

**GOLD SHELL-ISOLATED NANOSTARS CONJUGATED WITH
DIMETHYLMETHYLENE BLUE FOR DIAGNOSIS OF OVEREXPRESSED
PROTEINS IN BREAST CANCER CELLS**

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Breast cancer remains one of the most prevalent and lethal diseases worldwide, with high mortality rates strongly associated with late-stage diagnosis and metastatic progression. Despite advances in imaging and molecular diagnostics, current approaches still rely on invasive procedures, exogenous markers and techniques with limited sensitivity, particularly for deep-tissue detection. In this context, nanoplateforms based on plasmonic nanostructures have emerged as promising alternatives for the development of more sensitive, selective and minimally invasive diagnostic strategies. Gold shell-isolated nanostars (AuNS@SiO₂) are particularly attractive for biomedical applications due to their biocompatibility, localized surface plasmon resonance (LSPR) in the

near-infrared region, and strong electromagnetic field enhancement at their sharp tips, which can enhance optical phenomena such as fluorescence and photothermal conversion. In this work, we report the development and characterization of a nanoplatform composed of gold shell-isolated nanostars conjugated with the photosensitizer dimethylmethylen blue (DMMB), forming a sandwich-like architecture (AuNS@SiO₂-DMMB@SiO₂). Gold nanostars (AuNS) were synthesized via a seed-mediated growth method, yielding nanostructures with LSPR band centered at approximately 732 nm, spectrally overlapping with the absorption and emission bands of DMMB. The photosensitizer was covalently conjugated between the two silica shells of the nanoplatform, enabling control of the distance between the gold nanostars and DMMB while preventing fluorescence quenching. The resulting nanostructures were characterized by UV-Vis absorption spectroscopy, dynamic light scattering (DLS), and zeta potential measurements. The maximum LSPR band shifted from 732 nm for AuNS to 678 nm after the first silica coating (AuNS@SiO₂) and to 680 nm following DMMB conjugation and the second silica shell. The average hydrodynamic diameter increased from 47.87 ± 0.87 nm for AuNS to 73.99 ± 0.34 nm for AuNS@SiO₂, reaching 110.1 ± 1.5 nm for the nanoplatform AuNS@SiO₂-DMMB@SiO₂, confirming the successful formation of successive silica shells. Surface charge analysis revealed a transition in zeta potential from -21.38 ± 0.87 mV for AuNS to +24.35 ± 1.1 mV after the first silica coating, with a further increase to +31.2 ± 0.84 mV after DMMB conjugation and the second silica shell, indicating enriched colloidal stability for AuNS@SiO₂-DMMB@SiO₂. Overall, these results demonstrate the successful synthesis of a stable and optically active nanoplatform with controlled architecture, and a potential for fluorescence enhancement. This system provides a robust foundation for subsequent functionalization with targeting antibodies and hold strong potential for the highly sensitive detection of proteins overexpressed in breast cancer cells, contributing to the advancement of early and noninvasive diagnostic strategies.

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Palavras-chave: gold shell-isolated nanostars; fluorescence enhancement; dimethylmethylen blue; breast cancer diagnostic.