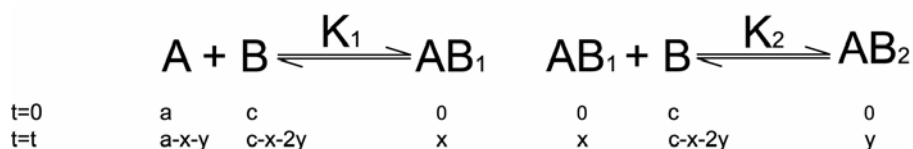


SUPPORTING INFORMATION

As the ATP aptamer has two ATP binding sites, we assume the following two equilibriums in the solution:



Then:

$$K_1 = \frac{x}{(a-x-y)(c-x-2y)} \dots\dots\dots(1)$$

$$K_2 = \frac{y}{x(c-x-2y)} \dots\dots\dots(2)$$

Where A stands for the ATP aptamer/[Ru(phen)₂(dppz)]²⁺ complex. B stands for ATP. AB₁ and AB₂ stand for the binding of one or two ATP molecules to the aptamer/[Ru(phen)₂(dppz)]²⁺ respectively. a is the initial concentration of the ATP aptamer, which is also considered as the initial concentration of the aptamer/[Ru(phen)₂(dppz)]²⁺ complex in the presence of extra amount of [Ru(phen)₂(dppz)]²⁺. c is the added total concentration of ATP. x and y are the concentrations of AB₁ and AB₂ at equilibrium respectively.

Since A, AB₁, AB₂ are all luminescent, F₀, F₁ and F₂ are defined as the luminescence intensity **per mole of** A, AB₁ and AB₂ respectively. F is the measured luminescence intensity of the solution **when different concentrations of ATP are added**. Obviously,

$$F = (a - x - y)F_0 + xF_1 + yF_2$$

$$= aF_0 + (F_1 - F_0)x + (F_2 - F_0)y \quad \Rightarrow$$

$$aF_0 - F = (F_0 - F_1)x + (F_0 - F_2)y \dots\dots\dots(3)$$

$$\frac{aF_0 - F}{aF_0} = \frac{F_0 - F_1}{aF_0}x + \frac{F_0 - F_2}{aF_0}y,$$

$$\text{Let } z = \frac{aF_0 - F}{aF_0}, f_1 = \frac{F_1}{F_0}, f_2 = \frac{F_2}{F_0}$$

then

$$z = \left(\frac{1}{a} - \frac{f_1}{a}\right)x + \left(\frac{1}{a} - \frac{f_2}{a}\right)y \dots\dots\dots(4)$$

Let

$$x = \frac{1}{m}, y = \frac{n}{m} \dots\dots\dots(5)$$

from equation (1) , (2) and (5), equation (6) can be obtained:

$$m = \frac{K_2 + K_1n + K_1n^2}{aK_1n} \dots\dots\dots(6)$$

from (5) , (6) and (2), a cubic equation of one variable is deduced:

$$n^3 + (1 - K_2c + 2aK_2)n^2 + \left(\frac{K_2}{K_1} - K_2c + aK_2\right)n - \frac{cK_2^2}{K_1} = 0 \dots\dots\dots(7)$$

In equation (7), n is a dependent variable, c is an independent variable, and a , K_1 , K_2 are constants (K_1 , K_2 unknown). Therefore, n can be expressed as a function of c by solving the cubic equation (7).

From (4), (5) and (6), we get:

$$z = \frac{(1-f_1)K_1n}{K_2 + K_1n + K_1n^2} + \frac{(1-f_2)K_1n^2}{K_2 + K_1n + K_1n^2} \dots\dots\dots(8)$$

Therefore, the luminescence change (z) in the titration experiment can be expressed as a function of n , thus a function of the added ATP concentration, c .

In the following, Cardan's method is used to solve equation (7)

$$\text{With the substitution } n = \alpha - \frac{1}{3}(1 - K_2c + 2aK_2) \dots\dots\dots(9)$$

equation (7) becomes

$$\alpha^3 + p\alpha + q = 0 \dots\dots\dots(10)$$

where

$$p = -\frac{1}{3}(1 - K_2c + 2aK_2)^2 + \left(\frac{K_2}{K_1} - K_2c + aK_2\right) \dots\dots\dots(11)$$

$$q = 2\left(\frac{1 - K_2c + 2aK_2}{3}\right)^3 - \frac{1}{3}(1 - K_2c + 2aK_2)\left(\frac{K_2}{K_1} - K_2c + aK_2\right) - \frac{cK_2^2}{K_1} \dots\dots\dots(12)$$

There are three possibilities for the solution of equation (10), depending on the value of the parameter W .

$$W = \frac{q^2}{4} + \frac{p^3}{27}$$

$$D = \sqrt{W}$$

Case 1. $W>0$: equation (10) has one real root and a pair of imaginary roots

$$\alpha_1 = \sqrt[3]{-\frac{q}{2} + D} + \sqrt[3]{-\frac{q}{2} - D} \quad (\text{real root})$$

$$\alpha_2 = -\frac{1}{2}(\sqrt[3]{-\frac{q}{2} + D} + \sqrt[3]{-\frac{q}{2} - D}) + i\frac{\sqrt{3}}{2}(\sqrt[3]{-\frac{q}{2} + D} - \sqrt[3]{-\frac{q}{2} - D})$$

$$\alpha_3 = -\frac{1}{2}(\sqrt[3]{-\frac{q}{2} + D} + \sqrt[3]{-\frac{q}{2} - D}) - i\frac{\sqrt{3}}{2}(\sqrt[3]{-\frac{q}{2} + D} - \sqrt[3]{-\frac{q}{2} - D})$$

Case 2. $W=0$, equation (10) has three real roots and two of them are the same:

$$\alpha_1 = 2\sqrt[3]{-\frac{q}{2}}$$

$$\alpha_{2,3} = -\sqrt[3]{-\frac{q}{2}}$$

Case 3. $W<0$, equation (10) has three different real roots:

$$\alpha_1 = \sqrt[3]{-\frac{q}{2} + D} + \sqrt[3]{-\frac{q}{2} - D}$$

$$\alpha_2 = \sqrt[3]{-\frac{q}{2} + D\varepsilon} + \sqrt[3]{-\frac{q}{2} - D\varepsilon^2}$$

$$\alpha_3 = \sqrt[3]{-\frac{q}{2} + D\varepsilon^2} + \sqrt[3]{-\frac{q}{2} - D\varepsilon} \quad \text{where} \quad \varepsilon = -\frac{1}{2} + i\frac{\sqrt{3}}{2}$$

Once n is determined from one of the above roots, z in equation (8) is expressed as a function of c , with the unknown constants of K_1 , K_2 , f_1 and f_2 . The experiment data in Figure 3A are then used to fit equation (8) to solve the unknown constants using nonlinear least squares fitting by the software Origin6.0.

Since n is the concentration ratio of AB_2 to AB_1 ($n=y/x$ from equation (5)), we first ignored the imaginary roots for n (in case 1). Then we checked all the 7 real roots of

equation (10) by replacing the derived n to equation (8) and carried out the fitting one by one. It is found that except for the two roots, the fitting of the experiment data to equation (8) with the other four roots was all failed. This indicates that only two solutions of n are possible for the experiment system. The fitting results with the two roots are shown in Table 1.

Then we analyzed whether the two sets of the results in Table 1 are the true results for our experiment. In the first set, f_1 and f_2 are 0.00071 and 0.41. This indicates that the aptamer/ $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ with one ATP bound almost has no luminescence (99.9% of decrease in molar luminescence) and the intercalation of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ to the aptamer is almost destroyed. Then the binding of the second ATP greatly enhances the intercalation of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and results in the substantial luminescence recovery of the aptamer/ $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ (the ratio of the molar luminescence intensities of the complex with two ATP bound to that with one ATP bound is 577 (0.41/0.00071)). This is quite unreasonable. Moreover, the previous reports^{6,14} all suggested that the two association constants for ATP binding with the aptamer are close, while there is a big difference between K_1 and K_2 in the first set of the results. On the other hand, the second set of results of K_1 , K_2 , f_1 , f_2 are all reasonable and comparable to the other reports^{6,14}. Therefore, we discarded the first set of the results, and determined K_1 , K_2 , f_1 , f_2 to be $1.0 \times 10^7 \text{M}$, $5.0 \times 10^6 \text{M}$, 0.85 and 0.32 respectively.

Table 1: The fitting results of the possible real roots

Roots		K_1	K_2	f_1	f_2	R^2	χ^2
$W > 0$	α_1	$1.2 \times 10^5 \text{M}$	$1.0 \times 10^9 \text{M}$	0.071%	41%	0.998	0.00051
$W < 0$	α_1	$1.0 \times 10^7 \text{M}$	$5.0 \times 10^6 \text{M}$	85%	32%	0.999	0.00034

(R is the correlation coefficient; χ^2 is the residual error)