Supporting Information for

Global analysis of fluorometric titration curves in the presence of excited-state association and quenching

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The Supporting Information contains the Maple scripts used in the main paper.

1. Ground-state dissociation constant K_d from a single fluorometric titration curve (eq 14)

```
> restart;
> with(linalg):
```
Set of equations for the rate constants (eq 11)
```
> x0 := kd*k01*(k02+k12);
> x1 := (kd*(k01*kq2+k21*k02)+(k01+kd*kq1)*(k02+k12))/x0;
> x2 := (k01*kq2+kq1*k12+(kd*kq2+k02)*(kq1+k21))/x0;
> x3 := kq2*(kq1+k21)/x0;
```
Roots of the third-degree polynomial (eq 13)
```
> solve({x3*_Z^3-x2*_Z^2+x1*_Z-1=0},{_Z});
```

2. Identifiability of the ground-state dissociation constant K_d

```
> restart;
> with(linalg):
```
Set of equations for the rate constants (eq 11)
```
> x0 := kd*k01*(k02+k12);
> eq1 := (kd*(k01*kq2+k21*k02)+(k01+kd*kq1)*(k02+k12))/x0=x1;
> eq2 := (k01*kq2+kq1*k12+(kd*kq2+k02)*(kq1+k21))/x0=x2;
> eq3 := kq2*(kq1+k21)/x0=x3;
```
Set of equations for the alternative rate constants (eq 16)
```
> x0_ := kd_*k01_*(k02_+k12_);
> x1 := (kd_*(k01_*kq2_+k21_*k02_)+(k01_+kd_*kq1_)*(k02_+k12_))/x0_;
> x2 := (k01_*kq2_+kq1_*k12_+(kd_*kq2_+k02_)*(kq1_+k21_))/x0_;
> x3 := kq2_*(kq1_+k21_)/x0_;
```
Combine eqs 15a and 15b at one emission wavelength and (at least two) different excitation wavelengths
```
> eq41 := (kq2+t1*k21)/(k02+k12)=(kq2_+t1_*k21_)/(k02_+k12_);
```
Combine eqs 15b and 15c at the same emission wavelength and (at least two) different excitation wavelengths

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> eq51 := (k01+k12/t1)/(kq1+k21)=(k01_+k12_/t1_)/(kq1_+k21_);
Combine eqs 15a and 15b at another emission wavelength and (at least two) different excitation wavelengths
> eq42 := (kq2+t2*k21)/(k02+k12)=(kq2_+t2_*k21_)/(k02_+k12_);
Combine eqs 15b and 15c at the same emission wavelength and (at least two) different excitation wavelengths
> eq52 := (k01+k12/t2)/(kq1+k21)=(k01_+k12_/t2_)/(kq1_+k21_);
Solution of the obtained set of algebraic equations
> w := solve({eq1,eq2,eq3,eq41,eq51,eq42,eq52},
{k01,k02,k12,k21,kd,kq1,kq2,t1,t2});

3. Determination of the ground-state dissociation constant K_d, (eqs 17 and 25)

> restart:
> with(linalg):
Elimination of the coefficients e (epsilon) and c in eq 10
Define a new variable x := e1/e2, the excitation ratio taken at a certain excitation wavelength
Equation 10a with the explicit dependence on the emission wavelength
> eq0 := i->y0.i=x*e2*c1.i/k01;
Equation 10b with the explicit dependence on the emission wavelength
> eq1 := i-
>y1.i=e2*(x*kd*(c1.i*kq2+c2.i*k21)+(c2.i*k01+c1.i*k12))/(kd*k01*(k02+k12));
Equation 10c with the explicit dependence on the emission wavelength
> eq2 := i->y2.i=e2*c2.i*(kq1+k21)/(kd*k01*(k02+k12));
Extract the coefficients c1 and c2 from eqs 10a and 10c at two different emission wavelengths
> assign(solve(eq0(1),{c11})); assign(solve(eq2(1),{c21}));
assign(solve(eq0(2),{c12})); assign(solve(eq2(2),{c22}));
Substitute the obtained coefficients in eq 10b
> eu1 := collect(factor(eq1(1)),[y01,y21,x]); eu2 :=
collect(factor(eq1(2)),[y02,y22,x]);
To simplify derivations, define a set of new variables, incorporating the rate constants and the excitation ratio x
> s := {(kd*kq2*kq1+kd*kq2*k21)/((kq1+k21)*kd*(k02+k12))+
(k12*kq1+k12*k21)/((kq1+k21)*kd*(k02+k12))/x=z0,
(kd^2*k21*k02+kd^2*k21*k12)*x/((kq1+k21)*kd*(k02+k12))+
(kd*k01*k02+kd*k01*k12)/((kq1+k21)*kd*(k02+k12))=z1};
Solve the simplified set of equations with respect to the new variables z0 and z1
> solve({subs(s,eu1),subs(s,eu2)},{z0,z1});
Eliminate x from z0 and z1 (*)
> collect(factor(solve(s,{x,z0})),[z1,kq2]);
The same relations can be obtained for another excitation wavelength
> restart;
> with(linalg):
Determination of the ground-state dissociation constant Kd (eqs 17 and 25)
Set of equations for the rate constants (eq 11)
> x0 := kd*k01*(k02+k12);
> eq1 := (kd*(k01*kq2+k21*k02)+(k01+kd*kq1)*(k02+k12))/x0=x1;
> eq2 := (k01*kq2+kq1*k12+(kd*kq2+k02)*(kq1+k21))/x0=x2;

> eq3 := kq2*(kq1+k21)/x0=x3;

Additional equations from eqs 10 and 19 written for two excitation wavelengths [see also (*) from Elimination of the coefficients e (epsilon) and c in eq 10]

> eq4 := z01*(k02+k12)=kq2+k12*k21/(z11*(kq1+k21)-k01); eq5 := z02*(k02+k12)=kq2+k12*k21/(z12*(kq1+k21)-k01);

Solve a set of 5 equations eq1, ... ,eq5

> assign(solve({eq1,eq4,eq5},{k12,kq2,k21})):

> eq2:=algsubs(kd^2=ce,factor(eq2)*kd^2):

> eq3:=algsubs(kd^2=ce,factor(eq3)*kd^2):

> assign(solve({eq2,eq3},{kd,ce}));

The final result for the ground-state dissociation constant Kd (eq 17)

> kd := collect(factor(kd),[x1,x2,x3]);

Check the result using the coefficients p1, p2 and p3 (eq 18)

> p1 := z01*z11-z02*z12;

> p2 := (z02-z01)*z11*z12+z12-z11;

> p3 := (z11-z12)*z02*z01-z02+z01;

Reproduce eq 17

> kd_ := (p1^2*x1+p2*p1*x2+p2^2*x3-p3*p1)/(p1^2*x1^2+(p2*p1*x2+p2^2*x3-2*p3*p1)*x1-p2*p3*x2+p2*p1*x3+p3^2);

Expect 0 - no difference

> factor(kd-kd_);

Model without quenching (eq 25)

> factor(eval(kd,x3=0));