Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Miniaturized Sensor for Electroanalytical and Electrochemiluminescent Detection of Pathogens enabled through Laser-Induced Graphene Electrodes embedded in Microfluidic Channels

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Plasma treatment

Substrates were treated with a PlasmaFlecto10 (plasma technology, Herrenberg-Gültstein, Germany) using the following setting: 0.2 mbar process pressure, 90 s gas input time, X min process time, 300 W power, 5 s purge time, 100% O_2. Substrates were treated with a piezobrush® PZ2 or PZ3 (relyon plasma, Regensburg, Germany) positioned a few millimeter above the substrates for the respective time. In case of the PZ3, 100% power was used all the time. Modules/nozzles were chosen according to the substrate material. Substrates were treated with an UVO-Cleaner® Model 144AX-220 (Jelight Company, Irvine, CA, USA) for X minutes using a total time of 15 minutes and an exhaust time of (15-X) minutes.

Determination of carboxylation density

The carboxylation densities on PMMA, Kapton® and LIG were determined using a toluidine blue O (TBO) staining assay adjusted from Nugen *et al.*[1] After plasma treatment, the substrates were incubated for 4 hours with 0.5 mmol L^{-1} TBO solution, pH 10. After removing the TBO solution, the substrates were washed 3-times with Milli-Q water pH 10 and vortexed for 10 s in 400 µL 50 wt% acetic acid to desorb the dye. The solutions were transferred into polystyrene half-micro cuvettes and the absorbance measured at 633 nm using a Cary 50 UV-Visible Spectrophotometer (Varian, Inc.).

Assay on PMMA (not successful)

PMMA was treated for 60 s using a piezobrush PZ3. Afterwards, a mask (holes, Ø 5,64 mm) was applied and the surface activated for 40 min with 40 µL 50 mmol L^{-1} EDC, 100 mmol L^{-1} NHS in 0.1 mol L^{-1} MES buffer pH 5.5. The substrates were subsequently incubated for 3 h with 25 µL 1.5 µmol L^{-1} NH_2-modified capture probe in 10 mmol L^{-1} Tris-HCl pH 7.5 + 1 mmol L^{-1} EDTA, for 1 h with 1% BSA in PBS buffer and for 30 min with 15 µL target DNA in hybridization buffer, always followed by two washing steps with Milli-Q water in between. Finally, they were incubated with 15 µL 1.05 mmol L^{-1} total lipid $Ru(bpy)_3Cl_2$ liposomes in HSS buffer, washed three times with HSS buffer and lysed for 15 min in ECL lysing cocktail. Lysates were transferred into microplates and measured with fluorescence.

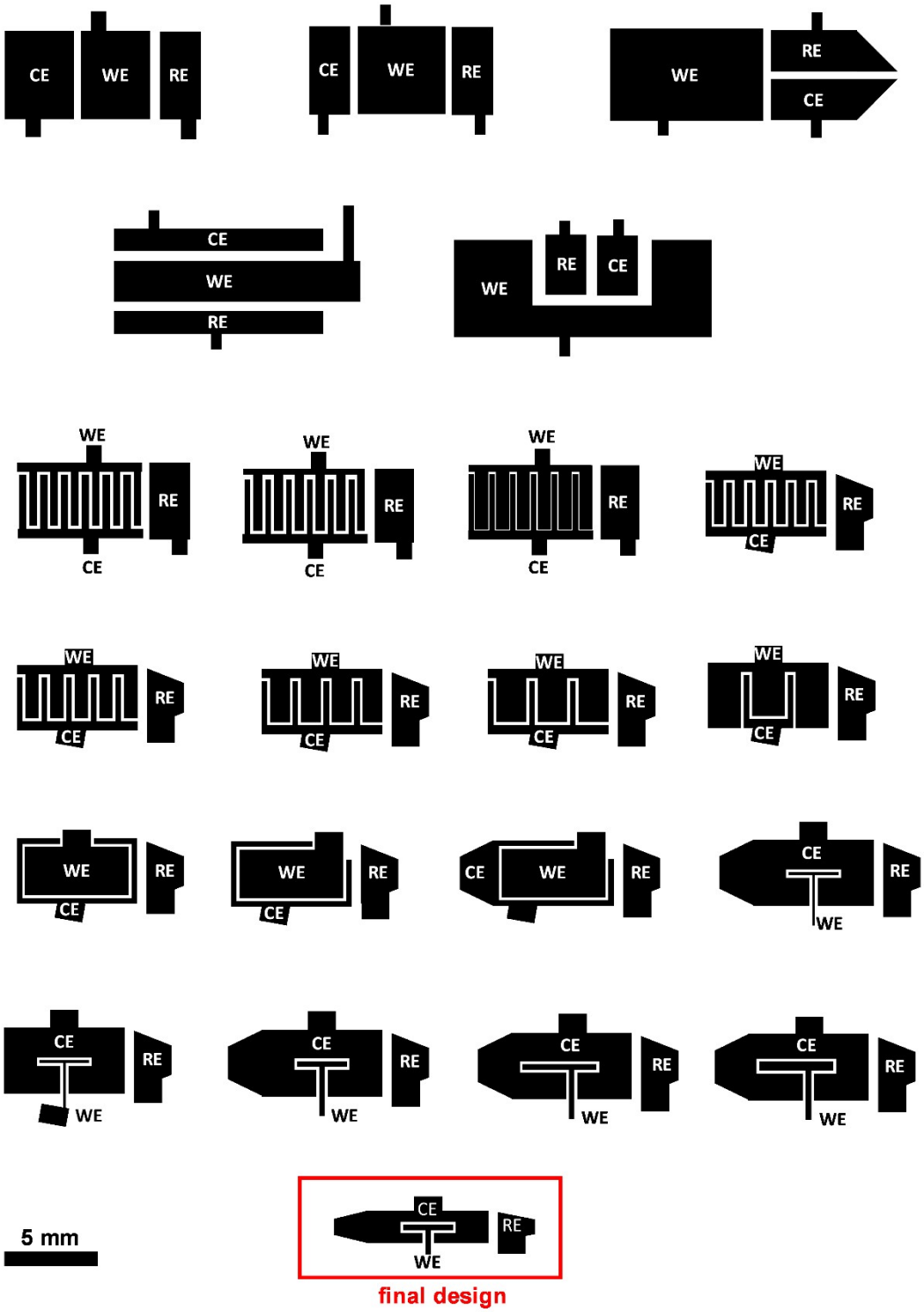

Figure S1. *Overview over all tested electrode layouts in chronological order from top to bottom. The individual electrodes are labeled with working electrode (WE), counter electrode (CE) and reference electrode (RE). Scale bar is 5 mm.*

Table S1. *Material costs for one microfluidic chip in a lab-scale production (without taxes).*

Material	Amount	Price / €
Polyimide (Kapton®)	2.5 cm × 4 cm	0.083
Silver paint	3 µL	0.014
Copper tape	3 mm × 12 mm	0.011
Adhesive tape	2.5 cm × 4 cm	0.013
PMMA	2.5 cm × 4 cm	0.020
Total		0.141

Table S2*. Liposome characterization data.*

liposome encapsulant	Size average / nm	Zeta potential / mV	PdI	Total lipid concentration / mmol L^{-1}	Encapsulant concentration[a] / µmol L^{-1}	Encapsulant concentration outside[a] / µmol L^{-1}
$Ru(bpy)_3Cl_2$	179 ± 4	-25 ± 3	0.08 ± 0.02	10.5 ± 0.6	241 ± 12	43 ± 8
$K_4[Fe(CN)_6]$	198 ± 3	-21 ± 1	0.11 ± 0.02	14.5 ± 0.2	175 ± 36	2.0 ± 0.6

[a] per 1 mmol L^{-1} total lipid concentration

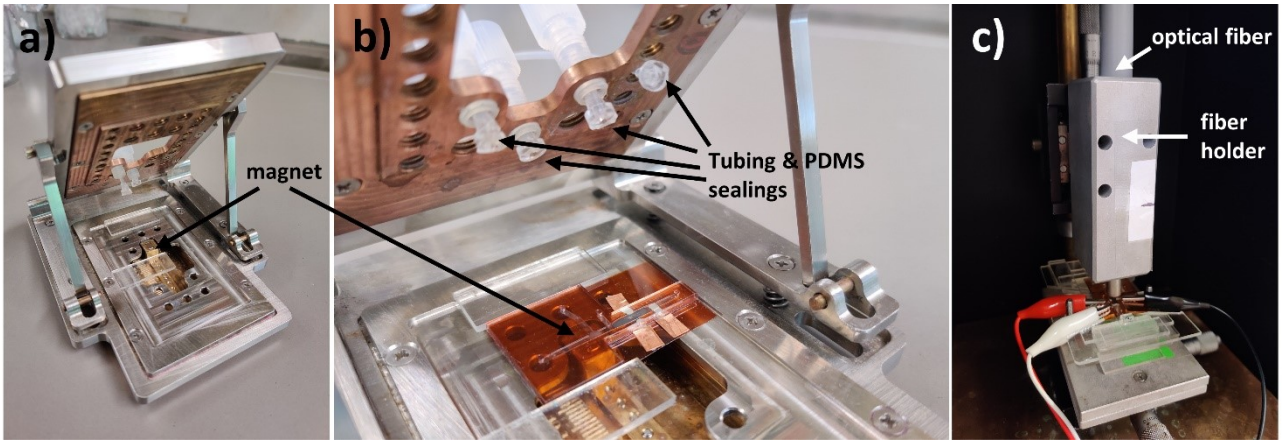

Figure S2. *a) Open chipholder with tubing conncetions and magnet b) Magnification of an open chipholder with inserted microfluidic chip c) Optical fiber positioned above a microfluidic chip with the help of a x-,y-,z-stage. Electrical connection established to the chip through crocodile clamps. Setup is positioned inside a dark box.*

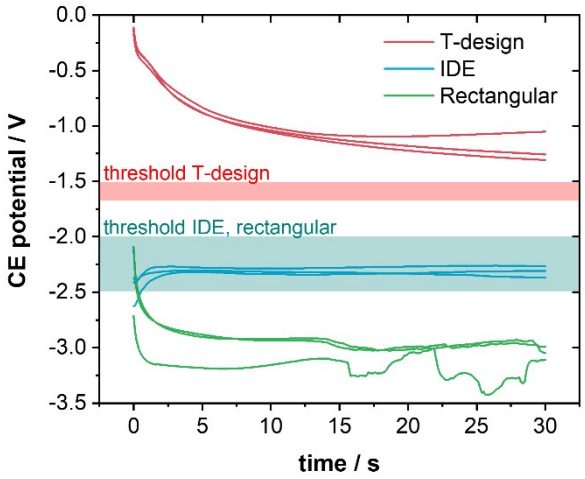

***Figure S3**. Counter electrode potentials of different electrode designs during a 30 s ECL measurement at 1.2 V. Thresholds represent the potentials were visible hydrogen generation occurred at the counter electrode during a CV scan with 50 mV s^{-1} (n=3).*

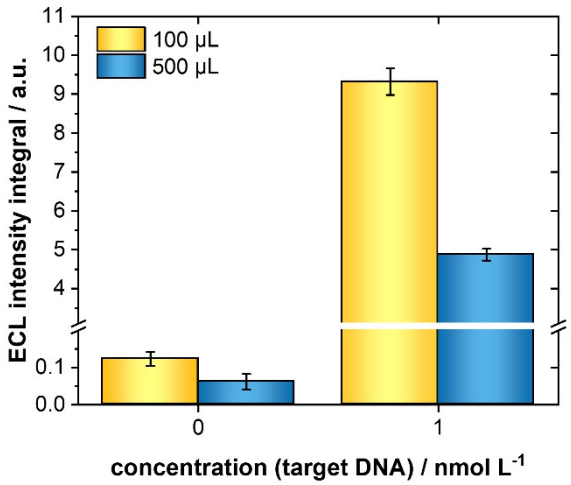

***Figure S4**. ECL responses from one-step assays with different incubation (and injection) volumes, while maintaining constant amounts of reagents (n=3).*

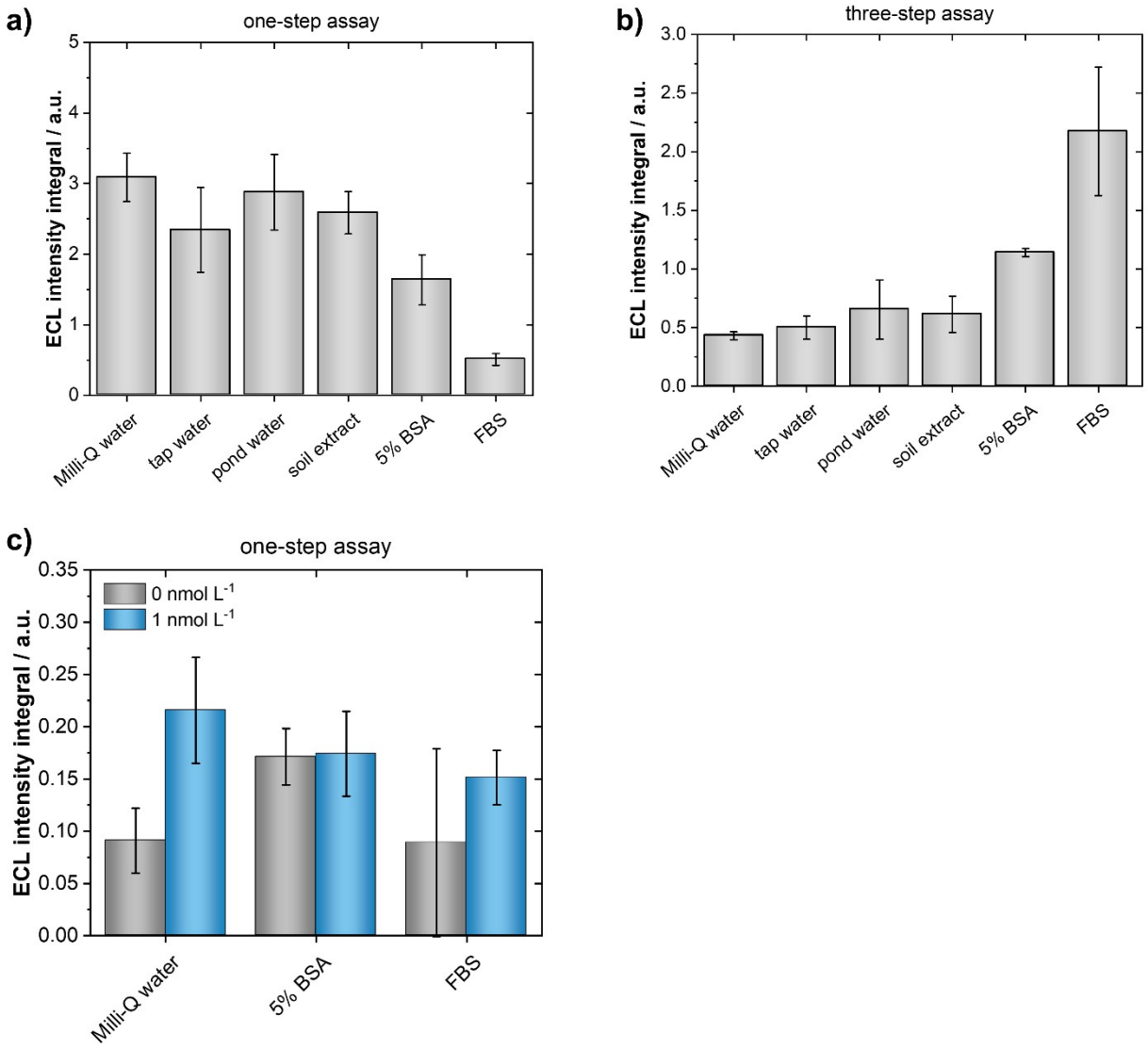

Figure S5. *ECL signals obtained from a one-step assay (a) and three-step assay (b) with target DNA spiked into different matrices (tap water, pond water, soil extract, 5 % BSA in PBS and fetal bovine serum(FBS)). Target DNA spiked into Milli-Q water served as reference. The final concentration of target DNA in the assay mix was 1 nmol L^{-1} in all cases. c) Signal comparisons between 0 and 1 nmol L^{-1} from one-step assays performed in Milli-Q water, 5% BSA in PBS and FBS (n=3).*

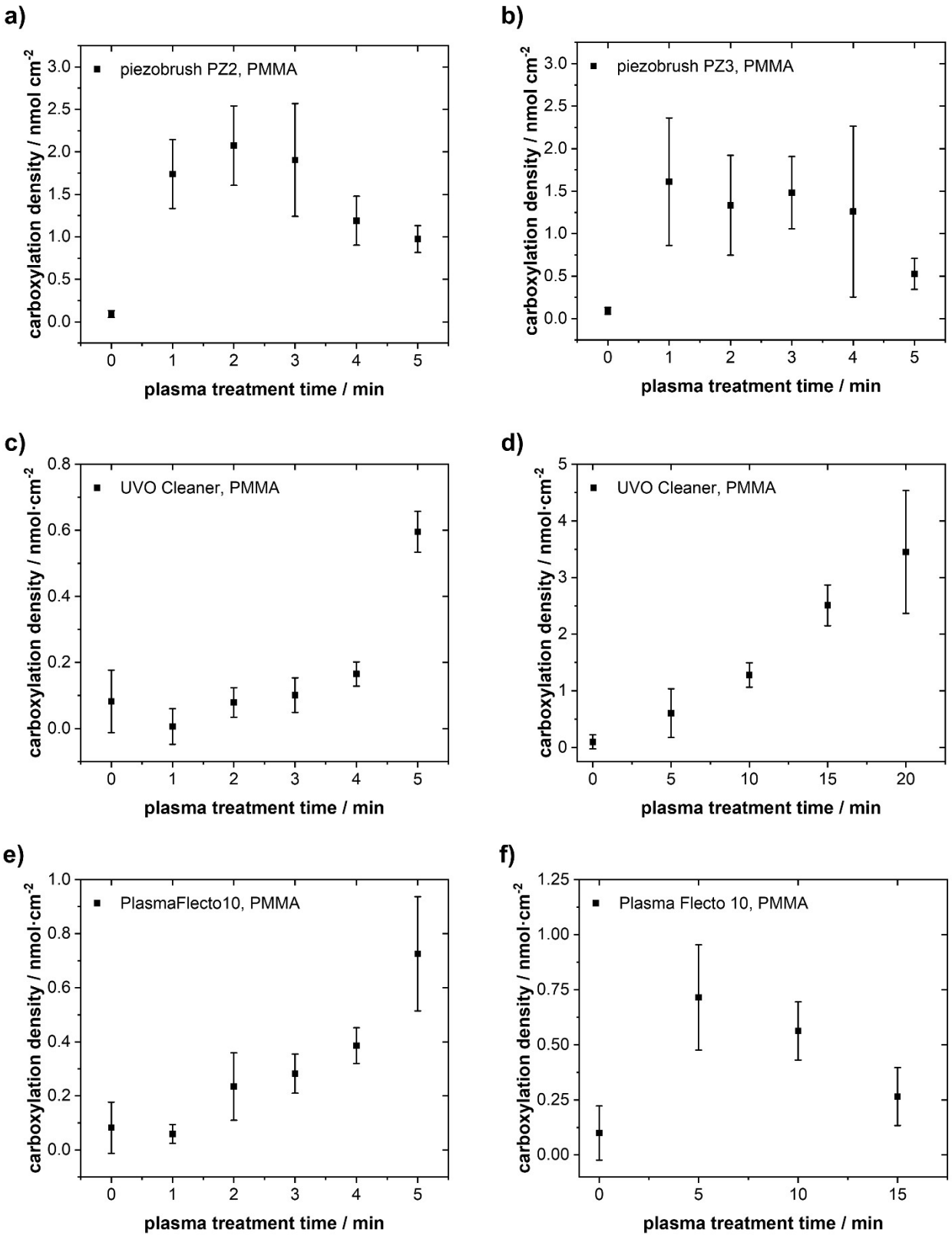

Figure S6. *Carboxylation densities on PMMA determined with a TBO staining assay after different treatment times with a piezobrush PZ2 (a), piezobrush PZ3 (b), UVO Cleaner (c,d) or PlasmaFlecto 10 (e,f) (n = 3).*

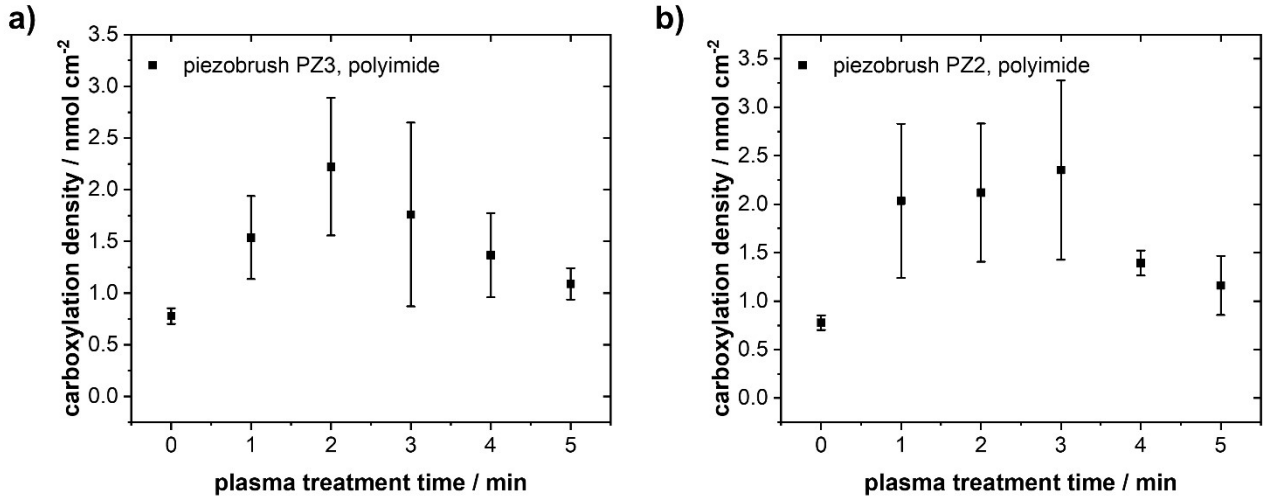

Figure S7. *Carboxylation densities on polyimide determined with a TBO staining assay after different treatment times with a piezobrush PZ3 (a) or piezobrush PZ2 (b) (n= 3).*

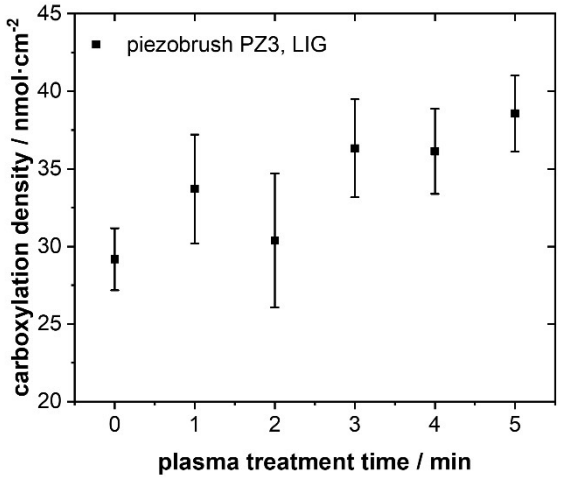

Figure S8*. Carboxylation densities on LIG determined with a TBO staining assay after different treatment times with a piezobrush PZ3 (n=3).*

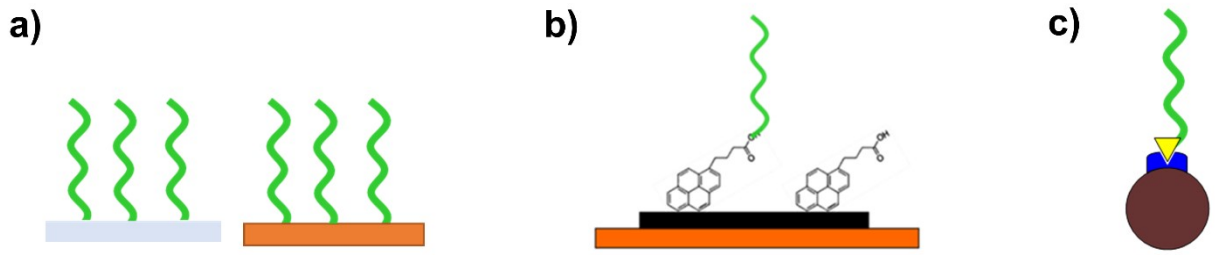

***Figure S9**. Schematic illustration of the three investigated immobilization strategies of the capture probe: a) on plasma activated PMMA or polyimide via EDC/NHS chemistry b) on LIG using 1-pyrenebutyric acid and EDC/NHS chemistry c) on streptavidin coated magnetic beads using biotinylated capture probe.*

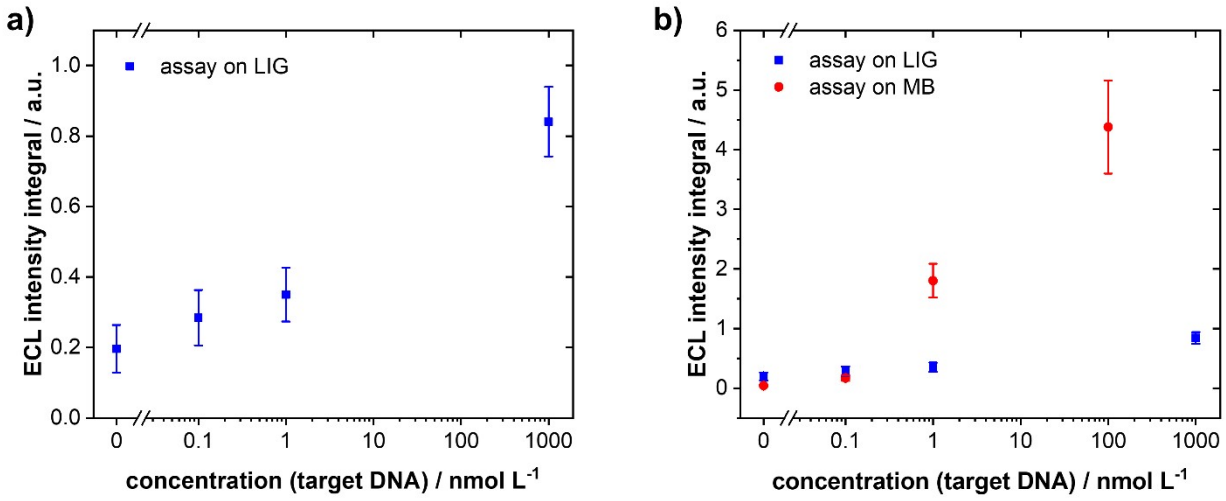

***Figure S10.** (a) Dose-response curve for C. parvum target DNA (0, 100 pmol L^{-1}, 1 nmol L^{-1}, 1 µmol L^{-1}) from an assay with capture probe immobilized on LIG/1-pyrenebutyric acid. (b) Comparison of the dose-response curve from (a) to parts of a dose-response curve (0, 100 pmol L^{-1}, 1 nmol L^{-1}, 100 nmol L^{-1}) of a three-step assay with biotinylated capture probe immobilized on streptavidin coated magnetic beads (n=4).*

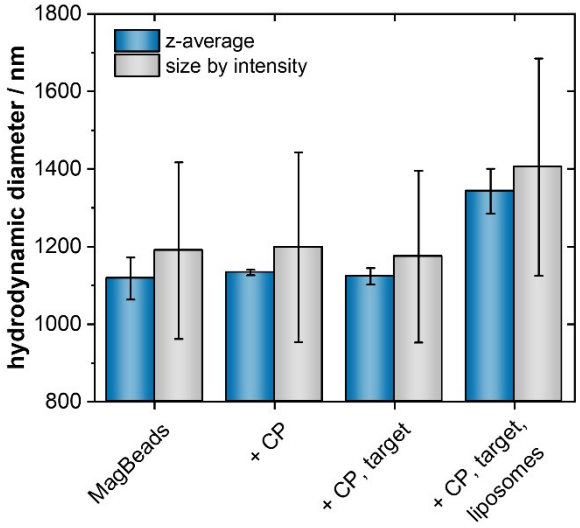

Figure S11. Hydrodynamic diameter of magnetic beads, magnetic beads with capture probe (CP), magnetic beads with CP and target DNA and magnetic beads with CP, target and liposomes (n=3).

References

1 S. R. Nugen, P. J. Asiello, J. T. Connelly, A. J. Baeumner, *Biosens. Bioelectron*., 2009, **24**, 2428-2433.