Detection of mismatched DNAs *via* the binding affinity of MutS using a gold nanoparticle-based competitive colorimetric method†

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A gold nanoparticle-based competitive colorimetric assay can detect mismatched DNAs using MutS, a mismatch binding protein, and determine their relative binding affinities for this protein by a simple color change and the melting temperature of DNA-functionalized nanoparticle assemblies.

Highly sensitive and selective detection of sequence-specific DNAs, such as single nucleotide polymorphisms (SNPs), is essential for understanding human molecular-based diseases and many therapeutic applications. Most genetic diseases are caused by changes in a particular DNA sequence.[1] Thus, SNPs can be an important marker for diagnosing genetic diseases at an early stage. The methods generally used to identify such mutations require immobilization involving multiple steps, labeling with fluorescent, chemiluminescent or radioactive molecules, enzymatic cleavage and ligation.[2] Although such methods are very sensitive and widely used, they are time-consuming and labor-intensive. As nanobiotechnology has developed, the use of gold nanoparticles (AuNPs) in biodiagnosis applications has rapidly increased. The unique properties of AuNPs, including a large surface area, easy conjugation of biomaterials, high absorption and scattering coefficient, allow the detection of SNPs or mutations associated with disease.[3] In particular, the colorimetric method based on aggregated AuNPs by DNA hybridization offers a rapid, easy, and low-cost detection system.[4] Recent reports on AuNP-based colorimetric detection of oligonucleotides, proteins, metal ions and small organic molecules demonstrated these advantages.[4a,b,5]

Herein, we demonstrate a simple AuNP-based colorimetric assay that can detect mismatched DNAs using MutS, a mismatch repair protein, and at the same time determine their relative binding affinities for this protein. To the best of our knowledge, this is the first example of SNP detection using a DNA–AuNP assembly system based on MutS specificity for mismatched DNAs. So far, most SNP detection by a colorimetric method has been based on enzyme activity, such as DNA ligase and nuclease.[6] However, experiments with such an enzymatic reaction need careful control of the reaction conditions, including pH, temperature and salt concentration. MutS is a promising tool to manipulate this problem. Since MutS specifically recognizes and binds all possible single-base mismatches, insertions and deletions, it has been widely exploited for specific detection of mismatched DNAs.[2a,b,7] In this study, we used *Thermus aquaticus* (*Taq*.) MutS among its homologs because its activity is stable under a wide range of pH and temperatures.[8] Thus, a series of colorimetric assays and melting temperature (T_m) measurements according to increasing temperature can be performed under the stable activity of protein. *Taq*. MutS also allows rapid and convenient experiments, and can be applied in sensors capable of working beyond normal physiological conditions.

In the present work, 13-nm AuNPs (each 1.7 nM) were functionalized with 5′ thiol-modified ssDNAs that contained T and G mismatch sites, respectively. When the DNAs were hybridized in 50 mM Tris (pH 7.0) buffer with 300 mM NaCl and 2 mM $MgCl_2$, DNA-functionalized AuNPs aggregated and formed a cross-linked network. This process caused a color change from red to purple due to the red-shifted plasmon resonance of the AuNPs. When these assemblies were heated, melting transition occurred due to network breakage upon DNA dehybridization. The binding of MutS to a mismatched site on DNA-functionalized AuNPs stabilized the DNA–AuNP assemblies and increased their T_m (ΔT_m = 14.7 °C). However, in the presence of MutS, the T_m of DNA–AuNP assemblies with a matched sequence was hardly different from that of assemblies in the absence of MutS, because MutS binds selectively to mismatched DNAs (ΔT_m = 2.8 °C) (Fig. S1†). Thus, the results of T_m measurement suggested that mismatched DNAs could be successfully detected by this DNA–AuNP assembly system using MutS.

To further investigate the relative selectivity of MutS for different mismatch types, we introduced an additional mismatched DNA containing different mismatched pairs, such as GT, CT and CC pairs, as a competitor for the DNA binding of MutS (Scheme 1). When a competitor DNA is added, the melting curve depends on the relative affinity of MutS for the competitor, because the DNA sequence (GT mismatch) on the functionalized AuNP assemblies does not change, and the extent of MutS binding to DNA–AuNP assemblies is constant. Fig. 1A shows that the T_m of DNA–AuNP assemblies decreases with increasing concentration of a competitor containing a GT mismatched pair. Because MutS can bind to either the DNA–AuNP assemblies or the competitor, the extent of the decrease in T_m depends on the competitor concentration. As shown by the plot of T_m *versus* competitor concentration, T_m was almost constant over a competitor concentration of 14 μM (Fig. 1B). On the other

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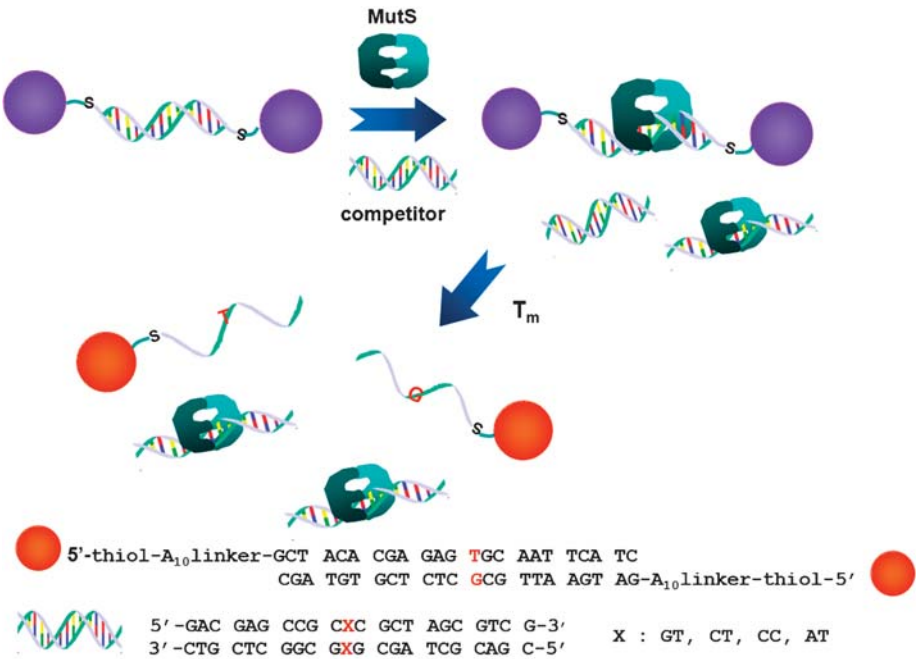

Scheme 1 A schematic representation of the detection of mismatched DNAs and comparison of their binding affinities for MutS using a DNA–AuNP assembly system. MutS binds to a mismatched site on DNA-functionalized AuNPs and the competitor. As the temperature increases above T_m, AuNPs are released, and a red color is represented depending on the relative affinity of MutS for the competitor.

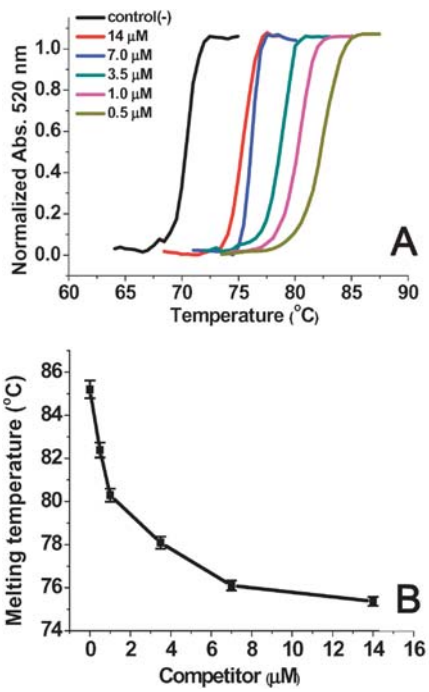

Fig. 1 (A) Normalized melting curve of DNA–AuNP assemblies for different concentrations of a GT competitor. (B) Correlation curve between competitor concentration and melting temperature. Control (–) represents DNA–AuNP assemblies without any competitors and MutS.

hand, the competitor containing a GT mismatched pair still works well at 500 nM in this system.

To determine the MutS selectivity for mismatched DNAs, a competitor and MutS were used in a 1 : 2 molar ratio (7 μM and 14 μM, respectively) because the active form of MutS is a dimer.

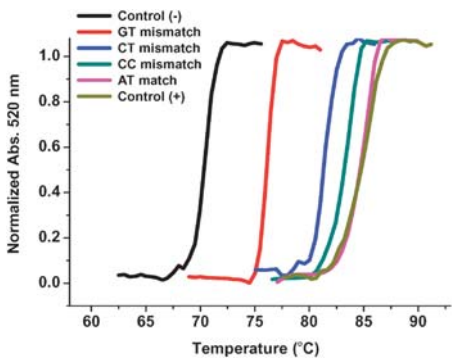

Fig. 2 Normalized melting curve of DNA–AuNP assemblies for different types of competitors according to MutS binding. Control (–) represents DNA–AuNP assemblies without any competitors and MutS. Control (+) represents DNA–AuNP assemblies in presence of MutS without any competitors.

Fig. 2 shows melting curves for DNA–AuNP assemblies with different competitors monitored at 520 nm (corresponding to the gold plasmon resonance). When MutS bound to a GT mismatched site of the DNA–AuNP assemblies, a T_m of 85.2 °C was observed. Addition of a competitor decreased this value. The greatest decrease was observed for a competitor containing a GT mismatched pair (T_m = 76.1 °C), while the lowest decrease was observed for a competitor containing a CC mismatched pair (T_m = 83.5 °C). In the case of an AT matched pair, the T_m was almost the same as in the absence of a competitor (85.1 °C and 85.3 °C, respectively) (Table S1†). This indicates that MutS forms the strongest complexes with GT mismatched DNA but the weakest complexes with CC mismatched DNA. The strong affinity of MutS for GT mismatched DNA leads to greater binding for a competitor

containing a GT mismatched pair. As a result, the T_m for the DNA–AuNP assemblies greatly decreased since MutS has relatively weak binding to the DNA–AuNP assemblies in comparison to those with no competitor. On the other hand, since the weak affinity of MutS for a competitor containing a CC mismatched pair induces relatively strong binding to the GT mismatched site of the DNA–AuNP assemblies, the T_m for the DNA–AuNP assemblies decreased slightly. According to our experiments, the order for the binding affinity of MutS is as follows: GT > CT > CC > AT, which confirms the previous results obtained using other methods.[2a,b,9]

To confirm the specificity of this system using the MutS protein, the T_m of DNA–AuNP assemblies was measured using a non-specific binding protein, BSA (14 μM). However, no change in T_m values was observed for different types of competitor (7 μM) (Fig. S2†). Therefore, in contrast to other approaches for detecting SNPs, our system based on MutS, retaining high selectivity and specificity to mismatched DNAs, clearly shows that direct distinction of various mismatches is possible, as well as detection of mismatched DNAs from significant changes in T_m.

In addition, mismatched DNAs were detected by a color change in the DNA–AuNP assemblies on MutS binding (Fig. 3). As the temperature increases, the color of DNA–AuNP assemblies changes from purple to red at a temperature that reflects the T_m value. As a result, DNA–AuNP assemblies with a GT mismatch competitor turned to red at 78 °C, while the color change for a CC mismatch competitor occurred at 82 °C. DNA–AuNP assemblies with an AT match competitor were the most stable and remained purple in color at 84 °C, similar to assemblies in the absence of a competitor. This colorimetric detection allows easy and simple distinction of mismatched DNAs and confirms the T_m results.

In conclusion, we detected mismatched DNAs using an AuNP-based competitive colorimetric assay. This is the first report of SNP detection *via* MutS selectivity to mismatched DNAs using an AuNP colorimetric method. The assay allows determination of the binding affinity of MutS to mismatched DNAs by a simple color change and T_m measurements. This simple AuNP-based approach using the ability of MutS provides a promising opportunity for the rapid and high-throughput screening of genetic mutations and SNPs, and for the clinical diagnosis of genetic diseases. Finally, it has potential to be widely applicable for easy detection of interactions between various proteins and their targets based on their binding affinity.

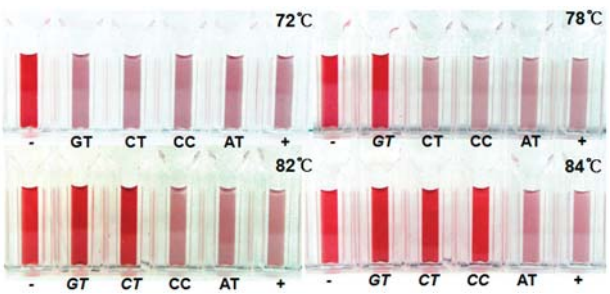

Fig. 3 Color change at various temperatures for DNA–AuNP assemblies with no competitor and no MutS (−), with various competitors (GT, CT, CC and AT) in presence of MutS, and with no competitor in presence of MutS (+).

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