Electronic Supplementary Material (ESI) for Analyst.

SUPPORTING INFORMATION

Signal Enhancement in Ligand-Receptor Interactions using Dynamic Polymers at Quartz Crystal Microbalance Sensors

Gunnar Dunér,[a,b] Henrik Anderson,[b,c] Zhichao Pei,[b] Björn Ingemarsson,[b] Teodor Aastrup,[b] and Olof Ramström [a,]*

[a] Department of Chemistry, KTH - Royal Institute of Technology, Teknikringen 30, S-10044, Stockholm, Sweden. [b] Attana AB, Björnnäsvägen 21, S-11419, Stockholm, Sweden. [c] Uppsala University, Ångström Laboratory, Solid State Electronics, P.O. Box 534, S-75121 Uppsala, Sweden.
ramstrom@kth.se

General

Acrylic acid (AAc) from Fluka, sodium diethyldithiocarbamate trihydrate (NaDEC) from Aldrich, and poly(vinylbenzyl chloride) (PVBC) from Aldrich (60/40 mixture of 3- and 4- isomers, average M_n ~55,000 and average M_w ~100,000 as determined by GPC), were used as received. ^{1}H-NMR spectra were recorded with a Bruker DMX 500 instrument at 298K in $CDCl_3$, using the residual signal from $CHCl_3$ (^{1}H: δ = 7.28 ppm) as internal standard. Photopolymerization was performed with a Lightningcure LC8 photosource equipped with a Hg-Xe UV-lamp from Hamamatsu Photonics. QCM experiments were performed with Attana 100, Attana 200 and A100 instruments from Attana, Stockholm, Sweden. Gold-plated QCM crystals, and crystals coated with carboxyl-terminated SAMs (Attana Carboxyl Surface), were from Attana. Spin-coated crystals were prepared using a Spin Coater, model P6700 series (Specialty Coating Systems). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 3-sulfo-*N*-hydroxysuccinimide (sulfo-NHS) for immobilization were from Attana. (+)-Biotinyl 3,6,9-trioxaundecanediamine used for functionalization was from Molecular BioSciences. Anti-biotin F_{ab} fragments [Goat] and lysozyme for interaction studies were purchased from Rockland and Sigma respectively. Acetic acid and ethanolamine used in the functionalization step were from Sigma, as were sodium hydroxide and hydrochloric acid for adjusting pH-solutions.

Synthesis of iniferter-derivatized poly(vinylbenzyl chloride) ($PVBD_{501}$)

PVBC (1.0 g, 6.6 mmol) and NaDEC (30 mg, 0.13 mmol) were dissolved in THF (50 mL) and stirred at ambient temperature overnight. After evaporation, the resulting material was dissolved in chloroform (75 mL) and water-soluble substances were extracted twice with deionized water. Evaporation and drying *in vacuo* at 40 °C yielded 0.95 g (95%) of $PVBD_{501}$ as a film. ^{1}H-NMR ($CDCl_3$): δ 7.07-6.49 (m, 20H, C_6H_4), 4.45 (s, 10H, C_6H_4-CH_2), 4.01 (m, 2H, NCH_2), 3.72 (m, 2H, NCH_2), 2.2-1.2 (m, 21H, $CHCH_2$, $CHCH_2$, CH_2CH_3)

Fabrication of sensor surfaces

AAc (1350 µL, 19.7 mmol) was dissolved in a 1:1 (v/v) mixture (50 mL) of deionized water and ethanol, which was deaerated prior to polymerization. Following spin coating of $PVBD_{501}$,[1] the

QCM crystals were placed in Pyrex glass vessels with the monomer feed solution, and a stream of nitrogen was bubbled through the solution. Polymerization was initiated by UV-irradiation at a measured intensity of 13.3-13.5 mW/cm^2 at 360 nm, and irradiation was allowed to proceed for 70 min. The crystals were thoroughly rinsed in deionized water to remove any unreacted monomer.

Derivatization of the pAAc- and carboxyl-terminated SAM sensors with (+)-biotinyl 3,6,9-trioxaundecanediamine was carried out *in situ*. An activation solution containing EDC (0.2 M) and sulfo-NHS (0.5 M) was first injected over the sensor surfaces, immediately followed by a solution of the biotin derivative (0.24 mM) in acetate buffer (10 mM, pH 5.0). Residual sulfo-NHS esters were subsequently deactivated using an aqueous ethanolamine solution (1 M) with pH adjusted to 8.5 using HCl.

QCM analyses

For all QCM experiments a modified sample loading loop of 400 µL and a flow rate of 25 µL/min were used. The running buffer was prepared from a stock solution of HBS-T 10X buffer (100 mM HEPES-HCl, 1.5 M NaCl, 0.05% Tween 20, pH 7.4, Attana), diluted 1:9 (v/v) with milliQ-water, pH-adjusted and deaerated prior to use. Resistance measurements were carried out with a modified Attana 200 system.[2] Evaluation of the biotin-functionalized QCM sensors was performed using F_{ab} fragments (25 µg/mL) of affinity-purified anti-biotin antibodies diluted in running buffer. Regeneration of the sensors was performed with glycine buffer (10 mM) adjusted to pH 1.5 with HCl. A signal drift of < 0.2 Hz·min^{-1} was recorded for the pAAc sensor surfaces, indicating high stabilities during the experiments.

pH-dependence of pAAc surfaces

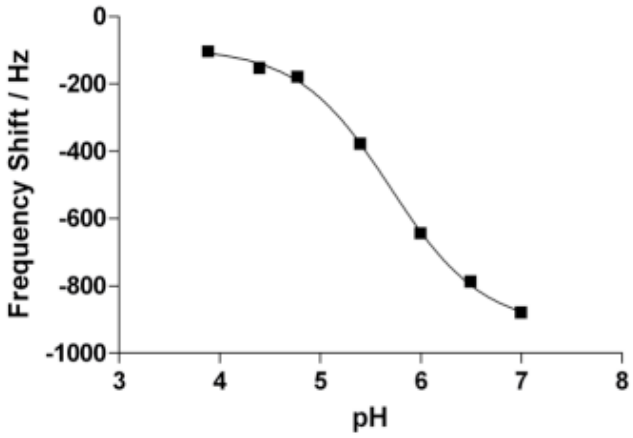

Figure S1. Typical frequency response for 100% pAAc-functionalized sensor surfaces. Analyzed using an Attana A200 instrument. Sensors were stabilized in acidified PBS buffer solution at a typical flow rate of 25 µL·min^{-1}, until a drift < 0.2 Hz·min^{-1} was reached. PBS-samples of increasing pH were subsequently injected and the responses recorded. Apparent pK_a = 5.16 ± 0.27 (n = 3).

References

1. G. Dunér, H. Anderson, A. Myrskog, M. Hedlund, T. Aastrup and O. Ramström, *Langmuir*, 2008, **24**, 7559–64.
2. H. Anderson, G. Wingqvist, T. Weissbach, D. Wallinder, I. Katardjiev and B. Ingemarsson, *Sensors Actuators B Chem.*, 2011, **153**, 135–144.