Supporting Information

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Ultrasensitive DNA-biomacromolecule sensor for the detection application of clinical cancer samples

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Figure S1. To obtain the maximum signal gain, we optimized the assembly concentration of the helper probe at the interface. Since both high and low density of the helper probe on the electrode surface were unfavorable, so 0.75 μM was chosen as the optimal incubation concentration to realize medium density. We defined signal gain as:

\[
Signal \ Gain \ (\%) = \frac{(I - I_0)}{I_0}
\]

Equation 1

Where, I is the peak current obtained in the presence of the target, and I_0 is the peak current obtained in the blank solution.
Figure S2. The ionic strength in the PBS buffer solution was optimized. When the concentration of NaCl was 1000 mM, the maximum signal gain was achieved, so 1000 mM was confirmed to be the optimal concentration. Data presented are means ± S.D. from 3 independent experiments.
Figure S3. To improve the signal gain of the BRE-AB sensor, we have optimized the concentration ratio between the aptamer and signal probe. When the ratio was 1:1, the signal gain was largest. Therefore, 1:1 was confirmed to be the optimal ratio to synthesize the aptamer/signal duplexes.
Figure S4. Signal gain at different concentration of aptamer/signal duplex in PBS buffer.
Since frequency is a key influence parameter for the sensor, we applied four concentrations (100 pM, 10 nM, 100 nM and 1 μM) of LH to investigate the signal gain under different SWV frequencies. The results indicated that 50 Hz presented the maximum signal gain at four concentrations and was selected as the test frequency. Considering scan time and current value, lower frequencies were ignored.
Figure S6. The Gibbs free energy of secondary structure of aptamer was estimated to be -0.10 kcal/mol by NUPACK software. (Web for reference: http://www.nupack.org/).
**Figure S7.** The delta G of aptamer binding to signal probe was calculated to be -24.52 kcal/mol by IDT software. (Web for reference: [http://sg.idtdna.com/pages](http://sg.idtdna.com/pages)).
Figure S8. The SWV current responses of the BRE-AB sensor before and after NGAL (a) and NCL (b). The concentration of NGAL and NCL was 100 nM.
Figure S9. a) The regenerability and reusability of the BRE-AB sensor were verified for four cycles. Data are presented as mean ± s.d., n = 6 independent experiments. ns, nonsignificant. (Student’s t-test). b) Four cycles of SWV voltammograms obtained before, after adding 100 nM LH and after regeneration.
**Figure S10.** SWV voltammograms obtained before and after adding $10^{-10}$, $10^{-9}$, $10^{-8}$, $10^{-7}$, $10^{-6}$, $10^{-5}$ M LH in 50% whole blood.
Figure S11. a) Dose-response curves of LH in 50% whole blood. b) The current signal of 100 pM was higher than the background signal plus 3-fold standard deviation and then the detection limit of the BRE-AB sensor was determined to be 100 pM in 50% whole blood.
Figure S12. SWV voltammograms obtained before and after adding $10^{-10}$, $10^{-9}$, $10^{-8}$, $10^{-7}$, $10^{-6}$, $10^{-5}$ M LH in undiluted plasma.
Figure S13. a) Dose-response curves of LH in undiluted plasma. The inset figure showed that the dynamic range was from 1 nM to 1 µM. b) The current signal of 100 pM was higher than the background signal plus 3-fold standard deviation and then the detection limit of the BRE-AB sensor was determined to be 100 pM in undiluted plasma.
Figure S14. The standard curve of ELISA method was drawn from the absorbance of the standard LH working solution, and the LH concentrations were as follows: 10, 5, 2.5, 1.25, 0.63, 0.31, 0.16, 0 IU/L.
Figure S15. LH detection with ELISA method in clinical samples.
Table S1. Energy type distribution of binding

| Energy type               | In H₂O (0.1 M NaCl) |
|---------------------------|---------------------|
|                           | KJ/mol              |
| van der Waal energy       | -241±20             |
| Electrostatic energy      | -6069±153           |
| Polar solvation energy    | 1118±87             |
| SASA energy               | -26±2               |
| SAV energy                | 0                   |
| WCA energy                | 0                   |
| Binding energy            | -5217±135           |
| Detection technology | Aptamer-based | Detection limit | Regenerability and reusability | Target type | Reference |
|----------------------|---------------|-----------------|--------------------------------|-------------|-----------|
| Optical spectroscopy | yes           | 6.7 nM          | N/A                            | Tau protein | Anal. Bioanal. Chem. 2020, 412, 1193-1201 |
| Electrochemical      | no            | 10 nM           | N/A                            | Antibody and streptavidin | J. Am. Chem. Soc. 2015, 137, 15596-15599 |
| Electrochemical      | yes           | 25 nM           | N/A                            | Ranibizumab | Sens. Actuators, B 2020, 312, 127941 |
| Microcantilever      | yes           | 1 nM            | N/A                            | Nucleolin   | Talanta 2016, 146, 727-731 |
| Optical spectroscopy | yes           | 0.2 nM          | N/A                            | Lipocalin 1 | Biosens. Bioelectron. 2020, 169, 112607 |
| Electrochemistry     | yes           | 3 nM            | N/A                            | Insulin     | Biosens. Bioelectron. 2021, 180, 113124 |
| Electrochemistry     | yes           | 10.9 nM         | 1                              | LH          | Nat. Commun. 2019, 10, 852 |
| Colorimetry          | no            | 1.5 nM          | N/A                            | Metallothionein | Anal. Chem. 2020, 92, 2080-2087 |
| Field effect transistors | yes           | 1.75 nM         | N/A                            | HPV-16 E7 protein | Anal. Bioanal. Chem. 2021, 413, 779-787 |
| Electrochemistry     | yes           | 10 pM           | 4                              | LH          | This work |

*This work*
**Table S3.** Probe sequences used in the study.

| Probe name   | Sequence (5'-3')                      |
|--------------|---------------------------------------|
| NGAL aptamer | AGCAGCACAGAGGTTCAGATGCGCTGGATAAGCAGCATCAGGT |
|              | TATCATCGTAAACCCTATGCGTGCTACCGTGAA     |
| NGAL-SP      | CATCCCATCTGAGAC-MB                    |
| NGAL-HP      | SH-C₆-AGGTCAGATGGCGCT                |
| Nucleolin aptamer | GGTGGTGGTGTTGTGGTGTTGGTGTTT        |
| NCL-SP       | TTCAACCACCGTATG-MB                   |
| NCL-HP       | SH-C₆-GTGGTGTTGTTG                  |
**MD simulation method**

Molecular docking: The Autodocktools software\(^{[1]}\) was used to obtain the proper aptamer/LH complex to start MDs with. Semi-flexible docking mode was confirmed using LH as the receptor and the aptamer as the ligand. For achieving a reasonable docking, a crystal structure of the beta subunit of LH (6p57) with PDB format was downloaded from the protein database (https://www.rcsb.org/structure/6P57). Before docking, this 3D-structure complex was processed to remove H\(_2\)O and small molecules with PyMol. The 3D-structure modeling of aptamer was carried out using RNAfold and RNAComposer software and then made an energy minimization step using GROMACS software.\(^{[2-4]}\) QGRS Mapper software was used to predict if there may form a G-quadruplex structure.\(^{[5]}\) Molecular dynamics simulation: GROMACS 2019.6 package with an Amber force field of FF99SB-ILDN was used for a systematical simulation. Five steps were mainly involved. 1. The proper aptamer/LH complex was solvated with a SPC216 H\(_2\)O model using a cubic box with 10 Å of aptamer to the box wall. 2. The GMX commands, genion and -conc, were repeatedly applied to acquire a simulation system of H\(_2\)O containing 0.1 M NaCl. The system was kept neutral with using appropriate number of Na\(^+\) to replace H\(_2\)O molecules. 3. A steepest descent algorithm of 50000 steps was carried out for optimizing simulation environment. 4. Both 300 K and 1 bar were set for the constant temperature and pressure, respectively. 5. 50 ns was performed for a complete simulation. MD simulation analysis: Appropriate time frames were extracted for an analysis of the interaction of aptamer to LH using Discovery studio and PyMOL. A g_mmpbsa method was used to calculate the binding free energy between the aptamer and LH.\(^{[6]}\)
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