## A versatile electrochemical sensing receptor based on a molecularly imprinted polymer

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## **Supporting Information**

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#### 1. Materials

Ethylene glycol dimethacrylate (EDMA), Acetylferrocene, Anthracene (Ant), Fluorene (FI), Phenanthrene (Phe) and tetrabutylammonium hexafluorophosphate were purchased from Acros. Benzo(a)pyrene (BaP), Chrysene (Chr), Fluoranthene (Flu), Pyrene (Py), Dibenzo(a,h)anthracene (Dba) were purchased from Aldrich and Azobisisobutyronitrile (AIBN) from Fluka. Toluene and acetonitrile were analytical grade and purchased from VWR and used without any further purification. Ultrapure water (Millipore) was used for analysis. Acetonitrile used for HPLC was HPLC grade.

### 2. Apparatus

All the determinations of PAH compounds in solution were performed using a LaChrom Elite HPLC (VWR Hitachi) which comprised an L-2130 quaternary pump, an L-2200 autosampler and an L-2300 column oven. Detection was performed with an L2455 photodiode array detector. PAHs separation was achieved using an analytical reversed-phase column (Lichrospher® PAH, 250  $^{\circ}$  3 mm, 5 µm; Merck) maintained at 30°C. Scanning Electron Microscope (SEM) images were taken using Supra40 VP (Gemini®). Infrared spectra were recorded with a Nexus-thermonicolet. All experiments in electrochemical study were carried out with a potentiostat/galvanostat model 2743 (EG&G Princeton Applied Research). Elemental analyses were performed in the CNRS Laboratory of Vernaison.

## 3. Synthesis of e-MIPs

#### Synthesis of vinylferrocene (VFc)

Vinylferrocene was prepared in two steps by reduction of acetylferrocene followed by a deshydratation according to a procedure described in the literature (SchemeS1) (K. M. Joly, R. M. Gleixner, S. M. E. Simpkins, D. M. Coe, L. R. Cox, *Tetrahedron* **2007**, *63*, 761–767).



Scheme S1. Synthesis pathway of vinylferrocene, (a) NaBH<sub>4</sub>, EtOH, (b) CuSO<sub>4</sub>, hydroquinone, toluene.

# Synthesis of Electrochemical Molecularly Imprinted Polymers (e-MIPs) and Non-Imprinted Polymers (e-NIPs)

To prepare e-MIPs, the template BaP (0.159 mmol), VFc (0.780 mmol), and EDMA (4.000 mmol) were dissolved in a mixture of acetonitrile and toluene (40 mL) in a 100 mL flask and sonicated for 10 minutes

for dissolution of solids. Then the solution was purged with gentle flow of nitrogen for 30 minutes in an ice-water container in order to prevent solvent evaporation. Free-radical initiator AIBN (0.014 mmol) was added to the solution. The solution was purged again by nitrogen for 10 minutes. The flask was connected to a Dean-Stark apparatus full of the mixture of solvents equipped with a condenser. Polymerization was carried out by submerging the flask in an oil bath pre-heated at 80°C. The solution was agitated with low speed (100 rpm) and the temperature was maintained at 80°C for 30 minutes. The temperature was then raised up to 110°C within 10 minutes. At this stage, the solution became cloudy indicating the beginning of precipitation. The temperature was maintained for 30 minutes to allow solvent reflux. The Dean-Stark was then emptied allowing the solvent to be condensed and distilled out of the polymerization medium. Half of the solvent was distilled within 20 minutes.

The polymers were recovered by centrifugation (10 000 rpm, 10 minutes) washed several times with acetonitrile and then with a mixture of acetonitrile/toluene (1/1, v/v) and a mixture of acetonitrile/dichloromethane (1/1, v/v) in order to get rid of all the template and unreacted monomers. In general 10 cycles of washing-centrifugation were needed. All supernatants were analyzed by HPLC-UV to confirm the efficiency of the washing. More than 99% of BaP was released. The polymers were dried under vacuum at 30°C overnight. Non-Imprinted Polymers (e-NIPs) were prepared under identical conditions except for omission of the template BaP. The different synthesis conditions are summarized in Table S1.

Polymer	BaP	VFc	EDMA	AIBN	Toluene	Acetonitrile	Yield
					% v/v	% v/v	
e-MIP20	0.159 mmol	0.780 mmol	4.000 mmol	0.013 mmol	80	20	47%
e-NIP20	0	0.780 mmol	4.000 mmol	0.013 mmol	80	20	40%
e-MIP30	0.159 mmol	0.780 mmol	4.000 mmol	0.013 mmol	70	30	33%
e-NIP30	0	0.780 mmol	4.000 mmol	0.013 mmol	70	30	37%

Table S1. Polymerization conditions of imprinted and non-imprinted poly VFc-co-EDMA

## 4. Polymers characterization

### 4.1 Scanning electron microscope (SEM)

Small amount of polymer was deposed on a SEM support and metalized with gold. The images were registered under the following conditions: 3kV, secondary electron detector, diaphragm 30 (Figures S1 to S3).



Figure S1. SEM images of e-MIP (a) and e-NIP (b) synthesized in pure acetonitrile.

The coefficients of variation (CV) were calculated on the basing of 200 particles according to Equation S1. Table S2 shows the average size and the CV of each polymer.

$$CV = \frac{Standard \ deviation}{average \ diameter} \times 100 = \frac{\left(\sqrt{\frac{\sum (d-D_n)^2}{N}}\right)}{D_n} \times 100$$
 [Equation S1]

Polymers	eMIP-20	e-NIP20	e-MIP30	e-NIP30
D <sub>n</sub> (μm)	2.4	1.7	1.5	1.8
CV (%)	25	39	26	45

Table S2. Average diameters ( $\mathsf{D}_{\mathsf{n}}$ ) and coefficients of variation (CV)



Figure S2. SEM images of e-MIP20 (a), e-NIP20 (b)



Figure S3. SEM images of **e-MIP30** (c) and **e-NIP30** (d)

#### 4.2 Elemental analysis

Elemental analysis was carried out by using an acid digestion and performed on e-NIPs and leached e-MIPs. The experimental values are listed in Table S3.

%w/w		%0	%C	%Fe	%Н	%mol VFc	%mol EDMA
Theory		26.77	61.51	4.37	7.35	16	84
	e-MIP20	25.90	62.60	4.53	6.97	16	84
Experiment	e-NIP20	25.64	62.10	5.46	6.80	20	80
Experiment	e-MIP30	25.37	62.41	5.13	7.09	18	82
	e-NIP30	25.39	62.45	5.10	7.06	18	82

Table S3. Percentage of element values obtained by elemental analysis and compared to theoretical values.

The experimental values are close to the theoretical ones determined from the initial quantities. This proves that the redox probe (VFc) and the cross-linker (EDMA) were well incorporated in the polymers and with the desired quantities.

## 5. Quantification of PAHs by HPLC-UV

All separations were achieved on using an isocratic system when only BaP was present or a gradient system for the mixtures of several PAHs. The former consisted of pure acetonitrile while the latter consisted of a mixture of acetonitrile-water starting with 55% acetonitrile for 10 minutes, then ascending linearly to 100% acetonitrile within 25 minutes, and being maintained for 20 minutes before finally dropped down to 55% within 5 minutes. In each case, the flow rate was fixed at 0.5 mL.min<sup>-1</sup> and a 50  $\mu$ L sample volume was injected into the chromatographic system.

Calibration curves were obtained using a series of standard solutions containing mixture of PAHs at five different concentrations. Three replicated injections for each concentration were made to ensure accurate and reproducible responses. Linearity was evaluated by using least square method. All calibration curves were linear over a wide concentration range as shown by the values of the coefficient of determination ranging from 0.9844 to 0.9995. The theoretical limits of detection (LOD) at a signal/noise ratio of 3 and theoretical limits of quantification (LOQ) at a signal/noise ratio of 10 for all PAHs were in the range of 0.04-0.79 and 0.13-2.64  $\mu$ g.L<sup>-1</sup>, respectively. Table S4 shows the data related to the validation of the method and the selected detection wavelengths. Reproducibility and accuracy of the method were checked by injection of the standard solutions of PAHs and measured from three replicated injections for within-day reproducibility and after 6 months, respectively.

	LOD,µg.L <sup>-1</sup>	LOQ,µg.L <sup>-1</sup>	λ,nm
Fluorene	0.36	1.19	262
Phenanthrene	0.04	0.13	254
Anthracene	0.23	0.78	254
Fluoranthene	0.10	0.33	286
Pyrene	0.42	1.39	242
Chrysene	0.07	0.23	267
Benzo(a)pyrene	0.79	2.64	296
Dibenzo(a,h)anthracene	0.23	0.76	296

Table S4. Limits of detection and quantification for each PAH.

#### 6. Batch binding experiment

10 mg of polymer were placed in a 30 mL glass vial and mixed with 10 mL of BaP solution of different concentrations varying from 0.01 to 10 mg.L<sup>-1</sup>. BaP solutions were prepared in water/acetonitrile (99/1, v/v). Then the vial was shaken under horizontal stirring at 200 rpm at room temperature until equilibrium (24 hours). The supernatants were collected. The amounts of adsorbed BaP were calculated by subtracting the supernatant concentrations from the initial concentrations of BaP in solution (Equation S2). The imprinting factor was determined as the ratio between the maximum binding capacity of the e-MIP and that of the e-NIP (Equation S3). The binding isotherm experiment was carried out in duplicate.

$$Q = \frac{adsorbed quantity (mg or \mu mol)}{polymer weight (g)}$$
[Equation S2]

 $or \ Q = \frac{(Initial \ concentration - supernantant \ concentration)x \ volume}{polymer \ weight \ (g)}$ 

$$IF = \frac{Q, e - MIP}{Q, e - NIP}$$
 [Equation S3]

#### 7. Cross-selectivity experiment

10 mg of polymer were placed in a 30 mL glass vial and mixed with 10 mL of solution containing BaP and an interferent PAH. The PAHs used were Fluorene (fl), Fluoranthene (Flu), Anthracene (Ant), Phenanthrene (Phe), Chrysene (Chr), Pyrene (Py), and Dibenzo(a,h)anthracene (Dba). The concentration of BaP and the interfering PAH was 0.4  $\mu$ g.L<sup>-1</sup> each. The vial was then shaken horizontally at 200rpm at room temperature for 24 hours. The supernatant was analyzed by HPLC-UV.

A distribution coefficient ( $K_d$ ) was determined for each PAH and for each polymer (Equation S4). This coefficient is the ratio between the binding capacity of each species and the equilibrium concentration. The selectivity coefficient (k) is calculated by Equation S5. And finally, the selectivity of e-MIP can be compared to the e-NIP by the relative selectivity coefficient (k') (Equation S6). The selectivity and relative selectivity coefficients are shown in the Table S5.

Distribution coefficient ( $K_d$ ) is the ratio between binding capacity ( $Q_e$ ) of a polymer to the equilibrium concentration of the target ( $C_e$ ):

$$K_d = \frac{Q_e}{C_e}$$

[Equation S4]

Selectivity coefficient (k) is the ratio of the distribution coefficient ( $K_d$ ) of a polymer for the BaP to that of the PAH interferent:

$$\boldsymbol{k} = \frac{K_{d} B a P}{K_{d} P A H}$$
 [Equation S5]

Relative selectivity coefficient k' is the ratio of the selectivity coefficient (k) of a MIP to that of the corresponding e-NIP:

$$k' = \frac{k_{\text{MIP}}}{k_{\text{NIP}}}$$
 [Equation S6]

Table S5. Selectivity coefficients and relative selectivity coefficients obtained by cross-selectivity experiments on e-MIP20/e-NIP20 and e-MIP30/e-NIP30.

	k		k'	' k		k'
	e-MIP20	e-NIP20		e-MIP30	e-NIP30	
Fluorene	0.56	0.58	0.97	0.66	0.64	1.03
Phenanthrene	2.86	1.59	1.80	2.31	1.42	1.62
Anthracene	0.45	0.38	1.20	1.61	0.40	4.01
Fluoranthene	0.72	0.55	1.31	0.80	0.43	1.85
Pyrene	0.55	0.48	1.14	0.40	0.66	0.61
Chrysene	0.62	0.58	1.08	0.57	0.57	0.99
Dibenzo(a,h)anthracene	1.87	1.58	1.18	2.44	1.47	1.65

## 8. Electrochemical study

The polymer was mixed with a carbon paste (Gwent group, C10903P14) (1/4, w/w). The mixture was homogenized with a small amount of dichloromethane and filled in a hollow working 5 mm diameter electrode (Metrohm, RDE.BLANK 80184). The electrode was then dried under vacuum at 30°C overnight and the surface was polished with a sand paper. The surface of the electrode observed by SEM is shown in Figure S5. The dry polymer and carbon paste weighed 11.4 mg. The working electrode containing polymer was then placed in an electrolytic cell in presence of a reference electrode (Hg/Hg<sub>2</sub>Cl<sub>2</sub>/KCl sat) and a counter electrode (Pt, 1mm diameter). The electrolyte used was 0.1M tetrabutylammonium hexafluorophosphate in acetonitrile.



Figure S4. SEM image displays a typical morphology of e-MIP20-carbon paste working electrode. It can be seen that on the surface the layer of irregular flakes of graphite powder was present.

Squaro wayo voltammetry (SM/V) parameters:	Differential Pulse Voltammetry (DPV) parameters:				
Square wave voltammetry (Svvv) parameters.	Differential Pulse Voltaninetry (DPV) parameters.				
<ul> <li>Initial potentiel (E<sub>1</sub>): 0.0 V (vs SCE)</li> </ul>	<ul> <li>Initial potentiel (E<sub>1</sub>): 0.0 V (vs SCE)</li> </ul>				
- Vertex potentiel (E <sub>v</sub> ): 1.0 V (vs SCE)	- Vertex potentiel (E <sub>v</sub> ): 1.0 V (vs SCE)				
<ul> <li>Final potentiel (E<sub>F</sub>): 0.0 V (vs SCE)</li> </ul>	<ul> <li>Final potentiel (E<sub>F</sub>): 0.0 V (vs SCE)</li> </ul>				
- Pulse (PH/PW): 0.15 V	- Pulse (PH/PW): 0.05 V				
- Pulse duration: 50 ms	- Pulse duration: 50 ms				
- Pulse height (SH): 2 mV	- Pulse height (SH): 5 mV				
- Scan rate: 20 mV/s	- Scan rate: 20 mV/s				

At the beginning of the experiment, the solution was purged 5 minutes with an argon flow. The blank measurement was registered after 30 minutes of electrode submersion in order to get a stabilized signal. Then 100  $\mu$ L of BaP solution at 0.2 mg.L<sup>-1</sup> was added into the electrolyte solution. Voltammograms were registered over 24 hours. Before each measurement a flow of argon was purged over the solution in order to prevent oxygen dissolution and the reference electrode surface was renewed.



Figure S5. Cyclic voltammograms of **e-MIP20CPE** and **e-NIP80CPE** in 0.1 M *n*-Bu<sub>4</sub>NPF<sub>6</sub> in acetonitrile at various scan rates (5-500 mV. s<sup>-1</sup>). Plot of anodic and cathodic peak currents of *vs* v or v<sup>1/2</sup>. Plot of charge *vs* log(v).



Figure S6. Cyclic voltammograms of **e-MIP30CPE** and **e-NIP30CPE** in 0.1 M *n*-Bu<sub>4</sub>NPF<sub>6</sub> in acetonitrile at various scan rates 5-500 mV.s<sup>-1</sup>. Plot of anodic and cathodic peak currents *vs* v or v<sup>1/2</sup>. Plot of charge *vs* log(v).



Figure S7. Evolution of DPV scans for e-MIP20CPE/e-NIP20CPE (left) and SWV scans for e-MIP30CPE/e-NIP30CPE (right) after addition of BaP. Acetonitrile, 0.1 M *n*-Bu<sub>4</sub>NPF<sub>6</sub>.