SUPPORTING INFORMATION

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

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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. 1H-NMR spectra were recorded with a Bruker DMX 500 instrument at 298K in CDCl3, using the residual signal from CHCl3 (1H: 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 Fab 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) (PVBD501)

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 PVBD501 as a film. 1H-NMR (CDCl3): δ 7.07-6.49 (m, 20H, C>H), 4.45 (s, 10H, C>H-C>H), 4.01 (m, 2H, NC>H), 3.72 (m, 2H, NC>H), 2.2-1.2 (m, 21H, C>HCH, CHC>H, CH2CH3)

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 PVBD501, 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/cm² 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-trioxaundecanediame 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. Evaluation of the biotin-functionalized QCM sensors was performed using F\textsubscript{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\textsuperscript{-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\textsuperscript{-1}, until a drift < 0.2 Hz·min\textsuperscript{-1} was reached. PBS-samples of increasing pH were subsequently injected and the responses recorded. Apparent pK\textsubscript{a} = 5.16 ± 0.27 (n = 3).](image)

References