from K=(xr)/(x-xr)(r-xr)

One site:

$$r = r_F + \frac{r_B - r_F}{2[RNA]}\left(\left(K_d + x + [RNA]\right) - \sqrt{(K_d + x + [RNA])^2 - 4[RNA]x}\right)$$

or:

$$\theta = \frac{r - r_F}{r_B - r_F} = \frac{1}{2[RNA]}\left(\left(K_d + x + [RNA]\right) - \sqrt{(K_d + x + [RNA])^2 - 4[RNA]x}\right) =$$

where:
θ is the fraction bound
x is the protein concentration
r is the observed anisotropy at the ith titration
r_F is the anisotropy at zero protein concentration
r_B is the anisotropy at saturating protein concentration (floated in fit at top, calculated in fit at bottom)

We also found a simpler binding equation, which seems not to be valid:

$$f_b = \frac{nK_a x}{1 + K_a x}$$

where:

f_b is the fraction bound, $\frac{r - r_F}{r_B - r_F}$

x is the protein concentration

This assumes x(free) is equal to x(total)

Derivation of equations for fitting fluorescence intensity changes during ligand binding:

Where $[S_F]$ is the free concentration of fluorophore-containing macromolecule in cuvette, and $[U_F]$ is the free concentration of unlabeled binding partner that is being added during the titration, the overall reaction and equation for equilibrium association constant K_a are as follows:

S + U ↔ SU $\qquad K_a = \frac{[SU]}{[S_F][U_F]} \qquad (1)$

We express the free concentrations ($[S_F]$ and $[U_F]$) in known concentrations, total S ($[S_T]$) and total U ($[U_T]$):

$$[S_F] = [S_T] - [SU] \qquad [U_F] = [U_T] - [SU] \qquad (2)$$

Substituting these values into equation (S.1):

$$K_a = \frac{[SU]}{([S_T] - [SU])([U_T] - [SU])} \qquad (3)$$

This can be rearranged as a quadratic equation in terms of $[SU]$:

$$K_a = \frac{[SU]}{([S_T][U_T] - [S_T][SU] - [U_T][SU] + [SU]^2)} \qquad (3a)$$

$$[S_T][U_T]K_a - [S_T][SU]K_a - [U_T][SU]K_a + [SU]^2 K_a = [SU] \qquad (3b)$$

$$0 = [SU]^2 K_a + (-[S_T]K_a - [U_T]K_a - 1)[SU] + [S_T][U_T]K_a \qquad (4)$$

Recall the solution to a quadratic equation $0 = ax^2 + bx + c$:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Solving this quadratic equation gives us:

$$[SU] = \frac{([U_T]K_a + [S_T]K_a + 1) - \sqrt{([U_T]K_a + [S_T]K_a + 1)^2 - 4[U_T][S_T]K_a^2}}{2K_a} \qquad (5)$$

The concentration of bound complex [*SU*] is related to changes in the fluorescence intensity. For each titration point (*i*), emission intensity was integrated from 320.0 – 410.0 nm to obtain the observed total emission intensity, $I_{obs}(i)$, due to the dramatic blue shift of the wavelength of maximum emission. Otherwise, the maximum intensity of the fluorescence emission spectrum is used for $I_{obs}(i)$. Before minimization, $I_{obs}(i)$ is corrected to obtain $I_{corr}(i)$, if (1) if sample is diluted >10% during the course of titration, and (2) buffer solution shows background fluorescence at wavelength of excitation.

Both free (*S*) and bound (*SU*) fluorophore-containing ligand contribute to the fluorescence signal. If the fluorescence intensity of free form is I_F and complexed form is I_C, the following equation fits the observed fluorescence intensity to the fractions arising from the free or bound forms:

$$I_{corr}(i) = \left(\frac{[S_T] - [SU(i)]}{[S_T]} \right) I_F + \left(\frac{[SU(i)]}{[S_T]} \right) I_C \qquad (6)$$

Equation (5) is substituted or linked to the [*SU*] in equation (6), and nonlinear least squares fit using software such as Kaleidegraph, Prism, SigmaPlot while floating K_a.

→The dissociation constant ($K_D = 1/K_a$) is usually reported for biological applications, since it corresponds to the concentration of titrated ligand required for 50% saturation of the binding partner.

→The simpler treatment $\left(\frac{[SU(i)]}{[S_T(i)]} \right) = \left(\frac{[U(i)]K_a}{1+[U(i)]K_a} \right)$ can only be used if $[S_F] <<< K_D$, because the derivation assumes $[S_T] \approx [S_F]$. Given the small changes being detected, this is hardly ever the case for a steady-state fluorescence titration.

Fluorescence anisotropy changes also can be fit to measure the affinity of ligand binding.

Here, 'S' remains the fluorophore-containing component, and 'U' is the ligand titrated into the cuvette. The fraction of bound 'S' (θ) is the concentration of complex divided by the total S concentration:

$$\theta = \frac{[SU]}{[S_T]}$$

The anisotropy change indicates SU complex formation. Where r_i is the anisotropy measured for the ith titration, r_f is the anisotropy of the fluorophore-containing component before addition of ligand (the 'blank'), and r_S is the anisotropy of the saturated solution with all fluorophore-containing component engaged in complex formation:

$$\theta = \frac{r_i - r_f}{r_S - r_f}$$

Recall equation (5) from the fitting of fluorescence intensity changes:

$$[SU] = \frac{([U_T]K_a + [S_T]K_a + 1) - \sqrt{([U_T]K_a + [S_T]K_a + 1)^2 - 4[U_T][S_T]K_a^2}}{2K_a}$$

Substitution for [SU] gives:

$$r_i = r_f + \frac{(r_S - r_f)\{([U_T]K_a + [S_T]K_a + 1) - \sqrt{([U_T]K_a + [S_T]K_a + 1)^2 - 4[U_T][S_T]K_a^2}\}}{2K_a[S_T]}$$

This equation is fit using the known r_i to obtain K_a, with the option of floating r_S .

The above equation assumes that there is no intensity change on binding. If observed, a correction is needed for Stokes' shift or quenching effects. The ratio of the fluorescence from the bound and free fluorophore-containing ligands $\frac{F_S}{F_f}$ can be used to normalize the anisotropy of the free state with that of the bound state:

$$\theta = \frac{[SU]}{[S_T]} = \frac{[SU]}{[S_F] + [SU]} = \frac{r_i - r_f}{\frac{F_S}{F_f}(r_S - r_i) + (r_i - r_f)}$$

so that substitution for [*SU*] gives:

$$r_i = r_f + \frac{\{\frac{F_S}{F_f}(r_S - r_i) + (r_i - r_f)\}\{([U_T]K_a + [S_T]K_a + 1) - \sqrt{([U_T]K_a + [S_T]K_a + 1)^2 - 4[U_T][S_T]K_a^2}\}}{2K_a[S_T]}$$