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Kissing Interactions for the Design of a Multicolour Fluorescence Anisotropy Chiral Aptasensor

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Abstract

The development of enantioselective assays and sensors has received much attention for the determination of enantiomeric impurities. Herein, we demonstrated that the previously reported aptamer kissing complex (AKC) assay strategy can be implemented for designing a chiral tool that allows both the simultaneous enantiomer quantification and the enantiopurity analysis. D- and L-arginine vasopressin (AVP) were employed as model enantiomeric targets. The D- and L-AVP engineered aptamers (aptaswitch) were used as recognition units whereas the Fluorescein or Texas Red labeled D- and L-hairpin probes (aptakiss) served as probes of the enantiomer-dependent AKC formation. The orthogonal fluorescence anisotropy signaling scheme at two different emission wavelengths permitted the concomitant sensing of the AVP enantiomers in a single sample, under a high-throughput microplate format. It was also shown that the AKC-based enantioselective sensor allowed the enantiomeric impurity detection at a level as low as 0.01 %.

Key-words: Aptamer Kissing complex, Fluorescence anisotropy, Orthogonal detection, Enantiomeric purity

1. Introduction

Chiral features of nucleic acids have been extensively used in numerous scientific fields ranging from enantioselective analysis to synthesis [1]. In the peculiar analytical area, a variety of studies has demonstrated that DNA and RNA molecules can serve as efficient chiral selectors. Most of their applications are found in the separation sciences by using liquid chromatography [2-6], capillary electrophoresis [7-9] or other technologies [10]. Simpler approaches rather focus on the development of chiral biosensing devices of high-throughput capability. As representative examples, both electrochemical and optical methods have been successfully developed for highly enantioselective aptamer-based sensors (aptasensors) allowing the enantiomeric impurity determination [11-15]. However, all these previously reported oligonucleotide-based biosensors do not allow the quantification of the two enantiomers in the same sample, limiting the accuracy of the methods.

To overcome this drawback, Heemstra and co-workers have recently described one elegant approach that permits the concomitant measurement of both the concentration of each enantiomer and the enantiomeric impurity in one single sample [16]. The general concept is based on the chiral inversion principle where both D- and L-aptamer mirror-image elements are present in the solution, providing the simultaneous recognition of their corresponding target enantiomers in the sample. An orthogonal fluorescent transduction strategy is applied, for which the two enantiomeric recognition units are initially labeled by two different fluorophores without spectral overlap. The approach then allows reporting in parallel the presence of the two enantiomers upon their respective binding to the D- and L-aptamers [16].

Although the general concept opens new opportunities in the DNA-based chiral analysis field, the described methodology suffers from limitations. First, it relies on the involvement of a structure-switching mechanism for the signal transduction. The approach is based on a partially complementary strand that is released from the aptamer upon target binding. As the result of its competitive nature, the structure-switching approach leads to intrinsic limitations in terms of sensitivity. In the case of the tyrosinamide enantioselective sensing, a loss in the binding affinity by a factor of about 200 [16] was observed as compared with the original binding properties of the tyrosinamide aptamer [13]. Second, the signaling strategy is based on the use of both a quencher-labelled complementary strand and a fluorophore-tagged aptamer. An increase in the fluorescence signal is then observed upon the enantiomer binding to the recognition element. However, the aptamer labelling by a fluorophore can greatly impact the binding features of the recognition element and then modify their response to the presence of

each enantiomer in relation to the kind of fluorophore used [17]. In addition, the tagging of the two DNA partners is relatively expensive and makes the identification of efficient orthogonal probe pairs more difficult than with a single labelling methodology.

In the present work, we aimed at developing a novel approach for the design of a chiral inversion-based enantioselective aptasensor that provides the simultaneous detection of both enantiomers in a single sample. The method relied on the use of the aptamer kissing complex (AKC) strategy instead of the structure-switching binding route. The AKC concept has been established few years ago [18] to detect the presence of small targets (including adenosine, GTP and theophylline) through a variety of platforms that range from biosensors [19-22] to smart nano-architectures [23, 24]. It relies on the ability of an engineered hairpin aptamer (aptaswitch) to engage loop-loop interactions (kissing complex) with another hairpin (aptakiss) only upon the small target binding to the aptaswitch (Figure 1). For the numerous AKC methodologies designed to date, the target binding affinity of the aptaswitch unit is found to be maintained relative to the parent aptamer [18, 20]. Furthermore, by using a single fluorophore-tagged aptakiss and the fluorescence anisotropy (FA) technique as transduction method, the target interaction with the aptaswitch can be signaled without any modification/labelling of the binding unit [18, 20]. Indeed, the aptakiss binding to the binary target-aptaswitch complex is able to produce a FA signal increase as the simple consequence of the enhancement in the probe molecular mass [17].

The proof-of-principle of the chiral inversion-based enantioselective AKC assay was established by using the arginine vasopressin-aptamer pair as a model system. The aptakiss and aptaswitch couple sequences for the arginine vasopressin have been designed in one earlier work [25]. This previous study was dedicated to the elaboration of a biostable sensing system based on an all-L configuration kissing complex. It was demonstrated that the natural L-arginine vasopressin could be detected without interferences in samples containing up to 10% of untreated serum. Herein, we focused our attention on the development of a multiplexed chiral sensor relying on the simultaneous formation of enantiomeric D- and L-systems that were signaled by spectrally orthogonal Fluorescein and Texas Red labels, respectively. The approach allowed the concomitant monitoring of the two stereoisomeric events through a multicolour FA detection scheme (see Figure 1 for the representative scheme of the orthogonal chiral sensing).

2. Experimental and methods

2.1. Chemicals and apparatus

The D- and L- arginine vasopressin (D-AVP and L-AVP) trifluoroacetate salt enantiomers were obtained from Bachem (Weil am Rhein, Germany). Tris-HCl was supplied by Sigma Aldrich (Saint-Quentin, France). Magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) was purchased from Roth (Lauterbourg, France), methanol was obtained from Carlo Erba (Val de Reuil, France).

All oligonucleotides were synthesized and HPLC-purified by Eurogentec (Angers, France).

D-DNA- and L-DNA aptaswitches (D-Vasosw, L-Vasosw respectively) showed the following sequence:

5'-TCACGTGCATGATA**ACACTCCCATTCT**GAGTTGCTGTGTGCCGATGCACGTGA-3'

D-RNA and L-RNA aptakiss hairpins (5'-CGAGCC**UGGGAGCUCG**-3') were labelled either in 3' position by Fluorescein or in 5' position by Texas Red (respectively named F-D-kiss and TR-L-kiss). The apical loop sequences of both aptaswitch and aptakiss are presented in bold-face type.

2.2. Methods

The binding buffer for multicolour FA detection assays, previously optimized, consisted in 10 mM Tris, pH 7.4, 5 mM MgCl_2 and 18.75% methanol buffer. The oligonucleotide stock solutions were prepared in PCR water at 10^{-3} M and stored at -20°C . Intermediate dilutions in water were prepared at 10^{-4} M or 10^{-5} M. For multiplex analyses, premixed working solutions of aptaswitches (D-Vasosw and L-Vasosw) or aptakisses (F-D-kiss and TR-L-kiss) were prepared from the individual stock solutions by 2-fold dilution with concentrated binding buffer (20 mM Tris-HCl, pH 7.4, 10 mM MgCl_2). Aptakiss or Vasoswitch solutions for controls were prepared individually in the same way. A stock solution of D- or L-AVP was prepared at 1845 μM in water. After preparation, this parent stock solution was diluted using the 2X concentrated buffer and an appropriate volume of methanol to obtain a final concentration of 37.5%.

Appropriate wells were established by mixing the solution of the aptaswitch(es), of the kiss(es) and the different amounts of target solutions (final volume = 100 μL), resulting in aptaswitch(es) and kiss(es) final concentrations of 300 nM and 10 nM, respectively. Blank wells were prepared by addition of 100 μL of the binding buffer with 18.75 % methanol in the microplate. In cross reactivity studies, D and L-AVP were fixed in wells at 600 nM final concentrations. For multicolour FA detection calibration curves, similar concentrations of D-

and L-AVP ranging from 0.036 to 2400 nM were prepared in same wells with L- and D-aptakiss-aptaswitch complexes. For the enantiomeric purity analysis, solutions containing various proportions of D- and L-AVP (0, 25, 50, 75 and 100 %) for a total oligopeptide concentration fixed at 200 nM were prepared. The determination of enantiomeric impurity (defined in a non-racemic sample as the ratio of the minor enantiomer to the major enantiomer expressed as a per cent) was realized by using L-AKC sensor and by varying the D-AVP concentration (0.1 and 1 mM) in presence of 100 nM of L-arginine vasopressin.

For all the analyses, the microplate was placed into the microplate device for the reading of anisotropy after 90 minutes of incubation at 4°C in the dark. For the reading, excitation was set at 485 ± 20 nm and 590 ± 20 nm, emission was collected with 535 ± 25 nm and 635 ± 35 nm bandpass filters for the F-and TR-labelled aptakiss probes, respectively.

The anisotropy (r) was calculated by the instrument software, as classically reported:

$$r = \frac{I_{vv} - GI_{vh}}{I_{vv} + 2GI_{vh}} \quad (1)$$

I_{vv} and I_{vh} are the vertically and horizontally polarized components of the emission after excitation by vertically polarized light. The instrumental correction factor G was determined according to the manufacturer's instructions.

The r_0 value was the anisotropy of the probe in the presence of aptaswitch only and the r value was the tracer anisotropy measured in the presence of both aptaswitch and target. The Δr parameter was calculated as follows:

$$\Delta r = r - r_0 \quad (2)$$

The mean and standard deviation (error bar) values were calculated from triplicate experiments. The apparent dissociation constants (K_d) were estimated by using a Langmuir-type model equation for fitting the standard curves, as previously described [13].

3. Results and discussion

3.1. Cross-reactivity study for the AKC recognition systems

In the first stage of the work, we focused our attention on the chiral recognition features of the two enantiomeric AKC systems. Several earlier works have established that the arginine vasopressin aptamer binds its target enantiomer with a very high enantioselectivity (of about 1000) [26-28]. These enantioselective properties were confirmed for the two L- and D-AKC systems (Figure 2a). The Fluorescein-tagged D-aptakiss hairpin (F-D-kiss) formed the kissing complex with the D-aptaswitch (D-Vasosw) in presence of D-arginine vasopressin (D-AVP).

Indeed, due to the mass enhancement of the F-D-kiss probe, the FA signal increased upon addition of D-AVP in a solution containing both D-Vasosw and F-D-kiss elements while no significant anisotropy variation was observed after addition of the L-enantiomer to the reaction solution. Conversely, by using the Texas Red-labelled L-aptakiss (TR-L-kiss) as probe and the L-Vasosw as L-AVP binding unit, the formation of the L-AKC assembly is detected in presence of target L-enantiomer but not with the D-AVP species. To note, the maximal FA response was slightly different between the two enantiomeric systems likely due to the difference in the FA signaling properties of the two dyes [13]. Nevertheless, these data confirm that the general principle of the chiral inversion, *i.e.* an enantiomer recognizes a chiral selector with the same features than the other one with the mirror-image of the receptor, is effective in the ternary kissing assembly.

In order to adapt the chiral inversion sensing approach to the AKC strategy, additional cross-reactivity studies need to be performed. Indeed, the simultaneous detection in a single solution containing all the D- and L-nucleic acid partners must fulfil the following criteria: the F-D-kiss probe should not recognize the L-AVP:L-Vasosw complex while the TR-L-kiss should not interact with the mirror image D-AVP:D-Vasosw association. As shown in Figure 2b, we did not detect any significant FA signal change for the RNA probes in presence of a target-aptaswitch complex of opposite chirality. This result clearly indicates the lack of cross-reactivity between the two enantiomeric AKC entities. Moreover, such data are consistent with earlier works that have established that mirror-image nucleic acids with complementary sequence are unable to hybridize through Watson-Crick pairing [29]. Finally, as can be seen in Figure 2b, no change in the FA response of the probes was also observed in solutions that contained other combinations of the different involved species such as the F-D-kiss, L-Vasosw, D-AVP and the TR-L-kiss, D-Vasosw, L-AVP mixtures.

3.2. Multi-colour detection of enantiomers

Once the lack of cross-reactivity between the D- and L-AKC enantiorecognition systems was demonstrated, we subsequently investigated the potential orthogonality of the reporting probes. The fluorescein and Texas-Red fluorophores were chosen as they display a very limited potential of spectral overlap and similar excited state lifetimes [30] (Table 1).

FA measurements of both TR-L-kiss and F-D-kiss upon binding to their corresponding target complexes were then performed in a single solution, with a dual signal detection at the two emission wavelengths (Figure 3). We prepared samples that contained the D- and L-Vasosw/kiss (300 nM/10 nM) mixture and a racemic mixture of D- and L-AVP enantiomers

(the concentration for each enantiomer varied from 0.037 to 2400 nM). Due to the modest maximal FA change observed upon target binding (Δr in the 0.015-0.020 range at saturating AVP concentration), error bars appeared of relatively significant magnitude in the calibration curves. The implementation of previously described mass-based amplification approaches could significantly improve the FA response and then the assay precision [17, 31], however at the cost of the sensor design simplicity. Nevertheless, the apparent dissociation constants were extracted from the standard curves of these orthogonal detection experiments. The K_d values were found to be $0.044 \mu\text{M} \pm 0.014$ and $0.021 \mu\text{M} \pm 0.004$ for L-AKC and D-AKC, respectively. Such values are identical to those determined for the individual L-AKC or D-AKC recognition systems using an aptakiss probe tagged with the Texas Red fluorophore (K_d in the 0.025-0.040 μM range) [25]. Detection limits (20 nM and 40 nM for L-AKC and D-AKC, respectively) extracted from the multi-colour experiments are close to the analytical data determined from the individual AKC assays (LOD in the 10-25 nM range). Linear dynamic ranges (from 2 to 150 and 600 nM) were also observed for the two types of experiments. These results indicate that the substitution of the Texas Red label by the Fluorescein fluorophore on the same aptakiss (*i. e.* D-aptakiss) does not significantly change both the stability of the AKC and the assay performances, suggesting the lack of susceptibility of the kissing system to the type of aptakiss labelling. They also reveal that (1) the formation of the D-AKC system can be specifically monitored at a given emission wavelength (535 nm) without any signal interference of the Texas Red-signaled L-AKC and (2) the FA increase at 635 nm upon L-AVP binding to the L-Vasosw is not affected by the simultaneous formation of the D-AKC system. The potentiality of our approach to quantify simultaneously the D- and L-AVP enantiomers in a single solution is thus established. It is also of interest to notify that the AKC binding features were significantly improved under our optimized operating conditions as compared with those reported for the original AVP-aptamer binary complex (apparent K_d in the sub-micromolar range) [26]. It was previously demonstrated that the presence in the reaction solution of both Mg^{2+} as stabilizing divalent cation and methanol as cosolvent favours the formation of the ternary complex [25].

3.3. Enantiomeric purity analysis

We subsequently assessed the ability of our AKC sensing platform to measure the enantiomeric excess of samples by using the dual detection scheme. In this context, we prepared solutions containing various proportions of D- and L-AVP (0, 25, 50, 75 and 100 %), for a total oligopeptide concentration fixed at 200 nM. The variation of the AVP enantiomer

ratio was plotted against the relative fluorescence anisotropy variation for the two reporting systems present in the solution (Figure 4a). The two experimental curves were fit to the model equation used above for the determination of the apparent dissociation constants. From the fitting parameters, the proportions of L- and D-AVP enantiomers were calculated and compared with the actual data (Figure 4b). A good linear regression was obtained ($R^2 \geq 0.98$), revealing the accuracy of the enantiomeric purity method. In addition, the current approach provides the detection of enantiomeric targets under a microplate format, with a total analysis time for screening 96 samples of about 3 min. The high-throughput feature of the sensing platform constitutes a great advantage over the typical chiral techniques such as HPLC, SFC or capillary electrophoresis that suffer from low parallel analysis capabilities.

The highly enantioselective potential of the chiral AKC aptasensor was finally established. As a representative example, the L-AVP (100 nM) sensing using the L-AKC system was performed in the presence of high excess of non-target enantiomer (D-AVP) in the sample. No significant interference in the L-AVP detection was obtained in presence of either 0.1 or 1 mM of D-AVP. This is exemplified by the L-AVP recoveries of $99.6 \pm 0.2 \%$ and $101.0 \pm 1 \%$ that were respectively achieved within these two experiments. The present assay then allowed the detection of an enantiomeric impurity down to 0.01% in a non racemic sample.

Wang and co-workers have previously described optical and electrochemical enantioselective aptamer-based biosensors for determining the D-AVP amount in the presence of an L-enantiomer excess [14, 32]. They reported detection limits in the 0.1-10 % range for the enantiomeric impurity, higher than that retrieved with the current AKC aptasensor. Moreover, our aptasensing approach allows the minor enantiomer detection with at least one order of magnitude lower than the 0.1-1% detection limits that are attainable with common chiral analysis separation-based tools [33].

4. Conclusion

In summary, we have described a novel approach for the design of a multicolour aptamer-based sensing approach that provides the simultaneous fluorescence anisotropy detection of enantiomers in a single solution as well as the determination of the enantiomeric impurity. This work constitutes the first example of an enantioselective assay based on the AKC approach, extending the scope of the kissing assembly strategy to the DNA-based chiral analysis field. As compared with the previously reported orthogonal method from Heemstra and co-workers [16], the present assay provides a simpler detection scheme with very limited

potential interferences originating from the fluorescent labelling. In addition, it displays higher sensitivity due to the retained affinity features of the reported AKC relative to the binary target-aptamer complex. We also demonstrate that even trace amounts of the minor enantiomer (0.01%) can be detected in a non racemic mixture. The present AKC-based approach could be potentially applied to the chiral recognition analysis of other kinds of small chiral compounds for which hairpin aptamers have been isolated, including nucleosides/nucleotides and histidine, tyrosine or tryptophan amino acids [12, 34, 35].

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References

- [1] G. Roelfes, B. L. Feringa, DNA-Based Asymmetric Catalysis, *Angew. Chem. Int. Ed.* 44 (2005) 3230-3232.
- [2] J. R. Aldrich-Wright, I. Greguric, R. S. K. Vagg, Vickery, P. A. Williams, Development of DNA-immobilised chromatographic stationary phases for optical resolution and DNA-affinity comparison of metal complexes, *J. Chromatogr. A* 718 (1995) 436-443.
- [3] R. B. Kotia, L. Li, L. B. McGown, Separation of nontarget compounds by DNA aptamers, *Anal. Chem.* 72 (2000) 827-831.
- [4] A. Brumbt, C. Ravelet, C. Grosset, A. Ravel, A. Villet, E. Peyrin, Chiral stationary phase based on a biostable L-RNA aptamer, *Anal. Chem.* 77 (2005) 1993-1998.
- [5] X. Su, F. L. Qin, Kong, J. Ou, C. Xie, H. Zou, Characterization of enantioselective binding of racemic natural tetrahydropalmatine to DNA by chromatographic methods, *J. Chromatogr. B* 845 (2007) 174-179.
- [6] C. Ravelet, R. Boulkedid, A. Ravel, C. Grosset, A. Villet, J. Fize, E. Peyrin, A L-RNA aptamer chiral stationary phase for the resolution of target and related compounds, *J. Chromatogr. A* 1076 (2005) 62-70.
- [7] L. Tohala, F. Oukacine, C. Ravelet, E. Peyrin, Chiral Resolution Capabilities of DNA Oligonucleotides, *Anal. Chem.* 87 (2015) 5491–5495.
- [8] J. P. Schaeper, L. A. Nelsen, M. Alyson Shupe, B. J. Herbert, N. A. P. Kane-Maguire, J. F. Wheeler, Capillary electrophoresis as a probe of enantiospecific interactions between photoactive transition metal complexes and DNA, *Electrophoresis* 24 (2003) 2704-2710.
- [9] J. Ruta, S. Perrier, C. Ravelet, B. Roy, C. Perigaud, E. Peyrin, Aptamer-modified micellar electrokinetic chromatography for the enantioseparation of nucleotides, *Anal. Chem.* 81 (2009) 1169-1176.
- [10] L. Zhang, M. Song, Q. Tian, S. Min, Chiral separation of L,D-tyrosine and L,D-tryptophan by ct DNA, *Sep. Purif. Technol.* 55 (2007) 388-391.
- [11] Y. Du, S. Guo, H. Qin, S. Dong, E. Wang, Target-induced conjunction of split aptamer as new chiral selector for oligopeptide on graphene-mesoporous silica-gold nanoparticle hybrids modified sensing platform, *Chem. Commun.* 48 (2012) 799-801.
- [12] S. Perrier, C. Ravelet, V. Guieu, B. Roy, C. Perigaud, E. Peyrin, Rationally designed aptamer-based fluorescence polarization sensor dedicated to the small target analysis, *Biosens. Bioelectron.*, 25 (2010) 1652-1657
- [13] J. Ruta, S. Perrier, C. Ravelet, J. Fize, E. Peyrin, Noncompetitive fluorescence polarization aptamer-based assay for small molecule detection, *Anal. Chem.* 81 (2009) 7468-7473.
- [14] J. Ren, J. Wang, J. Wang, N. W. Luedtke, E. Wang, Enantioselective and label-free detection of oligopeptide via fluorescent indicator displacement, *Biosens. Bioelectron.* 35 (2012) 401-406.

- [15] L. Challier, F. Mavré, J. Moreau, C. Fave, B. Schöllhorn, D. Marchal, E. Peyrin, V. Noël, B. Limoges, Simple and highly enantioselective electrochemical aptamer-based binding assay for trace detection of chiral compounds, *Anal. Chem.* 84 (2012) 5415-5420.
- [16] T. A. Feagin, D. P. V. Olsen, Z. C. Headman, J. M. Heemstra, High-throughput enantiopurity analysis using enantiomeric DNA-based sensors, *J. Am. Chem. Soc.* 137 (2015) 4198-4206.
- [17] S. Perrier, V. Guieu, C. Ravelet, E. Fiore, E. Peyrin, Panoply of fluorescence polarization/anisotropy signaling mechanisms for functional nucleic acid-based sensing platforms, *Anal. Chem.* 90 (2018) 4236-4248.
- [18] G. Durand, S. Lisi, C. Ravelet, E. Dausse, E. Peyrin, J. J. Toulmé, Riboswitches based on kissing complexes for the detection of small ligands, *Angew. Chem. Int. Ed.* 126 (2014) 7062-7065.
- [19] B. Chovelon, G. Durand, E. Dausse, J. J. Toulmé, P. Faure, E. Peyrin, C. Ravelet, ELAKCA: enzyme-linked aptamer kissing complex assay as a small molecule sensing platform, *Anal. Chem.* 88 (2016) 2570-2575.
- [20] G. Durand, E. Dausse, E. Goux, E. Fiore, E. Peyrin, C. Ravelet, J. J. Toulmé, A combinatorial approach to the repertoire of RNA kissing motifs; towards multiplex detection by switching hairpin aptamers, *Nucleic Acids Res.* 44 (2016) 4450-4459.
- [21] K. Zhang, K. Wang, X. Zhu, M. Xie, A label-free kissing complexes-induced fluorescence aptasensor using DNA-templated silver nanoclusters as a signal transducer, *Biosens. Bioelectron.* 78 (2016) 154-159.
- [22] E. Goux, E. Dausse, V. Guieu, L. Azéma, G. Durand, M. Henry, L. Choisnard, J. J. Toulmé, C. Ravelet, E. Peyrin, A colorimetric nanosensor based on a selective target-responsive aptamer kissing complex, *Nanoscale* 9 (2017) 4048-4052.
- [23] Y. Takeuchi, M. Endo, Y. Suzuki, K. Hidaka, G. Durand, E. Dausse, J. J. Toulmé, H. Sugiyama, Single-molecule observations of RNA-RNA kissing interactions in a DNA nanostructure, *Biomater. Sci.* 4 (2016) 130-135.
- [24] L. Azéma, S. Bonnet-Salomon, M. Endo, Y. Takeuchi, G. Durand, T. Emura, K. Hidaka, E. Dausse, H. Sugiyama, J. J. Toulmé, Triggering nucleic acid nanostructure assembly by conditional kissing interactions, *Nucleic Acids Res.* 46 (2018) 1052-1058.
- [25] B. Chovelon, E. Fiore, P. Faure, E. Peyrin, C. Ravelet, Mirror-image aptamer kissing complex for arginine-vasopressin sensing, *Anal. Chim. Acta* 1001 (2018) 143-150.
- [26] K. P. Williams, X. H. Liu, T. N. Schumacher, H. Y. Lin, D. A. Ausiello, P. S. Kim, D. P. Bartel, Bioactive and nuclease-resistant L-DNA ligand of vasopressin, *Proc. Natl. Acad. Sci. U. S. A.* 94 (1997) 11285-11290.
- [27] M. Michaud, E. Jourdan, A. Villet, A. Ravel, C. Grosset, E. Peyrin, A DNA aptamer as a new target-specific chiral selector for HPLC, *J. Am. Chem. Soc.* 125 (2003) 8672-9679.
- [28] J. Ren, J. Wang, J. Wang, E. Wang, Colorimetric enantio-recognition of oligopeptide and logic gate construction based on DNA aptamer-ligand-gold nanoparticle interactions, *Chem-Eur. J.* 19 (2013) 479-483.

- [29] J. T. Sczepanski, G. F. Joyce, Binding of a structured D-RNA molecule by an L-RNA aptamer, *J. Am. Chem. Soc.* 135 (2013) 13290-13293.
- [30] G. Gokulrangan, J. R. Unruh, D. F. Holub, B. Ingram, C. K. Johnson, G. S. Wilson, DNA aptamer-based bioanalysis of IgE by fluorescence anisotropy, *Anal. Chem.* 77 (2005) 1963–1970.
- [31] A. V. Samokhvalov, I. V. Safenkova, S. A. Eremin, A. V. Zherdev, B. B. Dzantiev, Use of anchor protein modules in fluorescence polarisation aptamer assay for ochratoxin A determination, *Anal. Chim. Acta* 962 (2017) 80-87.
- [32] H. Qin, J. Liu, C. Chen, J. Wang, E. Wang, An electrochemical aptasensor for chiral peptide detection using layer-by-layer assembly of polyelectrolyte-methylene blue/polyelectrolyte-graphene multilayer, *Anal. Chim. Acta* 712 (2012) 127-131.
- [33] M. A. A. Ragab, E. El-Kimary, High performance liquid chromatography with photo diode array for separation and analysis of naproxen and esomeprazole in presence of their chiral impurities: Enantiomeric purity determination in tablets, *J. Chromatogr. A* 1497 (2017) 110–117.
- [34] I. Majerfeld, D. Puthenvedu, M. Yarus, RNA Affinity for Molecular L-Histidine; Genetic Code Origins, *J. Mol. Evol.* 61 (2005) 226–235.
- [35] C. Ravelet, R. Boulkedid, A. Ravel, C. Grosset, A. Villet, J. Fize, E. Peyrin, A L-RNA aptamer chiral stationary phase for the resolution of target and related compounds, *J. Chromatogr. A* 1076 (2005) 62–70.

Table 1. Common spectral features of the two fluorophores used in the multicolour FA detection scheme of the current chiral sensor.

	Fluorescein	Texas-Red
Excitation wavelength	485 nm	590 nm
Emission wavelength	535 nm	635 nm
Excited state lifetime [30]	~ 4 ns	~ 4 ns

Figure 1. Schematic representation of the chiral inversion-based enantioselective AKC FA sensor. The putative kissing interaction between the two apical loops is depicted in light blue.

Figure 2. (a) FA changes for the enantiomeric aptakiss-aptaswitch pairs in presence of 600 nM of D-AVP (hatched bars) or L-AVP (blank bars). (b) FA changes of other aptakiss-aptaswitch pairs in presence of 600 nM of D-AVP (black bars) or L-ADVP (blank bars). Experimental conditions: [Aptakisses] = 10 nM, [Aptaswitches] = 300 nM, in 10 mM Tris, pH 7.4, 5 mM MgCl₂ and 18.75% methanol buffer. Reading after 90 minutes of incubation at 4°C in the dark (triplicate experiments).). $\lambda_{Em} = 585$ nm for the F-D-kiss probe and $\lambda_{Em} = 635$ nm for the TR-L-kiss probe.

Figure 3. Calibration curves obtained in solutions containing increasing amounts of a racemic mixture of D-AVP (red circle) and L-AVP (black circle) (concentration of each enantiomer varying from 0.037 to 2400 nM), in presence of the two L-aptakiss-aptaswitch and D-aptakiss-aptaswitch interacting pairs. Theoretical curves are created using parameters from the Langmuir-type model equation. Experimental conditions: [Aptakisses] = 10 nM, [Aptaswitches] = 300 nM, in 10 mM Tris, pH 7.4, 5 mM MgCl₂ and 18.75% methanol buffer. Reading after 90 minutes of incubation at 4°C in the dark (triplicate experiments). $\lambda_{Em} = 585$ nm for the F-D-kiss probe and $\lambda_{Em} = 635$ nm for the TR-L-kiss probe.

Figure 4. (a) Enantiopurity analysis of samples containing various enantiomer ratios (0, 25, 50, 75, 100 %) for a fixed total AVP concentration of 200 nM. Theoretical curves are created using parameters from the Langmuir-type model equation. (b) Comparison between calculated and actual proportions of L- and D-AVP enantiomers. Experimental conditions: [Aptakisses] = 10 nM, [Aptaswitches] = 300 nM, in 10 mM Tris, pH 7.4, 5 mM MgCl₂ and 18.75% methanol buffer. Reading after 90 minutes of incubation at 4°C in the dark (triplicate experiments). $\lambda_{Em} = 585$ nm for the F-D-kiss probe and $\lambda_{Em} = 635$ nm for the TR-L-kiss probe.

Figure 1

Figure 2

(a)

(b)

Figure 3

Figure 4

Kissing Interactions for the Design of a Multicolour Fluorescence Anisotropy Chiral Aptasensor

Graphical abstract: