

Supporting Information

Molecularly Imprinted Polymer (MIP) Film with Improved Surface Area Developed by Using Metal-Organic Framework (MOF) for Sensitive Lipocalin (NGAL) Determination

Zofia Iskierko,[†] Piyush Sindhu Sharma,[†] Daniel Prochowicz,[†] Krzysztof Fronc,[‡]

Francis D'Souza,[§] Diana Toczyłowska,^{||} Filip Stefaniak,^{||} Krzysztof Noworyta,^{ †}*

[†]Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224
Warsaw, Poland

[‡]Institute of Physics, Polish Academy of Sciences, 32/46 Al. Lotników, 02-668 Warsaw, Poland

[§]Department of Chemistry, University of North Texas, Denton, Texas 76203-5017, United
States.

^{||} Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and
Cell Biology in Warsaw, Ks. Trojdena 4, 02-109 Warsaw, Poland

*E-mail: knoworyta@ichf.edu.pl

Experimental:

Chemicals:

NGAL peptide, zinc nitrate hexahydrate, terephthalic acid, 2,2'-bithiophene, 2,2'-bithiophene-5-carboxylic acid, **1**, toluene (anhydrous), acetonitrile (anhydrous) were purchased from Aldrich and used as received. Tetra-n-butylammonium perchlorate (electrochemical grade) was purchased from Fluka. The *p*-bis(2,2'-bithien-5-yl)methyl-o-catechol monomer, **2**, was synthesized according to procedure described earlier.¹

Equipment and procedures

Electrochemical deposition experiments were performed in three-electrode electrochemical cell using Autolab PGSTAT20 (Eco Chemie, The Netherlands) potentiostat/galvanostat driven by dedicated GPES v.4.3 software. The MIP film was deposited by potentiodynamic electropolymerization in the potential range from 0 to 1.4 V with the scan rate of 50 mV s⁻¹. The solution for polymerization was prepared by dissolving the functional **2** and cross-linking **3** monomers (Scheme 1) together with the NGAL template at the molar ratio of 100 : 400 : 1, respectively. The mixed toluene and acetonitrile solvent solution at the volume ratio of 7 : 3 was used to dissolve the monomers and the template. The 0.1 M tetrabutylammonium perchlorate was used as a supporting electrolyte. For immobilization of the protein template, the Au plated glass slide coated with MOF-5 crystals was immersed in the pre-polymerization solution for 1 h.

The protein template and MOF removal has been performed by washing samples for 1 h in 0.1 M HCl.

The simulation of NGAL docking was conducted using Autodock Vina (version 1.1.2) with exhaustiveness parameter set to 64 with the box size 16x18x20. The center of the box was set to the geometric center of native ligand.

In order to obtain carboxy-terminated Au electrode an electrochemical polymerization of 2 mM 2,2'-bithiophene-5-carboxylic acid monomer was performed under potentiodynamic conditions in 0.1 M (TBA)ClO₄, acetonitrile solution with scan rate of 50 mV s⁻¹ (Fig. S1). One potentiodynamic cycle was used for thin polymer film deposition. In the next step, the carboxy-terminated Au electrode was placed in a solution of 0.335 g (1.12 mmol) Zn(NO₃)₂×6H₂O and 0.042 g (0.25 mmol) terephthalic acid in 5 mL dimethylformamide (DMF) to obtain film of MOF-5. The mixture was heated for 6 h at 120 °C, and then the temperature was raised to 150 °C until crystallization began. Finally, the sample was repeatedly washed with pure DMF and CHCl₃, and kept for drying.

Scanning electron microscopy images of polymers deposited on Au-coated glass slides were recorded with Nova NanoSEM 450 microscope of FEI (USA).

Powder X-Ray diffraction (XRD) data were collected on Empyrean diffractometer (PANalytical, The Netherlands). Measurements employed Ni-filtered Cu K α radiation of a copper sealed tube charged with 40 kV voltage and 40 mA current and Bragg-Brentano geometry with beam divergence of 1 deg. in the scattering plane. Diffraction patterns were measured in the range of 4-50 degrees of scattering angle by step scanning with step of 0.02 degree.

Electrochemical impedance spectra of deposited films were recorded in solution containing 5 mM K₃Fe(CN)₆/5 mM K₄Fe(CN)₆ redox probe and 0.1 M KNO₃ as supporting electrolyte. All measurements were performed using SP-300 potentiostat/galvanostat of Bio-Logic (France) equipped with frequency response analyzer module. The impedance spectra were recorded at

open circuit potential with 10 mV potential amplitude and frequency in the range from 500 kHz to 50 mHz.

Polarization modulated reflection-absorption IR spectroscopy (PM-IRRAS) measurements were performed using Vertex 80v spectrophotometer of Bruker (Germany) equipped with PMA accessory with nitrogen-cooled MCT detector. Sample was illuminated at the angle of 83°. Diffuse reflectance IR spectra were recorded with Tensor II spectrophotometer of Bruker equipped with Praying Mantis accessory of Harrick (U.S.A.) and DLaTGS detector.

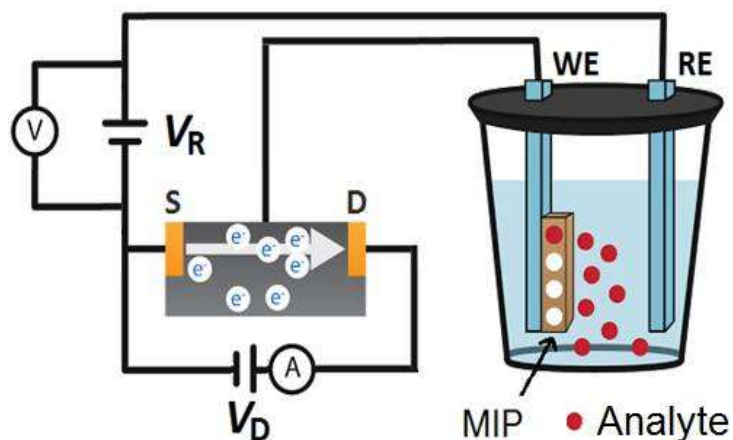
The extended-gate field effect transistor (EG-FET) sensing setup consisted of two main units (Scheme S1). The recognition unit was made of a thin MIP-NGAL (or surface developed MIP on MOF) film with the surface area of 21 mm², deposited electrochemically on Au-glass slide. This extended recognition unit was electrically connected to the transduction unit, i.e., the gate of a commercial MOSFET model CD4007UB. A Keithley 2636A (Keithley Instruments, Inc. OH, USA) dual-channel source meter was used for recording the MIP- or NIP-coated EG-FET characteristics.

Table S1. Binding pockets of the NGAL protein with their volumes, depths, drug scores, and docking scores determined by examination of docking of **2** in these pockets.

Pocket symbol	Volume [nm ³]	Depth [nm]	Drug score	Docking score [kJ/mol]
P0	0.393	1.382	0.65	-28.9
P1	0.315	1.060	0.51	-25.9
P2	0.218	1.090	0.43	-21.8
P3	0.164	1.029	0.35	-22.6
P4	0.134	0.972	0.32	-5.0
P5	0.130	0.845	0.28	7.1
P6	0.120	0.819	0.25	7.9

Table S2. Analytical parameters of the EG-FET based MIP-NGAL chemosensor for two different gate voltages.

Analytical parameter	Gate voltage	
	1.5 V	2.0 V
Limit of detection (LOD) for thin MIP (at S/N = 3)	0.27 μM	0.24 μM
Limit of detection (LOD) for the MIP-MOF (at S/N = 3)	0.12 μM	0.15 μM
Sensitivity, thin MIP	2.24 nA μM^{-1}	1.06 $\mu\text{A } \mu\text{M}^{-1}$
Sensitivity, MIP-MOF	5.10 nA μM^{-1}	2.33 $\mu\text{A } \mu\text{M}^{-1}$
Sensitivity, thin NIP	2.08 nA μM^{-1}	1.14 $\mu\text{A } \mu\text{M}^{-1}$
Sensitivity, NIP-MOF	2.29 nA μM^{-1}	1.04 $\mu\text{A } \mu\text{M}^{-1}$
Apparent imprinting factor (AIF) for thin MIP film	1.08	0.93
Apparent imprinting factor (AIF) for surface enhanced MIP film	2.22	2.23



Scheme S1. A sketch of the experimental setup based on the EG-FET design. An Au-plated glass slide coated with the NGAL-templated MIP or NIP, both thin, and surface developed films, was used as the gate (working electrode, **WE**), and a Pt wire as the reference electrode (**RE**). The **G**, **D**, and **S** symbols stand for the gate, drain, and source components of the FET structure, respectively.

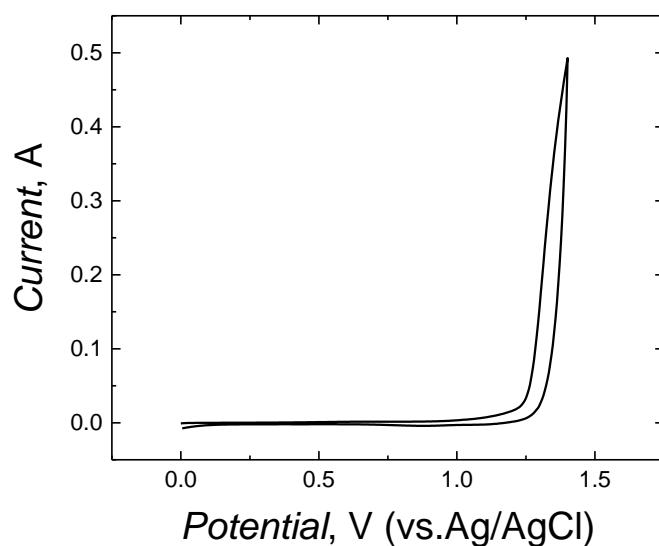


Figure S1. Electropolymerization of 2,2'-bithiophene-5-carboxylic acid **1** in acetonitrile under potentiodynamic conditions resulting in deposition of a poly(2,2'-bithiophene-5-carboxylic acid) supporting layer for the MOF growth. Concentration of **1** was 2 mM. The potential scan rate was 50 mV s⁻¹.

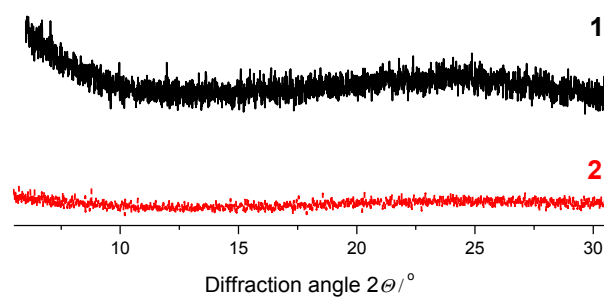


Figure S2. A powder X-ray diffraction (PXRD) patterns for (1) Au-coated glass slide and (2) poly(2,2'-bithiophene-5-carboxylic acid) supporting layer deposited on Au-coated glass slide.

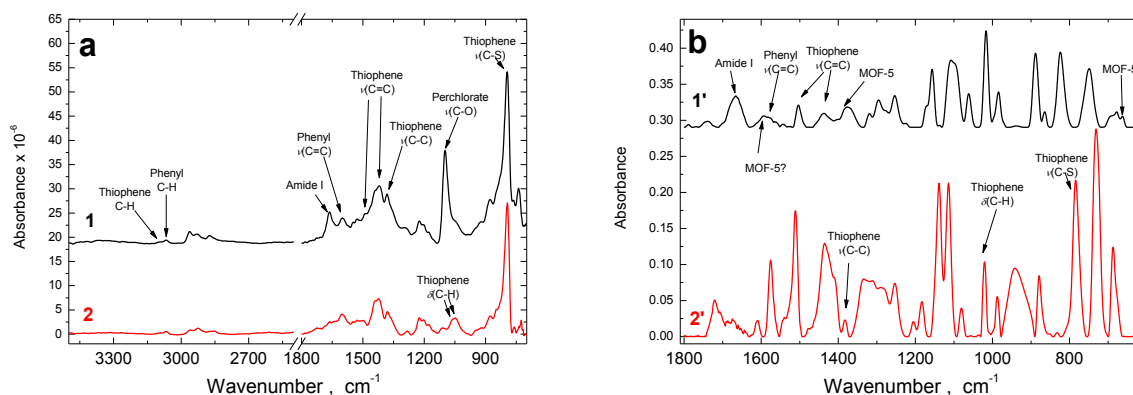


Figure S3. (a) PM-IRRA spectra of thin (1) NGAL templated MIP and (2) NGAL extracted MIP films electrodeposited on Au-coated glass slides recorded at 83° incidence angle. (b) Diffuse reflectance FTIR spectra of surface enhanced (1') NGAL templated MIP and (2') NGAL extracted MIP films electrodeposited on MOF-5 film grown on Au-coated glass slides.

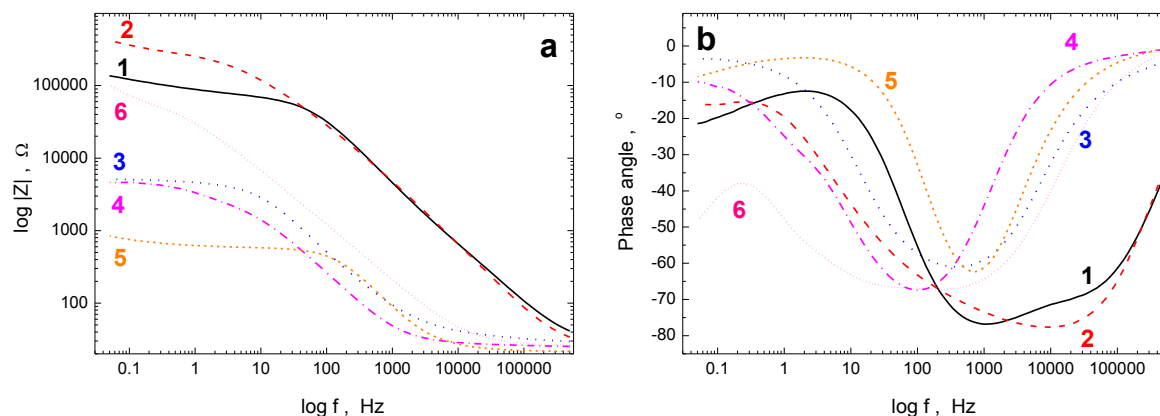


Figure S4. (a) Impedance modulus and (b) phase angle plots for (1) extracted MIP and (2) NIP films deposited directly on Au slide, as well as (3) extracted MIP and (4) NIP film deposited on MOF-modified Au slide. Bode plot for clean Au slide (5) and MOF-5 grown on Au slide (6) are added for comparison. Impedance spectra were recorded at open circuit potential in 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ /5 mM $\text{K}_4\text{Fe}(\text{CN})_6$ solution in 0.1 M KNO_3 . Applied potential frequency ranged from 500 kHz to 50 mHz with 10 mV potential amplitude.

Bibliography:

1. Pietrzyk A.; Suriyanarayanan S.; Kutner W.; Maligaspe E.; Zandler M. E.; D'Souza F., Molecularly Imprinted Poly[bis(2,2'-bithienyl)methane] Film with Built-in Molecular Recognition Sites for a Piezoelectric Microgravimetry Chemosensor for Selective Determination of Dopamine. *Bioelectrochemistry*, **2009**, 80, 62-72.