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## Exo-III assisted amplification strategy through target recycling of $Hg^{2+}$ detection in water: A GNP based label-free colorimetry employing T-rich hairpin-loop metallo base

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Due to deleterious environmental and health effects of the $Hg^{2+}$ ions, various online, detection methods apart from the traditional analytical tools have been developed by researchers. Biosensors especially, label, label-free, colorimetric and optical sensors have advanced with sensitive detection. However, there remains a gap of ultrasensitive quantification as noise interacts significantly in the AuNP based label-free colorimetry. This study reported an amplification strategy using Exo-III enzyme for target recycling of $Hg^{2+}$ ions in a T-rich hairpin loop metallo base label-free colorimetric nanosensor with an improved sensitivity using unmodified gold nanoparticles (uGNPs) as an indicator. The two T-rich metallo base hairpin loop structures as 5'- CTT TCA TAC ATA GAA AAT GTA TGT TTG -3 (HgS1), and 5'- GGC TTT GAG CGC TAA GAA A TA GCG CTC TTT G -3' (HgS2) were tested in the study. The thermodynamic properties of HgS1 and HgS2 were calculated using online tools. The lab scale synthesized uGNPs were utilized in the analysis. The DNA sequence had T-rich bases on both tails end, which in the presence of $Hg^{2+}$ forms a T-$Hg^{2+}$-T mismatch, promoting the formation of dsDNA. Later, the Exo-III incubation enables the enzyme to cleave stepwise mononucleotides from the 3' end until the structure become single-stranded. These ssDNA fragments then adsorb on the surface of AuNPs in their presence and protect AuNPs from the induced salt aggregation. The visible change in color from blue (aggregation stage in the absence of $Hg^{2+}$) and pink (dispersion state in the presence of $Hg^{2+}$ and adsorption of ssDNA fragments) can be observed and analyzed through UV spectrometry. An ultrasensitive quantitative nanosensor employing Exo-III assisted target recycling of mercury ions through label-free colorimetry with nanomolar detection using uGNPs have been achieved and is further under the optimization to achieve a picomolar range by avoiding the influence of the environmental matrix. The proposed strategy will supplement in the direction of uGNP based ultrasensitive, rapid, onsite and label-free colorimetric detection.

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