Supporting information

Biotinyl moiety-selective polymer films with highly ordered macropores

Subramanian Suriyanarayanan, Luigi Petrone, Thomas Ederth and Ian A. Nicholls

Bioorganic & Biophysical Chemistry Laboratory, Linnaeus University Centre for Biomaterials Chemistry, Linnaeus University, SE-391 82 Kalmar, Sweden.
Division of Molecular Physics, Department of Physics, Chemistry and Biology, Linköping University, SE-581 83 Linköping, Sweden.
Department of Chemistry - BMC, Uppsala University, Box 576, SE-751 23 Uppsala, Sweden

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1 Materials and methods

1.1 Chemicals

Monodisperse polystyrene, latex beads (in diameters of 0.1, 0.3 and 0.8 µm and as 10 wt% aqueous solutions or suspensions), biotin (1), p-aminobenzoic acid (2), pyrrole (3), sodium sulfate, sodium chloride, phosphoric acid, sodium hydroxide, and sulfuric acid were purchased from Sigma-Aldrich (Steinheim, Germany). Biotin methyl ester (4) has been prepared by a procedure reported elsewhere. Hydrogen peroxide (30%) was obtained from Fluka (Buchs, Switzerland). Ultrapure water (resistance value < 18.2 MΩ) was obtained using a Milli-Q gradient water filtration system (Millipore, MA, USA), and was used for solution preparation and rinsing of substrates.

1.2 Instrumentation and protocols

1.2.1 Electrochemistry and quartz crystal microbalance studies

Cyclic voltammetry (cv) was used for sensor fabrication (see section 1.2.5) employing a potentiostat, (Reference 600, Gamry Instruments, Warminster, PA, USA) which was controlled using the software supplied by the manufacturer. The Ag|AgCl electrode used as the reference electrode and the platinum wire acting as the counter electrode were both procured from Gamry Instruments.

Piezoelectric microgravimetric measurements were accomplished with an Attana Cell 200 quartz crystal microbalance (QCM) system (Attana AB, Stockholm, Sweden). The QCM was equipped with a dual channel flow injection analysis (FIA) setup and was controlled with software supplied by the manufacturer. AT-cut, polished quartz resonators (10 MHz, 8 mm diameter, Attana AB, Stockholm, Sweden), sputtered with 140 nm gold on both the sides (adhered with an underlying layer of 10-nm Ti or Cr) were used both as the working electrode and sensor substrate. Substrates were cleaned by immersion in piranha solution (1:3, v/v; H₂O₂:H₂SO₄) for 1 min (caution: piranha solution reacts violently with organic compounds).
and is dangerous in contact with the skin or eyes). The substrate was then rinsed exhaustively with ultrapure water, dried under a stream of N₂ gas and stored under vacuum.

1.2.2 Reflection absorption infrared spectroscopy (RAIRS)

RAIR spectra of the polymer film coated Au/quartz surfaces were recorded on a Bruker Hyperion 3000 IR microscope coupled to a Tensor 27 IR spectrometer and computer-steered sample stage. The infrared beam was double surface reflected at angles of 52° and 83° to the surface normal using a grazing angle objective. The spectra were obtained from acquisitions of 1000 interferograms collected using a single element mercury-cadmium-telluride (MCT) detector with a resolution of 4 cm⁻¹. Throughout measurements, the sample chamber was maintained under an inert atmosphere by purging with nitrogen gas at positive pressure. A three-term Blackmann–Harris apodization function was applied to the interferograms, prior to the Fourier transformation. An unmodified Au/quartz resonator was used to measure the background spectra.

1.2.3 Scanning electron microscopy (SEM)

SEM analyses were performed using a Leo 1550 Gemini instrument equipped with a field emission electron gun. The polymer particles were placed on a black carbon tape attached to alumina stubs and coated with a thin layer of platinum by a platinum sputtering unit (LEICA EM SCD 500) before being inserted in the SEM instrument. A 3 kV potential was applied to the electron gun to generate the electron beam used to scan the polymer particles.

1.2.4 Flow injection analysis (FIA)

The analytical performance of the polymer film coated Au/quartz resonators was evaluated under flow injection analysis (FIA) conditions. The substrates were mounted in the flow cell holders provided by the manufacturer. Phosphoric acid solution (10 mM containing 150 mM of NaCl at pH 3.3) was used as the carrier buffer. A dual piston peristaltic pump, in-built within the QCM instrument, was used to pump the carrier buffer solution over the
polymer film-coated substrate at the desired flow rate. The polymer film was equilibrated with the buffer solution under these conditions until a change of $\leq \pm 0.5$ Hz in the resonant frequency was obtained for a period of at least 400 seconds. An aliquot (180 $\mu$L) of the analyte in the carrier buffer was injected into the flow cell using the 6-point injection valve provided in the instrument.

1.2.5 Sensor fabrication and characterization

Cell chip holders (Attana AB, Stockholm, Sweden) were clamped horizontally and loaded with the piranha-cleaned Au/quartz (working electrode) substrate facing upwards. Aqueous polystyrene bead solution (25 $\mu$L of 0.25 %) was placed on top of the gold surface. The solvent (water) was then allowed to evaporate by placing the setup in a desiccator (12 h). The surface was then flushed with nitrogen gas and used as the working electrode for electropolymerization. Polymer films were prepared by electropolymerization on the bead-covered gold-coated quartz transducers (Au/quartz). Initially, pre-polymerization solutions were prepared by mixing template (1), cross-linkable functional monomers (2) and (3) in the ratio 1 : 4 : 25, dissolved in ultrapure water containing 0.23 M of Na$_2$SO$_4$ (supporting electrolyte). pH of this solution was adjusted to 3 using 0.5 M H$_2$SO$_4$ The solution was allowed to equilibrate (5 min). A drop (100 $\mu$L) of the pre-polymerization solution was placed over the working electrode. The counter and reference electrodes were then placed in contact with the drop solution close to the gold surface, though without touching each other.

The MIP recognition film was deposited by scanning the potential from -0.5 to 1.45 V at 50 mV/s. The growth of the polymer could be governed by regulating the number of cycles. Subsequently, the polymer film was rinsed in ultrapure water to remove physisorbed species. The sacrificial polystyrene bead layer was removed by soaking the polymer film in dry toluene for 12 h. The biotin template was extracted from the MIP film with 5 mM NaOH for 6
h. A reference polymer film was prepared in the absence of biotin (1) and/or the sacrificial polystyrene beads using the procedure described above.
Scheme 1-SI. Schematic representation of self-assembled sacrificial bead (polystyrene bead)-layered molecularly imprinted polymers on Au-coated quartz surfaces.
Scheme 2-SI. Structures of the template (Biotin, 1) monomers: \(p\)-aminobenzoic acid (4-ABA) (2) and pyrrole (3) employed in the polymer film preparation. Analyte used to evaluate the sensor performance: biotin methyl ester (BtOMe, 4).
**Figure 1-SI.** Scanning electron micrographs of 100 nm-diameter polystyrene bead-layered on Au/quartz surfaces.
Figure 2-SI. Cyclic voltammograms recorded for the electrochemical co-polymerization of 16 mM of (2) and 100 mM of (3) in the (i) presence and (ii) absence of 4 mM biotin, in 0.2 M Na₂SO₄ aqueous solution at pH 3.0 on the (A) unmodified, (B) 100 nm, (C) 300 nm and (D) 800 nm-diameter sacrificial (non-conducting) polystyrene bead coated Au/quartz electrodes. Potential scan rate was 0.05 V/s. Curves 1 and 2 are from the first and fifth cycles of the cyclic voltammogram, respectively, illustrating the polymer growth on each surface.
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Figure 3-SI. Scanning electron micrographs of an electrochemically deposited biotin-imprinted polymer film grown through (A) 100, (B) 300 and (C) 800 nm diameter self-assembled polystyrene bead layers on Au/quartz surfaces. The polymer film was prepared as shown in Scheme 1-SI and Figure 2-SI
Figure 4-SI. Surface profile of the biotin imprinted polymer film grown with 800 nm diameter polystyrene bead layers. Point ‘A’ refers the polymer film coated on Au/quartz resonator and ‘B’ refers to the quartz surface. Inset is the picture of the electrodeposited polymer film on Au/quartz surface. The black line in the inset indicates the path travelled by the profilometer probe.
Figure 5-SI. (A) QCM trace for the gold-coated quartz resonator coated with biotin- (i) imprinted and (ii) non-imprinted (REF) electropolymerized film grown in the presence of 100 nm diameter polystyrene beads, upon injection of analyte (4) under FIA conditions. Concentration of the analyte injected is indicated on the trace. (B) Corresponding FIA calibration plots for (6) on (i) MIP (ii) REF films. Volume of the injected analyte was 180 µL. Phosphoric acid solution (0.01 M) containing 150 mM NaCl at pH = 3.3 was used as carrier solution (flow rate of 100 µL/min). MIP films were prepared as shown in Scheme 1-SI and Figure 2-SI.
Figure 6-SI. Resonant frequency change with time, upon injection of 10 mM analyte (6) under FIA conditions (section 1.2.4-SI), for biotin imprinted polymer films electrodeposited on Au/quartz surface (i) with and (ii) without 100-nm diameter sacrificial bead layer.
**Figure 7-SI.** FIA calibration plots for (1) BtOMe, (2) thiamine, (3) pyridoxamine, and (4) urea for the biotin-imprinted electropolymerized film, grown through 800-nm diameter polystyrene bead layer Au/quartz surface. Volume of the injected analyte was 180 µL. Phosphoric acid solution (0.01 M) containing 150 mM NaCl at pH = 3.3 was used as the carrier solution (flow rate of 20 µL/min). MIP films were prepared as shown in Scheme 1-SI and Figure 2-SI. Sensitivity of the MIP film toward each analyte is given within parentheses.
**Figure 8-SI.** Resonant frequency changes (three injections, error bars reflect standard error of the mean) for the FIA-binding (see section 1.2.4-SI) of BtOMe on the biotin-MIP film grown through 300-nm diameter polystyrene bead layer, BtOMe concentrations used were 5 mM (red) and 10 mM (blue). The MIP-sensor chips were stored in the dry state between measurements.

**Table 1-SI** Mass of the biotin imprinted polymers on the sensor surfaces as determined by piezoelectric microgravimetric analysis.

<table>
<thead>
<tr>
<th>Sensor Surface</th>
<th>Total mass of deposited film, µg</th>
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<tbody>
<tr>
<td>Au/quartz/MIP</td>
<td>3.11 ± 0.12^a</td>
</tr>
<tr>
<td>Au/quartz/100-nm latex bead/MIP</td>
<td>2.54 ± 0.10</td>
</tr>
<tr>
<td>Au/quartz/300-nm latex bead/MIP</td>
<td>1.38 ± 0.04</td>
</tr>
<tr>
<td>Au/quartz/800-nm latex bead/MIP</td>
<td>0.80 ± 0.09</td>
</tr>
</tbody>
</table>

^a standard error of the mean

**References-SI**
