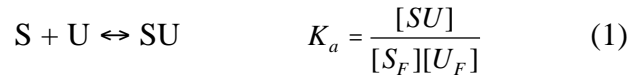


Derivation of equations for fitting fluorescence intensity changes during ligand binding:

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:



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

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

Substituting these values into equation (S.1):

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

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

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.

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