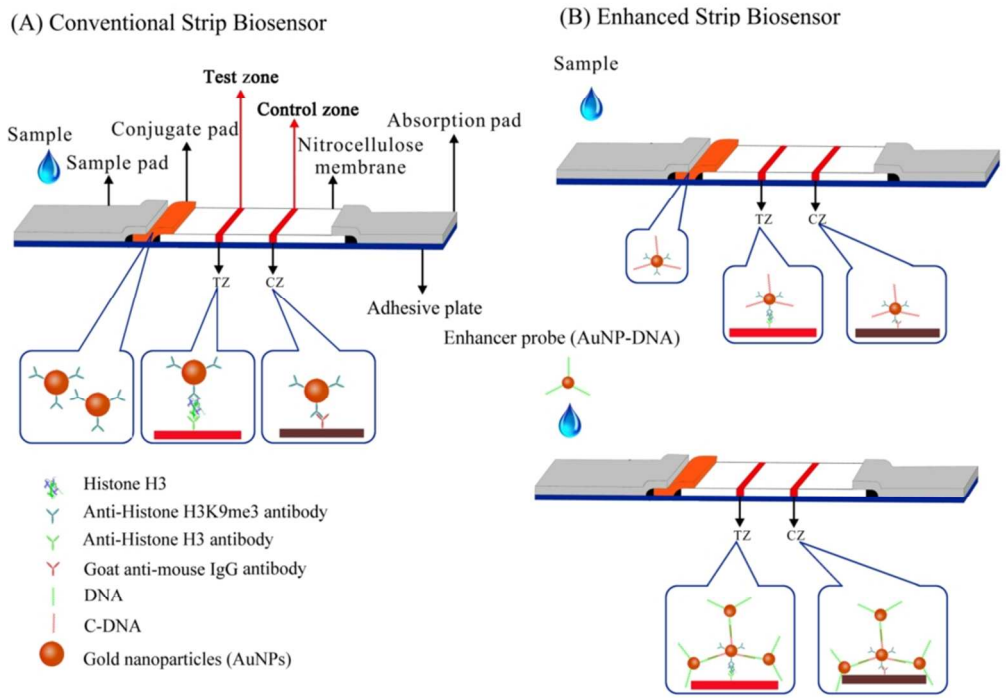
Article

An enhanced strip biosensor for rapid and sensitive detection of histone methylation

Chenchen Ge, Luxin Yu, zhiyuan fang, and Lingwen Zeng

Anal. Chem., **Just Accepted Manuscript** • DOI: 10.1021/ac402202x • Publication Date (Web): 26 Aug 2013



Scheme 1. (A) In the conventional strip biosensor for H3K9me3 detection, AuNPs on the conjugate pad are only labelled with an anti-Histone H3K9me3 monoclonal antibody. (B) In the enhanced strip biosensor, the AuNPs on the conjugate pad are labelled with an anti-Histone H3K9me3 antibody and c-DNA simultaneously. Mouse monoclonal antibody against histone H3 and goat anti-mouse IgG antibody are immobilized on the NC membrane to form the test zone and control zone respectively. In the presence of histone extract two red bands are observed and the dual labelled AuNPs are immobilized on the test zone and control zone, respectively. Ten minutes later, AuNP-DNA solution is loaded onto the strip to hybridize with the c-DNA on the surface of dual labelled AuNPs. The detection results can be observed within 15 min by the naked eye.
266x186mm (96 x 96 DPI)

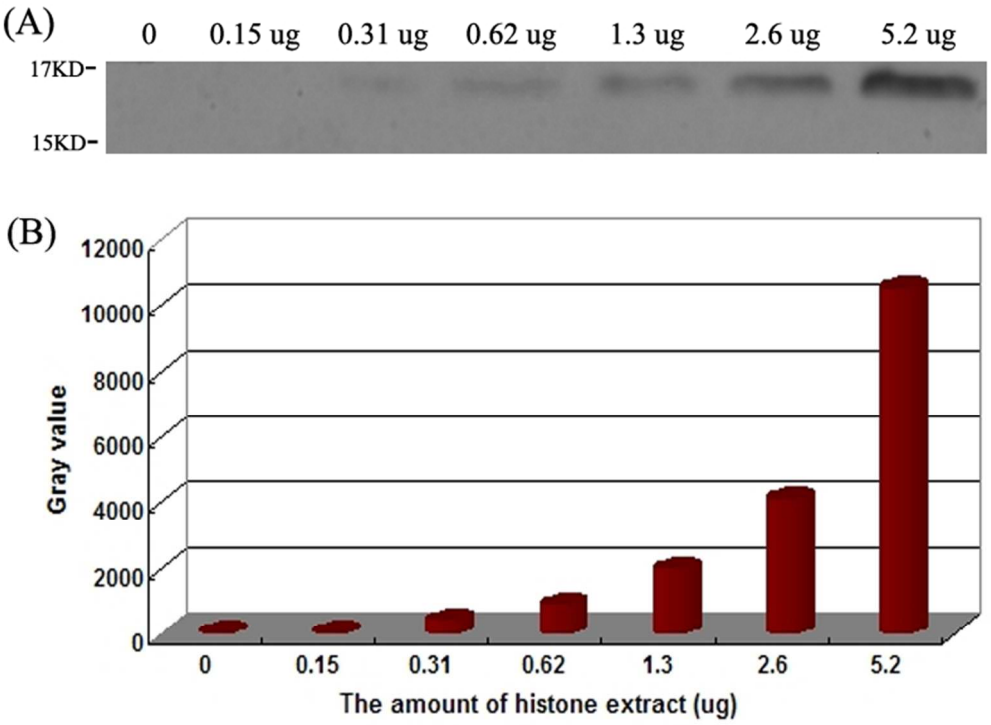


Figure 1. (A) Detection of H3K9me3 in HeLa cells using western blot. The amounts of histone extract from left to right were 0, 0.15, 0.31, 0.62, 1.3, 2.6 and 5.2 μg, respectively. (B) The gray values of the bands on the film corresponding to different amount of histone extract.
281x207mm (72 x 72 DPI)

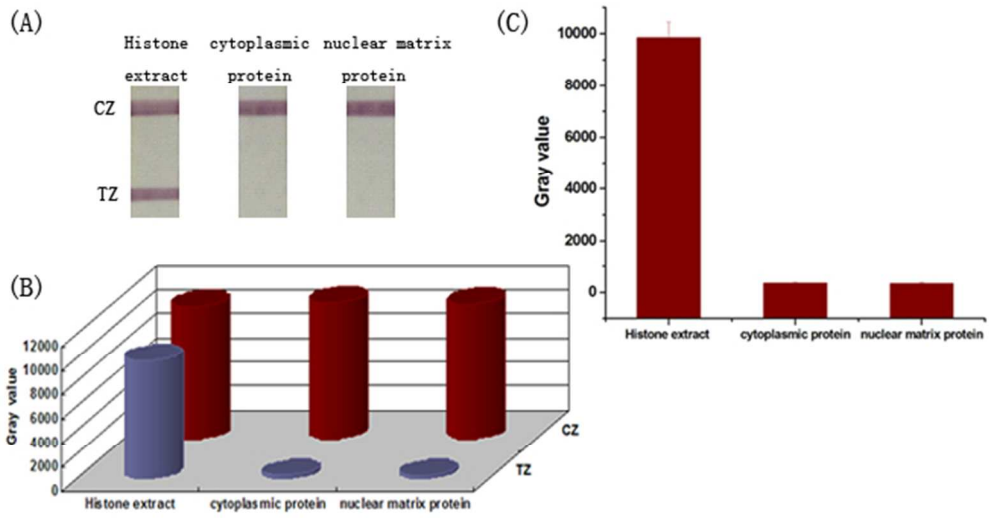


Figure 5. (A) Photo images and (B) their corresponding optical responses of the enhanced strip loaded with 1.2 μg of histone extract, 12 μg of cytoplasmic protein extract and nuclear matrix protein extract, respectively. The gray values of red bands were calculated using the image processing software ImageJ 1.37v. (C) Histogram representing gray values of the test zones responding to 1.2 μg of histone extract, 12 μg of cytoplasmic protein extract and nuclear matrix protein extract, respectively. The error bars represent the standard deviation of three independent measurements.
232x126mm (72 x 72 DPI)

An enhanced strip biosensor for rapid and sensitive detection of histone methylation

Chenchen Ge,[†, ‡] Luxin Yu,[†] Zhiyuan Fang,[†] and Lingwen Zeng *[, †]

[†] Key Laboratory of Regenerative Biology, South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China.

[‡] School of life sciences, University of Science and Technology of China, Hefei, 230026, China.

ABSTRACT: Histone methylation is a crucial epigenetic modification of chromosomes. In this work, we describe an enhanced strip biosensor using oligonucleotide functionalized gold nanoparticles as an enhancer probe (AuNP-DNA) for rapid and sensitive detection of histone methylation. In conventional strip biosensor, methylated histone is captured on the test zone through the formation of antibody/methylated histone/antibody labelled AuNP sandwich structures. Whereas in the enhanced strip biosensor, the AuNPs in the sandwich structures are dual labelled with an antibody and another oligonucleotide (c-DNA). The sequence of the c-DNA is complementary to the oligonucleotide on the enhancer probe. The enhancer probe, AuNP-DNA, hybridizes with the c-DNA on the dual labelled AuNPs and the color intensity of the red band on the test zone is then enhanced dramatically. The enhanced strip biosensor has been used for the visual detection of tri-methylated lysine 9 of histone H3 (H3K9me3) in 20 ng of histone extract from HeLa cells within 15 min. The detection limit is 10-fold and 15-fold lower than the conventional strip biosensor and western blot, respectively.

INTRODUCTION

In eukaryotic cells, DNA does not exist naked within nucleus but wraps around histone octamer in the form of a nucleosome structure.[1] The nucleosome, which is the repeating unit of chromatin, consists of two copies each of the core histones H2A, H2B, H3 and H4.[2] Meanwhile, nucleosomes are thought to carry epigenetically inherited information through DNA and core histone modification, such as DNA methylation, histone acetylation, phosphorylation, ubiquitination, sumoylation and methylation,[3] which have important functions in gene regulation, chromosome condensation (mitosis), DNA recombination and repair.[4] The core histones contain flexible N-terminal tails that protrude from the nucleosome core, which can be covalently modified at

several places. The commonly modified residues are lysine (K) and arginine (R).The Lysine side chain can be mono-, di- or tri-methylated, whereas the arginine side chain can be mono- or di-methylated.[5] The most-studied sites of lysine methylation lie in the N-terminal of histone H3 (K4, K9, K27, K36) and H4 (K20).[6] Different types of histone methylation are present within certain types of chromatin (either euchromatin or heterochromatin) and have specific meanings. For example, mono- or tri-methylation of H3K4 often exists in euchromatin, which is a loosely packed and transcriptionally active chromatin domain. While di- or tri-methylation of H3K9 (H3K9me2 or H3K9me3) is present mainly in heterochromatin, which is a condensed and transcriptionally silent chromatin domain.[7] Here, we focus on H3K9me3 because the level of tri-methylated H3K9 is not only crucial to establishing and maintaining the stability of heterochromatin structure,[12] it is also closely related to various important biological events, such as cellular senescence,[8] tumorigenesis [9,10] and determination of the differentiation direction of human bone marrow mesenchymal stem cells (MSCs).[11]

The widely used method to evaluate the level of H3K9me3 is western blot. Although this assay is effective, it requires the use of costly apparatus, experienced operators and long manipulation times. In addition, some of the reagents used in the assay are toxic, hence, a rapid, simple and inexpensive method for H3K9me3 detection is needed.

Recently, strip biosensors have received extensive attention because of their short assay time, low-cost, user-friendly format and long-term stability. Moreover, the strip platform minimizes the requirements for highly qualified personnel and eliminates complex analysis procedures requiring expensive equipment. The detection result can be obtained by visually observing the color intensity of red band on the test zone. Our group and others have successfully developed strip biosensors for the detection of nucleic acids,[13, 14] proteins,[15, 16] cancer cells,[17] and small molecules.[18, 19] In 2009, Zeng and co-workers confirmed the presence of H3K9me3 in HeLa cells.[20] Herein, we developed an enhanced strip biosensor using AuNP-DNA for rapid detection of H3K9me3 in HeLa cells.

The enhanced strip biosensor consists of four parts: a sample pad, a conjugate pad, a NC membrane and an absorbent pad. The conjugate pad is sprayed with an anti-Histone H3K9me3 monoclonal antibody and a c-DNA dual labelled AuNP. An anti-Histone H3 monoclonal antibody and a goat anti-mouse IgG antibody were immobilized on the nitrocellulose (NC) membrane

simultaneously to form the test zone and control zone, respectively. In the presence of histone extract, the dual labelled AuNPs accumulate on the test zone through the formation of a sandwich structure of antibody/H3K9me3/dual labelled AuNP, and a red band can be observed on the test zone first. The remaining dual labelled AuNPs continue to migrate towards the control zone and are captured by the IgG antibody. Ten minutes later, AuNP-DNA solution was added to the biosensor to hybridize with c-DNA on the surface of the dual labelled AuNPs on the test zone and control zone, and two darker red bands were observed. Qualitative analysis was performed by observing the color change of the test zone and semi-quantitative analysis was conducted by calculating the gray values of the red bands on the test zone using image processing software ImageJ 1.37v.

EXPERIMENTAL SECTION

Chemicals and Materials. Sodium citrate, bovine serum albumin (BSA), streptavidin (SA) and DMSO (dimethylsulfoxide) were purchased from Sigma–Aldrich (Steinheim, Germany). NHS-LC-Biotin was purchased from Pierce Biotechnology (Rockford, USA). Mouse monoclonal antibody against H3 and H3K9me3 were purchased from Abcam (Cambridge, USA). Goat anti-mouse IgG antibody was purchased from Cellway-Lab (Luoyang, China). Nonidet P-40 (NP-40), protease and phosphatase inhibitors, phenylmethanesulfonyl fluoride (PMSF) and dithiothreitol (DTT) were purchased from Guangzhou WhiGa Technology Ltd. (Guangzhou, China). The sequence of thiolated DNA is 5'-SH-ATCATAAGCTCATACAATCACTAA-3'. The biotinylated c-DNA sequence, which is complementary to the DNA, is 5'-biotin-TTAGTGATTGTATGAGCTTATGAT-3'. All oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology (Shanghai, China). NC membrane was purchased from Shantou ealon (Shantou, China). Fiberglass and absorbent paper were purchased from Shanghai Kinbio (Shanghai, China). All buffer solutions used in this study were prepared in our lab. Other chemicals were purchased from standard commercial sources and were of analytical grade purity.

Preparation of AuNP-DNA Conjugates. AuNP with a diameter of 15 nm was prepared

using citrate reduction method, [21] and the AuNP-DNA conjugates were obtained using the previously reported method.[15] Briefly, AuNP solution was concentrated four times by centrifugation at 13.4×10^3 g for 20 min. Then 1.0 OD of thiolated DNA was added to 1 mL of concentrated AuNP solution and shaken gently overnight at 4 °C. The solution was subjected to "aging" by adding 110 μL of 100 mM phosphate buffer (pH 7.0, containing 1% sodium dodecyl sulfate and 1.5 M NaCl) and kept at 4 °C for 12 h. AuNP-DNA solution was centrifuged (13.4×10^3 g, 20 min) and rinsed three times with rinsing buffer (20 mM Na_3PO_4, 5% BSA, 0.25% Tween-20, 10% sucrose and 0.1 % NaN_3) to remove any unbound DNA. The AuNP-DNA pellets were resuspended in 100 μL of rinsing buffer and then stored at 4 °C before use.

Preparation of AuNP-SA Conjugates. Firstly, 0.1 M K_2CO_3 (6 μL) was added into the 1 mL of AuNP (15 nm) solution to adjust the pH value. Ten minutes later, 10 μg of SA was added to mix with the AuNPs for 30 min. Then, 100 μL of 10 % BSA was added to block the non-specific sites on the surface of AuNPs for 30 min. The solution was centrifuged at 13.4×10^3 g for 20 min (4 °C). The AuNP-SA pellets were resuspended in 100 μL of borate saline buffer (0.2M, pH 9.0) and stored at 4 °C before use.

Preparation of Biotinylated Anti-Histone H3K9me3 Monoclonal Antibody. NHS-LC-Biotin was immediately dissolved in DMSO at a concentration of 10 mM before use. 50 ug of anti-Histone H3K9me3 monoclonal antibody was added into PBS, then NHS-LC-Biotin was added to obtain a 5-fold molar excess of biotinylation reagent over the antibody. The mixture was incubated at room temperature (RT) for 1 h. Unreacted NHS-LC-Biotin and reaction by-products were removed using dialysis against PBS at 4 °C overnight. The concentration of biotinylated antibody was determined by Bradford protein assay (Figure S1). [15]

Preparation of the Antibody and c-DNA Dual Labelled AuNPs. Different molar ratios of biotinylated anti-Histone H3K9me3 monoclonal antibody and biotinylated c-DNA (1/1, 2/1, 3/1, 4/1, 5/1) were added into AuNP-SA solution, respectively. The mixture was shaken gently for 30 min at RT and centrifuged at 13.4×10^3 g for 15 min (4 °C) twice to remove the unbound

antibody and c-DNA. The dual labelled AuNP pellets were concentrated and resuspended in 50 μL of borate saline buffer (0.2M, pH 9.0) and stored at 4 °C before use.

Construction of the Strip Biosensor. Mouse monoclonal antibody against H3 (0.67 mg mL^{-1}, 30 μL) and goat anti-mouse IgG antibody (0.5 mg mL^{-1}, 30 μL) solutions were dispensed onto the NC membrane simultaneously to form the test zone and control zone with the aid of a lateral flow dispenser (Shanghai Kinbio, Shanghai, China). Fiberglass was soaked in sample pad buffer (1% BSA, 2% Triton, 2% PEG4000, 20 mM Tris-Ac, 50 mM NaAc) and used as a sample pad. Dual labelled AuNP solution (9 μL cm^{-1}) was dispensed onto the fiberglass to form a conjugate pad. The sample pad, conjugate pad, NC membrane and absorbent pad were attached along the long axis of an adhesive plate with an overlap of 2–3 mm in the order to allow the sample to flow. Then they were cut into 4 mm-wide strips using a strip cutter (Shanghai Kinbio, shanghai, China) and stored under dry conditions.

Preparation of Histone Extract. HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS) , resuspended in lysis buffer (10 mM Hepes, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, and 0.5 mM beta-mercaptoethanol, plus protease and phosphatase inhibitors) and incubated on ice for 20min. Then 5μL of NP-40 was added and the mixture was centrifuged at 13.4×10^3 g for 15 min, the supernatant is cytoplasmic protein extract. The nuclei were collected and lysed in nucleus lysis buffer (10 mM Tris-HCl, pH 7.6, 420 mM NaCl, 0.5% NP-40, and 1mM DTT, 1mM PMSF, 2mM $MgCl_2$, plus protease and phosphatase inhibitors) for 20 min on ice and centrifuged at 13.4×10^3 g for 15 min, the extract is nuclear matrix protein extract. The pellets were then resuspended in 80 μL of 0.25 M HCl overnight at 4°C and centrifuged at 13.4×10^3 g for 10 min. The supernatant was the histone extract and the protein concentration of histone extract was determined using Bradford protein assay.

Western Blot Analysis of H3K9me3 in HeLa Cells. Different amounts of histone extract (0, 0.15, 0.31, 0.62, 1.3, 2.6 and 5.2 μg) were separated in 15% SDS-PAGE, electric-transferred to a PVDF membrane and probed with an antibody against H3K9me3 (diluted at 1:2000). The membrane was then incubated with horseradish peroxidase modified goat anti-mouse IgG

antibody (diluted at 1:4000) and the bands on the PVDF membrane were developed using ECL detection reagents (Amersham Biosciences) according to the manufacturer's instructions.

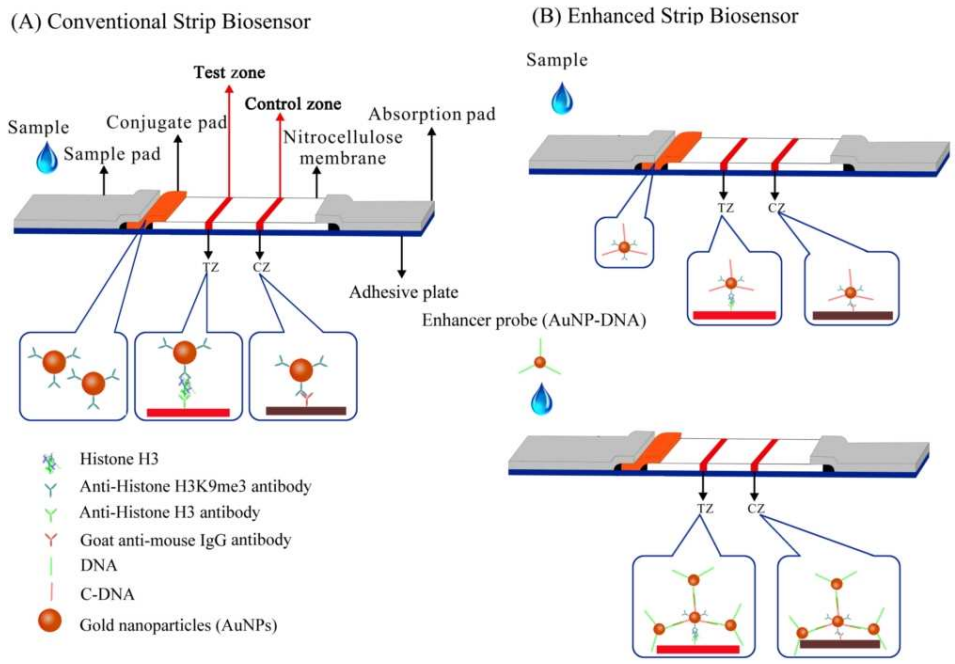
Preparation of Escherichia coli (*E.coli*) Protein Extract. *E.coli* was grown in Luria–Beritani (LB) broth in a shaker incubator for 12 h at 220 rpm (37 °C). Cells were harvested by centrifugation at 2.3×10^3 g for 10 min. The pellets were collected and lysis buffer (1 mM EDTA, 100 mM NaCl, 50 mM Tris-Cl, pH 8.2) was added to the resuspend *E.coli*. After an ultrasonication step, the *E.coli* lysate was centrifuged at 13.4×10^3 g for 15 min, the protein concentration in the supernatant was determined using the Bradford protein assay.

Detection of H3K9me3 Using Enhanced Strip Biosensor. Different amounts of histone extract were mixed with PBS to reach a final volume of 60 μL. The mixture was then loaded onto the sample pad. Ten minutes later the AuNP-DNA solution with a final volumn of 20 μL in PBS was applied to the biosensor. The red bands on the test zone and control zone were observed by the naked eye, and the gray values of red bands were calculated using image processing software ImageJ 1.37v.

RESULTS AND DISCUSSION

Principle of H3K9me3 Detection Using Enhanced Strip Biosensor. As shown in Scheme 1B, the principle of enhanced detection of H3K9me3 in HeLa cells is based on the formation of the antibody/H3K9me3/dual labelled AuNP sandwich structure and DNA duplexes between the DNA and c-DNA on the surface of AuNP-DNA and the dual labelled AuNPs on the test zone. Monoclonal antibody against H3 and goat anti-mouse IgG antibody were immobilized on the NC membrane simultaneously to form the test zone and the control zone, respectively. Anti-Histone H3K9me3 monoclonal antibody and c-DNA dual labelled AuNP solution were dispersed onto the fiberglass as the conjugate pad. When the histone extract was loaded onto the sample pad, the solution began to flow along the long axis of the strip due to the capillary effect. The dual labelled AuNPs migrated to the test zone and were immobilized through the formation of the antibody/H3K9me3/dual labelled AuNP sandwich structure. The excess dual labelled AuNPs

continue to migrate to the control zone and were captured by goat anti-mouse IgG antibody. Ten minutes later the AuNP-DNA solution was added onto the biosensor to hybridize with the c-DNA on the surface of dual labelled AuNPs. In this case, the red bands on the test zone and control zone deepened significantly due to accumulation of more AuNPs. In the absence of histone extract there was only one red band on the control zone, which indicated that the strip biosensor functioned correctly. Qualitative analysis was performed by observing the color change on the test zone, and semi-quantitative analysis was conducted by calculating the gray values of the red bands on the test zone using the image processing software ImageJ 1.37v.



Scheme 1. (A) In the conventional strip biosensor for H3K9me3 detection, AuNPs on the conjugate pad are only labelled with an anti-Histone H3K9me3 monoclonal antibody. (B) In the enhanced strip biosensor, the AuNPs on the conjugate pad are labelled with an anti-Histone H3K9me3 antibody and c-DNA simultaneously. Mouse monoclonal antibody against histone H3 and goat anti-mouse IgG antibody are immobilized on the NC membrane to form the test zone and control zone respectively. In the presence of histone extract two red bands are observed and the dual labelled AuNPs are immobilized on the test zone and control zone, respectively. Ten minutes later, AuNP-DNA solution is loaded onto the strip to hybridize with the c-DNA on the surface of dual labelled AuNPs. The detection results can be observed within 15 min by the naked eye.

Western Blot Analysis of H3K9me3 in Histone Extract. The presence of H3K9me3 in HeLa cells was initially confirmed by western blot. As shown in Figure 1A, the color intensity of gray bands on the film increased with a corresponding increase in the amount of histone extract, and no gray band was observed when using 0 or 0.15 μg of histone extract. The gray band on the film is quite visible even at 0.31 μg of histone extract, which can be used as the threshold for the determination of H3K9me3 using western blot. The gray values of the bands on the film were further calculated using the image processing software ImageJ 1.37v (Figure 1B).

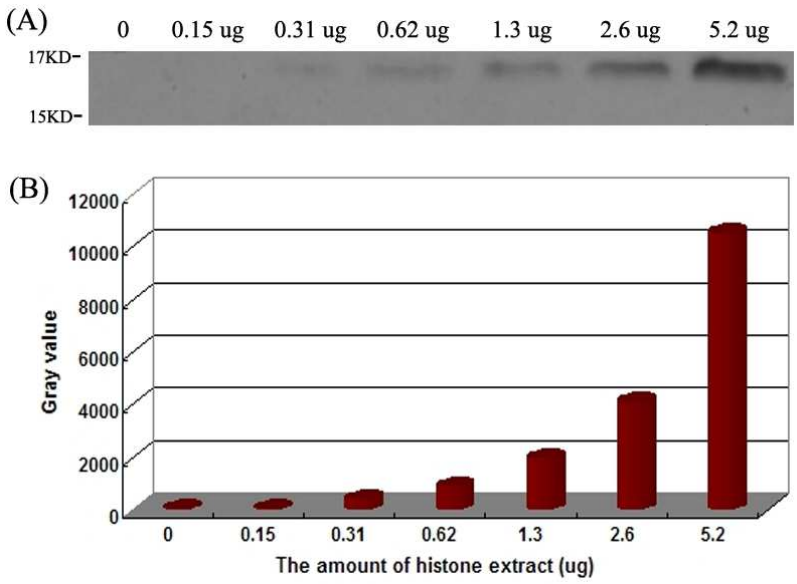


Figure 1. (A) Detection of H3K9me3 in HeLa cells using western blot. The amounts of histone extract from left to right were 0, 0.15, 0.31, 0.62, 1.3, 2.6 and 5.2 μg, respectively. (B) The gray values of the bands on the film corresponding to different amount of histone extract.

Optimization of the molar ratio of antibody to c-DNA on the surface of AuNP-SA. In order to investigate the effect of the concentration of biotinylated antibody and biotinylated c-DNA on the stability of AuNP-SA, different molar ratios (1/1, 2/1, 3/1, 4/1, 5/1) of biotinylated antibody to c-DNA were added into 1 mL of AuNP-SA solution, respectively. The concentration of biotinylated c-DNA was 4×10^{-10} M. As shown in Figure 2A, the red color of concentrated dual labelled AuNP solution became gradually deeper with the increasing molar ratio of biotinylated antibody to c-DNA (insert), and the absorption spectra of the solution increased accordingly. This

result indicated that the AuNP solution became more stable with an increase in the molar ratio. The corresponding absorbance at 520 nm was also recorded in Figure 2B, and the absorbance of the AuNP solution at 520 nm enhanced when the molar ratio increased in the range of 1/1 to 4/1, and almost leveled off thereafter. Therefore, 4/1 was select as the optimal molar ratio of biotinylated antibody to biotinylated c-DNA for H3K9me3 detection.

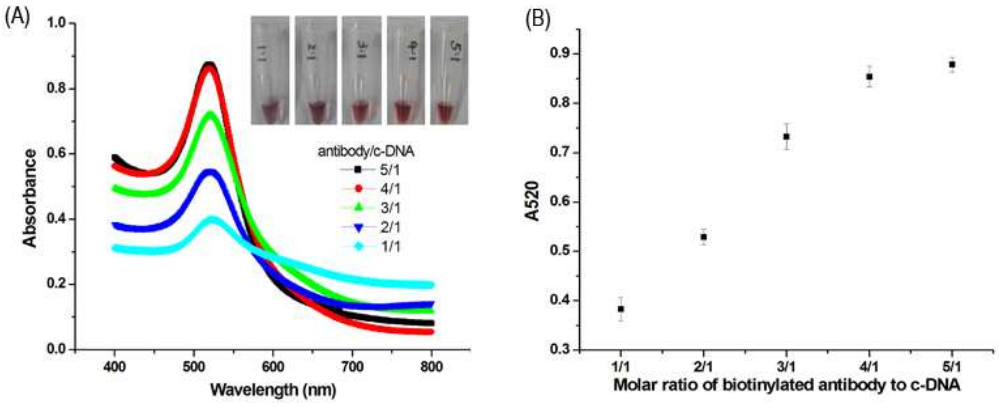


Figure 2 (A) The absorption spectra and (B) the absorbance of dual labelled AuNP solution corresponding to different molar ratios of biotinylated antibody to biotinylated c-DNA (1/1, 2/1, 3/1, 4/1, 5/1), respectively. The error bars represent the standard deviation of three independent measurements.

Optimization of the Volumn of AuNP-DNA solution for H3K9me3 Detection. The intensity of red band on the test zone is closely correlated to the volumn of concentrated AuNP-DNA solution. As shown in Figure 3A, in the presence of 1.0 μg of histone extract, the intensity of the red bands on the test zone increase gradually as the volumn of AuNP-DNA solution increases from 0 to 2.4 μL. However, the intensity of red band does not increase significantly when the volumn of AuNP-DNA solution increases up to 2.4 μL or higher. The corresponding optical responses were confirmed by calculating the gray values of the red bands on the test zone using the image processing software ImageJ 1.37v (Figure 3B).

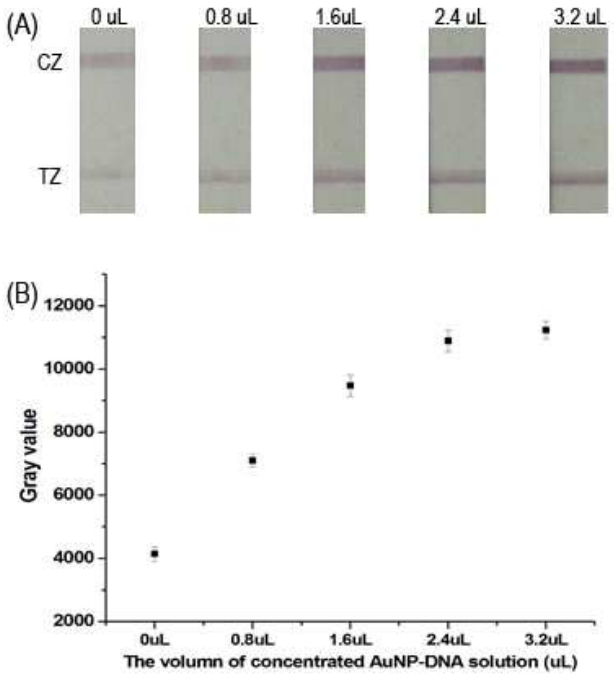


Figure 3 (A) Photo images of the strips with different volumns of concentrated AuNP-DNA solution (0, 0.8, 1.6, 2.4 and 3.2 μL). (B) The gray values of red bands on the test zone were calculated using the image processing software ImageJ 1.37v. The error bars represent the standard deviation of three independent measurements.

Detection of H3K9me3 Under Optimal Experimental Conditions. To evaluate the sensitivity and dynamic range of the enhanced strip biosensor for H3K9me3 detection in HeLa cells, different amounts of histone extract were loaded onto the strip biosensor. As shown in Figure 4A (b), the color intensity of red bands on the test zone increased with the increase of the amounts of histone extract. In the absence of histone extract, there was no red band observed on the test zone. Their corresponding optical responses were further confirmed by recording the gray values of the red bands on the test zone (Figure 4B). The resulting calibration curve shows that the gray value is proportional to the amount of histone extract in the range of 0.02 μg to 0.5 μg (Figure 4C). The red band on the test zone is visible even at 0.02 μg of histone extract, which can be used as the threshold for the visual determination of H3K9me3 in HeLa cells without instrumentation.

We also detected H3K9me3 using a conventional strip biosensor. As shown in Figure 4A (a), the color intensity of red bands on the test zone and control zone were much weaker than the enhanced biosensor even when more histone extract was used. The color intensity of red bands on the test zone increased gradually with increasing the amounts of histone extract from 0.2 μg to 2.0 μg. When 0 or 0.1μg of histone extract was used, there was no red band observed on the test zone. Hence, the detection limit for H3K9me3 using conventional biosensor was 0.2 μg of histone extract.

As a result, the detection limit for H3K9me3 in HeLa cells of the enhanced strip was 10-fold and 15-fold lower than the conventional strip and western blot, respectively.

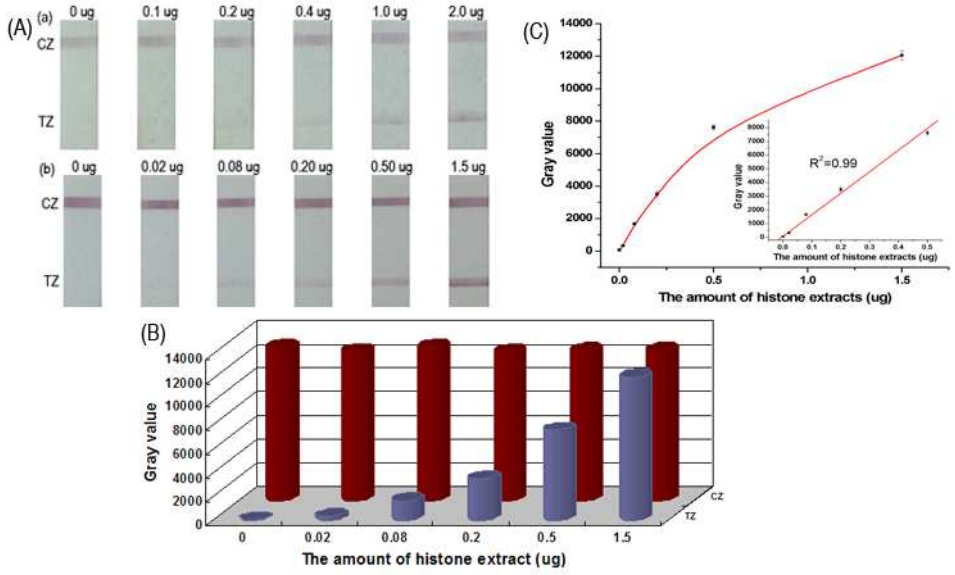


Figure 4. (A) Photo images of the conventional (a) and enhanced strip (b) with different amount of histone extract. (B) The corresponding optical response of the enhanced strip. The gray values of red bands were calculated using the image processing software ImageJ 1.37v. (C) Plots of the gray values of red bands on the test zone vs. the amount of histone extract. Inset: calibration curve of the gray values of red bands on the test zone vs. the amount of histone extract. The error bars represent the standard deviation of three independent measurements.

Specificity of the Enhanced Strip Biosensor. To validate the specificity of this assay for H3K9me3 detection, cytoplasmic protein extract and nuclear matrix protein extract of HeLa cells were used as negative control. The detection results were showed in Figure 5. In the presence of

1.2 μg of histone extract, the red band on the test zone can be observed obviously. However, when 12 μg of cytoplasmic protein extract and nuclear matrix protein extract were loaded onto the strip, only a red band can be observed on the control zone. The experimental results indicated that this method can detect H3K9me3 in histone extract from HeLa cells successfully without interference from other constituents.

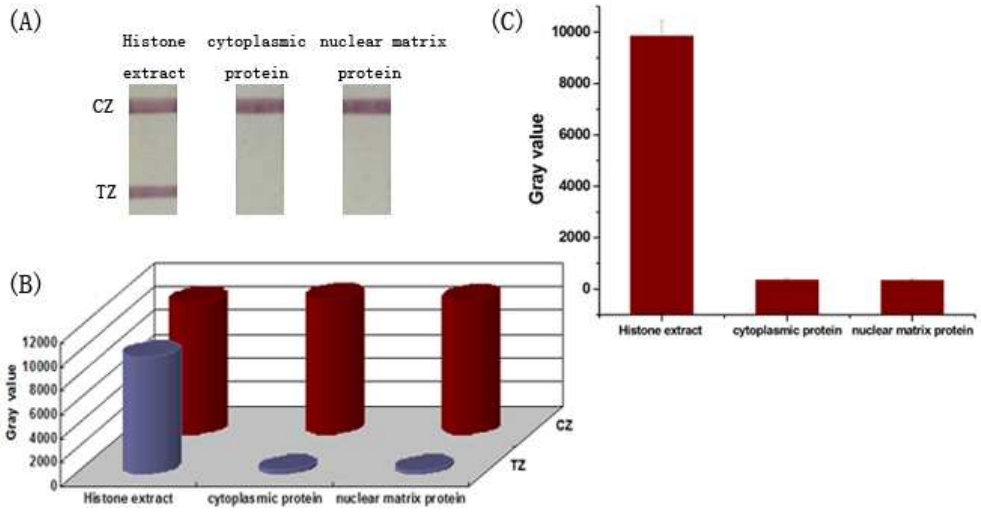


Figure 5. (A) Photo images and (B) their corresponding optical responses of the enhanced strip loaded with 1.2 μg of histone extract, 12 μg of cytoplasmic protein extract and nuclear matrix protein extract, respectively. The gray values of red bands were calculated using the image processing software ImageJ 1.37v. (C) Histogram representing gray values of the test zones responding to 1.2 μg of histone extract, 12 μg of cytoplasmic protein extract and nuclear matrix protein extract, respectively. The error bars represent the standard deviation of three independent measurements.

CONCLUSION

In summary, we have successfully constructed an enhanced strip biosensor using AuNP-DNA as an enhancer probe for rapid and sensitive detection of histone methylation, and H3K9me3 in HeLa cells is chosen as our target. The mechanism of enhanced detection of H3K9me3 is based on the formation of the antibody/H3K9me3/dual labelled AuNP sandwich structure and DNA duplexes between DNA and c-DNA on the surface of AuNP-DNA and dual labelled AuNP on the test zone. With this biosensor, we can visually detect H3K9me3 in 20 ng of histone extract from

HeLa cells in 15 min without instrumentation, which is 10-fold and15-fold lower detection limit than the conventional strip biosensor and western blot, respectively. In addition, the principle of the enhanced strip biosensor can be applied to detect other types of histone methylation, such as mono-, di- or tri-methylated H3K4 and H3K9, as well as other analytes, such as nucleic acids, proteins, virus and microorganisms.

AUTHOR INFORMATION

Corresponding Author

* E-mail: zeng6@yahoo.com; Fax: +86 20 32015245; Tel: +86 20 32015312.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

Financial support was provided by the Ministry of Science and Technology 973 Project (2013CB967100).

REFERENCES

(1) Peterson, C. L.; Laniel, M. A. Curr. Biol. **2004**, *14*, R546-R551.

(2) Wigle, T. J.; Provencher, L. M.; Norris, J. L.; Jin, J.; Brown, P. J.; Frye, S. V.; Janzen, W. P. Chemistry & biology **2010**, *17* , 695-704.

(3) Berger, S. L. Curr. Opin. Genet. Dev. **2002**, *12* , 142-148.

(4) Rodriguez-Collazo, P.; Leuba, S. H.; Zlatanova, J. Nucleic Acids Res. **2009**, *37*, e81.

(5) Bannister, A. J.; Kouzarides, T. Nature **2005**, *436*, 1103-1106.

(6) Lachner, M.; O'Sullivan, R. J.; Jenuwein, T. Journal of cell science **2003**, *116*, 2117-2124.

(7)Fischle, W.; Tseng, B. S.; Dormann, H. L.; Ueberheide, B. M.; Garcia, B. A.; Shabanowitz, J.; Hunt, D. F.; Funabiki, H.; Allis, C. D. Nature **2005**, *438*, 1116-1122.

(8) Elgin, S. C. R.; Grewal, S. I. S. Curr. Biol. **2003**, *13*, R895-R898.

(9) Feser, J.; Tyler, J. Febs. Lett. **2011**, *585*, 2041-2048.

(10) Braig, M.; Lee, S.; Loddenkemper, C.; Rudolph, C.; Peters, A. H. F. M.; Schlegelberger, B.; Stein, H.; Dorken, B.; Jenuwein, T.; Schmitt, C. A. Nature **2005**, *436*, 660-665.

(11) Peters, A. H. F. M.; O'Carroll, D.; Scherthan, H.; Mechtler, K.; Sauer, S.; Schofer, C.; Weipoltshammer, K.; Pagani, M.; Lachner, M.; Kohlmaier, A.; Opravil, S.; Doyle, M.; Sibilia, M.; Jenuwein, T. Cell **2001**, *107*, 323-337.

(12) Ye, L.; Fan, Z. P.; Yu, B.; Chang, J.; Hezaimi, K. A.; Zhou, X. D.; Park, N. H.; Wang, C. Y. Cell Stem Cell. **2012**, *11*, 50-61.

(13) Xiao, Z.; Lie, P. C.; Fang, Z. Y.; Yu, L. X.; Chen, J. H.; Liu, J.; Ge, C. C.; Zhou, X. M.; Zeng, L. W. Chem. Commun. **2012**, *48*, 8547-8549.

(14) He, Y. Q.; Zeng, K.; Zhang, S. Q.; Gurung, A. S.; Baloda, M.; Zhang, X. B.; Liu, G. D. Biosens. Bioelectron. **2012**, *31*, 310-315.

(15) Fang, Z. Y.; Ge, C. C.; Zhang, W. J.; Lie, P. C.; Zeng, L. W. Biosens. Bioelectron. **2011**, *27*, 192-196.

(16) Xu, H.; Mao, X.; Zeng, Q. X.; Wang, S. F.; Kawde, A. N.; Liu, G. D. Anal. Chem. **2009**, *81*, 669-675.

(17) Liu, G. D.; Mao, X.; Phillips, J. A.; Xu, H.; Tan, W. H.; Zeng, L. W. Anal. Chem. **2009**, *81*, 10013-10018.

(18) Mazumdar, D.; Liu, J. W.; Lu, G.; Zhou, J. Z.; Lu, Y. Chem. Commun. **2010**, *46*, 1416-1418.

(19) Chen, J. H.; Zhou, X. M.; Zeng, L. W. Chem. Commun. **2013**, *49*, 984-986.

(20) Zeng, W.; de Greef, J. C.; Chen, Y. Y.; Chien, R.; Kong, X.; Gregson, H. C.; Winokur, S. T.; Pyle, A.; Robertson, K. D.; Schmiesing, J. A.; Kimonis, V. E.; Balog, J.; Frants, R. R.; Ball, A. R., Jr.; Lock, L. F.; Donovan, P. J.; van der Maarel, S. M.; Yokomori, K. Plos Genet. **2009**, *5*, e1000559.

(21) Mao, X.; Ma, Y. Q.; Zhang, A. G.; Zhang, L. R.; Zeng, L. W.; Liu, G. D. Anal. Chem. **2009**, *81*, 1660-1668.