Colorimetric Screening

DOI: 10.1002/ange.200605249

A Gold-Nanoparticle-Based Real-Time Colorimetric Screening Method for Endonuclease Activity and Inhibition**

*Xiaoyang Xu, Min Su Han, and Chad A. Mirkin**

Nucleic acids act as the carriers of genetic information, and most organisms contain a type of enzymes called endonucleases,[1] which can hydrolyze the phosphodiester linkages in the nucleic acid backbone. These nucleases are important for many processes involving the replication, repair, and recombination of nucleic acids. Endonucleases such as DNA gyrase and virus integrase play key roles in biological process such as the topological altering of DNA and the insertion of proviral DNA into host chromosomal DNA.[2] Molecules that inhibit endonucleases are therefore considered candidates for a variety of antimicrobial and antiviral drugs. As such, methods for the high-throughput screening of different nuclease inhibitors have become a central part of the drug-development process.[3]

The most widely used assays include those based on the use of chromogenic or fluorogenic substrates to produce a spectrophotometric signal.[4] In many cases, however, it is desirable to measure the reaction between an enzyme and a well-defined nucleic acid substrate of interest, as opposed to a fluorogenic or chromogenic derivative of that substrate. Historically, endonuclease activity has been screened by viscometry, radioactive labeling, and gel electrophoresis, in addition to the more recent fluorescence-based approaches.[5] Most of these protocols are time consuming and do not provide a measure of endonuclease activity in real time. Of these methods, only fluorescence is appreciably used for high-throughput screening, and the fluorescence-based approach has just recently been implemented.[6]

Herein, we report an operationally simple colorimetric endonuclease-inhibition assay, which enables the real-time monitoring of endonuclease activity and the simultaneous determination of the efficiencies of endonuclease inhibitors (e.g., DNA-binding molecules).[7] The new method relies on polymeric aggregates of DNA-functionalized gold nanoparticles (DNA-AuNPs) with DNA-duplex interconnects.[8] DNA-AuNPs have been used previously to detect DNA, proteins, metal ions, and DNA-binding molecules.[9] Others have used gold nanoparticles and electrochemical approaches to evaluate enzymatic activity.[10] To our knowledge, we report the first example of the use of DNA-AuNPs as colorimetric indicators to evaluate enzymatic activity and to screen enzyme inhibitors. In principle, this method can be used to screen libraries of inhibitors of endonucleases in a high-throughput fashion by using either the naked eye or a simple colorimetric reader.

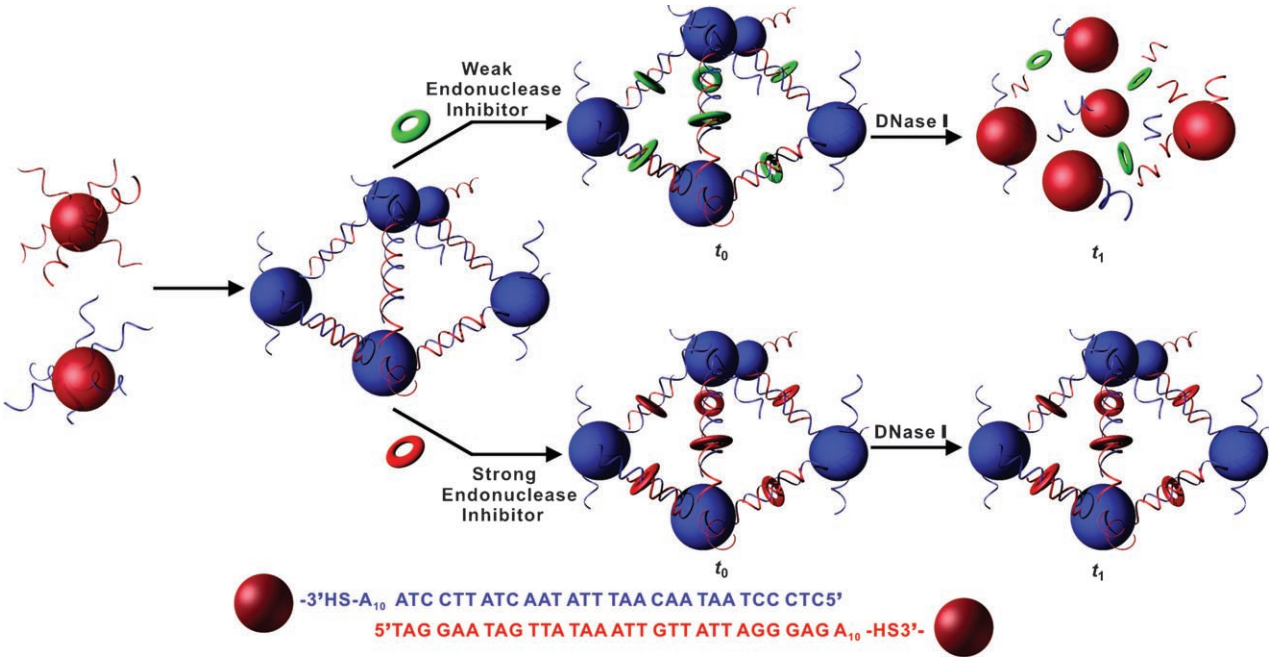
Probes were prepared by functionalizing two separate batches of 13-nm gold particles with two different thiol-modified oligonucleotide strands, DNA-1 (5′-CTCCCTAA-TAACAATTTATAACTATTCCTA-A_{10}-SH-3′) and DNA-2 (5′-TAGGAATAGTTATAAATTGTTATTAGGGAG-A_{10}-SH-3′). These functionalized particles are denoted DNA-AuNP-1 and DNA-AuNP-2. DNA-1 and DNA-2 are complementary to each other. Therefore, DNA-AuNP-1 and DNA-AuNP-2 can hybridize to form a cross-linked network of nanoparticles, which is purple in color owing to the red-shifted plasmon band of the gold nanoparticles (13 nm). This red-shifting is a well-understood process and is a highly diagnostic feature of aggregate formation.[11] These aggregates can then be used as colorimetric indicators of endonuclease activity (Scheme 1). As the endonuclease degrades the DNA-duplex interconnects, particles are released, regenerating a red color due to the dispersed nanoparticles. The color can be observed with the naked eye, or the absorbance (520 nm) can be measured by UV/Vis spectroscopy.

The DNA-AuNP aggregates were used to evaluate the enzymatic activity of deoxyribonuclease (DNase) I. In a typical experiment, DNase I, at a predetermined concentration (10, 15, 20, 30, or 40 units mL^{-1}), was added to a solution of the aggregates. The color of the solution gradually changed from purple to red. By measuring the absorbance at 520 nm, we could quantitatively follow the nucleic acid hydrolysis catalyzed by DNase I (Figure 1 a). The reaction rate increases with increasing enzyme concentration and can be followed in real time.

In addition to screening enzyme activity, one can easily use the assay to evaluate the efficiency of inhibitors of DNase I. In a typical screening experiment, DNase I (15 units mL^{-1}) was added to solutions of the DNA-AuNP aggregates in the presence of one of the following DNA-binding molecules (1 μM): amsacrine (AMSA), anthraquinone-2-carboxylic acid (AQ2A), 9-aminoacridine (9-AA), ellipticine (EIPT), daunorubicin (DNR), ethidium bromide (EB), or 4′,6-diamidino-2-phenylindole (DAPI). These DNA-binding molecules are known to inhibit DNase I.[7,12] The absorbance at 520 nm was monitored as a function of time (sample scan rate = 5 min^{-1}; Figure 1 b), and the color of the solution was followed with the naked eye (Figure 2). The time

[*] X. Xu,[+] Dr. M. S. Han,[#][+] Prof. C. A. Mirkin
Department of Chemistry and
International Institute for Nanotechnology
Northwestern University
2145 Sheridan Road, Evanston, IL 60208-3113 (USA)
Fax: (+1) 847-467-5123
E-mail: chadnano@northwestern.edu
[#] Current address:
Department of Chemistry, Chung-Ang University
Seoul 156-765, Korea
[+] These authors equally contributed to this work.

[**] This work was supported by AFOSR, DARPA, NCI/CCNE, and NSF. C.A.M. is grateful for a NIH Director's Pioneer Award.



Scheme 1. Illustration of the aggregation and dissociation of the DNA-AuNP probes used in the colorimetric screening of endonuclease inhibitors. The probes consist of gold nanoparticles (spheres) functionalized with two complimentary oligonucleotides (blue and red ribbons). The individual nanoparticles (red) aggregate into a cross-linked network of nanoparticles (blue) through the hybridization of their oligonucleotide chains. Upon addition of DNase I, the aggregates remain intact longer in the presence of a strong endonuclease inhibitor.

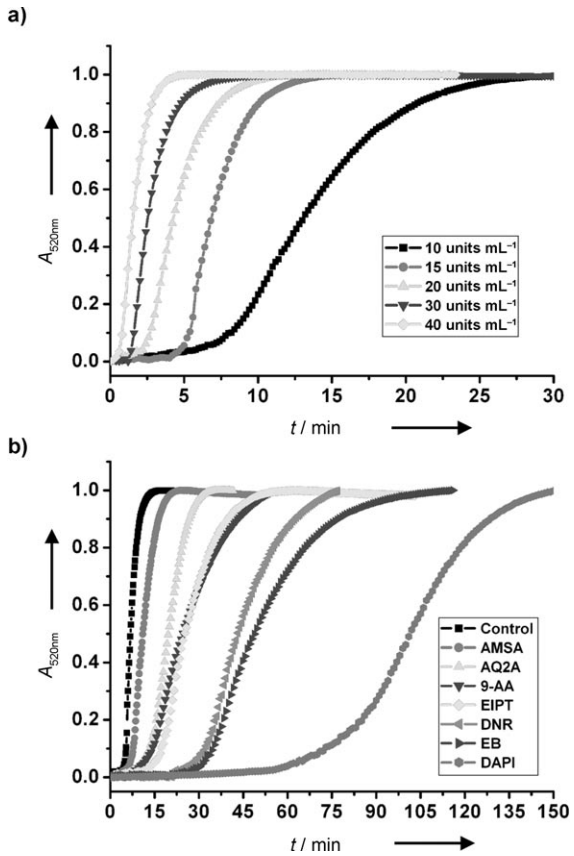


Figure 1. Normalized dissociation curves for the DNA-AuNP aggregates a) at different DNase I concentrations, and b) in the absence and presence of endonuclease inhibitors (1 μM) at a DNase I concentration of 15 units mL^{-1}. The change in the absorbance at 520 nm in the UV/Vis spectra was monitored.

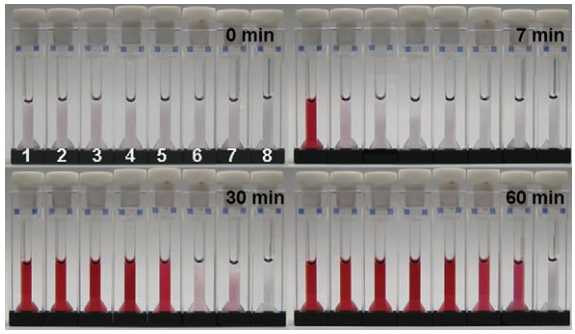


Figure 2. Colors of solutions of the DNA-AuNP aggregates in the absence (1: control) or presence of endonuclease inhibitors (2: AMSA, 3: AQ2A, 4: 9-AA, 5: EIPT, 6: DNR, 7: EB, 8: DAPI) at specific time points after the addition of DNase I.

at which 50% of the aggregates are hydrolyzed (T_H) can be used as a measure of inhibition. The inhibitors decrease the DNase I activity, and increase the T_H and, therefore, the corresponding time required for a solution color change. The inhibitors studied exhibit the following trend in T_H values: DAPI > EB > DNR > 9-AA, EIPT, AQ2A > AMSA (Figure 1b, Table 1). This order is consistent with the relative binding affinities of the inhibitors to DNA, as determined by measuring the melting temperature of the duplex DNA in the presence of each DNA-binding molecule (Table 1). This approach can also be used for the high-throughput screening of endonuclease inhibitors through visual inspection, whereby the relative degree of endonuclease inhibition can be differentiated easily (Figure 2).

In conclusion, we have developed a new colorimetric assay for screening endonuclease activity and determining the

Table 1: Melting temperatures (T_m) of duplex DNA in the presence of endonuclease inhibitors and hydrolysis times (T_H) for the DNA-AuNP aggregates in the presence of the inhibitors and DNase I.

Inhibitor	T_m[a] [°C]	T_H[b] [min]
control	61.0	6.8
AMSA	61.5	11.0
AQ2A	62.5	19.6
9-AA	62.5	24.6
EIPT	63.0	25.2
DNR	65.5	43.2
EB	65.5	48.8
DAPI	77.0	101.2

[a] Conditions: DNA duplex (2.0 μM) in sodium phosphate buffer (10 mM, pH 7.0) containing sodium chloride (100 mM), in the presence of the inhibitor (5.0 μM). [b] T_H is the time at which 50% of the aggregates are hydrolyzed, as determined by monitoring the absorbance at 520 nm in the UV/Vis spectra. See Experimental Section for conditions.

relative inhibitory potencies of potential inhibitors by monitoring the kinetics of DNA-AuNP aggregate dissociation. This screening approach is simpler than other assays: it is easy to monitor and provides a rapid qualitative indication of relative inhibition capabilities. For high-throughput screening, a way of qualitatively measuring differences in endonuclease activity is extremely useful.[13]

Experimental Section

Preparation of DNA-AuNP aggregates: DNA-AuNP-1 and DNA-AuNP-2 were prepared by functionalizing gold nanoparticles with two complementary 3′-thiol-modified 30-mer oligonucleotides, DNA-1 (5′-CTCCCTAATAACAATTTATAACTATTCCTA-A10-SH-3′) and DNA-2 (5′-TAGGAATAGTTATAAATTGTTATTAGGGAG-A_{10}-SH-3′), using previously reported methods.[14] After combining 1-mL aliquots of the two probes DNA-AuNP-1 and DNA-AuNP-2 (each 3 nM) in hybridization buffer (tris(hydroxymethyl)aminomethane (Tris)–HCl buffer (50 mM, pH 7.0) containing $MgCl_2$ (2 mM)), the mixture was heated to 90 °C and held at this temperature for 10 min. The solution was cooled to room temperature, which resulted in hybridization of the particles and the concomitant diagnostic red-to-purple color change.

Colorimetric screening assay: The as-prepared DNA-AuNP aggregates were washed three times with DNase I buffer (sodium phosphate buffer (10 mM, pH 7.0) containing $MgCl_2$ (0.75 mM)). They were then resuspended in 1 mL of DNase I buffer. A 10-μL aliquot of each of the endonuclease inhibitors (0.1 mM) was then added to a solution (1 mL) of the aggregates and incubated for 10 min. The assay was initiated by adding DNase I, and the hydrolysis was monitored by UV/Vis spectroscopy (Cary 5000, Varian). The solution was continuously stirred with a magnetic stir bar at room temperature to keep the aggregates suspended.

Received: December 28, 2006
Published online: March 27, 2007

Keywords: endonucleases · high-throughput screening · inhibitors · nanoparticles · oligonucleotides

[1] E. S. Rangarajan, V. Shankar, *FEMS Microbiol. Rev.* **2001**, *25*, 583.
[2] a) L. A. Mitscher, *Chem. Rev.* **2005**, *105*, 559; b) H. Zhao, N. Neamati, S. Sunder, H. Hong, S. Wang, G. W. A. Milne, Y. Pommier, T. R. Burke, Jr., *J. Med. Chem.* **1997**, *40*, 937.
[3] a) P. A. Johnston, *Drug Discovery Today* **2002**, *7*, 353; b) D. L. Boger, J. Desharnais, K. Capps, *Angew. Chem. Int. Ed.* **2003**, *115*, 4270; *Angew. Chem. Int. Ed.* **2003**, *42*, 4138; c) S. Wang, T. B. Sim, Y.-S. Kim, Y.-T. Chang, *Curr. Opin. Chem. Biol.* **2004**, *8*, 371.
[4] a) E. D. Matayoshi, G. T. Wang, G. A. Krafft, J. Erickson, *Science* **1990**, *247*, 954; b) J.-L. Reymond, D. Wahler, *ChemBioChem* **2002**, *3*, 701.
[5] a) M. Laskowski, M. D. Seidel, *Arch. Biochem.* **1945**, *7*, 465; b) E. P. Geiduschek, A. Daniels, *Anal. Biochem.* **1965**, *11*, 133; c) R. Kohen, M. Szyf, M. Chevion, *Anal. Biochem.* **1986**, *154*, 485.
[6] K. Eisenschmidt, T. Lanio, A. Jeltsch, A. Pingoud, *J. Biotechnol.* **2002**, *96*, 185.
[7] R. A. Ikeda, P. B. Dervan, *J. Am. Chem. Soc.* **1982**, *104*, 296.
[8] C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607.
[9] a) R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger, C. A. Mirkin, *Science* **1997**, *277*, 1078; b) L. He, M. D. Musick, S. R. Nicewarner, F. G. Salinas, S. J. Benkovic, M. J. Natan, C. D. Keating, *J. Am. Chem. Soc.* **2000**, *122*, 9071; c) D. J. Maxwell, J. R. Taylor, S. Nie, *J. Am. Chem. Soc.* **2002**, *124*, 9606; d) T. Niazov, V. Pavlov, Y. Xiao, R. Gill, I. Willner, *Nano Lett.* **2004**, *4*, 1683; e) V. Pavlov, Y. Xiao, B. Shlyahovsky, I. Willner, *J. Am. Chem. Soc.* **2004**, *126*, 11768; f) J. Liu, Y. Lu, *J. Am. Chem. Soc.* **2003**, *125*, 6642; g) M. S. Han, A. K. R. Lytton-Jean, B.-K. Oh, J. Heo, C. A. Mirkin, *Angew. Chem.* **2006**, *118*, 1839; *Angew. Chem. Int. Ed.* **2006**, *45*, 1807; h) M. S. Han, A. K. R. Lytton-Jean, C. A. Mirkin, *J. Am. Chem. Soc.* **2006**, *128*, 4954.
[10] a) T. Niazov, V. Pavlov, Y. Xiao, R. Gill, I. Willner, *Nano Lett.* **2004**, *4*, 1683; b) Z. Wang, R. Levy, D. G. Fernig, M. Brust, *J. Am. Chem. Soc.* **2006**, *128*, 2214; c) G. Cristian, P. Lucia, D. F. Vincenzo, S. Paolo, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 3978; d) A. G. Kanaras, Z. Wang, A. D. Bates, R. Cosstick, M. Brust, *Angew. Chem.* **2003**, *115*, 201; *Angew. Chem. Int. Ed.* **2003**, *42*, 191.
[11] N. L. Rosi, C. A. Mirkin, *Chem. Rev.* **2005**, *105*, 1547.
[12] a) S. M. Forrow, M. Lee, R. L. Souhami, J. A. Hartley, *Chem.-Biol. Interact.* **1995**, *96*, 125; b) P. G. Baraldi, A. Bovero, F. Fruttarolo, D. Preti, M. A. Tabrizi, M. G. Pavani, R. Romagnoli, *Med. Res. Rev.* **2004**, *24*, 475.
[13] J. Bajorath, *Nat. Rev. Drug Discovery* **2002**, *1*, 882.
[14] J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, *120*, 1959.

