

## Supporting Information

### **Molecularly imprinted polymers electrochemical sensing: the effect of inhomogeneous binding sites on the measurements. Comparison between imprinted polyaniline versus nanoMIP-doped polyaniline electrodes for the EIS detection of 17 $\beta$ -estradiol.**

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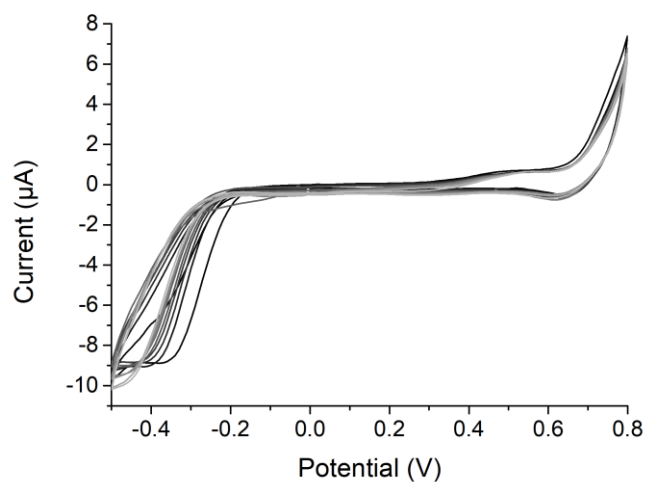
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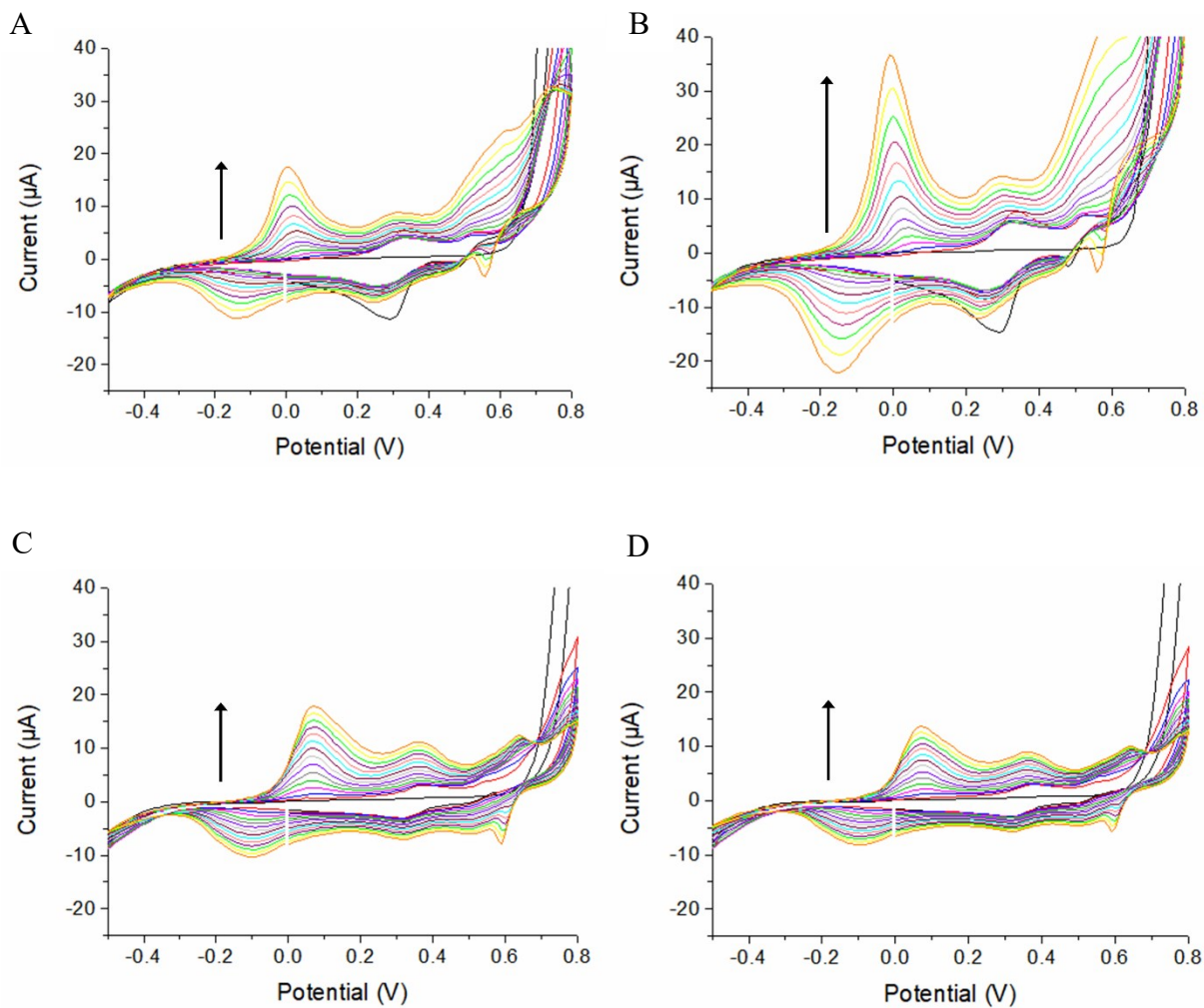
## 1. Investigation of electrochemical behaviour of E2

Preliminary investigation of the electrochemical behaviour of the target analyte 17 $\beta$ -estradiol (E2) at the bare screen-printed gold electrode was assessed by CV. Ideally the target analyte/template used to prepare electropolymerized MIPs should be inert and it should not actively participate in the MIP layer formation. Figure S1 shows a flat voltammogram without any peaks indicating that in the range of -0.5 V and +0.8 V the analyte is not a redox compound. This is crucial as it indicates that any resulting polyaniline layers are void of any interferences from the template.



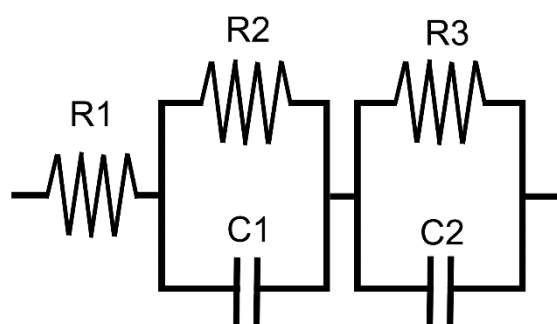
**Figure S1.** CV of E2.

## 2. CVs of electrodeposition of aniline for imprinted PANI electrode and nanoMIPs-doped PANI electrode



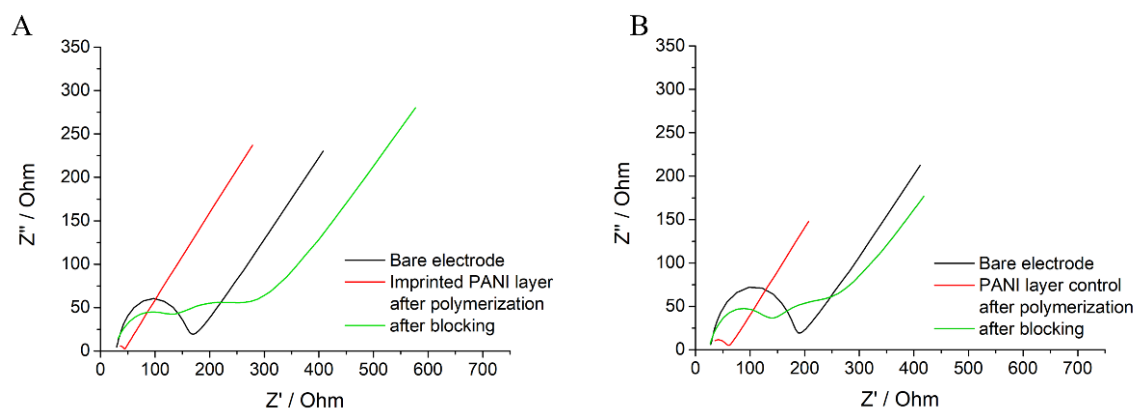
**Figure S2.** CV of (A) imprinted PANI layer, (B) PANI control, (C) nanoMIPs-doped PANI and (D) NPs control.

### 3. Fitting with PStTrace software



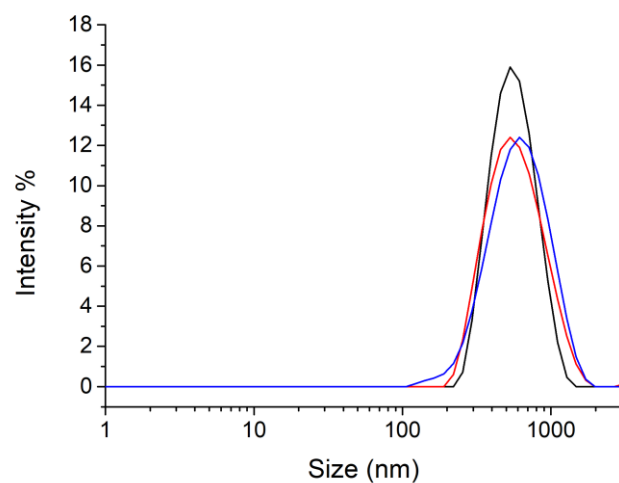
**Figure S3.** Equivalent circuit used for the fitting of Nyquist plot in PStTrace Software.

#### 4. Preparation of imprinted PANI layer and PANI control



**Figure S4.** Nyquist plot of Imprinted PANI layer (A) and PANI control (B) after the electropolymerization and after the blocking step.

## 5. Dynamic Light Scattering (DLS) of nanoMIPs

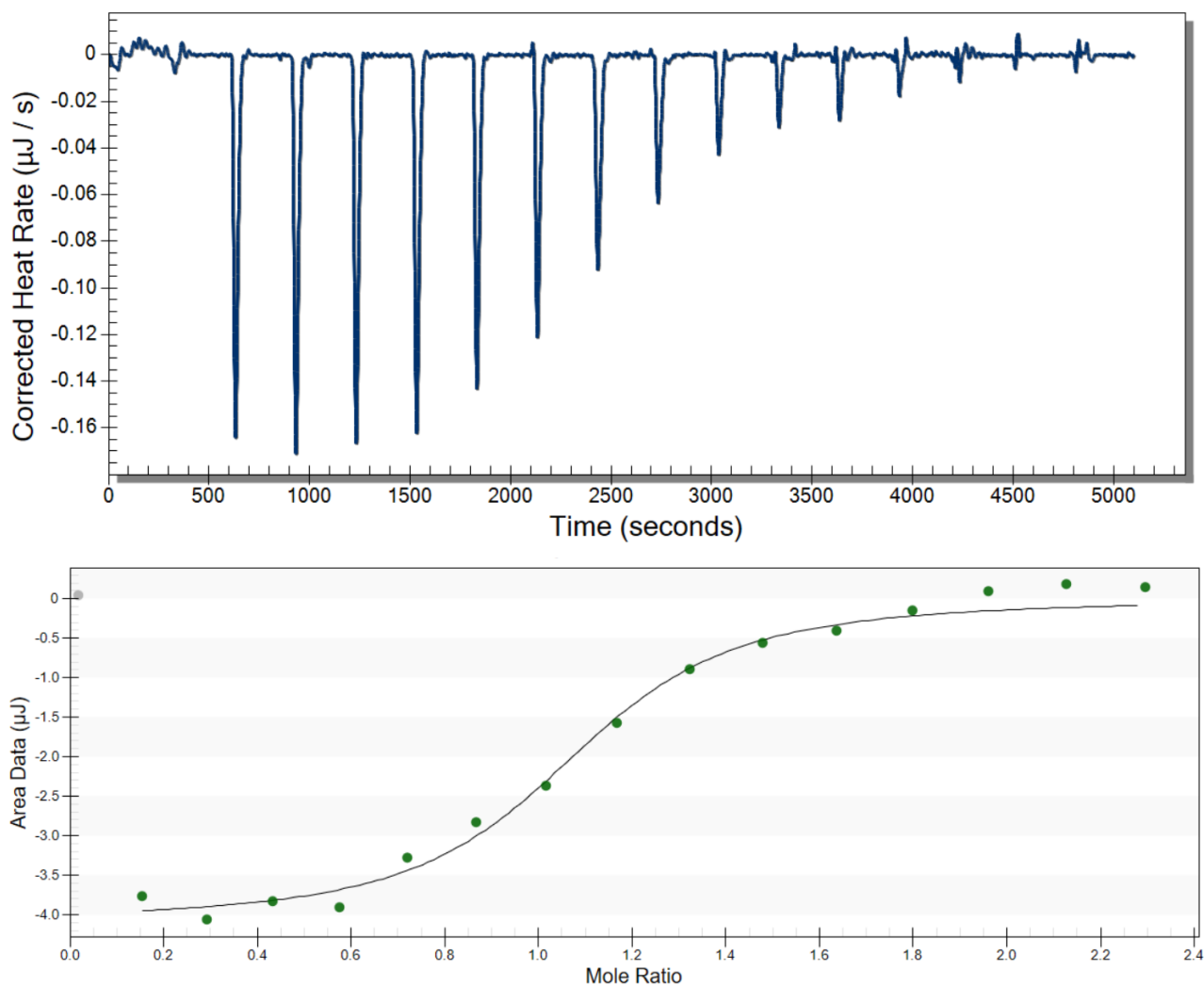


**Figure S5.** Example of DLS measurements of nanoMIP.

**Table S1.** Parameters obtained from DLS measurements of nanoMIP.

	<b>Value</b>
<b>Z-average (d.nm)</b>	553.3
<b>PdI</b>	0.275
<b>Intercept</b>	0.937

## 6. Isothermal Titration Nano Calorimetry (ITC) of nanoMIP



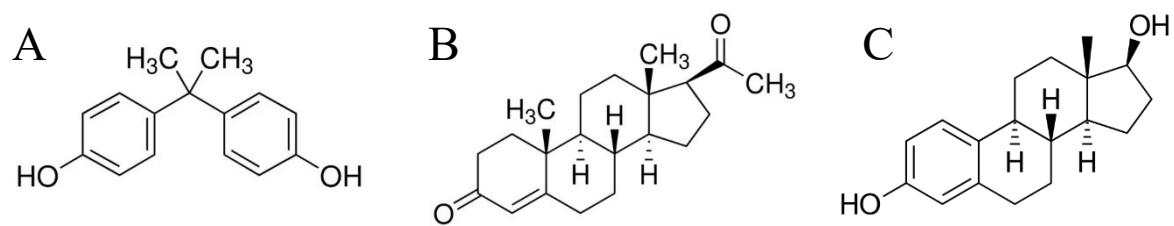
**Figure S6.** Upper panel: Raw ITC profile of nanoMIPs titrated with the template E2. Lower panel: integrated heats plotted as a function of the molar ratio nanoMIPs/E2 (green circles) and fitting of the data with an independent sites model.

**Table S2.** ITC fitting parameters.

Parameters	Value
$K_d$ (M)	$1.172 \times 10^{-8}$
$n$	1.029
$\Delta H$ (kJ/mol)	-380.8
$\Delta S$ (J/mol·K)	-1125



## 7. Structure of interferents



**Figure S7.** Molecular structure of Bisphenol A (A), Progesterone (B) and 17β-Estradiol (C).

## 8. Real sample measurements

**Table S3.** Normalized Rct of NanoMIP-doped PANI electrode tested in real sample

	<b>E2 (ng/mL)</b>	<b>Normalized Rct</b>
<b>PBS</b>	0.1	$0.151 \pm 0.0032$
	1	$0.249 \pm 0.0037$
	10	$0.370 \pm 0.0032$
<b>Wastewater</b>	0.1	$0.144 \pm 0.0024$
	1	$0.240 \pm 0.0049$
	10	$0.350 \pm 0.0042$