Supporting Information

Nanoparticle-based colorimetric biosensor for detection of telomerase activity based on peroxidase mimicking activity of nanogold

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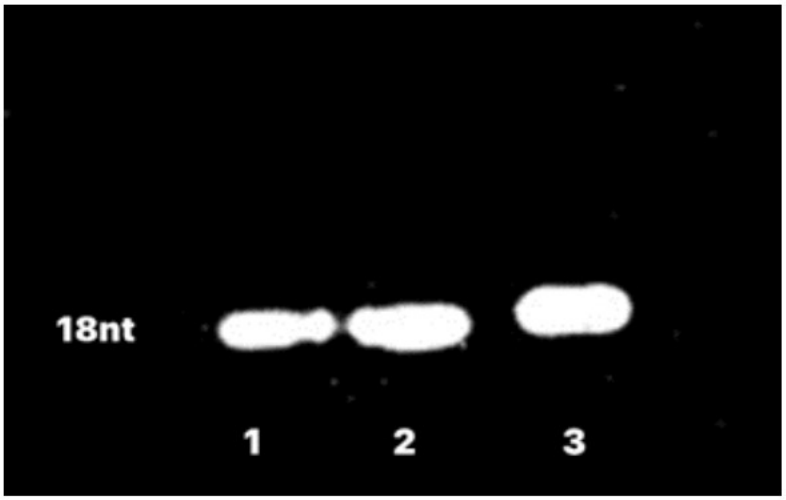

Figure S1: Analysis of the capacity of the TS primer and TS complementary primer to generate a duplex DNA using agarose gel electrophoresis. TS primer (lane 1), TS complementary primer (lane 2), and duplex DNA derived from TS primer and TS complementary primer (lane 3).

The TS primer (100 µM) was hybridized with the TS complementary primer (100 µM) by heating at 90°C for 2 min, followed by incubation at 60°C for 30 min to allow primer extension. The migration positions of the individual primers and the TS primer-TS complementary primer duplex were visualized under UV illumination using a Western blot imaging system after electrophoresis on a 1% agarose gel at 100 V for 35 min. Gel electrophoresis results show that the TS primer and TS complementary primer migrated to the same position, consistent with their identical predicted length of 18 nucleotides. In contrast, the hybridized duplex product in lane 3 displayed a higher-molecular-weight band, reflecting its double-stranded structure, which migrated more slowly through the gel.

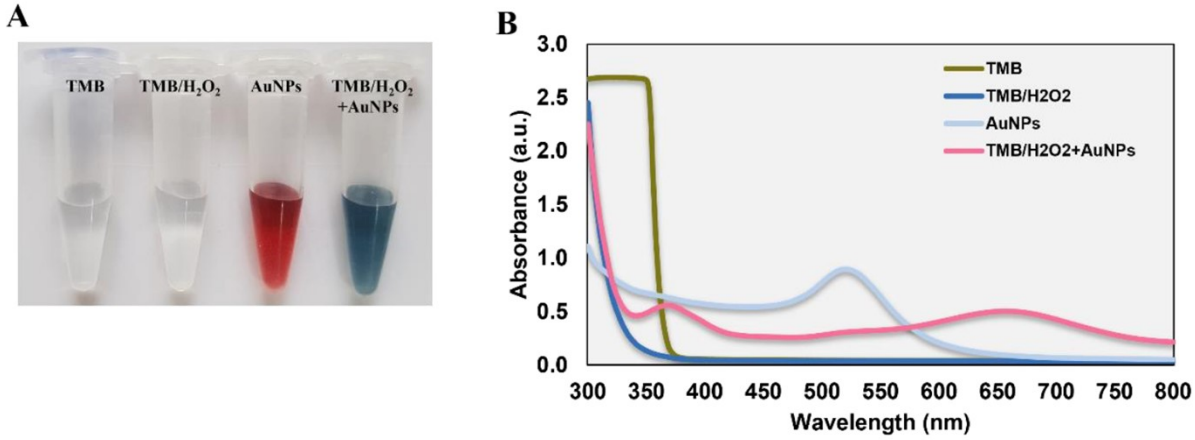

Figure S2: (A) Color photographs of the catalyzed reaction of 1.0 mM TMB/0.3% H_2O_2 solution in the presence and absence of AuNPs. (B) UV-Vis spectroscopic analysis of the catalyzed reaction of TMB/H_2O_2 in the absence and presence of AuNPs.

The peroxidase-like catalytic activity of AuNPs, acting as effective nanozyme mimetics, was verified as shown in Figure S2. In the presence of 1 mM TMB and 0.3% H_2O_2, a distinct blue coloration was observed only when AuNPs were present, confirming their ability to catalyze the oxidation of TMB via a peroxidase-mimicking mechanism. In contrast, solutions containing TMB alone or TMB/H_2O_2 in the absence of AuNPs remained colorless, indicating that spontaneous oxidation of TMB does not occur under these conditions.

UV-Vis spectroscopic analysis further supports this conclusion. Upon addition of the TMB/H_2O_2 substrate to AuNPs, two characteristic absorption peaks at approximately 370 nm and 650 nm appeared (Figure S2B), corresponding to the formation of oxidized TMB. These spectral features were absent in all control samples lacking AuNPs, confirming that the observed colorimetric response originates from AuNP-catalyzed oxidation rather than nonspecific or matrix-driven reactions.

The hydrogen peroxide concentration was set at 0.3% H_2O_2, which is consistent with commonly reported conditions for AuNP-based nanozyme assays and was selected to balance catalytic efficiency with assay specificity. Higher H_2O_2 concentrations are known to increase the risk of nonspecific TMB oxidation, particularly in complex biological matrices such as cell lysates. Under the optimized condition of 0.3% H_2O_2, no background oxidation was observed in control experiments (TMB/H_2O_2 without AuNPs or lysate), ensuring that the colorimetric signal arises exclusively from AuNP nanozyme activity. This concentration therefore provides sufficient oxidizing power for sensitive detection while minimizing false-positive signals.