Supporting Information for

**The Role of Analyte Concentration in Accelerated Reaction Rates in Evaporating Droplets**

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**Table of Contents**

**Section S1. Determining concentrations of DCIP and L-AA in the initially formed droplet using the internal standard peptides in each barrel to determined relative flow rates.**
The concentrations of DCIP and L-AA in the initially formed droplet were determined from the known concentrations of these reactants in each barrel of the theta-glass emitter and was corrected for minor differences in flow rates. Differences in flow rates between the two channels were measured using the relative abundances of the peptides used as internal standards in each channel using the following equations:

$$A_{r,L} = \frac{A_L}{A_M} \tag{S1}$$

$$A_{r,M} = 1 \tag{S2}$$

where $A_L$ and $A_M$ are the abundances of protonated Leu-enk ($m/z$ = 556) and Met-enk ($m/z$ = 574), respectively. $A_{r,L}$ is the relative abundance of protonated Leu-Enk to protonated Met-Enk, making $A_{r,M}$ equal to 1. The relative ionization efficiency of the two peptides was determined in a separate experiment in which a 1:1 ratio of Leu-enk and Met-enk was mixed and resulted in an abundance ratio of 0.8:1.0 Leu-enk:Met-enk. The relative flow rates were determined from the measured abundances of these two peptides, corrected for their different ionization efficiencies using the following equations:

$$Flow_{r,L} = \frac{A_{r,L}}{0.8} \tag{S3}$$

$$Flow_{r,M} = \frac{A_{r,L}}{1} \tag{S4}$$

where $Flow_{r,L}$ and $Flow_{r,M}$ are the relative flow rates of each barrel. The initial concentration of DCIP/L-AA in the droplet is then corrected based on the relative flow rates of each barrel and the initial, known concentration of each reagent using the following equation:

$$[DCIP]_{droplet} = \frac{Flow_{r,L}}{Flow_{r,L} + Flow_{r,M}} \times [DCIP]_{emitter} \tag{S5}$$

where $[DCIP]_{droplet}$ is the concentration of DCIP in the initially formed droplet, and $[DCIP]_{emitter}$ is the known concentration of the solution in the emitter. The initial droplet concentration of L-AA can be found in the same manner using $[L\text{-}AA]_{emitter}$ and $Flow_{r,M}$ in place of $[DCIP]_{emitter}$ and $Flow_{r,L}$ in Eq. S5. The difference between the initial known concentration and the concentration in the droplet that is corrected for minor differences in flow rate are shown in Table S1. These values differ by between 0% and 10%.

| Concentration added (µM) in 1.84 µm emitters | Concentration of DCIP in the nanodrop (µM) | Concentration of L-AA in the nanodrop (µM) | Concentration added (µM) in 684 nm emitters | Concentration of DCIP in the nanodrop (µM) | Concentration of L-AA in the nanodrop (µM) |
|---|---|---|---|---|---|
| 0.005 | 0.005 ± 0.0002 | 0.005 ± 0.0002 | 0.050 | 0.050 ± 0.001 | 0.050 ± 0.001 |
| 0.0125 | 0.0121 ± 0.0004 | 0.0129 ± 0.0004 | 0.125 | 0.124 ± 0.001 | 0.126 ± 0.001 |
| 0.020 | 0.020 ± 0.0003 | 0.020 ± 0.0003 | 0.150 | 0.150 ± 0.002 | 0.150 ± 0.002 |
| 0.025 | 0.025 ± 0.0002 | 0.025 ± 0.0002 | 0.200 | 0.198 ± 0.006 | 0.202 ± 0.006 |
| 0.0375 | 0.0369 ± 0.0005 | 0.0381 ± 0.0005 | 0.250 | 0.248 ± 0.002 | 0.252 ± 0.002 |
| 0.050 | 0.050 ± 0.001 | 0.050 ± 0.001 | 0.375 | 0.378 ± 0.006 | 0.372 ± 0.007 |
| 0.125 | 0.126 ± 0.001 | 0.124 ± 0.001 | 0.500 | 0.483 ± 0.007 | 0.517 ± 0.002 |
| 0.25 | 0.25 ± 0.00 | 0.25 ± 0.00 | 1.00 | 1.02 ± 0.00 | 0.98 ± 0.00 |
| 0.50 | 0.48 ± 0.01 | 0.52 ± 0.02 | 1.50 | 1.49 ± 0.02 | 1.51 ± 0.02 |
| 1.25 | 1.28 ± 0.02 | 1.22 ± 0.02 | 2.00 | 1.97 ± 0.02 | 2.03 ± 0.01 |
| 2.50 | 2.48 ± 0.05 | 2.52 ± 0.06 | 2.50 | 2.57 ± 0.03 | 2.43 ± 0.03 |
| 5.00 | 4.97 ± 0.10 | 5.03 ± 0.11 | 3.75 | 3.75 ± 0.05 | 3.75 ± 0.05 |
| 25.0 | 25.3 ± 0.2 | 24.7 ± 0.3 | 5.00 | 5.06 ± 0.17 | 4.94 ± 0.16 |
| 50.0 | 49.3 ± 1.8 | 50.7 ± 1.8 | 25.0 | 25.1 ± 0.5 | 24.8 ± 0.5 |
| 250.0 | 277 ± 11 | 223 ± 11 | 50.0 | 48.3 ± 0.4 | 51.7 ± 0.4 |
| 500.0 | 492 ± 9 | 508 ± 9 | 250.0 | 250.4 ± 1.6 | 249.5 ± 1.6 |
|  |  |  | 500.0 | 504 ± 5 | 496 ± 5 |

**Table S1.** Known concentrations of DCIP and L-AA added to the emitters and concentrations of DCIP and L-AA in the droplet determined from these values corrected for relative flow rates based on the abundances of the two peptide internal standards. Differences between the concentrations in the emitter and in the nanodrop differ by 0-10%. The method used to determine these values are provided in Section S1.
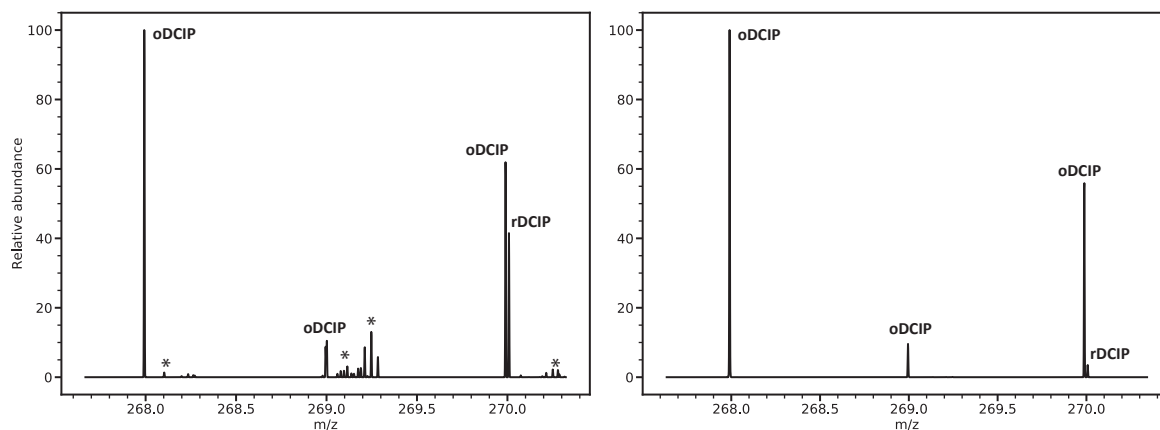
**Figure S1.** Representative mass spectra obtained with a 1.84 μm emitter from a) a 25 nM: 25 nM solution and b) a 500 μM: 500 μM solution in the region around oxidized DCIP (*m/z* 267.99). An A+2 isotope of DCIP is at *m/z* 269.96 and reduced DCIP (product) appears at *m/z* 270.08. Starred peaks are background contaminants in the pH 3 water solution.

| Concentration added (µM) in 1.84 µm emitters | Conversion ratio | Concentration added (µM) in 684 nm emitters | Conversion ratio |
|---|---|---|---|
| 0.005 | <0.003* | 0.050 | <0.003* |
| 0.0125 | <0.002* | 0.125 | 0.008 ± 0.010 |
| 0.020 | 0.217 ± 0.009 | 0.150 | 0.062 ± 0.031 |
| 0.025 | 0.414 ± 0.033 | 0.200 | 0.136 ± 0.011 |
| 0.0375 | 0.346 ± 0.059 | 0.250 | 0.154 ± 0.022 |
| 0.050 | 0.373 ± 0.025 | 0.375 | 0.124 ± 0.012 |
| 0.125 | 0.316 ± 0.060 | 0.500 | 0.122 ± 0.012 |
| 0.250 | 0.300 ± 0.009 | 1.000 | 0.107 ± 0.003 |
| 0.500 | 0.291 ± 0.005 | 1.500 | 0.076 ± 0.010 |
| 1.250 | 0.269 ± 0.019 | 2.000 | 0.071 ± 0.021 |
| 2.500 | 0.234 ± 0.055 | 2.500 | 0.068 ± 0.008 |
| 5.000 | 0.173 ± 0.060 | 3.750 | 0.066 ± 0.009 |
| 25.00 | 0.157 ± 0.010 | 5.000 | 0.064 ± 0.013 |
| 50.00 | 0.077 ± 0.004 | 25.00 | 0.072 ± 0.003 |
| 250.0 | 0.099 ± 0.021 | 50.00 | 0.079 ± 0.028 |
| 500.0 | 0.073 ± 0.008 | 250.0 | 0.048 ± 0.019 |
| | | 500.0 | 0.050 ± 0.020 |

**Table S2.** Tabulated values of the conversion ratios for 1:1 reaction of DCIP and LAA obtained with 1.84 µm and 684 nm emitters at various concentrations. This conversion ratio was determined using Eq. 1 in the main text. Starred entries in the table represent the detection limit for these experiments where no product abundance was seen above the detection limit.

| Concentration added (µM) in 1.84 µm emitters | Acceleration factor | Concentration added (µM) in 684 nm emitters | Acceleration factor |
|---|---|---|---|
| 0.005 | N/A | 0.050 | N/A |
| 0.0125 | N/A | 0.125 | $1.63\times10^5 \pm 0.64\times10^5$ |
| 0.020 | $1.12\times10^7 \pm 0.04\times10^7$ | 0.150 | $1.09\times10^6 \pm 0.36\times10^6$ |
| 0.025 | $2.31\times10^7 \pm 0.21\times10^7$ | 0.200 | $1.86\times10^6 \pm 0.11\times10^6$ |
| 0.0375 | $1.19\times10^7 \pm 0.19\times10^7$ | 0.250 | $1.72\times10^6 \pm 0.18\times10^6$ |
| 0.050 | $9.68\times10^6 \pm 0.64\times10^6$ | 0.375 | $8.95\times10^5 \pm 0.61\times10^5$ |
| 0.125 | $3.13\times10^6 \pm 0.56\times10^6$ | 0.500 | $6.62\times10^5 \pm 0.51\times10^5$ |
| 0.250 | $1.39\times10^6 \pm 0.05\times10^6$ | 1.000 | $2.84\times10^5 \pm 0.23\times10^5$ |
| 0.500 | $6.64\times10^5 \pm 0.19\times10^5$ | 1.500 | $1.29\times10^5 \pm 0.04\times10^5$ |
| 1.250 | $2.38\times10^5 \pm 0.15\times10^5$ | 2.000 | $9.09\times10^4 \pm 0.79\times10^4$ |
| 2.500 | $1.03\times10^5 \pm 0.18\times10^5$ | 2.500 | $6.85\times10^4 \pm 0.11\times10^4$ |
| 5.000 | $3.58\times10^3 \pm 0.97\times10^3$ | 3.750 | $4.46\times10^4 \pm 0.40\times10^4$ |
| 25.00 | $5.99\times10^3 \pm 0.30\times10^3$ | 5.000 | $3.28\times10^4 \pm 0.46\times10^4$ |
| 50.00 | $1.36\times10^3 \pm 0.06\times10^3$ | 25.00 | $3.69\times10^3 \pm 0.13\times10^3$ |
| 250.0 | $3.64\times10^2 \pm 0.62\times10^2$ | 50.00 | $7.04\times10^3 \pm 1.30\times10^3$ |
| 500.0 | $1.27\times10^2 \pm 0.10\times10^2$ | 250.0 | $4.83\times10^2 \pm 1.21\times10^2$ |
|  |  | 500.0 | $2.52\times10^2 \pm 0.69\times10^2$ |

**Table S3.** Tabulated values of the acceleration factors for 1:1 reaction of DCIP and L-AA obtained with 1.84 µm and 684 nm emitters at various concentrations determined from Eq. 2 in the main text. Acceleration factors for low concentrations where no product abundance was seen above the noise level are listed as N/A in the table.

| Emitter Tip size | Flow rate from spray + evaporation (nL/min) | Flow rate from evaporation (nL/min) | Flow rate from spray (nL/min) |
|---|---|---|---|
| 1.84 μm | 45 ± 8 | 10 ± 6 | 36 ± 3 |
| 684 nm | 29 ± 13 | 11 ± 2 | 18 ± 11 |

**Table S4.** Solution flow rates were determined by measuring the masses of the theta-glass emitters before and after spraying solution for 15-30 minutes in the same position and under the same conditions used in all of the experiments. To determine the contribution from evaporation, the emitters were placed in the same position in front of the instrument inlet for 15-30 minutes without any applied voltage. This was done to ensure equivalent temperature resulting from heat from the mass spectrometer interface. The mass was converted to a volume by using the density of water at room temperature, 997.05 kg m$^{-3}$.
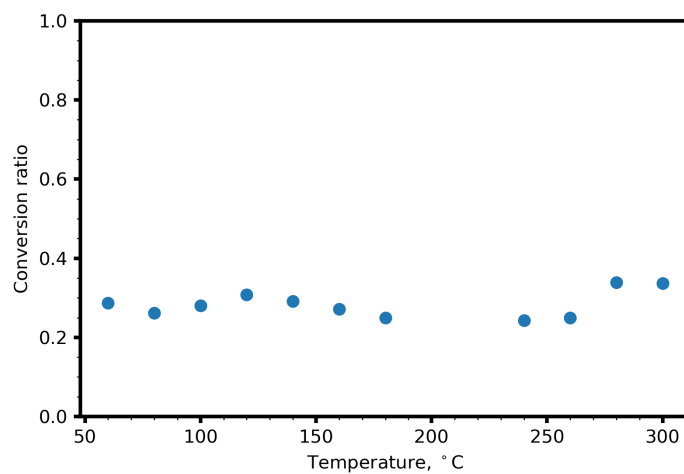
**Figure S2.** Effects of the capillary inlet temperature on the conversion ratio obtained using 1 µM DCIP + 1 µM L-AA rapidly mixed using a theta-glass emitter with a 1.84 µm emitter. There is no significant change in the conversion ratio with capillary inlet temperature over this range of values.

| | Volume of droplet containing two molecules (nL) | Droplet diameter (nm) | $\dfrac{D_{droplet}}{D_{emitter}}$ |
|---|---|---|---|
| 1.84 μm emitters (20 nM) | $8.3 \times 10^{-8}$ | 541 | 0.37 |
| 684 nm emitters (100 nM) | $1.7 \times 10^{-8}$ | 317 | 0.46 |

**Table S5.** The volume and diameter of a spherical droplet containing two molecules at the total solution concentration corresponding to the midpoint of the low-concentration drop-off in rate acceleration (see text) is given in this table. A droplet containing two molecules has an equal probability of containing two DCIP molecules, two L-AA molecules or one of each assuming complete mixing in the Taylor cone prior to initial droplet formation. Reactions should be significantly reduced at and below these two molecules per droplet limit. Concentration values were 20 nM and 100 nM for the 1.84 μm and 684 nm emitters, respectively. The solution volume was determined from the following equation:

$$V = \frac{2\ molecules}{M_{total} \times N_A} \times 10^9 \tag{S6}$$

where V is the volume, $M_{total}$ is the total concentration of reagents in the droplet, $N_A$ is Avogadro's number, and $10^9$ is a conversion from liters to nanoliters. The droplet diameter was determined assuming a spherical shape. The ratio of the droplet diameter to the emitter diameter in the long dimension is also provided in the Table.