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Non-equilibrium capillary electrophoresis of equilibrium mixtures – appreciation of kinetics in capillary electrophoresis

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Supplementary Material

Determination of K_d for non-covalent interaction between a protein and DNA

Here we derive the expression for K_d, as a function of the total concentrations of protein and DNA and the ratio between the equilibrium concentrations of free protein and protein-DNA complex.

Lets consider the following equilibrium between SSB, fDNA, and their complex, SSB•fDNA:

$$\mathrm{SSB + fDNA} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{SSB{\bullet}fDNA} \qquad (1s)$$

Lets assume that the ratio, R, of equilibrium concentrations of free fDNA, $[\mathrm{fDNA}]_{eq}$, and the complex, $[\mathrm{SSB{\bullet}fDNA}]_{eq}$, is known from measuring the areas of peaks on the electropherogram:

$$\mathrm{R} = \frac{[\mathrm{fDNA}]_{eq}}{[\mathrm{SSB} \bullet \mathrm{fDNA}]_{eq}} \qquad (2s)$$

From expression (2s) we get:

$$[\mathrm{SSB} \bullet \mathrm{fDNA}]_{eq} = \frac{[\mathrm{fDNA}]_{eq}}{\mathrm{R}} \qquad (3s)$$

Using the conservation of mass principle for fDNA and expression (3s) we obtain:

$$[\mathrm{fDNA}]_0 = [\mathrm{fDNA}]_{eq} + [\mathrm{SSB} \bullet \mathrm{fDNA}]_{eq} = [\mathrm{fDNA}]_{eq} + [\mathrm{fDNA}]_{eq}/\mathrm{R} = [\mathrm{fDNA}]_{eq}(1 + 1/\mathrm{R}) \qquad (4s)$$

From expression (4s) we can derive:

$$[\mathrm{fDNA}]_{eq} = \frac{[\mathrm{fDNA}]_0}{(1 + 1/\mathrm{R})} = \frac{[\mathrm{fDNA}]_0 \mathrm{R}}{(1 + \mathrm{R})} \qquad (5s)$$

From the conservation of mass principle for fDNA and expression (5s) we get:

$$[\mathrm{SSB} \bullet \mathrm{fDNA}]_{eq} = [\mathrm{fDNA}]_0 - [\mathrm{fDNA}]_{eq} = [\mathrm{fDNA}]_0 - \frac{[\mathrm{fDNA}]_0 \mathrm{R}}{(1 + \mathrm{R})} = [\mathrm{fDNA}]_0 \left(1 - \frac{\mathrm{R}}{1 + \mathrm{R}}\right) =$$

$$= [\mathrm{FDNA}]_0 \left(\frac{1}{1 + \mathrm{R}}\right) = \frac{[\mathrm{FDNA}]_0}{1 + \mathrm{R}} \qquad (6s)$$

Using the conservation of mass principle for SSB and expression (6s) we can write:

$$[\mathrm{SSB}]_{eq} = [\mathrm{SSB}]_0 - [\mathrm{SSB} \bullet \mathrm{fDNA}]_{eq} = [\mathrm{SSB}]_0 - \frac{[\mathrm{fDNA}]_0}{1 + \mathrm{R}} = \frac{[\mathrm{SSB}]_0 (1 + \mathrm{R}) - [\mathrm{fDNA}]_0}{1 + \mathrm{R}} \qquad (7s)$$

Finally, from the definition of K_d and expressions (5s)-(7s) we derive:

$$\boldsymbol{K}_d = \frac{[\mathrm{SSB}]_{eq}[\mathrm{fDNA}]_{eq}}{[\mathrm{SSB} \bullet \mathrm{fDNA}]_{eq}} = \frac{\frac{[\mathrm{SSB}]_0 (1 + \mathrm{R}) - [\mathrm{fDNA}]_0}{1 + \mathrm{R}} \times \frac{[\mathrm{fDNA}]_0 \mathrm{R}}{1 + \mathrm{R}}}{\frac{[\mathrm{fDNA}]_0}{(1 + \mathrm{R})}} = \frac{[\mathrm{SSB}]_0 (1 + \mathrm{R}) - [\mathrm{fDNA}]_0}{1 + 1/\mathrm{R}} \qquad (8s)$$

Determination of the relative quantum yield of fDNA in free and SSB-bound forms.

fDNA is present only in the free form in CE; therefore, the peak area in the CE experiment is:

$$A_{CE} = b\,\varphi_{fDNA} \tag{9s}$$

where b is a constant.

The presence of SSB in the run buffer during ACE results in the equilibrium between free and SSB-bound forms of fDNA. Since the quantum yield of fluorescence is an additive function, the peak area of fDNA in ACE is the sum of two components corresponding to free fDNA and SSB-bound fDNA:

$$A_{ACE} = b\left\{\varphi_{fDNA}\frac{[fDNA]_{eq}}{[fDNA]_0} + \varphi_{SSB\bullet fDNA}\frac{[SSB\bullet fDNA]_{eq}}{[fDNA]_0}\right\} \tag{10s}$$

Using the conservation of mass principle for fDNA and expression (10s) we get:

$$A_{ACE} = b\left\{\varphi_{fDNA}\frac{[fDNA]_{eq}}{[fDNA]_0} + \varphi_{SSB\bullet fDNA}\left(1-\frac{[fDNA]_{eq}}{[fDNA]_0}\right)\right\} \tag{11s}$$

Dividing expression (11s) by (9s) and rearranging the result leads to the desired expression:

$$\frac{\varphi_{fDNA}}{\varphi_{SSB\bullet fDNA}} = \frac{1-[fDNA]_{eq}/[fDNA]_0}{A_{ACE}/A_{CE}-[fDNA]_{eq}/[fDNA]_0} \tag{12s}$$

The fraction on non-bound fDNA, $[fDNA]_{eq}/[fDNA]_0$, can be found from fluorescence anisotropy experiments. Similarly to the quantum yield, fluorescence anisotropy is an additive function. Thus, the anisotropy in the ACE experiment is a sum of two components corresponding to free and SSB-bound fDNA:

$$r_{ACE} = r_{fDNA}\frac{[fDNA]_{eq}}{[fDNA]_0} + r_{SSB\bullet fDNA}\frac{[SSB\bullet fDNA]_{eq}}{[fDNA]_0} \tag{13s}$$

Using the conservation of mass principle for fDNA and expression (13s) we get:

$$r_{ACE} = r_{fDNA}\frac{[fDNA]_{eq}}{[fDNA]_0} + r_{SSB\bullet fDNA}\left(1-\frac{[fDNA]_{eq}}{[fDNA]_0}\right) \tag{14s}$$

Rearranging of expression (14s) leads gives a formula for the fraction of non-bound fDNA:

$$\frac{[fDNA]_{eq}}{[fDNA]_0} = \frac{r_{SSB\bullet fDNA}-r_{ACE}}{r_{SSB\bullet fDNA}-r_{fDNA}} \tag{15s}$$

Finally, by substituting (15s) into (12s) we get an expression for the relative quantum yield as a function of peak areas and fluorescence anisotropy:

$$\frac{\varphi_{fDNA}}{\varphi_{SSB\bullet fDNA}} = \frac{1-(r_{SSB\bullet fDNA}-r_{ACE})/(r_{SSB\bullet fDNA}-r_{fDNA})}{A_{ACE}/A_{CE}-(r_{SSB\bullet fDNA}-r_{ACE})/(r_{SSB\bullet fDNA}-r_{fDNA})}$$