Fast Mixing and Reaction Initiation Control of Single-Enzyme Kinetics in Confined Volumes

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Supporting Information

Photolithography

PDMS molds were fabricated with multilayer photolithographic techniques. Briefly, negative tone photomasks for the control and mixer layers were printed on transparency films at 20,000 dots per inch resolution by CAD/Art Services (Bandon, OR). A chrome mask with arrays of 5 μm dots was purchased from MEMS and Nanotechnology Exchange (Reston, VA). To make the mold for the mixer/chamber layer, SU8 2005 was first spun at 2000 rpm on a piece of isopropanol-cleaned silicon. After soft baking (1 min at 65°C and 2 min at 95°C), the 5 μm dot arrays as well as alignment marks were transferred from the chrome mask to the resist by UV exposure at 4.5 mW/cm^2 for 90 sec, followed by post-exposure baking (1 min at 65°C and 1 min at 95°C), and development in SU8 developer. 5 μm dot patterns were further stabilized by hard baking at 150°C for 5 min. Another layer of 25 μm thick SU8 2025 photoresist was then spin-coated on top of the dot pattern and soft baked (1 min at 65°C and 3 min at 95°C). Each 5 μm chamber had a volume of approximately 100 femtoliters (4.4 μm diameter with 6.5 μm height), as inferred from scanning electron microscopy (SEM) images of the microchambers.

To align the staggered herringbone mixer patterns relative to the array of dots, four corners of the silicon chip were carefully cleared with SU8 developer to reveal the alignment marks on the mold. The mixer patterns were then transferred to the second resist layer through the transparency mask by UV

exposure at 4.5 mW/cm^2 for 1 min. After post exposure baking (1 min at 65°C and 3 min at 95°C) and development in SU8 developer, the patterns were hard baked at 150°C for 5 min. The mold for the control layer was made separately in a similar manner using single-step photolithography.

The main channel layer was also made using SU8 2025 photoresist. A piece of clean, dry # 1 coverglass (Fisher Scientific, Pittsburgh, PA) was coated with a uniform layer of 35 μm thick SU8 2025. After soft baking (1 min at 65°C and 3 min at 95°C), the coated coverglass was exposed to UV illumination under the transparency mask for the channel layer at 4.5 mW/cm^2 for 1 min. Post-exposure baking and development in SU8 developer revealed negatively defined channels in resist layer on the glass coverslip.

Multilayer Soft-lithography and Device Assembly

The final device consists of three layers: a main channel layer (bottom), an intermediate mixer/microchamber layer (middle) and a control layer (top). The top two layers were assembled using multilayer soft lithography[16]. Before application of the PDMS elastomer, all of the silicon molds were treated with chlorotrimethylsilane vapor for 5 min in order to prevent strong adhesion between the elastomer and photoresist after curing. The elastomer used in this experiment was RTV 615 from General Electric Silicones. A silicone elastomer mixture with excess polymer base (20:1 mass ratio of polymer base and curing agent) was spin-coated onto the microchamber/mixer mold at 2,400 rpm for 60 sec and cured in an oven at 80°C for 45 min. The control layer was prepared by pouring silicone elastomer with deficient polymer base (5:1 mass ratio of polymer base and curing agent) onto the control layer mold and baking at 80°C for 45 min after the elastomer was degassed. The control layer was peeled from the mold and aligned with the PDMS coated microchamber/mixer mold. The two-layer PDMS assembly was then baked for 2 hours to bond the two layers together into a single structure, which was then peeled from the microchamber/mixer mold. The gradient of curing agents at the interface of the two layers drives their migration from the top layer to the bottom, ensuring that the two layers are strongly bonded together by covalent cross-linking at the interface[16]. Finally, the bottom layer

and top two layers were activated by oxygen plasma cleaning (plasma cleaner PDC-32G, Harrick Scientific Corp., Ossining, NY) and bonded together by heat treatment in an oven at 80°C overnight.

Stopped-flow measurements

The kinetics of reference β-Gal/RGP reactions in bulk solution were monitored with a stopped-flow instrument (SX.18MV, Applied Photophysics, Surrey, UK). Final concentration of 0.19 nM β-Gal with 100 μM of RGP was mixed and the fluorescence increase due to the enzymatic generation of resorufin was measured. A typical plot of resorufin concentration increase as a function of time is shown in figure S1. The mean and standard deviation from five measurements gave the initial rate of resorufin formation as 288 ± 33.2 per sec per enzyme, which is close to the rate from a single enzyme in a 100 fL chamber: 243 resorufin molecules per sec per enzyme.

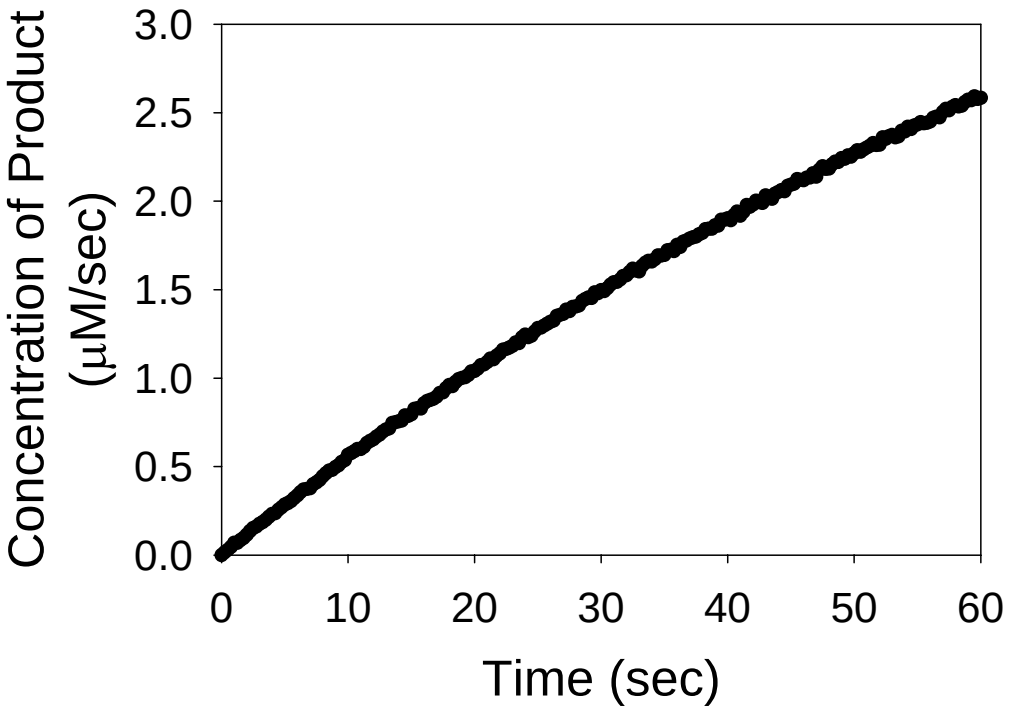

Figure S1. Bulk measurement of β-Gal enzyme activities by stopped-flow.