Recognition and Sensing of Nucleoside Monophosphates by a Dicopper(II) Cryptate

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SUPPLEMENTARY SUPPORTING INFORMATION

- Calculation details, general procedures and Figures S1-S3 showing the fitting of [R] and [A] data on equation (XV) for calculating K_{A1} and K_{A2}. Data refer to the titration with GMP(S1), TMP (S2) and UMP (S3).

- Figures S4-S6: concentration profiles of the species present at the equilibrium, over the course of the spectrofluorimetric titration experiment (indicator displacement). Data refer to the titration with TMP(S4), UMP (S5) and CMP (S6).

- Figure S7: ESI simulated spectra of the species $[Cu_2^{II}(\mathbf{1})(GMP)]^{2+}$(a) and $\{[Cu_2^{II}(\mathbf{1})GMPH]^{3+},CF_3SO_3^-\}^{2+}$(b).

- Figures S8, S9: cyclic voltammetry studies carried out on a solution of 1×10^{-3} M in $[Cu_2^{II}(\mathbf{1})]^{4+}$ in H2O:MeOH mixture buffered to pH 7 with HEPES 0.05M and $[Bu_4N]NO_3$ 0.05 M (S8) and MeCN (S9).

- Figures S10, MM$^+$ structure calculated for the "empty" cage $[Cu_2^{II}(\mathbf{1})]^{4+}$.

- Figures S11, S12: EPR spectra of the $[Cu_2^{II}(\mathbf{1})]^{4+}$ specie without GMP (S12) and with 4 eqv of GMP(S13).

1.   Determination of the receptor/analyte equilibrium constants. The receptor/analyte binding constants were obtained from indicator displacement spectrofluorimetric titrations of the type illustrated in Figure 3. The experimental data were treated by taking into account the receptor (R) / fluorogenic indicator (In) and the receptor (R) / analyte (A) following equilibria.

$$R + In \rightleftharpoons RIn \qquad (I)$$

$$R + RIn \rightleftharpoons R_2In \qquad (II)$$

$$R + A \rightleftharpoons RA \qquad (III)$$

$$R + RA \rightleftharpoons R_2A \qquad (IV)$$

Equilibrium constants are K_{F1}, K_{F2}, K_{A1} and K_{A2}, respectively.
The total molar concentration of the fluorophore ($[In]_0$) and of the receptor ($[R]_0$) are:

$$[In]_0 = 2.0 \times 10^{-7} \text{ M}$$

$$[R]_0 = 2.0 \times 10^{-5} \text{ M}$$

From mass balances, we obtain equations (V) and (VI)

$$[In]_0 = [In] + [RIn] + [R_2In] \qquad (V)$$

$$[R]_0 = [R] + [RIn] + 2[R_2In] + [RA] + 2[R_2A] \qquad (VI)$$

In a typical experiment, the indicator concentration is 1/100 with respect to the receptor; thus, we could assume that RIn and R_2In concentrations are negligible with respect to [R], [RA] and $[R_2A]$. Therefore, the mass balance (VI) can be simplified to:

$$[R]_0 \cong [R] + [RA] + 2[R_2A] \quad (VI')$$

In the course of the indicator displacement titrations, we could determine the molar concentration of the free fluorophore [In] from its fluorescent emission intensity (I) through equation (VII) (I_0 is the fluorescent intensity of the indicator 2.0×10^{-7} M solution, in the absence of the receptor).

$$[In] = [In]_0 \frac{I}{I_0} \qquad (VII)$$

The concentration of the indicator, bound to the receptor's cavity, can be determined from eqn. (VIII)

$$[In]_{bound} = [RIn] + [R_2In] = [In]_0 - [In] \qquad (VIII)$$

The concentration of species RIn and R_2In can be calculated from the known equilibrium constants, K_{F1} and K_{F2}.
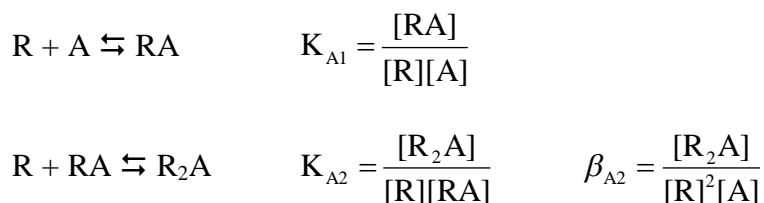
$$[RIn] = K_{F1}[R] [In] \qquad (IX)$$

$$[R_2In] = K_{F1}K_{F2}[R]^2 [In] \qquad (X)$$

The free receptor ([R]) concentration is determined from equation (XI), which derives from the substitution of (IX) and (X) in eqn. (VIII).

$$[R] = \frac{-K_{F1}[In] + \sqrt{(K_{F1}^2[In]^2 + 4K_{F1}K_{F2}[In]([In]_0 - [In]))}}{2K_{F1\backslash}K_{F2}[In]}$$

$$= \frac{-K_{F1} + \sqrt{(K_{F1}^2 + 4K_{F1}K_{F2}([In]_0 - [In])/[In])}}{2K_{F1}K_{F2}} \qquad (XI)$$

[R] is the concentration of the receptor, which is not involved in the interaction with the fluorophore and it is therefore available to anion binding. The [R] value can be obtained for each addition of the analyte, from the fluorescence intensity and from the known values of K_{F1} and K_{F2}. The equilibrium constants, relative to anion binding, are

$$R + A \leftrightarrows RA \qquad K_{A1} = \frac{[RA]}{[R][A]}$$

$$R + RA \leftrightarrows R_2A \qquad K_{A2} = \frac{[R_2A]}{[R][RA]} \qquad \beta_{A2} = \frac{[R_2A]}{[R]^2[A]}$$

Since the added anion (A) is in excess with respect to the receptor (R), we can neglect the fraction of anion bound to the receptor from the total concentration ([A]$_0$).
[RA] and [R$_2$A] can be obtained from the K_{A1} and K_{A2} definitions:

[RA] = K_{A1} [R] [A]   (XII)

[R$_2$A] = K_{A1} K_{A2} [R]^2[A]   (XIII)

By substituting eqn. (XII) and (XIII) in eqn. (VI'), the second order equation (XIV) is obtained. Its solution gives [R], as a function of the analyte concentration and of the binding constants K_{A1} and K_{A2} (see eqn XV).

[R]$_0 \cong$ [R] + [RA] + 2[R$_2$A] = [R] + K_{1A} [R] [A] + 2 $K_{1A} \times K_{1A}$ [R]^2 [A]   (XIV)

$$[R] = \frac{-K_{A1}[A] - 1 + \sqrt{((K_{A1}[A]+1)^2 + 8\beta_{A2}[A][R]_0)}}{4\beta_{A2}[A]} \qquad (XV)$$

Thus, the following quantities can be obtained for each titration point:
[R] = concentration of the receptor, available for anion binding; it derives from eqn (XI)
[A] = total concentration of the added anion, obtained from the anion displacement titration data
Finally, [R] and [A] are fitted with respect to equation (XV) through a non-linear least-squares procedure and pertinent values of K_{A1} and β_{A2} (= $K_{A1} \times K_{A2}$) are calculated. As an example, curve fitting related to the titration of Figure 3 (A = GMP) is shown in Figure S1.
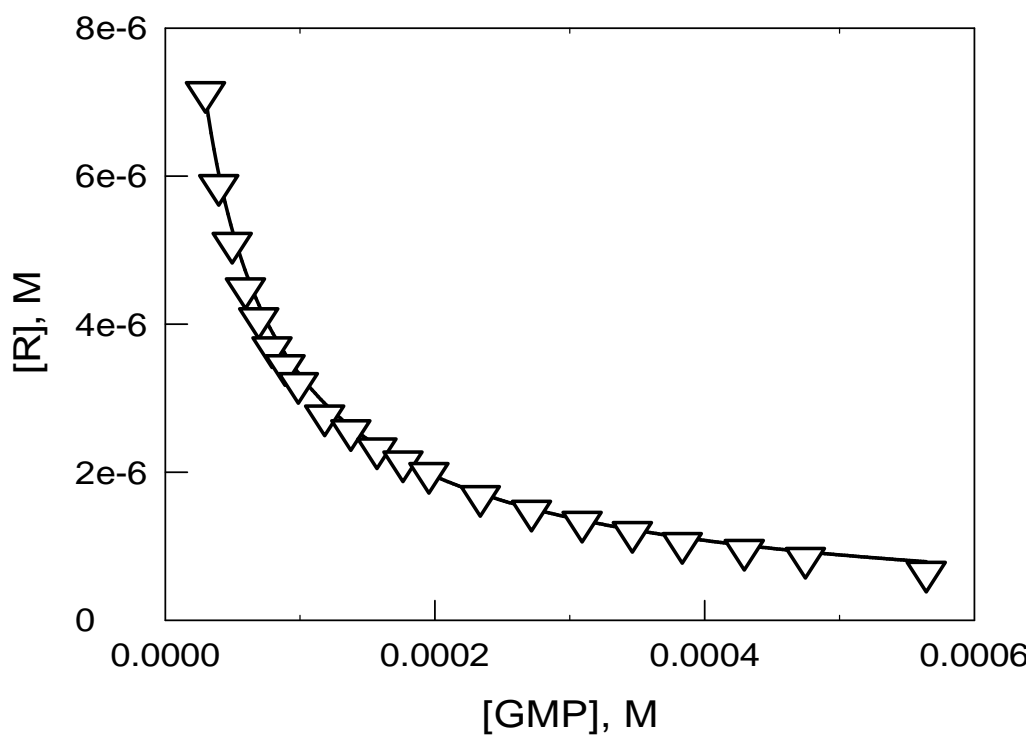
Figure S1. Fitting of [R] *vs.* [A] data on equation (XV) for calculating K_{A1} and K_{A2}. Data refer to the titration with GMP.
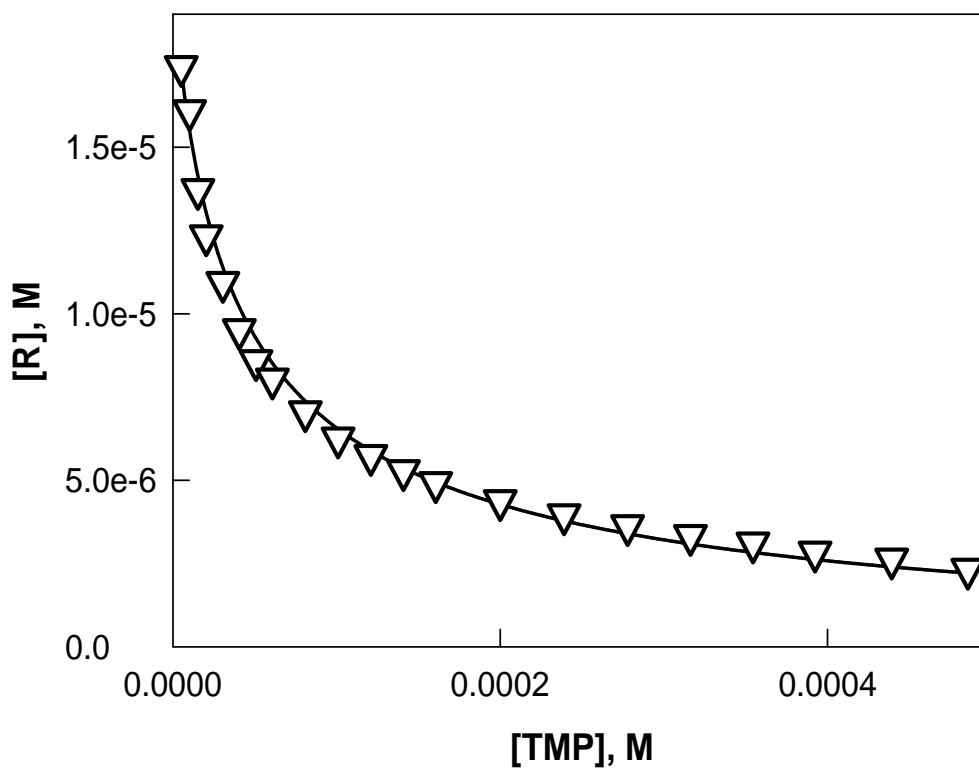

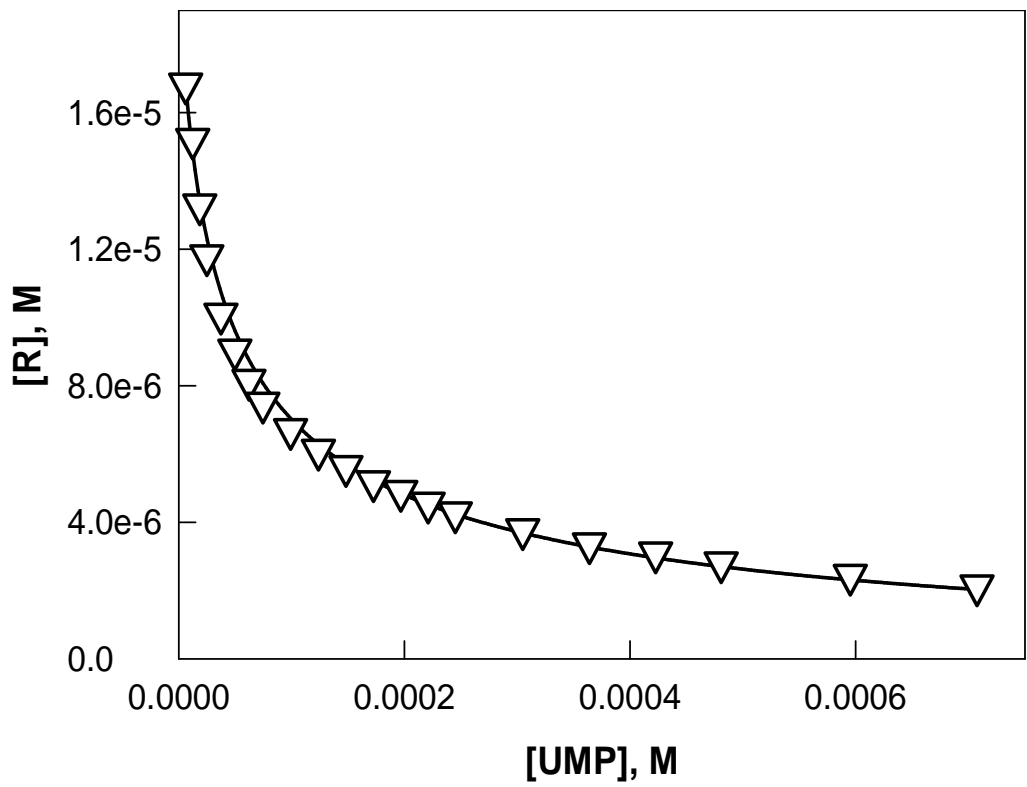**Figure S2.** Fitting of [R] and [TMP] data on equation (XV) for calculating K_{A1} and K_{A2}.

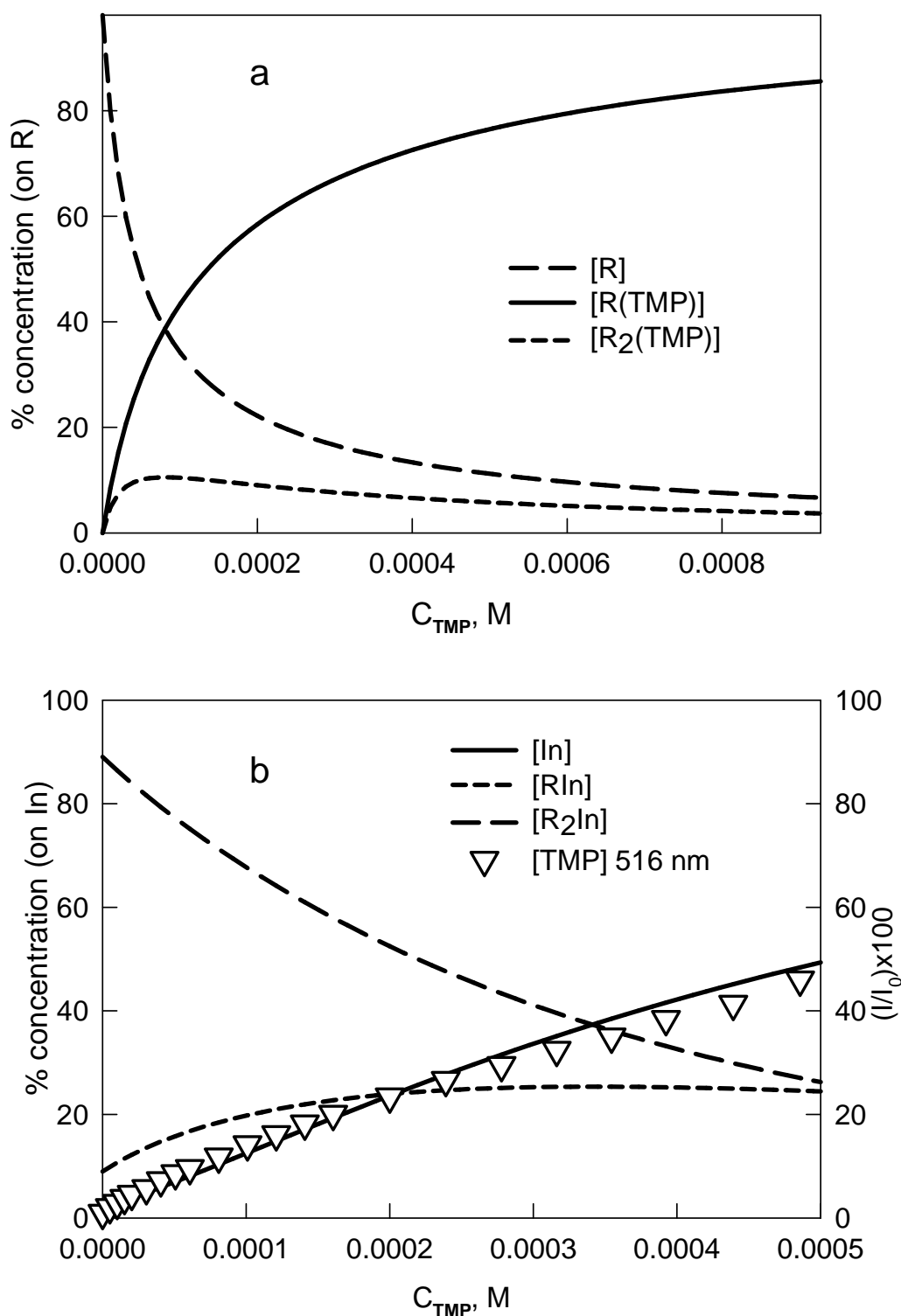**Figure S3.** Fitting of [R] and [UMP] data on equation (XV) for calculating K_{A1} and K_{A2}.

Figure S4. (a): concentration profiles of the species present at the equilibrium, over the course of the spectrofluorimetric titration experiment with TMP; concentration (in %) is referred to the total concentration of the receptor R (= $[Cu_2^{II}(\mathbf{1})]^{4+}$): $2.0{\times}10^{-5}$ M ; (b) concentration (in %) is referred to the total concentration of the Indicator In (= $\mathbf{6}$)): $2.0{\times}10^{-7}$ M; symbols: normalized intensity of the emission at 516 nm of the released indicator, right vertical axis.
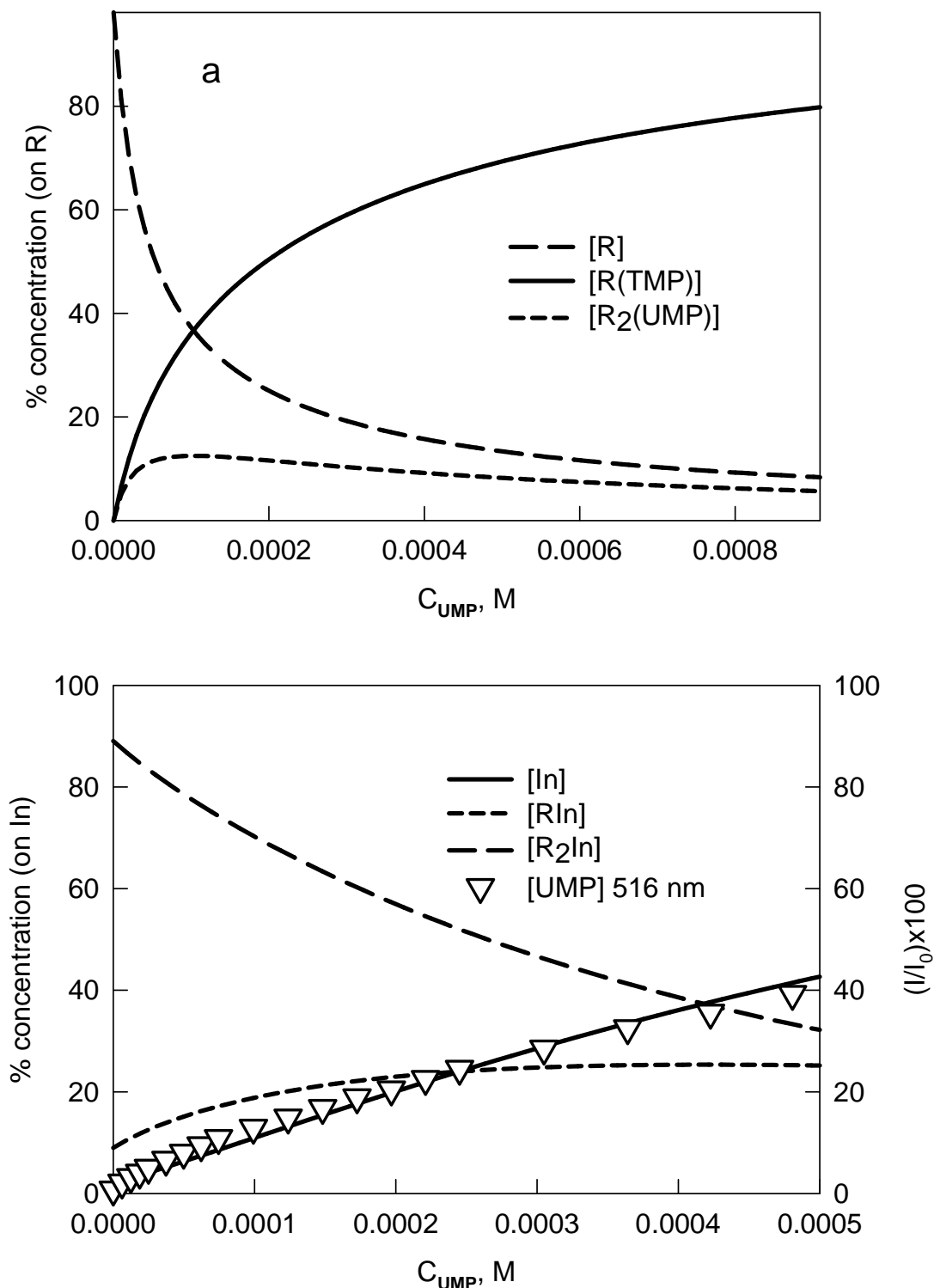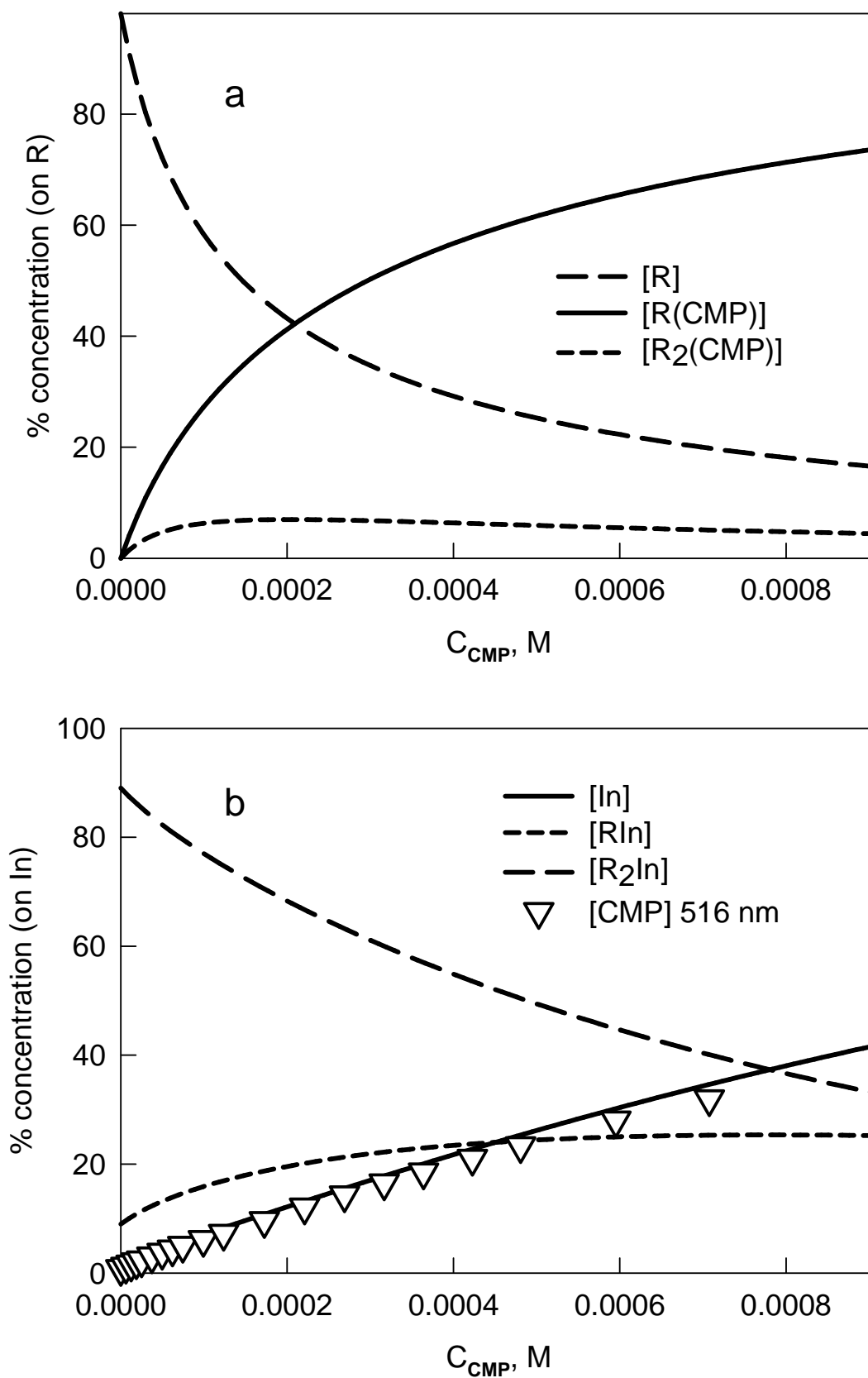
Figure S5. (a): concentration profiles of the species present at the equilibrium, over the course of the spectrofluorimetric titration experiment with UMP; concentration (in %) is referred to the total concentration of the receptor R (= $[Cu_2^{II}(\mathbf{1})]^{4+}$): 2.0×10^{-5} M ; (b) concentration (in %) is referred to the total concentration of the Indicator In (= **6**)): 2.0×10^{-7} M; symbols: normalized intensity of the emission at 516 nm of the released indicator, right vertical axis.

Figure S6. (a): concentration profiles of the species present at the equilibrium, over the course of the spectrofluorimetric titration experiment with CMP; concentration (in %) is referred to the total concentration of the receptor R (= $[Cu_2^{II}(\mathbf{1})]^{4+}$): 2.0×10^{-5} M ; (b) concentration (in %) is referred to the total concentration

of the Indicator In (= **6**)): 2.0×10^{-7} M; symbols: normalized intensity of the emission at 516 nm of the released indicator, right vertical axis.
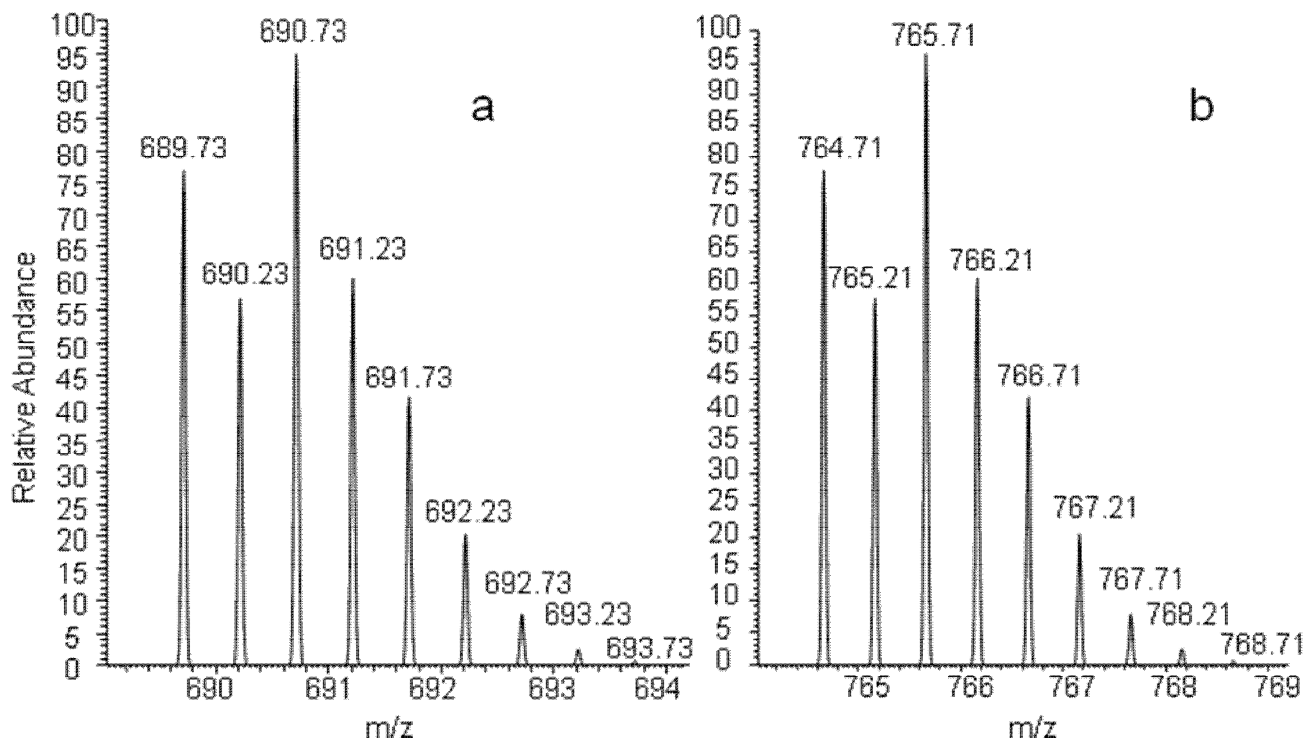

Figure S7. ESI mass simulated spectra. Portion (a) displays a double positive charge peak at In particular, portion (a) displays a double positively charged peak at 690.6 m/z, corresponding to the species: $[Cu_2^{II}(\mathbf{1})(GMP)]^{2+}$. The isotope pattern is tipycal of two Cu^{II} ions, while the peak-to-peak separation of 0.5 m/z indicates the presence of a double positive charge of the complex. the portion (b) of the spectrum shows a peak at 765.5 m/z, corresponding to the complex species: $\{[Cu_2^{II}(\mathbf{1})GMPH]^{3+}, CF_3SO_3^-\}^{2+}$.
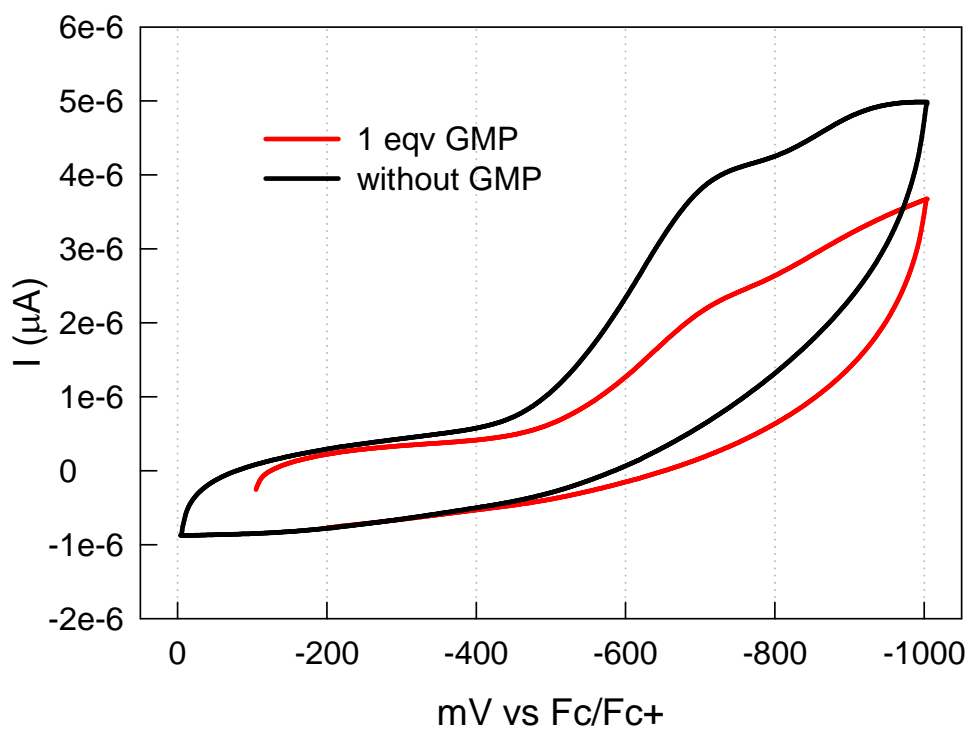
Figure S8. Cyclic voltammetry of 1×10^{-3} M in $[Cu_2^{II}(\mathbf{1})]^{4+}$ H2O:MeOH mixture solution, buffered to pH 7 with HEPES 0.05M and $[Bu_4N]NO_3$ 0.05 M, in absence (black line) and presence (red line) of GMP. Scan rate 50 mV/s. Potential is referred to ferrocene/ferrocenium couple (internal standard).
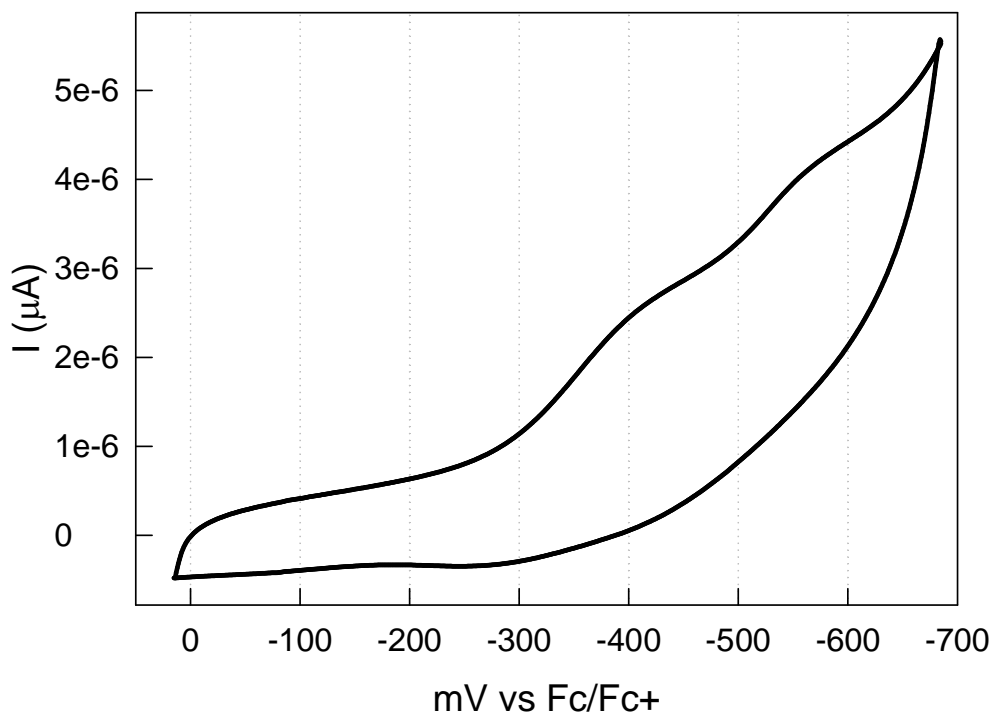

Figure S9. Cyclic voltammetry of 1×10^{-3} M in $[Cu_2^{II}(\mathbf{1})]^{4+}$ MeCN solution. Scan rate 50 mV/s. Potential is referred to ferrocene/ferrocenium couple (internal standard).
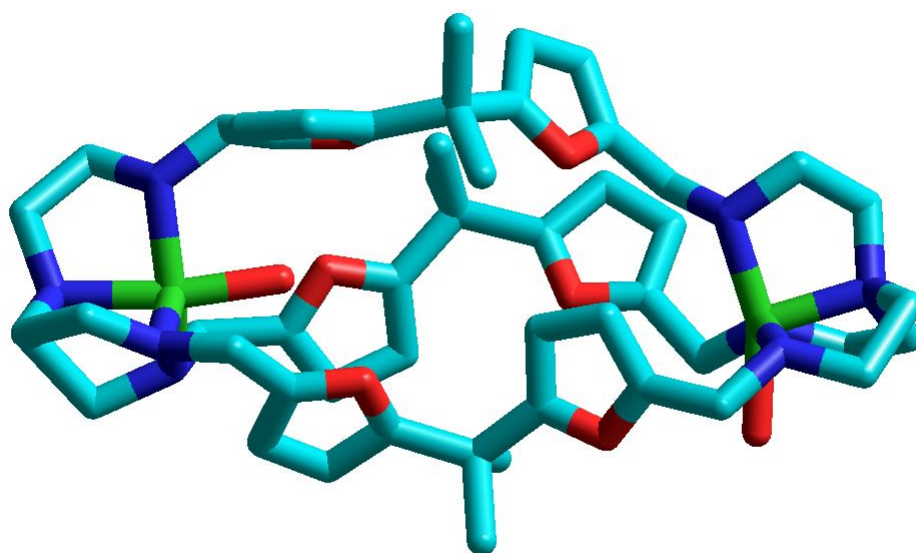

Figure S10. MM$^+$ calculated structure of the "empty" cage $[Cu_2^{II}(\mathbf{1})]^{4+}$. An OH$^-$ ion is coordinated to each Cu^{II} center. It has to be noted that in the calculated structure Cu–N and Cu–O distances results ca. 0.1 Å

lower than observed in crystallographically investigated $[Cu^{II}(tren)(H_2O)]^{2+}$ (Schatz, M.; Becker, M.; Walter, O.; Liehr, G.; Schindler, S. *Inorg. Chim. Acta* **2001**, *324*, 173–179 and $[Cu_2^{II}(bistren)(H_2O)_2]^{4+}$ (Boiocchi, M.; Bonizzoni, M.; Fabbrizzi, L.; Piovani, G.; Taglietti, A. *Angew. Chem. Int. Ed.* **2004**,*116*, 3935-3940) complexes. However, such differences do not affect the substance of the geometrical discrimination between different nucleotides, whose bite difference is of the order of 1 Å or more.
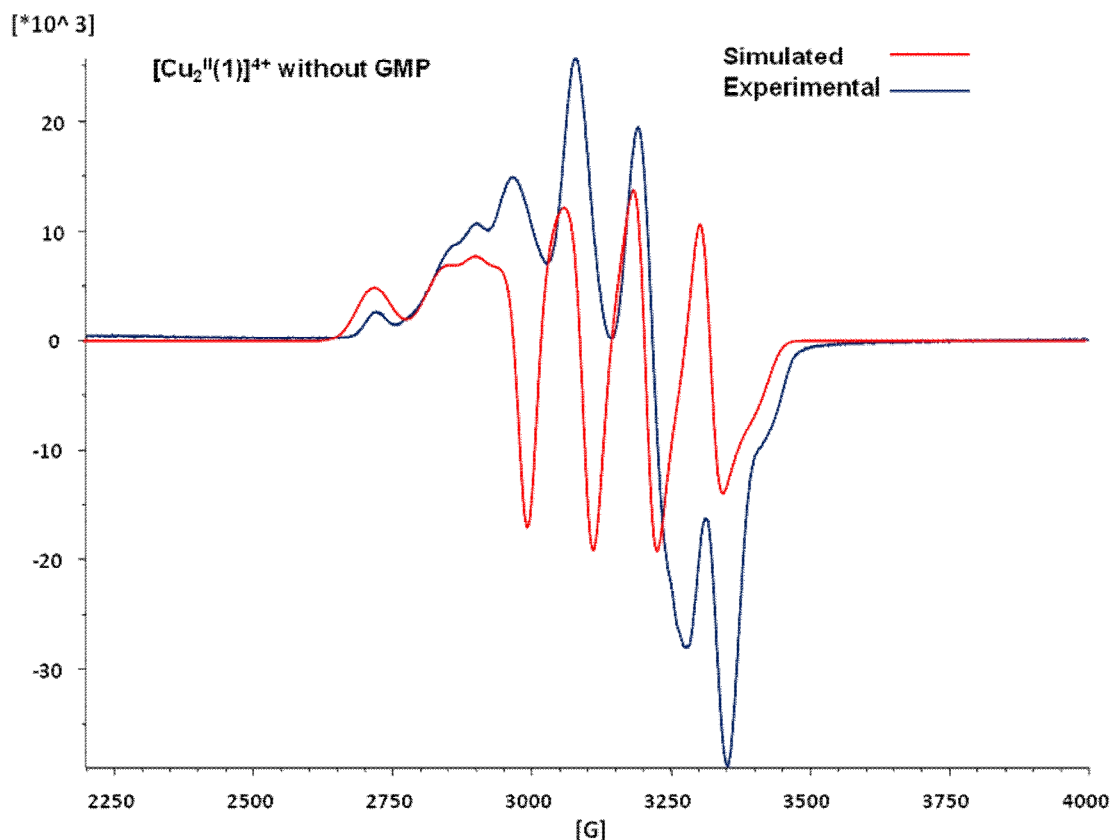

Figure S11. EPR spectrum of a 1×10^{-3} M in $[Cu_2^{II}(1)]^{4+}$ H_2O:MeOH mixture solution, buffered to pH 7 with HEPES 0.05M in absence of GMP, frozen at 77K. The blue one is the sperimental spectrum, the red one is simulated. The EPR parameters obtained from the simulation of the experimental spectrum are: 1. Principal values of the g-tensor g_{xx} = 2.105, g_{yy}= 2.110, g_{zz}= 2.295; 2. Principal values of the Copper hyperfine tensor A_{XX} = 414, A_{YY}= 327, A_{ZZ}= 395 (in MHz) and A_{XX} hyperfine tensor component of one Nitrogen nucleus A_{XX} = 88 MHz; 3. Line width tensor L_{XX}= 4, L_{YY}= 4, L_{ZZ}= 6 (in mT).
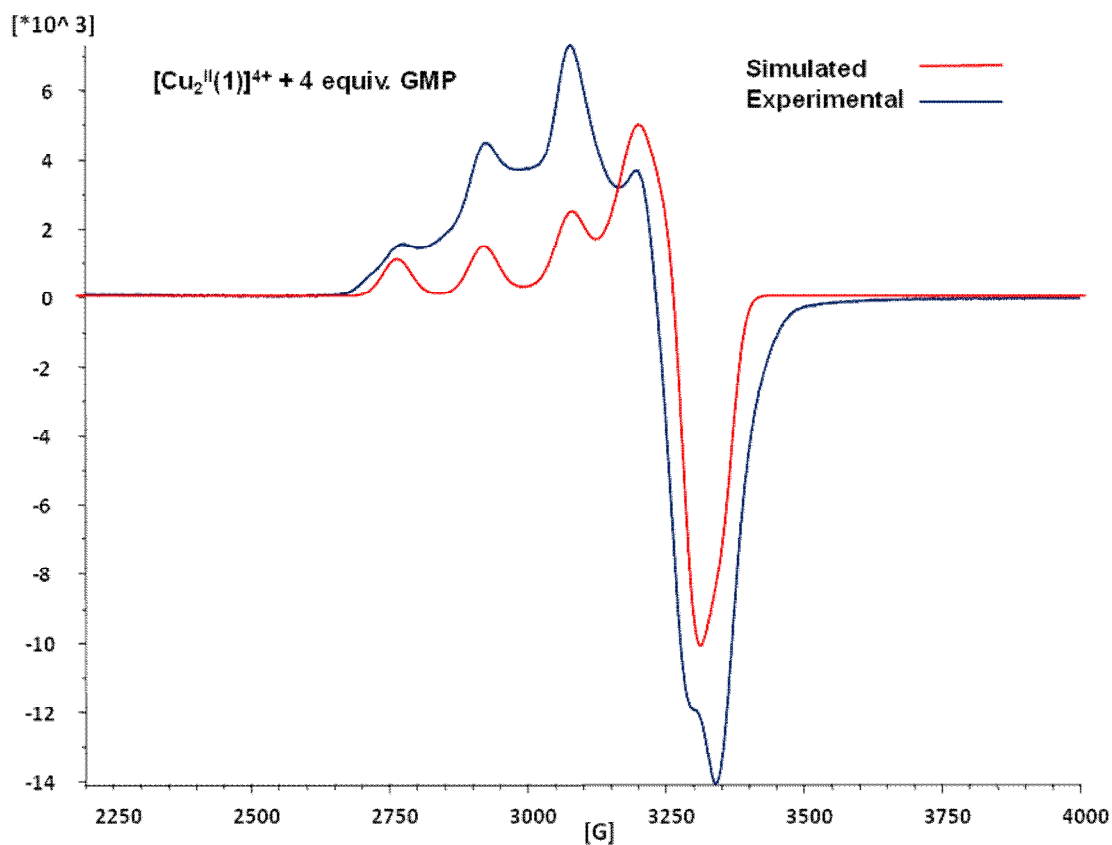
Figure S12. EPR spectrum of a 1×10^{-3} M in $[Cu_2^{II}(1)]^{4+}$ H2O:MeOH mixture solution, buffered to pH 7 with HEPES 0.05M with 4 equiv. added of GMP, frozen at 77K. The blue one is the sperimental spectrum, the red one is simulated. The EPR parameters obtained from the simulation of the experimental spectrum are: 1. Principal values of the g-tensor $g_{xx} = 2.017$, $g_{yy} = 2.075$, $g_{zz} = 2.220$; 2. A_{ZZ} component of the Copper hyperfine tensor $A_{ZZ} = 478$ MHz; 3. Line width tensor $L_{XX} = 4$, $L_{YY} = 5$, $L_{ZZ} = 5$ (in mT).