

Reviewer 1:

The authors reported novel electrochemical sensors using a splitted dopamine DNA aptamer for a sensitive and selective detection, claiming the lowest LOD of 0.5 fM.

1. While the strategy of splitting DNA aptamer in order to lower the background is innovative, it is not convincing if two splitted aptamer fragments form a correct quadruplex structure in the presence of its target, dopamine. Is these splitted aptamer sequences novel in this manuscript? If not, please include a corresponding reference. If it is novel, major concerns are below with some minor comments at the end.

Response: Thank you for your comment. The split aptamer sequence used as dopamine receptor in this manuscript has been reported in our previous work. [T. Guo *et al.*, Phys. Status Solidi A 2020, 1900924] The corresponding modification has been made in the revised manuscript in red.

“Split aptamers were employed to get rid of the electrochemical response of the blank sample in which one receptor fragment (aptamer1) is covalently attached to the surface of gold electrode, as described in our previous work [31]” (Page 2, line 63-66)

2. Although author showed a data in Fig 1a that there was no signal in the absence of dopamine, they missed another very critical control experiment, which is monitoring current change with dopamine concentration variations in the absence of aptamer 2.

Response: I agree with the reviewer that a control experiment should be provided to exclude the influence of dopamine concentration on the current change of amperometric sensor. A corresponding modification was made in the revised manuscript and the supplemental materials in red.

“while there was an obvious increase of the Faraday current with rising dopamine concentrations registered at the peak potential of around -250 mV (vs. Ag/AgCl) in the presence of the aptamer 2 fragment. A control experiment was performed to characterized the response of the aptamer 1-modified gold electrode to different concentrations of dopamine without the presence of aptamer 2, where no current response was observed (Figure S2), indicating that the specific binding between the split aptamer and dopamine causes the current response.” (Page 4, line 174-179)

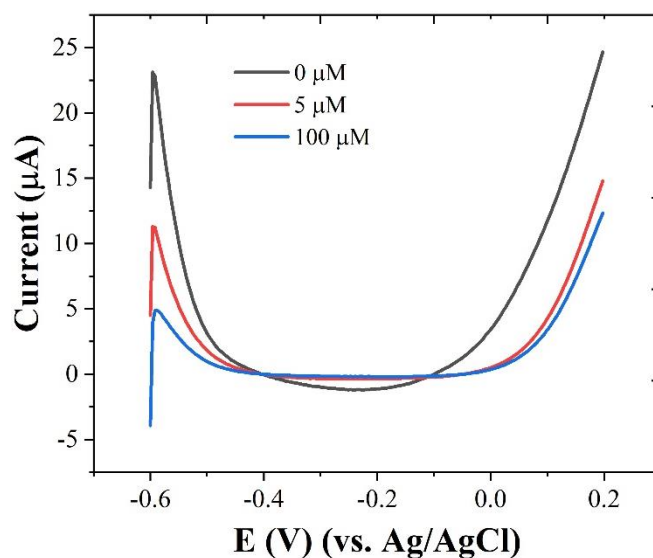


Figure S3. The SWV response of amperometric aptasensor to different concentrations of dopamine without the addition of aptamer 2 molecule.” (Supplementary materials, page 2)

3. What is the design strategy in splitting the original ssDNA aptamer into two fragments without defecting its secondary structure which is critical for its selective dopamine binding? Does sandwiching of two aptamer fragments maintain a compatible affinity compared with the original ssDNA aptamer?

Response: This design strategy refers to our previously reported work that utilized ratiometric electrochemical biosensor based on the split aptamer. In that work, we report on the splitting of the original ssDNA aptamer into two fragments, which exhibited a strong dopamine affinity and formed a complex with this analyte molecule. [T. Guo *et al.*, Phys. Status Solidi A 2020, 1900924] The principle for selecting the cut site is by inspection of aptamer’s predicted secondary structure and testing that the selected site does not impede target binding. [X. Zuo *et al.*, J. Am. Chem. Soc. 2009, 131, 6944–6945]

In the present work, we didn’t compare the affinity of the split aptamer fragments with that of the original ssDNA aptamer. According to previously reported work for ATP detection, [X. Zuo *et al.*, J. Am. Chem. Soc. 2009, 131, 6944–6945] sandwich assays can exhibit a significantly higher signal gain and improved response time in comparison with the corresponding full aptamer-based electrochemical sensor. Therefore, we assume that in the present work, the dopamine may drive the sandwich formation between two dissociated parts by binding the complex with high affinity, which may be comparable to that with the original ssDNA aptamer.

4. The reported K_D of original ssDNA aptamer, assuming the sequence was from reference #6, was 150 nM. What was the K_D of this sandwiched aptamer? How many dopamine molecules bind per aptamer molecule? It is unlikely that the electric current increased over 100 μ M when the dopamine was subjected with aptamer 2 at constant concentration of 0.5 μ M.

Response: We assume that the aptamer binds one analyte molecule, same as the full aptamer. There is no reason to presume that the cutting is considerably changing the binding complex. For this kind of detection system, the measured K_D differs from the theoretical K_D since the actual measured K_D value depends not only on the binding affinity of the receptor to its ligand but also on the sensitivity of the transducer by which he converts the binding event into a detectable signal. We estimated the measured K_D to be approximately 5 μ M for the amperometric transducer. As you can see later, this value is very different from that of the potentiometric transducer, although exactly the same chemical equilibrium system was studied, measured by two different transducer systems resulting in different K_D values.

A corresponding comment was added to the SI in red:

“The dissociation constant (K_D) of the split aptamer sequences with dopamine target can be obtained by fitting the dependence curves of the electrochemical current on dopamine concentration using a Langmuir equation [1], which is described as:

$$I_{\text{target}} = I_0 + \left(\frac{C_{\text{target}}(I_{\text{max}} - I_0)}{C_{\text{target}} + K_D} \right)$$

where I_{target} and I_0 are the signal current with and without the presence of dopamine, respectively. I_{max} is the maximum current, C_{target} is the target concentration. It is noteworthy, that we determined two calibration curves for exactly the same chemical equilibrium system, which was read out by two independent transducer systems delivering two very different K_D values, being $K_D = 5 \mu\text{M}$ and $K_D = 7 \text{ pM}$ for the amperometric and the potentiometric transducer, respectively. This finding emphasizes that the measured K_D differs from the theoretical K_D since the actual measured K_D value depends not only on the binding affinity of the receptor to its ligand but also on the sensitivity of the transducer, which converts the binding event into a detectable signal for this kind of detection system.

Reference

1. Andrea, I.; Claudio, P.; Gabriel, O.; W., P. K., Calibration-free measurement of phenylalanine levels in blood using an electrochemical aptamer-based sensor suitable for point-of-care applications. *ACS Sens.* **2019**, *4* (12), 3227–3233. [<https://doi.org/10.1021/acssensors.9b01703>] ” (Page 5)

5. The reference #2 was cited to support the current drop over 117.2 μM of dopamine but I can't see the reference is explaining dopamine polymerization at higher concentration. Dopamine dose dependency data in reference #6 showed a plateau once the assay was saturated. How does dopamine self-polymerize at higher concentration?

Response: We are sorry for the mistake. The related reference has been cited for dopamine polymerization at high concentration. Because of the presence of oxygen in the buffer solution, the oxygen can work as oxidant to induce the oxidation of the catechol groups into quinone and the subsequent polymerization. The polymerization kinetics can speed up by increasing the initial concentration of dopamine. [V. Ball *et al.*, J. Colloid Interf. Sci., 2012, 386, 366 – 372] A corresponding modification was done in the revised manuscript in red.

“which may on the one hand interfered the formation of aptamer/analyte sandwich structures and on the other hand hindered the charge transfer between redox probes and electrode by fouling the electrode surface [36].”(Page 5, line 185-187)

6. According to Nakatsuka, et. al., (reference #6), conformational change of ssDNA aptamer in the presence of dopamine induced moving of the substation portion of the negatively charged backbone of aptamer closer to the electrode surface, resulting charge transfer. Therefore, it should be clarified that the signal change observed in this manuscript is not due to the reorientation of immobilized aptamer 1 in the presence of dopamine, regardless of aptamer 2.

Response: we totally agree with the reviewer and we clarified the detection mechanism of split aptamer to dopamine using the charge transfer between the redox probe at the distal end of aptamer and electrode surface, which is consistent with reference 6. “the target can on the one hand induce the association of the two aptamer fragments into an aptamer1/dopamine/aptamer 2 sandwich structure, which may increase the concentration of redox probe at the electrode surface, and on the other hand, decrease the distance between gold-electrode and the redox group, which therefore facilitates the charge transfer and generates a detectable electrochemical signal via an amperometric transducer principle [32].” (Page 2, line 68-72)

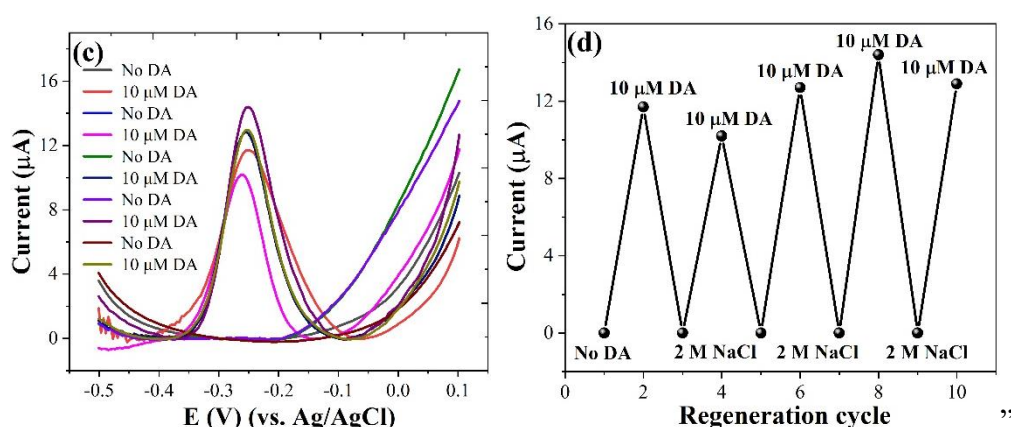
7. Selectivity validation: although the authors cited reference #6 to support the selectivity against structurally related catecholamines, such as norepinephrine and epinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC), vanillic acid (VA), 3-methoxytyramine (3-MT), or tyramine, it should be validated in this sensor platforms because of different aptamer strategy. Sandwiching and MB labeling to a part of aptamer can affect sensitivity and/or selectivity.

Response: In present manuscript, the main purpose is to distinguish dopamine to some other kinds of neurotransmitter, which have a similar chemical structure and molecular weight as dopamine but have not been tested before to provide the greatest possible

novelty. It is likely that the cutting has relatively little influence on the selectivity since it is mainly affecting the entropy but not so much the energy. The main problem related to the electrochemical detection of dopamine is that its oxidation potential is close to some other interfering molecules, such as uric acid and ascorbic acid, which usually leads to poor selectivity in dopamine detection. Gamma-aminobutyric acid and glutamic acid as two important neurotransmitters, have similar chemical groups as dopamine, such as $-NH_2$ and $-OH$, which make it crucial to distinguish them from dopamine when the sensor is applied to detect dopamine in the central nervous system.

8. Regeneration and Fig 2d: The current looks continuously increasing from the 2nd washing unlike other sensors which normally show decreasing efficiency. About 17% increase after 3 washing cycle is significant. To prove a reproducible regeneration, this experiment needs more repeats, ~10 times.

Response: The regeneration test with five cycles is shown to evaluate the reproducibility of the amperometric aptasensor by SWV measurement. The detected current is formed by the charge transfer between the probe redox at the distal end of aptamer and the surface of electrode, which depends on the conformation of the aptamer molecule. Due to the flexibility of the linear ssDNA macromolecule, a slight variation of molecule conformation may cause the observed marginal unstable signal. To prove a reproducible regeneration, one more cycle was applied on the employed amperometric aptasensor. Even after 4 washing cycle, the resulting current is almost similar to that of the first cycle. The corresponding modification has been made in the revised manuscript. Every reader will understand that an increase of experimental repeats reduces the error, however this will not any value to the manuscript which reports on a novel transducer system for dopamine detection with a fempto molar detection limit.



9. Incubation time: 10 min incubation was mention in Figure 4. Is 10 min sufficient for aptamer sandwiching? Incubation time dependency needs to be validated.

Response: We agree with the reviewer that the optimization of incubation time should be provided. The corresponding modification has been made in the revised manuscript and supplementary materials.

“Subsequently, the aptamer1-modified gold electrode was incubated in the electrolyte for 10 min, which showed highest current response to 10 μ M dopamine (Figure S1),” (Page 4, line 136-137)

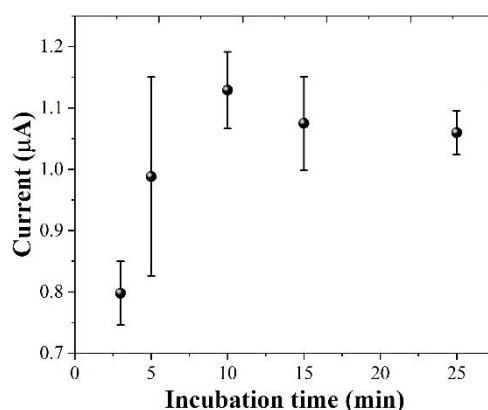


Figure S1. Optimization of the incubation time of amperometric aptasensor with target molecule dopamine (50 μ M).” (Supplementary materials, Page 1)

Minor corrections:

10. A reference for dopamine aptamer sequence should be cited.

Response: We agree with the reviewer that a reference about dopamine aptamer sequence should be cited. The corresponding modification has been made in the revised manuscript in red.

11. It would be informative to readers if references stating the advantages of aptamer receptors-based biosensors such as high affinity, selectivity, low cost, and easy-fabrication are included.

Response: We agree with the reviewer that the advantages of aptamer-based biosensors should be stated in the introduction part. The corresponding modification has been made in the revised manuscript in red.

“Aptamer, as one of the target recognition component of electrochemical sensor, has attracted plenty of attention because of its high selectivity, flexibility, high affinity[16], low cost, and easy-fabrication [17].” (Page 1, line 40-42)

12. Throughout the manuscript, the reference citation is not consistent. Please check the citation format.

Response: We are sorry for the mistake. The corresponding modification has been made in the revised manuscript in red.

13. tris-EDTA buffer: change to TE buffer

Response: The corresponding modification has been made in the revised manuscript in red.

“10mM TE buffer (pH 8.0) was used to prepare the stock solutions of both DNA probes,” (Page 3, line96-97)

14. H2SO4: change to H₂SO₄ (numbers should be subscript)

Response: We are sorry for the mistake. The corresponding modification has been made in the revised manuscript.

“Oxidation and reduction scans were performed in 50 mM H₂SO₄ solution over the potential range of −0.15 V to 1.55 V with a scan rate of 1 V/s and step width of 0.01 V for final electrochemical annealing”. (Page 3, line 110-112)

15. tris: change to Tris (capital T)

Response: The corresponding modification has been made in the revised manuscript in red.

16. Line 43, liquors: change to fluids

Response: The corresponding modification has been made in the revised manuscript in red.

“However, due to the variable distribution of dopamine concentrations in different body fluids and tissues” (Page 1, line 42-43)

17. Line 135, targets binding events: change to target-binding events

Response: The corresponding modification has been made in the revised manuscript in red.

“Cyclic voltammogram and square wave voltammetry (SWV) were recorded by an Autolab potentiostat PGSTAT302 in 0.5 μM aptamer 2 solution dissolved in 10 mM Tris buffer.”. (Page 3, line130-132)

18. Line 121, tris(hydroxymethyl)aminomethane buffer: change to Tris buffer

Response: The corresponding modification has been made in the revised manuscript in red.

“10 mM high salt Tris buffer (Tris, 1.5 M NaCl, pH=7.4), overnight.” (Page 3, line 116-117)

19. Line 182, “concentrations, Figure 2a, which may on the one hand interfered the formation of aptamer/analyte..” Figure 2a is not relevant with the description.

Response: We are sorry for the mistake. The corresponding modification has been made in the revised manuscript in red.

“This observation can most likely be attributed to the formation of polydopamine at high monomer concentrations, Figure 1c,” (Page 4, line 184-185)

20. Lines 211-212, please cite corresponding references for clinical concentrations in each different system (adrenal chromaffin cells, human serum, and brain) instead of copying from other’s citation.

Response: We agree with the reviewer that corresponding references for clinical concentrations should be cited. The corresponding modification has been made in the revised manuscript in red.

“and can be as small as a few fM in single adrenal chromaffin cells [38], 1 nM in human serum [39], or 10 nM in the brain [40]” (Page 6, line 215-216)

21. Line 238, “channel current was determined, Figure 3c [20].” Is the reference #20 relevant?

Response: We are sorry for the mistake. The corresponding modification has been made in the revised manuscript in red.

“The transconductance, which represents the capability to convert changes of the gate potential into variations of the source-drain channel current [22], was determined, Figure 3c.” (Page 7, line 244-246)

22. Line 301, “that the immobilization of the split aptamer2 strand together with the analyte...” Analyte and aptamer 2 were incubated not immobilized, so they can be washed out.

Response: We agree with the reviewer. The corresponding modification has been made in the revised manuscript in red.

“These results in combination with the high signal amplification observed here suggest that the incubation of the split aptamer2 strand together with the analyte is advantageous

over full aptamer receptors for OECT sensors, where only the analyte itself is bound to the surface tethered receptor molecules.” (Page 9, line 306-309)