Electronic Supplementary Material (ESI) for Analytical Methods.


**Electronic Supplementary Information**

# Incorporation of Thermal Gels for Facile Microfluidic Transient Isotachophoresis

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## 1 Analyte Structures

The structures of the three fluorescent dyes used in this study are illustrated in Figure S1. All dyes have comparable excitation and emission wavelengths enabling each to be detected with a single optical filter set.

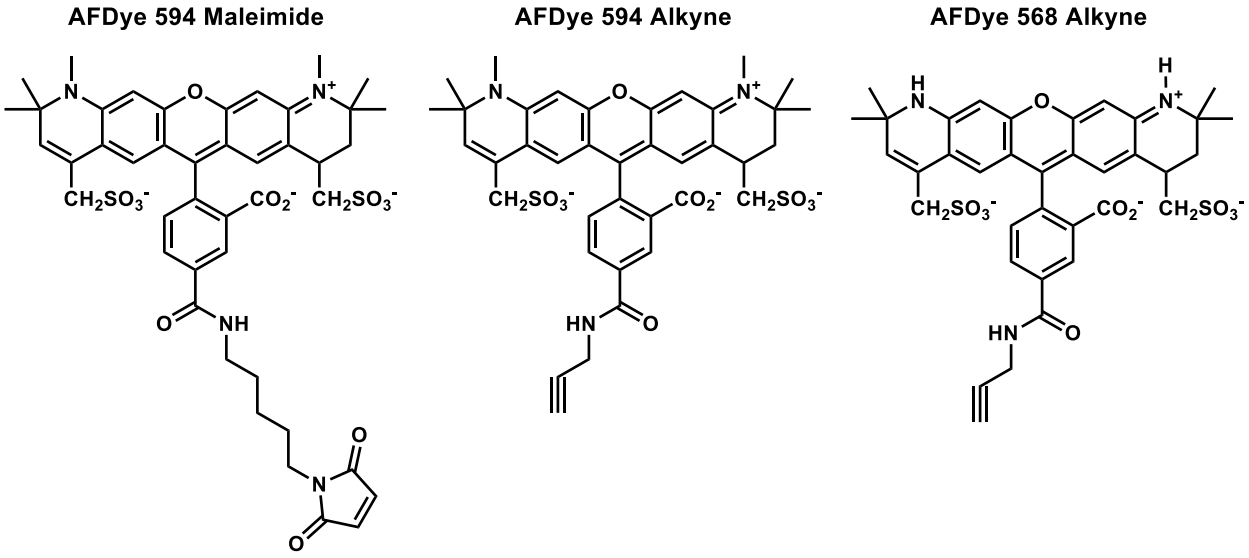


**Figure S1**. Chemical structures of AFDye 594 Maleimide, AFDye 594 Alkyne, and AFDye 568 Alkyne.

## 2 Device Operation

Voltages were applied using a high voltage power supply to drive fluid flow during priming, injection, and separation steps. Specific voltages and times are reported in Table S1 for tITP conditions analyzing dyes and DNA and for gated MGE experiments.

**Table S1.** Priming, injection, and separation voltages (kV) and times for tITP experiments and for gated MGE.

| Reservoir | tITP, Dyes | | | Gated MGE, Dyes | | | tITP, DNA | | |
|---|---|---|---|---|---|---|---|---|---|
| | Prime | Inject | Separate | Prime | Inject | Separate | Prime | Inject | Separate |
| R1 | -0.5 | -1 | 0 | -0.3 | -2 | -0.3 | -1 | -1 | 0 |
| R2 | 0 | 0 | -1.5 | -0.8 | 0 | -0.8 | 0 | 0 | -1 |
| R3 | 0.5 | 0 | 0 | 0.4 | 0 | 0.4 | 0 | 0 | 0 |
| R4 | 0 | 1 | 1.5 | 1.7 | 2 | 1.7 | 1 | 1 | 1 |
| Time (s) | 60 | 5 | 120 | 60 | 2 | 120 | 24 | 5 | 120 |

## 3 tITP Characterization

### 3.1 Preconcentration Factor

Analyte preconcentration using a finite ITP injection scheme was determined using AFDye 594 Maleimide as the model analyte. A calibration curve (5 nM–1,000 nM) of the dye was obtained by filling device channels with dye (without conducting ITP) and integrating the fluorescence signal using MicroManager. Fluorescence intensity was plotted against dye concentration in the channel to generate a calibration curve (Figure S2). ITP was then used to focus analytes analogous to the tITP studies discussed in the main manuscript. The fluorescence of the post-ITP focused band was integrated and its apparent concentration determined from the pre-ITP calibration curve. The preconcentration factor was calculated by dividing the post-ITP dye concentration by the initial pre-ITP concentration (Table S2). Preconcentration factors of up to 2,300-fold were obtained. A limitation of this study is a lack of high-end sensitivity due to saturation of the camera. Preconcentration factors of higher concentration samples (>10 nM) were artificially under-reported because their true post-ITP concentrations extended beyond the linear calibration range.

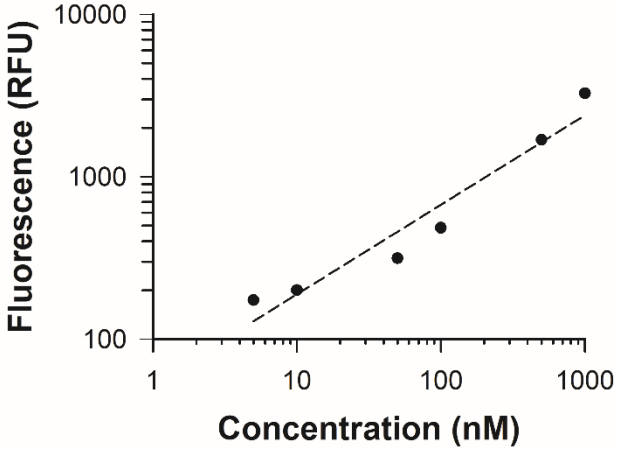


**Figure S2**. Calibration curve of AFDye 594 Maleimide ($y = 3.09x + 163.2$, $R^2 = 0.999$). The curve contains six calibration points (n=3 per point). Error bars represent ±1 standard deviation (smaller than the data point circles).

**Table S2**. Table of pre-ITP AFDye 594 Maleimide concentrations (n=3) and their corresponding post-ITP concentrations and preconcentration factors.

| Pre-ITP Concentration (nM) | Post-ITP Concentration (nM) | Preconcentration Factor |
|---|---|---|
| 0.01 | 23 | 2300 |
| 0.1 | 86 | 860 |
| 1 | 310 | 310 |
| 10 | 830 | 83 |
| 100 | 1100 | 11 |
| 1000 | 1300 | 1.3 |

### 3.2 tITP Calibration Curves

Calibration curves for AFDye 594 Maleimide, AFDye 594 Alkyne, and AFDye 568 Alkyne were generated after thermal gel tITP separations (Figure S3). Dyes were loaded at concentrations of 0.05, 0.1, 0.5, 1, 5, 10, and 100 nM. Fluorescence signal intensity was measured and MicroManager was used to generate electropherograms from the processed image files. The 100 nM sample was determined to be the high end of the linear dynamic range before camera saturation occurred. The Chromophoreasy software package was used to integrate peaks allowing us to measure peak areas, migration times, and peak widths to calculate peak resolutions and separation efficiencies. The equations used to calculate resolution (R) and separation efficiency (N) are shown below where $t_m$ is the analyte migration time and $w_{0.5}$ is the width of the peak at half-height.

$$R = 1.175\,\frac{t_{m,2} - t_{m,1}}{w_{0.5,1} + w_{0.5,2}} \qquad N = \frac{5.545\,{t_m}^2}{{w_{0.5}}^2}$$

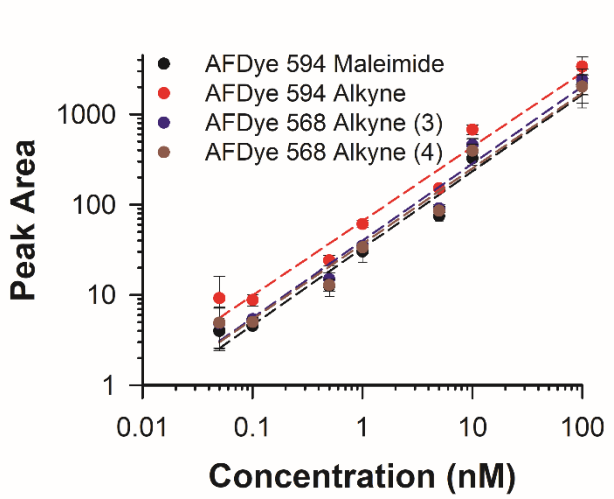


**Figure S3**. Calibration curves for AFDye 594 Maleimide (y = 20.183x + 31.58, $R^2$ = 0.991), AFDye 594 Alkyne (y = 33.478x + 58.762, $R^2$ = 0.990), and AFDye 568 Alkyne (Peak 3 [y = 23.941x + 32.375, $R^2$ = 0.991] and Peak 4 [y = 21.085x + 15.769, $R^2$ = 0.997]) following finite ITP injections. Each calibration curve is constructed from seven dye concentrations (n=3 replicates per point). Error bars represent ±1 standard deviation.

## 4 Gated Injection MGE Characterization

### 4.1 Calibration Curves

Calibration curves were generated using dye concentrations of 1, 5, 10, 50, 100, 500, and 1000 nM (n = 3 replicates) for AFDye 594 Maleimide, AFDye 594 Alkyne, and AFDye 568 Alkyne from the gated injection scheme (Figure S4). Images were processed with MicroManager and separation metrics were calculated with Chromophoreasy, as described above.

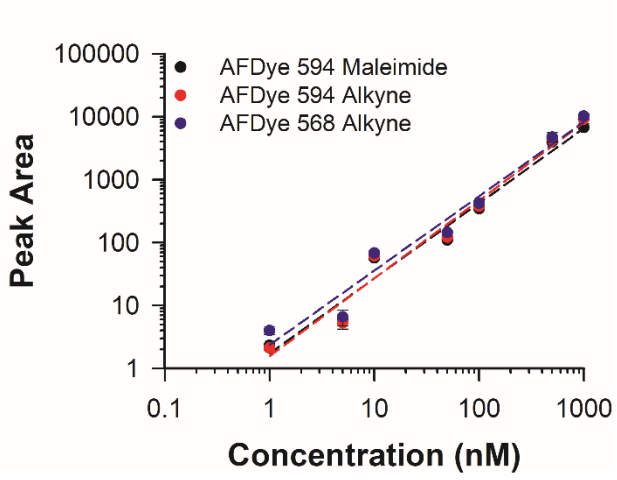


**Figure S4**. Calibration curves for AFDye 594 Maleimide ($y = 7.002x – 65.745$, $R^2 = 0.989$), AFDye 594 Alkyne ($y = 9.198x – 197.58$, $R^2 = 0.997$), and AFDye 568 Alkyne ($y = 10.277x – 223.19$, $R^2 = 0.996$) following gated injections. Each calibration curve is constructed from seven concentrations (n=3 replicates per point). Error bars represent ±1 standard deviation.

### 4.2. Separation Characterization

Separation efficiencies (at half-height) and peak resolutions are presented in Table S3 for each dye analyzed in the gated MGE separations. Plate numbers were not as high as in tITP but were comparable to electrophoretic separations in PDMS devices. All dyes were baseline resolved; however, AFDye 568 Alkyne variants did not split into separate peaks under these conditions. A comparison of electropherograms obtained using tITP and gated electrophoresis is shown in Figure S5 analyzing 5 nM dye samples. This figure depicts the power of our tITP method compared to gated microchip electrophoresis.

**Table S3**. Separation efficiencies and resolutions from gating studies in the thermal gel. Values reflect averages from electropherograms used to generate the calibration curve (n = 15) across the calibration range (n = 5 concentrations). Values are reported ±1 standard deviation.

| Peak | N x $10^3$ (plates $m^{-1}$) | Resolution |
|---|---|---|
| 1 | 234 ± 1 | 4.9 ± 0.5 |
| 2 | 286 ± 2 | 1.6 ± 0.1 |
| 3 | 227 ± 1 | – |

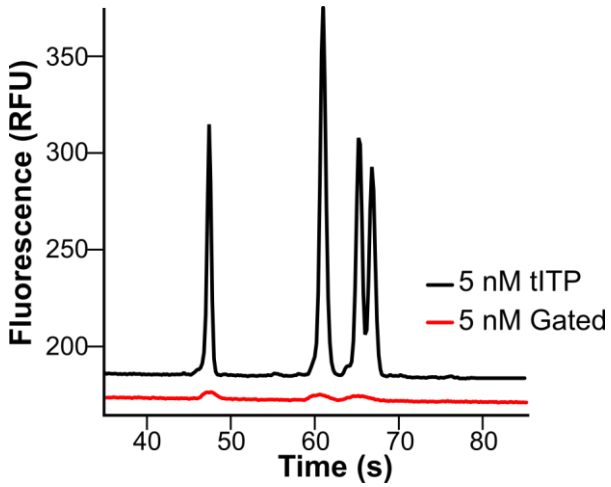


**Figure S5.** Comparison between tITP and gated MGE separations of 5 nM dyes in 30% PF-127.