Highly sensitive fluorescent detection of p53 protein based on DNA functionalized Fe_3O_4 nanoparticles

Qunfang Xu, Kaixin Liang, Jiang Ouyang, Wangsong Chen, Liu Deng, You-Nian Liu

www.elsevier.com/locate/talanta

PII: S0039-9140(18)30469-7
DOI: https://doi.org/10.1016/j.talanta.2018.05.009
Reference: TAL18649

To appear in: *Talanta*

Received date: 6 December 2017
Revised date: 25 April 2018
Accepted date: 2 May 2018

Cite this article as: Qunfang Xu, Kaixin Liang, Jiang Ouyang, Wangsong Chen, Liu Deng and You-Nian Liu, Highly sensitive fluorescent detection of p53 protein based on DNA functionalized Fe_3O_4 nanoparticles, *Talanta,* https://doi.org/10.1016/j.talanta.2018.05.009

Highly sensitive fluorescent detection of p53 protein based on DNA functionalized Fe_3O_4 nanoparticles

Qunfang Xu[a,b,#], Kaixin Liang[a,#], Jiang Ouyang[a], Wangsong Chen[a,b], Liu Deng[a,b,*] and You-Nian Liu[a,b,*]

[a] *College of Chemistry and Chemical Engineering Central South University, Changsha, Hunan 410083, P. R. China*

[b] *Hunan Provincial Key Laboratory of Efficient and Clean Utilization of Manganese Resources, Central South University, Changsha, Hunan 410083, P. R. China*

Abstract

The accurate quantification of p53 protein expression level is of great importance for cancer diagnosis. Here, a highly sensitive fluorescent sensor based on DNA functionalized magnetic nanoparticles was developed for the detection of p53 protein expression. Instead of a monoclonal antibody, a consensus DNA was employed to capture p53 protein. Meanwhile the fluorescent dye tethered DNA was used as the signal output instead of enzyme tagged nanoparticle or antibody. Consequently, our developed method is cost-effective for both the p53 capture and detection by compared with the conventional immunoassay. The biosensor developed by the above strategy was used to quantitatively detect p53, which yields a detection limit of 8 pM with the linear range of 50 pM to 2 nM. The sensitive for specific p53 detection was

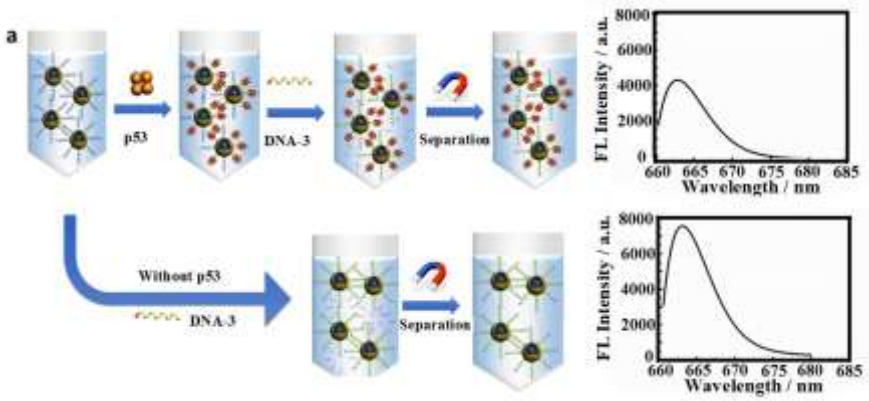
[#] These two authors are contributed equally to this work.

∗ Corresponding authors. Tel/Fax: +86-731-8887-9616.

E-mail address: dengliu@csu.edu.cn (L. Deng); liuyounian@csu.edu.cn (Y.-N. Liu).

achieved due to the facile magnetic separation from the complex condition, and the reduced non-specific absorption effect by dextran. Moreover, the method is able to measure p53 from real cell lysate without extensive sample pretreatment/separation. The developed p53 biosensor has high sensitivity, good selectivity and reliable accuracy. It demonstrates great potential in clinical cancer diagnosis and early detection of cancer.

Graphical Abstract



A fluorescent sensor for p53 protein expression was developed by combination of functional consensus DNA and magnetic nanoparticles. The sensor can realize ultrasensitive detection of p53 protein in real cell lysate.

Keywords: p53 protein; fluorescent biosensor; magnetic nanoparticles; DNA; biomarker.

Introduction

The incidence of cancer is increasing over the years and it has become one of the major disease that threaten human health [1]. It is estimated that about 30% of cancer mortality can be significantly reduced if the cancer can be detected and treated at an early stage. Thus, the early detection of cancer biomarkers have drawn great attention for cancer screening and prognosis [2, 3]. As tumor suppressor and transcription factor protein, p53 protein plays important role in the control of cell cycle, apoptosis and DNA repair [4, 5]. It is reported that the mutation of p53 gene can be increased to approximately 50% in all cancers, and leads to the accumulation of mutated p53 protein and decrease of wild-type p53 protein in a variety of human cancers [6, 7]. Therefore, the effective determination of p53 protein has been a major focus in clinical research and early diagnosis of cancers.

Many efforts have been devoted to developing strategy for sensitive detection of p53 protein. Various techniques, including surface enhanced Raman scattering (SERS) [8], chemiluminescence [9], electrochemical method [10] and glucose meter [11] have been utilized for p53 protein detection. However, most of the reported methods for p53 determination are immunoassay based on specific binding interaction between the antibody and antigen. The high cost and complicated modification of antibody limit their clinic applications. Recently, the wild-type p53 protein was found to be able to bind to the sequence-specifically sites in consensus DNA and the mutated p53 protein is otherwise [12], which has been proved an appealing alternative of antibodies to detect p53 protein. For example, the combination of consensus DNA and ferrocene-modified Au nanoparticles (NPs) was developed as electrochemical probe for the p53 protein detection [13]. Wang's group combined carboxymethylated dextran surface with consensus ds-DNA to capture p53 protein and obtained a sensitive SPR p53 sensor [14]. Our group has developed a self-powered, quantitative sensor for p53 protein based in the fact that the interaction between p53 protein and DNA can block the electron transfer process on electrode surface [15]. However, efficient and reliable approaches for sensitive and selective determination of p53

protein detection in clinical diagnostics still remains a great challenge. On the other hand, the fluorescent-based strategy has gained great attention in the biomarker detection, taking the advantages of simple operation, low cost and easy signal quantification [16-18]. To the best of our knowledge, utilizing the highly specific binding properties of DNA to p53 for detecting p53 protein through fluorescence method in cell lysates has not been reported.

Considering the importance of selectivity and sensitivity towards the efficiency of detection approach, magnetic NPs are usually employed to develop economical and stable separation strategy for the complex clinic sample [19, 20]. To improve the stability, biocompatibility and functionality of the magnetic NPs, various polymers have been utilized to modify the surface of NPs [21–23]. Among them, dextran has drawn much attention due to its unique features, such as hydrophilicity, biocompatibility, biodegradability and the ability to reduce the non-specific absorption [24, 25]. Especially, dextran can be easily functionalized with amino/carboxyl groups to facilitate the conjugation of targeting ligands [26]. Thus, the dextran modified magnetic NPs could facilitate the immobilization of DNA and form an ideal platform for sensitive assay of p53 protein.

Herein, we propose a novel biosensor for the sensitive and selective detection of p53 in cell lysate. Aminated-dextran was deposited onto Fe_3O_4 NPs via layer-by-layer assembly. Then consensus DNA, which can capture p53, was immobilized on aminated-dextran modified Fe_3O_4 NPs through amine coupling chemistry. Due to the thermodynamically preferred DNA replacement between partially complementary DNA and fully complementary DNA, Cy-5 tagged DNA can be tethered to magnetic NPs surface, as a fluorescent signal this replacement process can be affected by the interaction between p53 protein and DNA. Hence, the captured p53 protein can result in the decrease of fluorescent emission. Instead of monoclonal antibody, a consensus DNA was used to recognize p53 protein. Meanwhile, instead of antibody conjugated to an enzyme, a fluorescence dye-DNA was utilized for signal output. Compared with the conventional immunoassay, the developed biosensor based on DNA functionalized Fe_3O_4 NPs is cost-effective for both the p53 capture and detection.

Moreover, the facile magnetic separation and specific recognition facilitate the sensitive quantitative measurement of wild-type p53 protein in normal or cancer cell lysate.

2. Experimental

2.1 Reagents and materials

Ferric chloride, ethylene glycol, sodium acetate anhydrous, polyacrylic acid (PAA; average M_w =18000), dextran 5000, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), dithiothreitol (DTT), $MgCl_2$, KH_2PO_4, and K_2HPO_4 were acquired from Sigma (St. Louis, MO). DNA samples were purchased from Sangon Co., Ltd. (Shanghai, China). The aminated-dextran was synthesized according to the previous report [27]. To immobilize the consensus ds-DNA onto the dextran surface, a carboxyl oligonucleotide probe with a sequence of COOH-$(CH_2)_6$-TTT TTT TCC GGG CAT GCC CGG CAT TGC-3′ (DNA-1) and its partial complementary sequence of 5′-GGG CAT GCC C-3′ (DNA-2) were used. Cy5-5′-GCA ATG CCG GGC ATG CCC GCA-3′ (DNA-3) labeled by fluorescent dye Cy5, was used to replace DNA-2. The sequences for the production of the nonconsensus ds-DNA is 5′-GGG TTT TCCC-3′ (DNA-4, three mismatching base). Recombinant p53 sample was purchased from BD Biosciences Pharmingen (San Diego, CA). Normal and cancer cell lysates was obtained from Xiangya Hospital of Central South University (Changsha, China). All stock solutions were prepared daily with deionized water treated with a water purification system (Simplicity 185, Millipore Corp. Billerica, MA).

2.2 Preparation of aminated-dextran functionalized Fe_3O_4 NPs

Fe_3O_4 NPs were prepared by solve-thermal method according to previously reported procedures [28]. Briefly, $FeCl_3 \cdot 6H_2O$ (1.0 g) was dissolved in ethylene glycol (20 mL), and then added NaAc (3.0 g) and polyethylene glycol (0.7 g). After stirred vigorously for 30 min, the mixture was sealed in a Teflon-lined stainless-steel autoclave. The autoclave was maintained at 200 °C for 8 h and allowed to cool to room temperature. After washed with ethanol several times and dried at 60 °C, the

Fe_3O_4 NPs was obtained. The modification of Fe_3O_4 NPs with polyelectrolytes was carried out by the layer-by-layer assembly technique. To 10 mL of 1 mg mL^{-1} PAA aqueous solution, 1 mL of Fe_3O_4 NPs suspension (1 mg mL^{-1}) was added. The mixture was incubated for 15 min with vibrator before collected by a magnet. After washed with water for several time, the PAA coated Fe_3O_4 NPs were obtained by magnetic separation. After that, a 10 mL aminated-dextran solution (1 mg mL^{-1}) was added to 1 mL PAA-coated Fe_3O_4 solution and incubated for 15 min with vibrator. Finally, the dextran/PAA/Fe_3O_4 NPs were prepared after 3 times wash cycles.

2.3 Preparation of DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs

First, 150 μL EDC and 150 μL NHS (0.1 mM/L) were added to the dextran/PAA/Fe_3O_4 NPs solution. After 15 min, a 5 OD DNA-1 aqueous solution was introduced to the above mixture. Then, the DNA-1 functionalized dextran/PAA/Fe_3O_4 NPs were collected by the magnet after 5 h reaction. Finally, the partially complementary sequence DNA-2 hybridized to DNA-1 was carried out at 90 °C for 5 min. The DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs were collected by magnet after 3 times wash cycles.

2.4 Detection of p53 protein

A p53 protein was diluted with phosphorous buffer solution (PBS) comprising 20 mM DTT, then added to react with the DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs for 30 min. Then the p53 protein attached DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs was washed with PBS for 3 times, and collected by the magnet. After p53 protein was captured, the Cy5-labeled DNA-3 was added to replace DNA-2, which is complementary sequence of DNA-1. After heated for 4 h at 37 °C, the resulted NPs were collected and re-dispersed in PBS to evaluate their fluorescence. The normal cell lysates were diluted 5 times for the real sample analysis.

2.5. Instruments

F-4600 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) was used to determine the fluorescence intensity. Transmission electron microscopy (TEM) was performed using a JEOL2000 transmission electron microscopy (Jeol, Boston, MA)

with an accelerating voltage of 200 kV. The sample for TEM characterization was prepared by placing a drop of prepared solution on a carbon-coated copper grid and dried at room temperature. Zeta potential measurements were performed using a Zeta Sizer Nano ZS (Malvern Instruments, UK).

3. Results and Discussion

3.1 Characterization of DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs

The consensus DNA functionalized magnetic NPs were fabricated as schematically outlined in Fig. 1a. The magnetic NPs were functionalized with aminated-dextran via layer-by-layer assembly. PAA on the surface of Fe_3O_4 NPs endow the negative charge of the surface, and improve the stability of the NPs. The negatively charged surface would be favorable for the further deposition of positively charged amino-dextran. As the surface charge of outermost layer would influence the ζ potential of the NPs, ζ potential measurements were carried out to monitor the sequential polyelectrolyte layer deposition on Fe_3O_4 NPs. As shown in Fig. 1d, the bare Fe_3O_4 NPs have a positive ζ potential of 6.5 mV. After the negatively charged PAA deposition, the ζ potential alternate to negative values (–39 mV), and further deposition of dextran will reverse the ζ potential to negative values (25 mV). The ζ potential alternation indicated that the successful assemble of polymer layer growth occurred on Fe_3O_4 NPs surface. Then the aminated-dextran functionalized Fe_3O_4 NPs were mixed with DNA-1. DNA-1 was attached to the magnetic nanoparticle surfaces via the amine coupling chemistry. After DNA-1 anchored onto the surface of Fe_3O_4 NPs, the partially complementary DNA-2 was introduced to form consensus ds-DNA. The functionalized NPs were characterized by TEM. As shown in Fig. 1b, the bare Fe_3O_4 NPs are with diameter about 50 nm, while the DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs are monodisperse with a similar mean diameter to that of Fe_3O_4 NPs (Fig. 1c), indicating that the functionalization did not cause the agglomeration of the NPs. The hydrodynamic size (D_h) of DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NP was measured to be 86.1 nm with PdI of 0.19 by dynamic light scatting (DLS), while that of Fe_3O_4 NPs is about 62.5 nm. This significant size increase also confirms the successful

modification of consensus DNA on Fe_3O_4 NPs surface.

Fig. 1

3.2 Detection of p53 protein

To measure p53 protein, a fluorescent assay was carried out as shown in Fig. 2a. The wild-type p53 solution was first added to DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs, and captured by the consensus DNA domain in DNA-1/DNA-2 duplex. The most stable binding configuration of p53 protein on DNA was the attachment of p53 tetramers to the full binding sites of individual ds-DNA [29]. Then a magnet was used to separate the p53 attached DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs from the solution. After the magnetic separation, Cy5-labeled DNA-3, which is complementary sequence of DNA-1, was added to p53 attached DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs. Since the DNA-2 is partially complementary sequence of DNA-1, DNA-3 would replace DNA-2 thermodynamically if no p53 protein attached to DNA-2/DNA-1. In that case, the fluorescent dye, Cy5 would immobilize on the magnetic NPs surface by this DNA replacement process. Consequently, fluorescent emission of Cy5 at 633 nm can be observed after the magnetic separation from the mixture (Fig. 2c). Otherwise, no DNA-3 replacement could occur when p53 protein bind on the consensus DNA domain in DNA-2/DNA-1 duplex. Then, a decreased emission intensity would be found after the separation. To investigate the feasibility of the p53 protein detection with this proposed strategy, 2 nM p53 protein (50 μL) was added into the DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs solution. After the DNA replacement and magnetic separation, a distinct decrease of the fluorescent emission in presence of p53 protein is observed (Fig. 2b), suggesting that the p53 protein can be used to inhibit the fluorescence dye tagged DNA replacement on the surface of the magnetic NPs. Thus, the amount of p53 in the samples was able to convert to the fluorescent emission difference between blank sample and p53 contained samples.

Fig. 2

To explore the specificity of the binding capability for consensus DNA, mismatched DNA sequences (DNA-4) was introduced to produce the nonconsensus ds-DNA. As shown in Fig. 3a, the fluorescent intensity for DNA-4/DNA-1/dextran/PAA/Fe_3O_4 NPs with 1 nM p53 protein increased 47%, compared with that of DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs. It indicates that the binding of p53 to the consensus sequence is highly specific and in accordance with the previous studies [12, 29].

Fig. 3

3.3 Optimization of experiment condition

In order to maximize the detection performance of the proposed p53 protein biosensor, various conditions were optimized by single factor experiments. As the source of the fluorescence, the exchange time for DNA-3 to DNA-2 on magnetic NPs surface is a key factor in fluorescence signal output. The influence of exchange time on the fluorescence output was monitored from 10 to 50 min. As shown in Fig. 3b, the fluorescence output increase with the increment of exchange time, and become to plateau after 40 min. Hence, an exchange time of 40 min was selected for DNA-3 and DNA-2 replacement process. The incubation time for p53 protein anchored on consensus DNA is another important parameter for the sensitive detection. As shown in Fig. 3c, the fluorescence decline amplitude become a steady value after p53 protein incubated with DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs for 30 min, and longer incubation time doesn't enhance the signal intensity. This phenomena indicates a thorough capturing of the p53 protein by consensus DNA on the NPs surface. Therefore, the optimal incubation time for the p53 protein capture was 30 min.

The coverage of the consensus DNA on magnetic NPs surface is another important factor for the sensitive detection. Therefore, different amounts of DNA-1 ranging

from 1 to 7 OD were separately added to 1 mL dextran/PAA/Fe_3O_4 NPs to produce DNA-1 functionalized magnetic NPs, and the same amount of DNA-2 as DNA-1 were hybridized with DNA-1. Then 1 nM p53 protein, DNA-3 and DNA-1 were added in sequence. As shown in Fig. 3d, the optimum output occurred at a DNA-1 concentration of 5 OD. The results indicated that more consensus DNA immobilized on Fe_3O_4 NPs would provide more p53 protein binding sites to capture p53 protein. But after reaching the optimum coverage, this effect stopped due to the space hindrance for the p53 capture and DNA exchange by the saturation of the consensus DNA on magnetic NPs surface, which eventually lead to a decreased response. Therefore, 5 OD per 1 mg Fe_3O_4 NPs is the optimal ratio between DNA to magnetic NPs.

3.4 Performance of the fluorescence detection of p53 protein

Under the optimal conditions, the p53 protein fluorescence sensor was used to quantitatively determine wild-type p53 protein. Fig. 4a shows the fluorescent emission of the developed assay in the presence of various concentrations of wild-type p53 protein. It is obvious that fluorescence emission displays a dependence upon the concentration of target p53, and the emission intensity decreases with the increasing p53 concentration. As shown in Fig. 4b, the difference in emission intensity at 663 nm between blank sample and p53 containing samples was proportionable to p53 concentration from 50 pM to 2 nM (R^2 = 0.999) with the detection limit of 8 pM. The reproducibility of our developed biosensor was examined by detecting 1 nM p53 with five different sensors. The relative standard deviation (RSD), estimated from the slopes of the calibration plots of five different and freshly prepared p53 sensors was 5.6%, suggesting the good precision and reproducibility of the biosensor. The storage stability of the biosensors in solution was evaluated at 4 ^{o}C. The response to 1 nM p53 protein of 5 biosensors, stored at 4 ^{o}C when not in use and tested every 4 days for 8 weeks showed an average decrease of 10% of the initial current value.

Fig. 4

To further investigate the specificity, several possible proteins, such as bovine serum albumin (BSA), human serum albumin (HSA) and α-fetoprotein (AFP) were detected. As shown in Fig. 5, no distinguishable intensity decrease appears toward non-target proteins. More importantly, the introduction of interfering materials in the target p53 samples does not cause the significant change in the intensity by comparison with p53 alone. These results clearly demonstrate the high specificity of the developed biosensor.

Moreover, wild-type p53 protein in cell lysates was measured to demonstrate the feasibility of the developed biosensor for practical detection. The wild-type p53 protein concentration level was 3.467 ± 0.039 nM in normal epithelial kidney cell lysates, and 0.066 ± 0.007 nM in colorectal cancer cell lysates, which is also highly consistent with previous reports [15]. This obvious decline of the wild-type p53 concentration is ascribed to the elevation of the mutant p53 gene, which usually occurs in cancer cells. Therefore, the developed p53 biosensor is amenable to the quantification of wild-type p53 levels in normal and cancer cells.

Fig. 5

4. Conclusion

In summary, consensus DNA/dextran/PAA/Fe_3O_4 NPs was explored to fabricate a novel fluorescent biosensor for p53 protein quantification. To reduce the nonspecific absorption of protein or other species present in cell lysate, the aminated-dextran modified Fe_3O_4 NPs was chosen for consensus DNA functionalization. The wild-type p53 protein could immobilize on the DNA/dextran/PAA/Fe_3O_4 NPs surface by the specific interaction between consensus DNA domain and wild-type p53 protein. The signal probe, Cy-5 tagged DNA can be anchored to magnetic NPs surface by the thermodynamically preferred DNA replacement between partially complementary

DNA and fully complementary DNA. The decrease of fluorescent emission would be observed, owing to the inhibition of DNA replacement process by the captured p53 protein on consensus DNA domain. The novelty of this strategy relies on: (1) by converting the quantification of target biomarkers to the detection of the fluorescent emission; (2) cost-effectiveness, due to the utilization of consensus DNA as a recognize probe instead of monoclonal antibody, and the fluorescence dye-DNA as the signal probe instead of another antibody conjugated to an enzyme; (3) high specificity and sensitivity, owing to the combination of DNA recognize the target and magnetic separation from the complex body fluid. The successful measurement of p53 from normal cell and cancer cell lysate was also realized by our developed method. Most importantly, the method is able to measure p53 from real samples without extensive sample pretreatment/separation or specialized instruments. This novel sensing protocol is able to provide a cost-effective, facile and sensitive analysis of p53 protein, and will have great potential as a clinical strategy for detection of p53 in normal and cancer cells for clinic diagnosis.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 21636010 and 21476266) and the Hunan Provincial Science and Technology Plan Project, China (No. 2016TP1007).

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2016, CA: Cancer J. Clin. 66 (2016) 7-30.

[2] R.V. Devi, M. Doble, R.S. Verma, Nanomaterials for early detection of cancer biomarker with special emphasis on gold nanoparticles in immunoassays/sensors, Biosens. Bioelectron. 68 (2015) 688-698.

[3] A.B. Chinen, C.M. Guan, J.R. Ferrer, S.N. Barnaby, T.J. Merkel, C.A. Mirkin, Nanoparticle probes for the detection of cancer biomarkers, cells, and tissues by fluorescence, Chem. Rev. 115 (2015) 10530-10574.

[4] A.J. Levine, p53, the cellular gatekeeper for growth and division, Cell 88 (1997) 323–331.

[5] P.A. J. Muller, K.H. Vousden, p53 mutations in cancer, Nat. Cell. Biol. 15 (2013) 2–8.

[6] M. Toshiyuki, J.C. Reed, Tumor suppressor p53 is a direct transcriptional activator of the human bax gene, Cell 80 (1995) 293–299.

[7] M. Yamamoto, M. Hosoda, K. Nakano, S.S. Jiia, K.C. Hatanaka, E. Takakuwa, Y. Hatanaks, Y. Matsuno, H. Yamashita, p53 accumulation is a strong predictor of recurrence in estrogen receptor-positive breast cancer patients treated with aromatase inhibitors, Cancer Sci. 105 (2014) 81 – 88.

[8] L. Wu, Z.Y. Wang, S.F. Zong, H. Chen, C.L. Wang, S.H. Xu, Y.P. Cui, Simultaneous evaluation of p53 and p21 expression level for early cancer diagnosis using SERS technique, Analyst 138 (2013) 3450–3456.

[9] H. Afsharan, F. Navaeipour, B. Khalilzadeh, H. Tajalli, M. Mollabashi, M.J. Ahar, M.R. Rashidi, Highly sensitive electrochemiluminescence detection of p53 protein using functionalized Ru–silica nanoporous@gold nanocomposite, Biosens. Bioelectron. 80 (2016) 146–153.

[10] R. Elshafey, M. Siaj, A.C. Tavares, Au nanoparticle decorated graphene nanosheets for electrochemical immunosensing of p53 antibodies for cancer prognosis, Analyst 141 (2016) 2733–2740.

[11] Y.T. Zhao, D. Du, Y.H. Lin, Glucose encapsulating liposome for signal

amplification for quantitative detection of biomarkers with glucometer readout, Biosens. Bioelectron. 72 (2015) 348–354.

[12] W.S. el-Deiry, S.E. Kern, J.A. Pietenpol, K.W. Kinzler, B. Vogelstein, Definition of a consensus binding site for p53, Nat. Genet. 1 (1992) 45–49.

[13] J.X. Wang, X. Zhu, Q.Y. Tu, Q. Guo, C.S. Zarui, J. Momand, X.Z. Sun, F.M. Zhou, Capture of p53 by electrodes modified with consensus DNA duplexes and amplified voltammetric detection using ferrocene-capped gold nanoparticle/streptavidin conjugates, Anal. Chem. 80 (2008) 769–774.

[14] Y.C. Wang, X. Zhu, M.H. Wu, N. Xia, J.X. Wang, F.M. Zhou, Simultaneous and label-free determination of wild-type and mutant p53 at a single surface plasmon resonance chip preimmobilized with consensus DNA and monoclonal antibody, Anal. Chem. 81 (2009) 8441–8446.

[15] Y.J. Han, J.M. Chabu, S.Q. Hu, L. Deng, Y.-N. Liu, S.J. Guo, Rational tuning of the electrocatalytic nanobiointerface for a “turn-off” biofuel-cell-based self-powered biosensor for p53 protein, Chem. Eur. J. 21 (2015) 13045 – 13051.

[16] L. Kurzawa, M. Pellerano, J.B. Coppolani, M.C. Morris, Fluorescent peptide biosensor for probing the relative abundance of cyclin-dependent kinases in living cells, PLoS ONE 6 (2011).

[17] W.H. Zhang, W. Ma, Y.T. Long, Redox-mediated indirect fluorescence immunoassay for the detection of disease biomarkers using dopamine-functionalized quantum dots, Anal. Chem. 88 (2016) 5131–5136.

[18] Z.J. Liu, W.S. Chen, Y.J. Han, O.Y. Jiang, C.Min, S.Q. Hu, L. Deng, Y.N. Liu, A Label-free Sensitive Method for Membrane Protein Detection Based on Aptamer and AgNCs Transfer, Talanta. 174 (2017) 470-476.

[19] Y.J. Sung, H.J. Suk, H.Y. Sung, T. Li, H. Poo, M.G. Kim, Novel antibody/gold nanoparticle/magnetic nanoparticle nanocomposites for immunomagnetic separation and rapid colorimetric detection of Staphylococcus aureus in milk, Biosens. Bioelectron. 43 (2013) 432–439.

[20] B. Liu, Y.Y. Jia, M. Ma, Z.Y. Li, H.N. Liu, S. Li, Y. Deng, L.M. Zhang, Z.X. Lu, W. Wang, High throughput SNP detection system based on magnetic

nanoparticles separation, J. Biomed. Nanotechnol. 9 (2013) 247–256.

[21] S. Kyeong, C. Jeong, H. Kang, H.J. Cho, S.J. Park, J.K. Yang, S. Kim, H.M. Kim, B.H. Jun, Y.S. Lee, Double-layer magnetic nanoparticle-embedded silica particles for efficient bio-separation, PLoS ONE 10 (2015).

[22] Q.M. Kainz, O. Reiser, Polymer-and dendrimer-coated magnetic nanoparticles as versatile supports for catalysts, scavengers, and reagents, Acc. Chem. Res. 47 (2014) 667–677.

[23] Y.J. Tang, Z.Y. Li, N.Y. He, L.M. Zhang, C. Ma, X.L. Li, C.Y. Li, Z.F. Wang, Y. Deng, L. He, Preparation of functional magnetic nanoparticles mediated with PEG-4000 and application in Pseudomonas Aeruginosa rapid detection, J. Biomed. Nanotechnol. 9 (2013) 312–317.

[24] V. Ayala, A.P. Herrera, M. Latorre-Esteves, M. Torres-Lugo, C. Rinaldi, Effect of surface charge on the colloidal stability and in vitro uptake of carboxymethyl dextran-coated iron oxide nanoparticles, J. Nanopart. Res. 15 (2013).

[25] Z.Y. Li, H.W. Yang, N.Y. He, W.B. Liang, C. Ma, M.A.A. Shah, Y.J. Tang, S. Li, H.N. Liu, H.S. Jiang, Solid-phase hybridization efficiency improvement on the magnetic nanoparticle surface by using dextran as molecular arms, J. Biomed. Nanotechnol. 9 (2013) 1945-1949.

[26] L. Josephson, C.H. Tung, A. Moore, R. Weissleder, High-efficiency intracellular magnetic labeling with novel superparamagnetic-Tat peptide conjugates, Bioconjugate Chem. 10 (1999) 186–191.

[27] Y. Perez, A. Valdivia, L. Gomez, B.K. Simpson, R. Villalonga, Glycosidation of Cu, Zn-superoxide dismutase with end-group aminated dextran. pharmacological and pharmacokinetics properties, Macromol. Biosci. 5 (2005) 1220–1225.

[28] Y.M. Zhai, J.F. Zhai, Y.L. Wang, S.J. Guo, W. Ren, S.J. Dong, Fabrication of iron oxide core/gold shell submicrometer spheres with nanoscale surface roughness for efficient surface-enhanced Raman scattering, J. Phys. Chem. C 113 (2009) 7009–7014.

[29] K.G. McLure, P.W.K. Lee, p53 DNA binding can be modulated by factors that alter the conformational equilibrium, EMBO J. 18 (1999) 763–770.

Figures Caption

Fig. 1 (a) Schematic illustration of the preparation of DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs. The TEM image of Fe_3O_4 NPs (b) and DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs (c). (d) The Zeta potential of the Fe_3O_4 NPs (1), PAA/Fe_3O_4 NPs (2) and dextran/PAA/Fe_3O_4 NPs (3).

Fig. 2 (a) Schematic illustration of the p53 protein measurement principle based on consensus DNA functionalized Fe_3O_4 NPs. (b) The fluorescent emission of the proposed sensor in presence of 2 nM p53 protein. (c) The fluorescent emission of the proposed sensor without p53 protein.

Fig. 3 (a) Fluorescent emission intensity of the proposed method based on DNA-2/DNA-1/dextran/PAA/Fe_3O_4 NPs and DNA4/DNA-1/dextran/PAA/Fe_3O_4 NPs. Dependence of fluorescent emission of the proposed method on an exchange time (b), incubation time (c) and DNA-1 amount (d).

Fig. 4 (a) Fluorescent emission intensity of the proposed method toward p53 protein with various concentrations. (b) Calibration curve for the detection of p53 protein.

Fig. 5 Fluorescent emission intensity under different interfering agents.

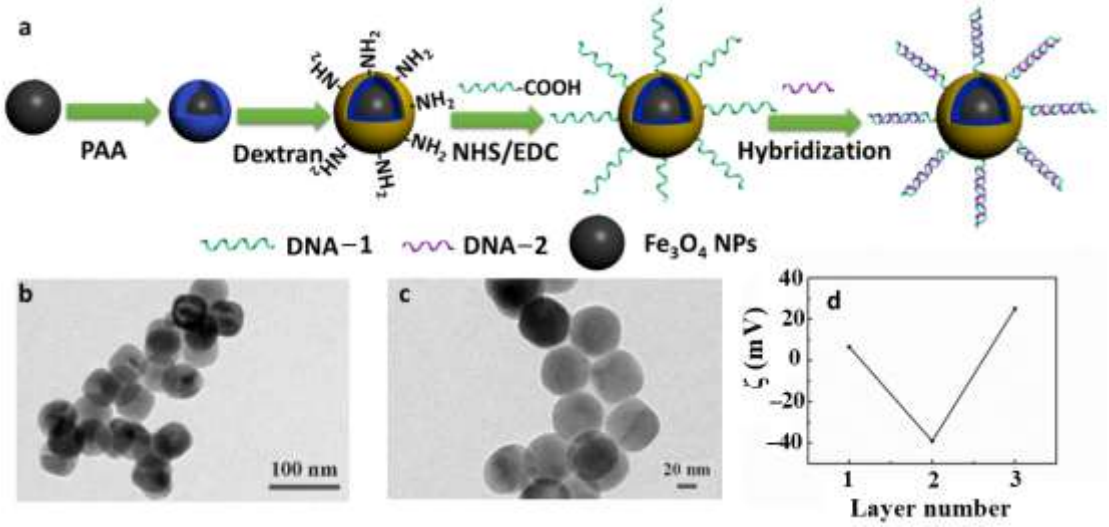


Fig. 1

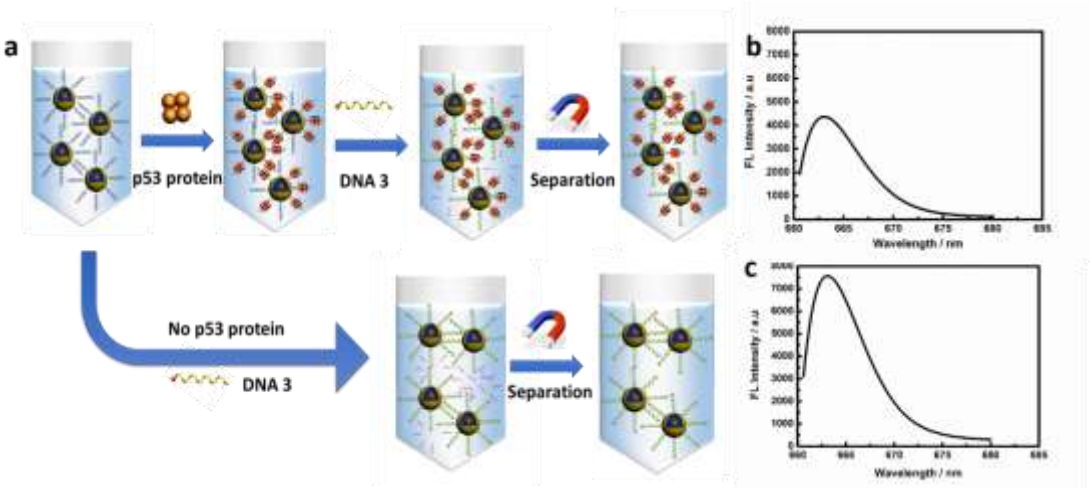


Fig. 2

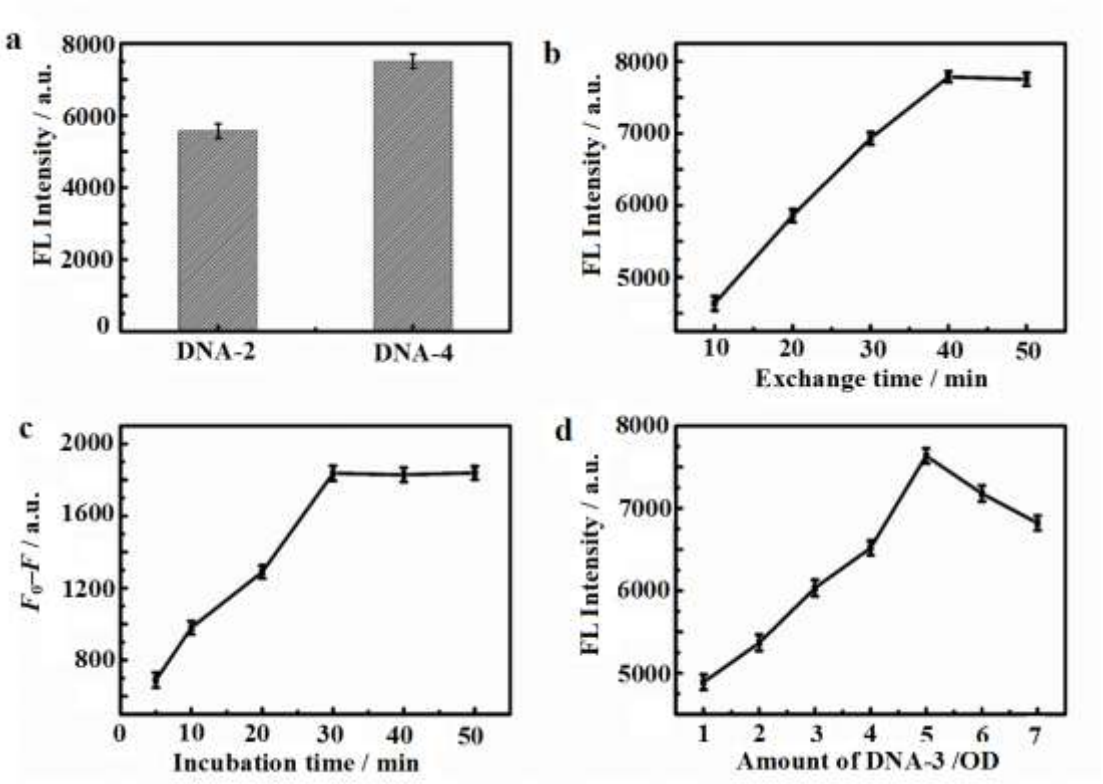


Fig. 3

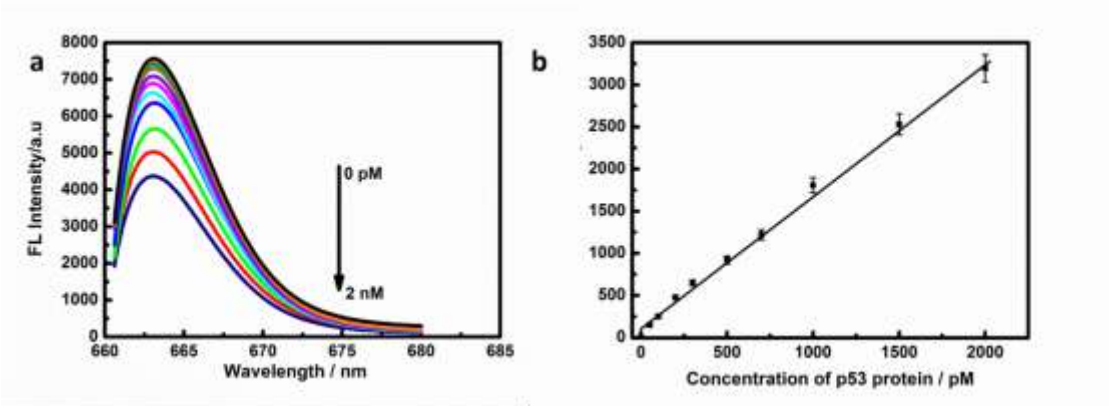


Fig. 4

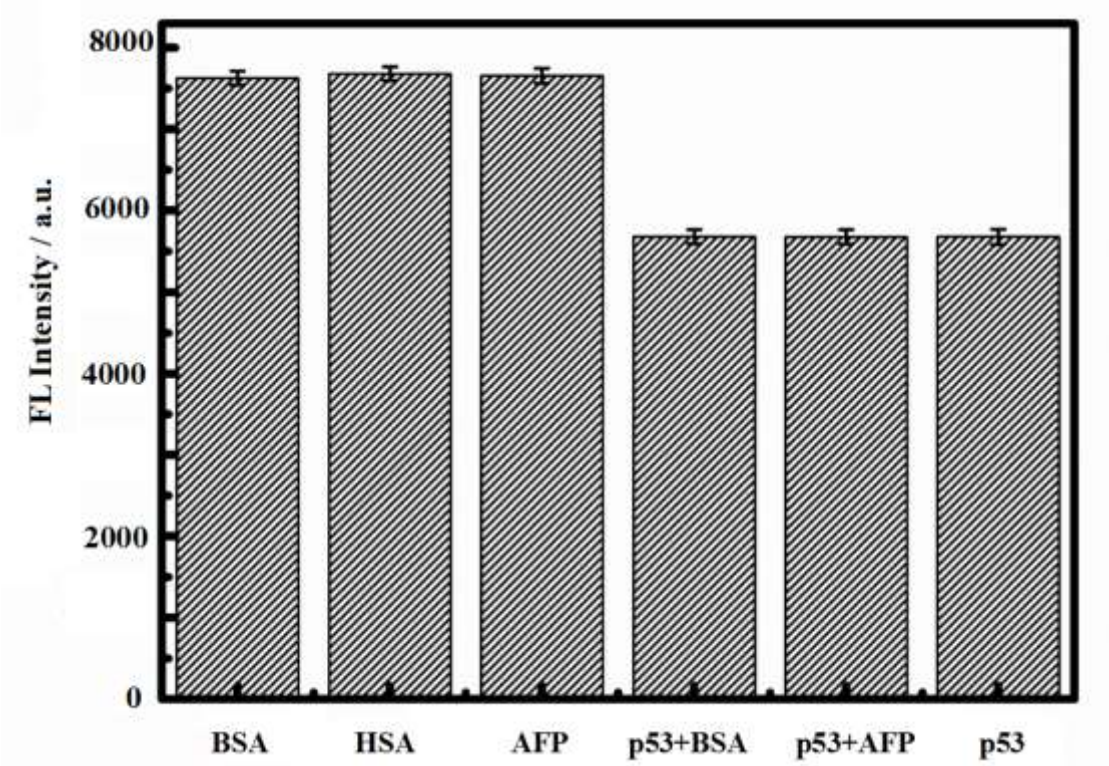


Fig. 5

Highlights

- The DNA functionalized magnetic nanoparticles was developed for p53 accurate quantitative measurement.
- Facile measurement of p53 in complex environment was accomplished.
- The expression levels of p53 in cancer cells can be monitored successfully.
-