Gold Nanoparticle Assists SNP Detection at Room Temperature in the NanoBioArray Chip

Abootaleb Sedighi and Paul C. H. Li
Department of chemistry, Simon Fraser University, Burnaby, British Columbia, Canada
Email: asedighi@sfu.ca, paulli@sfu.ca

***Abstract*—This study employs a NanoBioArray (NBA) chip for single base-pair discrimination between the sequence of the wild type Kras gene and three of its common codon 12 mutants, which are frequently observed in the adenocarcinoma diseases. Here we use gold nanoparticles (AuNPs) in order to perform the single nucleotide polymorphism (SNP) assay without the need of temperature stringency. In the NBA chip, an array of 20-mer oligonucleotide probes was first created on the glass surface, and then a trace amount (2.5 fmol) of target oligonucleotides (20-mer and 60-mer) were introduced through the PDMS microchannels. DNA hybridization between the targets and their complementary probes was achieved at the intersections between microchannels and probe lines. The target oligonucleotides were previously loaded on the AuNP surfaces which established a competition between the comlementary probes and the AuNP surface for binding to the targets. This competition, in turn, enhances the hybridization stringency which disfavor sthe mismatch binding thus facilitates the SNP detection abilities. Using this technique fast and high-throughput multiple discrimination of Kras gene codon 12 was achieved at room-temperature using the NBA chip and the specificity of the method was proved to be as high as the temperature stringency method.**

***Index Terms*—single nucleotide polymorphism (SNP), kras gene, gold nanoparticle, target DNA, microarray, NanoBioArray chip.**

I. Introduction

The drugs targeting epidermal growth factor receptor (EGFR) have been approved for clinical practice for many patients with colorectal cancer [1]. However, the patients carrying a single nucleotide polymorphism (SNP) in their Kras gene have been reported to have resistance to the therapy. Given that around 40% of patients with metastatic colorectal cancer harbor the SNP sites in the Kras gene, prior detection of these SNPs is critical for the selection of the type of therapy [2]. Most of the methods currently available for SNP detection of Kras gene are based on DNA sequencing or real-time PCR. DNA sequencing is known as the gold standard technique for the SNP detection; however this approach is expensive, labor-intensive and low-throughput [3]. Real-time PCR is another approach, but this technique suffers from non-linear amplification [2]. DNA bioarrays, on the other hand, provide a high-throughput platform for multiple analyses of SNPs [4]. Combined with the nanofluidic technology, this approach makes the SNP detection faster and cheaper with a high potential of automation [5], [6]. The requirement of temperature optimization for detecting individual sequences, however, imposes a critical limitation for the multiple mutation detection using this technique [7].

Gold nanoparticles (AuNPs) possess unique chemical and physical properties that make them attractive for various DNA analysis applications [8]-[11]. While most of the DNA-biosensing applications involving AuNPs employ the chemisorption of thiol-modified oligonucleotides on the AuNP surface, Li et al. reported in 2004 that AuNPs adsorb non-thiolated single-stranded DNA (ssDNA), but not double-stranded dsDNA, on their citrate-capped particle surfaces [12]. This selective adsorption of ssDNA on the AuNP has been utilized in several DNA analysis sensors. Wang et al. observed that AuNP-loaded complementary target DNAs (AuNP-targets) hybridized to the surface-immobilized probe, i.e. perfectly-matched (PM), but the AuNP-loaded non-complementary target did not, i.e. single base-pair mismatched (MM) [13]. There was no such discrimination when the targets were in the random-coil format (free targets).

In this report, we aim to develop the AuNP-assisted method for SNP analysis of the Kras gene codon 12. Here we utilize NanoBioArray (NBA) chip in order to perform the SNP analysis in a faster and cheaper way. We manage to discriminate the PM and MM targets using both probes.

II. Experimental Section

A. Reagents and Materials

Gold nanoparticles (5 nm, stabilized with tannic acid) were purchased from Sigma life science. Sodium dodecyl sulfate (SDS), 3-Aminopropyltriethoxysilane (APTES), 25% glutaraldehyde and Triton X-100 were purchased from Sigma-Aldrich. Negative photoresist (SU-8 50) and its developer were purchased from MicroChem Corp. (Newton, MA). The glass microscope slides of 3 in. × 2 in. were obtained from Fisher Scientific Co. (Ottawa, ON, Canada). All oligonucleotides were synthesized and modified by Integrated DNA Technologies (Coralville,

Manuscript received August 8, 2013; revised September 22, 2013.

doi: 10.12720/ijmse.1.1.45-49

IA). Target oligonucleotides (60-mer) with the sequence of the Kras gene were modified with biotin at the 5'- end. The sequences were designed in such a way that the codon 12 sequence (the SNP site) was located at the center of the oligonucleotide strands. The SNP site either have the wild-type (W) nucleotide or one of 3 mutant ones. The mutant strands are G12A (A), G12D (D) and G12V (V). Four different 20-mer oligonucleotides, each of them complementary to one of the targets were designed. The probes were modified with an amine group and a C12 spacer at the 5'-end.

B. NBA Chip Fabrication

Fabrication of PDMS slab (2 in. × 2 in.) with 16 parallel channels has been described elsewhere [7]. The width of straight channels was 200 μm and the height was 35 μm. A flat-end syringe needle was used to punch out the solution reservoirs (1 mm in diameter) at both ends of the channels on the 2 mm-thick PDMS slab. Glass slides were aldehyde-functionalized using an established procedure [7]. PDMS slab and the modified glass slide were reversibly sealed together to create the NBA chip (Fig. 1A).

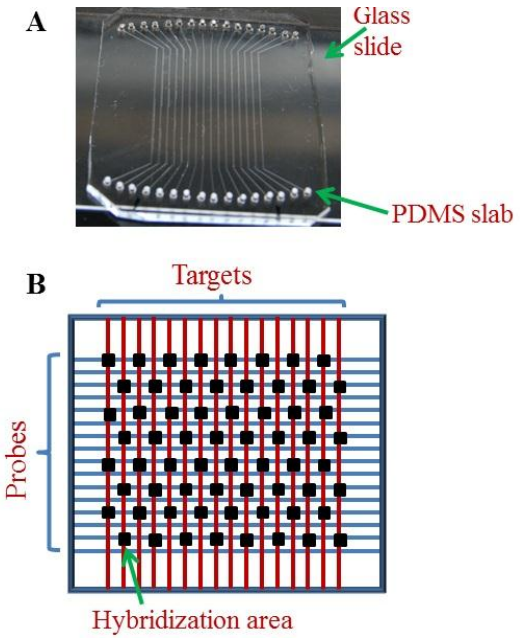


Figure 1. An image of the NBA chip (A), and a schematic of the analysis procedure (B).

C. Probe Immobilization

The probes were printed with a procedure similar to a previous report [6], [7]. Briefly, 0.5 μl of probe solution (in 1.0M NaCl + 0.15M $NaHCO_3$) was added to the inlet reservoir of each of the 16 horizontal channels of the NBA chip and the solution was then filled in the channel using suction applied at the outlet reservoir. The probe solution was pumped out of the channel after 30 min. of incubation at room temperature. Subsequent to washing of the channel, the PDMS slab was peeled off, leaving behind 16 probe lines on the glass slide. The glass slide was rinsed and dried.

D. DNA Hybridization

Another PDMS slab with 16 channels was sealed against the glass slide pre-printed with the probe lines. The straight channels were orthogonal to the probe lines on the glass slide surface. Before hybridization to the probe, the target oligonucleotides were conjugated non-covalently to the surface of gold nanoparticles. In order to achieve this, gold nanoparticle solution was added to the target solution so that the ratio of the gold nanoparticle to the target was 1:1. The mix was incubated at 95 ℃ for 5 min. Control solutions (free-target), which were not mixed with the AuNP solutions, were prepared similar to the AuNP-target solutions. The conjugate (target-AuNP) was then prepared in a hybridization buffer (1X SSC+0.2% SDS) to produce a final concentration of 10 nM. AuNP-target solution (0.5 μl) was added to the inlet reservoir and then filled in the channel using suction. The hybridization of the targets to the complementary probes occurred at the intersection of target channels with the probe lines, resulting in hybridization patches of 200 × 200 μm^2 in dimensions (Fig. 1B). The target solutions were pumped out from the channels after an incubation time of 20 min. (unless noted otherwise) at room temperature (22 ℃). Immediately after hybridization, the channels were washed by 2 μl of hybridization buffer. In order to detect the oligonucleotide duplexes formed at the hybridization patches, streptavidin-Cy5 solution (50 μg/ml in 1X PBS buffer) was added to the channels. After incubation for 15 min, the channel was rinsed using a wash solution (1X PBS, Tween-20 0.1%) and then the PDMS slab was peeled off from the glass slide.

E. Detection

Following rinsing and drying, the glass slide was scanned on a confocal laser fluorescent scanner (Typhoon 9410, GE Healthcare) at 10 μm resolution, as previously described [7], [14]. The excitation and emission wavelengths were 633 and 670 nm, respectively. The photomultiplier tube voltage was set to 600 V. The scanned image was analyzed by IMAGEQUANT 5.2 software. The average fluorescent signals were measured in relative fluorescent unit.

F. Bead-Based Assay

In the bead-based method, different probe DNAs were first immobilized on the color-coded beads (Luminex). The hybridization of the target DNAs to the bead-immobilized probes were monitored via a Luminex 200 instrument. The target DNAs were either free-targets or previously loaded on the surface of 5 nm AuNP with DNA-to-AuNP concentration ratios of 1:1, 2:1 and 3:1.

III. Results and Discussion

The schematic diagram in Fig. 2 shows how the AuNP-targets discriminate between the PM probes and the MM probes. At a low temperature (e.g. room temperature) and fairly high salt concentration (NaCl 0.15 M), which we used in our experiments, free-target solution have a high tendency to hybridize to the complementary probes on the surface. In such a low stringent condition the

hybridization energy is high enough to compensate for the destabilizing mismatch base-pair at the center of the strand, and therefore results in a high concentration of MM duplexes formed on the surface. This makes the SNP detection very difficult. Unlike the bases of the free-target strands, which can freely hybridize to the probe bases, the bases of AuNP-targets are bound to the AuNP surfaces, rendering them less available. This provides a selection mechanism for the hybridization to occur between the PM, but not the MM strands.

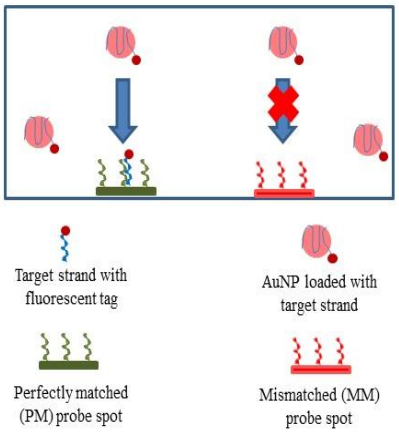


Figure 2. Schematic diagram of single base-pair discrimination of AuNP-targets

Fig. 3 shows the results of NBA chip hybridization. The scanned image in Fig. 3A shows the spots resulted from hybridization of two 60-mer targets (W' and D') to their corresponding PM and MM probes (W and D), performed in duplicate. The histogram (Fig. 3B) was created based on measured signal intensities of the spots along the vertical target channel line. The discrimination ratio, calculated based on the ratio of the mean signal intensities of PM and MM, is shown above the bars in Fig. 3B. We observe that when targets are free, there is no significant difference between the signal intensities of the PM and MM spots or the discrimination ratio is close to 1.0. A significant improvement is observed in case of AuNP-targets, in which the PM/MM discrimination ratios are 2.8 ± 0.2 and 2.4 ± 0.2 for W' and D' targets, respectively.

These results show that using the AuNP-assisted method in the NBA chip, the targets can effectively discriminate between their PM probes and the MM probes at room-temperature at a short hybridization time of 20 min. [15], [16].

Following the AuNP-assisted method using wild-type and one of its mutants (G12D) of the Kras gene sequences, the method was evaluated fully using all 3 of the codon 12. Other than the NBA chip, we have tried to combine the AuNP-assisted method with the bead-based method, which is a solution-based single base-pair discrimination technique [17]. Fig. 4 shows the discrimination ratios resulted from target hybridizations to the bead-immobilized probes. The discrimination ratios are calculated based on the hybridization signal intensities of each target with its PM probe divided by the average signal resulted from its hybridization to the 3 MM probes. As shown in Fig. 4, the discrimination ratios from the targets loaded on the AuNP surfaces with various ratios are not found to be different from those obtained from the free-targets. The failure of AuNP binding in enhancing the discrimination ratios in the bead-based assay may be attributed to the differences in the DNA hybridization conditions in the solution phase from the hybridization on the surface of the NBA chip [18].

Fig. 5A and 5B show the image and the corresponding histogram of room-temperature hybridization of free-target as well as AuNP-targets of all Kras mutant sequences in the NBA chip. The numbers above each group of PM and MM column are the discrimination ratio of each target which are the signal of PM spot divided by the average of the signals of other 3 MM spots. Although the discrimination ratios of W and D were similar to those shown in Fig. 3, the ratios of A and V were higher. This shows the discrimination was enabled by AuNPs only in the NBA chip but not in the bead-based assay. Since the AuNP-targets show lower signal intensities than their corresponding free-target spots in the NBA chip, further optimizations will be needed.

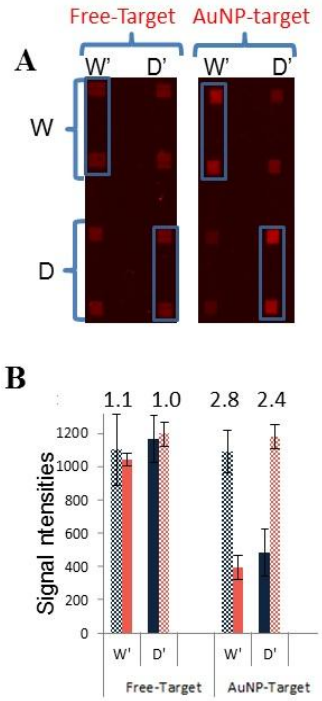


Figure 3. A) Scanned image with the spots resulted from hybridization of 60-mer targets (W' and D') with their corresponding PM and MM probes at 22 ℃ for 20 min. The targets were either free or previously conjugated with AuNPs (5 nm). The boxed regions are the expected true positive binding regions, corresponding to the hollow bars in the histogram. B) The histogram showing the signal intensities of the spots obtained along the vertical target channels. The column bars show the average of signal intensities of the spots, measured at the intersection of horizontal probe lines and the vertical target lines. Error bars show the standard deviation of 2 measurements. The number above each column shows the discrimination ratio which is determined by dividing the intensity of the column corresponds to the spots at the PM probes (W'-W, D'-D) by the signal intensities of the column correspond to the spots at the MM probes (W'-D, D'-W).

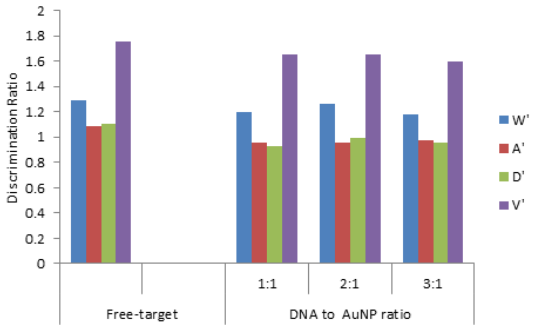


Figure 4. The discrimination ratios resulted from bead-based assays of the wild type target(W'), and the mutants G12A(A'), G12D (D') G12V (V') at room temperature. The targets are either free-targets or loaded on the AuNP surfaces with different ratios.

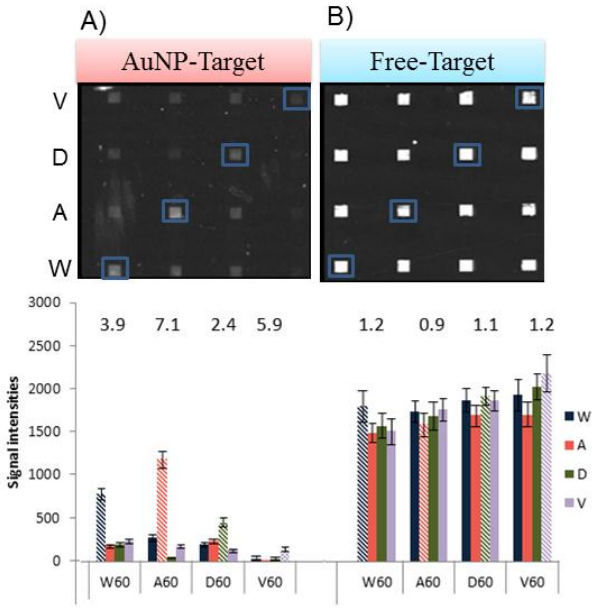


Figure 5. Original scanned image and resulted histogram of (A) 5-nm AuNP-target and (B) free-target (60-mer) of the wild type target (W'), and the mutants G12A(A'), G12D (D') G12V (V'). For other conditions see Fig. 3.

ACKNOWLEDGEMENT

We gratefully acknowledge financial support from the Natural Sciences and Engineering Research Council of Canada for a Discovery Grant. We thank Dr. John Brunstein (BC Children's Hospital) for useful suggestion in detection of the Kras mutation, and Dr. Dipankar Sen (SFU Department of Molecular Biology and Biochemistry) for useful advice and technical support on PCR amplification.

REFERENCES

[1] D. French, A. Smith, M. P. Powers, and A. H. B. Wu, "KRAS mutation detection in colorectal cancer by a commercially available gene chip array compares well with Sanger sequencing," *Clinica Chimica Acta, International Journal of Clinical Chemistry*, vol. 412, no. 17–18, pp. 1578–81, Aug. 2011.

[2] L. van der Weyden and D. J. Adams, "The Ras-association domain family (RASSF) members and their role in human tumourigenesis," *Biochimica Et Biophysica Acta*, vol. 1776, no. 1, pp. 58–85, Sep. 2007.

[3] K. Sato, M. Onoguchi, Y. Sato, K. Hosokawa, and M. Maeda, "Non-cross-linking gold nanoparticle aggregation for sensitive detection of single-nucleotide polymorphisms: Optimization of the particle diameter," *Analytical Biochemistry*, vol. 350, no. 1, pp. 162–4, Mar. 2006.

[4] A. Sedighi and P. C. H. Li, "Challenges and future trends in DNA microarray analysis," in *Fundamentals of Advanced Omic Technologies: From Genes to Metabolites*, C. Simó, A. Cifuentes, V. Garcá-Cañas, eds. Elsevier Science, 2013.

[5] A. Sedighi, L. Wang, and P. C. H. Li, "2D Nanofluidic bioarray for nucleic acid analysis," in *Nanopatterning and Nanoscale Devices for Biological Applications*, K. Iniewski and S. Selimovic eds. Tylor & Fransis, CRC press 2013.

[6] L. Wang, P. C. H. Li, H.-Z. Yu, and A. M. Parameswaran, "Fungal pathogenic nucleic acid detection achieved with a microfluidic microarray device," *Analytica chimica acta*, vol. 610, no. 1, pp. 97–104, Mar. 2008.

[7] L. Wang and P. C. H. Li, "Flexible microarray construction and fast DNA hybridization conducted on a microfluidic chip for greenhouse plant fungal pathogen detection," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 26, pp. 10509–10516, Dec. 2007.

[8] K. Saha, S. S. Agasti, C. Kim, X. Li, and V. M. Rotello, "Gold nanoparticles in chemical and biological sensing," *Chemical Reviews*, vol. 112, no. 5, pp. 2739–79, May 2012.

[9] T. a Taton, C. a Mirkin, and R. L. Letsinger, "Scanometric DNA array detection with nanoparticle probes," *Science (New York, N.Y.)*, vol. 289, no. 5485, pp. 1757–60, Sep. 2000.

[10] X. Zhao, R. Tapec-Dytioco, and W. Tan, "Ultrasensitive DNA detection using highly fluorescent bioconjugated nanoparticles," *Journal of the American Chemical Society*, vol. 125, no. 38, pp. 11474–11475, Sep. 2003.

[11] P. Tiwari, K. Vig, V. Dennis, and S. Singh, "Functionalized gold nanoparticles and their biomedical applications," *Nanomaterials*, vol. 1, no. 1, pp. 31–63, June 2011.

[12] H. Li and L. Rothberg, "Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles," in *Proc. of the National Academy of Sciences of the United States of America*, vol. 101, no. 39, Sep. 2004, pp. 14036–14039.

[13] L. Wang and P. C. H. Li, "Gold nanoparticle-assisted single base-pair mismatch discrimination on a microfluidic microarray device," *Biomicrofluidics*, vol. 4, no. 3, p. 32209, Jan. 2010.

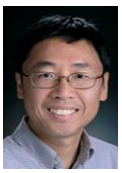
[14] L. Wang and P. C. H. Li, "Optimization of a microfluidic microarray device for the fast discrimination of fungal pathogenic DNA," *Analytical Biochemistry*, vol. 400, no. 2, pp. 282–8, May 2010.

[15] M. Maekawa, T. Nagaoka, T. Taniguchi, H. Higashi, H. Sugimura, *et al.*, "Three-dimensional microarray compared with PCR-single-strand conformation polymorphism analysis/DNA sequencing for mutation analysis of K-ras codons 12 and 13," *Clinical Chemistry*, vol. 50, no. 8, pp. 1322–1327, Aug. 2004.

[16] J. H. Park, I. J. Kim, H. C. Kang, Y. Shin, and H. W. Park, "Oligonucleotide microarray-based mutation detection of the k-ras gene in colorectal cancers with use of competitive DNA hybridization," *Clinical Chemistry*, vol. 50, no. 9, pp. 1688-1691, Sep. 2004.

[17] S. A. Dunbar, "Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection," *Clinica Chimica Acta; International Journal of Clinical Chemistry*, vol. 363, no. 1–2, pp. 71–82, Jan. 2006.

[18] D. Irving, P. Gong, and R. Levicky, "DNA surface hybridization: Comparison of theory and experiment," *The Journal of Physical Chemistry. B*, vol. 114, no. 22, pp. 7631–40, July 2010.



Paul C.H. Li obtained his Ph.D. degree in Analytical Chemistry in the University of Toronto of Canada in 1995. He conducted postdoctoral research at the University of Alberta of Canada. He started his independent academic career at City University of Hong Kong, and joined Simon Fraser University in 1999, and became full professor in 2010. He developed the

microfluidic lab-on-a-chip and conducted single-cell analysis for analyzing the effects of chemical compounds on individual cancer cells. His research interests also include on-chip nucleic acid analysis, protein assays, and radiochemical synthesis. He has published a monograph: Fundamentals of lab on a chip for biological analysis and discovery in 2010. Dr. Li is associate editor of the international journal called "Canadian Journal of Pure and Applied Sciences". He is the inventor of 4 issued patents and 5 pending patents, and he is the founder of ZellChip Technologies Inc. specializing in microfluidic-based instrument for cellular and DNA analysis.

Abootaleb Sedighi obtained his B.Sc. degree in Chemistry at Yasouj University of Iran in 2004. In 2007, he obtained the M.Sc. degree in Analytical Chemistry at Shahid Beheshti University of Iran. He is currently a Ph.D. candidate at the Department of Chemistry of Simon Fraser University. His research interests are in nanoparticle-assisted nucleic acid detection using a nanobioarray chip, and thermodynamic and kinetic studies on nucleic acid hybridization. His research experiences include design and development of various steps of nucleic acids analysis including primer and probe designs, various amplification techniques and bioarray detection, either performed on separated devices or integrated in a Lab-on-a-chip platform.

