

Supporting Information

Mn²⁺ - nucleotide coordination at the myosin active site as detected by pulsed EPR.

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Structures of the nucleotides studied in this work

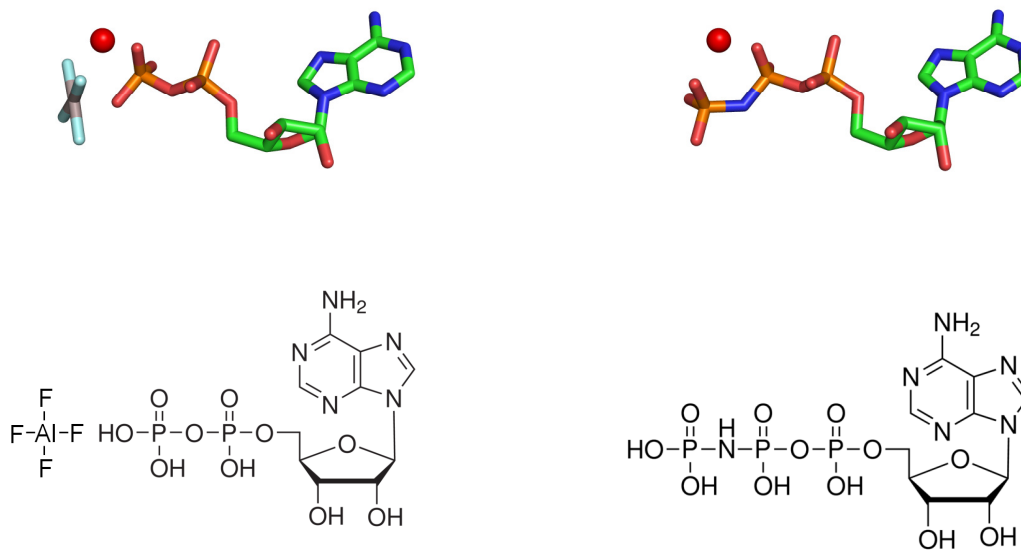


Figure S1. The structure of ADP·AlF₄ (C₁₀H₁₅N₅O₁₀P₂AlF₄) with the metal ion, coordinating β phosphate and AlF₄.

Figure S2. The structure of AMPPNP (C₁₀H₁₇N₆O₁₂P₃) with the metal ion, coordinating γ and β phosphates.

Interpretation of the ligand hfi parameters observed in the complexes of high-spin transition metal ions.

Although the relationship between the observed (effective) *hfi* constant and the true one-electron *hfi* constant in high-spin systems is well-known [1-3], the necessity to appropriately rescale the ^{31}P and $^{14(15)}\text{N}$ isotropic *hfi* constants of coordinated phosphates and nitrogens in the Mn.nucleotide complexes was missed in all of the high-resolution EPR works where such complexes were studied [4-10]. Therefore, as a reminder, we present here a simple derivation that demonstrates the general approach to obtaining such scaling factors. Let us consider the specific system at hand, the Mn^{2+} center, which has the d_5 electronic configuration with the total spin $S = 5/2$. This total spin represents a sum of the individual spins of each of the d -electrons:

$$S = \sum_j S_j \quad (\text{S1})$$

where $S_j = 1/2$ are the individual spins, and the simple summation implies that the multiplet with the highest possible spin is formed. The states with positive total S_Z are described by:

$$\begin{aligned} |5/2\rangle &= \alpha\alpha\alpha\alpha\alpha \\ |3/2\rangle &= \frac{1}{\sqrt{5}}(\alpha\alpha\alpha\alpha\beta + \alpha\alpha\alpha\beta\alpha + \alpha\alpha\beta\alpha\alpha + \alpha\beta\alpha\alpha\alpha + \beta\alpha\alpha\alpha\alpha) \\ |1/2\rangle &= \frac{1}{\sqrt{10}}(\alpha\alpha\alpha\beta\beta + \alpha\alpha\beta\alpha\beta + \alpha\beta\alpha\alpha\beta + \beta\alpha\alpha\alpha\beta + \alpha\alpha\beta\beta\alpha + \\ &\quad + \alpha\beta\alpha\beta\alpha + \beta\alpha\alpha\beta\alpha + \alpha\beta\beta\alpha\alpha + \beta\alpha\beta\alpha\alpha + \beta\beta\alpha\alpha\alpha) \end{aligned} \quad (\text{S2})$$

and those with the negative S_Z are obtained from the corresponding positive ones by interchanging the single-electron functions α and β . The average values of single-electron operators $\mathbf{S}_{\mathbf{z}k}$ (where the index k indicates any one of the five electrons) obtained from eq S2 are:

$$\begin{aligned} \langle S_{\mathbf{z}k} \rangle_{5/2} &= \langle 5/2 | \mathbf{S}_{\mathbf{z}k} | 5/2 \rangle = 1/2 = (5/2) \times 1/5 \\ \langle S_{\mathbf{z}k} \rangle_{3/2} &= \langle 3/2 | \mathbf{S}_{\mathbf{z}k} | 3/2 \rangle = 3/10 = (3/2) \times 1/5 \\ \langle S_{\mathbf{z}k} \rangle_{1/2} &= \langle 1/2 | \mathbf{S}_{\mathbf{z}k} | 1/2 \rangle = 1/10 = (1/2) \times 1/5 \end{aligned} \quad (\text{S3})$$

etc. The factor of 1/5 in eq S3 equals to $2S$, and the average single-electron values can be expressed as:

$$\langle S_{Zk} \rangle_{S_z} = \langle S_Z \rangle / (2S) \quad (S4)$$

Although the derivation of eq S4 was based on considering the specific case of $S = 5$, it is actually general and applies to the highest spin multiplets formed by any number of individual spins $S_j = 1/2$. In particular, it is relevant for the half-filled orbital shells of atoms and ions, *e.g.*, p_3 (N^0), d_5 (Mn^{2+}), or f_7 (Gd^{3+}).

The ligand isotropic *hfi* constants can be considered based on the model where only one of the five *d*-orbitals predominantly interacts with a given ligand, and thus only one unpaired electron delocalizes on that ligand and results in the isotropic *hfi* with the ligand nucleus. The isotropic *hfi* constant in such a model can either be expressed through the total electron spin (the experimentally observable effective *hfi* constant a_{ef}) or using the single-electron operators (the true one-electron *hfi* constant a):

$$H_{iso} = a_{ef} \mathbf{S}_Z \mathbf{I}_Z = a \mathbf{S}_{Zk} \mathbf{I}_Z \quad (S5)$$

where a high-field approximation is assumed. Substituting the operators by the expectation values and using eq S4, one immediately obtains:

$$a = 2S \cdot a_{ef} \quad (S6)$$

For the anisotropic *hfi*, two contributions should be considered. The first contribution comes from the (one-electron) spin density delocalized on the ligand and is treated similar to the isotropic *hfi* (eqs S5 and S6), *e.g.*,

$$H_{aniso}^{ligand} = T_{ZZef}^{ligand} \mathbf{S}_Z \mathbf{I}_Z = T_{ZZ}^{ligand} \mathbf{S}_{Zk} \mathbf{I}_Z \quad (S7)$$

(and similar terms for T_{ZX} and T_{ZY}), which results in

$$T_{ZZ}^{ligand} = 2S \cdot T_{ZZef}^{ligand} \quad (S8)$$

The second contribution is the through-space magnetic dipole interaction of the ligand nucleus with the spin density on the central ion. In this case, all $N = 2S$ individual unpaired electrons interact with the ligand nucleus approximately equally:

$$H_{aniso}^{central} = T_{ZZef}^{central} \mathbf{S}_Z \mathbf{I}_Z = \left(\sum_{k=1}^{2S} T_{ZZ}^{central} \mathbf{S}_{Zk} \right) \cdot \mathbf{I}_Z = T_{ZZ}^{central} \left(\sum_{k=1}^{2S} \mathbf{S}_{Zk} \right) \cdot \mathbf{I}_Z \quad (\text{S9})$$

(and similar terms for T_{ZX} and T_{ZY}). Substituting the average values of the spin projections and using eq S4, one immediately arrives at:

$$T_{ZZ}^{central} = T_{ZZef}^{central} \quad (\text{S10})$$

Taking into account the conversion factors given by eqs S6, S8, and S10 allows one to meaningfully compare the *hfi* parameters obtained for the ligands of high-spin ions with those observed for the ligands of the $S = 1/2$ ions. In addition, using the true isotropic *hfi* constant (eq S6) rather than the effective one results in more accurate estimates of the spin density delocalization on the ligands.

As an example, for the phosphate ligand at Mn^{2+} ion we obtained, on average, $a_p \sim 23$ MHz (see Table 1 in the main text). Taking into account that the unpaired electron on the $3s$ orbital of phosphorus results in the *hfi* constant of about 1.33×10^4 MHz [11] and that the s -character of the phosphorus electronic orbitals in phosphate is about $1/4$ (sp_3 orbitals), we can estimate the spin density on phosphorus as $\rho_p \sim 0.007$. Such a spin density will result in the anisotropic *hfi* constant $T_{\perp}^{ligand} \sim (-367 \text{ MHz}) \times (3/4) \times \rho_p \sim -1.9 \text{ MHz}$, where -367 MHz is the value of T_{\perp} that would be obtained for a pure p -orbital and $\rho_p = 1$ [11], and $3/4$ is the p -character of the sp_3 orbital. The effective (observable) anisotropic *hfi* from this source is thus $T_{\perp ef}^{ligand} = T_{\perp}^{ligand} / 2S \sim -0.4 \text{ MHz}$. The ‘‘through-space’’ anisotropic *hfi* with the central ion at the distance $R_{\text{MnP}} = 3.2\text{-}3.3 \text{ \AA}$ is $T_{\perp ef}^{central} = T_{\perp}^{central} \sim -0.9 \text{ MHz}$. Adding the two contributions together (with the proper account of the geometry of the Mn-O-P fragment) results in a slightly rhombic tensor $(T_{11}, T_{22}, T_{33})_{ef} \sim (-1.1, -1.36, 2.46) \text{ MHz}$. The two smaller components obtained by this estimate are somewhat larger than the experimental value of $T_{\perp ef} = -0.9 \text{ MHz}$. This discrepancy can be corrected by taking into account the spin delocalization from the Mn ion to

the ligands (*e.g.*, assuming $\rho_{\text{Mn}} = 0.7$ results in the calculated $T_{\perp\text{ef}}$ similar to the experimental one).

The purpose of the above discussion was only to demonstrate the general approach to the analysis of the anisotropic and isotropic *hfi* for the ligands of the high-spin ions, in particular, Mn^{2+} . While this analysis is rather approximate, it is qualitatively better than simply estimating the Mn – P distance using a point dipole approximation. As follows from this analysis, the fact that the point-dipolar anisotropic *hfi* (for $\rho_{\text{Mn}} = 1$) is similar to the experimental one is purely coincidental because the spin density delocalization into the phosphate orbitals gives a noticeable (~40%) contribution into the total anisotropic *hfi*.

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