# Supplementary information

# Designed miniaturization of microfluidic biosensor platforms using the stop-flow technique

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### On-chip tetracycline assay preparation

In this microfluidic biosensor platform, the assay incubation is realized only on the immobilization area, which can be easily filled with reagents by capillary forces (i.e., pipetting drops of reagents on the channel inlet). A hydrophobic stopping barrier between the measurement cell and the immobilization capillary enables a passively metering of the fluids and prevents any contamination of electrodes with the employed reagents. After the immobilization of biomolecules, the excess reagents are removed again through the channel inlet by using a vacuum.

The working principle of the repressor protein-based tetracycline assay is displayed in figure 1. At the first step of the assay immobilization, the channel surface is functionalized with 50 µg ml$^{-1}$ anti-fluorescein antibodies (Sigma-Aldrich, Germany) for 1 hour which is followed by a subsequent blocking step with 1 % bovine serum albumin (BSA) for another hour to prevent any non-specific binding. Consequently, 0.1 µM of the operator DNAs (*TetO*) with fluorescein labeling (Sigma-Aldrich, Germany) are immobilized on the microfluidic capillary for 15 minutes.

Upon the assay preparation, the protein solution containing 1 µg ml$^{-1}$ avidin-GOx (Biomol, Germany), 0.2 µg ml$^{-1}$ biotinylated repressor (bTetR) and 50 mM magnesium chloride $MgCl_2$ (Sigma-Aldrich, Germany) are introduced into the biosensor chip for only 5 minutes. The presence of $MgCl_2$ is crucial for the assay functionality. Magnesium ions ($Mg^{2+}$) form a complex with tetracyclines. The tetracycline-$Mg^{2+}$ complexes bound to bTetR undergo a conformational change by decreasing their affinity for the cognate DNA operators. Thus, this results in the release of bTetRs from their operator DNAs if a class-specific antibiotic is present in the sample solution. After a final washing step with 300 µl wash buffer

(physiological buffer containing 0.05 % Tween®), the assay signal is read out electrochemically by the amperometric stop-flow detection within 10 min.

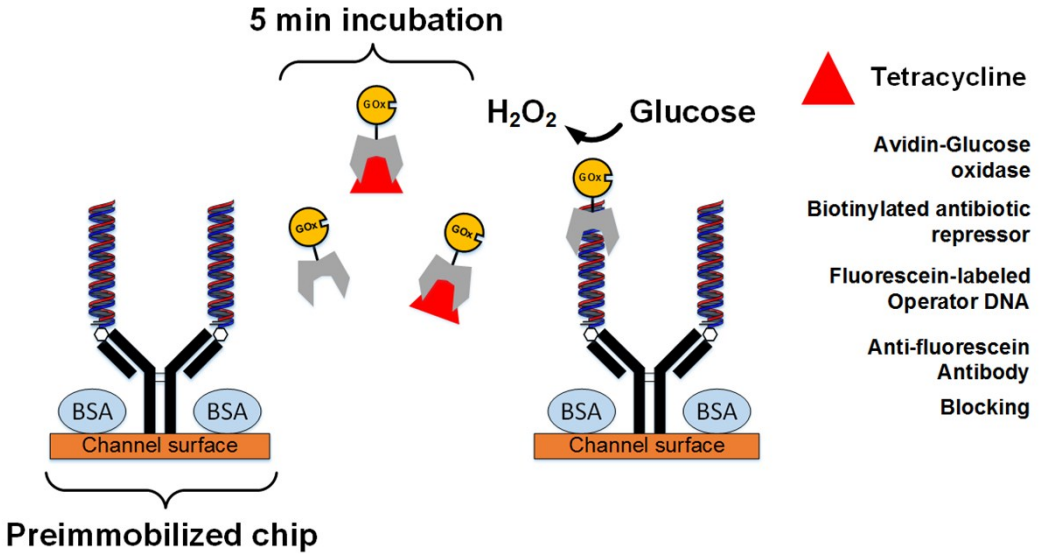


Figure 1. The graphical abstract of the repressor protein-based tetracycline assay.

## On-chip electrochemical measurements

For the proof-of-principle experiments, the utilized measurement setup includes a custom-made chip holder for fluidic and electrical sensor connections, a four-channel bipotentiostat (Jobst Technologies GmbH, Germany) and a syringe pump PHD2000 (Harvard Apparatus, USA). Herein, microfluidic chips were fastened in duplicates into the custom-made chip adapter. It enables an easy connection and simultaneous measurement of up to four microfluidic biosensor chips. To establish a constant flow as well as the stop-flow protocols, the syringe pump was employed in withdrawal mode with four individual 2.5 ml syringes (Hamilton Gastight, USA) and silicone tubes.

The electrochemical cell of the microfluidic biosensors comprises a Ag/AgCl reference electrode, Pt counter and Pt working electrodes. The hydrogen peroxide oxidation at the working electrode takes place at 450 mV vs. on-chip Ag/AgCl pseudo-reference electrode. The amperometric signal readout of the stop-flow experiments were realized using the four-channel bipotentiostat and visualized with the BioMON software (Jobst Technologies GmbH, Germany) on a notebook. The utilized chemicals were purchased from Sigma-Aldrich, Germany or otherwise as stated in the text. All solutions used in this work were prepared in ultra-pure HPLC-grade water (Alfa Aesar, USA).

The stop-flow measurement starts in phosphate buffer saline (PBS, pH = 7.4) at a constant flow rate of 20 µl $min^{-1}$. When the measured signal reaches a steady-state current (i.e. signal baseline), a 40 mM glucose substrate in 0.1 M PBS is introduced into the microfluidic capillary and electrochemical stop-flow measurements are carried out for different stop-intervals.