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# A simple modular aptasensor platform utilizing cucurbit[7]uril and a ferrocene derivative as an ultrastable supramolecular linker†

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**A simple modular aptamer-based sensor (aptasensor) platform was prepared by combining the merits of the rapid and efficient preparation of a self-assembled monolayer of cucurbit[7]uril (CB[7] SAM) and the strong and specific binding affinity of CB[7] to ferrocenemethylammonium (FA), as an ultrastable supramolecular linker, to immobilize aptamers on CB[7] SAM.**

Aptamers are the artificial nucleotide sequences that fold into secondary and tertiary structures with high selectivity and specificity to target molecules including proteins, peptides and small organic molecules.[1] Along with these features, aptamers have shown extraordinary promise in analytical applications due to their easy chemical synthesis and modifications with reporter molecules, linkers, and other functional groups.[2] Many researchers have thus explored the aptamers as highly selective biorecognition components in bioassays and biosensing.[2,3] Apart from the inherent advantages of biosensors, aptamer-based biosensors (aptasensors) offer the advantage of reusability utilizing reversible structural-switching.[4] Furthermore, their small size and chemical functionalities allow efficient immobilization at high density,[5] which is of vital importance in multiplexing miniaturized systems. In spite of these rapid advances, aptamer-based bioassays are still immature compared to antibody-based immunoassays, thus reflecting the limited availability of aptamer types and the relatively poor knowledge of surface-immobilization technologies for aptamers.[6] In this regard, the multiple processing steps for immobilization of aptamers on surfaces is a major concern in the development of such systems.[7] The streptavidin–biotin binding pair[8] has extensively been used as an efficient linker system to immobilize aptamers on surfaces[5b,9] because of their strong and specific noncovalent interactions (binding constant $\sim 10^{13}$ $M^{-1}$). The multiple processing steps include the pretreatment of additional chemical reagents, such as alkane thiols, functionalized dextrans, polyethyleneglycol thiols (PEG-SH) and also requires a chemical modification of proteins with delicate handling.[7,10] Each step in this process claims to be a crucial parameter in the analytical performance of aptasensors. Such limitations therefore necessitate a new aptasensor platform that can simplify the processing steps using a chemically stable binding pair which could be convenient in terms of storage and handling.

Earlier, we and other researchers reported that cucurbit[7]uril (CB[7]), a member of the host family cucurbit[*n*]uril (CB[*n*], $n$ = 5–8, 10, 14)[11,12] with a hydrophobic cavity and two identical carbonyl-fringed portals, binds the ferrocenemethylammonium (FA) ion with an exceptionally high binding affinity with a binding constant of $\sim 10^{12}$ $M^{-1}$ in aqueous solution.[13] This exceptionally strong binding affinity of CB[7] to FA led us to develop a synthetic system successfully employed, as an alternative artificial system to a streptavidin–biotin binding pair, in biological applications such as immobilization of biomolecules on a solid surface and membrane protein isolation.[14] Recently, Li and others reported a method for the formation of a self-assembled monolayer of CB[7] on a gold surface by dipping a gold surface into a solution of CB[7].[15] It was observed that CB[7] molecules on the gold surface are uniform in their orientation and hold carbonyl portals perpendicular to the plane of the surface to maintain the guest binding and recognition properties of CB[7]. Further, Brunsveld *et al.* demonstrated simple and reversible immobilization of FA-conjugated yellow fluorescent protein (FA-YFP) on CB[7].[16] Although aptamers have become increasingly important molecular tools in the biosensing field because of the inherent advantages mentioned above, the immobilization of aptamers on CB[7] has not been reported yet.

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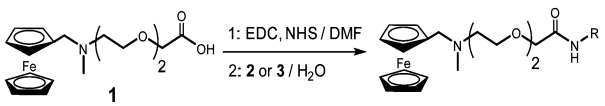
Described herein is a simple modular aptasensor platform obtained by combining the merits of both simple preparation of CB[7] SAM and highly strong and selective binding affinity of CB[7] to FA. We utilize the FA–CB[7] binding pair system as an ultrastable supramolecular linker, with the help of which various FA-conjugated aptamers can efficiently be immobilized on CB[7] SAM for surface plasmon resonance (SPR) studies (Scheme 1). Our present aptasensor system bears unique features including (1) easy preparation of CB[7] SAM by either direct injection of CB[7] onto a gold surface or dipping a gold surface into a solution of CB[7], (2) versatility of CB[7] SAM for immobilization of various FA-conjugated aptamers, (3) resistance to harsh conditions such as elevated temperature or unwanted enzymatic degradation, and (4) long-term durability due to the usage of stable synthetic molecules,[14b] instead of (strept)avidin proteins used in conventional aptasensors. This FA–CB[7] linker-based aptasensor may act as a simple and efficient bio-sensor including proteins and thus widen the practical applications of aptasensors.

CB[7] SAM was prepared by simply dipping a gold surface into a solution of CB[7] (1.0 mM in deionized $H_2O$) for 6 h as per the literature reports[15a] and conveniently kept at RT until it was ready to be used as a sensor chip. Direct injections of CB[7] solution (1.0 mM in deionized $H_2O$, 20 min, 20 μL $min^{-1}$) onto a gold surface mounted in a SPR machine were also conducted as an alternative method. After 5 sequential injections, we observed approximately 500 RU (1000 RU = 1 ng $mm^{-2}$)[17] of sensor response (Fig. S1, ESI†) which turned out to be 0.43 pmol of CB[7] on 1 $mm^2$. This result suggested that *ca.* 55% of a plain gold surface is covered with CB[7] by considering the theoretical density of densely packed CB[7] (of diameter 1.6 nm)[12b] on a plane surface (0.75 pmol $mm^{-2}$). In AFM images (Fig. S2, ESI†), small dots approximately 1 nm in height and only little aggregation appeared on a gold surface after treatment of CB[7]. The SPR and AFM results suggest that even simple direct injections of CB[7] solution to a gold surface can lead to the formation of CB[7] SAM on a gold sensor chip. Since CB[7] is a stable molecule synthesized under highly acidic conditions at high temperature,[11a,b] and it has higher resistance to harsh conditions and better long-term durability compared to (strept)avidin, which suggests that CB[7] SAM can be useful as a robust aptasensor platform.

In the present work, a carefully studied aptamer (TBA, 15-mer DNA-based) that binds strongly and selectively to thrombin was chosen as the model probe.[18] By exploiting FA–CB[7] interactions for the immobilization of TBA on CB[7] SAM, it was first "ferrocenylated"[14a] by conjugation of carboxylated FA (**1**)[14b] to aminohexyl group at the 5′ terminus using EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) coupling (Scheme 2). Thus formed ferrocenylated TBA (FA-TBA) was further purified and characterized using HPLC and ESI-mass spectroscopy, respectively (Fig. S4, ESI†). A binding constant of chemically modified TBA ($\sim 10^9$ $M^{-1}$) measured *via* a filter binding assay[19] was noted to be comparable to that of unmodified TBA. This implies that the ferrocenylation barely affects the binding affinity of TBA to its target protein, thrombin.[20]

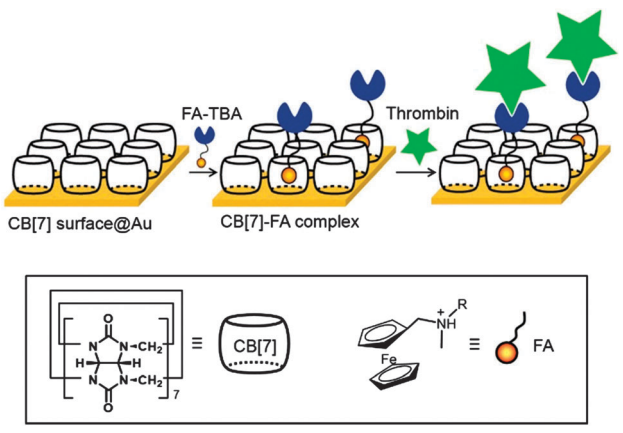
After 25 min of injection of FA-TBA and TBA into each channel on the CB[7] sensor chip, ~700 RU and 30 RU, respectively, were observed at 1920 s (Fig. 1). The higher RU value (~23-fold) for FA-TBA compared to TBA proves its importance for effective immobilization of aptamers. The converted density of FA-TBA on CB[7] SAM from the RU value (0.12 pmol $mm^{-2}$), suggested that approximately one out of four CB[7] molecules on average seemed to capture one FA-TBA. This result suggested successful immobilization of FA-TBA on CB[7] SAM by making the use of strong and specific interactions between CB[7] and FA. To check the versatility of CB[7] SAM for immobilization of various




Amine terminated thrombin DNA aptamer: 5′-$NH_2C_6H_{12}$-GGT TGG TGT GGT TGG-3′ (**2**)
Amine terminated scrambled DNA sequences: 5′-$NH_2C_6H_{12}$-GGT GGT GGT TGT GGT-3′ (**3**)

R= Thrombin DNA aptamer: -$C_6H_{12}$-GGT TGG TGT GGT TGG-3′ (**FA-TBA**)
Scrambled DNA sequences: -$C_6H_{12}$-GGT GGT GGT TGT GGT-3′ (**FA-SDNA**)

Scheme 2 Conjugation of ferrocenemethylammonium to thrombin binding aptamer (FA-TBA) and scrambled DNA sequences (FA-SDNA).




Scheme 1 Immobilization of the ferrocenemethylammonium-conjugated thrombin binding aptamer (FA-TBA) on a CB[7] SAM using the strong biding affinity of CB[7] to FA, and its application as an aptasensor chip for sensing thrombin.

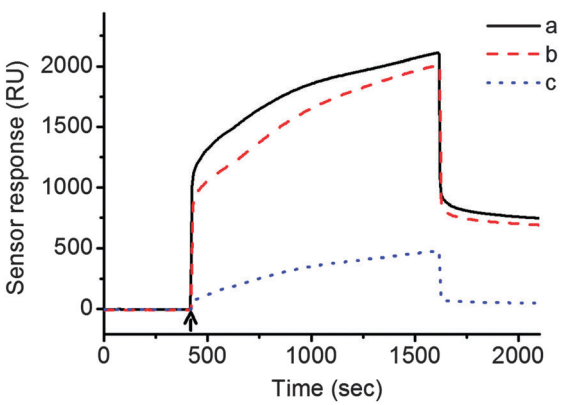



Fig. 1 SPR sensorgrams of CB[7] SAM treated with (a) FA-TBA, (b) FA-SDNA and (c) TBA (the arrow indicates injection of samples).

aptamers, we prepared FA-scrambled DNA (FA-SDNA) with the same molecular weight as FA-TBA but with different DNA sequences (Scheme 2). The observed RU value *i.e.* 670, for FA-SDNA injection was almost in line with that of FA-TBA. This finding clearly shows that CB[7] SAM can not only immobilize FA-TBA but also other various aptamers as long as they are conjugated with FA utilizing the strong and specific interaction between CB[7] and FA as a supramolecular linker.

Having established FA-aptamer@CB[7] SAM as a simple and modular aptasensor system, we examined the feasibility of FA-TBA@CB[7] SAM as a model SPR sensor chip. To confirm the selective recognition of thrombin, we injected it and bovine serum albumin (BSA, as a negative control) into a different channel on the FA-TBA@CB[7] sensor chip. Accordingly, after 15 min of injection, we observed 340 RU for thrombin and 35 RU for BSA (Fig. S5, ESI†). These values signify the specific recognition and non-specific adsorption, respectively. Moreover, the higher sensor response of thrombin as compared to BSA, by one order of magnitude, denotes its selective recognition. Furthermore, a concentration dependent sensor response was observed by injection of different thrombin concentrations to a channel of the FA-TBA@CB[7] sensor chip (Fig. 2). These recorded responses ranged from the sub-ppm to the ppm level, thus showing the sensing of target protein in nanomolar concentrations. Even though this noted analytical performance of FA-TBA@CB[7] sensor chip is comparable to that of the streptavidin–biotin binding pair based system reported earlier,[7] the former comes up with the benefits of effortless preparation, resistance to harsh conditions, long term durability and reusability by utilizing either reversible structural-switching of aptamers[4] or reversible noncovalent binding properties of CB[7] to FA[12g,21] as previously demonstrated for proteins.[16]

In summary, we have successfully demonstrated the efficient and versatile immobilization of FA-conjugated aptamers with FA-TBA and FA-SDNA on CB[7] SAM by taking advantage of its facile formation on a gold surface and also ultrastable binding of CB[7] to FA. The profound analysis of a target protein using FA-TBA@CB[7] SAM proved the utility of the FA–CB[7] binding pair as an efficient linker system providing a new aptasensor platform. Furthermore, the optimization of the formation of CB[7] SAM and immobilization of FA-aptamers using functionalized CB[7],[22] which can further control the density of CB[7] on a gold surface, may result in the enhanced analytical performance of CB[7]-based aptasensors.

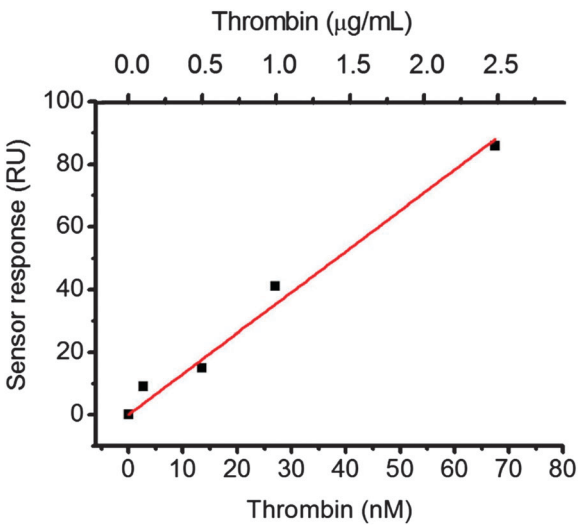



Fig. 2 Sensor responses as a function of the thrombin concentration on the CB[7] SAM SPR sensor chip (15 min, 5 μL $min^{-1}$).

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## Notes and references

1 (*a*) A. B. Iliuk, L. Hu and W. A. Tao, *Anal. Chem.*, 2011, **83**, 4440–4452; (*b*) D. S. Wilson and J. W. Szostak, *Annu. Rev. Biochem.*, 1999, **68**, 611–647; (*c*) S. M. Shamah, J. M. Healy and S. T. Cload, *Acc. Chem. Res.*, 2008, **41**, 130–138.
2 E. Luzi, M. Minunni, S. Tombelli and M. Mascini, *TrAC, Trends Anal. Chem.*, 2003, **22**, 810–818.
3 (*a*) S. D. Jayasena, *Clin. Chem.*, 1999, **45**, 1628–1650; (*b*) W. Zhou, P. J. Huang, J. Ding and J. Liu, *Analyst*, 2014, **139**, 2627–2640; (*c*) S. P. Song, L. H. Wang, J. Li, J. L. Zhao and C. H. Fan, *TrAC, Trends Anal. Chem.*, 2008, **27**, 108–117; (*d*) W. Mok and Y. F. Li, *Sensors*, 2008, **8**, 7050–7084; (*e*) C. Tuerk and L. Gold, *Science*, 1990, **249**, 505–510; (*f*) S. Tombelli, M. Minunni and M. Mascini, *Biomol. Eng.*, 2007, **24**, 191–200; (*g*) K. Sefah, J. A. Phillips, X. Xiong, L. Meng, D. Van Simaeys, H. Chen, J. Martin and W. Tan, *Analyst*, 2009, **134**, 1765–1775; (*h*) S. Tombelli, M. Minunni and M. Mascini, *Biosens. Bioelectron.*, 2005, **20**, 2424–2434.
4 (*a*) Y. L. Zhang, Y. Huang, J. H. Jiang, G. L. Shen and R. Q. Yu, *J. Am. Chem. Soc.*, 2007, **129**, 15448–15449; (*b*) Z. S. Wu, M. M. Guo, S. B. Zhang, C. R. Chen, J. H. Jiang, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2007, **79**, 2933–2939.
5 (*a*) J. F. Lee, G. M. Stovall and A. D. Ellington, *Curr. Opin. Chem. Biol.*, 2006, **10**, 282–289; (*b*) M. Liss, B. Petersen, H. Wolf and E. Prohaska, *Anal. Chem.*, 2002, **74**, 4488–4495.
6 A. Bini, M. Minunni, S. Tombelli, S. Centi and M. Mascini, *Anal. Chem.*, 2007, **79**, 3016–3019.
7 V. Ostatna, H. Vaisocherova, J. Homola and T. Hianik, *Anal. Bioanal. Chem.*, 2008, **391**, 1861–1869.
8 (*a*) P. C. Weber, D. H. Ohlendorf, J. J. Wendoloski and F. R. Salemme, *Science*, 1989, **243**, 85–88; (*b*) N. M. Green, *Methods Enzymol.*, 1990, **184**, 51–67; (*c*) C. E. Chivers, A. L. Koner, E. D. Lowe and M. Howarth, *Biochem. J.*, 2011, **435**, 55–63.
9 (*a*) A. Tahiri-Alaoui, L. Frigotto, N. Manville, J. Ibrahim, P. Romby and W. James, *Nucleic Acids Res.*, 2002, **30**, e45; (*b*) R. Kirby, E. J. Cho, B. Gehrke, T. Bayer, Y. S. Park, D. P. Neikirk, J. T. McDevitt and A. D. Ellington, *Anal. Chem.*, 2004, **76**, 4066–4075.
10 (*a*) D. T. Tran, K. Knez, K. P. Janssen, J. Pollet, D. Spasic and J. Lammertyn, *Biosens. Bioelectron.*, 2013, **43**, 245–251; (*b*) J. Ashley and S. F. Y. Li, *Biosens. Bioelectron.*, 2013, **48**, 126–131; (*c*) J. W. Lee, S. J. Sim, S. M. Cho and J. Lee, *Biosens. Bioelectron.*, 2005, **20**, 1422–1427.
11 (*a*) J. Kim, I. S. Jung, S. Y. Kim, E. Lee, J. K. Kang, S. Sakamoto, K. Yamaguchi and K. Kim, *J. Am. Chem. Soc.*, 2000, **122**, 540–541; (*b*) A. Day, A. P. Arnold, R. J. Blanch and B. Snushall, *J. Org. Chem.*, 2001, **66**, 8094–8100; (*c*) S. Liu, P. Y. Zavalij and L. Isaacs, *J. Am. Chem. Soc.*, 2005, **127**, 16798–16799; (*d*) X. J. Cheng, L. L. Liang, K. Chen, N. N. Ji, X. Xiao, J. X. Zhang, Y. Q. Zhang, S. F. Xue, Q. J. Zhu, X. L. Ni and Z. Tao, *Angew. Chem., Int. Ed.*, 2013, **52**, 7252–7255.
12 (*a*) K. Kim, *Chem. Soc. Rev.*, 2002, **31**, 96–107; (*b*) J. W. Lee, S. Samal, N. Selvapalam, H. J. Kim and K. Kim, *Acc. Chem. Res.*, 2003, **36**, 621–630; (*c*) E. Masson, X. X. Ling, R. Joseph, L. Kyeremeh-Mensah and X. Y. Lu, *RSC Adv.*, 2012, **2**, 1213–1247; (*d*) S. Gadde and A. E. Kaifer, *Curr. Org. Chem.*, 2011, **15**, 27–38; (*e*) L. Isaacs, *Chem. Commun.*, 2009, 619–629; (*f*) J. Lagona, P. Mukhopadhyay, S. Chakrabarti and L. Isaacs, *Angew. Chem., Int. Ed.*, 2005, **44**, 4844–4870; (*g*) A. E. Kaifer, *Acc. Chem. Res.*, 2014, **47**, 2160–2167.

 

13 (*a*) W. S. Jeon, K. Moon, S. H. Park, H. Chun, Y. H. Ko, J. Y. Lee, E. S. Lee, S. Samal, N. Selvapalam, M. V. Rekharsky, V. Sindelar, D. Sobransingh, Y. Inoue, A. E. Kaifer and K. Kim, *J. Am. Chem. Soc.*, 2005, **127**, 12984–12989; (*b*) M. V. Rekharsky, T. Mori, C. Yang, Y. H. Ko, N. Selvapalam, H. Kim, D. Sobransingh, A. E. Kaifer, S. Liu, L. Isaacs, W. Chen, S. Moghaddam, M. K. Gilson, K. Kim and Y. Inoue, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 20737–20742; (*c*) S. Liu, C. Ruspic, P. Mukhopadhyay, S. Chakrabarti, P. Y. Zavalij and L. Isaacs, *J. Am. Chem. Soc.*, 2005, **127**, 15959–15967.

14 (*a*) I. Hwang, K. Baek, M. Jung, Y. Kim, K. M. Park, D. W. Lee, N. Selvapalam and K. Kim, *J. Am. Chem. Soc.*, 2007, **129**, 4170–4171; (*b*) D. W. Lee, K. M. Park, M. Banerjee, S. H. Ha, T. Lee, K. Suh, S. Paul, H. Jung, J. Kim, N. Selvapalam, S. H. Ryu and K. Kim, *Nat. Chem.*, 2011, **3**, 154–159.

15 (*a*) Q. An, G. T. Li, C. G. Tao, Y. Li, Y. G. Wu and W. X. Zhang, *Chem. Commun.*, 2008, 1989–1991; (*b*) E. Blanco, C. Quintana, L. Hernandez and P. Hernandez, *Electroanalysis*, 2013, **25**, 263–268; (*c*) A. Gomez-Casado, P. Jonkheijm and J. Huskens, *Langmuir*, 2011, **27**, 11508–11513.

16 J. F. Young, H. D. Nguyen, L. Yang, J. Huskens, P. Jonkheijm and L. Brunsveld, *ChemBioChem*, 2010, **11**, 180–183.

17 X. Q. Cui, R. J. Pei, X. Z. Wang, F. Yang, Y. Ma, S. J. Dong and X. R. Yang, *Biosens. Bioelectron.*, 2003, **18**, 59–67.

18 (*a*) L. C. Bock, L. C. Griffin, J. A. Latham, E. H. Vermaas and J. J. Toole, *Nature*, 1992, **355**, 564–566; (*b*) R. F. Macaya, P. Schultze, F. W. Smith, J. A. Roe and J. Feigon, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 3745–3749.

19 R. Conrad and A. D. Ellington, *Anal. Biochem.*, 1996, **242**, 261–265.

20 E. Baldrich, A. Restrepo and C. K. O'Sullivan, *Anal. Chem.*, 2004, **76**, 7053–7063.

21 (*a*) D. Sobransingh and A. E. Kaifer, *Org. Lett.*, 2006, **8**, 3247–3250; (*b*) W. Li and A. E. Kaifer, *Langmuir*, 2012, **28**, 15075–15079.

22 (*a*) S. Y. Jon, N. Selvapalam, D. H. Oh, J. K. Kang, S. Y. Kim, Y. J. Jeon, J. W. Lee and K. Kim, *J. Am. Chem. Soc.*, 2003, **125**, 10186–10187; (*b*) K. Kim, N. Selvapalam, Y. H. Ko, K. M. Park, D. Kim and J. Kim, *Chem. Soc. Rev.*, 2007, **36**, 267–279.