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Review

# Gel electrophoresis as a nanoseparation tool serving DNA nanotechnology

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ABSTRACT

The past years have witnessed a rapid development of DNA nanotechnology in nanomaterials science with a central focus on programmable material construction on the nanoscale. An efficient method is therefore highly desirable (but challenging) for analytical/preparative purification of DNA-conjugated nano-objects and their DNA-assemblies. In this regard, agarose gel electrophoresis, a traditional technique that has been invented for biomacromolecule separation, has found many innovative uses. This includes shape, size, charge, and ligand-valence separations of nanoparticle building blocks as well as monitoring a self-assembly process towards product identification and purification.


## 1. Introduction

With the development of nanotechnology and related disciplines, the demand for nanostructured materials with designable structures and functions is rapidly growing [1]. Despite many research efforts, to obtain nanomaterials with definable structures and properties by chemical synthesis is highly challenging. DNA nanotechnology as one of the most attractive research frontiers in nanoscience and nanotechnology has a great potential to meet the above need through DNA-guided material assembly [2]. This mainly benefits from the programmable DNA base-pairing coming from the strictest natural selection in living systems. However, to guarantee the accuracy of DNA-programmable assembly, a nanoseparation technique is required for both building block development and product purification.

Gel electrophoresis is a simple and widely adopted tool for biomolecule (*e.g.* nucleic acids and proteins) isolation. It is heavily relied in molecular biology and genetics laboratories to enable the studies of specific DNA or proteins. In addition to DNA and proteins, the inherent nano-porosity of an agarose gel should allow for nanoseparation applications involving inorganic colloids [3]. In fact, researchers of DNA nanotechnology have been using gel electrophoresis as a simple, cheap, and versatile tool for the separation of DNA-conjugated inorganic nanoparticles and their super-assemblies. This mini review covers some of these innovative applications, including: (1) size and shape separation of colloidal materials; (2) valence separations of DNA-conjugated nanoparticles; and (3) identification and purification of DNA-programmable nanomaterials.

## 2. Size and shape sorting of colloidal nanomaterials

One nice work adopting gel electrophoresis for shape and size discrimination of nanomaterials was demonstrated by Hanauer *et al.* [4]. As-synthesized silver and gold nanoparticles in mixed shapes (sphere, rod, and triangular plate) were stabilized by polymeric PEG ligands before being loaded on a 0.2% agarose gel. The PEG molecule had a molecular weight of 5000, which was covalently linked with a thiol and a carboxylic group at its two ends. The thiol group provided a strong anchoring of the PEG ligand on the nanoparticles and thus introduced a high density of carboxylic groups. The resulting PEG-capped nanoparticles were well protected from aggregation due to strong electrostatic and steric repulsions. After a voltage was applied on the gel, the silver nanomaterials gradually ran into a broadened band featuring a spectrum of colors (Fig. 1). The different colors stemmed from the shape-dependent plasmonic properties of the metal nanomaterials, allowing a visual observation of the separation process. This meant that nanoparticles with different shapes (and sizes) migrated at slightly different speeds and finally got sorted in the gel. There was also a clear separation between gold spheres (red) and gold rods (green) (Fig. 1). It was important that a suitable

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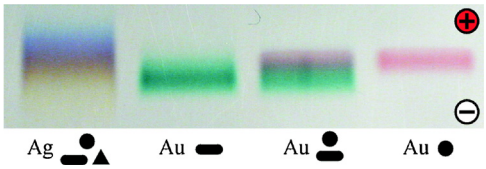


**Fig. 1.** True color agarose gel electrophoretic photograph of Au and Ag nanomaterials with various shapes. Reproduced with permission from Ref. [4].

terminating group should be chosen for the PEG-loaded nanoparticles in order to realize a successful separation. In this case, –COOH terminated PEG displayed the best separation for the nanoparticles.

In the above case, nanoparticles were sorted in an order determined by a mixed size and shape effect (assuming their surface charges were similar). For spherical nanoparticles, it will be much easier to predict their migration order based on their sizes: large particles usually get more retarded in the gel matrix. Therefore, it is possible to show the size resolution of a gel separation for spherical particles. Another important issue that needs to be addressed is how to scale up the separation for preparative purposes. Xu *et al.* answered these two questions in their research [5]. They tried a preparative column gel to isolate mixed gold nanoparticles with diameters of 5, 15, and 20 nm, and achieved a good separation (Fig. 2a). As shown on a regular slab gel, gold nanoparticles with a diameter difference as small as 2–3 nanometers could be readily resolved (Fig. 2b). In addition, the authors employed the column gel for a shape discrimination of gold nanomaterials. As shown in Fig. 2c, the crude sample was separated into two distinct bands corresponding to spheres and plates (mixed with short rods). Very long rods could not migrate into the gel, which were easily recoverable from the top of the gel. Before all separations, the samples were ligand-exchanged by 11-mercaptoundecanoic acid to produce well-defined gel bands.

## 3. Valence separation of DNA-nanoparticle conjugates

### 3.1. DNA–AuNP conjugates

DNA-conjugated nanoparticles are important building blocks for DNA-programmable nanofabrication. One fundamental prerequisite is that the nanoparticles should have a strictly defined DNA valence (number of DNA ligands per particle) in order to realize an accurate assembly control. Unfortunately, these ideal building blocks are hardly achievable in a DNA-conjugation reaction. Instead, a wide distribution of DNA valences are normally obtained for the DNA-nanoparticle conjugates. The introduction of a nanoseparation technique may help to solve this problem.

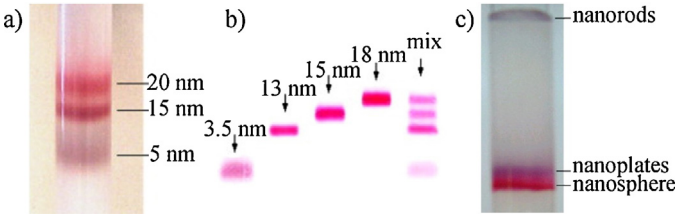


**Fig. 2.** (a) Preparative column agarose gel electrophoretic separation of 5 nm, 15 nm, and 20 nm AuNPs; (b) slab gel electrophoresis of mixed AuNPs of 5 nm, 13 nm, 15 nm, and 18 nm diameters; (c) column gel separation of nanospheres (red), nanoplates (purple), and long nanorods (light brown). Reproduced with permission from Ref. [5].

The isolation of Au nanoparticles (AuNPs) decorated with a specific number of DNA oligonucleotides was shown by Zanchet *et al.* in 2001 [6]. Because the gel mobility of a DNA-nanoparticle conjugate is dictated by its size and surface charge, it is important that unconjugated AuNPs should have a stable and negative surface charge close to or higher than DNA to realize a DNA valence separation. This condition can be met after modifying the AuNPs with a suitable capping agent. In this way, the introduction of a DNA strand on an AuNP will cause a decreased gel mobility due to an increase of its hydrodynamic size. As a result, DNA–AuNP conjugates with mixed valences could be separated into a ladder of gel bands (Fig. 3a). It should be emphasized that monovalent DNA–AuNP products are of special interest for DNA-templated assembly, though all other valences can have different uses. For example, AuNP dimers could be assembled in very high yields by hybridizing two DNA–AuNP mono-conjugates (Fig. 3b) [7]. Following this pioneering work, complicated AuNP superstructures have been made based on DNA programming [2,8].

### 3.2. DNA–AgNP conjugates

It is now known that nanoparticles should have a narrow size distribution, a good stability, and a high surface charge in order to realize a gel-based separation. These restrictions have caused an obvious delay in adapting the agarose gel electrophoretic separation to other metal materials including Ag nanoparticles (AgNPs). Despite the unique chemical, electronic, and optical properties of AgNPs, the weak chemical and colloidal stabilities coupled with the synthetic difficulties of monodisperse AgNPs have made their DNA conjugation quite tricky [9–11]. Zheng *et al.* [12] synthesized 2 nm AgNPs with excellent stability by employing shortened fish sperm DNA (FSDNA) as a nucleation template. Thanks to the existence of the surface-adsorbed FSDNA, a very sharp gel band was observed for the AgNPs. More importantly, the as-obtained AgNPs could be easily decorated with thiolated DNA, based on which a clear DNA valence separation was realized (Fig. 4a). Besides, the gel results clearly revealed that a single thiol

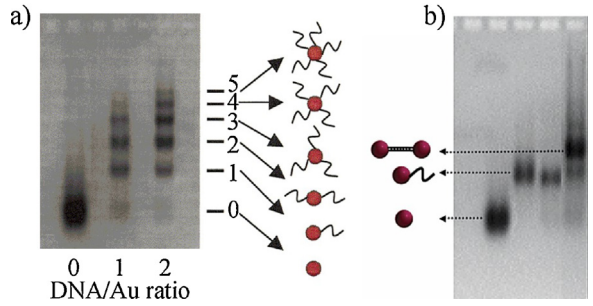


**Fig. 3.** (a) Electrophoretic valence separation of 5 nm DNA–AuNP conjugates; (b) gel purification of AuNP dimers. Reproduced with permissions from Refs. [6,7].

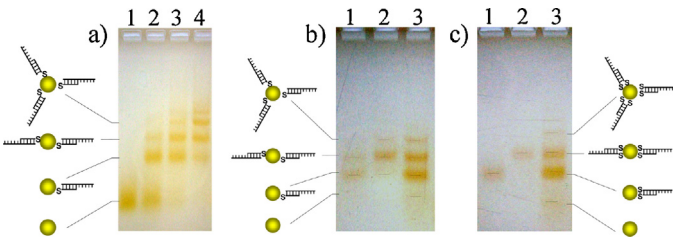


**Fig. 4.** (a) Gel electrophoresis of DNA conjugated AgNPs (lanes 2–4 corresponding to increased DNA:nanoparticle ratio); (b and c) purity check of isolated monovalent (lane 1) and divalent (lane 2) DNA–AgNPs. One and two thiols were appended to the DNA ligands in (b) and (c), respectively. Reproduced with permission from Ref. [12].