Supporting Information

Deconvoluting Binding Sites in Amyloid Nanofibrils using Time-Resolved Spectroscopy

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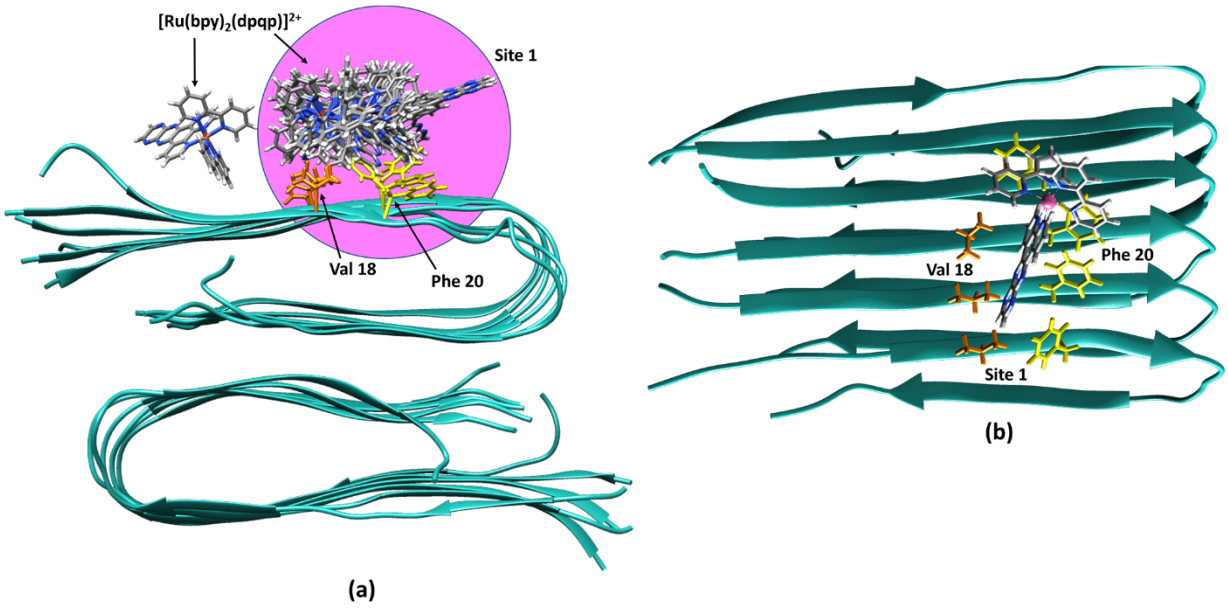


Figure S1. (a). Docking results of the $[Ru(bpy)_2(dpqp)]^{2+}$ on the surface of 2-fold Aβ fibril. (b) Binding site 1 used for MD simulation (docking pose).

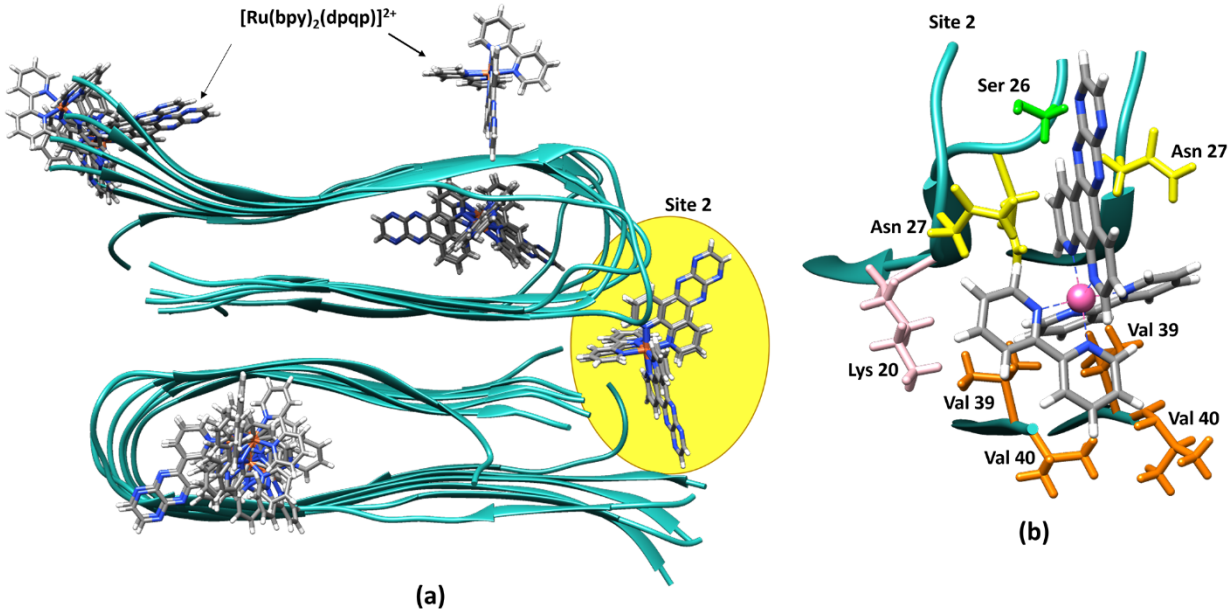


Figure S2. Docking results of the $[Ru(bpy)_2(dpqp)]^{2+}$ on the whole 2-fold Aβ fibril. (b) Binding site 2 used for MD simulation (docking pose). Molecules bound above and below the fibril are discarded as these binding sites are infrequent in comparison with sites 1 and 2.

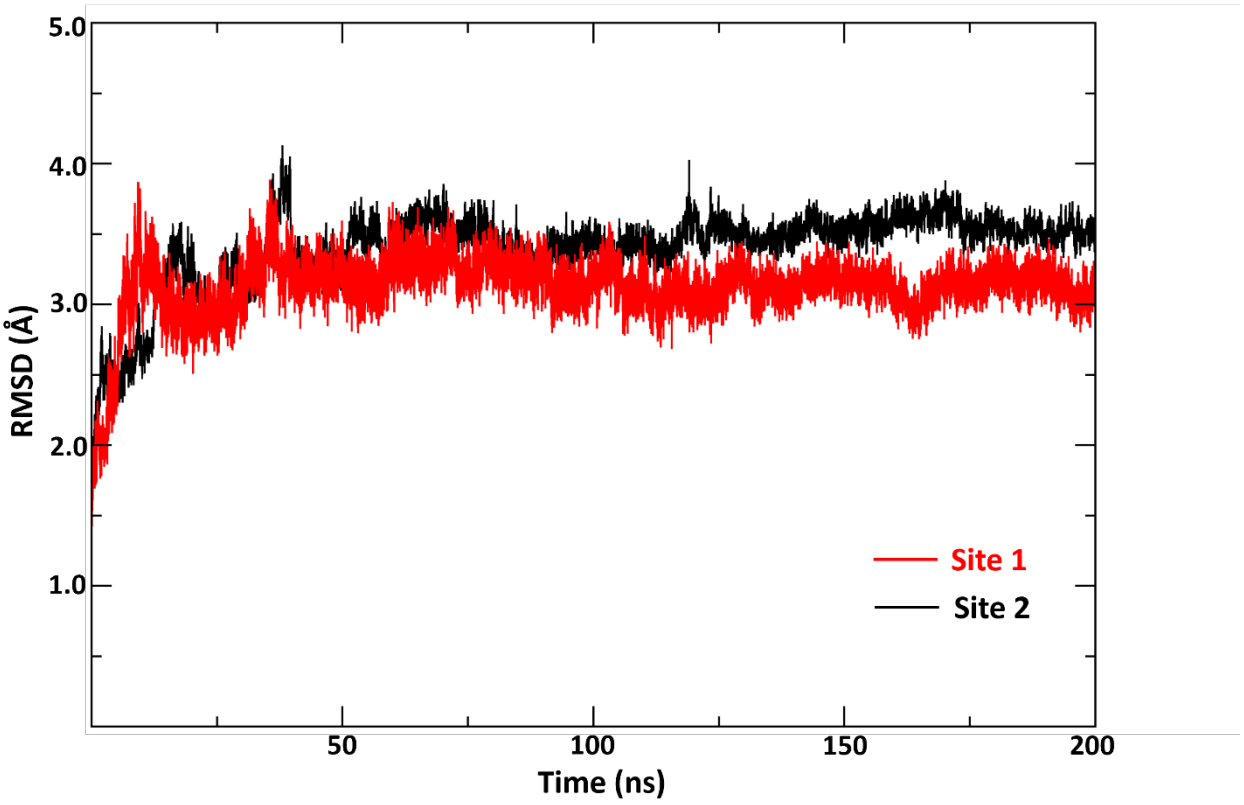


Figure S3. Per-residue root mean square deviation (RMSD) for site 1 and 2 during MD simulations.

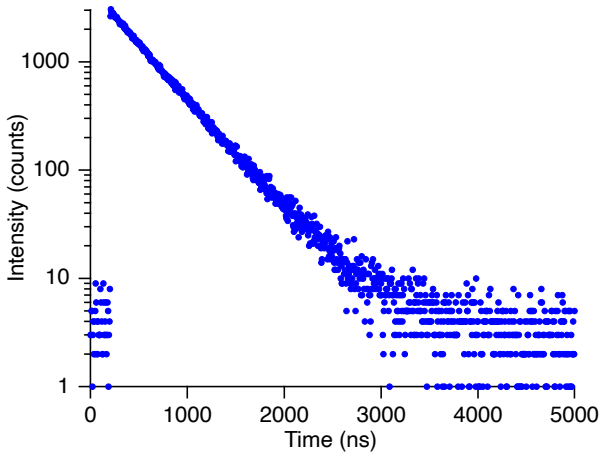


Figure S4. Decay curve for $[Ru(bpy)_2(dpqp)]^{2+}$ in the presence of 50 μM soluble Aβ.

(a) (b)

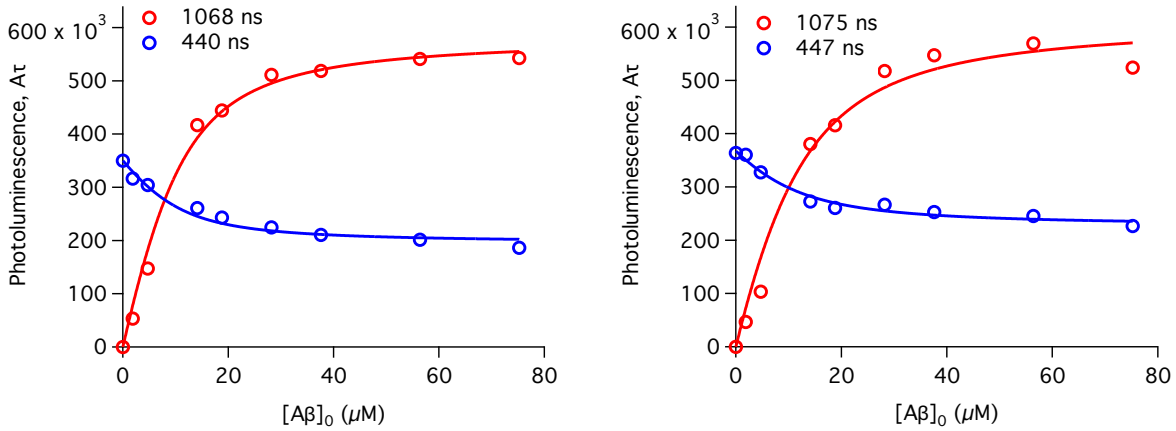


Figure S5. Titration curves of two additional independent sets of titration experiments between $[Ru(bpy)_2(dpqp)]^{2+}$ and Aβ (a) and (b). The red circles represent the intensity of the longer lifetime component (ca. 1082 ns). The blue circles represent the intensity of the shorter lifetime component (ca. 446 ns). Red and blue solid lines represent non-linear least-square fits to equations 11 and 12 respectively.

(a)

Parameter	Values
K_{d1}	4 ± 1 μM
K_{d2}	2.5 ± 0.4 μM
δ_1	$(2.3 \pm 0.3) \times 10^{-6}$ M
δ_2	$(10.5 \pm 0.4) \times 10^{-6}$ M

(b)

Parameter	Values
K_{d1}	2.0 ± 0.4 μM
K_{d2}	1.6 ± 0.2 μM
δ_1	$(3.0 \pm 0.2) \times 10^{-6}$ M
δ_2	$(11.4 \pm 0.4) \times 10^{-6}$ M

(c)

Parameter	Values
K_{d1}	4 ± 1 μM
K_{d2}	2.4 ± 0.5 μM
δ_1	$(2.5 \pm 0.4) \times 10^{-6}$ M
δ_2	$(10.9 \pm 0.5) \times 10^{-6}$ M

Table S1. Parameters of the binding of $[Ru(bpy)_2(dpqp)]^{2+}$ to Aβ fibrils determined by fitting the data sets shown in (a) Figure 2a (b) Figure S5a (c) Figure S5b with equations 11 and 12.

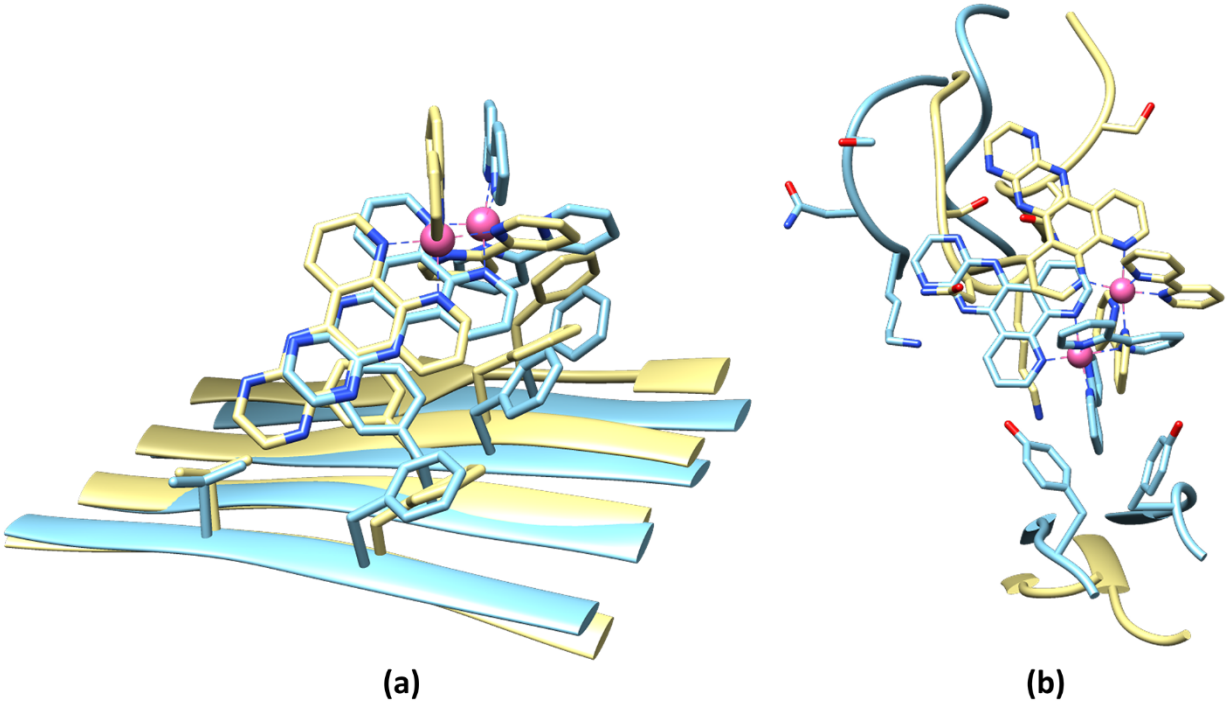


Figure S6. Overlay of the binding sites after MD simulations with AMBER 03/TIP3P (khaki color) and AMBER 99-ILDN/TIP4P-EW (sky blue color) methods for (a) Site1 and (b) Site2.

Binding Site	Binding Free Energy (kcal/mol)
1	-7.4
2	-18.4

Table S2. Calculated binding free energies by λ-particle approach.

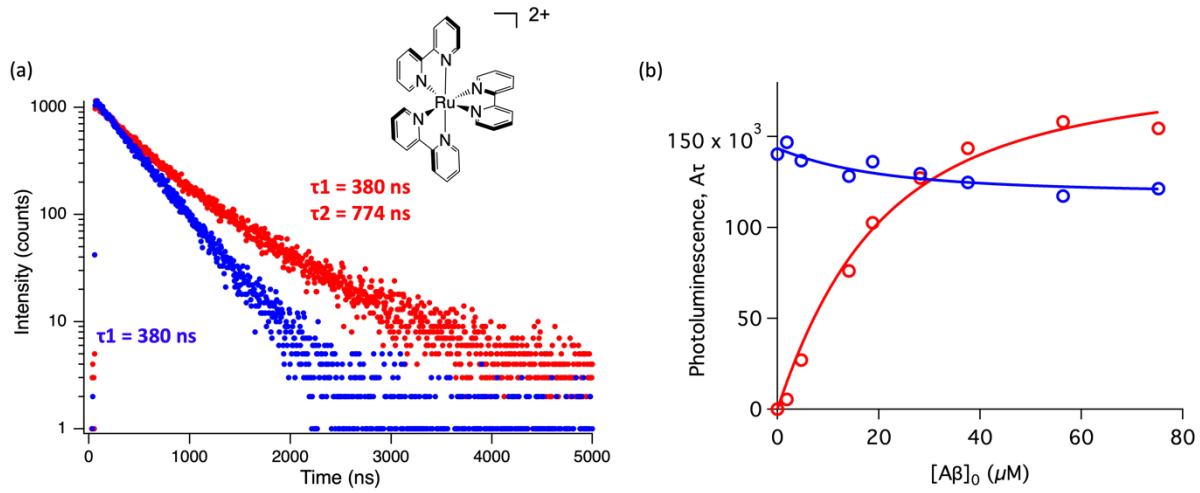


Figure S7. Photoluminescence lifetime of $[Ru(bpy)_3]^{2+}$ with Aβ fibrils. (a) Time decay curves of $[Ru(bpy)_3]^{2+}$ in aqueous solution (blue) and in the presence of Aβ fibrils (red). (b) Titration curve showing the different components of the decay curves of $[Ru(bpy)_3]^{2+}$ with different concentrations of Aβ fibrils. Curves are fits to equations 11 and 12.

(a)

Parameter	Values
K_{d1}	21 ± 6 μM
K_{d2}	4.6 ± 0.6 μM
δ_1	(3.8 ± 0.9) × 10^{-6} M
δ_2	(27.9 ± 0.7) × 10^{-6} M

(b)

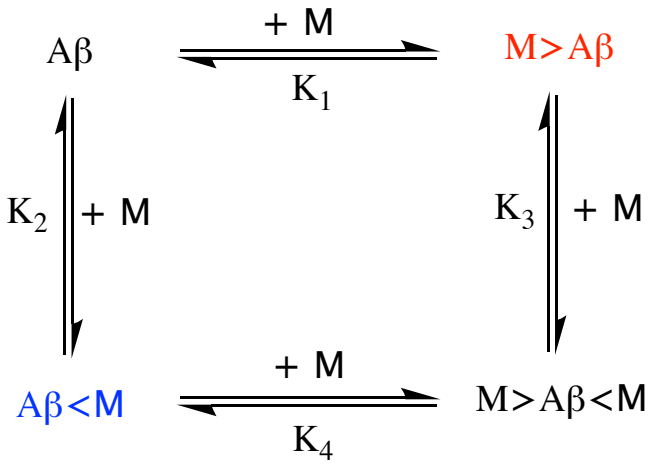
Parameter	Values*
K_{d1}	9 ± 1 μM
K_{d2}	1.8 ± 0.3 μM
δ_1	(157 ± 70) × 10^{-6} M
δ_2	(3 ± 2) × 10^{-6} M
δ_3	(7 ± 4) × 10^{-6} M

Table S3. (a) Parameters of the binding of $[Ru(bpy)_3]^{2+}$ to Aβ fibrils were determined by fitting with equations 11 and 12. (b) Parameters of the binding of $[Ir(ppy)_2(bpy)]^{+}$ to Aβ fibrils were determined by fitting with equations S27-S29.

* Averaged values and standard errors of three sets of experiments.

Appendix 1

Let us consider the following equilibria:[1]



where, for two binding sites that are independent from each other:

$$K_1 = \frac{[A\beta][M]}{[M{>}A\beta]} \qquad \text{(S1)}$$

$$K_2 = \frac{[A\beta][M]}{[A\beta{<}M]} \qquad \text{(S2)}$$

$$K_3 = \frac{[M{>}A\beta][M]}{[M{>}A\beta{<}M]} \qquad \text{(S3)}$$

$$K_4 = \frac{[A\beta{<}M][M]}{[M{>}A\beta{<}M]} \qquad \text{(S4)}$$

and therefore

$$K_1 = K_4 = K_{d1} \qquad \text{(S5)}$$

$$K_2 = K_3 = K_{d2} \qquad \text{(S6)}$$

from the manuscript we get that the free metal complex is given by:

$$[M] = -\frac{a}{3} + \frac{2}{3}\left(\sqrt{a^2 - 3b}\right)\cos\left(\frac{\theta}{3}\right) \quad \text{(S7)}$$

$$\theta = \arccos\left(\frac{-2a^3 + 9ab - 27c}{2\sqrt{(a^2 - 3b)^3}}\right) \quad \text{(S8)}$$

$$a = K_1 + K_2 + \frac{[A\beta]_0}{n} + \frac{[A\beta]_0}{m} - [M]_0 \quad \text{(S9)}$$

$$b = K_1K_2 + K_2\frac{[A\beta]_0}{n} + K_1\frac{[A\beta]_0}{m} - (K_1 + K_2)[M]_0 \quad \text{(S10)}$$

$$c = -K_1K_2[M]_0 \quad \text{(S11)}$$

From eq S1 and S5 we get:

$$K_{d1} = \frac{[A\beta][M]}{[M{>}A\beta]} \quad \text{(S12)}$$

and from eq S3 and S6 we get:

$$K_{d2} = \frac{[M{>}A\beta][M]}{[M{>}A\beta{<}M]} \quad \text{(S13)}$$

By multiplying S12 and S13 we get:

$$(K_{d1})(K_{d2}) = \frac{[A\beta][M]}{[M{>}A\beta]}\frac{[M{>}A\beta][M]}{[M{>}A\beta{<}M]} = \frac{[A\beta][M]^2}{[M{>}A\beta{<}M]} \quad \text{(S14)}$$

and solving for [M>Aβ<M] we obtain:

$$\boldsymbol{[M > A\beta < M] = \frac{[A\beta][M]^2}{(K_{d1})(K_{d2})}} \quad \text{(S15)}$$

The two mass action equations are

$$[A\beta]_0 = [A\beta] + [M > A\beta] + [A\beta < M] + [M > A\beta < M] \quad \text{(S16)}$$

$$[M]_0 = [M] + [M > A\beta] + [A\beta < M] + 2[M > A\beta < M] \quad \text{(S17)}$$

Where [Aβ] and [M] are the concentration of free Aβ and free M.

Subtracting S17 – S16 we get:

$$[M]_0 - [A\beta]_0 = [M] + [M > A\beta < M] - [A\beta] \quad \text{(S18)}$$

Rearranging:

$$[M]_0 - [A\beta]_0 - [M] = [M > A\beta < M] - [A\beta] \quad \text{(S19)}$$

And substituting S15:

$$[M]_0 - [A\beta]_0 - [M] = \frac{[A\beta][M]^2}{(K_{d1})(K_{d2})} - [A\beta] \quad \text{(S20)}$$

Solving for [Aβ]:

$$\frac{[A\beta][M]^2}{(K_{d1})(K_{d2})} - [A\beta] = [M]_0 - [A\beta]_0 - [M]$$

$$[A\beta]\left(\frac{[M]^2}{(K_{d1})(K_{d2})} - 1\right) = [M]_0 - [A\beta]_0 - [M]$$

$$[A\beta] = \frac{[M]_0 - [A\beta]_0 - [M]}{\left(\frac{[M]^2}{(K_{d1})(K_{d2})} - 1\right)} \quad \text{(S21)}$$

Substituting S21 in S15 we obtain:

$$[M > A\beta < M] = \frac{([M]_0 - [A\beta]_0 - [M])([M]^2)}{\left(\frac{[M]^2}{(K_{d1})(K_{d2})} - 1\right)(K_{d1})(K_{d2})} \quad \text{(S22)}$$

Also, substituting S21 in S12 we obtain:

$$[M > A\beta] = \frac{[A\beta][M]}{K_{d1}} = \frac{\left(\frac{[M]_0 - [A\beta]_0 - [M]}{\left(\frac{[M]^2}{(K_{d1})(K_{d2})} - 1\right)}\right)[M]}{K_{d1}} \quad \text{(S23)}$$

substituting S21 in S2 we obtain:

$$[A\beta < M] = \frac{[A\beta][M]}{K_{d2}} = \frac{\left(\frac{[M]_0 - [A\beta]_0 - [M]}{\left(\frac{[M]^2}{(K_{d1})(K_{d2})} - 1\right)}\right)[M]}{K_{d2}} \quad \text{(S24)}$$

Therefore, for $[Ru(bpy)_2(dpqp)]^{2+}$ the saturation curve for the ca. 1082 ns component can be fitted to the sum of eq S15 and S12 where $[M]_{b1}$ is the concentration of $[Ru(bpy)_2(dpqp)]^{2+}$ bound to site 1:

$$A_1\tau_1 = \frac{[M]_{b1}}{\delta_1} = \frac{[M > A\beta] + [M > A\beta < M]}{\delta_1} = \frac{1}{\delta_1}\left(\frac{[A\beta][M]}{K_{d1}} + \frac{[A\beta][M]^2}{(K_{d1})(K_{d2})}\right) = \frac{1}{\delta_1}\left(\frac{[M]}{K_{d1}}\right)[A\beta]\left(1 + \frac{[M]}{K_{d2}}\right)$$

Now substituting [Aβ] for equation S21 we get:

$$A_1\tau_1 = \frac{1}{\delta_1}\left(\frac{[M]}{K_{d1}}\right)\left(\frac{[M]_0 - \frac{[A\beta]_0}{n} - [M]}{\left(\frac{[M]^2}{(K_{d1})(K_{d2})} - 1\right)}\right)\left(1 + \frac{[M]}{K_{d2}}\right) \quad \text{(S25)}$$

where δ_1 is a proportionally constant that relates the intensity of the ca. 1082 ns component with its concentration and [M] is given by S7. Similarly, the saturation curve for the ca. 446 ns component (composed of free and bound ligand) can be fitted to:

$$A_2\tau_2 = \frac{[M]_{b2} + [M]}{\delta_2} = \frac{[A\beta < M] + [M > A\beta < M] + [M]}{\delta_2} = \frac{1}{\delta_2}\left(\left(\frac{[M]}{K_{d2}}\right)\left(\frac{[M]_0 - \frac{[A\beta]_0}{m} - [M]}{\left(\frac{[M]^2}{(K_{d1})(K_{d2})} - 1\right)}\right)\left(1 + \frac{[M]}{K_{d1}}\right) + [M]\right) \quad \text{(S26)}$$

Here $[M]_0$ and $[A\beta]_0$ are the total concentration of the metal complex and Aβ respectively and [M] is given by equation S7. Including the binding stoichiometry requires that all values of $[A\beta]_0$ in equations S22, S23 and equations S22, S24 to be substituted for $[A\beta]_0/n$ and $[A\beta]_0/m$ where n and m are the number of monomers that form binding site 1 and 2 respectively.

For the binding or $[Ir(ppy)_2(bpy)]^+$, the following equations were used:

$$A_1\tau_1 = \frac{1}{\delta_1}[M] \quad \text{(S27)}$$

$$A_2\tau_2 = \frac{1}{\delta_2}\left(\frac{[M]}{K_{d1}}\right)\left(\frac{[M]_0 - \frac{[A\beta]_0}{n} - [M]}{\left(\frac{[M]^2}{(K_{d1})(K_{d2})} - 1\right)}\right)\left(1 + \frac{[M]}{K_{d2}}\right) \quad \text{(S28)}$$

$$A_3\tau_3 = \frac{1}{\delta_3}\left(\frac{[M]}{K_{d2}}\right)\left(\frac{[M]_0 - \frac{[A\beta]_0}{m} - [M]}{\left(\frac{[M]^2}{(K_{d1})(K_{d2})} - 1\right)}\right)\left(1 + \frac{[M]}{K_{d1}}\right) \quad \text{(S29)}$$

1. S.-C. Tso, Q. Chen, S. A. Vishnivetskiy, V. V. Gurevich, T. M. Iverson and C. A. Brautigam, Using Two-Site Binding Models to Analyze Microscale Thermophoresis Data, Anal. Biochem., **2018**, 540–541, 64–75, DOI: 10.1016/j.ab.2017.10.013.