Electrochemical sensor based on molecularly imprinted polymer for the detection of cefalexin

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Supplementary information

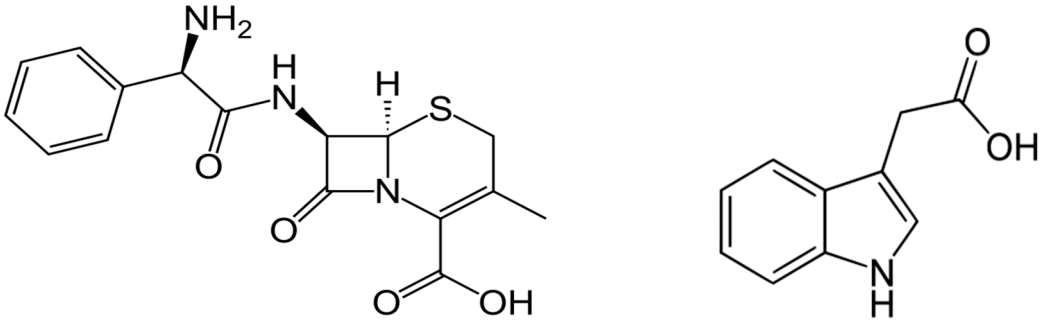

Figure S1. CFX (left) and I3AA (right) structures

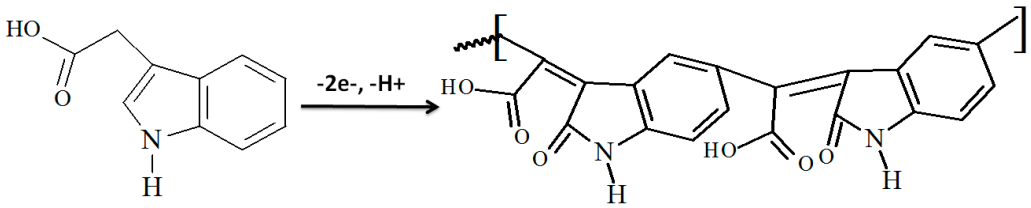

Figure S2. The polymerization mechanism

Table S1. Redox probe for the MIP-modified GCE

Polymerization: 1 mM I3AA, 0.5 mM CFX, 5 cycles; Extraction: NaOH 0.1 M, 30 min; Incubation: 0.5 µM CFX, water, 30 min						
Redox probe	**GCE**					
	MIP			**NIP**		
	S_{polym}	**S_{extr}**	**S_{incub}**	**S_{polym}**	**S_{extr}**	**S_{incub}**
$[Ru(NH_3)_6]^{3+}$	0.885	9.413	0.027	0.87	8.725	0.023
$[Fe(CN)_6]^{3-/4-}$	**0.995**	**6.320**	**0.436**	**0.995**	**1.138**	**0.176**
1,1'-ferrocenedimethanol	0.925	12.235	0.022	0.916	11.374	0.021

Table S2. Redox probe for the MIP-modified BDDE

Polymerization: 1 mM I3AA, 0.5 mM CFX, 5 cycles; Extraction: NaOH 0.1 M, 30 min; Incubation: 0.5 µM CFX, water, 30 min						
Redox probe	**BDDE**					
	MIP			**NIP**		
	S_{polym}	**S_{extr}**	**S_{incub}**	**S_{polym}**	**S_{extr}**	**S_{incub}**
$[Ru(NH_3)_6]^{3+}$	0.835	7.635	0.015	0.85	7.881	0.024
$[Fe(CN)_6]^{3-/4-}$	**0.986**	**8.348**	**0.532**	**0.993**	**0.735**	**0.123**
1,1'-ferrocenedimethanol	0.934	12.483	0.03	0.945	11.856	0.018

Table S3. Optimization of polymerization – DPV signal, on the GCE electrode

Extraction: NaOH 0.1 M, 30 min; Incubation: 0.5 µM CFX, water, 30 min							
Parameter	**Value**	**GCE**					
		MIP			**NIP**		
		S_{polym}	**S_{extr}**	**S_{incub}**	**S_{polym}**	**S_{extr}**	**S_{incub}**
C_{mon} (mM) (C temp – 0.05 mM)	0.1	0.907	5.210	0.311	0.957	2.425	0.284
	1	**0.932**	**4.870**	**0.358**	**0.978**	**2.310**	**0.292**
	5	0.981	3.920	0.243	0.99	1.485	0.287
C_{temp} (mM) (C_{mon} – 1 mM)	0.01	0.942	3.460	0.303	**0.978**	**2.310**	**0.292**
	0.05	0.932	4.870	0.358			
	0.1	0.921	6.650	0.398			
	0.5	**0.973**	**8.820**	**0.501**			
	1	0.872	9.750	0.373			
No of cycles	2	0.93	7.240	0.382	0.963	2.670	0.294
	5	**0.973**	**8.820**	**0.501**	**0.978**	**2.310**	**0.292**
	10	0.987	6.230	0.238	0.985	1.860	0.107

Table S4. Optimization of polymerization – DPV signal, on the BDDE electrode

Extraction: NaOH 0.1 M, 30 min; Incubation: 0.5 µM CFX, water, 30 min							
Parameter	**Value**	**BDDE**					
		MIP			**NIP**		
		S_{polym}	**S_{extr}**	**S_{incub}**	**S_{polym}**	**S_{extr}**	**S_{incub}**
C_{mon} (mM) (C temp – 0.05 mM)	0.1	0.915	6.930	0.327	0.948	1.432	0.271
	1	**0.927**	**6.340**	**0.398**	**0.965**	**1.235**	**0.257**
	5	0.972	5.280	0.289	0.987	0.845	0.238
C_{temp} (mM) (C_{mon} – 0.05 mM)	0.01	0.958	5.670	0.297	**0.965**	**1.235**	**0.257**
	0.05	0.927	6.340	0.398			
	0.1	0.949	7.860	0.475			
	0.5	**0.987**	**9.530**	**0.604**			
	1	0.898	10.230	0.412			
No of cycles	2	0.953	7.830	0.432	0.924	1.023	0.246
	5	**0.987**	**9.530**	**0.604**	**0.965**	**1.235**	**0.257**
	10	0.991	8.010	0.367	0.982	0.723	0.209

Table S5. Optimization of polymerization – EIS signal, on the GCE electrode

Extraction: NaOH 0.1 M, 30 min; Incubation: 0.5 µM CFX, water, 30 min							
Parameter	**Value**	**GCE**					
		MIP			**NIP**		
		S_{polym}	**S_{extr}**	**S_{incub}**	**S_{polym}**	**S_{extr}**	**S_{incub}**
C_{mon} (mM) (C temp – 0.05 mM)	0.1	10.763	0.839	0.467	22.267	0.708	0.397
	1	**13.701**	**0.827**	**0.558**	**45.167**	**0.698**	**0.413**
	5	52.34	0.794	0.325	97.023	0.598	0.403
C_{temp} (mM) (C_{mon} – 0.05 mM)	0.01	16.241	0.774	0.434	**45.167**	**0.698**	**0.413**
	0.05	13.701	0.827	0.558			
	0.1	11.94	0.867	0.672			
	0.5	**39.034**	**0.901**	**1.011**			
	1	9.835	0.908	0.597			
No of cycles	2	14.015	0.879	0.619	26.027	0.723	0.416
	5	**39.034**	**0.901**	**1.011**	**45.167**	**0.698**	**0.413**
	10	79.678	0.863	0.315	66.687	0.651	0.121

Table S6. Optimization of polymerization – EIS signal, on the BDDE electrode

Extraction: NaOH 0.1 M, 30 min; Incubation: 0.5 µM CFX, water, 30 min							
Parameter	**Value**	**BDDE**					
		MIP			**NIP**		
		S_{polym}	**S_{extr}**	**S_{incub}**	**S_{polym}**	**S_{extr}**	**S_{incub}**
C_{mon} (mM) (C temp – 0.05 mM)	0.1	10.775	0.874	0.487	18.345	0.589	0.372
	1	**12.743**	**0.865**	**0.662**	**28.572**	**0.563**	**0.347**
	5	34.716	0.842	0.407	75.94	0.46	0.314
C_{temp} (mM) (C_{mon} – 0.05 mM)	0.01	22.812	0.85	0.423	**28.572**	**0.563**	**0.347**
	0.05	12.743	0.865	0.662			
	0.1	18.609	0.888	0.905			
	0.5	**76.345**	**0.907**	**1.525**			
	1	8.813	0.911	0.7			
No of cycles	2	21.276	0.893	0.763	12.346	0.508	0.329
	5	**76.345**	**0.907**	**1.525**	**28.572**	**0.563**	**0.347**
	10	102.123	0.891	0.583	55.321	0.421	0.267

Table S5. Optimization of extraction – DPV signal

Polymerization: 1 mM I3AA, 0.5 mM CFX, 5 cycles; Extraction: NaOH 0.1 M, 30 min; Redox probes:10 mM Fe^{3+} – DPV, 10 mM $[Fe(CN)_6]^{-3/-4}$ -EIS									
Solvent	**Time (minutes)**	**GCE**				**BDDE**			
		MIP		**NIP**		**MIP**		**NIP**	
		S_{extr}	S_{incub}	S_{extr}	S_{incub}	S_{extr}	S_{incub}	S_{extr}	S_{incub}
MeOH	5	6.410	0.352	1.813	0.284	7.762	0.415	1.627	0.203
	15	7.560	0.391	2.840	0.312	8.853	0.521	2.154	0.225
	30	9.750	0.448	3.152	0.287	10.236	0.557	3.231	0.248
	60	11.380	0.37	3.750	0.279	12.053	0.456	3.689	0.235
NaOH 0.1M	5	4.510	0.345	1.423	0.289	5.387	0.423	0.926	0.25
	15	6.330	0.404	1.873	0.29	7.325	0.527	1.085	0.253
	30	**8.820**	**0.501**	**2.310**	**0.292**	**9.530**	**0.604**	**1.235**	**0.257**
	60	9.280	0.435	2.458	0.283	9.864	0.461	1.367	0.239
PBS, pH 7.4	5	2.320	0.345	0.856	0.285	3.213	0.423	0.763	0.244
	15	4.530	0.404	1.341	0.283	4.345	0.525	0.959	0.248
	30	6.210	0.467	1.557	0.278	5.847	0.585	1.183	0.245
	60	6.850	0.374	1.625	0.277	6.329	0.46	1.348	0.247

Table S6. Optimization of extraction – EIS signal

Polymerization: 1 mM I3AA, 0.5 mM CFX, 5 cycles; Extraction: NaOH 0.1 M, 30 min; Redox probe: 10 mM $[Fe(CN)_6]^{-3/-4}$									
Solvent	**Time (minutes)**	**GCE**				**BDDE**			
		MIP		**NIP**		**MIP**		**NIP**	
		S_{extr}	S_{incub}	S_{polym}	S_{extr}	S_{polym}	S_{polym}	S_{extr}	S_{polym}
MeOH	5	0.866	0.544	0.644	0.398	0.886	0.709	0.624	0.252
	15	0.885	0.647	0.741	0.456	0.899	1.091	0.683	0.29
	30	0.908	0.81	0.759	0.404	0.911	1.257	0.764	0.331
	60	0.92	0.588	0.792	0.387	0.925	0.839	0.787	0.306
NaOH 0.1M	5	0.818	0.527	0.587	0.407	0.843	0.732	0.481	0.333
	15	0.863	0.679	0.653	0.409	0.881	1.114	0.52	0.337
	30	**0.899**	**1.011**	**0.697**	**0.414**	**0.905**	**1.525**	**0.553**	**0.348**
	60	0.903	0.771	0.71	0.395	0.908	0.854	0.577	0.314
PBS, pH 7.4	5	0.696	0.527	0.461	0.399	0.763	0.732	0.433	0.323
	15	0.821	0.677	0.572	0.395	0.813	1.104	0.489	0.331
	30	0.863	0.876	0.609	0.385	0.854	1.408	0.542	0.321
	60	0.873	0.597	0.619	0.384	0.867	0.849	0.574	0.328

Table S7. Optimization of incubation (DPV, EIS)

Polymerization: 1 mM I3AA, 0.5 mM CFX, 5 cycles; Extraction: NaOH 0.1 M, 30 min; Redox probes:10 mM Fe^{3+} – DPV, 10 mM $[Fe(CN)_6]^{-3/-4}$ - EIS									
Solvent	**Time (minutes)**	**GCE**				**BDDE**			
		MIP		**NIP**		**MIP**		**NIP**	
		DPV	**EIS**	**DPV**	**EIS**	**DPV**	**EIS**	**DPV**	**EIS**
Water	5	0.369	0.601	0.205	0.253	0.435	0.772	0.179	0.221
	15	0.430	0.777	0.237	0.306	0.546	1.203	0.213	0.268
	30	**0.501**	**1.020**	**0.292**	**0.412**	**0.604**	**1.560**	**0.257**	**0.347**
	60	0.505	1.023	0.298	0.420	0.609	1.540	0.261	0.351
PBS, pH 7.4	5	0.372	0.610	0.204	0.261	0.440	0.792	0.205	0.258
	15	0.435	0.792	0.276	0.381	0.553	1.237	0.231	0.300
	30	0.506	1.035	0.293	0.414	0.612	1.567	0.262	0.352
	60	0.508	1.041	0.301	0.433	0.615	1.624	0.265	0.357

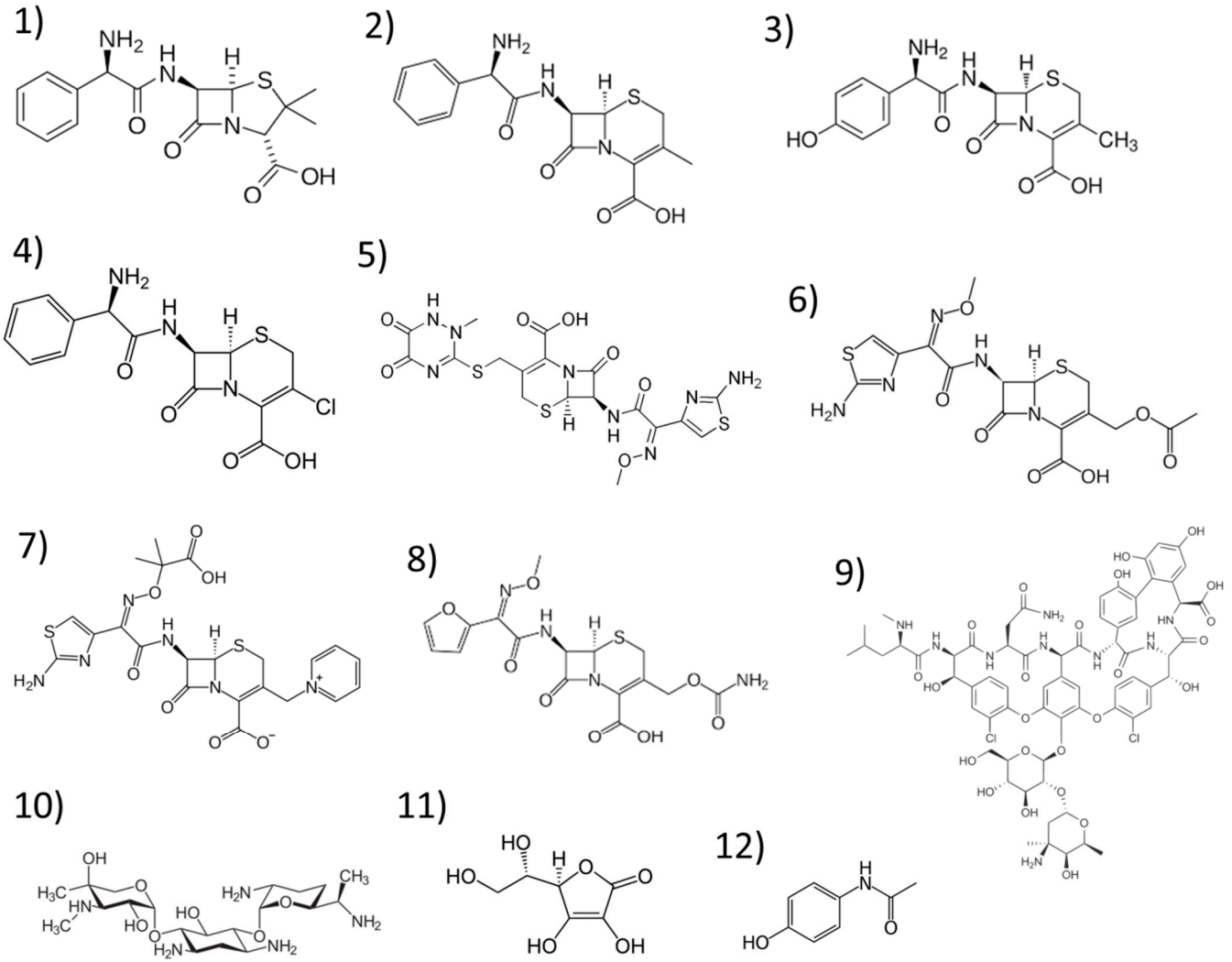

Figure S3. The structures of the molecules for which the MIP sensor was tested: 1) Ampicillin (AMP), 2) Cefalexin (CFX), 3) Cefadroxil (CFD), 4) Cefaclor (CFC), 5) Ceftriaxone (CFTXO), 6) Cefotaxime (CFTX), 7) Ceftazidime (CFTZ), 8) Cefuroxime (CFOX)), 9) Vancomycin (VAN), (10) Gentamicin (GEN), 11) Ascorbic acid (AA) and 12) Acetaminophen (APAP)

Table S8. Comparative analytical performance of different methods for CFX detection

No.	Method of detection	Linear range (ng/mL)	LOD (ng/mL)	Interferences	Real-life samples	Observations	Ref.
1.	Visualized microarray	8.20–68.60	8.20	Melamine, aflatoxin	Milk	Complex work protocol Requires use of monoclonal antibodies (expensive, liable)	(1)
2.	MIP for SPE+ HPLC			Ceftazidime, ampicillin and tetracycline hydrochloride	Milk	Complex work protocol Requires qualified staff Interferences on C18 SPE column due to non-specific interactions	(2)
3.	MISPE-UHPLC–MS/MS		2.24	Cefthiofur, cefazolin, cefquinome, cephapirin, cephalexin and cephalonium fluoroquinolones, doxycyciline	Milk	Toxic reagents (ACN, MeOH) Complex work protocol Requires qualified staff Low recovery rates for cephalexine Similar recovery rates between analytes and interferences	(3)
4.	Spectrophotometric UV	800.00–2800.00	168.00	Starch, lactose, glucose, sucrose, and gum acacia	Pharm. form.	Requires derivatisation of the probe with 1,2-naftoquinone-4-sulphonic	(4)
5.	DPV on bare BDDE	173.80-243313.00	34.74	acetaminophen, ascorbic acid, glucose, other cephalosporins	River water, pharm. form., human urine	DPV peak for cephalexin decreases in the presence of other cephalosporins	(5)
6.	SWV on heated glassy carbon electrode	208.55-17379.50	52.00	Na+ , K+ , Ca2+, Cu2+, Mg2+, Cu2+, Pb2+, Zn2+, CO3 2−, SO4 2−, NO3 − , Cl− , dextrin, amylum glucose, 100 NH4 + , Fe2+, SO3 2−, lactose, glucose and sucrose, and citric acid, urea and uric acid	Pharm. form.	Requires alkaline hydrolysis of the analyte before the method can be applied	(6)

7.	HPLC with UV detection	25.00 to 1600.00	10.00	-	Bovine milk	Harmful reagents (acetonitrile, glutaraldehyde) Requires a protein exclusion step, using two columns and a column switching system	(7)
8.	LC–ESI–MS/MS	2.00–100.00	0.50	Simultaneous detection with Cefminox, cefotaxime, cefetametplvoxll, ceftazidime, cephalonium cefixime cefadroxil, cefazolin, cefuroxime, cefalotin, cefradine, cefapirin cefaclor cefonicid, cefamandole, cefepime, ceftriaxone, cefpirome, cefoperazone, ceftiofur, cefquinome	Pork muscle	Harmful reagents Qualified staff	(8)
9.	Microbiological system	-	128.00	-	Ovine milk	Lacks selectivity Requires Geobacillus stearothermophilus spores, which can contaminate the work environment	(9)
10.	Electrochemical oxidation by CV using the boron-doped diamond thin-film electrode	In the range of mM	-	-	-	Requires microwave plasma-assisted chemical vapor deposition of film Deposition lasts about 10 h	(10)
11	Our MIP-based sensor	3.47 - 347.38	1.11 (BDDE) 1.70 (GCE)	Cefadroxil, cefaclor, ceftriaxone, cefotaxime, ceftazidime, cefuroxime, ampicillin, gentamicin, vancomycin, ascorbic acid, acetaminophen	Pharm. form., river water	-	This work

Pharm. form - pharmaceutical formulations; LOD –Limit of detection

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