Supplementary material for the paper by

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Lab-on-chip FIA system without an external pump and valves and integrated with an in line electrochemical detector.

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Passive wash of the microchannel

In passive pump driven microfluidic devices only a fraction of the pumping sample is transported from the sampling port to the reservoir port of the device. Consequently, the analysis channel has to be cleaned from the residues of the sample between injections. In our work between the passive pump driven pumping/analysis steps passive pump driven washing steps were applied. Once the measurement of a sample was completed, i.e., the flow stopped, 2µL solution was withdrawn from reservoir port by a pipette. Immediately after the removal of the waste, 2µL background electrolyte solution was loaded at the sample port and pumped through the channel passively. This washing solution withdrawal/pumping steps with 2 µL aliquots of background electrolyte were performed up to 5 times. Between the individual cleaning steps the background electrolyte was left in the channel for 40 s. The efficacy of this washing procedure (the clearance of the analyte) was followed by recording the chronoamperometric current representing the analyte concentration in the channel. As shown in Figure S1, after 3-5 washing steps the current decayed to its background value.

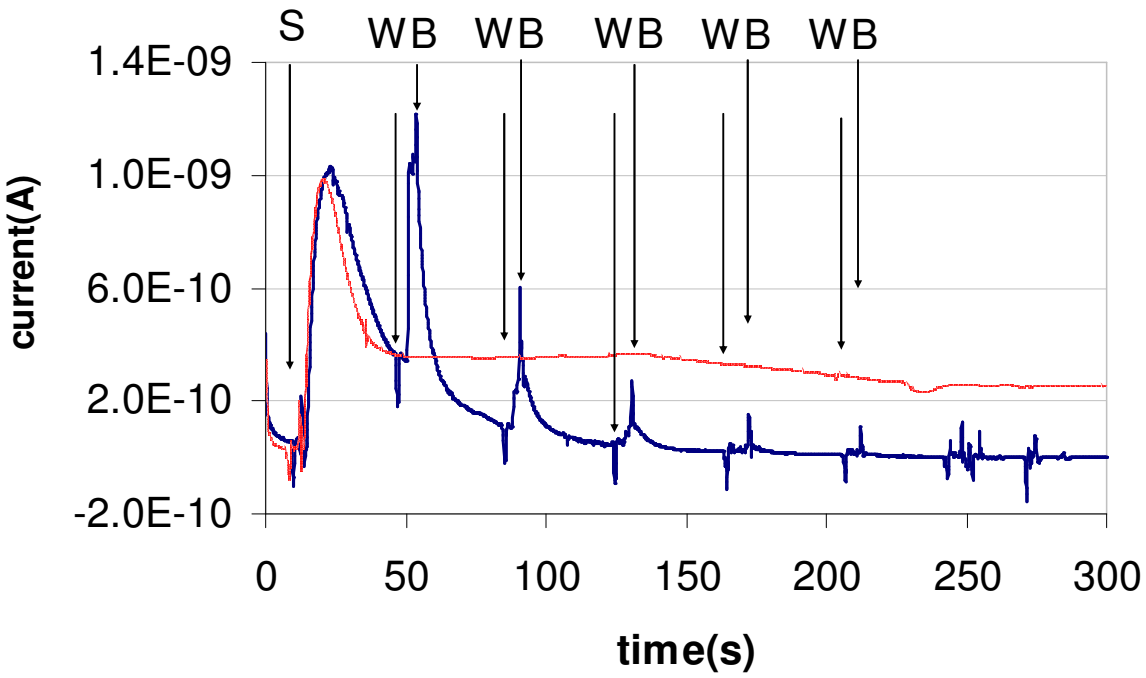

Figure S1: Chronoamperometric transients recorded following the injection of 2 µL, 10^{-5} M hexacyanoferrate (II) solution and the consequent washing steps with 2 µL aliquots of background electrolyte. The arrows indicate the introduction of the sample (S), withdrawal of the waste (W) and the injection (loading) of 2 µL background electrolyte (B) as wash solution. Thin, dotted red line represents the injection of 2 µL sample without wash. Solid black line represents the injection of 2 µL sample with 5 withdrawal/washing steps. Working electrode: 600 µm long IDA array with 5 µm wide fingers and 5 µm finger to finger distance. Applied potentials, 0.35 V and -0.1 V vs. Ag/AgCl reference electrode (0.1 mM NaCl).

Measurement of the electrochemically active product of surface confined ALP

With these preliminary data we intend to show the feasibility of using the passive pump driven microfluidic system beyond the simple injection of an electrochemically active analyte, e.g., hexacyanoferrate (II), or ascorbic acid. Toward this goal alkaline phosphatase (ALP) enzyme has been immobilized to the surface of the gold microband working electrodes as a streptavidin-biotinylated alkaline phosphatase scaffold.[1, 2] This system can serve as a model for an in line enzyme sensor or as a model for the detection step of a microfluidic enzyme immunoassay in which the surface confined enzyme label activity provides the analytically relevant information. When ALP is exposed to an ascorbic acid phosphate (AAP) solution, ascorbic acid (AA) is generated, which can be oxidized on the working electrode surface. The generated current measures the rate of the AA generation which, beyond other experimental parameters, is a function of the AAP concentration in the solution and the immobilized enzyme activity.

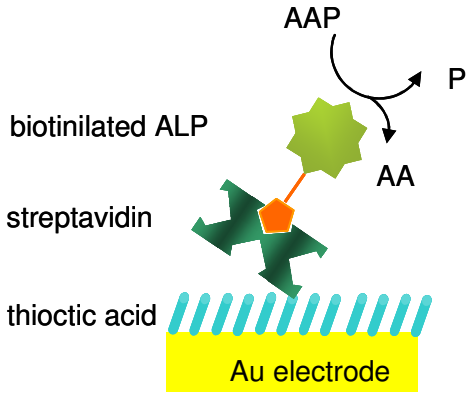

Figure S2: Model of a sandwich ELISA in which biotinylated ALP is coupled to covalently immobilized streptavidin on a gold electrode surface. The ALP enzyme activity is measured through the electrochemical oxidation of AA generated from AAP in the ALP catalyzed reaction.

Chemicals/Protocols:

To build the streptavidin-biotinylated alkaline phosphatase construct (Figure S2), first the gold electrodes patterned onto the glass microscope slide were cleaned in the Harrick Sci. plasma cleaner. Next, they were placed into 5 mM thioctic acid (Sigma-Aldrich) solution (in 5% acetic acid) for 12 hours and rinsed thoroughly with 5% acetic acid solution. After that, the PDMS-based flow channel was placed over the thioctic acid modified gold electrode surfaces and the channel was connected to a syringe pump, i.e., the subsequent steps of the surface modifications were performed in the fully assembled flow channel. The channel was filled with 10 mM pH 4.5 acetic acid and the gold electrodes were treated with 0.5 V amplitude potential pulses (3 pulses each 10s) to

facilitate the proper orientation of the thiol molecules on the Au surface. Then, the carboxylic functional groups of the thioctic acid monolayer were activated by pumping 50mM EDC (N-3-dimethylaminopropyl-N'-ethyl-carbodiimide, Fluka) and 50mM NHS (N-hydroxysuccinimide, Aldrich) solution through the channel. After 10 minutes incubation the excess EDC/NHS is washed away with acetate-buffer and the electrodes were exposed to 50µg/ml streptavidin (Promega, Madison, WI) solution in 10 mM acetate-buffer. The non-specifically adsorbed streptavidin is washed away with pH 4.5 acetate buffer and pH 7.4 phosphate buffer saline (PBS) solutions and the EDC/NHS activated surface is deactivated with 1M ethanolamine (Fluka) solution. Next, the electrodes were washed with a PBS-based blocking buffer solution containing 1% bovine serum albumin (BSA, Sigma) and 1 % Tween 20 (Bio-Rad, Hercules, CA), to minimize nonspecific adsorption. In the final step of the ALP immobilization process the streptavidin modified electrode surface is exposed to 50µg/mL biotinylated ALP (New England Biolads, Boston, MA) solution in pH 7.4 PBS buffer for conjugation. The nonspecifically adsorbed biotinylated ALP is washed away with the pH 7.4 PBS buffer.

To assess the immobilized ALP enzyme activity the channel was filled with pH 9.5 borate buffer solution containing 1mM $MgCl_2$. Next the enzyme modified working electrode was polarized to 0.45 V vs. Ag/AgCl reference electrode (in these experiments downstream to the working electrode) and the current recording was started. After approximately 30 seconds, 2µL ascorbic acid 2-phosphate (Sygma) solutions were loaded on the sample port of the channel and the transient current related to the oxidation of ascorbic acid generated in the enzyme catalyzed reaction was recorded. The peak-shaped current-time traces are shown in Figure S3A. The shapes of these transients are a complex function of the rates of the enzyme catalyzed reaction, the mass transport of AAP, and the electrochemical oxidation of AA. However, by loading increasing concentrations of AAP onto the entry port of the passive pump-based FI device the transient peak heights increased proportionally as it is shown in the calibration curve constructed from the peak heights of the transients Figure S3B.

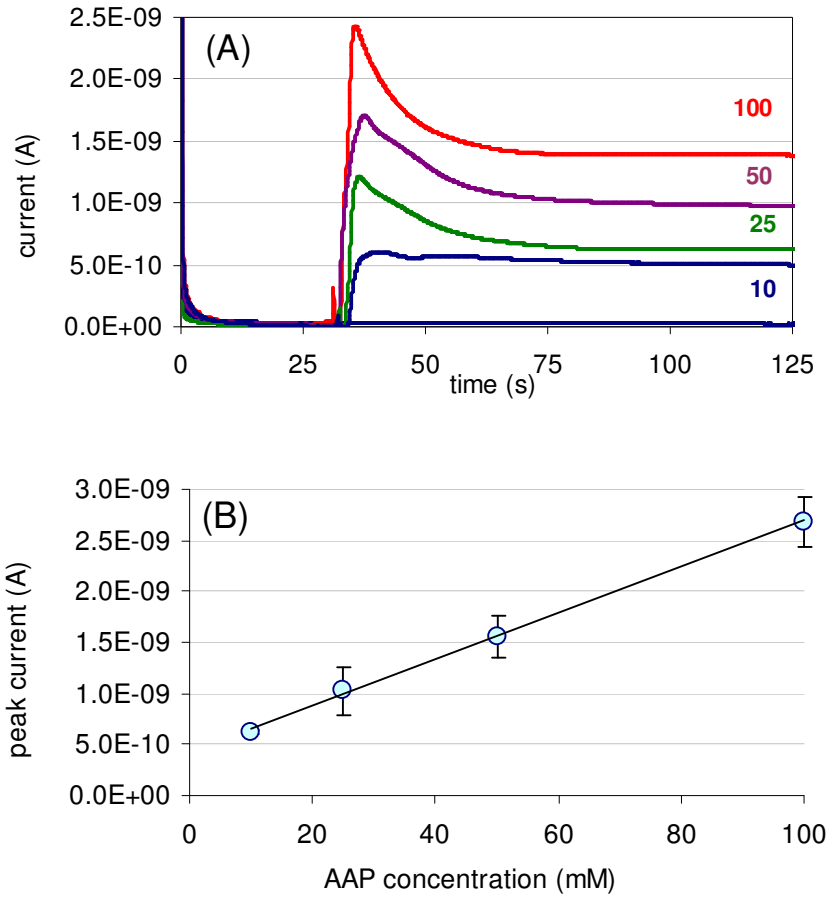

Figure S3: Response characteristics of the passive pump driven FIA system. (A) Chronoamperometric transients recorded with ALP modified gold microband electrodes following the passive pump driven injection of 2 µL AAP of $1 \cdot 10^{-2}$, $2.5 \cdot 10^{-2}$, $5 \cdot 10^{-2}$ and $1 \cdot 10^{-1}$ M concentrations. (B) Calibration curve constructed from the peak heights of the transients. The points represent the mean value of n = 3 parallel measurements. They are plotted with their standard deviation. Working electrode: ALP modified gold microband array with five interconnected 5 µm wide electrodes with 100 µm separations. The applied potential: 0.45 V vs. the Ag|AgCl in the channel. The background solution pH 9.5 borate buffer with 1 mM $MgCl_2$

1. Hermanson, G. T., Mallia, A. K., Smith, P. K., *Immobilized affinity ligand techniques*. Academic Press: San Diego, 1992.
2. Riccardi, C., Dahmouche, K., Santilli, C., Costa, C., Yamanaka, H., *Talanta* **2006**, *70*. 637-643.