

## Supporting Information

# Creation of Stepwise Concentration Gradient in Picoliter Droplets for Parallel Reactions of Matrix Metalloproteinase II and IX

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### Supplementary Figure Contents

**Figure S1.** Proteolytic cleavage of the peptide substrate, MMP substrate III.

**Figure S2.** Droplet in a rounded channel.

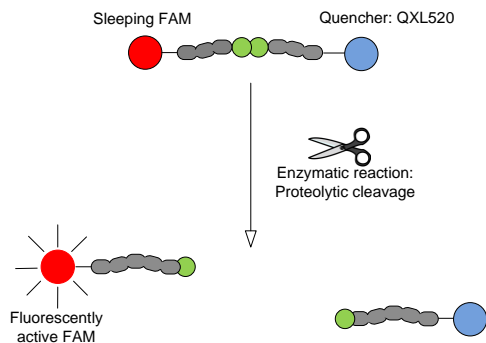
**Figure S3.** Standard curve of the fluorescent molecule, FAM, for off-chip experiments.

**Figure S4.** Time courses of the MMP-2 and MMP-9 reactions.

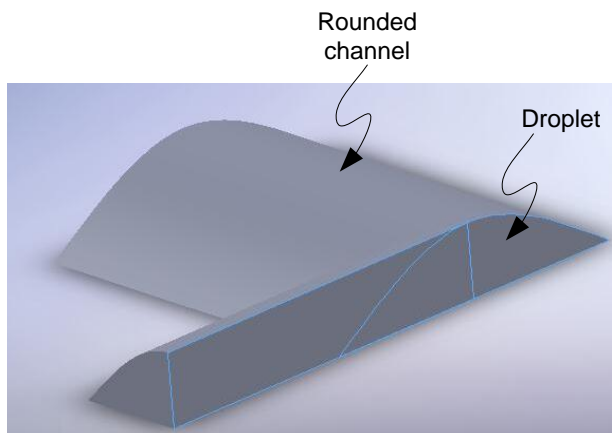
**Figure S5.** Schematic diagrams represent (a) Droplet scanning and (b) Plotting of fluorescence intensities of each droplet.

**Figure S6.** Kinetic parameters from off-chip experiments.

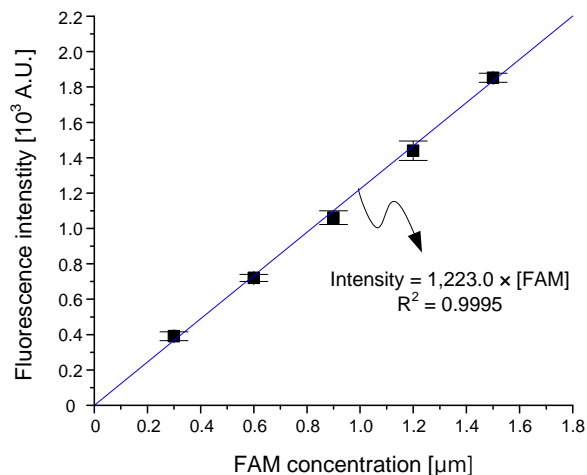
**Figure S7.** Photobleaching of FAM.



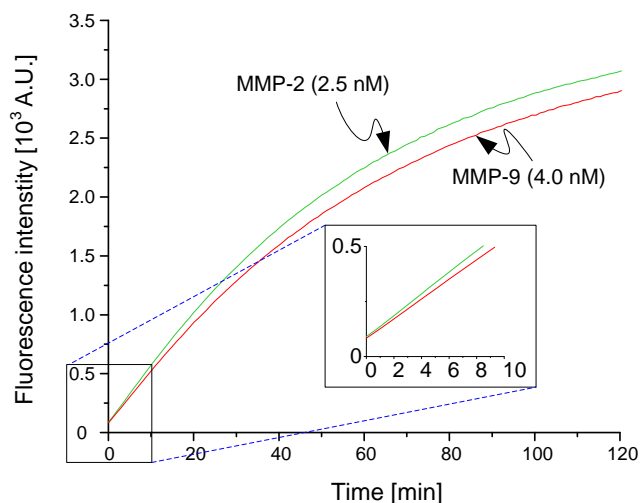
**Figure S1.** The peptide substrate contains a fluorophore, 5-carboxy-fluoresein-Pro-Leu-OH (FAM), and a quencher, QXL520. Upon proteolytic cleavage of the peptide substrate, the fluorescence of FAM is emitted, and the fluorescence signals are observed as an indicator of enzymatic reaction.



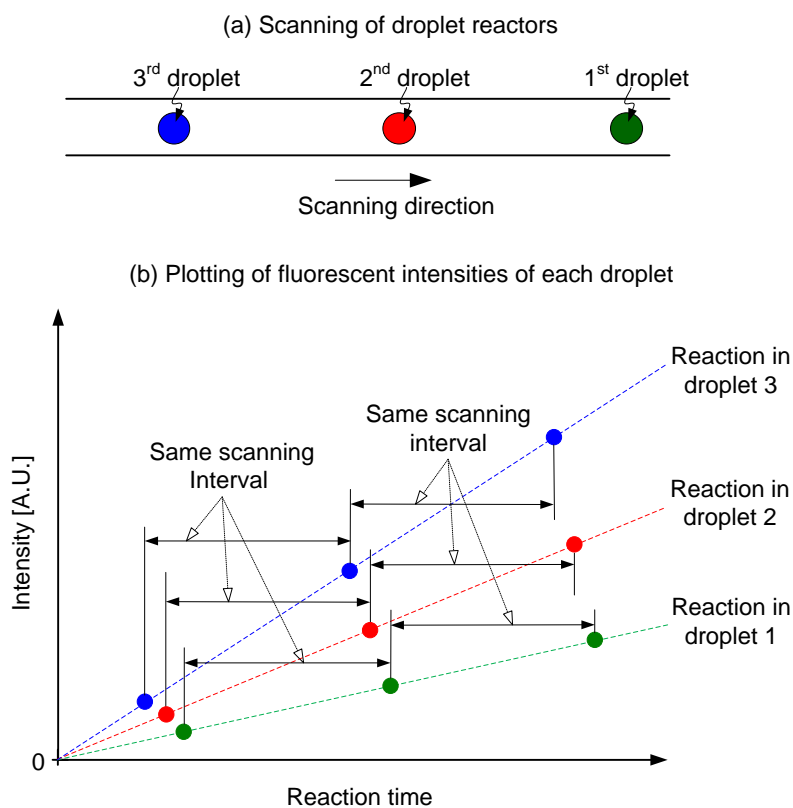
**Figure S2.** Variation of droplet height in the rounded channel.



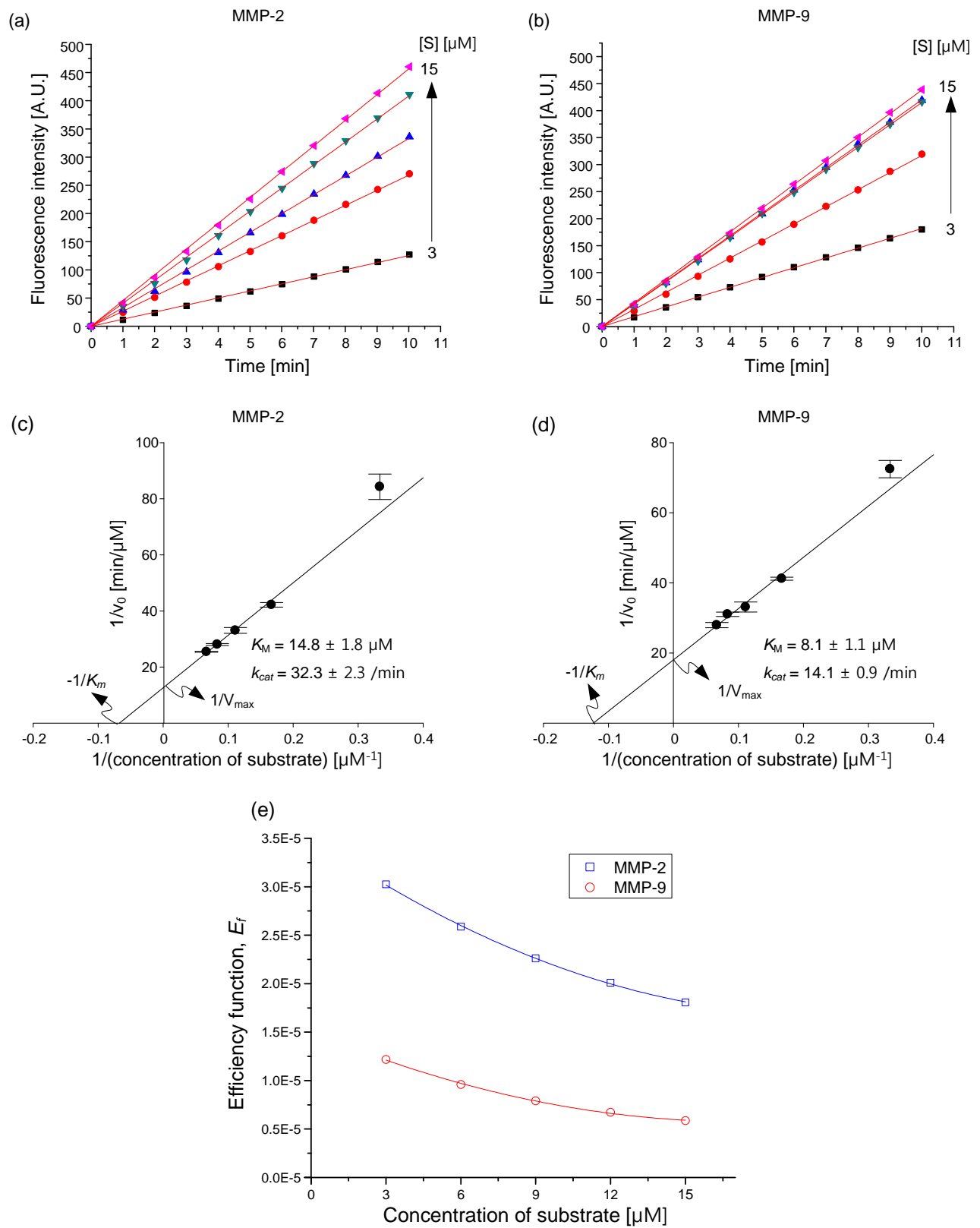
**Figure S3.** Standard curve of the fluorescent molecule, FAM (off-chip).



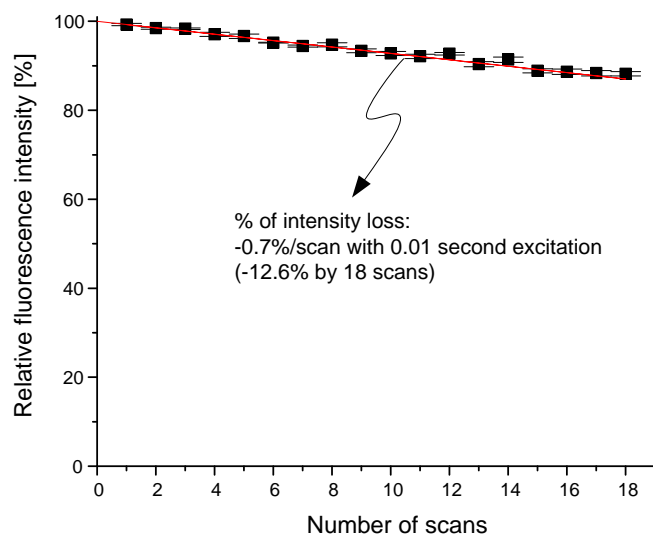
**Figure S4.** Time traces of enzymatic reaction of MMP-2 (2.5 nM) and MMP-9 (4.0 nM) with substrate concentration of 30  $\mu\text{M}$ . The velocities of these two reactions showed linear responses upto 10 min as shown in the inset figure.



**Figure S5.** Scanning droplets and plotting of fluorescent signals.



**Figure S6.** Determination of kinetic parameters using conventional, off-chip, methods. (a) and (b) The time scans of the MMP-2 and 9 reactions with different substrate concentrations. (c) and (d) Lineweaver-Burk kinetic plots. (e) Comparison of the efficiency function,  $E_f$ , of MMP-2 and MMP-9.



**Figure S7.** Effect of multiple scans on the photobleaching of FAM. About 0.7% of the fluorescent intensity was decreased with 0.01 s excitation at 490 nm.