanning Electron Microscopy and Agarose Gel Electrophoresis [1]. The bioconjugates, together with the sticky cellulose-based membrane functionalised with anti-horseradish peroxidase antibodies, were used for SERS applications, resulting in an immunoassay with demonstrated functionality for antigen detection. The sandwich immunoassay platform proved to be able to detect the immunocomplex formed and perform a multiplex assay through direct classical least squares method application [2]. The cellulose-based membrane allowed a good uniformity of SERS signal, and a good reproducibility when different functionalisation batches were tested (Relative Standard Deviation of 13%). Furthermore, these SERS platforms remained stable after 168h of storage and presented a regeneration capability of seven cycles. Since the antibody used was a generic IgG antibody, the subjacent principle of this platform can be applied to screen detection assays of other antibody-antigen systems.

## REFERENCES

- [1] Oliveira, M. J.; P. de Almeida, M.; Nunes, D.; Fortunato, E.; Martins, R.; Pereira, E.; J. Byrne, H.; Águas, H.; Franco, R., *Nanomaterials*, 9 (2019), 1561
- [2] Keating, M. E.; Bonnier, F.; Byrne, H. J., *Analyst*, 137 (2012) 5792–5802

## ACKNOWLEDGEMENTS

This work was supported by the Applied Molecular Biosciences Unit - UCIBIO and Associate Laboratory for Green Chemistry - LAQV which are financed by national funds from FCT/MCTES (UIDB/04378/2020 and UIDB/50006/2020), and grant PTDC/NAN-MAT/30589/2017. This research was also funded by Fundação para a Ciência e a Tecnologia (MCTES funds, Portugal) and European Union (European Social Fund and European Regional Development Fund), through grants UID/Multi/04378/2019 and UID/CTM/50025/2019 and POCI-01-0145-FEDER-007688 CENIMAT/i3N); and fellowship SFRH/BD/132057/2017, all from COMPETE and COMPETE 2020 programs and MIT Portugal PhD Program.