SUPPORTING INFORMATION

Making DNA Hybridization Assays in Capillary Electrophoresis Quantitative

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MATHEMATICAL APPENDIX

1. Fraction of SSB-bound probe as a function of migration times

Velocity is an additive function, therefore, the apparent velocity of the probe, v, in electrophoresis with SSB present in the run buffer (assuming fast equilibrium between free probe and SSB-bound probe) a sum of two terms contributed by free probe, P, and SSB-bound probe, SSB•P*:

$$v = v_{\mathrm{P}^*} f_{\mathrm{P}^*} + v_{\mathrm{SSB}\bullet\mathrm{P}^*} f_{\mathrm{SSB}\bullet\mathrm{P}^*} \tag{1.1}$$

where v_{P} and $v_{\mathrm{P}\bullet\mathrm{SSB}}$ are velocities of free and SSB-bound probe and f_{P^*} and $f_{\mathrm{SSB}\bullet\mathrm{P}^*}$ are fractions of free and SSB-bound probe. The fractions are defined through concentrations as:

$$f_{\mathrm{P}^*} = \frac{[\mathrm{P}^*]}{[\mathrm{P}^*]_0}, \quad f_{\mathrm{SSB}\bullet\mathrm{P}^*} = \frac{[\mathrm{SSB}\bullet\mathrm{P}^*]}{[\mathrm{P}^*]_0} \tag{1.2}$$

$$[\mathrm{P}^*]_0 = [\mathrm{P}^*] + [\mathrm{SSB}\bullet\mathrm{P}^*]$$

A sum of the fractions is equal to unity:

$$f_{\mathrm{P}^*} + f_{\mathrm{SSB}\bullet\mathrm{P}^*} = 1 \tag{1.3}$$

Using equation (1.3), we can rewrite equation (1.1) as:

$$v = v_{\mathrm{P}^*}(1 - f) + v_{\mathrm{SSB}\bullet\mathrm{P}^*} f \tag{1.4}$$

And express the fraction of bound probe through the velocities:

$$f = \frac{v - v_{\mathrm{P}^*}}{v_{\mathrm{SSB}\bullet\mathrm{P}^*} - v_{\mathrm{P}^*}} \tag{1.5}$$

It is more convenient to work with experimentally measured migration times, t, than with velocities. The velocity is reciprically proportional to migration times:

$$v = L/t, \quad v_{\mathrm{P}^*} = L/t_{\mathrm{P}^*}, \quad v_{\mathrm{SSB}\bullet\mathrm{P}^*} = L/t_{\mathrm{SSB}\bullet\mathrm{P}^*} \tag{1.6}$$

where L is the capillary length to the detector. Using equations (1.5) and (1.6) we ca express the fraction of the SSB-bound probe through the migration times:

$$f = \frac{1/t - 1/t_{\mathrm{P}^*}}{1/t_{\mathrm{SSB}\bullet\mathrm{P}^*} - 1/t_{\mathrm{P}^*}} = \frac{t_{\mathrm{SSB}\bullet\mathrm{P}^*}}{t} \frac{t_{\mathrm{P}^*} - t}{t_{\mathrm{P}^*} - t_{\mathrm{SSB}\bullet\mathrm{P}^*}} \tag{1.7}$$

2. Equilibration time

The equilibration time, t_{eq}, can be precisely found for a pseudo-first order reaction of probe, P*, binding to SSB. Let's assume that the concentration of SSB is greater than that of the probe: [SSB] >> [P*]. In our case of SSB present in the run buffer, this condition can be reduced to [SSB] ≥ [P*]. Indeed, since SSB fills the entire length of the capillary and P* fills only a short part of it, even if the local concentration of SSB in the vicinity of P* is reduced due to binding to

P*, the remaining SSB-unbound P* rapidly moves to a new volume with "untouched" SSB. We can thus assume that [SSB] = const even if [SSB] ≥ [P*].

Our goal is to find the characteristic equilibration time, t_{eq}, of the following reaction:

$$\mathrm{SSB} + \mathrm{P^*} \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} \mathrm{SSB \bullet P^*} \qquad (2.1)$$

where k_{on} is the rate constant of complex formation and k_{off} is the rate constant of complex dissociation. By definition, the characteristic equilibration time is reciprocal of the observed rate constant of changing either [P*] or [SSB•P*]:

$$t_{eq} = 1/k_{obs} \qquad (2.2)$$

Using the pseudo-first order approximation we can assume that:

$$k_{on}[\mathrm{SSB}] = \mathrm{const} = k_{on}^{app} \qquad (2.3)$$

We can then write for the rate of the reaction (2.1):

$$\text{Rate of reaction} = \frac{d[\mathrm{SSB \bullet P^*}]}{dt} = -\frac{d[\mathrm{SSB}]}{dt} = -\frac{d[\mathrm{P^*}]}{dt} = \\ = k_{on}[\mathrm{SSB}][\mathrm{P^*}] - k_{off}[\mathrm{SSB \bullet P^*}] = k_{on}^{app}[\mathrm{P^*}] - k_{off}[\mathrm{SSB \bullet P^*}] \qquad (2.4)$$

Lets solve equation (2.4) for d[SSB•P*]/dt and for the initial concentration of SSB•P* equal to zero and initial concentration of P* equal to $[\mathrm{P^*}]_0$. Mass balance requires that:

$$[\mathrm{P^*}] + [\mathrm{SSB \bullet P^*}] = [\mathrm{P^*}]_0 \qquad (2.5)$$

Using equation (2.5) we can write for the rate of reaction:

$$\frac{d[\mathrm{SSB \bullet P^*}]}{dt} = k_{on}^{app}[\mathrm{P^*}] - k_{off}[\mathrm{SSB \bullet P^*}] = \\ = k_{on}^{app}\left([\mathrm{P^*}]_0 - [\mathrm{SSB \bullet P^*}]\right) - k_{off}[\mathrm{SSB \bullet P^*}] = \\ = k_{on}^{app}[\mathrm{P^*}]_0 - k_{on}^{app}[\mathrm{SSB \bullet P^*}] - k_{off}[\mathrm{SSB \bullet P^*}] = \\ = k_{on}^{app}[\mathrm{P^*}]_0 - (k_{on}^{app} + k_{off})[\mathrm{SSB \bullet P^*}] \qquad (2.6)$$

From equation (2.6) we can obtain an ordinary differential equation with separated variables [SSB•P*] and t:

$$\frac{d[\mathrm{SSB \bullet P^*}]}{k_{on}^{app}[\mathrm{P^*}]_0 - (k_{on}^{app} + k_{off})[\mathrm{SSB \bullet P^*}]} = dt \qquad (2.7)$$

Equation (2.7) can be directly integrated using the following tabular intergal:

$$\int \frac{dx}{ax + b} = \int dt \qquad (2.8)$$

The solution of equation (2.7) is:

$$[\mathrm{SSB \bullet P^*}] = \frac{k_{on}[\mathrm{SSB}][\mathrm{P^*}]_0}{k_{on}[\mathrm{SSB}] + k_{off}}\left\{1 - e^{-(k_{on}[\mathrm{SSB}] + k_{off})t}\right\} = \frac{k_{on}[\mathrm{SSB}][\mathrm{P^*}]_0}{k_{obs}}\left\{1 - e^{-k_{obs}t}\right\} \qquad (2.9)$$

From equation (2.9) we can find the observed rate constant, k_{obs}:

$$k_{obs} = k_{on}[\mathrm{SSB}] + k_{off} \qquad (2.10)$$

Finally, an expression for the characteristic equilibration time is:

$$t_{eq} = 1/k_{obs} = 1/\left(k_{on}[\mathrm{SSB}]_0 + k_{off}\right) \qquad (2.11)$$

Equation (2.11) governs the equilibration between SSB-bound and SSB-unbound probe under an assumption of constant concentration of SSB.

SUPPORTING FIGURES

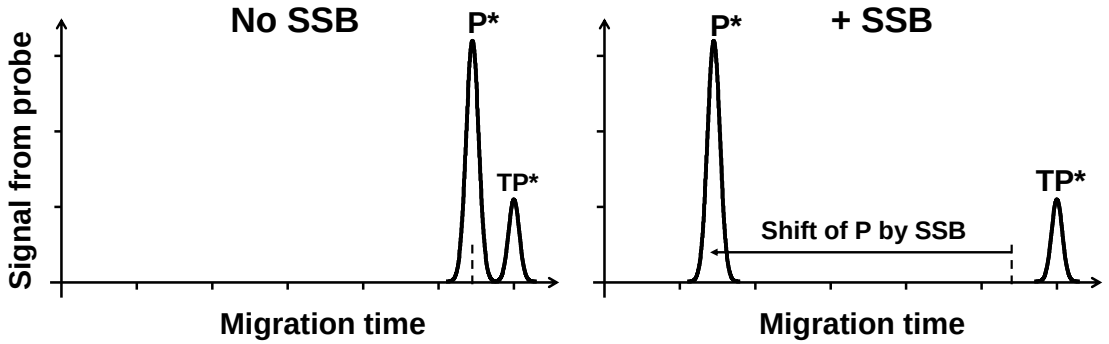

Figure S1. Conceptual illustration of the effect of SSB present in the run buffer on the capillary-electrophoresis separation of target-probe hybrid, TP*, form the excess of free probe, P*.

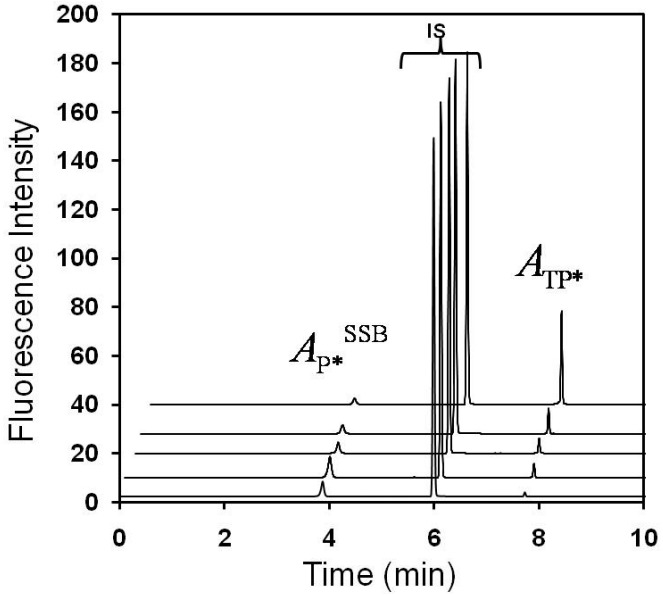

Figure S2. Electropherograms used for calculation of target, T, concentration in Fig. 7 in main text. The areas of the peaks, along with calculated quantum yields were used in determining target concentration. 100 nM DNA probe was incubated with (from bottom): 3.125, 6.25, 12.5, 25, and 50 nM target, respectively. The mixture was spiked with 100 nM fluorescein used as an internal standard (IS) and the components of the mixture were separated in the run buffer containing 50 nM SSB.