Gold Nanoparticle based colorimetric biosensing of various DNA types and ligands

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Abstract: The purpose of this study was to test the hypothesis that an array of unmodified gold nanoparticles and various DNA types and conformations could provide qualitative and quantitative information about DNA and DNA binding ligands. The array system was designed and optimized with DNA and a range of unmodified gold nanoparticle sizes (8, 10, 13, 20nm), different ionic strengths (0 -300mM) and various DNA binding ligands (Neutral red, Ethidium bromide, Actinomycin D). This system successfully detected various DNA types (ss -DNA, ds -DNA and quadruplex DNA), different sizes (22 base pairs to genomic length) and different concentrations (nanoMolar to miliMolar). Quantification of the DNA binding ligands was performed successfully in the presence of DNA and ionic strength by extracting RGB values of the arrays. Colorimetric method shows an outstanding capability of diagnosis of various DNA and DNA binding ligands and can be applied as fingerprints for diagnostics and genetic studies. Furthermore sensitive and specific ligand detection and quantification is applicable in developing and characterizing new potential drugs and detection of toxic small molecules contaminating the environment. These quantitative DNA and ligand arrays can be commercialized and used as diagnostic tools in future.

Keywords: Unmodified gold nanoparticle; colorimetric biosensor; DNA array; ligand quantification.

Introduction

Gold nanoparticle (AuNP) based colorimetric biosensors serve as a class of nanomaterials that have significant diagnostics applications in unaided visual readout in biological and pharmaceutical fields [1]. DNA functionalized AuNP conjugates (covalently linked AuNPs with probe DNAs or aptamers) have been used successfully as biosensors [2]. However their synthesis for commercialized applications remains as a major challenge [3]. The second broadly utilized approach is the use of unmodified gold nanoparticles [4-6]. These are bare spherical AuNPs without further surface modifications with unique optical properties [7]. AuNP based colorimetric biosensors, because of the visible color change have been used as real time detection of DNA and small molecules [1,8-9]. Different electrostatic properties of single stranded DNA (ss-DNA), double stranded DNA (ds-DNA) and folded (e.g. quadruplex) oligonucleotides with the surface of AuNPs result in changing the NaCl resistance and aggregation of AuNPs induced by NaCl as illustrated in figure 1 [1,3-4].

Application of unmodified gold nanoparticles emerges as a successful approach recently for detection of ss-DNA conformational change to ds-DNA and folded structures [1,3]. Recent development in nanomaterials has opened new opportunities for designing high-throughput screening processes for DNA ligands which possess potential anticancer effects; through convenient detection of the DNA conformational changes [1,10-11].

The central idea of this work was to develop an array method for easy, efficient and swift detection of various DNA types and DNA binding ligands.

Quantification of the ligands leading to extraction of more qualitative and quantitative information about small anticancer drug molecules was done in the present study for the first time according to the open literature.

This proposed method can be extended to different nucleic acids and non-nucleic acid targets such as various small molecules, proteins and ions.

Fig. 1 Different absorption properties of ss-DNA and ds-DNA on the surface of AuNPs [2]

Experimental

DNA synthesized by BioNEER–Dr. Takapouzist co. Iran (OD: 29, purification: BioRP). Our system included five types of DNA:

1. A 22-mer GCTATGGAATTCCCTCGTAGGCA random sequence (R-DNA); 2. A 22-mer AGGGTTAGGGTTAGGGTTAG quadruplex DNA which carries a piece of the human telomeric sequence (G-DNA); 3. A 22-mer i-motif DNA, complementary to...
the sequence number 2 (C-DNA); 4. A ds-DNA named GC-DNA; prepared by mixing equal amounts of G-DNA and C-DNA with same concentrations; 5. Calf Thymus DNA (genomic) purchased from Sigma (P-DNA).

All stock solutions of DNA types were prepared to become 1mM, in 20 mMolar (mM) Tris Buffer (Sigma, USA) plus 1mM EDTA (Sigma, USA), pH: 7.

The conformation of the model oligonucleotides was characterized by Circular dichroism (CD) spectroscopy, (AVIV, model: 215) at Tehran university of medical sciences. Gold nanoparticle (AuNP) synthesis was done by citrate reduction of Hydrogen tetrachloaurate (III) hydrate (HAuCl4.3H2O) purchased from Alfa Aesar (A Johnson Matthey Company, USA) [12]. The synthesized nanoparticles were analyzed by Dispersion Technology Software (DTS) (Ver. 5.02, Malvern Instruments Ltd, UK) at Zanjan university of medical sciences. For ligand detection, system setup was similar to the previous work done by authors of this study [13] by Neutral red (NR) and Ethidium bromide (EB) (Sigma, USA) as weak and strong DNA ligands. Actinomycin D (ActD) (Sigma, USA) was selected to be tested as an anticancer drug. For colorimetric analysis, High-resolution images of the array were taken by Sony Cyber-shot DSC-W220 12.1 mega pixels digital camera. Resulting images were digitized and processed using MATLAB software (ver. R2009a) for further quantification by means of averaged Red-Blue-Green quantities (RGBs).

**Results and Discussion**

The array system was designed and optimized with DNA and a range of unmodified gold nanoparticle sizes (8, 10, 13, 20nm), different ionic strengths (0-200mM) and various DNA binding ligands (NR, EB, ActD). The arrays were tested with and without the complementary DNA strands.

This system successfully detected various DNA types (ss-DNA, ds-DNA and quadruplex DNA), different sizes (22 base pairs to genomic length) and different concentrations (nanoMolar to miliMolar). A simple DNA array by AuNP 13nm and without any ligand, is presented in figure 2.

Detection and quantification of DNA binding ligands is the other application of this array system which we have reported previously for two DNA binding ligands (G-quadruplex intercalators); Neutral Red and Ethidium Bromide in interaction with telomere DNA (end chromosomal sequence which plays an important role in cancer disease) using a still digital camera and translated colors to RGB quantities for quantitative studies.

In the present study detection and quantification of an anticancer drug named Actinomycin D is reported which is widely used in chemotherapeutics. Quantification of Actinomycin D was performed in the presence of G-DNA and ionic strength and illustrated in the plots of extracted RGB values (Figure 3 and 4), and revealed some novel points about the drug mechanism of action.

Li and Rotberg’s DNA hybridization assay using unmodified gold nanoparticles as optical nanoprobe was the first line study. The advantages of their method were speed, simplicity, and no need for any modifications in AuNP probe or target [5]. All these advantages are found in methodology utilized in this study. Further we

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**Fig. 2 DNA detection by AuNP 13nm**

**Fig. 3 ActD Quantification via G-DNA and AuNP 13nm**

**Fig. 4 Three dimensional plot for ActD Quantification via G-DNA and AuNP 13nm with blue color quantities**
extended the study to further DNA targets different from ss-DNA, ds-DNA to folded DNA and combinations of these forms (GC-DNA).

Colorimetric recognition method for DNA intercalator molecules with unmodified gold nanoparticles was developed by Xin, et al. [11]. This method based on desorption of a short self complementary ss-DNA from AuNP surface by DNA intercalators was detected by a color change from red to blue(31). This similar study could be used for selection of potential anticancer agents from combinatorial libraries just on the basis of their binding affinities to ds-DNA which limits their applicability. Extension to other DNA types and ligands not only increased our method applicability, but also provided the facility of for more specific detection. Quantification of the ligand is a crucial point of the current study which was not achieved by any of the previous works.

Conclusions
An array system has been designed based on unmodified gold nanoparticles for the first time; which is simple, swift, requiring no complicated apparatus.

DNA detection was successfully performed by an array of different AuNP sizes and different NaCl concentrations. RGB values of the arrays were extracted and used as quantitative means of DNA detection and differentiation between various kinds and conformations. DNA array can be applied as a fingerprint for diagnostics (detection of disease biomarkers in biological samples), genetic studies (hybridization assay, gene studies, etc.).

A visual and quantitative array of DNA types and ionic strengths was designed for each ligand which presented as a key (fingerprint). These ligand arrays can be used for developing and characterizing new potential drugs in pharmacologic studies (ligand binding and conformational change studies to understand drug mechanism of action) and detection of toxic small molecules contaminating the environment.

This method can be commercialized in future and used in diagnostic devices.

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