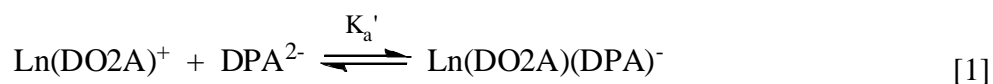


## APPENDIX B

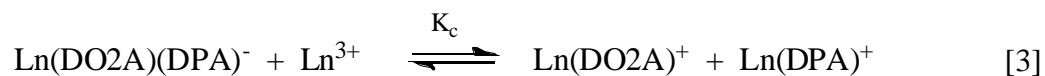
### Derivation of Model for Ln(DO2A)(DPA) Binding Affinity by Competition

Here we report details of the Binding Affinity by Competition (BAC) assay applied to determine the binding constant,  $K_a'$ , for DPA binding to the binary complex of Ln-(1,4,7,10-tetraazacyclododecane-1,7-diacetate) (i.e.,  $\text{Ln}(\text{DO2A})^+$ ), as shown in equilibrium [1] and depicted in the corresponding equilibrium expression [2].<sup>a</sup>



$$K_a' = \frac{[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{eq}}}{[\text{Ln}(\text{DO2A})^+]_{\text{eq}} [\text{DPA}^{2-}]_{\text{eq}}} \quad [2]$$

In order to determine  $K_a'$ , we performed a competitive binding experiment described by equilibrium [3] with equation [4], where the addition of excess  $\text{Ln}^{3+}$  to  $\text{Ln}(\text{DO2A})(\text{DPA})^-$  results in the equimolar formation of the two species  $\text{Ln}(\text{DO2A})^+$  and  $\text{Ln}(\text{DPA})^+$ .



$$K_c = \frac{[\text{Ln}(\text{DO2A})^+]_{\text{eq}} [\text{Ln}(\text{DPA})^+]_{\text{eq}}}{[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{eq}} [\text{Ln}^{3+}]_{\text{eq}}} \quad [4]$$

As  $\text{Ln}^{3+}$  is added, the shift in equilibrium concentrations of  $\text{Ln}(\text{DO2A})(\text{DPA})^-$  and  $\text{Ln}(\text{DPA})^+$  are monitored *via* a specific ligand field-sensitive transition using luminescence spectroscopy. A best fit of luminescence intensity titration data to a two-state thermodynamic model yields the competition equilibrium constant ( $K_c$ ), which in

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<sup>a</sup> Based on lifetime measurements of analogous Tb complexes, we assume that the remaining coordination sites of  $\text{Ln}(\text{DPA})^+$  and  $\text{Ln}(\text{DO2A})^+$  are occupied by water molecules.

conjunction with independent measurement of the  $\text{Ln}(\text{DPA})^+$  formation constant ( $K_a$ ) allows calculation of the ternary complex formation constant ( $K_a'$ ).

We measure the luminescence intensity ( $I_{\text{obs}}$ ) as a function of excess  $\text{Ln}^{3+}$  added ( $[\text{Ln}^{3+}]_{\text{xs}}$ ). The titration data are fit using a two state model [5], assuming that only the two DPA-bound species contribute to the observed luminescence intensities (*i.e.*,  $I_{\text{Ln}(\text{DO2A})(\text{DPA})}$  and  $I_{\text{Ln}(\text{DPA})} \gg I_{\text{Ln}(\text{DO2A})}$  and  $I_{\text{Ln}}^1$ ), and where  $I_{\text{max}}$  and  $I_{\text{min}}$  are the maximum and minimum observed intensities, and  $c_1$  and  $c_2$  are the respective fractions of  $\text{Ln}(\text{DO2A})(\text{DPA})^-$  and  $\text{Ln}(\text{DPA})^+$  at equilibrium (*i.e.*,  $c_1 = [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{eq}} / [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}}$ ,  $c_2 = [\text{Ln}(\text{DPA})^+]_{\text{eq}} / [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}}$ , and  $c_1 + c_2 = 1$ ).

$$\begin{aligned} I_{\text{obs}} &= c_1 I_{\text{max}} + c_2 I_{\text{min}} \\ &= \left( 1 - \frac{[\text{Ln}(\text{DPA})^+]_{\text{eq}}}{[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}}} \right) I_{\text{max}} + \left( \frac{[\text{Ln}(\text{DPA})^+]_{\text{eq}}}{[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}}} \right) I_{\text{min}} \end{aligned} \quad [5]$$

To fit the data for  $I_{\text{obs}}$  versus  $[\text{Ln}^{3+}]_{\text{xs}}$ , we derive an expression for  $[\text{Ln}(\text{DPA})^+]_{\text{eq}}$  in terms of  $[\text{Ln}^{3+}]_{\text{xs}}$  and  $K_a'$  from [2]. Towards this end, we consider the equilibrium of  $\text{Ln}^{3+}$  and  $\text{DPA}^{2-}$  expressed in [6] and depicted in [7].



$$K_a = \frac{[\text{Ln}(\text{DPA})^+]_{\text{eq}}}{[\text{Ln}^{3+}]_{\text{eq}} [\text{DPA}^{2-}]_{\text{eq}}} \quad [7]$$

The value of  $K_a$  has been previously determined through a dilution study at pH 7.5 (see Appendix A). Since  $K_c$  is related to  $K_a'$  by equation [8], and  $[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{eq}}$  and  $[\text{Ln}^{3+}]_{\text{eq}}$  can be expressed in terms of mass balance equations [9] and [10], respectively, we obtain an expression for  $[\text{Ln}(\text{DPA})^+]_{\text{eq}}$  in terms of  $K_a'$ ,  $K_a$ ,  $[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}}$ , and  $[\text{Ln}^{3+}]_{\text{T}}$ .

$$\begin{aligned}
K_c &= \frac{K_a}{K_a'} = \frac{[\text{Ln}(\text{DPA})^+]_{\text{eq}}^2}{[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{eq}} [\text{Ln}^{3+}]_{\text{eq}}} \\
&= \frac{[\text{Ln}(\text{DPA})^+]_{\text{eq}}^2}{\left( [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}} \right) \left( [\text{Ln}^{3+}]_{\text{T}} - [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}} \right)} \\
&\quad \left( -[\text{Ln}(\text{DPA})^+]_{\text{eq}} \right) \left( -[\text{Ln}(\text{DPA})^+]_{\text{eq}} \right)
\end{aligned} \tag{8}$$

In equation [8], we assume that the  $\text{DO2A}^{2-}$  ligand stays bound to  $\text{Ln}^{3+}$ , given that  $\log K_{\text{GdDO2A}} = 19.4$ .<sup>2</sup> As a result, the total ternary complex concentration  $[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}}$  at equilibrium can be expressed as the sum of all  $\text{Ln}(\text{DO2A})^+$ -containing species in equation [9]:

$$\begin{aligned}
[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}} &= [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{eq}} + [\text{Ln}(\text{DO2A})^+]_{\text{eq}} \\
&= [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{eq}} + [\text{Ln}(\text{DPA})^+]_{\text{eq}}
\end{aligned} \tag{9}$$

The total  $\text{Ln}^{3+}$  concentration ( $[\text{Ln}^{3+}]_{\text{T}}$ ) at equilibrium is defined as the sum of all  $\text{Ln}^{3+}$  containing equilibrated species as shown in equation [10]:

$$\begin{aligned}
[\text{Ln}^{3+}]_{\text{T}} &= [\text{Ln}^{3+}]_{\text{eq}} + [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{eq}} + [\text{Ln}(\text{DO2A})^+]_{\text{eq}} + [\text{Ln}(\text{DPA})^+]_{\text{eq}} \\
&= [\text{Ln}^{3+}]_{\text{eq}} + [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{eq}} + 2[\text{Ln}(\text{DPA})^+]_{\text{eq}}
\end{aligned} \tag{10}$$

Solving equation [8] for  $[\text{Ln}(\text{DPA})^+]_{\text{eq}}$ , we obtain equation [11]:

$$[\text{Ln}(\text{DPA})^+]_{\text{eq}} = \frac{[\text{Ln}^{3+}]_{\text{T}} + \left( \left\{ [\text{Ln}^{3+}]_{\text{T}} - 2(1 - K_a' \cdot K_a^{-1}) [\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}} \right\}^2 \right)^{1/2} + 4K_a' \cdot K_a^{-1} (1 - K_a' \cdot K_a^{-1}) ([\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}})^2}{2(1 - K_a' \cdot K_a^{-1})} \tag{11}$$

The total concentration of the ternary complex,  $[\text{Ln}(\text{DO2A})(\text{DPA})^-]_{\text{T}}$ , is experimentally fixed (in this case 1.0  $\mu\text{M}$ ), and the total  $\text{Ln}^{3+}$  concentration ( $[\text{Ln}^{3+}]_{\text{T}}$ ) is the sum of this

initial ternary complex concentration and any excess  $\text{Ln}^{3+}$  added ( $[\text{Ln}]_{\text{XS}}$ ) as shown in equation [12].

$$[\text{Ln}^{3+}]_{\text{T}} = [\text{Ln}(\text{DO2A})(\text{DPA})^{-}]_{\text{T}} + [\text{Ln}^{3+}]_{\text{XS}} \quad [12]$$

Substituting the expression for  $[\text{Ln}(\text{DPA})^{+}]_{\text{eq}}$  from equation [11] into equation [5], we obtain equation [13], the BAC assay two-state model expression used to calculate  $K_a'$ .

$$I_{\text{obs}} = \left( 1 - (2AB)^{-1} \left\{ A + [\text{Ln}^{3+}]_{\text{XS}} + \left( \left\{ A + [\text{Ln}^{3+}]_{\text{XS}} - 2AB \right\}^2 + 4A(1-A)B^2 \right)^{1/2} \right\} \right) I_{\text{max}} \quad [13]$$

$$+ \left( (2AB)^{-1} \left\{ A + [\text{Ln}^{3+}]_{\text{XS}} + \left( \left\{ A + [\text{Ln}^{3+}]_{\text{XS}} - 2AB \right\}^2 + 4A(1-A)B^2 \right)^{1/2} \right\} \right) I_{\text{min}}$$

where

$$A = [\text{Ln}(\text{DO2A})(\text{DPA})^{-}]_{\text{T}}$$

$$B = 1 - K_a' \cdot K_a^{-1}$$

This model is used to calculate  $K_a'$  from the data with the Curve Fitting Toolbox in Matlab® in the following format:

$$f(x) = (1 - (1e-06 + 10^x - ((1e-06 + 10^x - (2e-06)*(1 - (1/c))))^2 + (4/c)*(1 - (1/c))*(1e-06)^2)^{0.5}) / ((2e-06)*(1 - (1/c)))) * a + ((1e-06 + 10^x - ((1e-06 + 10^x - (2e-06)*(1 - (1/c))))^2 + (4/c)*(1 - (1/c))*(1e-06)^2)^{0.5}) / ((2e-06)*(1 - (1/c)))) * b$$

where  $x = [\text{Ln}^{3+}]_{\text{XS}}$  in M

$$a = I_{\text{max}}$$

$$b = I_{\text{min}}$$

$$c = K_a / K_a'$$

## B5

Each trial is fit independently to produce values for all three coefficients (a, b, and c). Assuming reasonable fits,  $K_a'$  is calculated from c using the  $K_a$  value from identical pH and temperature conditions, and the three values are averaged to produce the final reported value of  $K_a'$  with standard deviation.

**References**

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2. Kim, W. D.; Hrcir, D. C.; Kiefer, G. E.; Sherry, A. D., Synthesis, Crystal-Structure, And Potentiometry Of Pridine-Containing Tetraaza Macrocyclic Ligands With Acetate Pendant Arms. *Inorganic Chemistry* **1995**, 34, (8), 2225-2232.