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RNA aptamer binding to polyhistidine-tag

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ABSTRACT

Polyhistidine-tag (His-tag) is a powerful tool for purification of recombinant protein. His-tagged protein can be affinity-purified by using resins immobilizing Ni^{2+} or anti-His-tag antibodies. However, Ni^{2+}-affinity-purification is prevented by the presence of divalent cations. The purification with antibodies has contamination of antibody peptides, which interferes with following analysis. In the present study, we isolated RNA aptamers binding to His-tag. The best clone, named shot47, bound to the target with low picomolar dissociation constant. In the presence of divalent cations, shot47 was substitutable for antibodies against His-tag on ELISA, immunoprecipitation, and Western blotting. Shot47 can be synthesized easily by in vitro transcription. Thus, shot47 would be applicable as a useful and cost-effective tool for biochemical analyses.

An aptamer is a short RNA or DNA molecule that specifically binds to a target molecule. It is possible to produce an aptamer with a high affinity for a small molecule, such as a peptide or other molecular compound, against which antibodies (Ab) are difficult to obtain. Aptamers can make good molecular-targeted drug platforms because they are chemically synthesizable, poorly immunogenic, and often bind to a target molecule more strongly than do Ab [1,2]. Aptamers have also been utilized as a novel molecular sensor [3] against proteins [4–7], other molecules [8–10], and ions [11]. Aptamers used for biochemical detection [12] and affinity-purification [13,14] do not have any peptide contamination, which is valuable for biochemical analysis. Aptamer-purified targets can be eluted under milder conditions than Ab-purified targets [14]. Furthermore, producing an aptamer is more cost-advantageous than an Ab because it can be synthesized easily and in large quantities by in vitro transcription, PCR, or chemical synthesis. Thus, aptamers are useful and cost-effective tools for biochemical analyses.

Polyhistidine-tag (His-tag) is a powerful tool for molecular analysis and purification of recombinant protein. His-tagged recombinant protein can be affinity-purified by using resins immobilizing Ni^{2+} or Co^{2+}. However, this affinity-purification is prevented by the presence of divalent cations or divalent cation-binding molecules. Although anti-His-tag Ab is useful for detection and purification of His-tagged protein, the purification with Ab has contamination of Ab peptides, which interferes with following analysis.

In the present study, we isolated RNA aptamers bound to the polyhistidine-tag (His-tag). The best clone, named shot47, bound to the target with low picomolar dissociation constant. Shot47 was substitutable for Ab against His-tag on ELISA, immunoprecipitation, and Western blotting. Shot47 would be applicable as a useful and cost-effective tool without peptide contamination.

Materials and methods

Isolation of RNA aptamer against His-tag. RNA aptamers against His-tag was isolated by systematic evolution of ligands by exponential enrichment in which nucleic acids are amplified by transcription (SELEX-T). The detail was described in the companion paper. Antisense oligonucleotides for the RNA library consists of 40 random nucleotide sequences inserted between two fixed 20 nucleotide sequences and T7 promoter sequences (5′- ACTGCACGTCCAGGCACT GA N_{40} TGAGCGTACGTGAGCGTCCC TATAGTGAGTCGTATTA-3′). The RNA library was transcribed from of antisense oligonucleotides

Abbreviations: Ab, antibodies; GFP, green fluorescent protein; HBS-T, 10 mM HEPES buffered saline (pH 7.2) containing 0.01% Tween 20 and 0.1 mM magnesium acetate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; His–MIF, His-tagged macrophage migration inhibitory factor; His-tag, polyhistidine-tag; SELEX-T, systematic evolution of ligands by exponential enrichment in which nucleic acids are amplified by transcription.

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(50 pmol) and T7 promoter primer using heat-resistant T7 RNA polymerase (ScriptMAX Thermo T7 Transcription Kit, Toyobo Co., Ltd., Osaka, Japan). The RNA library was pre-absorbed with TALON Metal Affinity Resin (Takara Bio Inc., Shiga, Japan) in 50 μL of 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.2) containing 100 mM NaCl, 0.01% Tween 20, and 0.1 mM magnesium acetate with 0.4 U/mL RNase inhibitor and 500 μg/mL tRNA. The non-adsorptive RNA was separated by a membrane filter and incubated for 15 min at 22 °C with His-tagged macrophage migration inhibitory factor (His–MIF) (ATGen Co., Ltd., Gyeonggi-do, South Korea). The RNA–His–MIF complex was adsorbed with TALON Metal Affinity Resin, washed, and then eluted with buffer containing 150 mM imidazole. The RNA was transformed into cDNA, including the T7 promoter, with the OneStep RT-PCR kit (QIAGEN GmbH, Hilden, Germany). The RNA was transcribed with heat-resistant T7 RNA polymerase and used for the next round of selection. A total of 7 selection cycles was completed for this SELEX-T.

Biacore analysis. The dissociation constant of shot47 to His–MIF was measured by an analysis based on surface plasmon resonance using Biacore X (GE Healthcare UK Ltd., Buckinghamshire, UK). Shot47 plus a poly(A_{20}) tail was immobilized via 5′-biotinylated oligo(dT_{20}) onto the sensor tip with streptavidin (GE Healthcare UK Ltd.). A flow cell without 5′-biotinylated oligo(dT_{20}) was used as the reference cell. His–MIF was injected as an analyte with 10 mM HEPES buffered saline (pH 7.2) containing 0.01% Tween 20 and 0.1 mM magnesium acetate (HBS-T). The dissociation constant was calculated according to the manufacturer's instructions.

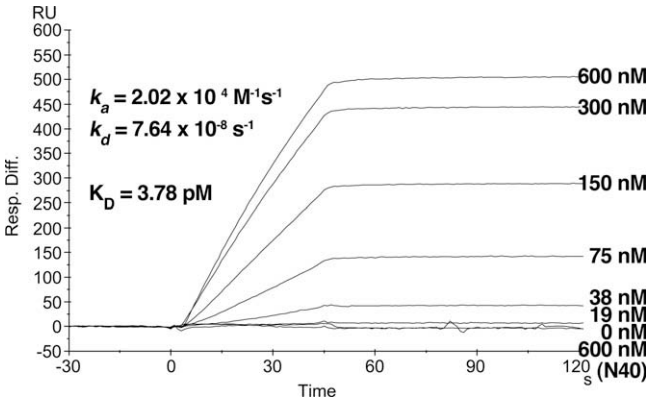
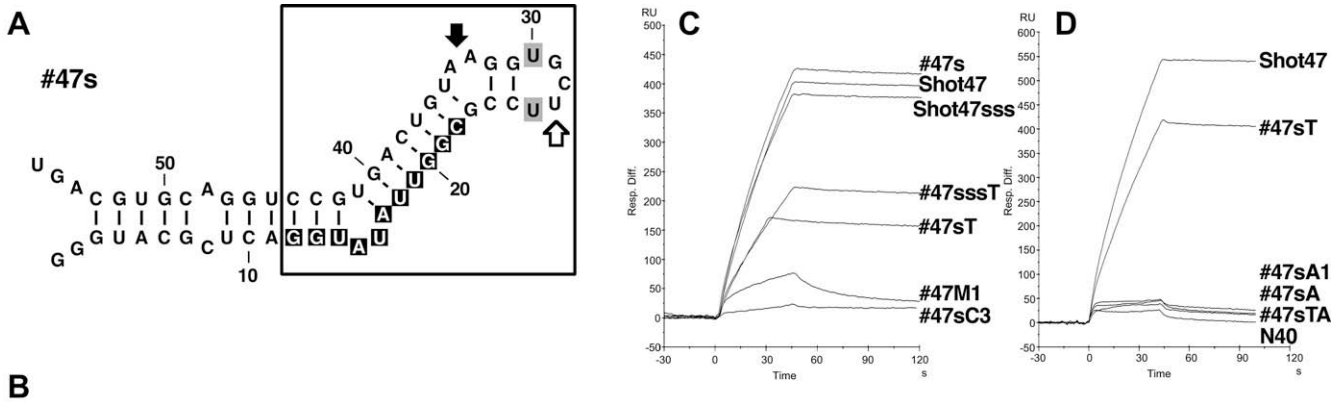


Fig. 1. The dissociation constant of shot47. The binding was measured by Biacore X analysis. Immobilized shot47 plus poly(A_{20}) tail was treated with a series of concentrations of His–MIF in HBS-T. The control RNA (N40) was treated with 600 nM His–MIF. The association rate constant (k_a) and the dissociation rate constant (k_d) were calculated according to the manufacturer's procedure. The dissociation content (K_D) is equal to k_d/k_a.

Enzyme-linked RNA aptamer assay. Production of tagged green fluorescent protein (GFP) is described in Supplementary Fig. S1. Anti-GFP (JL-8, Takara Bio Inc.), anti-His-tag (Penta-His antibody, QIAGEN GmbH), or anti-MIF (MAB289, R&D Systems Inc., Minneapolis, MN) was adsorbed onto 96-well plates. The plates were



Aptamer name	Sequences
Shot47	GGGACGCUCACGUACGCUCA CGUCCGAUCGAUACUGGUAUAUUGCGCGCCUUCGUGGAAUG UCAGUGCCUGGACGUGCAGU
#47s	ggGUACGCUCA ----------GGUAUAUUGGCGCCUUCGUGGAAUG UCAGUGCCUGGACGUGCAGU
#47sT	ggGUACGCUCA ----------GGUAUAUUGGCGCC-UCG-GGAAUG UCAGUGCCUGGACGUGCAGU
#47sC3	ggGUACGCUCA ----------GGUAUAUUGGCGCC-CCG-GGAAUG UCAGUGCCUGGACGUGCAGU
#47sA1	ggGUACGCUCA ----------GGUAUAUUGGCGCCUUCGUGGA-UG UCAGUGCCUGGACGUGCAGU
#47sA	ggGUACGCUCA ----------GGUAUAUUGGCGCCUUCGUGG--UG UCAGUGCCUGGACGUGCAGU
#47sTA	ggGUACGCUCA ----------GGUAUAUUGGCGCC-UCG-GG--UG UCAGUGCCUGGACGUGCAGU
Shot47sss	gGGUAUAUUGGCGCCUUCGUGGAAUG UCAGUGCCUGG
#47M1	gggUACUGGUAUAUUGGCGCCUUCGUGGAAUG UCAGUG
#47sssT	gGGUAUAUUGGCGCC-UCG-GGAAUG UCAGUGCCUGG

Fig. 2. Binding of shortened shot47 to His–MIF. (A) Secondary structure of shortened shot47, #47s. The conserved sequence is shown as black boxes. The deleted bases are shown as shaded characters or arrowed positions. The core sequence of shot47sss is indicated by a square box. (B) Sequences of shortened shot47. (C,D) Sensorgrams of surface plasmon resonance. Immobilized RNA aptamer plus poly(A_{20}) tail was treated with 600 nM His–MIF in HBS-T. Control RNA is shown as N40.

blocked with bovine serum albumin and then treated with His–MIF or bacterial extract containing tagged GFP. After washing with HBS-T, the plates were incubated with 50 μL of biotin-associated RNA aptamer. To produce the biotin-associated RNA aptamer, RNA aptamer plus a poly(A_{20}) tail (300 nM) was heat-denatured and preincubated for 30 min at 4 °C with 5′-biotinylated oligo(dT_{20}) (740 nM) in HBS-T containing 100 μg/mL tRNA and 0.16 units/mL RNase inhibitor. After washing, the plates were incubated with horseradish peroxidase–conjugated streptavidin (Thermo Fisher Scientific Inc., Rockford, IL), developed with 1-Step Ultra TMB substrates (Thermo Fisher Scientific Inc.). Tag-deleted MIF was prepared by cleaving His–MIF by enterokinase (Novagen, EMD Chemicals, Inc., San Diego, CA).

Pull-down assay. Biotin-associated RNA aptamer was prepared as described above in the section on enzyme-linked RNA aptamer assay. The biotin-associated RNA aptamer (50 μL) was mixed with His–MIF or bacterial extracts containing tagged GFP. After the first incubation, the mixed solution was further incubated with 5 μL of streptavidin–Sepharose (GE Healthcare UK Ltd.), and the gels were washed three times with HBS-T. The tagged GFP or His–MIF was resolved by SDS–PAGE, and transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore). The membrane was blocked with 5% nonfat milk and then treated with anti-MIF or anti-His-tag. After washing, the membrane was treated with horseradish peroxidase–conjugated sheep anti-mouse IgG (GE Healthcare UK Ltd.) and developed with ECL advance (GE Healthcare UK Ltd.).

Northwestern blotting. Biotin-associated RNA aptamer was prepared as described above in the section on enzyme-linked RNA aptamer assay. Serially-diluted His–MIF was resolved by SDS–PAGE and transferred to a polyvinylidene difluoride membrane. The membrane was blocked with 5% nonfat milk, and then treated for 3 h at 4 °C with 50 μL of the biotin-associated RNA aptamer in 5 mL of HBS-T containing 5% nonfat milk. After washing, the membrane was treated with horseradish peroxidase–conjugated streptavidin (GE Healthcare UK Ltd.) and developed with ECL advance.

Results and discussion

RNA aptamer binding to His–MIF

We isolated some RNA aptamers against His–MIF by SELEX-T (see the companion paper). The dissociation constant of the best clone, named shot47, was calculated at 3.78×10^{-12} M under physiological salt concentration by Biacore analysis (Fig. 1). This result indicated that shot47 had a higher affinity to His–MIF than anti-His-tag Ab, which had a dissociation constant of $>1 \times 10^{-9}$ M [15].

The conserved sequence [GGUN$_{1-3}$AYUGGY; Y, pyrimidine base] is important for binding to His–MIF (see the companion paper). The RNA aptamers against His–MIF have a similar bulge loop containing the conserved sequence on the predicted secondary structure. We designed clone #47s that formed a stable stem-loop structure with the conserved sequence (Fig. 2A and B). As shown in Fig. 2C, #47s bound to His–MIF as strongly as did shot47. To shorten the hairpin loop, two uridines were deleted from #47s (#47sT in Fig. 2B, shaded bases in Fig. 2A are deleted). This resulted in a decrease in the binding level of #47sT (Fig. 2C). When a cytidine was substituted for a uridine (#47sC3 in Fig. 2B, open arrow in Fig. 2A), the clone did not bind to His–MIF (Fig. 2C). Deletion

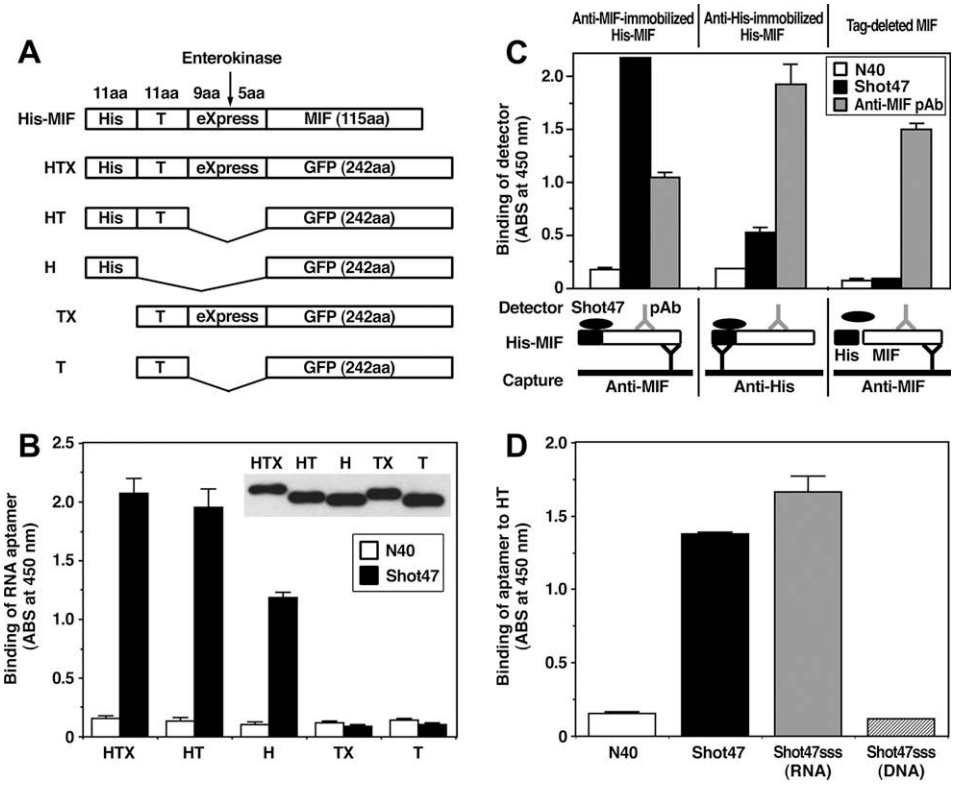


Fig. 3. Binding of shot47 to His-tag. Values represent the means ± SD of triplicate determinations. (A) Structure of tagged proteins. His-tag, T7 gene 10 leader, and eXpress tag are shown as His, T, and eXpress, respectively. (B) Binding of shot47 to tagged GFPs. Binding was measured by enzyme-linked RNA aptamer assay. Western blot of each tagged GFP with anti-GFP is shown in the right upper side. (C) Binding of shot47 to His–MIF on enzyme-linked RNA aptamer assay. Horseradish peroxidase-conjugated anti-MIF polyclonal antibodies (Anti-MIF pAb, R&D Systems Inc.) were used as a positive control for detection. (D) Binding of shot47sss and its DNA analog to His-tag. Shot47 and shot47sss (shot47sss [RNA]) were prepared as poly(A)-tailed RNAs. The DNA analog of shot47sss (shot47sss [DNA]) was synthesized as an oligodeoxynucleotide plus a poly(A) tail. GFP including His-tag and T7 gene 10 leader (HT) was used as the His-tagged protein. Abbreviations: HTX, GFP with a His-tag, T7 gene 10 leader, and eXpress tag; H, GFP with only a His-tag; TX, GFP with T7 gene 10 leader and eXpress tag; T, GFP with only a T7 gene 10 leader; N40, control RNA.

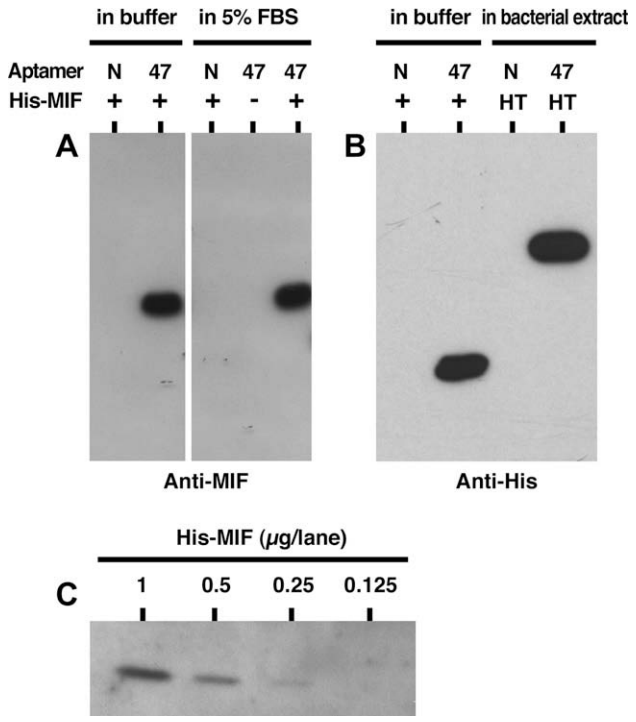


Fig. 4. Detection of His-tagged protein using shot47. (A) Pull-down assay of His–MIF with shot47. His–MIF was purified with shot47 (47), but not a control RNA (N), from HBS-T (buffer) or culture medium containing 5% fetal bovine serum (FBS). His–MIF was detected by Western blotting using anti-MIF. (B) Pull-down assay of His-tagged protein with shot47. His–MIF or His-tagged GFP (HT) was purified with shot47 (47), but not a control RNA (N), from HBS-T (buffer) or bacterial extract, respectively. His-tagged proteins were detected by Western blotting using anti-His. (C) Northwestern blotting using shot47. Serially-diluted His–MIF was resolved by nonreducing SDS–PAGE and transferred to a polyvinylidene difluoride membrane. Detection with shot47 is described in Materials and methods.

of adenosine in the bulge loop without the conserved sequence (#47sA1, #47sA, or #47sTA in Fig. 2B, closed arrow in Fig. 2A) completely eliminated affinity to His–MIF (Fig. 2D). These results suggested that the loop regions of shot47 were necessary for the high affinity binding of shot47 to His–MIF.

The smallest aptamer derived from shot47 (shot47sss in Fig. 2B, square box in Fig. 2A) bound to His–MIF as much as did shot47 or #47s (Fig. 2C). The deletion of two uridines in the hairpin loop (#47sssT in Fig. 2B) decreased the binding levels of His–MIF (Fig. 2C). The clone #47M1 is forward-shifted, and includes the conserved sequence and the loop region (Fig. 2B), but not the base pairs for the stable stem-loop structure. As shown in Fig. 2C, #47M1 interacted with His–MIF weakly, but it could not form an associative complex. These results suggested that shot47sss was the shortest sequence that retained a high affinity for His–MIF and that the stem structure of shot47sss was necessary to form a stable complex between shot47sss and His–MIF.

Binding of shot47 to His-tag

To investigate where shot47 binds to His–MIF, tagged proteins in which MIF was replaced with GFP were constructed (Fig. 3A and Supplementary Fig. S1). Shot47 bound to GFP with a His-tag, T7 gene 10 leader, and eXpress tag (HTX); GFP with a His-tag and T7 gene 10 leader (HT); and GFP with only a His-tag (H) (Fig. 3B). Shot47 did not bind to GFP without a His-tag (TX and T). Control RNA (N40) did not bind to any GFPs (Fig. 3B). Shot47 bound to His–MIF immobilized with anti-MIF more efficiently than did anti-His-tag; furthermore, shot47 did not recognize tag-deleted MIF (Fig. 3C). Interaction between shot47 and non-tagged MIF was not observed by Biacore analysis (data not shown). These results suggested that shot47 binds to His-tag. Additionally, shot47sss bound to HT as much as shot47, whereas the DNA analog of shot47sss did not bind to HT (Fig. 3D).

Detection of His-tagged protein using shot47

Shot47, but not the control aptamer, precipitated His–MIF (left sides in Fig. 4A and B). Shot47, without modification, collected His–MIF from a culture medium containing 5% fetal bovine serum (right side in Fig. 4A). Shot47 also purified HT from a bacterial extract (right side in Fig. 4B). These results indicated that shot47, without any modification, was capable of replacing anti-His-tag Ab for immunoprecipitation of His-tagged protein.

We also tested whether shot47 substituted for anti-His-tag Ab on Western blotting and found that shot47 recognized His–MIF transferred onto a membrane (Fig. 4C).

Conclusion

We produced His-tag-binding RNA aptamers that had a higher affinity for His-tagged protein than Ab. They were synthesizable easily and inexpensively; furthermore, they could substitute for anti-His-tag Ab in typical biochemical analyses in the presence of divalent cations. His-tag is a powerful tool for molecular analysis and purification of recombinant protein. Shot47 would be applicable as a useful tool in many biochemical analyses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.06.014.

References

[1] S.M. Nimjee, C.P. Rusconi, B.A. Sullenger, Aptamers: an emerging class of therapeutics, Annu. Rev. Med. 56 (2005) 555–583.
[2] E.W.M. Ng, D.T. Shima, P. Calias, E.T. Cunningham Jr., D.R. Guyer, A.P. Adamis, Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease, Nat. Rev. Drug Discov. 5 (2006) 123–132.
[3] T. Mairal, V.C. Ozalp, P. Lozano Sánchez, M. Mir, I. Katakis, C.K. O'Sullivan, Aptamers: molecular tools for analytical applications, Anal. Bioanal. Chem. 390 (2008) 989–1007.
[4] Y.C. Huang, B. Ge, D. Sen, H.Z. Yu, Immobilized DNA switches as electronic sensors for picomolar detection of plasma proteins, J. Am. Chem. Soc. 130 (2008) 8023–8029.
[5] C. Deng, J. Chen, Z. Nie, M. Wang, X. Chu, X. Chen, X. Xiao, C. Lei, S. Yao, Impedimetric aptasensor with femtomolar sensitivity based on the enlargement of surface-charged gold nanoparticles, Anal. Chem. 81 (2009) 739–745.
[6] F. Wei, C.M. Ho, Aptamer-based electrochemical biosensor for *Botulinum* neurotoxin, Anal. Bioanal. Chem. 393 (2009) 1943–1948.
[7] C.C. Huang, S.H. Chiu, Y.F. Huang, H.T. Chang, Aptamer-functionalized gold nanoparticles for turn-on light switch detection of platelet-derived growth factor, Anal. Chem. 79 (2007) 4798–4804.
[8] J.S. Swensen, Y. Xiao, B.S. Ferguson, A.A. Lubin, R.Y. Lai, A.J. Heeger, K.W. Plaxco, H.T. Soh, Continuous, real-time monitoring of cocaine in undiluted blood serum via a microfluidic, electrochemical aptamer-based sensor, J. Am. Chem. Soc. 131 (2009) 4262–4266.
[9] N. de-los-Santos-Alvarez, M.J. Lobo-Castañón, A.J. Miranda-Ordieres, P. Tuñón-Blanco, Modified-RNA aptamer-based sensor for competitive impedimetric assay of neomycin B, J. Am. Chem. Soc. 129 (2007) 3808–3809.
[10] L. Shen, Z. Chen, Y. Li, P. Jing, S. Xie, S. He, P. He, Y. Shao, A chronocoulometric aptamer sensor for adenosine monophosphate, Chem. Commun. (2007) 2169–2171.
[11] C.W. Liu, C.C. Huang, H.T. Chang, Highly selective DNA-based sensor for lead(II) and mercury(II) ions, Anal. Chem. 81 (2009) 2383–2387.

[12] Y. Yoshida, N. Sakai, H. Masuda, M. Furuichi, F. Nishikawa, S. Nishikawa, H. Mizuno, I. Waga, Rabbit antibody detection with RNA aptamers, Anal. Biochem. 375 (2008) 217–222.
[13] D.H. Bunka, P.G. Stockley, Aptamers come of age – at last, Nat. Rev. Microbiol. 4 (2006) 588–596.
[14] S. Miyakawa, Y. Nomura, T. Sakamoto, Y. Yamaguchi, K. Kato, S. Yamazaki, Y. Nakamura, Structural and molecular basis for hyperspecificity of RNA aptamer to human immunoglobulin G, RNA 14 (2008) 1154–1163.
[15] QIAexpress Detection and Assay Handbook, QIAGEN GmbH, Hilden, Germany, Oct, 2002, p. 15.