Supporting Information

Detection of the Carcinogenic Water Pollutant Benzo[a]pyrene with an Electro-Switchable Biosurface

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Synthesis of B[a]P-peptide conjugate

65 mg (0.029 mmol, 1 equiv.) of rink-amide resin (NovaPeg, Novabiochem, Merck, Darmstadt, Germany) were pre-swelled for 1 h in in 5 mL DMF, followed by three subsequent washing steps with 3 mL DMF. First, the peptide linker was synthesized and stored attached to the resin for subsequent coupling of B[a]P. The peptide sequence Lys-Gly-Ser-Gly was produced by pooling the corresponding Fmoc- and/or side chain-protected amino acid (0.143 mmol, 5 equiv.), 49.8 μL (0.286 mmol, 10 equiv.) *N,N'*-diisopropylethylamine (DIPEA), 3 mL DMF and 54.2 mg (0.143 mmol, 5 equiv.) of *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluroniumhexafluorophosphat (HBTU, Merck Millipore). The solution was then added to the pre-swelled resin. The mixture was bubbled under a gentle stream of nitrogen for 1 h and then rinsed 5 times with 3-mL portions of DMF. First, Fmoc-and Boc-protected lysine was coupled to the resin, starting at the carboxyl-end of the peptide chain. To check the coupling of the Fmoc-protected amino acid, a TNBS test (trinitrobenzenesulfonic acid solution, 1% in DMF, Sigma-Aldrich) was carried out. For this purpose, a small aliquot of the resin was removed from the column and reacted with a solution of TNBS + DIPEA (2 droplets of a 1% (v/v) TNBS in DMF plus same volume of a 10% (v/v) DIPEA in DMF). If coupling was complete, resin beads presented a transparent appearance, otherwise they turned red. In the case of the later, the coupling was performed second time. After satisfactory coupling, the Fmoc group was cleaved for further coupling with the next amino acid (in sequence Fmoc-Lys(tBu)-OH, Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-glycin, all from Merck Millipore) by bubbling 3 mL of a 14% (v/v) piperidine in DMF solution for 20 min. The TNBS test was also carried out on the deprotected resin beads after thorough washing with five 5-mL portions of DMF. In this case, deprotection was judged complete when the resin beads turned red immediately with the TNBS solution. After the last amino acid was coupled, the resin was successively rinsed several times with DMF, methanol and DCM.

For coupling of the hapten to the deprotected peptide resin, 339 mg (0.0289 mmol (1 equiv.)) of B[a]P butyric acid was dissolved in 3 mL DMF and 54.2 mg (0.143 mmol, 5 equiv.) HBTU was added. The mixture was given to the resin and bubbled with nitrogen for 2 h. After coupling, the resin was successively washed three times with 9 mL DMF, DCM and methanol, respectively, and dried under vacuum overnight. For cleavage and deprotection, the derivatized resin beads were worked up at different concentrations of trifluoroacetic acid (TFA). First, the resin was slurried for ca. 10 min with 5 mL of TFA/TIS (triisopropylsilane/water/DCM mixture (10:2.5:2.5:85, v/v). The solution was dropwise (10 droplets per min) collected in a 50-mL flask with tapering neck. Next, five times 2 mL of TFA/TIS/water/DCM (5:2.5:2.5:90, v/v) were allowed to percolate slowly through the resin and pooled with the previous 5 mL. The solution was evaporated to ca. 1.5 mL with a gentle stream of nitrogen. Finally, 5 mL of a more concentrated TFA/TIS/DCM solution (95:2.5:2.5, v/v) was given to the resin and allowed to stand for 30 min. The solution was pooled together with the two fractions and evaporated slowly by a nitrogen stream. The brownish residue was three times washed with 3 mL of cold diethyl ether and the white precipitate dried under vacuum.

Purification was performed by semi-preparative HPLC. Gradient elution was applied on a C18 column (Gemini, 5 µm, 150 x 10 mm ID, Phenomenex, Aschaffenburg, Germany) using methanol/water, containing 0.1% TFA, at a flow rate of 4.7 mL/min. The UV absorption was monitored at 350 nm (UV-Detector L-4250, Merck Millipore). A linear gradient from 30% methanol to 90% methanol within 12 min, followed by 10 min at 90% methanol was used for separation. The product was collected from 12.5 to 14 min. ESI-MS measurement (Orbitrap Exactive, Thermo Fisher, Bremen, Germany) shows $[M + H]^+ = 667.3214$.

MALDI-TOF-MS measurement of B[a]P-DNA conjugate

The MALDI-TOF-MS measurements were performed with an Ultraflex TOF/TOF (Bruker-Daltronics, Bremen, Germany) in positive ion mode using a 337 nm N_2-laser. 100 µL of the B[a]P-DNA conjugate (10 µM) was desalted using Amicon Ultra-0.5 Centrifugal Filter Devices (Merck Millipore) in three consecutive steps according to manufacturer's instructions and concentrated by a factor of two. 1.5 μL of a mixture of the prepared B[a]P-DNA conjugate, a citric acid solutions (50 mg/mL) and saturated 3-hydroxypicolinic acid (HPA) was applied to an aluminum-target and dried at room temperature before being analyzed by MALDI-TOF-MS.

The MALDI-TOF-MS measurements revealed a molecular mass of 15781 g/mol. Because the theoretical mass of the conjugate was calculated as 15794 g/mol, the peak in the spectrum could clearly be identified as the B[a]P-DNA conjugate. Furthermore, no impurities originating from uncoupled DNA with molecular mass reduced by 668 g/mol, could be observed.

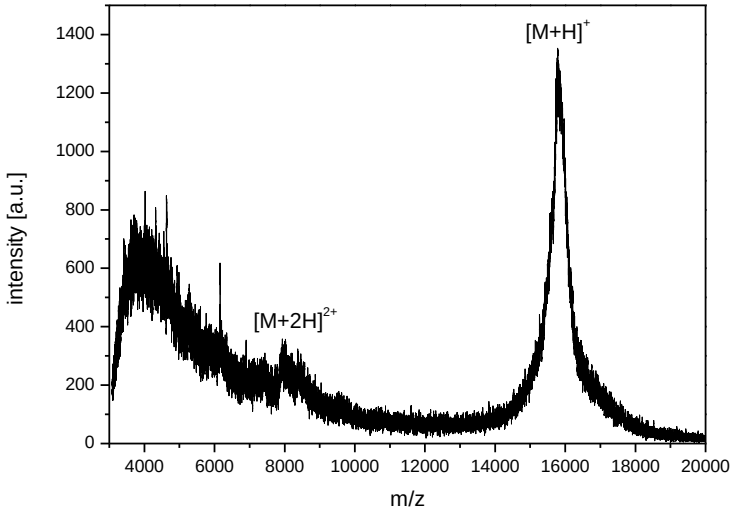

Figure S1. MALDI-TOF-MS measurement of the synthesized B[a]P-peptide conjugate.

Surface plasmon resonance measurements

For surface plasmon resonance (SPR) experiments, the Biacore X100 and Sensor Chip CM5 (GE Healthcare Europe GmbH, Freiburg, Germany) were used. The synthesized B[a]P-peptide was immobilized on the chip surface. Experiments were carried out at 25 °C and with a flow speed of 10 μL/min. Hepes buffered saline, containing 3 mM ethylenediaminetetraacetic acid (EDTA) and 0.05% P20 surfactant (HBS EP+ buffer; GE Healthcare) was used as running buffer. The chip was activated with a mixture (1/1, v/v) of 1-ethyl-3-(3-dimetylaminopropyl)carbodiimide (0.4 M) and *N*-hydroxysuccinimide (0.1 M) for 7 min. Afterwards a 0.01 M solution of B[a]P-peptide in sodium acetate buffer (pH 5.0) was passed over the chip surface for 2 min, causing an immobilization level of ca. 500 refractive units (RU). Finally, unreacted NHS-groups on the chip were deactivated using ethanolamine (1.0 M) for 7 min. The reference cell was activated and deactivated as well. For the

determination of the affinity constant of antibody 22F12, it was passed two times at each concentration of 1, 5, and 10 μg/mL over the chip for 4 min, followed by a dissociation time of 15 min. The regeneration was done by passing 50 mM sodium hydroxide (containing 5% acetonitrile) solution over the chip surface for 2 min. The association (k_a) and dissociation (k_d) rate constants were determined using the 1:1 binding model of the Biacore X100 Evaluation Software.

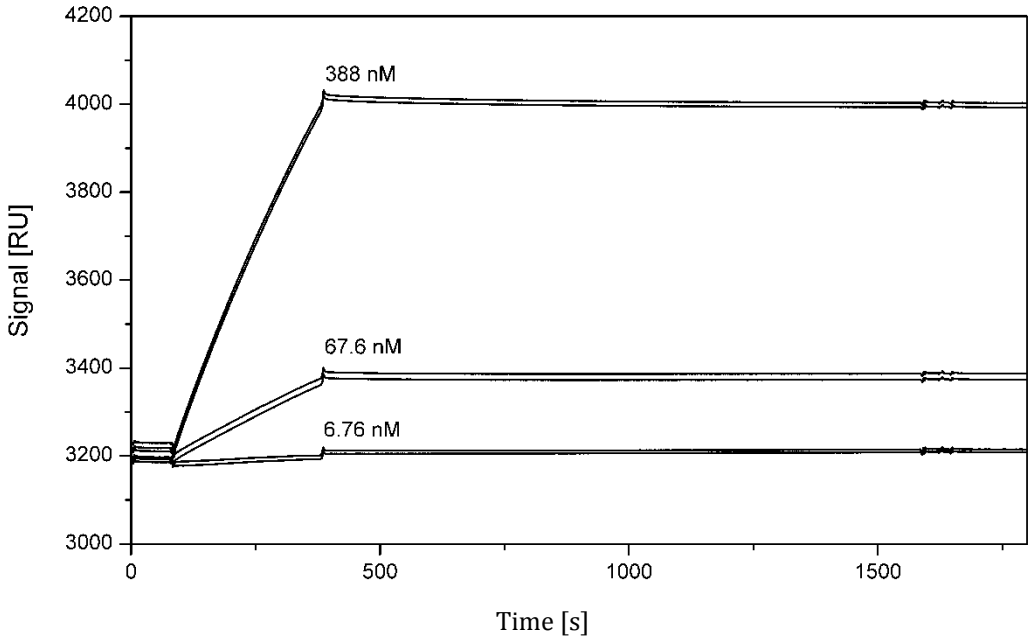

Figure S2. Binding kinetics of anti-B[a]P antibody 22F12 using different molar concentrations of antibody and B[a]P-peptide conjugate immobilized on Sensor Chip CM5.

ESB measurements: different antibody concentrations and reproducibility

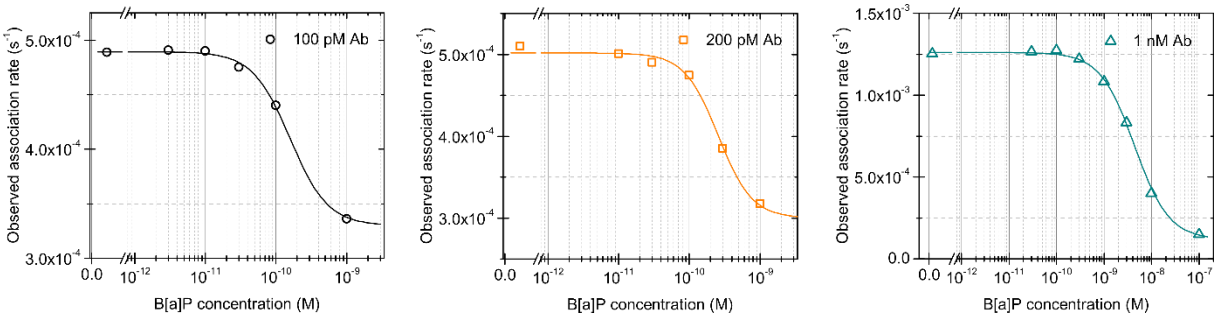

Figure S3. Observed association rate constants (k_{on}^{obs}) as a function of B[a]P concentration for different anti-B[a]P IgG concentrations.

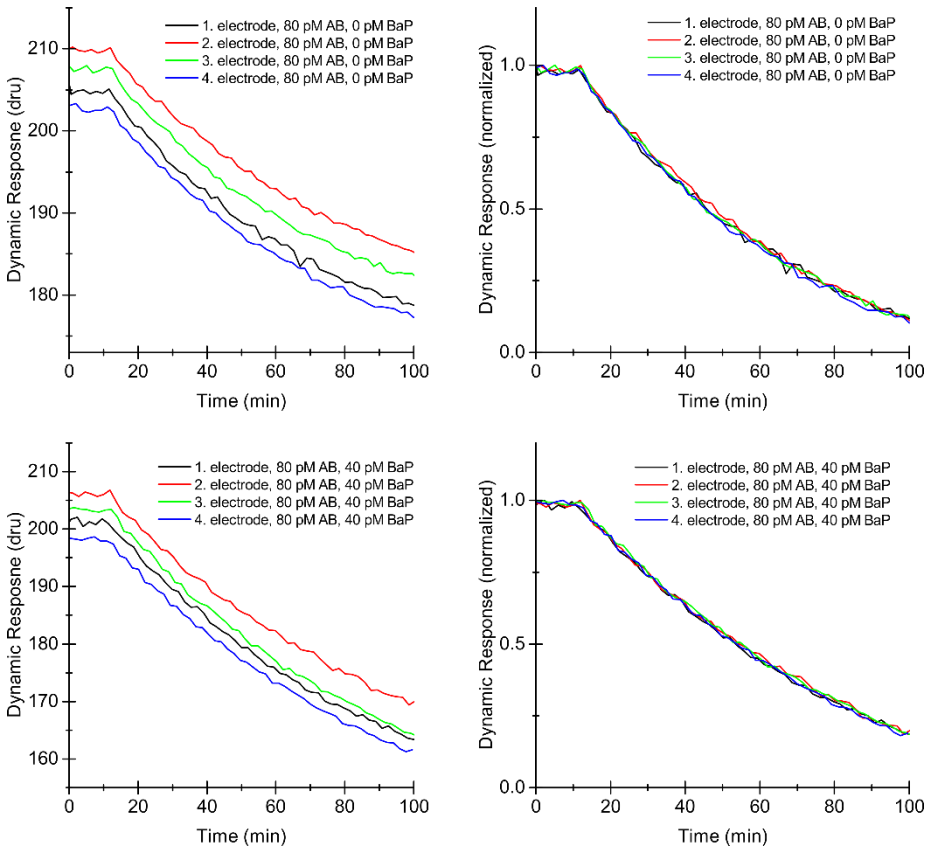

Figure S4. Association kinetics of 80 pM anti-B[a]P antibody to B[a]P-modified DNA layers in the absence (top) and presence (bottom) of solute 40 pM B[a]P.

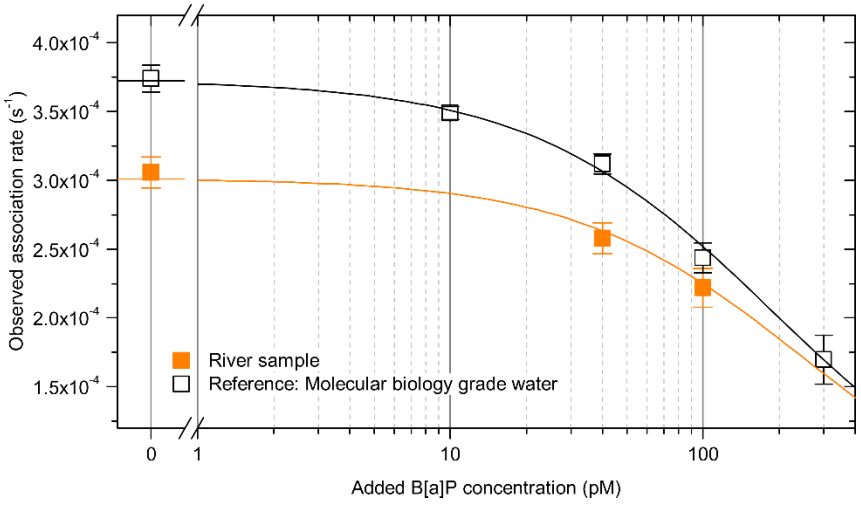

Figure S5. Measurement of a river water sample and molecular biology grade water (reference). The values of the neat samples are shown at "0" concentration, other B[a]P concentrations refer to added amounts. 100 pM anti-B[a]P was used.

Determination of PAHs using the U.S.-EPA method 8270

Table S1: Determination of 16-EPA PAHs in surface water sample using U.S.-EPA method 8270 and calculation of B[a]P-equivalent concentration based on antibody's cross-reactivity

PAH compound	Acro-nym	MW [g/mol]	Antibody cross-reactivity [%]	Concentration determined by method 8270 [ng/L]	B[a]P-equivalent concentration [ng/L]
Naphthalene	Nap	128	0	0	0
Acenaphthene	Acn	154	0	0	0
Acenaphthylene	Acy	152	0	0	0
Fluorene	Flu	166	0	0	0
Anthracene	Ant	178	1	0	0
Phenanthrene	Phe	178	1	12	0.12
Fluoranthene	Fla	202	15	18	2.7
Pyrene	Pyr	202	18	11	1.98
Benzo[a]anthracene	B[a]A	228	13	0	0
Chrysene	Chr	228	77	0	0
Benzo[b]fluoranthene	B[b]F	252	24	0	0
Benzo[k]fluoranthene	B[k]F	252	5	0	0
Benzo[a]pyrene	B[a]P	252	100	0	0
Dibenzo[ah]anthracene	DhA	278	0	0	0
Benzo[ghi]perylene	BgP	276	1	0	0
Indeno[1,2,3-cd]pyrene	IcP	276	45	0	0
					sum: 4.8