



Ultra-small octahedral PtNP-labeled antibodies as an ultrasensitive nanozyme probe for chemiluminescence detection in bioanalytics

Mauro Moglianetti¹, Luisa Stella Dolci², Barbara Roda^{2,3}, [Anna Scarsi](mailto:anna.scarsi@iit.it)^{4,5}, Valentina Marassi³, Deborah Pedone⁴, Pier Paolo Pompa⁴, Aldo Roda²

¹ HiQ-Nano s.r.l., Via Barsanti 73010 Arnesano (LE)

² INBB – Biostructures and Biosystems National Institute-Rome, Italy

³ Department of Chemistry G. Ciamician, Alma Mater Studiorum-University of Bologna, Italy

⁴ Nanobiointeractions & Nanodiagnostics, Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova, Italy

⁵ Department of Chemistry and Industrial Chemistry, University of Genova, 16146 Genova, Italy

anna.scarsi@iit.it

Keywords: ultrasensitive nanozyme CL-probe, antibody conjugated Pt-nanocrystal, PtNPs in bioassay

Chemiluminescence (CL)-based probes are one of the most sensitive detection principles in nanodiagnostics. A widely used system is the CL substrate for peroxidase (HRP) employed to label a variety of molecules ranging from small steroids to protein. The CL cocktail for HRP is based on luminol or its analogues, hydrogen peroxide, and an enhancer, allowing the detection of submolar concentrations of the enzyme-labeled analyte. The catalyst of the CL reaction, namely HRP, can be replaced by other more practical and highly stable nano-catalysts/nanozymes; among them, recent studies have demonstrated the superior performance of Pt nanomaterials [1]. In this work, we propose the use of ultra-small (3 nm) citrate-coated octahedral Pt nanocrystals prepared by a new wet chemical reduction method in aqueous environment and conjugated to a secondary human IgG antibody, as an ultrasensitive probe for luminol/hydrogen peroxide CL detection. Conjugates with different nanocrystal-to-antibody molar ratios were first fully characterized and purified by Field-Flow Fractionation (FFF) [2]. FFF confirms the homogeneous size of the conjugated which represents a fundamental parameter for their efficiency. The FFF-selected purified conjugates are homogeneous in size and highly concentrated, and readily available for downstream CL tests, an important requirement for the use of FFF as semi-prep step. First results demonstrate the applicability of Pt nanocrystals as probes for CL detection. Indeed the Pt nanocrystals-antibodies CL signal has been measured for the different nanoparticles-IgG molar ratios, showing an increasing signal as a function of nanoparticles concentration with the possibility to detect IgG down to 10^{-12} M, value close to that obtained using HRP [3]. In addition, the light signal reaches a steady state value for more than 30 minutes, thus facilitating the assay handling. These results paves the way to the use of Pt nanomaterials, highly monodisperse in size and shape and with easy-to-remove coating, for the production of highly efficient catalysts/nanozymes for CL applications and the development of simple and rapid new tests.

References

- 1 M. Moglianetti, E. De Luca, D. Pedone, R. Marotta, T. Catelani, B. Sartori, H. Amenitsch, S. F. Retta and P. P. Pompa, *Nanoscale*, 2016, **8**, 3739-3752
- 2 V. Marassi, D. Calabria, I. Trozzi, A. Zattoni, P. Reschiglian and B. Roda, *Journal of Chromatography A*, 2021, **1636**, 461739
- 3 A. Roda, F. Arduini, M. Mirasoli, M. Zangheri, L. Fabiani, N. Colozza, E. Marchegiani, P. Simoni and D. Moscone, *Biosensors and Bioelectronics*, 2020, **155**, 112093