Supplementary Information

pFe$^{3+}$ Determination of Multidentate Ligands by a Fluorescence Assay

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Structures of Ligands

L1

L2

L3
L13: \( R_1 = \text{CH}_3, \; R = \text{CH}_3 \)

L14: \( R_1 = \text{n-C}_3\text{H}_7, \; R = \text{C}_2\text{H}_5 \)

L15: \( R_1 = \text{CH}_3\text{O(\text{CH}_2\text{CH}_2\text{O})}_2\text{CH}_2, \; R_1 = \text{C}_2\text{H}_5 \)

L16: \( R_1 = \text{CH}_3, \; R = \text{n-C}_4\text{H}_9 \)

L17: \( R_1 = \text{n-C}_3\text{H}_7, \; R = \text{n-C}_4\text{H}_9 \)

L18: \( R_1 = \text{CH}_3\text{O(\text{CH}_2\text{CH}_2\text{O})}_2\text{CH}_2, \; R = \text{n-C}_4\text{H}_9 \)

L19: \( R_1 = \text{CH}_3\text{O(\text{CH}_2\text{CH}_2\text{O})}_2\text{CH}_2, \; R = \text{n-C}_6\text{H}_{13} \)

L20: \( R_1 = \text{n-C}_3\text{H}_7, \; R = \text{CH}_2\text{CH}_2\text{OH} \)

L21: \( R = \text{CH}_2\text{CH}_2\text{OH} \)
L22

L23

**L24**: Polymer of L23 and iron binding capacity at 291 µmol/g.
**Calculation of unknown pFe value of hexadentate ligand based on the pFe value of the competing ligand and the relative fluorescence**

Hexadentate ligands have simple equilibrium constants as indicated in Eq. 1 and 2. The competition between the two hexadentate ligands for iron is presented in Eq. 3.

\[ \text{Fe} + \text{L}_1 \xrightleftharpoons{K_1} \text{FeL}_1 \quad \text{Eq. 1} \]

\[ \text{Fe} + \text{L}_2 \xrightleftharpoons{K_2} \text{FeL}_2 \quad \text{Eq. 2} \]

\[ \text{FeL}_1 + \text{L}_2 \xrightleftharpoons{K} \text{L}_1 + \text{FeL}_2 \quad \text{Eq. 3} \]

The equilibrium constants \( K_1, K_2 \) and \( K \) can be written as follows:

\[ K_1 = \frac{[\text{FeL}_1]}{[\text{Fe}][\text{L}_1]} \]

\[ K_2 = \frac{[\text{FeL}_2]}{[\text{Fe}][\text{L}_2]} \]

\[ K = \frac{[\text{L}_1][\text{FeL}_2]}{[\text{FeL}_1][\text{L}_2]} \]

If \( \text{L}_1 \) represents CP691 and \( \text{L}_2 \) represents DFO, then based on the exponential curve in Figure 3, at 50% relative fluorescence, the ratio of \( [\text{L}_2]_{\text{total}} / [\text{L}_1]_{\text{total}} = 125 \). As \( [\text{L}_1]_{\text{total}} = [\text{Fe}]_{\text{total}} = 6 \mu\text{M} \), then \( [\text{L}_2]_{\text{total}} = 750 \mu\text{M} \), and the point of 50% fluorescence occurs at \( [\text{L}_1] = 3 \mu\text{M} \).

As \( [\text{L}_1]_{\text{total}} = [\text{L}_1] + [\text{FeL}_1] \),

\( [\text{FeL}_1] \) can be calculated by the equation \( [\text{FeL}_1] = [\text{L}_1]_{\text{total}} - [\text{L}_1] = 3 \mu\text{M} \)

As \( [\text{Fe}]_{\text{total}} = [\text{Fe}] + [\text{FeL}_1] + [\text{FeL}_2] \) and the ligands are in excess and [Fe] is very low, \( [\text{FeL}_2] \approx [\text{Fe}]_{\text{total}} - [\text{FeL}_1] = 3 \mu\text{M} \).

As \( [\text{L}_2]_{\text{total}} = [\text{L}_2] + [\text{FeL}_2] \), so \( [\text{L}_2] = [\text{L}_2]_{\text{total}} - [\text{FeL}_2] = 750 - 3 = 747 \mu\text{M} \).

Therefore, \( K = (3 \mu\text{M} \times 3 \mu\text{M})/(3 \mu\text{M} \times 747 \mu\text{M}) = 1 / 249 \)

As \( K = [\text{L}_1][\text{FeL}_2] / [\text{FeL}_1][\text{L}_2] = ([\text{L}_1](K_2[\text{Fe}]][\text{L}_2]) / (K_1[\text{Fe}][\text{L}_1][\text{L}_2]) = K_2 / K_1 \)
At the condition of $[L] = 10 \mu M$, $[\text{Fe}] = 1 \mu M$ and pH 7.4, $[\text{Fe}] = 2.5 \times 10^{-27} \mu M$ when $L = \text{DFO (pFe}^{3+} = 26.6)$,

Thus $K_2 = [\text{FeL}_2] / [\text{Fe}][\text{L}_2] = 1 \mu M / (2.5 \times 10^{-27} \mu M \times 9 \mu M)$

$K_1 = [\text{FeL}_1] / [\text{Fe}][\text{L}_1] = 1 \mu M / ([\text{Fe}]_{L_1} \times 9 \mu M) \ (\text{L}_1 = \text{CP691})$

So $[\text{Fe}]_{L_1} = 1 / (9K_1) = 1 / (9 \times (K_2 / K)) = K / (9K_2) = (1 / 249) / (9 \times (1 / (9 \times 2.5 \times 10^{-27} \mu M))) = 1 \times 10^{-29} \mu M$

Therefore, $p\text{Fe}_{L_1} = 29.0$. In fact, the $p\text{Fe}_{L_1}$ value can be calculated from any point on the exponential curve. The average value of the $p\text{Fe}$ calculated from the experimental ratio points is 28.8.