Colorimetric anticancer drug detection by gold nanoparticle-based DNA interstrand cross-linking†

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A low-cost, convenient approach, based on gold nanoparticles and DNA interstrand cross-linking has been developed for rapid colorimetric detection of anticancer drugs. This method also provides insight into the controlled assembly of DNA superstructures.

DNA interstrand cross-links (ICLs) in the form of covalent adducts present formidable blocks to DNA replication and transcription.¹ When left intact, these DNA ICLs can lead to either abnormal cell death due to an inability to make new DNA molecules or uncontrolled cell proliferation because of a lack of DNA repair processes.² It has been estimated that as few as 20 ICLs in a bacterial or mammalian genome can have severe consequences on cellular growth and metabolism of cells.¹ Importantly, the cytotoxic effect of ICLs has fueled the rapid development of many anticancer drugs.³

Many molecules,⁴ including bifunctional alkylating agents, mitomycin C, platinum-based complexes, and psoralen and its derivatives,⁴ can induce ICLs. Among them, psoralen and its derivatives have been widely used for causing ICLs upon ultraviolet photoactivation.⁴ The DNA strands required to interact with the psoralens are easily accessible, and the resulting cross-links are highly stable.⁴ Despite extensive investigation, the assessment of toxicity and biological response of these ICL-inducing drugs is difficult to establish. Conventional methods typically require laborious and time-consuming multistep procedures.⁵

In this work, we combine the concept of ICLs with gold nanoparticle amplification⁶,⁷ in an effort to develop a colorimetric method for identification of anticancer drugs. We demonstrate that by taking advantage of the plasmon resonance of gold nanoparticles, the extent of DNA interstrand cross-linking can be visualized. More importantly, this colorimetric detection system offers a sensitive assay method that facilitates rapid screening of anticancer agents.

The basic design of our assay method is shown in Scheme 1a. We first synthesized two sets of DNA-functionalized 14 nm gold nanoparticle probes (designated as probe A: 5′-HS-AAA TTT TCT GTA TAT AGA CAT AAA-3′; probe B: 5′-HS-AAA TTT ATG TCT ATA

Scheme 1  (a) Schematic representation of the colorimetric detection of anticancer agents through use of gold nanoparticles and interstrand cross-linking. (b) Schematic drawings showing interstrand cross-linking of a DNA duplex by 8-methoxypsoralen (8-MOP) between T-T base pairs.
TAC AGA AAA-3’). Probes A and B are complementary to each other. When mixed in a hybridization buffer solution, they can form a cross-linked network of nanoparticles. As a result, the solution color changes from red to purple/pink due to a bathochromic shift of the localized surface plasmon resonance. Notably, we can reverse the color change (or particle aggregation) by heating the particle solution above the melting temperature (calc. ~62 °C) of the as-prepared DNA duplex. However, upon addition of DNA cross-linking agents, such as 8-methoxypsoralen (8-MOP), and under controlled irradiation into an absorption band of the cross-linking molecule, covalent adducts can form with pyrimidine bases on both DNA strands (Scheme 1b). Because of the covalent cross-links with base-paired structures, we anticipate that the nanoparticles remain attached to each other even after heating at elevated temperatures.

As a proof-of-principle experiment, we first prepared DNA-modified gold nanoparticles following the previously reported procedures. This colorimetric assay began with the mixing of probe A- and B-modified gold nanoparticles (30 μL each, 8 nM) in 10 mM Tris (pH = 7.0) and 200 mM NaCl buffer. Upon incubation at room temperature overnight, 8-MOP (71.0 μg mL⁻¹) was added to the mixture and incubated in the dark for 1 h. The resulting mixture was illuminated for 3 h with ultraviolet 365 nm light (4 W). The reaction mixture was then heated at 90 °C to check the color change and further analyzed by UV-vis spectroscopy at an operating temperature of 90 °C. It should be noted that the buffer conditions and reaction time have been optimized (Fig. S1, ESI†). In the absence of 8-MOP, the heating of the solution at 90 °C resulted in redispersed nanoparticles as confirmed by the appearance of a sharp absorbance peak at 524 nm (Fig. 1a). This was further evidenced by the observation of a pink-to-red color change of the solution (Fig. 1b, panel 2). By comparison, when illuminated with 365 nm light in the presence of 8-MOP, the nanoparticle solution remained pink even after being heated at 90 °C (Fig. 1b, panel 3). We also observed a plasmon band shift and a reduction in absorption intensity, clearly indicating the 8-MOP-mediated formation of DNA ICLs. As a control experiment, the solution of gold nanoparticles incubated with 8-MOP but without ultraviolet photoactivation was also studied. Upon heating, the particle aggregates completely redispersed (Fig. 1b, panel 1), demonstrating the essential requirement of photoactivation for cross-link formation.

We further examined the extent of cross-linking by agarose gel electrophoresis analysis. As shown in Fig. 1c, the electrophoresis results show that the nanoparticles exposed to ultraviolet light and 8-MOP travel much slower on the gel plate than the nanoparticles without the treatment, which confirms the occurrence of the cross-linking reaction. The measured melting temperature for the nanoparticle-modified duplex also provided additional evidence of the cross-link formation by 8-MOP (Fig. S3, ESI†). As a separate note, we found that the desalting technique could be used to provide strong support for the presence of ICLs (Fig. S4, ESI†).

To test the sensitivity of our method, solutions of DNA-modified gold nanoparticles in the presence of different amounts of 8-MOP (0, 1.6, 3.2, 8.1, 12.9, 16.2, 80.8 μg mL⁻¹ each) were irradiated with 365 nm light for 3 h. Fig. 2 shows the corresponding colorimetric response of the reaction mixtures. As revealed in Fig. 2, we could detect 8-MOP at a low concentration of 8.1 μg mL⁻¹ (~37 μM) with the naked eye.

We further evaluated the selectivity of our method for different chemical compounds. Solutions containing a series of
organic molecules were screened under the same reaction conditions as in the case of 8-MOP. We found that only 4,5',8-trimethylpsoralen (TMP) and 8-MOP have comparable efficacy when used as cross-linking agents. After heating at 90 °C, no noticeable color changes were discerned for the reaction mixtures incubated with TMP and 8-MOP (Fig. 3a). Importantly, our approach could be used to colorimetrically differentiate the coupling efficiency of different anticancer agents for double-stranded DNA. One example is the case of 5-methoxypsoralen (5-MOP), a structural isomer of 8-MOP with the substituent in the 5-position. This substance has been known for relatively inefficient cross-linking. As expected, our colorimetric assays, under identical reaction conditions, revealed much less cross-linking efficiency of 5-MOP than its 8-MOP counterpart (Fig. 3a, tube 3). The reactivities of the anticancer agents TMP, 8-MOP and 5-MOP were further cross-checked by gel electrophoresis analysis (Fig. 3b).

In a further set of experiments, we investigated the effect of the cross-linked gold nanoparticles on cell proliferation. Human dermal fibroblast cells were incubated for 48 h with cross-linked gold nanoparticles and non-cross-linked gold nanoparticles, respectively. The cell viability assessed by MTT assays indicated no marked difference between the cross-linked nanoparticles and the controls.

In conclusion, we have developed a low cost and colorimetric method for detection of anticancer agents. The assay method is designed by a combination of self-assembly of oligonucleotide-modified gold nanoparticles with interstrand cross-linking. By taking advantage of the chemical specificity of interstrand cross-linking and enormous signal amplification through gold nanoparticles, we have demonstrated important analytical applications for sensing anticancer agents with high sensitivity and selectivity. We envision that the conceptual framework of this work may provide insights into designing strategies to visualize and modulate the effects of DNA cross-linking agents.

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Notes and references