Supporting Information

Behavior of Interacting Species in Capillary Electrophoresis Described by Mass Transfer Equation

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Figure S-1. CASE B, $\mu_P > \mu_A > \mu_C$, Scenario B-2.

The experimental parameters are listed in Table 1, which is identical to the table presented in the main body of this paper. In this scenario, most of the free analyte is complexed with the additive, and there is not enough free analyte to develop the front slope. As a result, the final analyte plug is not a Gaussian peak, but a peak with a plateau in the middle. The profile of free P (dash-dot line) has a similar shape with a height of 3.626×10^{-4} M or 101% of $[P]_0$. Although no Gaussian peak is obtained in Scenario B-2, the migration time of the rear edge (left hand side) of the analyte plateau can be used for the regression methods. The reason is that a steady-state condition is reached at the rear edge of the analyte plug shortly after the CE run begins, and [P] at the rear edge is nearly equal to $[P]_0$ for almost the entire process.

Figure S-2. CASE C, $\mu_C > \mu_A > \mu_P$, Scenario C-2.

In Scenario C-2, $[P]_0$ is increased by 10 times. As expected, [P] within the analyte plug is now closer to $[P]_0$ (~15.12 mM or 101%). The front edge (right hand side) of the peak is saturated with the additive from the start of the electrophoretic migration process, which means a steady state condition is reached for this edge for the entire process. The distance traveled by the front edge is the length from the inlet of the capillary to the detector minus the length of the injection plug.

Because [P] is much closer to $[P]_0$, the mobility difference between Peak 1 and the rest of the analyte plug is smaller. Peak 1 has yet to reach the outlet edge, and remains as a small bump over the top of the plateau with this set of simulation conditions.

Figure S-3. CASE D, $\mu_A > \mu_C > \mu_P$, Scenarios D-2 and D-3.

In Scenario D-2, $[P]_0$ is increased by 10 times. As more complex and less free analyte exist in the analyte plug, the rear slope is much smaller (Figure S-3A). In Scenario D-3, $[A]_0$ is increased by 10 times. It would take much longer time for the additive to sweep through the analyte plug. The

concentration of P in the analyte plug increases much slower. Therefore, a longer rear slope is created (Figure S-3B).

Figure S-4. CASE E, $\mu_A > \mu_P > \mu_C$, Scenarios E-2, E-3, and E-4.

In Scenario E-2, $[P]_0$ is increased to 50 mM while other conditions are kept the same as in E-1. The final peak for this scenario is displayed in Figure S-4A. The left peak is higher and sharper, and the right peak vanishes, because most of the analyte is in the form of complex and is lagging behind. In Scenario E-3, only the $[A]_0$ is reduced by 10 times. The free additive can get to the left side of the analyte band much more easily. The positive additive peak is very small, and the additive trough is narrower. As a result, in Figure S-4B, both the left and the right peaks become narrower.

In Scenario E-4, the only change is reducing the binding constant from 500 to 100. Because the free additive can now sweep through the analyte plug more easily, the left peak completely disappears in Figure S-4C. The only peak with a large rear slope looks similar to the one shown in Figure 8D (Scenario D-1). Keeping K at 100 M^{-1}, $[P]_0$ is again increased by 10 times in Scenario E-5 to increase the [C] to [A] ratio. The left peak reappears in Figure S-4D.

Figure S-5. CASE F, $\mu_C > \mu_P > \mu_A$, Scenario F-1.

Case A, C and F share one common property: $\mu_C > \mu_A$. Following our discussion of the similarities between the cases, we can expect Case F to show the characteristics of Case A + Case C. Scenario F-1 is simulated with the conditions listed in Table 1, and the profiles shown in Figure S-5 agree with our prediction.

Stage 1: The free additive enters the analyte plug to form the complex at the rear edge. At the same time, a small amount of the free additive mixes with the analyte at the front edge of the analyte plug to form a small Peak 1 (Figure S-5A) for the same reasons as mentioned in Case B. Peak 1 is visible throughout the process.

Stage 2: The free additive keeps moving into the analyte plug to form a plateau with a mixture of free and complexed analyte, and a sweeping effect, similar to the one in Case A, takes place to reduce the plug length, as shown in Figure S-5B. An additive gap is also formed in the process.

Stage 3: The faster migrating complex then moves out of the analyte plug and into the additive gap (Figure S-5C), and the length of the analyte plug increases. The analyte plug consists of two plateaus which are connected with a slope. Eventually, the additive gap disappears, and an additive valley with two sections, one V-shaped hole and one plateau, is formed (Figure S-5D). The left plateau on the analyte plug vanishes, while the right plateau becomes wider.

Stage 4: The complex at the front edge of the plug continues to move forward to produce another lower plateau where an additive plateau containing higher [P] than $[P]_0$ is also formed. The analyte plug keeps evolving and extending its length, and the profile reaching the detector is shown in Figure S-5F. The distance between the analyte plug and Peak 1 gets smaller because the right edge of the analyte plug migrates at a higher speed with higher additive concentration.

In this case, the left peak coexists with the negative additive trough, therefore, cannot be used to produce accurate results by the regression methods. The right peak develops after the gap of the free additive is filled. Once again, neither left nor right peaks are suitable for the regression methods. However, similar to Case E, if the additive concentration is high enough to fill the analyte plug quickly, it is possible to use the right peak in the regression methods to calculate approximate binding constants.

Table 1. The experimental conditions for 18 scenarios. $[A]_0$ and $[P]_0$ are the initial analyte and additive concentrations, respectively, μ_A, μ_P, and μ_C are the mobilities of free analyte, free additive, and the complex formed, respectively, and K is the binding constant.

	$[A]_0$ (mM)	$[P]_0$ (mM)	Length of Capillary (cm)	Length to Detector (cm)	Length of Injection Plug (cm)	μ_A	μ_P	μ_C	Voltage (kV)	K (M^{-1})
						($x10^{-4}cm^2/V{\cdot}s$)				
Scenario A-1	2.0	5.0	64.5	54.3	0.18	1.364	3.699	2.994	10	533
Scenario A-2	2.0	**50**	64.5	54.3	0.18	1.364	3.699	2.994	10	533
Scenario A-3	**0.2**	5.0	64.5	54.3	0.18	1.364	3.699	2.994	10	533
Scenario A-4	2.0	5.0	64.5	54.3	0.18	1.364	3.699	**1.600**	10	533
Scenario A-5	2.0	5.0	64.5	54.3	0.18	1.364	3.699	**3.500**	10	533
Scenario B-1	0.1	0.036	47	40	0.18	2.10	2.50	1.80	20	20000
Scenario B-2	0.1	**0.36**	47	40	0.18	2.10	2.50	1.80	20	20000
Scenario C-1	5.0	1.5	47	40	0.18	2.10	1.80	2.50	20	500
Scenario C-2	5.0	**15**	47	40	0.18	2.10	1.80	2.50	20	500
Scenario D-1	2.0	5.0	47	40	0.18	2.80	1.60	2.10	20	100
Scenario D-2	2.0	**50**	47	40	0.18	2.80	1.60	2.10	20	100
Scenario D-3	**20**	5.0	47	40	0.18	2.80	1.60	2.10	20	100
Scenario E-1	2.0	5.0	47	40	0.18	2.80	2.10	1.60	20	500
Scenario E-2	2.0	**50**	47	40	0.18	2.80	2.10	1.60	20	500
Scenario E-3	**0.2**	5.0	47	40	0.18	2.80	2.10	1.60	20	500
Scenario E-4	2.0	5.0	47	40	0.18	2.80	2.10	1.60	20	**100**
Scenario E-5	2.0	**50**	47	40	0.18	2.80	2.10	1.60	20	**100**
Scenario F-1	4.0	5.0	47	40	0.18	1.80	2.10	2.40	20	500

Note: The diffusion coefficients of all species are set to $1.0\times10^{-6}\,cm^2/s$. This table can also be found in the main body of this article. It is included here for readers' convenience.

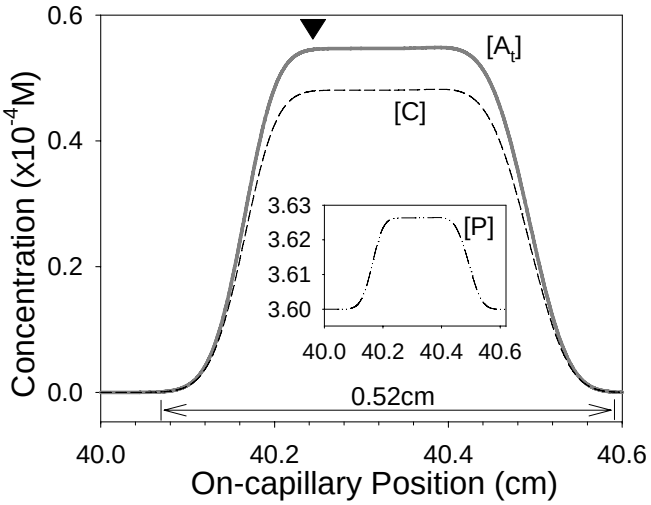

Figure S-1. The simulated concentration profiles for Scenario B-2. The black triangle indicates the position where the migration time of the analyte peak should be measured.

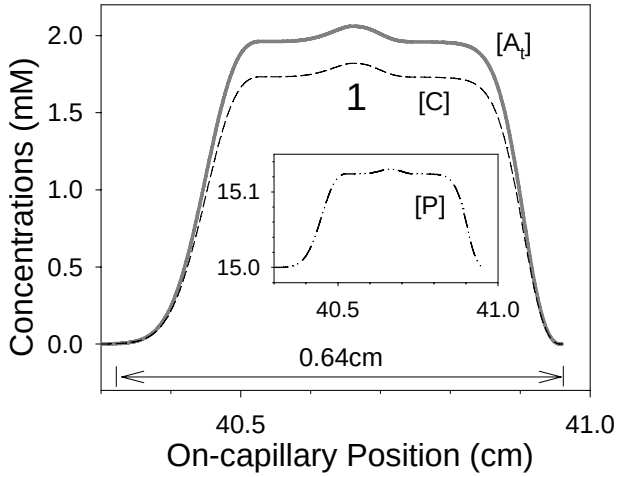

Figure S-2. The simulated concentration profiles for Scenario C-2. The peak labelled "1" is the complex formed at the front edge of the analyte plug boundary.

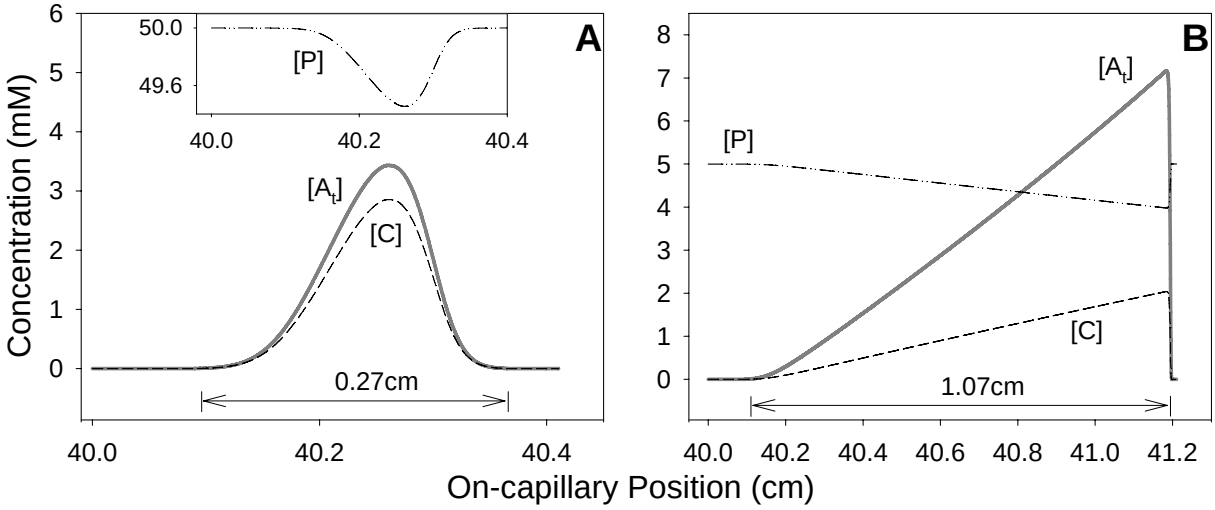

Figure S-3. The simulated concentration profiles for (A) Scenario D-2 and (B) Scenario D-3.

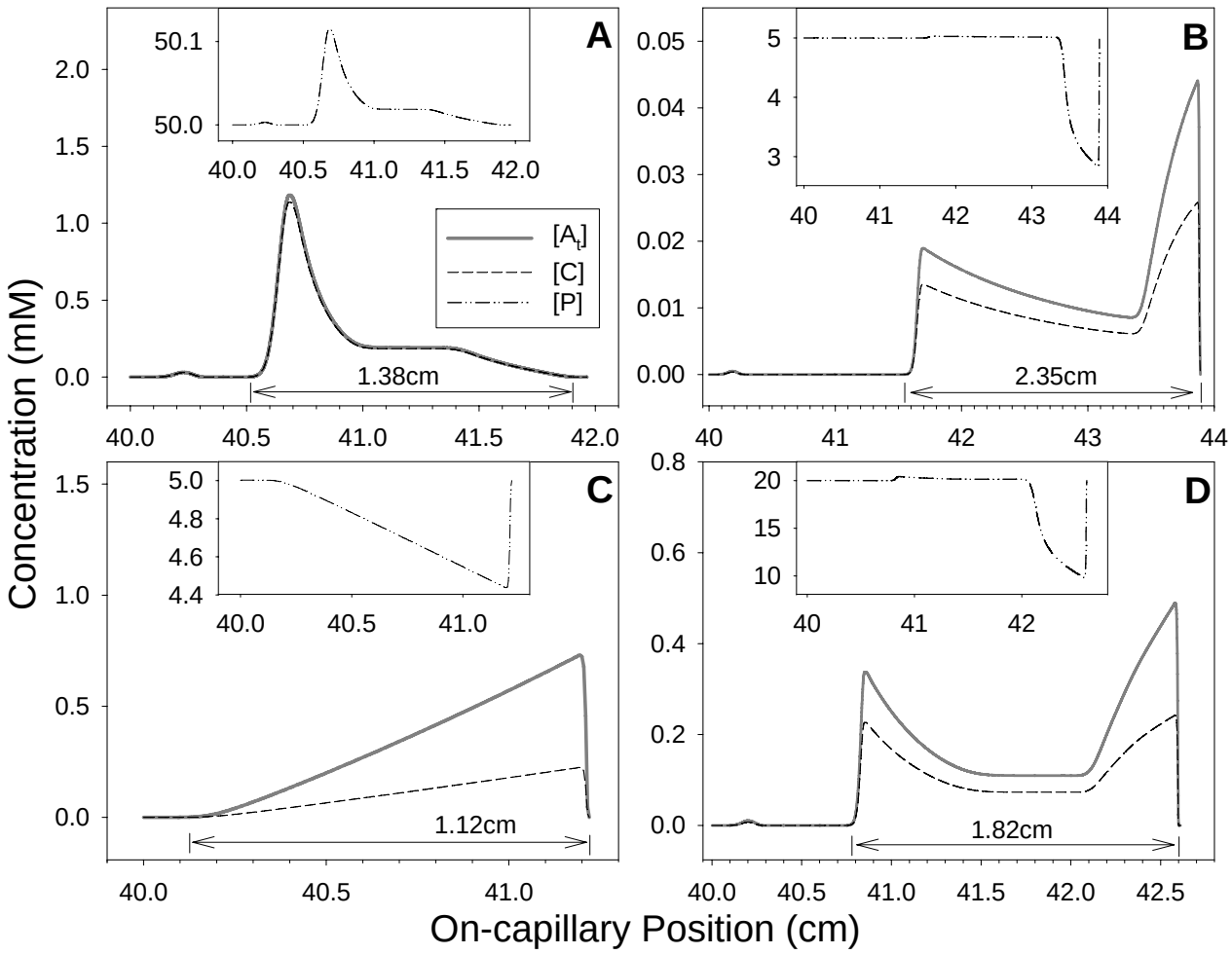

Figure S-4. The simulated concentration profiles for (A) Scenario E-2, (B) Scenario E-3, (C) Scenario E-4, and (D) Scenario E-5.

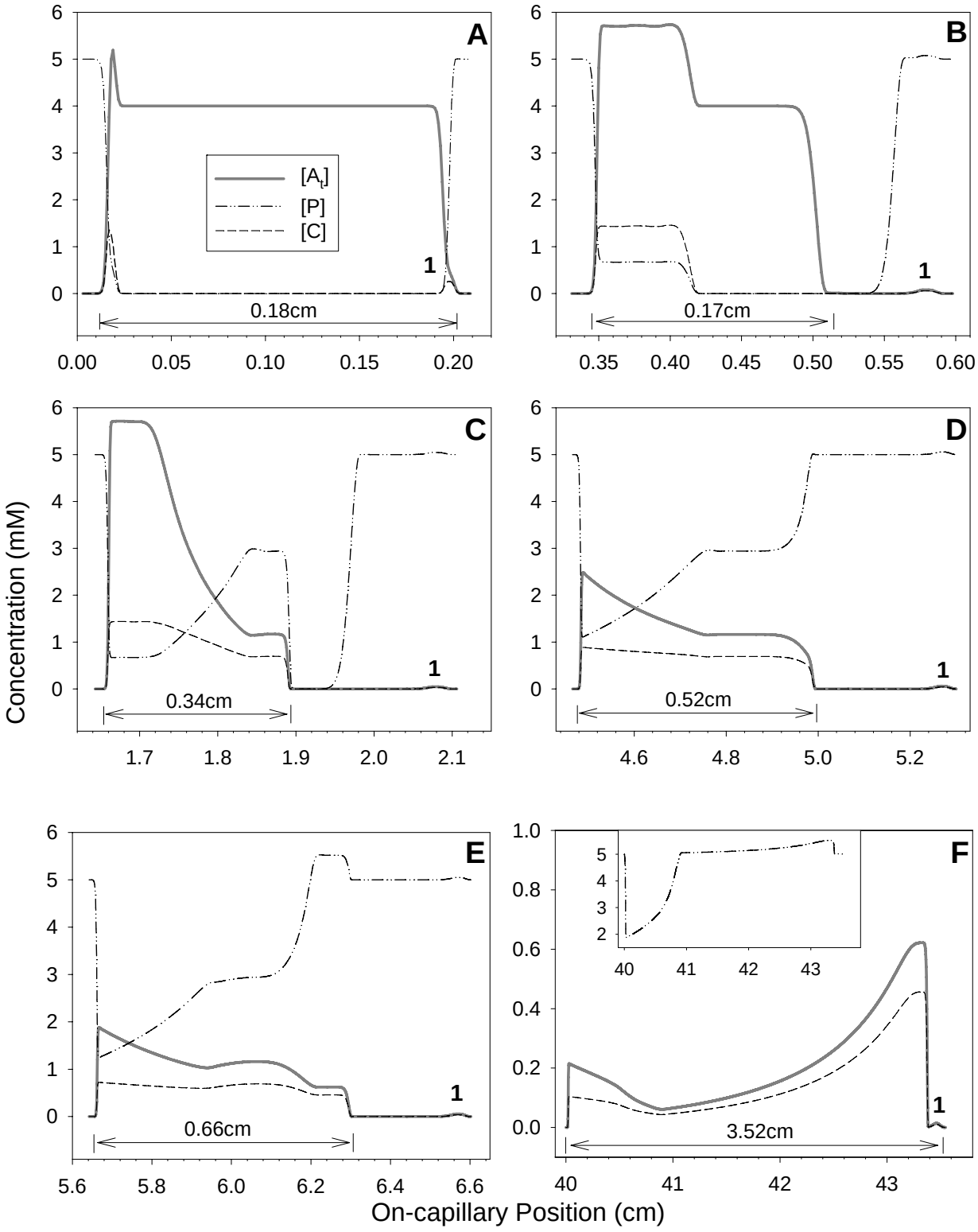

Figure S-5. The simulated concentration profiles for Scenario F-1. The peak labelled "1" is the complex formed at the front edge of the analyte plug boundary, as discussed in the text.