A new class of Thermochemiluminescent Polymer Nanoparticles as biosensors for ultrasensitive immunoassay applications

Luca Andronico*[1], Arianna Quintavalla[1], Marco Lombardo[1], Mara Mirasoli[1], Donato Calabria[2], Massimo Guardigli[1], Claudio Trombini[1], Daniel Chiu[3], and Aldo Roda[1]

[1]Department of Chemistry "Giacomo Ciamician", Alma Mater Studiorum University of Bologna – Italy
[2]Interdepartmental Center of Industrial Research (CIRI) – Energy and Environment, Alma Mater Studiorum, University of Bologna, Bologna, Italy – Italy
[3]Department of Chemistry, University of Washington, Seattle – United States

Abstract

Thermochemiluminescent (TCL) molecules are particularly advantageous in bioanalyses, as they enable reagentless chemiluminescence detection, since only a thermal trigger is required to elicit emission. Nevertheless, inclusion in nanoparticles is required to obtain signal amplification and sufficient TCL molecule stability over time [1]. Herein, we combined the Semiconductive Polymer Dots (Pdots) technology [2] with TCL based detection methods to synthesize new TCL nanoprobes (TCL-Pdots) suitable for fast and ultrasensitive immunoassay development.
Following a nanoprecipitation method, we easily obtained quite monodisperse TCL-Pdots (42 nm), employing CN-PPV as the polymer matrix and an acridin-1,2-dioxetane derivative as TCL substrate. Subsequently, NPs were functionalized with Streptavidin (SA) to generate universally applicable TCL nanolabels suitable for Biotinylated-antibody detection. The inclusion in nanoparticles remarkably increased the TCL label stability over time, both under storage conditions and during immunoassay execution.

Exploiting a highly efficient RET mechanism (F = 90%) occurring between thermally triggered 1,2-dioxetane derivative and the surrounding polymer matrix, the emission of TCL molecule was red-shifted (from 400 to 550 nm) and intensified thanks to the higher fluorescence of CN-PPV polymer.

The TCL-nanoprobes were tested in a model non-competitive immunoassay for IgG detection. Upon immunoassay completion, a heat shock (90∘C) was generated to trigger the TCL signal, which was proportional to the analyte concentration (LOD 13 nM).

TCL-Pdots based systems represent a new powerful luminescent probe for (bio)analytical analyses. Combining the high light-harvesting capacity, biocompatibility, and tunability of Pdots with the reagentless thermally-triggered light generation of TCL, a broad panel of ultrabright TCL nanoprobes could be designed for development of high sensitive ultra-fast immunoassays.

*Speaker

Roda, A.; Di Fusco, M.; Quintavalla, A.; Guardigli, M.; Mirasoli, M.; Lombardo, M.; Trombini, C. Analytical Chemistry 2012, 84, 9913–9919;
Wu, C.; Chiu, D. T. Angewandte Chemie International Edition 2013, 52, 3086–3109.

Keywords: Analytical detection method, 1, 2, dioxetane, Thermochemiluminescence, Semiconductive Polymer Dots, Immunoassay