

## Supplementary information

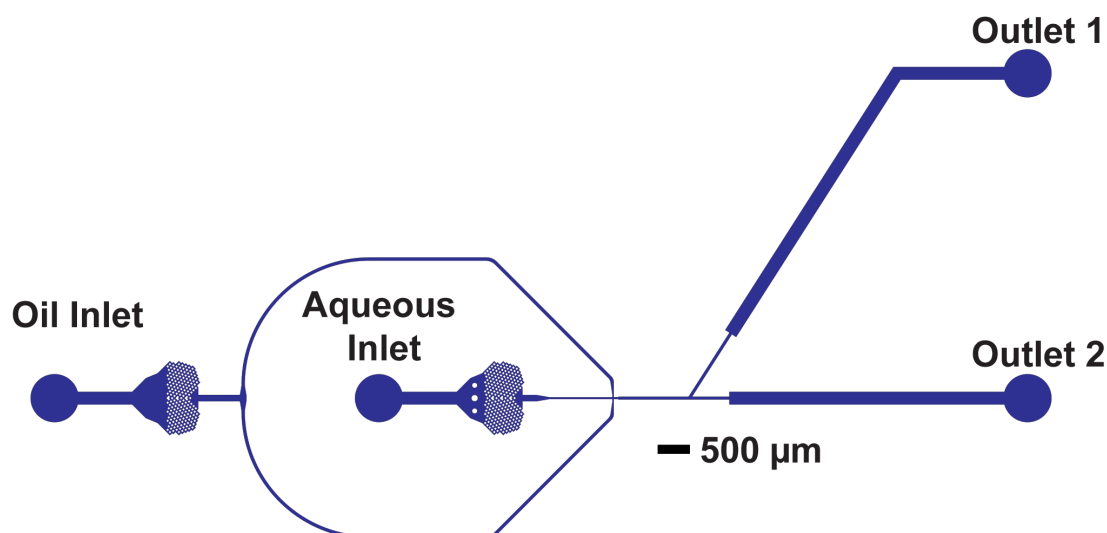
### Unit definition

One unit will hydrolyze 1.0  $\mu\text{mole}$  of o-nitrophenyl  $\beta$ -D-galactoside to o-nitrophenol and D-galactose per min at pH 7.3 at 37  $^{\circ}\text{C}$ .

### Data acquisition

A total of three replicate data sets were acquired for each of the five substrate concentrations using the microfluidic device, each data set consisting of twenty seconds of data gathered from each of the six measurement points. Each replicate data set was analyzed separately as described in the article. In total, one reaction velocity extract was produced for each combination of inhibitor concentration, substrate concentration and replicate number (for a total of 75 velocity values). All of the 25 triplicate velocity extracts were then compared internally and it was found that the average coefficient of variation within the triplicates was 3%.

## Supplementary information figures



*Figure S1: Schematic of the droplet generation circuit (circuit one) as seen from above. Blue indicates channels that are 30  $\mu\text{m}$  deep.*

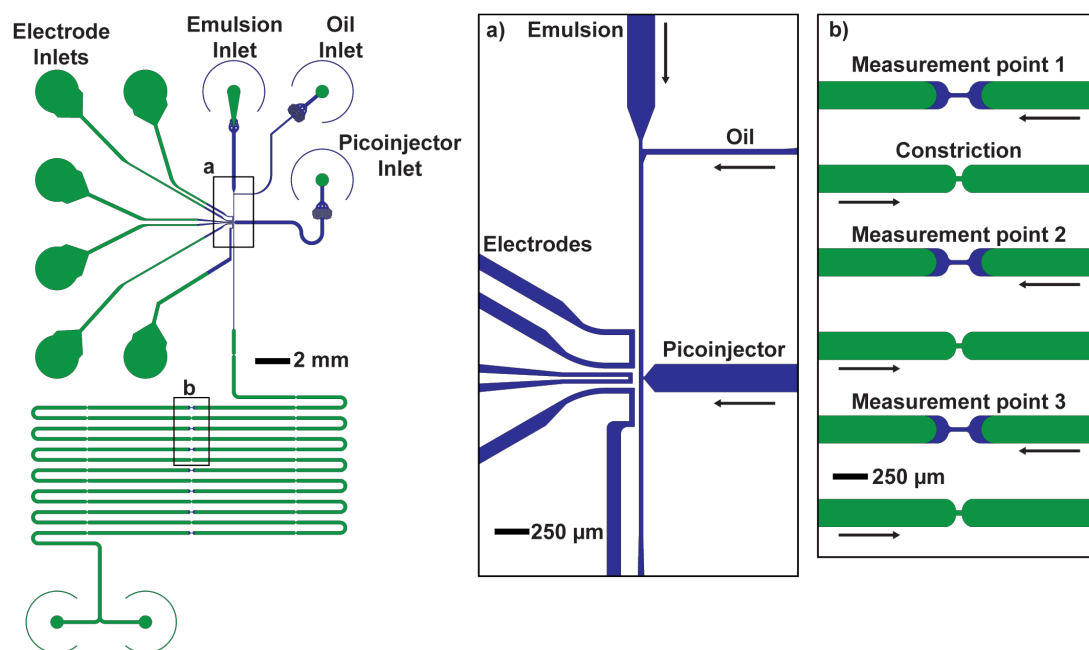


Figure S2. To the left: Schematic of the picoinjector incubation line circuit (circuit two) as seen from above. Green indicates 110 μm deep channels and blue indicates 30 μm deep channels. In the center: Zoom of emulsion reinjection nozzle and picoinjector module. To the right: Zoom of incubation channel with measurement point and constriction features. Note that measurement point contains a shallow channel to allow for single droplet resolution fluorescence measurements.

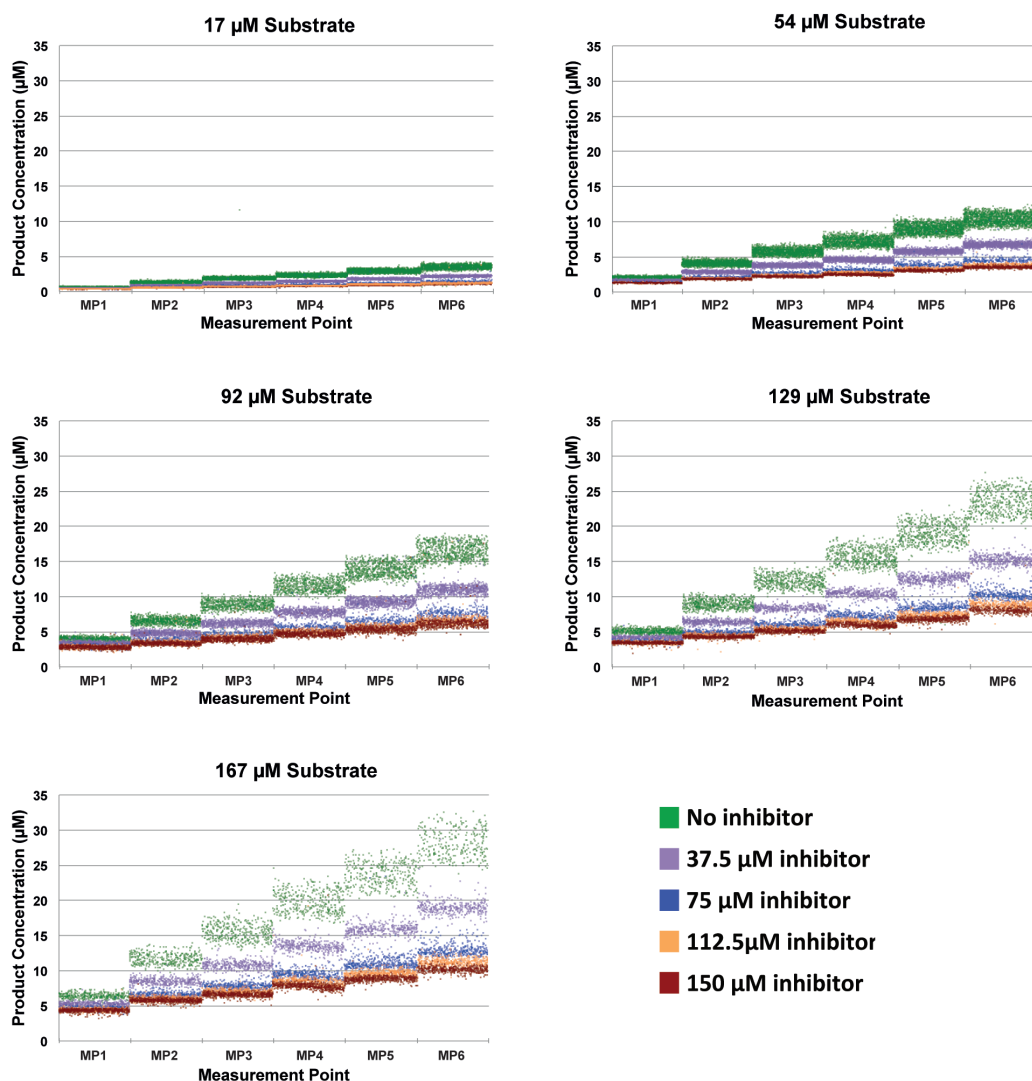


Figure S3. Reaction progress for the substrate concentrations of 17, 54, 92, 129 and 167  $\mu\text{M}$  RBG respectively. Y-axis shows product concentration as derived from the 593 nm fluorescence and the X-axis shows the measurement point. Each dot represents one droplet event and the color of dot indicates the inhibitor concentration of the droplet as gated according to the barcode fluorescent signal (525 nm) of the droplet.

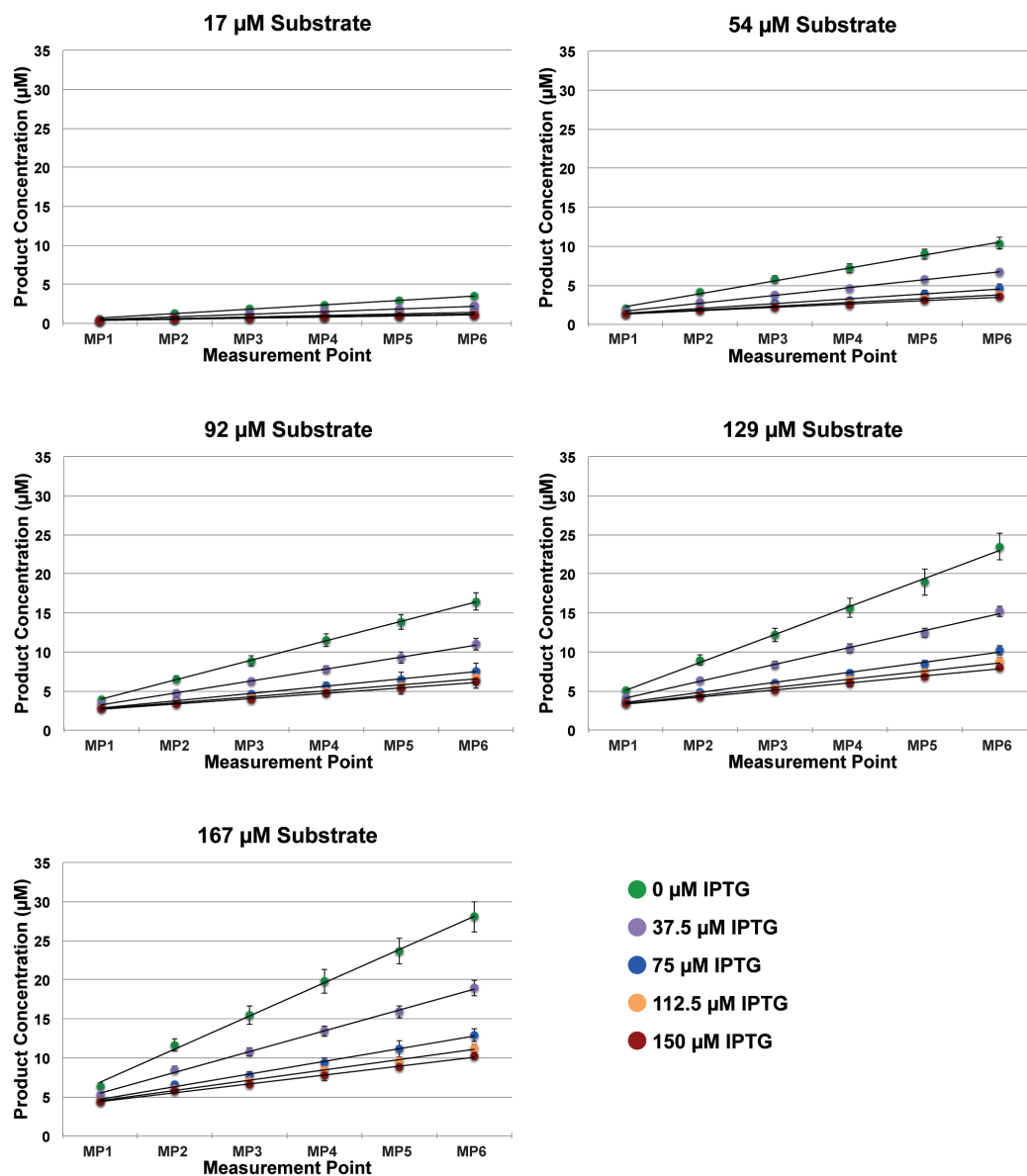


Figure S4. Extraction of reaction velocities for each combination of substrate and inhibitor concentration using simple linear regression. Each graph represents one substrate concentration and color indicates inhibitor concentration. Plotted data points show mean product concentration in droplets at respective measurement point. Error bars denote one standard deviation. Note that the linear regression was fit to all droplet events for respective inhibitor and substrate conc., even though the data are shown as a mean value and one standard deviation in the graph.