Supplementary Information

Nanoscale pipetting for controlled chemistry in small arrayed water droplets using a double barrel pipet

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Materials and Methods

SICM Apparatus. The apparatus used in this experiment (diagramed in Figure 1) was an inverted microscope in which pipets could be mounted and controlled and has been described extensively in previous publications.[1] Unlike the previous set-up, however, the SPM control was achieved using an M44 digital signal processing card (Innovative Integration, Simi Valley, CA) and software developed in-house. The M44 was used both for distance feedback control and to set the potential between the two barrels. Because, as shown in Figure 1, the M44 output is limited to 10 V, a relay was used to switch between the M44 and a DC power supply (Model PAB 350-0.1, Kikusui Electronics Corp., Yokohama, Japan) in order to provide higher voltage pulses. While these pulses could be triggered by the computer, their magnitude (on the DC Power Supply) and width (on a pulse generator, Model 555,

Berkeley Nucleonics Corp., Berkeley, CA) were set by hand. Droplets were created by bringing the pipet into control over the surface (ca. 100 nm), turning off the feedback control, triggering the voltage pulse, and withdrawing (ca. 20 μm) immediately after the pulse. Droplets were addressed in similar fashion, but without turning off feedback control and pulsing. Fluorescence data were collected by a CCD camera (Cascade II 512B, Photometrics, Tucson, AZ) with a dual-view image splitter (Optical Insights, Tucson, AZ) to provide real-time two-channel imaging. The image splitter is equipped with filters HQ535/50m and HQ700/60m (Chroma Thechnology Corp., Rockingham, VT) for the green and red channels respectively. Data were taken and processed with MetaMorph imaging software (Universal Imaging Corporation, Downington, PA). In the case of two-color images (Figure 5), data were obtained separately for the two channels, visualized on a linear scaling of either red or green, and overlaid by the software. The fluorescence microscope was calibrated for Alexa 488, and GFP experiments by measuring the fluorescence 5μm above the surface of bulk solutions of a known concentration of each species under the same measurement conditions as those used in the relevant experiments. Numbers of molecules presented were calculated using these calibrations to determine concentration data and multiplying by drop volume (assuming hemispherical caps).

Pipets. Pipets are routinely fabricated from 1.5 mm diameter glass capillaries using a pipet puller (Sutter Instruments, Model P-2000, Novato, CA). To create double-barrel pipets, capillaries with a central septum, theta glass, are used and this septum is preserved throughout the pulling process. In previous work with double-barrel pipets, the capillaries were oriented in the pipet puller in order to ensure that both barrels were heated equally, thus preserving the symmetry of the barrels in the final tip. However, asymmetric pipets were needed to create the droplets. To produce the asymmetric pipets used here, the capillaries were rotated by 90 degrees when clamped in the puller so that the septum was in the plane normal to the laser. Barrel sizes were characterized by measuring the resistance of each barrel against a bath electrode and calculating barrel radii as previously described for single-barrel

pipets (30). In drop-creation experiments, the pipets were filled with aqueous solutions of 250 mM NaCl, 0.1% Tween-20, and, in fluorescence experiments, 100 nM Alexa 488 or Alexa 647 dye. 0.1% Tween-20 was added to lower the surface tension of the droplets in order to promote the initial drop formation. Filled tips were treated to create hydrophobic outer walls by placing them in a covered dish with 100 μL of chlorotrimethylsilane (TMS) for 2 minutes (gas-phase reaction). Generally, droplets were produced on untreated glass coverslips. Pre-saturated mineral oil was obtained by simply mixing a few mL of organic with ca. 200 μL of Millipore purified water and withdrawing the organic layer to the sample dish.

Cell-Free Protein Expression. For the gene expression experiment, a GFP plasmid vector was prepared by Tim Craggs consisting of truncated-GFPuv containing three surface mutations from wildtype (which aid solubility) and truncated to Ile229 (the full length wildtype sequence being 238 aa) cloned into a PrBE vector (a derivative of the PrSET A,B,C system) engineered by Mark Proctor. This vector was used with a linked transcription:translation kit, PROTEINscript II (Ambion Inc., Austin, TX). The transcription reaction was performed with the provided reagents as described in the kit's instruction manual, while 0.1% Tween-20 and 250 mM NaCl were added to the translation master mix to allow for distance control and drop formation. Because fluorescence was used to monitor GFP expression, no radiolabeling was necessary so only the provided, unlabeled Methionine was used, at a final concentration of 40 μM. The reaction mixture was prepared on ice to inhibit translation and pipets were filled and used for drop production immediately after addition of the transcription product to the translation master mix. The droplet was incubated at room temperature (27 °C). Control experiments followed the same procedure with either DNA (–DNA) or Methionine (–MET) replaced with nuclease-free water (provided by the kit).

Enzyme experiments

For the enzyme experiment, biotin conjugated alkaline phosphatase (AP) was obtained from Rockland Immunochemicals, Inc. (Gilbertsville, PA) and fluorescein diphosphate (FDP) was obtained from Molecular Probes Europe BV (Leiden, The Netherlands). The FDP was purified to reduce fluorescein contamination as described previously.[2]

The activity of the enzyme alkaline phosphatase (AP) was tested on the substrate fluorescein diphosphate (FDP). When AP cleaves FDP, a fluorescent product fluorescein is produced and can be detected with the fluorescence microscope using 488 nm excitation. Because FDP and AP in different barrels of a pipet were observed to come out on the same potential (that is, at the same time), two separate pipets were used in this experiment. First, a pipet containing 1.35 μM FDP (in 0.1 M Tris at pH 8, containing 250 mM NaCl and 0.1% Tween-20) was used to produce two droplets with 50 V, 200 ms pulses. Because the FDP was slightly contaminated with the product fluorescein, these drops were photobleached before the addition of AP. A second tip, containing 5 nM AP (in the same buffer solution as the FDP), was used to address one of these drops for 40 s without pulsing to higher potential. As shown in Figure S1, this droplet's fluorescence intensity increased with time, having leveled off by the 3 hr. data point. The control drop (to which AP was not added) did not exhibit any increase in fluorescence (data not shown). The peak fluorescence measured here corresponds to a fluorescein concentration of 13 nM, which means there are approximately 2,000 product molecules in the 250 fL droplet. This experiment illustrates the activity of biomolecules in the droplets produced with the nanopipet is maintained and indicates the potential of this method for miniaturized biological assays.

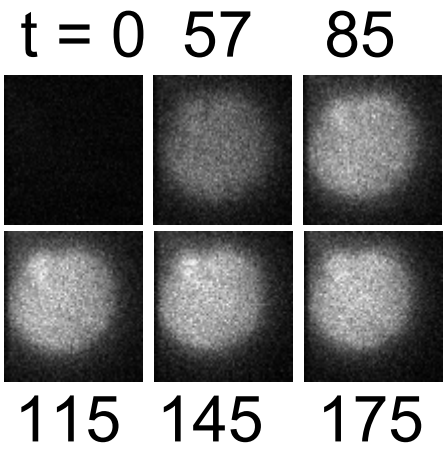

Figure S1. Fluorescein production by cleavage of Fluorescein Diphosphate (FDP) by Alkaline Phosphatase (AP). The images show production of Fluorescein over time (in minutes after the addition of AP) in a 10 μm diameter drop. The production levels off at a Fluorescein concentration of 13 nM, corresponding to approx. 2,000 molecules in the drop. The time after addition of AP is shown in minutes.

References

1. Ying, L. M.; Bruckbauer, A.; Zhou, D. J.; Gorelik, J.; Shevchuk, A.; Lab, M.; Korchev, Y.; Klenerman, D. *Phys. Chem. Chem. Phys.* **2005,** 7, 2859-2866.

2**.** Bruckbauer, A., Zhou, D. J., Ying, L. M., Abell, C. & Klenerman, D. (2004) *Nano Lett.* **4,** 1859-1862.

Movies – All three movies here are presented at 30 frames per second, four times faster than real-time (data was acquired at a rate of 8 fps).

Movie 1. Making a Drop.avi: This movie shows the process of drop production. After the pipet is brought into contact with the surface, a 2 s pulse to 50 V is applied to create the droplet. Immediately after the pulse, the tip is retracted quickly, leaving the droplet on the surface. The final drop size is 3.8 μm in diameter (13.7 fL volume); movie is approximately 20 μm on each side.

Movie 2. Row of Drops.avi: This movie illustrates the reproducibility and potential for arrayed analysis provided by this technique. Here, a row of five drops is created with 1 s pulses to 100 V. Mean drop size is 2.49 ± 0.09 μm in diameter (3.9 ± 0.4 fL volume); movie dimensions are 59.2 μm tall x 12.3 μm wide.

Movie 3. Adding Alexa 488.avi: This movie illustrates the addition of reagents to a droplet without changing its volume. Here, the fluorescent dye Alexa 488 (100 nM concentration in the pipet) is delivered into a pre-existing 4.4 μm diameter (21.3 fL) droplet for 20 seconds with no voltage pulse (tip is controlled at +1 V throughout the addition, as described in the text). The movie shows the droplet prior to addition, addition, retraction of the tip, and subsequent photobleaching of the added dye, stills of which are presented in Figure 4 in the main text; movie is approximately 20 μm on each side.