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DNA Hairpins and Stabilization of Gold Nanoparticles: Effect of Stem Length and Toehold Composition

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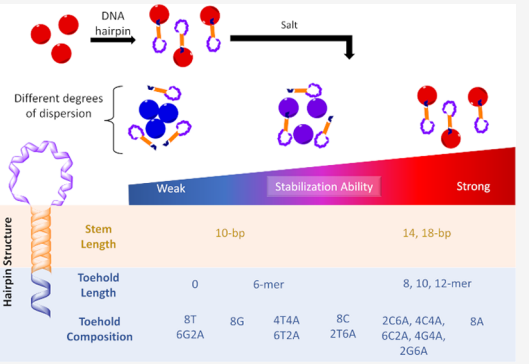
ABSTRACT: This study investigates the effect of DNA hairpins on the stabilization of gold nanoparticles (AuNPs) against salt-induced aggregation (SIA) in label-free colorimetric biosensors. AuNPs were incubated with DNA hairpins of varying stem lengths and toehold sequences, followed by the addition of NaCl, before being subjected to ultraviolet−visible (UV−vis) measurement. Results showed that hairpins with longer stems generally provide better stabilization of AuNPs (18-bp >14-bp >10-bp). No improvement was observed for 14- and 18-bp hairpins with a toehold beyond 8A, which may be attributed to saturated adsorption of hairpins on the gold surface. For 14-bp hairpins with an 8-mer homopolymeric toehold, we observed a stabilization trend of A > C > G > T, similar to the reported trend of ssDNA. For variants containing ≥50% adenine as terminal bases, introducing cytosine or guanine as preceding bases could also result in strong stabilization. As the proportion of adenine decreases, variants with guanine or thymine provide less protection against SIA, especially for guanine-rich hairpins (≥6G) that could form G-quadruplexes. Such findings could serve as guidelines for researchers to design suitable DNA hairpins for label-free AuNP-based biosensors.



INTRODUCTION

Gold nanoparticles (AuNPs) have been widely employed in label-free colorimetric biosensors. While many of these assays involve DNA-functionalized AuNPs, salt-induced aggregation (SIA) of unmodified AuNPs is another general principle of detection, whereby the latter often involves adsorption of single-stranded (ss) or double-stranded (ds) DNAs on the particles. Unmodified AuNPs are normally prepared using the citrate reduction method, which results in a layer of negatively charged ions surrounding the AuNP core. The overall repulsive negative charges of the AuNPs enable them to exist as dispersed particles in colloidal solution. Due to localized surface plasmon resonance, dispersed AuNPs with diameters of ∼10−40 nm exhibit strong absorption at around 520−530 nm and appear red to the naked eye.[1−3] SIA occurs when salt screens off these negative charges and reduces the interparticle repulsion, which causes the AuNPs to aggregate. This is accompanied by a red to purple or blue color change.

In their seminal study, Li and Rothberg showed that ssDNAs can adsorb negatively charged AuNPs at a length- and temperature-dependent rate, subsequently stabilizing the AuNPs against SIA.[4] ssDNAs have been shown to adsorb on the AuNPs via their nitrogenous bases with different degrees of affinity, where adenine (A) is widely reported to possess the highest affinity while thymine (T) has the lowest. As for cytosine (C) and guanine (G), there have been inconsistent findings, but the current consensus is that cytosine has a higher affinity than or similar affinity to guanine (A > C ≥ G > T).[5−8] Comparatively, dsDNAs show weaker adsorption due to electrostatic repulsion between the negatively charged phosphate backbone and citrate-coated AuNPs.[4]

A DNA hairpin is an interesting structural motif that comprises both dsDNA and ssDNA, as depicted in Figure 1. This conformation is kinetically trapped due to the presence of stem-loop. The toehold, typically found as a short and

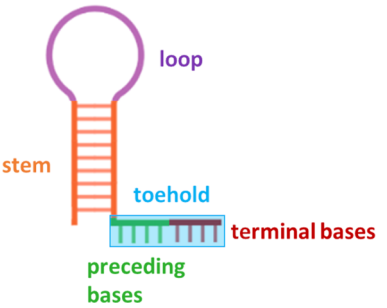


Figure 1. Different regions of a DNA hairpin.

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