Electronic Supplementary Material (ESI) for Lab on a Chip.
This journal is © The Royal Society of Chemistry 2014

Supplementary Information:

Supplementary 1 – Device Electrode Fabrication

The fabrication of the electrodes utilized a simple liquid-solder approach[1]. First, electrode channels were designed on our photo-mask for the fabrication of a master mold. In this way, the master mold and subsequently the PDMS microfluidic device would contain both the pico-injector array channels and empty electrode channels. After securing a tight bond of the PDMS microfluidic device to the PDMS-coated glass slide, the chip was heated on a hotplate to a temperature greater than 70˚C. Next, a low-melting point liquid alloy solder (Indium Corporation, USA) was injected into the empty electrode channels. Metal wires were inserted into the injection port to connect with the liquid solder. After the injection of liquid solder, the microfluidic chip was set aside for cooling such that the liquid electrodes could solidify into solid metal electrodes. The electrodes were observed under a microscope and tested with a multimeter to ensure proper functioning of the electrodes.

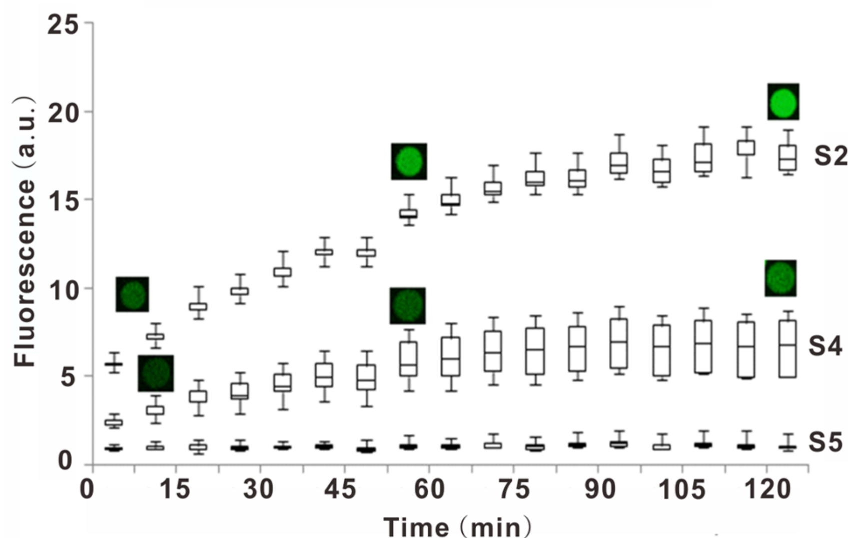
References:

1. Siegel AC, Bruzewicz DA, Weibel DB, Whitesides GM (2007). Microsolidics: Fabrication of three-dimensional metallic microstructures in poly(dimethylsiloxane). Adv Mater 19:727–733

Supplementary 2 – General Reagents

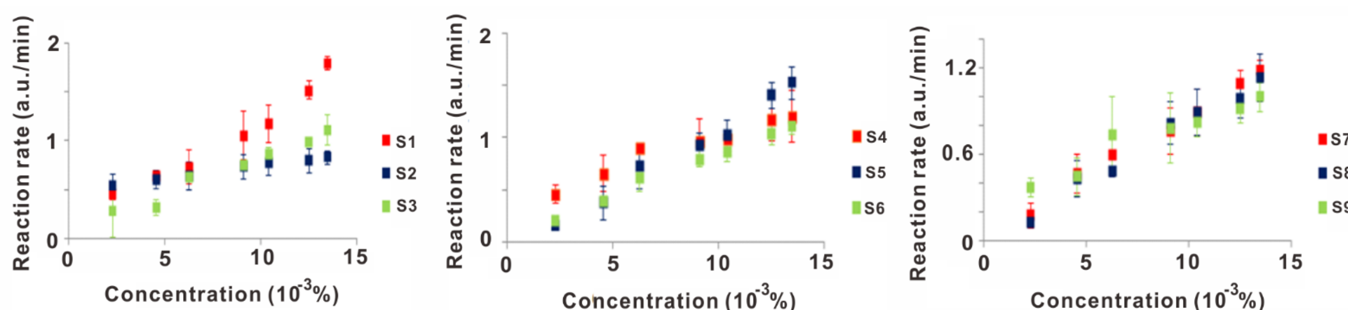
Nine FRET substrates were purchased from Biozyme, Inc; Apex, NC, and are listed as follows: S1: Dabcyl-Gly-Pro-Leu-Gly-Met-Arg-Gly-Lys(5-FAM)-NH2, S2: Dabcyl-Val-Asp-Leu-Phe-Tyr-Leu-Gln-Gln-Pro-Lys(5-FAM)-NH2, S3: Dabcyl-Ala-Pro-Arg-Trp-Ile-Gln-Asp-Lys(5-FAM)-NH2, S4: Dabcyl-Ser-Pro-Leu-Ala-Gln-Ala-Val-Arg-Ser-Ser-Lys(5-FAM)-NH2, S5: Dabcyl-Pro-Cha-Gly-Cys(Me)His-Ala-Lys(5-FAM)-NH2, S6: Dabcyl-His-Gly-Asp-Gln-Met-Ala-Gln-Lys-Ser-Lys(5-FAM)-NH2, S7: Dabcyl-Glu-His-Ala-Asp-Leu-Leu-Ala-Val-Val-Ala-Lys(5-FAM)-NH2, S8: Dabcyl-Ala-Pro-Phe-Glu-Met-Ser-Ala-Lys(5-FAM)-NH2, S9: Dabcyl-Leu-Ala-Gln-Ala-Homophenylalanine-Arg-Ser-Lys(5-FAM)-NH2. All of the substrates have excitation and emission wavelengths of 485 nm and 530 nm, respectively. All of the substrates were diluted in PBS 1x to 20 μM for library construction. Trypsin EDTA 10x (0.5%/0.2% in PBS) was purchased from GE Healthcare, USA. For the metalloproteinase (MMP) study, MMP-2, MMP-3 and MMP-9 were purchased from Enzo Lifescience, USA. Pure MMP recombinants were diluted in MMP buffer (50 mM Tris at pH 7.5, 150 mM NaCl, 2 mM CaCl2, 5 mM ZnSO4, and 0.01% Brij-35). MMP-2 inhibitor IV and MMP-9 inhibitor I were purchased from Merck Millipore, USA.

Supplementary 3 – Time-lapse study of droplets intensities



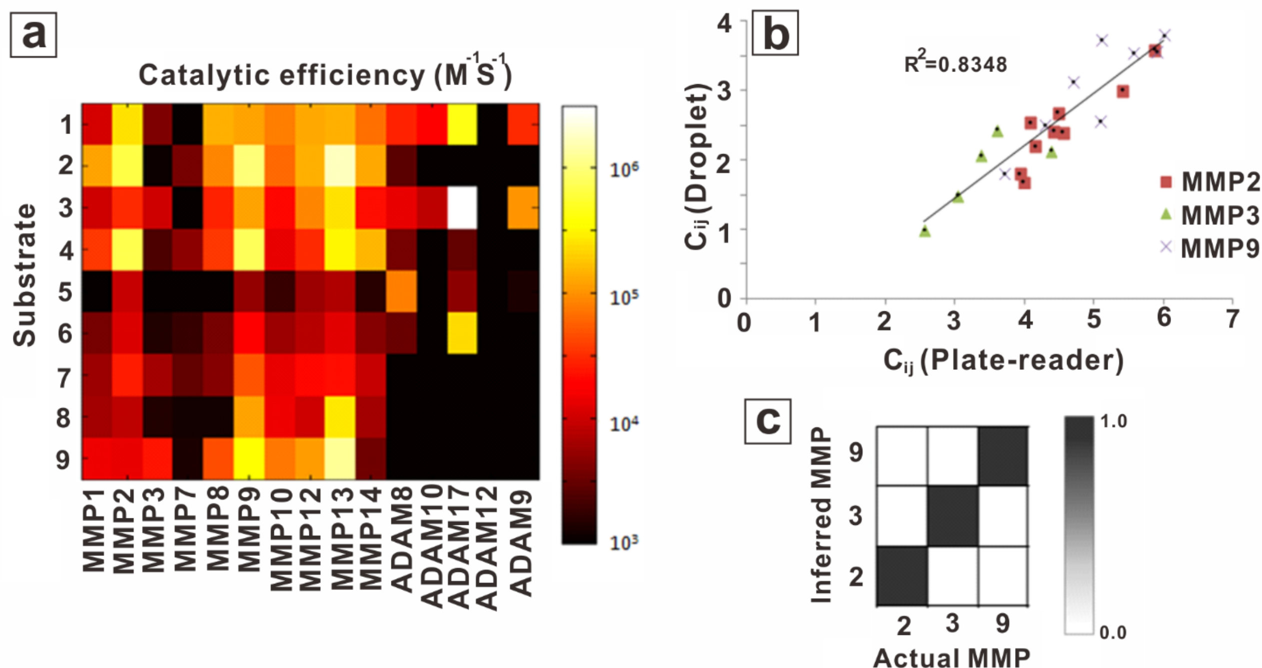
Added Supplementary-3: Time-lapse fluorimetry data of droplets of three substrates reacting with pure recombinant MMP-9. Each set of droplets were stabilized and observed for 2 hours. For illustration purpose, the droplet fluorescent intensity over time of three different substrates with distinct catalytic efficiencies with MMP-9 were recorded. Saturation of fluorescence was generally achieved at 1-1.5 hour mark. To estimate the reaction rates properly, the linear region of protease activities was recorded in the first 30 minutes of reactions (prior to saturation).

Supplementary 4 – Pico-injector array validation with trypsin solutions.



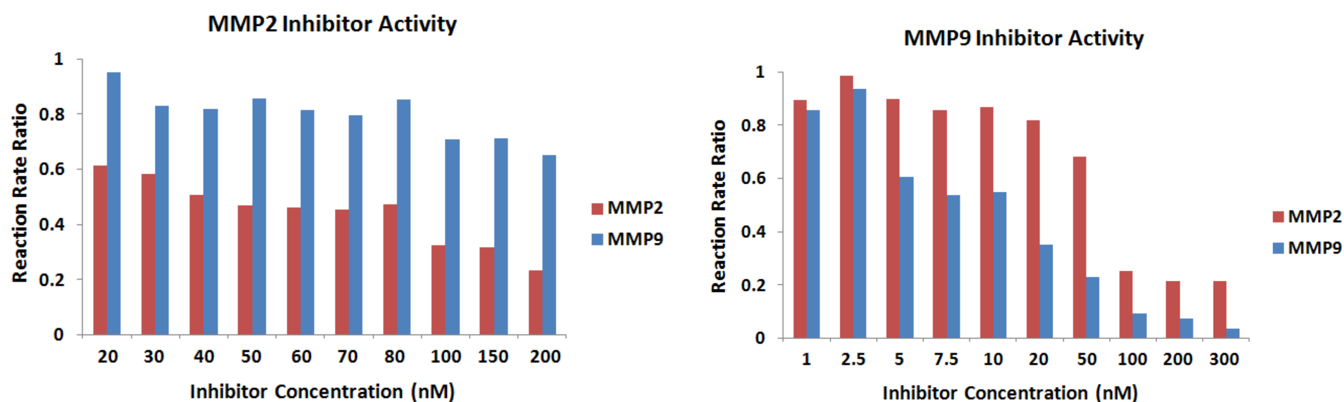
Reaction rates of substrates vs trypsin concentration. Using combinatorial injections, 7 sets of trypsin concentrations were achieved. The results showed a consistent pattern for all 9 substrates: the reaction rates of the substrates were higher when a higher trypsin concentration was injected into the droplets.

Supplementary 5 – PrAMA Calibration and Validation



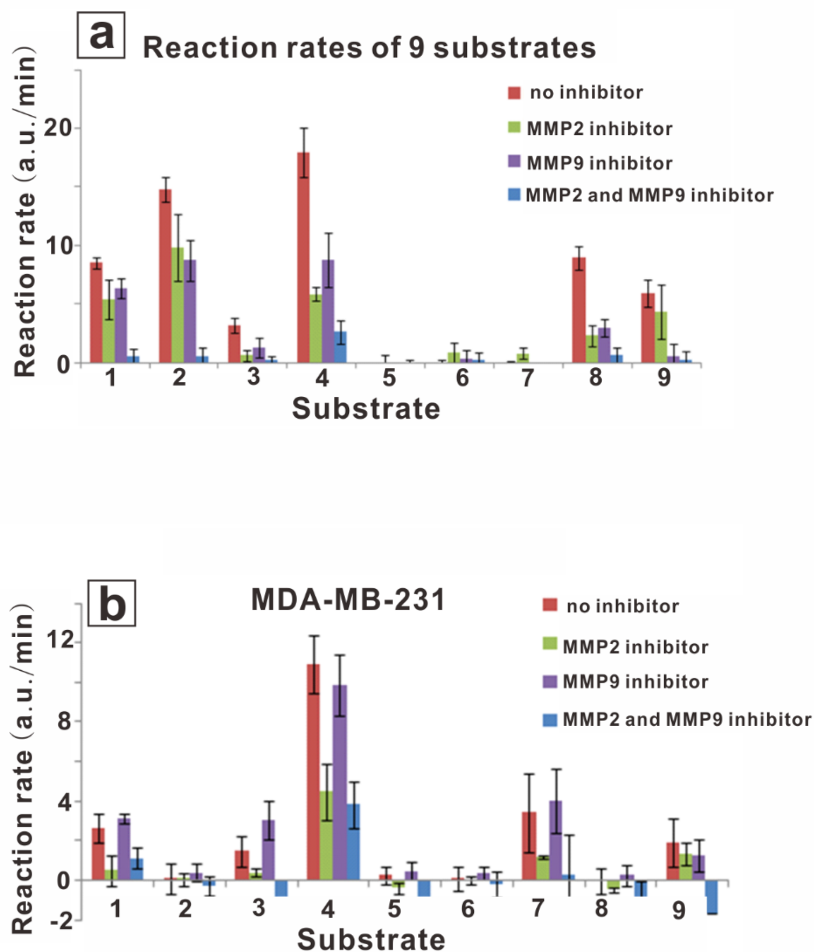
Calibration of droplet-based micro-PrAMA. (a) Heat map of the catalytic efficiency between pairs of metalloproteinases and substrates (previously reported in Miles et al [miles]). B) The catalytic efficiencies obtained using the droplet platform were compared with previously published measurements using a plate reader. The data are log-transformed. C) Validation of PrAMA using pure MMP recombinant. By setting a higher threshold, more false positive results were eliminated, and the inference results can achieve near 100% accuracy.

Supplementary 6 – MMP Inhibitors Calibration



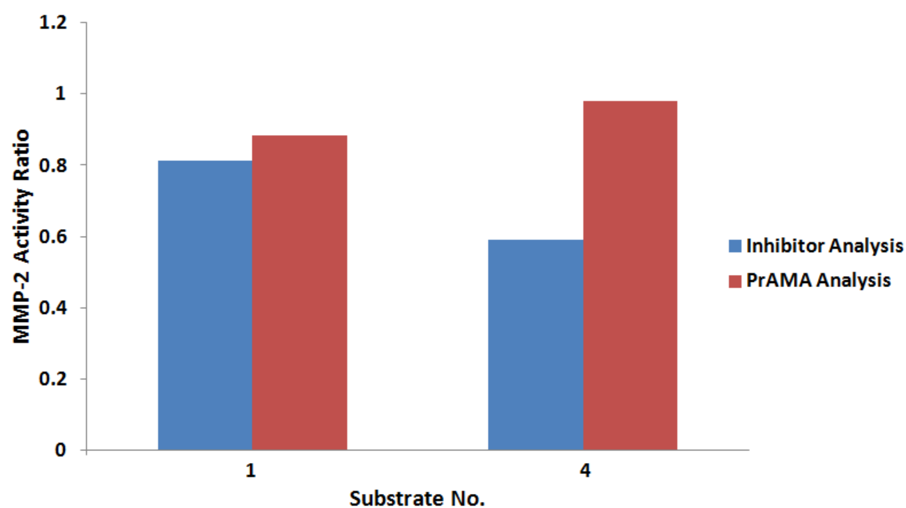
Calibration of MMP-2 inhibitor and MMP-9 inhibitor. For MMP-2 inhibitor, IC_{50} for MMP-2, MMP-8 and MMP-9 are 37nM, 320nM and above 1μM respectively. For MMP-9 inhibitor, IC_{50} for MMP-9, MMP-13 and MMP-1 are 5nM, 113nM and 1.05μM respectively. Our calibration results showed that MMP-2 inhibitor can cause a decrease in reaction rate ratio up to 60% (ratio of MMP-substrate reaction rate with inhibitor to MMP-substrate reaction rate without inhibitor ~40%) at a concentration above 100nM. For MMP-9 inhibitor, a concentration above 50nM can inhibit approximately 80% of activity. However, we observe that certain degree of cross-reactivity can be found if the MMP inhibitors concentrations are too high, especially for MMP-9 inhibitor at high concentration. To prevent excessive cross-reactivity while maintaining efficient inhibitory effect, MMP-2 inhibitor and MMP-9 inhibitor concentration were set at 100nM and 25nM respectively.

Supplementary 7 – Pico-injector array validation using pure MMP recombinants and MDA-MB-231 cells



(a) Reaction rates of substrates vs MMP-2+9 mixture. Significant decreases in the reaction rates can be observed for each substrate when inhibitors were introduced into the assay. MMP-2 and MMP-9 have very similar catalytic reaction profiles with the 9 FRET-based substrates used in this study. Only substrate-9 was significantly more reactive toward MMP-9 compared with MMP-2 (this result can be seen in the inhibitor effect: substrate-9 was well-cleaved by MMP-9 but not effectively cleaved by MMP-2; therefore, the MMP-9 inhibitor was able to drastically decrease the reaction rate of substrate-9 with the MMP-2 & MMP-9 mixture, whereas MMP-2 only caused a slight decrease). This result shows that MMP inhibitor plays a very important role in improving the specificity of the PrAMA inference. (b) Reaction rates of substrates vs MDA-MB-231 cell supernatant. Our results suggested that only MMP-2 activity was detected, and MMP-9 did not contribute to the cleavage of the FRET-based substrates. By using inhibitor, we are able to increase the specificity of the assay and improve the accuracy of the PrAMA inference.

Supplementary 8 – Verification of PrAMA inference with inhibitor analysis



The ratio of inferred MMP-2 activity to the overall inferred activity was compared to the fraction of MMP-2 activity which was inhibited by the MMP-2 inhibitor. Substrate 1 and 4 were analyzed as they were the most easily cleaved substrates by MMP-2 and they also had the highest activities with MDA-MB-231 supernatant. We obtained the ratio of MMP-2 activity to the total proteases activities by calculating the difference between reaction rates with and without MMP-2 inhibitor (inhibitor analysis). We then estimated the MMP-2 activity for each substrate by using PrAMA inference results and information from MMP-substrate catalytic efficiency (PrAMA analysis). Substrate 1 showed good correlation between two analysis results. For substrate 4, the MMP-2 activity ratio was lower in inhibitor analysis compared to that of PrAMA analysis, suggesting possible lack of specificity for either the inhibitor or PrAMA.