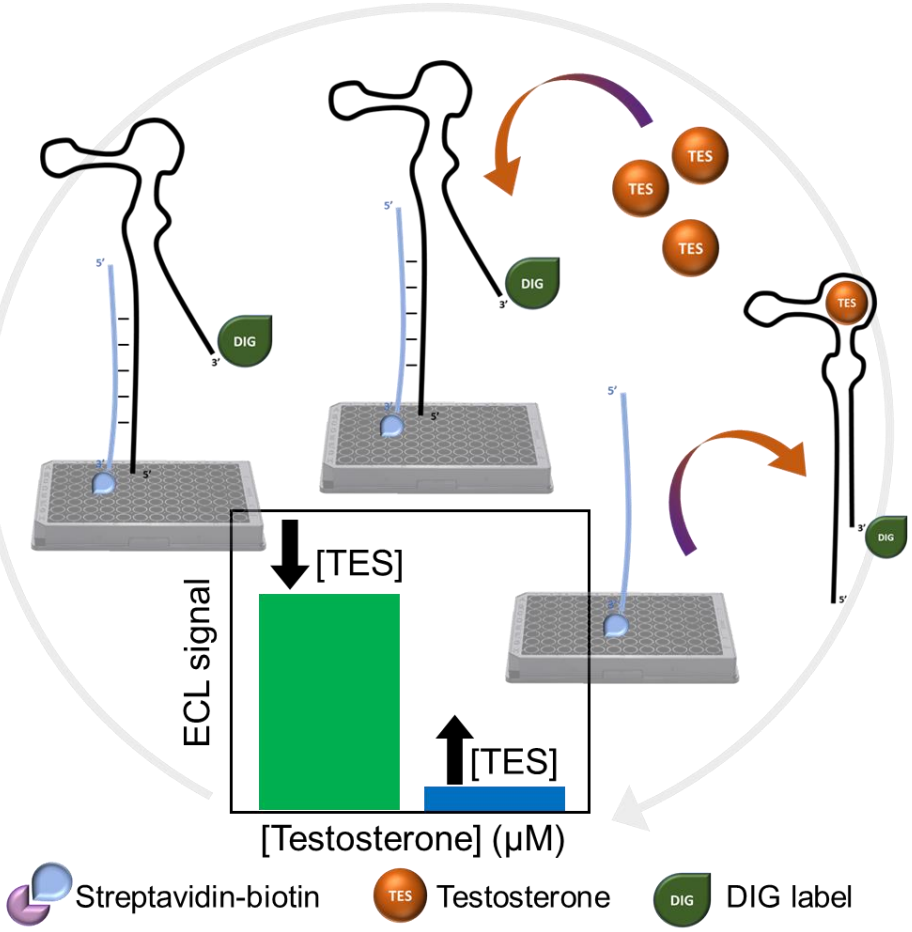
# Proof-of-concept electrochemiluminescent assay for the aptamer-based detection of small molecules

Rocío Cánovas[‡], Elise Daems[‡], Rui Campos[‡], Frank Sobott and Karolien De Wael*

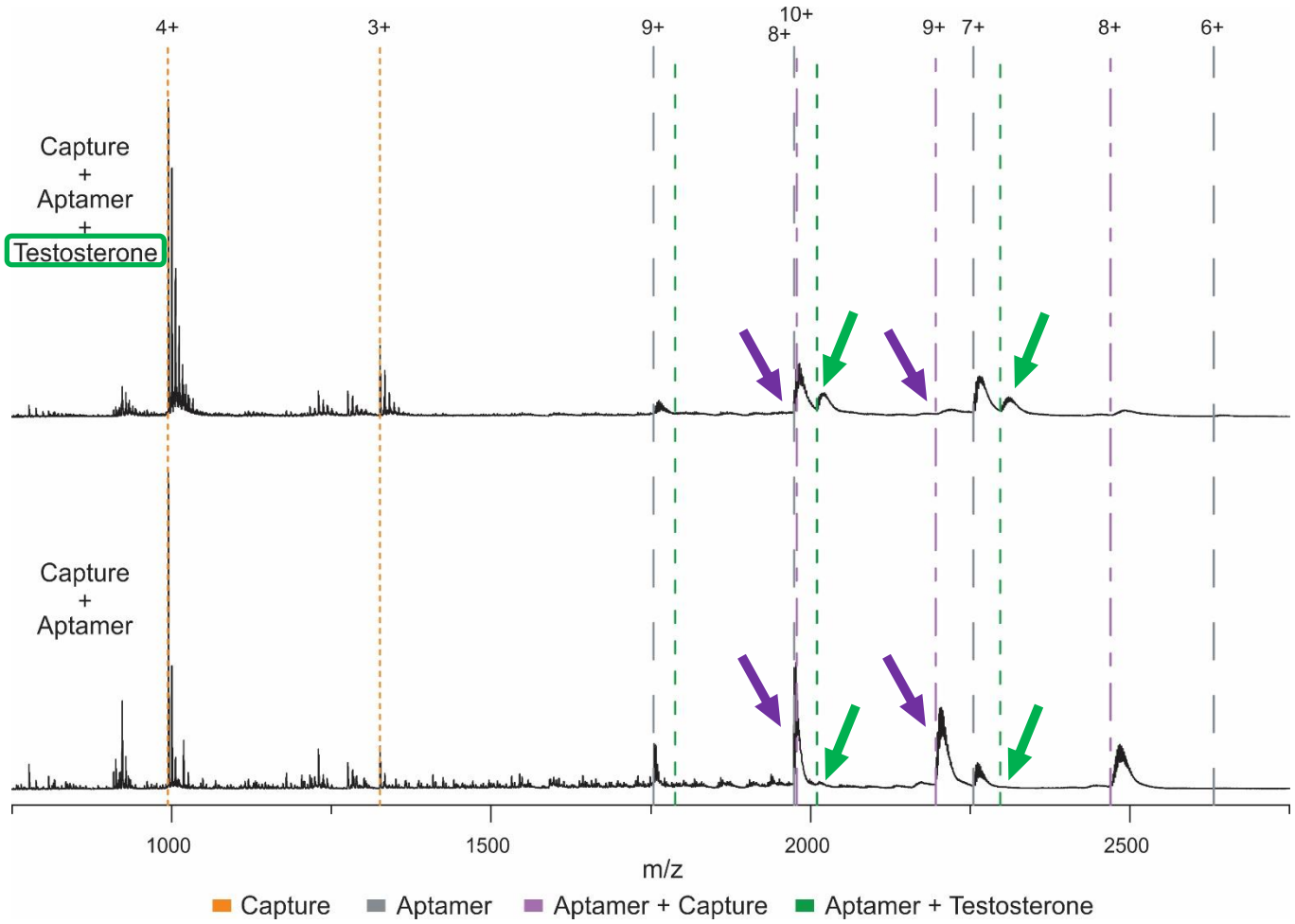
AXES
Antwerp X-ray Analysis, Electrochemistry & Speciation
University of Antwerp

# Competitive binding principle of the ECL assay




The TESS.1 aptamer carries the digoxigenin (DIG) label, to which the $Ru(bpy)_3^{2+}$-labelled anti-DIG antibody will bind. Hence, the aptamer, which is not immobilized, will be washed away in presence of the target. Therefore, the signal of the label is only observed when the aptamer is not bound to testosterone and decreases upon adding more target.

# Characterization of the TESS.1 aptamer by native mass spectrometry

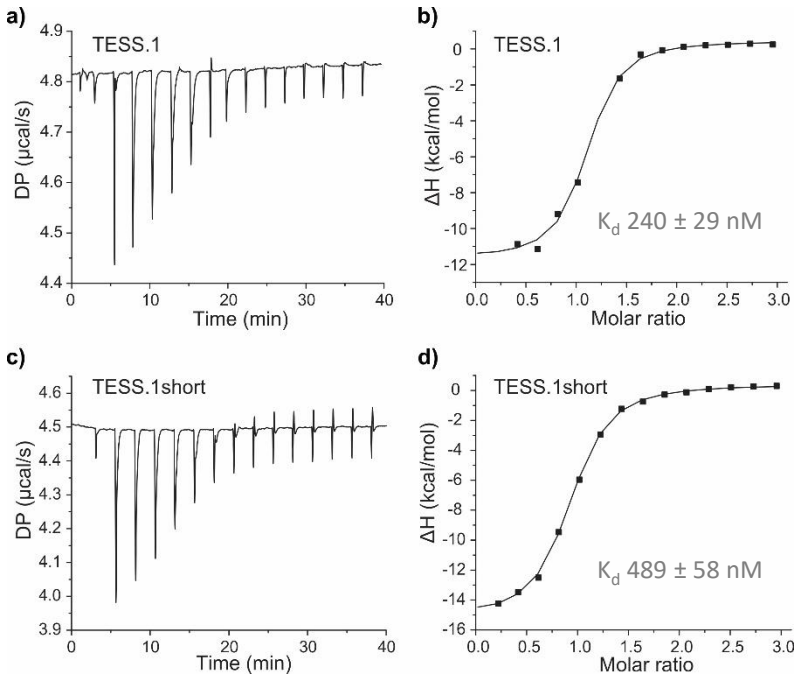



Upon addition of testosterone, the aptamer-capture interaction was disrupted, and an aptamer-testosterone complex was formed (green arrows).

In absence of target, a clear signal for the aptamer-capture complex is observed (purple arrows).

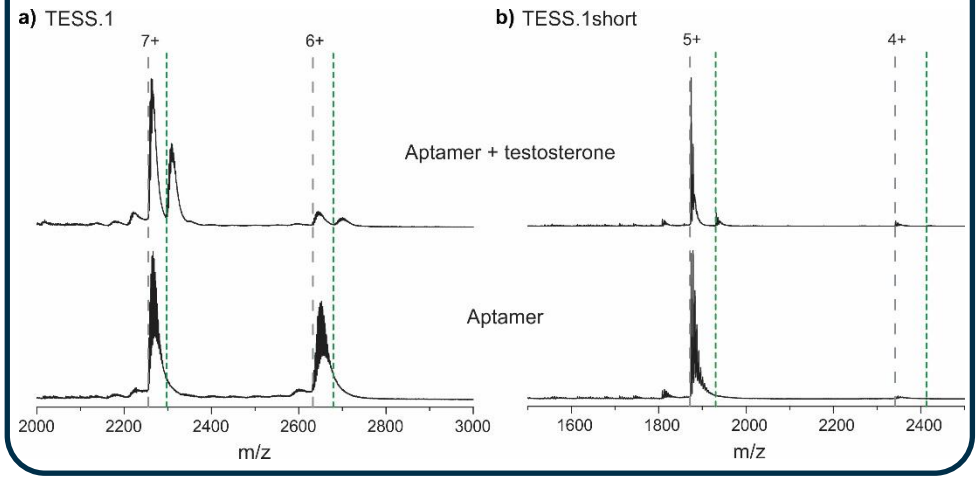
Native MS spectrum of the aptamer and capture in a 1:1 ratio (bottom) and the same mixture with testosterone added in a 1:10 ratio (top).

# Validation of the binding between TESS.1 and testosterone using ITC and native MS



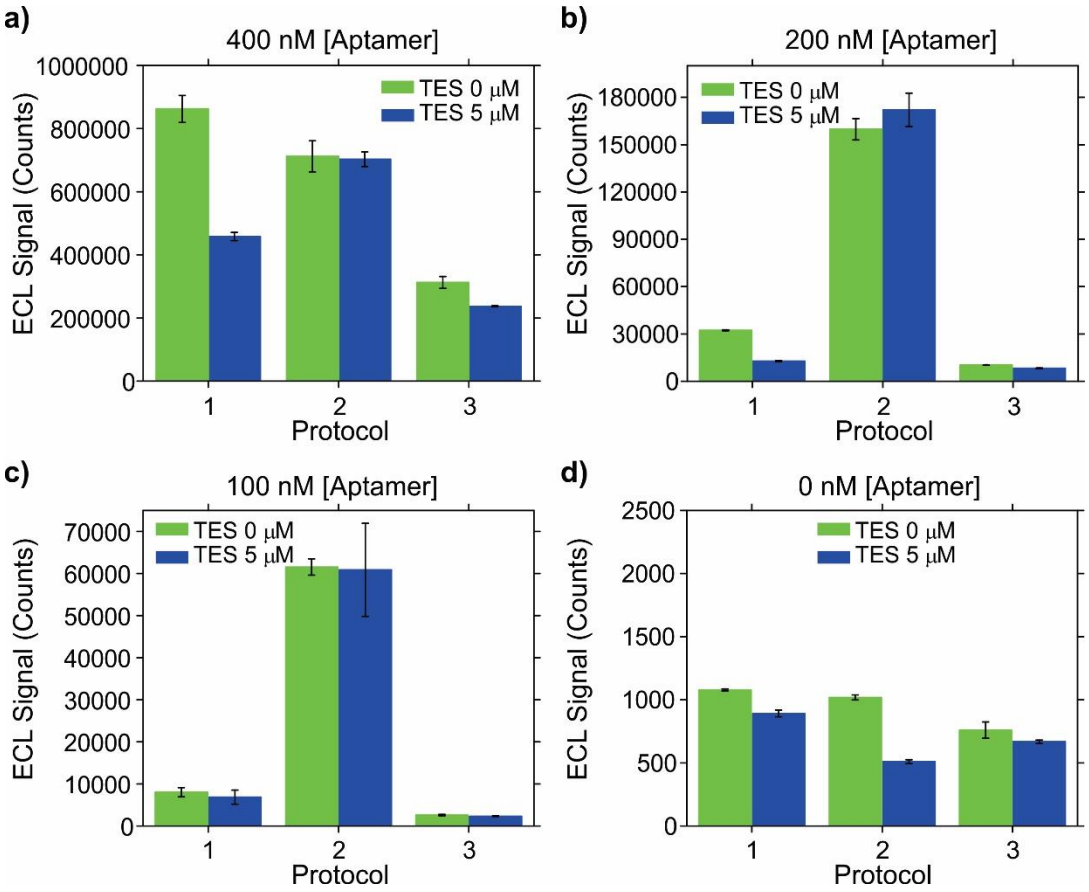

The thermogram for the ITC titration of TESS.1 with testosterone showed a clear exothermic binding process.

Native MS experiments were performed with the full-length TESS.1 aptamer and a shorter variant suggesting that even though the TESS.1short aptamer still contains the binding region, the stems of TESS.1 are a crucial structural part for the folding of the aptamer into its binding-competent state.




→ Even though theTESS.1short aptamer still contains the binding region, the stems of TESS.1 are a crucial structural part for the folding of the aptamer into its binding-competent state and allow high-affinity binding of testosterone

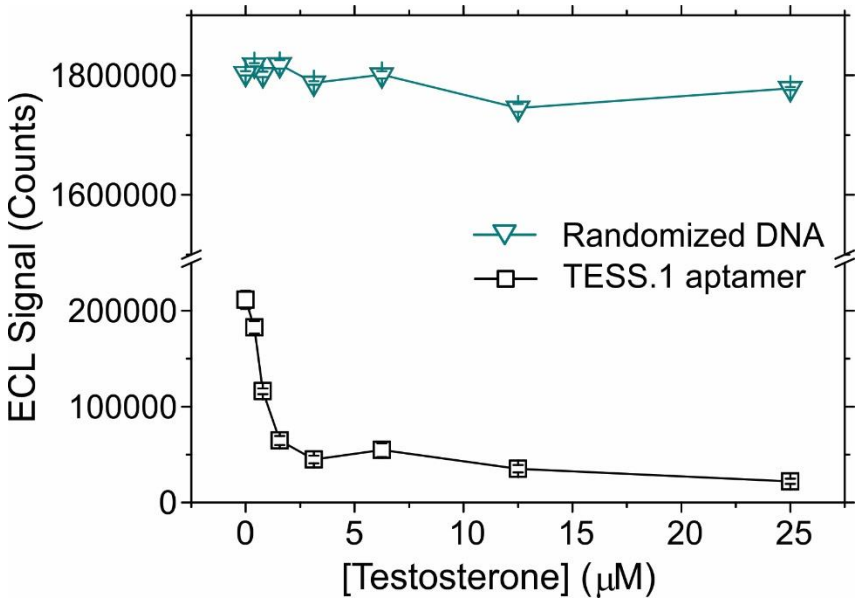
# Optimization of the incubation protocol



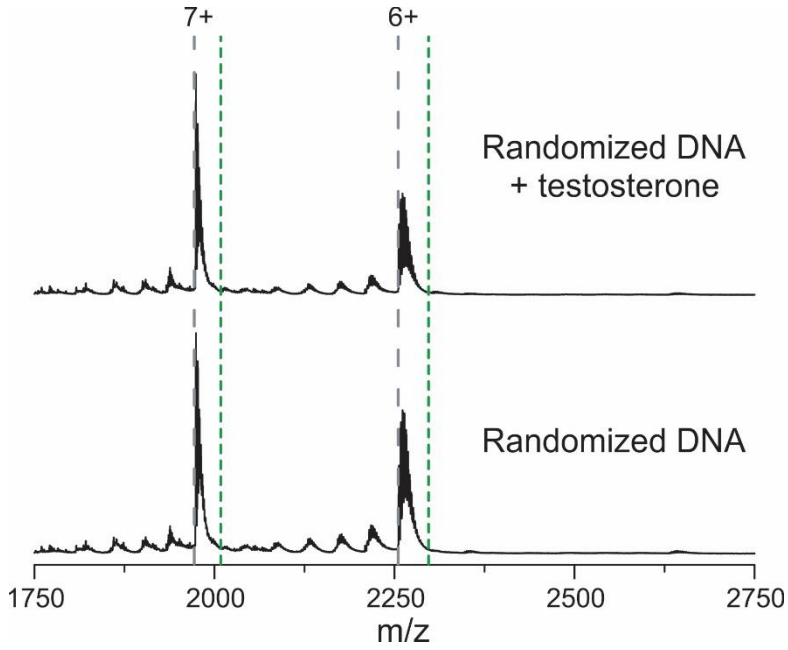

Parallel optimization of the ECL incubation protocol and the concentrations of the aptamer **a)** 400 nM, **b)** 200 nM, **c)** 100 nM and **d)** 0 nM, comparing the results with (5 µM) and without (0 µM) testosterone.

→ The concentration of 200 nM for the TESS.1 aptamer (and capture probe) and the protocol 1 were chosen as the optimal conditions since the decrease in signal intensity is the highest for this combination.

# ECL detection of testosterone and selective binding of testosterone to the aptamer




ECL data of testosterone (from 0 to 25 µM with a total of 8 concentrations, two-fold dilutions) in combination with the TESS.1 aptamer and randomized ssDNA sequence in the optimal concentration of 200 nM and following the protocol P1.




Native MS experiments with the randomized ssDNA were carried out to confirm and demonstrate that no binding occurs between this sequence and the target

→ Linear range from 0.39 to 1.56 µM and a limit of detection (LOD) of 0.29 µM.

# Conclusions

- A novel ECL assay based on aptamer recognition towards testosterone was introduced.
- The TESS.1 aptamer was used to demonstrate the novel methodology.
- The aptamer-target interaction was characterized and validated using complementary analytical techniques such as ITC and native nESI-MS.
- The analytical capability of the ECL array was studied and optimized using different incubation protocols.
- The selectivity and specificity of the array was assessed by performing negative control experiments.

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