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KC, Sanjaya ; Ranzoni, Andrea; Phetsang, Wanida; Hansen, Mikkel Fougt; Cooper, Matthew A.

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Title:

Composite magnetic-fluorescent nanoparticles for bioassays: CRP

Authors & affiliations:

Sanjaya KC^a, Andrea Ranzoni^a, Wanida Phetsang^a, Mikkel Fougth Hansen^b, Matthew A. Cooper^a

a) Institute for Molecular Bioscience, 306 Carmody Road, The University of Queensland, Brisbane 4072, QLD, Australia ; b) Department of Micro- and Nanotechnology, Technical University of Denmark, DTU Nanotech, Building 345 East, DK-2800 Kongens Lyngby, Denmark

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1. The title should be as brief as possible but long enough to indicate clearly the nature of the study. Capitalise the first letter of the first word **ONLY** (place names excluded). No full stop at the end.

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Nanoparticle-based sandwich assays, have successfully been used to detect biomarkers from complex biological fluids. We aim to exploit the catalytic properties of magnetic nanoparticles for extracting the target from complex biological fluids and simultaneous fluorescent labelling by replacing conventional magnetic nanoparticles with in-house synthesized hybrid magnetic-fluorescent nanoparticles. Polystyrene particles are doped with ferromagnetic grains and capped with a hydrophilic polymer that enables immobilization of reporter probes and the fluorescent agent (quantum dots, organic dye and HRP enzyme). Composite particles were prepared and tested on a model assay to detect exemplar biomarker CRP, clinically relevant for detection of inflammation.

Three different types of hybrid constructs were prepared; HY_QD, HY_FP and HY_HRP with magnetic core and quantum dots, fluorescent probe and HRP enzyme respectively on the outer polymer mesh. Iron grains (5 nm; Sigma Aldrich) were doped into polystyrene particles (200 nm; Polysciences Inc) to generate a magnetic core nanoparticle and further used to generate a polymer multi-layer trapping within quantum dots (Em: 750 nm; LifeTech™®), thus generating a hybrid nanoparticle. The polymer brush is loosely arranged in a hydrogel on the surface of the particle entrapping the QDs and hence facilitated increasing the fluorescent intensity of the particles.

1 µl of particle was added into 100 µl of serum spiked with CRP and incubated for 1 hour at room temperature. The particles were magnetically separated from serum and washed. These nanoparticles were then added to the wells of 96 well microplates (coated with anti-CRP antibodies) and incubated further for 1 hour. The final readout was performed on plate reader after removing the unbound particles by washing. The limit of detection was 320 pg/ml for HY_QDs and HY_FP and 10 µg/ml for HY_HRP.

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