Analytica Chimica Acta 696 (2011) 1–5

Contents lists available at ScienceDirect

Analytica Chimica Acta

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An ultrasensitive method for the detection of gene fragment from transgenics using label-free gold nanoparticle probe and dynamic light scattering

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ARTICLE INFO

Article history:
Received 24 January 2011
Received in revised form 30 March 2011
Accepted 2 April 2011
Available online 13 April 2011

Keywords:
Dynamic light scattering
Transgenic product
DNA
Gold nanoparticles

ABSTRACT

The detection of transgenic products is of great significance for the development of transgenic technique. In this paper, we developed a simple, rapid and ultrasensitive method for the detection of sequence-specific Nopaline synthase (NOS) gene from the transgenic plants using label-free gold nanoparticle (NP) probe and dynamic light scattering (DLS) technology. Gold NPs were stable in NaCl solution with the presence of NOS gene probe. On the contrary, they were aggregated in NaCl solution when the probe sequence was hybridized with target sequence. The change in the size of gold NPs can be detected by DLS technology with high sensitivity. Under the optimal conditions, the average hydrodynamic diameter of gold NPs was linear with the concentration of the target sequence ranging from 1.0×10^{-13} mol L^{-1} to 5.0×10^{-9} mol L^{-1}, with a detection limit of 3.0×10^{-14} mol L^{-1} (S/N = 3). The relative standard deviation (at 1.0×10^{-9} mol L^{-1} of target sequence) was 4.8% (n = 11). The result shows that gold NPs-based DLS method has great potential in the analysis of transgenic products.

1. Introduction

In recent years, the cultivation of the transgenic plants has increased at astonishing rate. The global area of transgenic plants has increased from 1.7 million hectares in 1996 to 134 million hectares in 2009 [1]. Transgenic plants have played an important role in solving many problems including the population explosion, food shortages, deficient energy sources and rampant diseases. However, the security of the transgenic plants is still a problem for the people around the world [2]. Therefore, it is important to develop a simple, rapid and ultrasensitive method for the detection of transgenic plants. Agrobacterium tumefaciens Nopaline synthase (NOS) is of bacterial origin and expressed in plant tissues to generate nopaline, which is secreted into the environment where Agrobacterium tumefaciens uses it as a nutrient source. It is generally used as a biomarker of transgenic plants [3,4]. At present, various methods have been developed for the assay of sequence-specific gene based on electrical [5–11], optical [12–15], and mechanical strategies [16,17]. Among these methods, gold nanoparticles (NPs) have been widely used due to their unique size and distance-dependent optical properties. Mirkin and co-workers first designed a cross-linking aggregation of single stranded DNA-modified gold NPs, in which a target sequence cross-linked two DNA-modified gold NPs by hybridization. The aggregation can be observed visually by color change or detected by ultraviolet visible (UV–vis) absorption spectroscopy, and the detection limit of the approach was about 1.0×10^{-8} mol L^{-1} [18]. To improve the sensitivity, a variety of detection techniques have been exploited including scanometric method [19,20] and surface-enhanced Raman spectroscopy (SERS) [21,22]. But these methods usually require labeling and immobilization procedures which make them complicated and time-consuming.

Dynamic light scattering (DLS) is a powerful tool for measuring slight changes in NP size. It has been applied to investigate the interaction between enzymes and quantum dots [23], and detect the cancer biomarker [24], arsenic [25] and DNA [26]. For example, Huo's group reported a one-step method for DNA detection using DLS technique and DNA-gold NPs, and the detection limit was around 1.0×10^{-12} mol L^{-1}.

In the present work, we developed a simple and sensitive method for detecting the transgenic sequence in a homogeneous solution using label-free gold NPs and DLS technology. The label-free gold NPs got together when the probe and target sequences were co-existence in the solution and the aggregation can be observed by DLS. Due to the strong light scattering property of gold NPs, a detection limit of 3.0×10^{-14} mol L^{-1} was obtained.

2. Experimental

2.1. Materials

All oligonucleotides related to NOS gene sequence were obtained from Augct Biotechnology Company (Beijing, China). Their base sequences are as follows:

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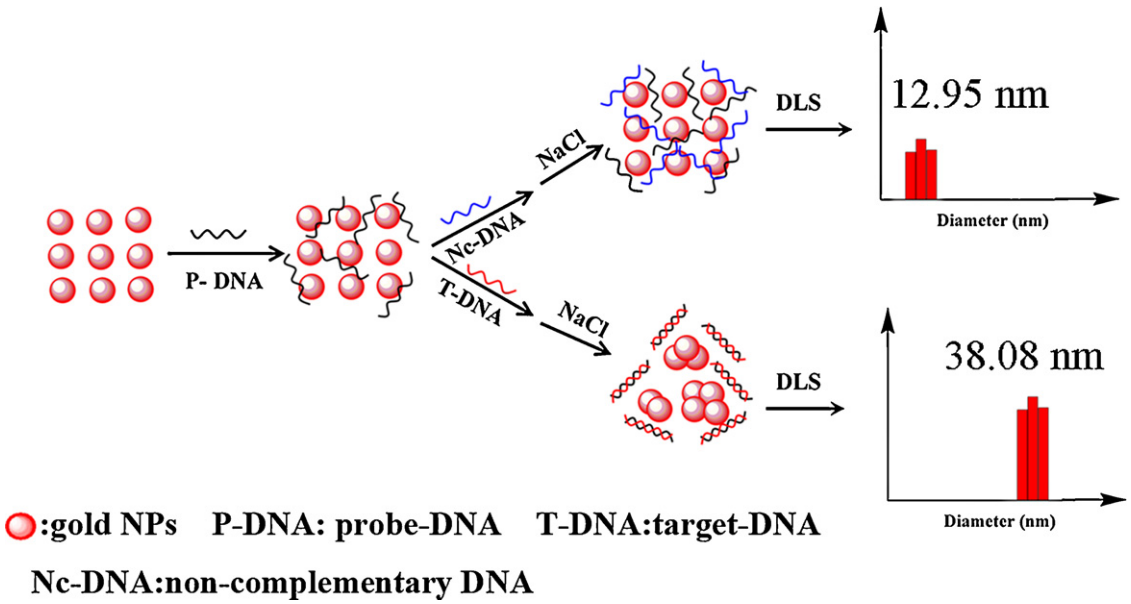
doi:10.1016/j.aca.2011.04.001



Scheme 1. Schematic diagram of the detection of transgenic sequences using gold NPs and DLS.

Probe sequence: 5′-AAT GTA TAA TTG CGG GAC TCT AAT C-3′;
Perfectly complementary target sequence: 5′-GAT TAG AGT CCC GCA ATT ATA CAT T-3′;
Non-complementary sequence: 5′-TGC CCA CAC TGA CGG CGC CAC TCC C-3′.

All oligonucleotide stock solutions were prepared using phosphate buffer solution (0.02 mol L^{-1}, pH 7.4). Chloroauric acid ($HAuCl_4$) was purchased from Sinopharm (Beijing, China). Sodium citrate and sodium chloride were purchased from Tianjin Chemical Reagent Company (Tianjin, China). Other reagents and chemicals were of analytical grade. Ultrapure water with 18.2 MΩ cm was used throughout.

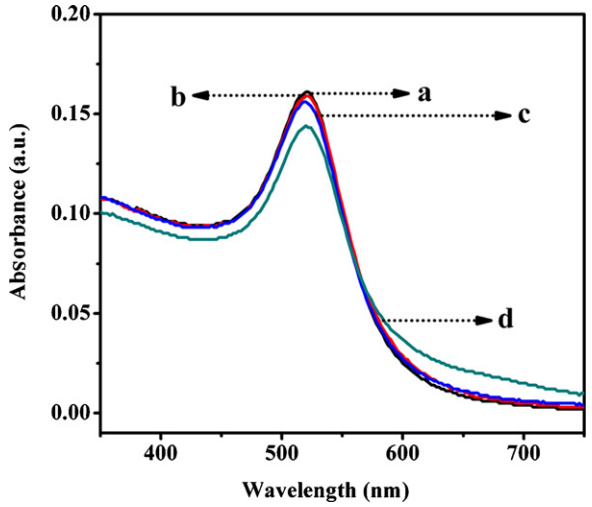


Fig. 1. UV–vis absorption spectra of gold NPs in the absence of DNA sequence (a) and in the presence of probe sequence (b), target sequence (c) and hybridization of probe sequence and target sequence (d), respectively (the concentration of DNA sequence is 1.0×10^{-6} mol L^{-1}).

2.2. Instrumentation

UV–vis absorption spectra were recorded on a Thermo Nicolet Corporation Model evolution 300 (America, Thermo Nicolet) equipped with a 5-mm quartz cuvette. Transmission electron microscopy (TEM) images were carried out on a Hitachi H-7650 TEM (Japan). Hydrodynamic diameters of gold NPs were measured using a Zetasizer Nano ZS90 DLS system (England, Malvern).

2.3. Preparation of gold NPs

The gold NPs were prepared by reducing $HAuCl_4$ with citrate [27]. Briefly, 100 mL of the 0.25 mM $HAuCl_4$ solution was heated until boiling with vigorous stirring. Rapid addition of 5.0 mL of a 1% (w/w) sodium citrate solution resulted in a continuous color change from colorless to red; stirring was continued for another 10 min after the color change ceased, and then the heating source was removed and continued stirring for the next 15 min. The final solution was stored at 4 °C. The particle size and shape were measured by the TEM.

2.4. Procedures for DLS detection of gold NPs aggregation

The DLS detection of gold NPs aggregation was divided into three steps. The first step was hybridization of probe sequence and target sequence. DNA hybridization was according to the following procedure. First, 25 μL 1×10^{-6} mol L^{-1} solution of probe sequence was mixed with equal volume of different concentrations of target sequence, and then the mixture was heated on a water bath at 95 °C for 5 min and hybridized at 55 °C for 30 min, at last the solution was slowly cooled to room temperature. Second, 25 μL of the above mixture and 25 μL of 0.5 mol L^{-1} NaCl solutions were added into 1 mL gold NP solution, respectively. At last, the DLS measurements were conducted. About 1 mL of the sample was transferred to a special dust-free light-scattering cell and the measurements were carried out at 25 °C. The experiments were started 2 min after the sample container was placed in the DLS instrument to maintain temperature balance. Each sample was conducted with a fixed 10 runs. A detection angle of 90° was chosen for the size measurement.

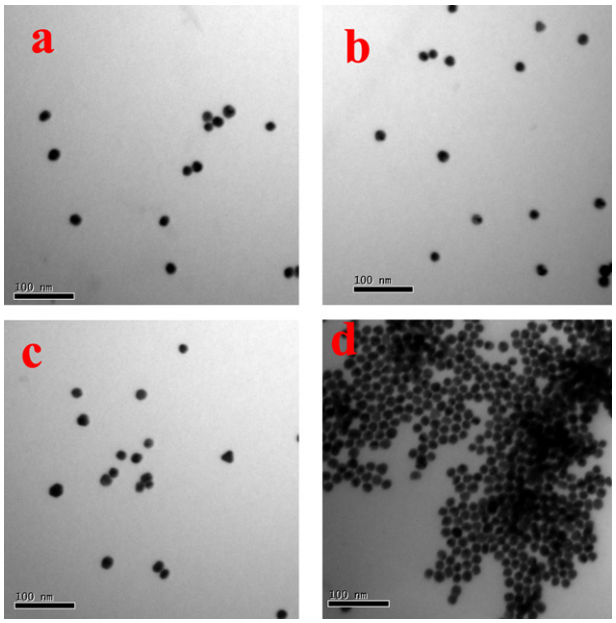


Fig. 2. TEM images of (a) gold NPs, (b) gold NPs and probe sequence, (c) gold NPs and target sequence, (d) gold NPs and hybridization of probe sequence and target sequence (the concentration of DNA sequence is 1.0×10^{-6} mol L^{-1}).

3. Results and discussion

3.1. Principle of the detection

As shown in Scheme 1, gold NPs were well dispersed in the salt solution after adding the non-complementary sequence solution. However, with the addition of the target sequence, they gathered together. The reason is that single-stranded and double-stranded DNA have different propensities to adsorb on gold NPs in colloidal solution [28]. Single-strand DNA (probe sequence or the non-complementary sequence) can uncoil sufficiently to expose its bases to the gold NPs and the negative charge on the backbone is distant, therefore, attractive van der Waals forces between the bases and the gold NPs are sufficient to cause single-strand DNA to stick to the gold NPs, however, double-strand DNA (after the hybridization of the target and probe sequence) possesses a stable double-helix geometry that always presents the negatively charged phosphate backbone, so the double-strand DNA could not be adsorbed on the gold NPs surface due to the electrostatic interaction between citrate ions and the charged phosphate backbone. The change in the size of gold NPs induced by the addition of target sequence can be measured by DLS technique.

3.2. Characterization of gold NPs

The synthesized gold NPs were characterized by UV–vis absorption spectra, TEM and DLS, respectively. Fig. 1 presents UV–vis absorption spectra of gold NPs before and after DNA hybridization. The as-prepared gold NPs displayed the characteristic absorbance peak at the wavelength of 520 nm (Fig. 1a). With the addition of single-stranded DNA (ss-DNA, probe sequence or target sequence), the intensity of the peak at 520 nm had nearly no change (Fig. 1b and c), whereas, it decreased at 520 nm and increased at 590 nm after hybridization of the two sequences (Fig. 1d). The results illustrated the gold NPs aggregated after the DNA hybridization.

TEM images of gold NPs are shown in Fig. 2. The synthesized gold NPs were spherical, well-dispersed and had an average diameter of

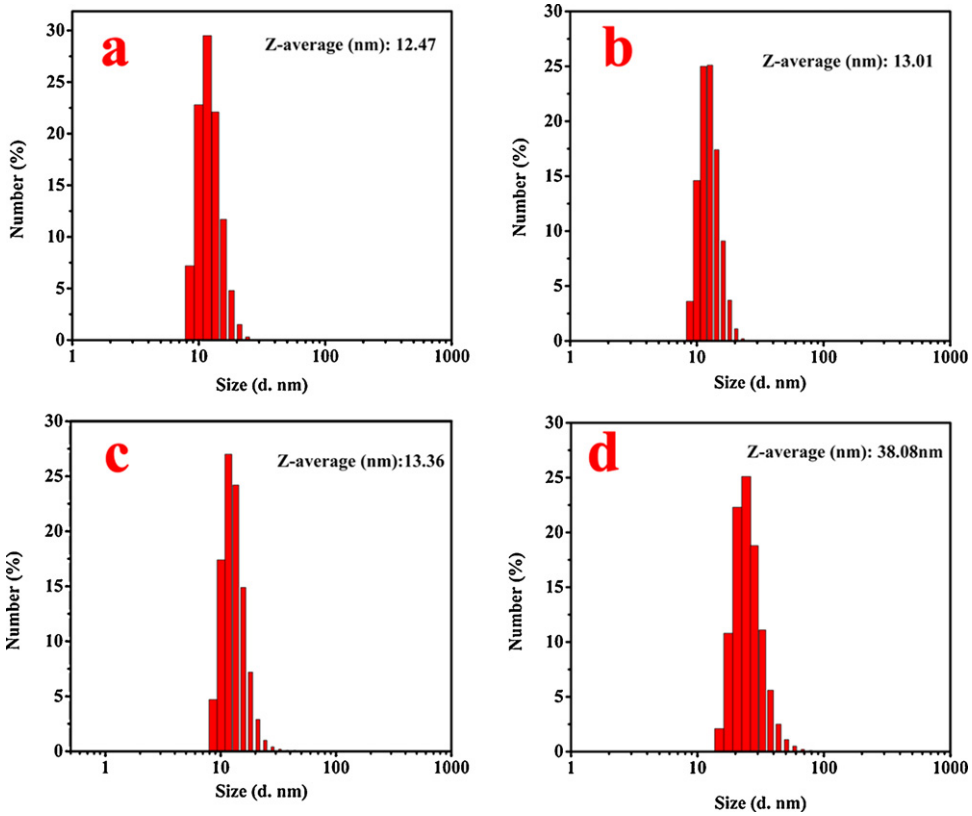


Fig. 3. Hydrodynamic diameters of the gold NPs without DNA sequence (a) and with probe sequence (b), target sequence (c) and hybridization of probe sequence and target sequence (d), respectively (the concentration of DNA sequence is 1.0×10^{-6} mol L^{-1}).

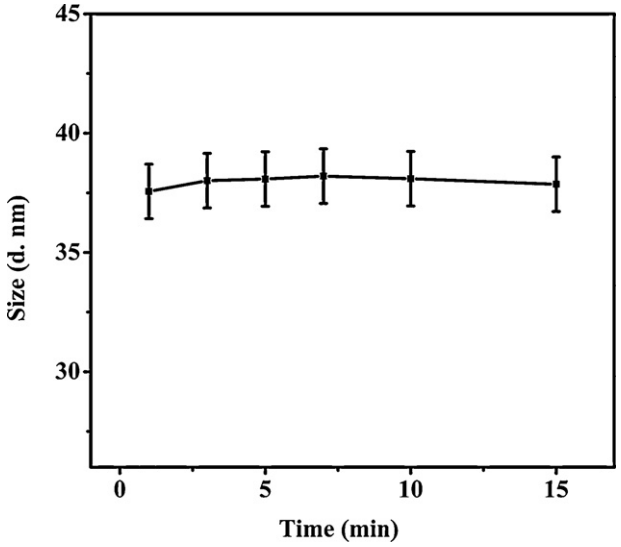


Fig. 4. The effect of aggregation time on the size of gold NPs.

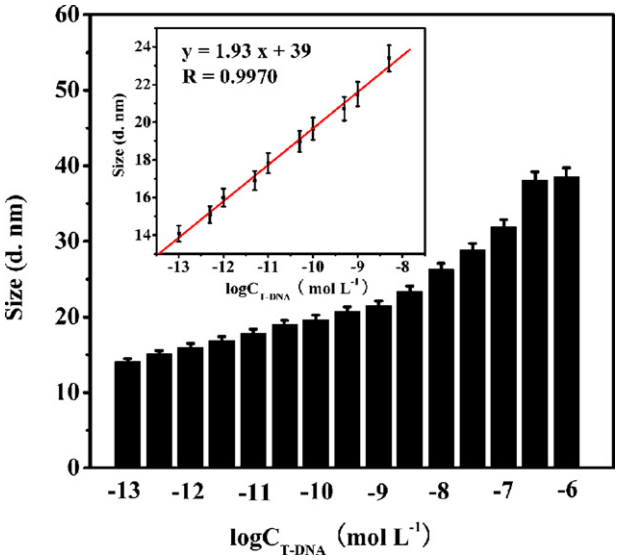


Fig. 5. The average hydrodynamic diameters of gold NPs in the presence of 1.0×10^{-6} mol L^{-1} probe sequence and hybridize with different concentrations of target sequence, insert: linear relationship between the concentration of target sequence and the average hydrodynamic diameters.

approximately 12 nm (Fig. 2a). They were still well dispersed in probe sequence (Fig. 2b) or target sequence (Fig. 2c), but they gathered together after hybridization of the two sequences (Fig. 2d). The results were consistent with the analysis of UV–vis absorption spectra.

DLS has been used to measure the size change of gold NPs induced by DNA hybridization in this system. The hydrodynamic diameter of the as-prepared gold NPs was 12.47 nm (Fig. 3a). In the presence of probe sequence or target sequence, it was 13.01 nm (Fig. 3b) and 13.36 nm (Fig. 3c), respectively, which was almost the same with the result of blank. However, after the hybridization of the probe and target, it increased dramatically from 12.47 nm to 38.08 nm (Fig. 3d). It indicated that gold NPs aggregated together after the hybridization of probe and target sequence.

3.3. Optimization the reaction time of gold NPs aggregation

The effect of the reaction time on the hydrodynamic diameter is also investigated by DLS. Fig. 4 demonstrated the gold NPs gathered together quickly after the hybridization of probe and target sequence in the salt solution. Therefore, the gold NPs exposed their surface after the hybridization. The salt solution screened the repulsive interactions of the citrate ions, so they aggregated quickly. The diameters do not have any change in 10 min. So a reaction time of 5 min is adopted in the following experiment.

Table 1
Comparison of the proposed method with those published previously for the detection of DNA hybridization.

Label	Method	Detection limit	Reference
Au NPS	Colorimetric	1×10^{-8} mol L^{-1}	[18]
Au chip	Surface plasmon resonance	1×10^{-9} mol L^{-1}	[29]
Au/polyaniline nanotube	Electrochemical impedance spectroscopic	3×10^{-13} mol L^{-1}	[30]
Quantum dots	Anodic stripping voltammetry	5×10^{-11} mol L^{-1}	[31]
ZnS and CdSe quantum dots	Fluorescence	2×10^{-9} mol L^{-1}	[32]
The proposed method	Dynamic light scattering	3×10^{-14} mol L^{-1}	

3.4. Sequence-specific detection of DNA

Previous results (Fig. 2b) have indicated that the average hydrodynamic diameter of the gold NPs was around 13.01 nm when the target sequence was absent. It increased to 14.07 nm when 1.0×10^{-13} mol L^{-1} target sequence was added in the sample. The average hydrodynamic diameters of the samples were presented in Fig. 5. It indicated that the average diameter of gold NPs increased as increasing concentration of the target sequence. A good linear relationship was found between the concentration of target sequence and the average hydrodynamic diameter of the sample ranging from 1.0×10^{-13} mol L^{-1} to 5.0×10^{-9} mol L^{-1}. The correlation equation was $y = 1.93x + 39$ (y (nm) is the average hydrodynamic diameter of the sample, x (mol L^{-1}) is the logarithm of the concentration of target sequence) and the correlation coefficient $R = 0.9970$. The detection limit (S/N = 3) was 3.0×10^{-14} mol L^{-1}.

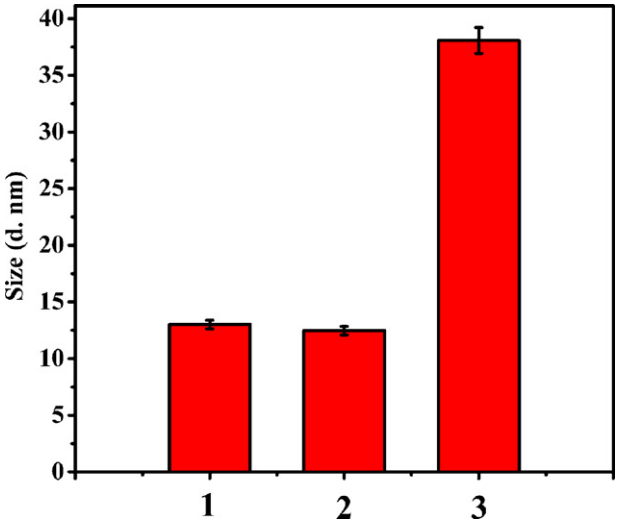


Fig. 6. Hydrodynamic diameters of gold NPs: (1) gold NPs and probe sequence (1.0×10^{-6} mol L^{-1}); (2) gold NPs and probe sequence mixed with non-complementary sequence (1.0×10^{-6} mol L^{-1}); (3) gold NPs and probe sequence hybrid with target sequence (1.0×10^{-6} mol L^{-1}).

The relative standard deviation (at 1.0×10^{-9} mol L^{-1} of target sequence) was 4.8% ($n = 11$). The comparison results of the proposed method and other reported techniques for the detection of DNA hybridization were listed in Table 1. It showed that the sensitivity of the proposed method was six orders of magnitude higher than that of the traditional colorimetric method [18], and also higher than surface plasmon resonance [29], electrochemical method [30,31] and fluorescence method [32].

3.5. Interference

The selectivity of the proposed method is evaluated by the analysis of the average hydrodynamic diameter changes arising from a complementary sequence and a non-complementary sequence. As shown in Fig. 6, compared to the blank, the size of the gold NPs was almost no change with the presence of non-complementary sequence. The non-complementary sequence cannot be hybrid with the probe sequence efficiently; therefore, the gold NPs were still protected by single-strand DNA. As a result, gold NPs were dispersed well in the salt solution. So the method is specific for the detection of NOS gene fragment.

4. Conclusions

In summary, a label-free, simple and sensitive method using DLS technique has been developed for the detection of transgenic sequence in homogenous solution. It shows that the proposed method has a low detection limit. Moreover, DLS assay also demonstrates its great potential in detecting the aggregation of NPs. It provides a new probability of detecting the transgenic plants rapidly, simply and sensitively.

Acknowledgements

The authors gratefully acknowledge the support for this research by genetically modified major projects (2009ZX08012-015B), National Natural Science Foundation of China (20975042), the program for academic pacesetter of Wuhan (200851430484) and the Fundamental Research Funds for the Central Universities of China (2010 PY 009, 2009JC005).

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