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

Supplementary information to:

An Automated Microreactor for Semi-Continuous Biosensor Measurements

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Figures S1-S5

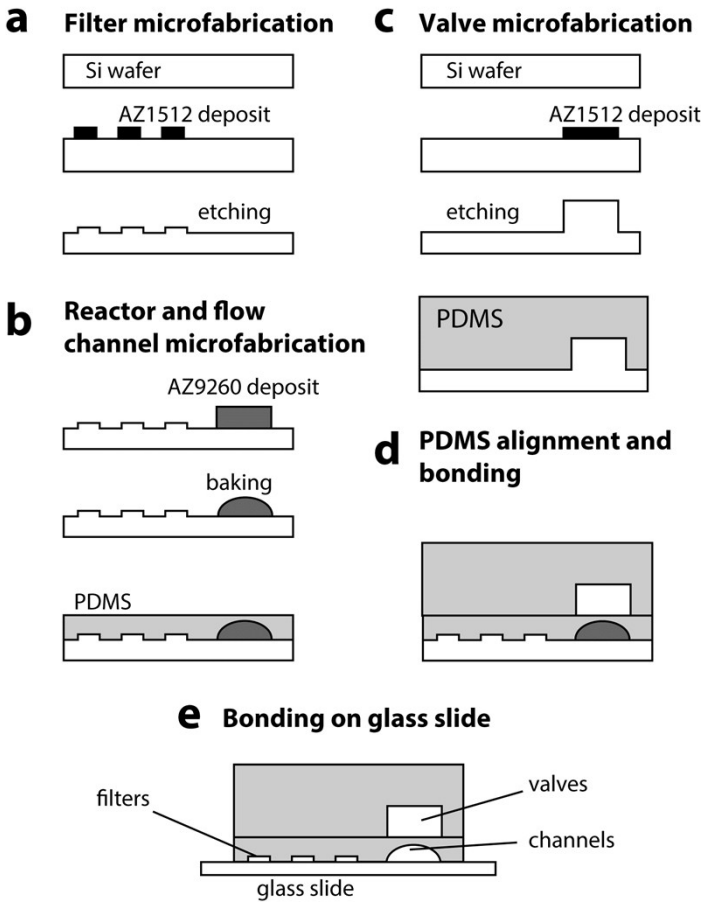

Figure S1: Microfluidic chip fabrication and assembly. **a** Microfabrication of the flow layer silicon mold (reactor, flow channel, filters and measurement zone). In a first step, the AZ1512 resist is deposited on the silicon wafer at the sites where microfilters have to form, which is next etched to lower the overall surface depth. **b** AZ9260 resist is deposited at the sites of formation of flow channels, measurement zone and reactor, which is baked to form slightly rounded surface structures. A thin layer (30 µm) of PDMS is poured by spin-coating to obtain a positive imprint. **c** On a separate Si wafer the AZ1512 resist is placed at the sites where valves and valve flow channels will appear. The wafer is next dry etched and PDMS is poured, providing the positive imprint of the valve control layer. **d** A block encompassing the biochip is cut out, peeled off from the mold and aligned with the thin PDMS layer on the other wafer. Bonding and curing attaches both PDMS layers permanently (up to 2.5 bar

overpressure). **e** The complete PDMS biochip block is now cut and peeled from bottom layer mold, entry holes are punched and the structure is mount and bonded to a microscope glass slide.

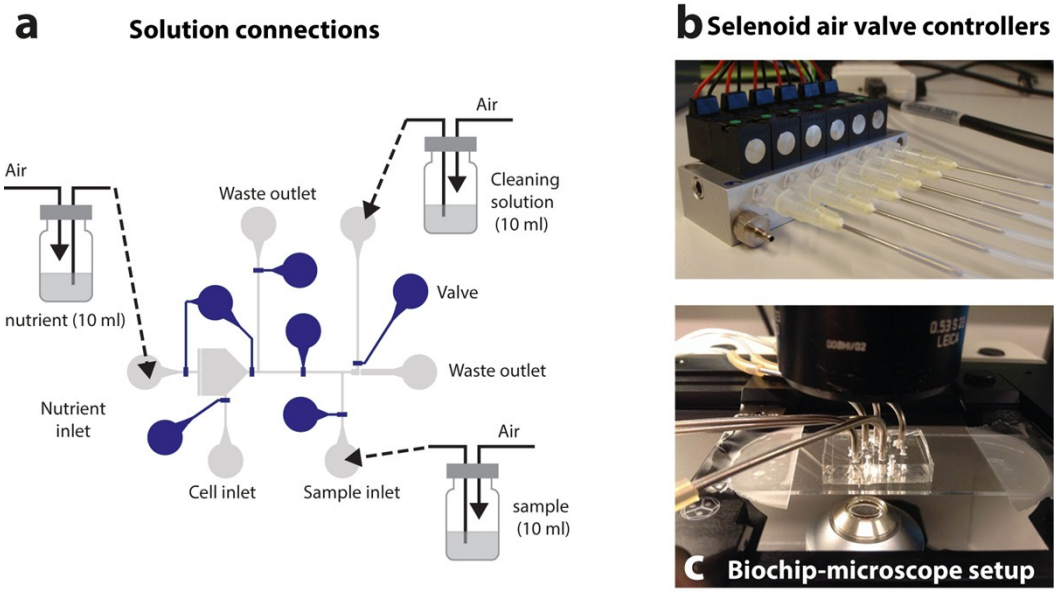

Figure S2: Set-up of the microfluidic reactor for constant growth and biosensor monitoring. **a** Sterile solutions and samples are connected to the respective inlets, and are driven by air-control overflow (0.3-0.5 bar air pressure). Other in- and outlets (apart from the cell inlet) are connected to 1 cm open tubes filled with 10 µl H_2O in order to avoid evaporation of liquid and formation of air bubbles in the channels. **b** A system of solenoid valves joined to pressurized air, which are connected by Teflon tubes and bent stainless-steel metal connectors to the biochip, controls the air pressure in the valves. The solenoid valves are operated by a LabView custom program. **c** Final set-up of the biochip reactor on a glass slide under an inverted epifluorescence microscope for continuous growth and measurements of

biosensor fluorescence. Visible are the stainless-steel adapters inserted in the biochip inlets.

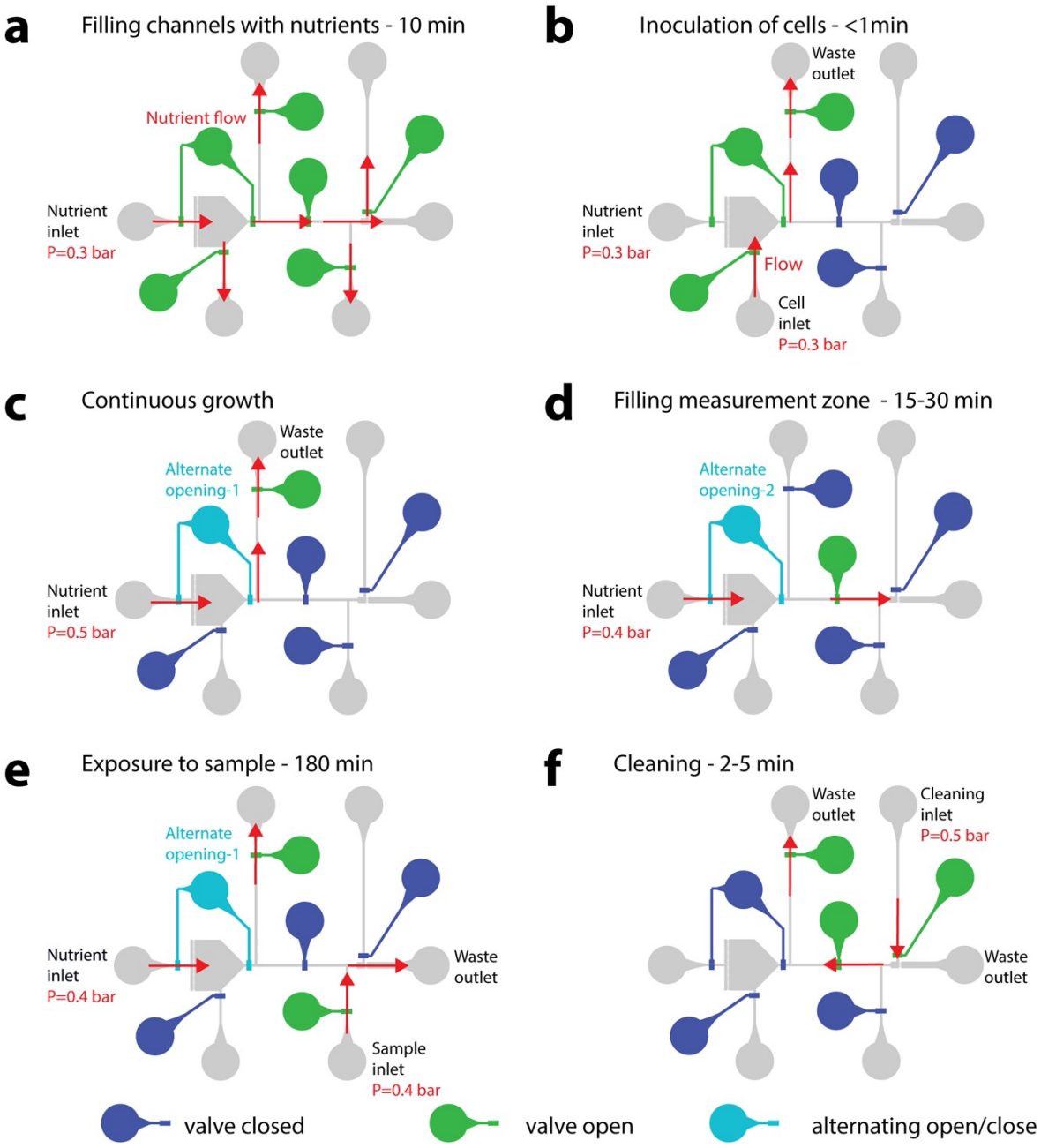

Figure S3: Operation principle of the microfluidic chip. **a** All control valves are open (indicated in green) and nutrient solution is allowed to wet all channels and inlets. Subsequently, the valves are closed by applying a pressure of 1.3 bar. **b** Cells are inoculated into the nl-reactor via the cell inlet under a driving pressure of 0.3 bar by

opening the valves indicated in green for a few seconds. Afterwards, the valve controlling the cell inlet channel is closed (indicated in dark blue). **c** Continuous culturing is started by simultaneously opening and closing the valves around the nl-reactor (driving pressure of 0.5 bar, opening frequency 15 h^{-1}, opening time 120 ms), and maintaining the valve to the waste outlet open. Cells flushed from the reactor are collected in the waste outlet. **d** For measurements, the valve to the cell waste outlet is closed, the valve in the middle channel is opened, and the nl-reactor valves continue to open and close but under an alternate regime (driving pressure of 0.4 bar, opening frequency 20 min^{-1}, opening time 120 ms). Outflowing cells are accumulated against the filter in the measurement zone. **e** As soon as sufficient cells are trapped, the middle valve is closed and continuous growth is resumed in the nl-reactor using the original flow settings. Captured cells are exposed to the sample during 180 min, by opening the sample inlet under a driving pressure of 0.4 bar. The fluorescence signal of the cells is recorded every 30 min. **f** After the measurement, the cage and channels are cleaned to remove accumulating cells by opening the cleaning inlet, middle valve and valve to the waste outlet (P = 0.5 bar) while closing the nl-reactor in- and outlets. After the cleaning, the system is operated by changing to step **c**. Before each subsequent measurement, step **f** is repeated, and the complete cycle in constant operation looks like: **c**, **f**, **d**, **e**, **f**, **c**, **f**, **d**, **e**, **f**, etc.

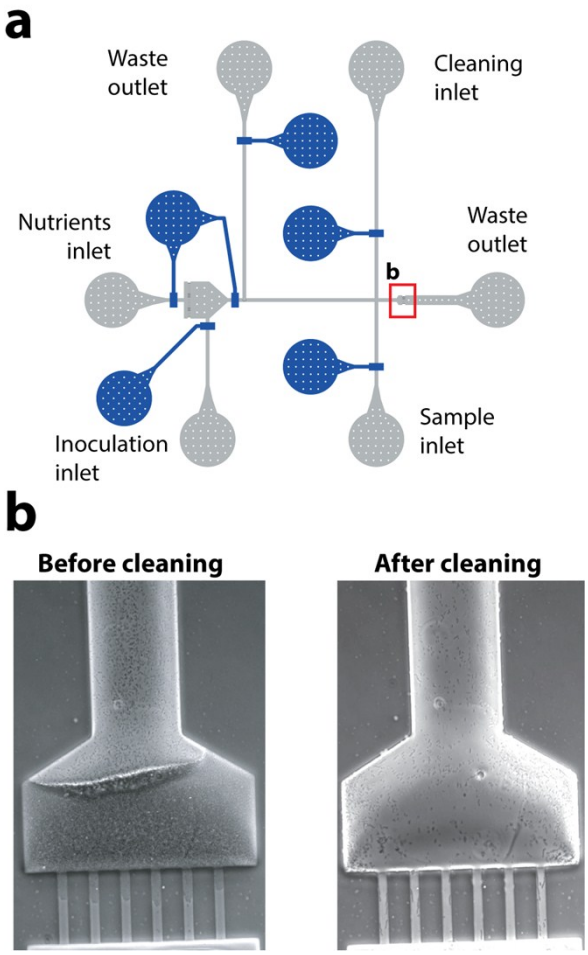

Figure S4: Scheme of the 13 nl reactor chip and the alternative measurement chamber. **a** 13 nl reactor layout with flow lines (in grey) and valve control (blue). For dimensions, see Figure 1. **b** Cell cleaning from the 13 nl reactor design proceeded by using 0.7 bar overpressure on the measurement cage, by which the filter lines deflect and let the cells pass. Pictures show the cage before cleaning (phase contrast image at 200x magnification with accumulated cells), and after cleaning. Because of the difficulties of precise manufacturing of filter line heights, this design was abandoned for a more robust one with back-flushing (see Fig. 1 and S3).

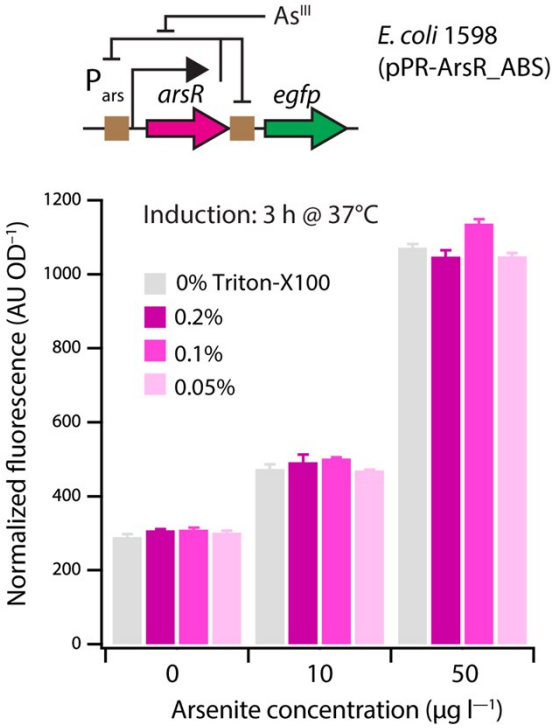

Figure S5. Effect of Triton X-100 on inducibility of the *E. coli* 1598 (pPR-ArsR_ABS) arsenic bactosensor. Cells were cultured in growth media with or without different Triton X-100 concentrations (*w/v*) to a culture turbidity of 1.3, washed and resuspended in induction medium (*Online Experimental Methods*). Data are averages from triplicate assays, normalized by the culture turbidity in the assay.

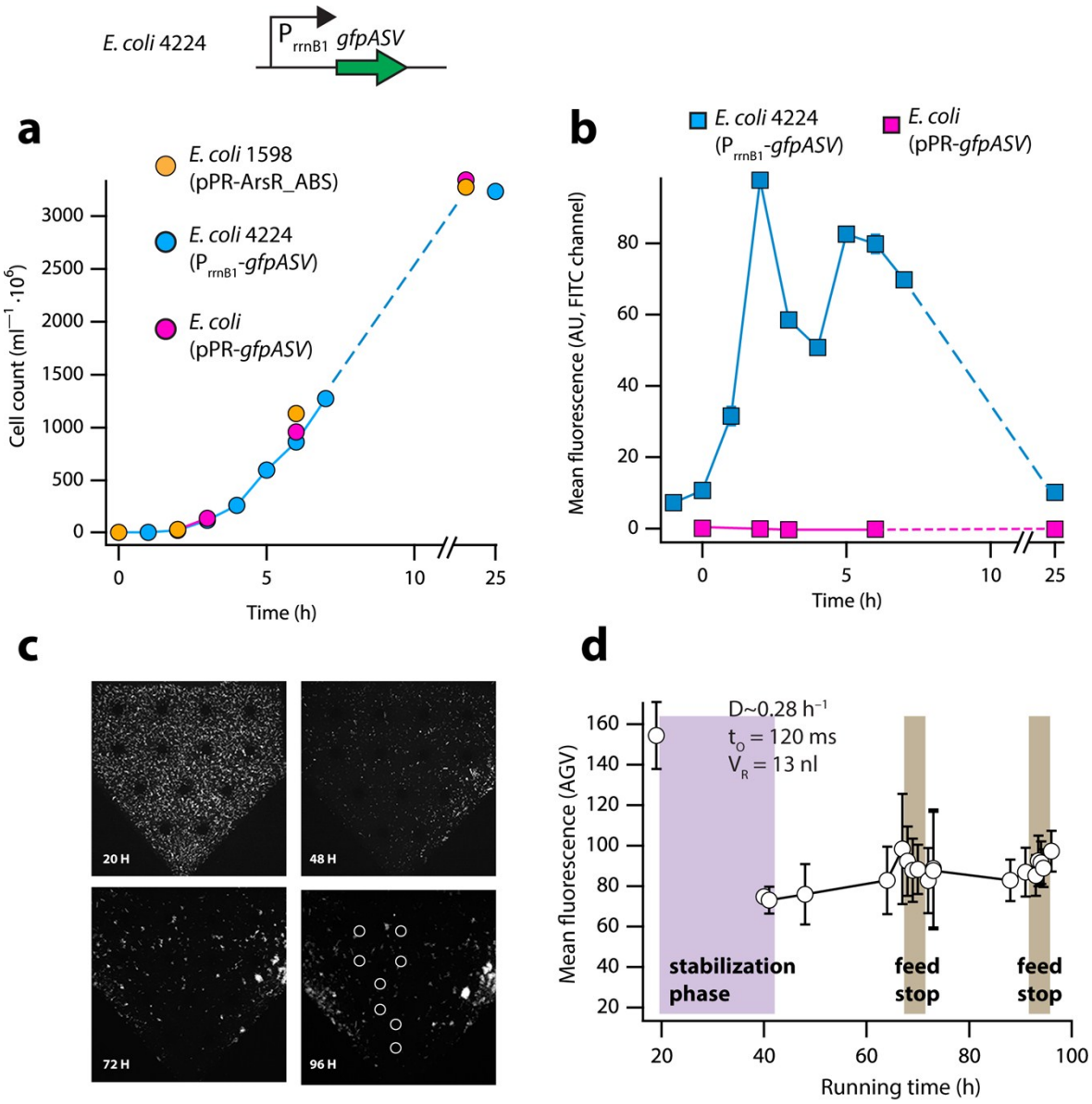

Figure S6. Physiology and induction of GFP fluorescence from *E. coli* 4224 carrying the growth-regulated *rrnB1* ribosomal promoter fusion to a *gfpASV* reporter (unstable GFP variant). **a** Cell counts by flow cytometry during growth in nutrient solution (*Online Experimental Methods*) at 37°C of *E. coli* 4224 in comparison to *E. coli* 1598 (pPR-ArsR_ABS) and *E. coli* with the empty vector pPR-*gfpASV*. **b** Mean fluorescence signal of *E. coli* 4224 cells at culture time points of the experiment in (a), showing activity of the promoter in exponentially growing but not stationary phase cells. **c** Reactor images of GFP fluorescence (scaled to same maximal intensity) at

different time points during a 4 day culturing of *E. coli* 4224 in the 13 nl reactor (D~0.28 h^{-1}). Circles at 96 h indicate the positions measured for GFP fluorescence in all reactor images for panel (d). **d** Mean reactor fluorescence of *E. coli* 4224 over time in the 13 nl reactor. Stabilization phase, start of continuous culturing; white zones, constant culturing; feed stop, no fresh nutrients.

Video S1 (still image): Actively growing *E. coli* 1598 cells in the 50 nl reactor. Left side shows the entry filter channels where the fresh nutrients are coming in. Middle "circle" shows a support pillar in the reactor. The video shows a 120 ms valve opening (at ~14 s in the movie) after which the cells are pushed back and then swim again in the direction of the filters. Note that some cells enter the filters despite the shallow height (estimated 600 nm), and in some cases even escape through the filters altogether. Because of the strong pressure difference upon nutrient entry, most cell growth remain in the reactor.