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SUPPLEMENTARY MATERIAL

Novel electrochemiluminescent assay for the aptamer-based detection of testosterone

Rocío Cánovas,[‡,a,b] Elise Daems,[‡,a,b,c] Rui Campos,[‡,a,b] Sofie Schellinck,[d] Annemieke Madder,[e] José C. Martins,[d] Frank Sobott,[c,f,g] Karolien De Wael*[,a,b]

[a] A-Sense Lab, Department of Bioscience Engineering, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.

[b] NANOlab Center of Excellence, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.

[c] BAMS Research Group, Department of Chemistry, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.

[d] NMR and Structure Analysis Research Group, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent 9000, Belgium

[e] Organic and Biomimetic Chemistry Research Group, Department of Organic and Macromolecular Chemistry, Ghent University, Ghent 9000,Belgium

[f] Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, UK.

[g] School of Molecular and Cellular Biology, University of Leeds, Leeds, LS2 9JT, UK.

‡ Sharing first authorship.

***Corresponding author**: Karolien De Wael (karolien.dewael@uantwerpen.be)

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Table S1. Parameters determined by ITC.

TESS.1	Kd (nM)	N	ΔG (kcal/mol)	ΔH (kcal/mol)	-TΔS (kcal/mol)
Average (3 titrations)	240	1.15	-9.04	-11	2
Standard deviation	29	0.09	0.07	1	1

TESS.1short	Kd (nM)	N	ΔG (kcal/mol)	ΔH (kcal/mol)	-TΔS (kcal/mol)
Average (3 titrations)	489	0.93	-8.61	-15.7	7.1
Standard deviation	58	0.07	0.07	0.6	0.6

K_d: dissociation constant, ΔG: Gibbs free energy change, ΔH: enthalpy change, ΔS: entropy change and N: stoichiometry

Table S2. Comparison of the detection limit (LOD) of the present strategy with reported works for determination of testosterone.

Technique/method	Analysis time	Linear Range	LOD	Ref.
Liquid chromatography-mass spectrometry (LC-MS)	90 min	86.7 pM – 8.7 nM	11.4 pM	[1]
Square-wave adsorptive stripping voltammetry (using glassy carbon electrode)	5 min	10–70 nM	1.18 nM	[2]
New automated electrochemiluminescence immunoassay	-	0.42 – 52 nM	6.9 nM	[3]
Biolayer interferometry (using double-stranded DNA fragments)	17 min	7.4 – 473.7 nM	0.09 nM	[4]
High performance liquid chromatography (HPLC) method	17–35 min	0.01–20 μM	0.05–0.1 μM	[5]
Capillary electrophoresis	-	27.7 nM – 3.3 μM	15.9 nM	[6]
Isotope dilution liquid chromatography/tandem mass spectrometry	120 min	1 – 29.5 nM	6.9 pM	[7]
Ultra-performance liquid chromatography/tandem mass spectrometric (UPLC/MS/MS)	4 min per sample	0.01 to 5 μM	0.01 μM *	[8]
Automated online in-tube solid-phase microextraction (SPME) coupled with liquid chromatography–tandem mass spectrometry (LC–MS/MS)	28 min	6.9 pM – 1.7 nM	1 pM 34.6 pM *	[9]
Electrochemistry using molecular imprinted polymers (MIPs)	-	0.34 pM – 0.34 nM	~pM	[10]
Electrochemical, recombinant Fab fragment-based immunosensor	-	1 – 138.7 nM	0.3 nM	[11]
Near-infrared spectroscopy	Few minutes	5.4 – 86.7 mM	1.7 mM	[12]
Double-layer structure molecularly imprinted polymer film (MIF) on the surface plasmon resonance (SPR) sensor chips	30 min	1 pM –1 nM	1 pM	[13]
Liquid chromatography-tandem mass spectrometry	5.5 min	13.9 pM – 3.5 nM	13.9 pM	[14]
Electrochemiluminescent assay	< 5 min 96-well plate	0.39−1.56 μM	0.29 μM	**This work**

* Limit of quantification in these cases.

2. MATERIAL AND METHODS

Isothermal Titration Calorimetry (ITC)

ITC experiments were performed on a MicroCal PEAQ-ITC instrument (Malvern Panalytical operated by MicroCal PEAQ-ITC control software. The binding experiments were performed using 150 µM of target which was titrated in 10 µM of aptamer solution. The assay buffer was a 10 mM phosphate buffer with 150 mM NaCl and 150 mM $MgCl_2$ at pH 7.0 and was degassed prior to use. The experiment consisted of 15 injections of 2.7 µL with a spacing of 150 s. The first injection was 0.4 µL to account for diffusion (initial delay 180 s). To correct for the dilution heat of the titrant, control titrations were performed consisting of injection of the target into the sample cell filled only with buffer. The reference power was set to 5 µcal/s and all titrations were performed at 25 °C. Data analysis was performed with the MicroCal PEAC-ITC Analysis software using a one-site binding model.

Figures

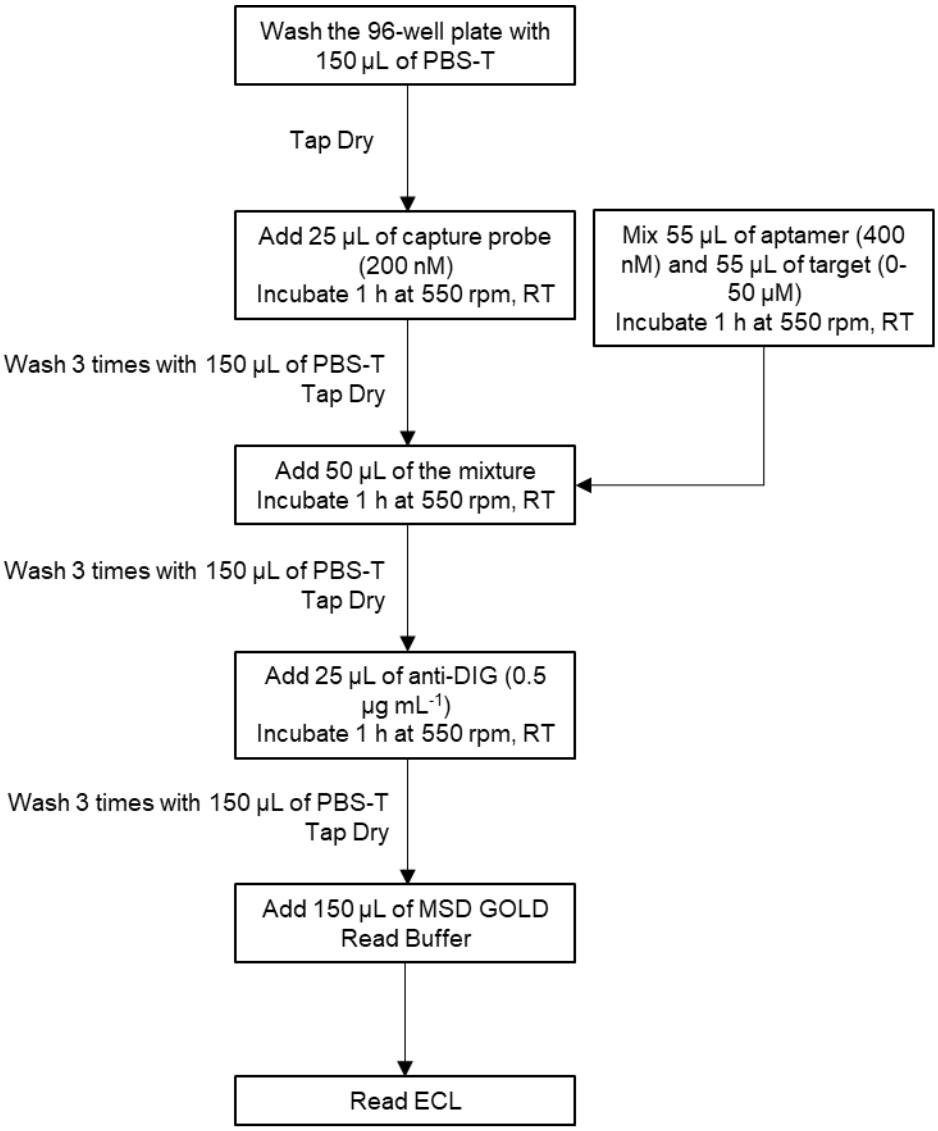

Fig. S1. Incubation protocol performed during the ECL measurements.

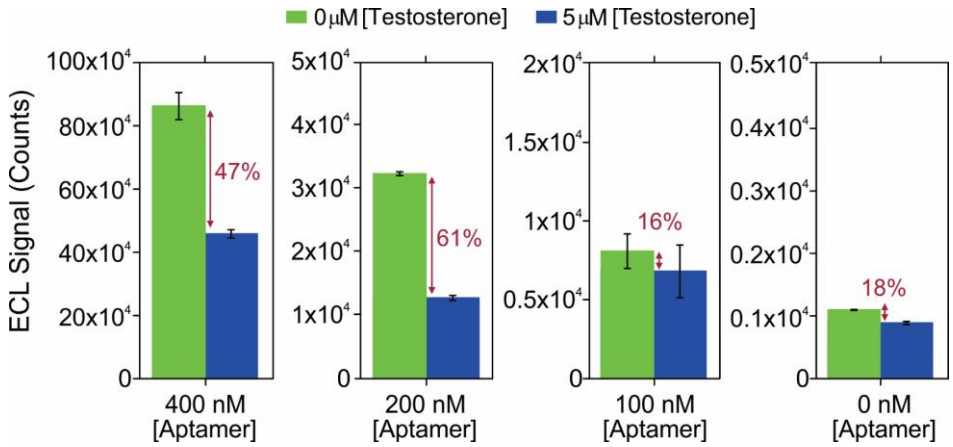

Fig. S2. Influence of the aptamer concentration during the incubation protocol. 400, 200, 100 and 0 nM concentrations of the TESS.1 aptamer were studied comparing the results with (5 µM) and without (0 µM) testosterone.

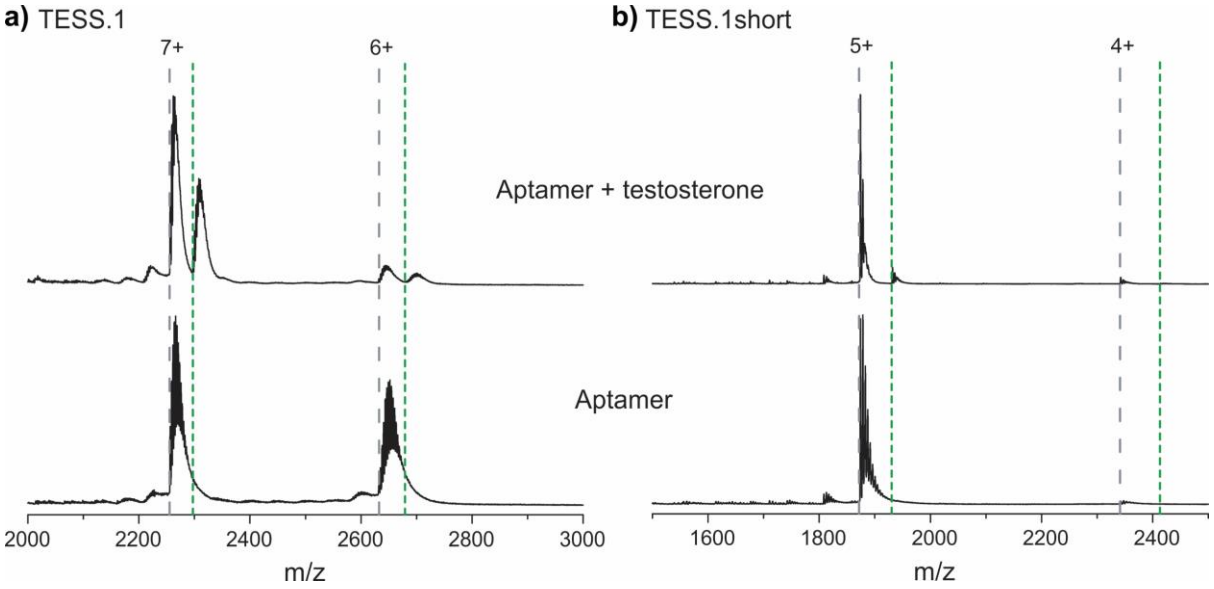

Fig. S3. Native MS spectra of the **a)** TESS.1 aptamer and **b)** TESS.1short aptamer. The bottom spectra are the aptamers alone, while the top spectra show the aptamer after testosterone addition (10-fold excess). The dashed grey and dotted green lines represent theoretical m/z-values of the aptamer and aptamer-testosterone complex, respectively.

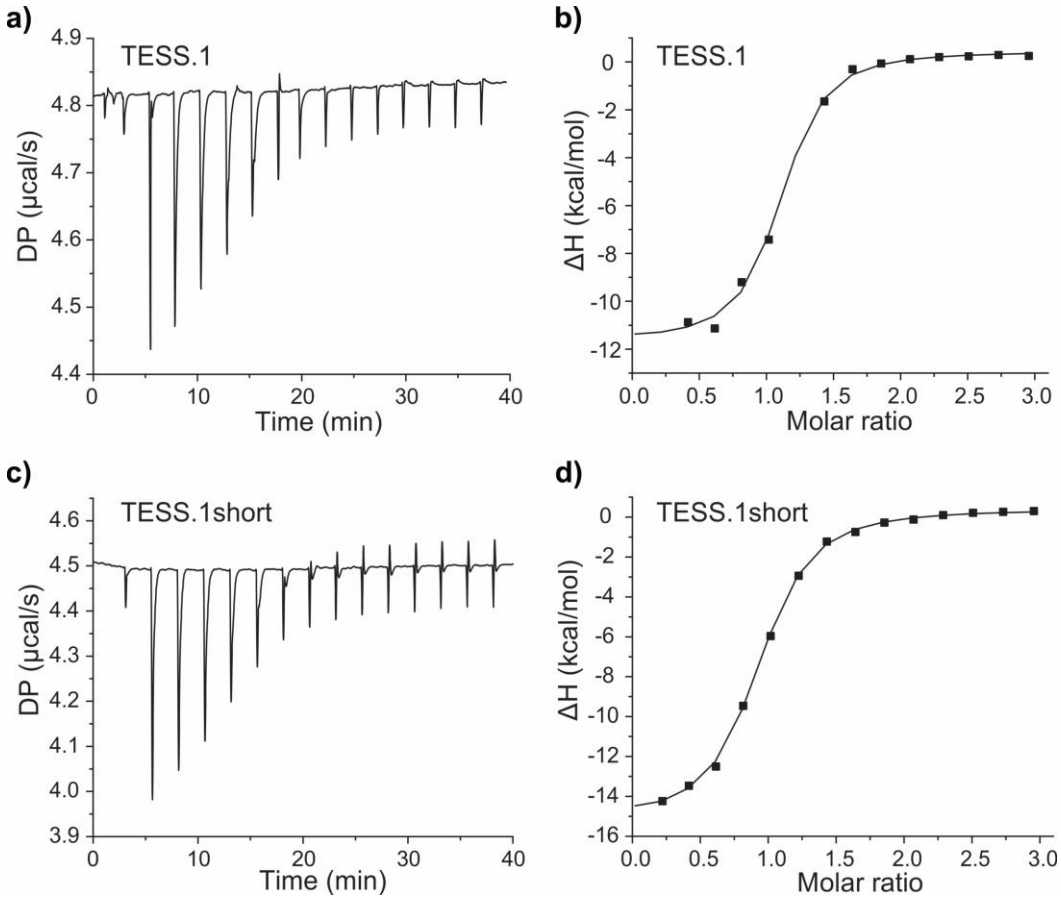

Fig. S4. **a)** Thermogram and **b)** binding curve of the ITC titration of 10 µM of the TESS.1 aptamer with 150 µM of testosterone. **c)** Thermogram and **d)** binding curve of the ITC titration of 10 µM of the TESS.1short aptamer with 150 µM of testosterone. The line in the binding curve represents the fitting with the "one set of binding sites" model.

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