ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Biotransformation of BMOV in the presence of blood serum proteins

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Experimental and Computational Section

Chemicals

Water was deionized prior to use through the purification system Millipore MilliQ Academic. VO^{2+} solutions were prepared from $VOSO_4 \cdot 3H_2O$ following literature methods.[1]

Human immunoglobulin G (IgG), human serum apo-transferrin (hTf) and human serum albumin (HSA) were purchased from Sigma. IgG (99%, Sigma G4386), hTf (98%, Sigma T4283) and HSA (97-99%, Sigma A9511), have a molecular weight of 155-160, 76-81 and 66 kDa, respectively. The concentration of the protein solutions was estimated from their UV absorption (ε_{280}(IgG) = 217 000 M^{-1} cm^{-1},[2] ε_{280}(hTf) = 92 300 M^{-1} cm^{-1},[3] and ε_{279}(HSA) = 35 300 M^{-1} cm^{-1} [4]). The solubility in water of immunoglobulin G, transferrin and albumin is 50 mg/mL (*ca*. 3.0×10^{-4} M), 20 mg/mL (*ca*. 2.5×10^{-4} M) and 50 mg/mL (*ca*. 7.5×10^{-4} M), respectively.

Maltol (Hma), 1-methylimidazole (1-MeIm), N-acetyl-histamine (Ac-his), $NaHCO_3$, KCl, NaCl and 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES), were of the highest grade available and were used as received.

Preparation of the solutions

The solutions were prepared dissolving in ultra-pure water bis(maltolato)oxovanadium(IV) (BMOV) in order to obtain a VO^{2+} concentration between 2.5×10^{-4} and 1.0×10^{-3} M. Argon was bubbled through the solutions to ensure the absence of oxygen and avoid the oxidation of VO^{2+} ion. To this solution, an appropriate amount of HEPES and NaCl was added. Subsequently, pH was raised to *ca*. 5.5 and, in the system with transferrin, $NaHCO_3$ was dissolved (the presence of $NaHCO_3$ as synergistic anion is necessary for vanadium coordination to hTf [5]). To 1 mL of this solution, again carefully purged with argon, an appropriate amount of IgG, hTf or HSA was added; readily, pH was adjusted to *ca*. 7.4.

The final concentration of HEPES, NaCl and $NaHCO_3$ was 1.0×10^{-1}, 1.5×10^{-1} (corresponding to the physiological osmolarity) and 2.5×10^{-2} M (corresponding to the concentration in the blood), respectively. EPR studies performed on model systems prove that HEPES, $NaHCO_3$ and NaCl do not interact with VO^{2+} ion in the conditions used for the experiments.

EPR spectra of the model systems (VO^{2+}/maltol and VO^{2+}/maltol/1-MeIm) were recorded as previously reported.[6]

EPR spectroscopy

EPR spectra were recorded with an X-band (9.4 GHz) Bruker EMX spectrometer. Anisotropic spectra were recorded on frozen solutions at 120 K. The addition of DMSO was not necessary and no improvement in the resolution of the spectra was obtained.

When the samples were transferred into the EPR tubes, the spectra were immediately measured. To increase the signal to noise ratio, signal averaging was used.[7] The number of scans, for the high field region of the anisotropic EPR spectra (Fig. 1 of the manuscript and Figs. S5-S7 of ESI), was 15 in the system with transferrin, 5 in the system with albumin and 15 in that with immunoglobulin G.

As usual for the analysis of the EPR spectra,[8] in all the figures reported in the text only the high field region, the part more sensitive to the identity and the amount of the several species in solution, is presented. The values of A_z were calculated through simulations with the computer program Bruker WinEPR SimFonia.[9] The estimated error in A_z is $\pm 0.3 \times 10^{-4}$ cm^{-1}.

Potentiometric measurements

The stability constants of the proton and VO^{2+} complexes were determined by pH-potentiometric titrations on 25.00 mL of samples at 25.0 ± 0.1 °C and at a constant ionic strength of 0.20 M KCl. The VO^{2+} concentration was in the range 1-8 mM while for the VO^{2+}/Hma (A)/1-MeIm (B) or VO^{2+}/Hma (A)/Ac-his (B) ternary systems 1:1:1, 1:2:2, 1:2:4 and 1:1:4 ternary system molar ratios were used. All of the titrations were performed over the pH range 2.0-11.0, or until precipitation occurred with a carbonate-free KOH of known concentration (*ca*. 0.2 M) under a purified argon atmosphere.[10] A Mettler Toledo T50 titrator equipped with a Metrohm combined electrode (type 6.0234.100) was used for the pH-metric measurements. The electrode system was calibrated according to Irving *et al*.,[11] and therefore the pH-metric readings were converted into hydrogen ion concentration. The water ionization constant, pK_w, was 13.76 ± 0.01 under the conditions employed. The reproducibility of the points included in the evaluation was within 0.005 pH unit in the whole pH range measured. The number of experimental points used in the calculations was about 200. The stability of the complexes, reported as the logarithm of the overall formation constant $\beta_{pqrs} = [(VO)_pA_qB_rH_s]/[VO]^p[A]^q[B]^r[H]^s$, where VO stands for VO^{2+} ion, A and B are the deprotonated forms of Hma and 1-MeIm or Ac-his, respectively, has been calculated with the aid of the PSEQUAD program.[12] Reported stability constants for the species formed in the VO^{2+}/Hma and VO^{2+}/1-MeIm systems and determined at identical conditions are used to estimate the stability

constants of the ternary complexes.[13,14] The uncertainties (3σ values) of the stability constants are given in parentheses. During the calculations the following hydroxo complexes of VO^{2+} were assumed: $[VO(OH)]^{+}$ ($\log\beta_{100-1}$ = –5.94), $[(VO)_2(OH)_2]^{2+}$ ($\log\beta_{200-2}$ = –6.95), with stability constants calculated from the data of Henry et al.[15] and corrected for the different ionic strengths by use of the Davies equation,[16] $[VO(OH)_3]^{-}$ ($\log\beta_{100-3}$ = –18.0) and $[(VO)_2(OH)_5]^{-}$ ($\log\beta_{200-5}$ = -22.0).[17]

DFT calculations

The calculations were performed with Gaussian 03 (revision C.02)[18] and ORCA (version 2.8.20)[19] softwares, and density functional theory (DFT) methods.[20] The hybrid exchange-correlation B3LYP,[21,22] PBE0,[23] and the half-and-half functional BHandHLYP as incorporated in the Gaussian 03 software, were used.

As demonstrated in the literature, DFT simulations are a valid tool for predicting EPR parameters of VO^{2+} complexes.[24] Using the BHandHLYP functional and 6-311g(d,p) basis set with Gaussian 03 and PBE0 functional and VTZ basis set with ORCA, it is possible to calculate the ^{51}V hyperfine coupling constant along the z axis (A_z) with a mean deviation from the experimental value lower than 3 and 4%, respectively.[25]

The geometry of *cis*-$[VO(ma)_2(1\text{-MeIm})]$ ((O_{ket}, O^{-}_{phen}); (O_{ket}, $O^{-}_{phen}{}^{ax}$); N_{imid} coordination), *cis*-$[VO(ma)_2(Ac\text{-His-}NH_2)]$ ((O_{ket}, O^{-}_{phen}); (O_{ket}, $O^{-}_{phen}{}^{ax}$); N_{His}), *cis*-$[VO(ma)_2(Ac\text{-Tyr-}NH_2)]$ ((O_{ket}, O^{-}_{phen}); (O_{ket}, $O^{-}_{phen}{}^{ax}$); O^{-}_{Tyr}), *cis*-$[VO(ma)_2(Ac\text{-Ser-}NH_2)]$ ((O_{ket}, O^{-}_{phen}); (O_{ket}, $O^{-}_{phen}{}^{ax}$); O^{-}_{Ser}), *cis*-$[VO(ma)_2(Ac\text{-Asp-}NH_2)]$ ((O_{ket}, O^{-}_{phen}); (O_{ket}, $O^{-}_{phen}{}^{ax}$); COO^{-}_{Asp}), was optimized at the B3LYP/6-311g level of theory; for *cis*-$[VO(ma)_2(Ac\text{-Cys-}NH_2)]$ ((O_{ket}, O^{-}_{phen}); (O_{ket}, $O^{-}_{phen}{}^{ax}$); S^{-}_{Cys} coordination) diffuse and polarization functions (6-311+g(d)) were added on S atom to ensure, as recently noticed,[25] that the calculated structures are closest to the experimental ones. Minimum was verified through frequency calculations.

The optimized structure was used to calculate the values of ^{51}V A_z at the level of theory BHandHLYP/6-311g(d,p) with Gaussian 03 and at the level PBE0/VTZ with ORCA, according to the procedures published in the literature.[26] It must be remembered that for a VO^{2+} species A_z value is usually negative, but in the literature its absolute value is usually reported. This must kept in mind when positive values are reported.

The ^{51}V HFC tensor $\boldsymbol{A}$ has three contributions: the isotropic Fermi contact (A^{FC}), the anisotropic or dipolar hyperfine interaction ($\boldsymbol{A}^{\mathrm{D}}$), and one second-order term that arises from SO coupling ($\boldsymbol{A}^{\mathrm{SO}}$):[19]

$$\boldsymbol{A} = A^{\mathrm{FC}}\mathbf{1} + \boldsymbol{A}^{\mathrm{D}} + \boldsymbol{A}^{\mathrm{SO}} \tag{1}$$

where $\mathbf{1}$ is the unit tensor; A^{FC} and the components $A^{\mathrm{D}}_{\mu\nu}$ and $A^{\mathrm{SO}}_{\mu\nu}$ of the tensors $\boldsymbol{A}^{\mathrm{D}}$ and $\boldsymbol{A}^{\mathrm{SO}}$ are given by the following equations:

$$A^{\mathrm{FC}} = \frac{4\pi}{3} g_{\mathrm{e}} g_{\mathrm{N}} \beta_{\mathrm{e}} \beta_{\mathrm{N}} \left\langle S_{\mathrm{z}} \right\rangle^{-1} \rho_{\mathrm{N}}^{\alpha-\beta} \tag{2}$$

$$A^{\mathrm{D}}_{\mu\nu} = \frac{1}{2} g_{\mathrm{e}} g_{\mathrm{N}} \beta_{\mathrm{e}} \beta_{\mathrm{N}} \left\langle S_{\mathrm{z}} \right\rangle^{-1} \sum_{k,l} P^{\alpha-\beta}_{k,l} \left\langle \Phi_k \left| \frac{\mathbf{r}^2 \delta_{\mu\nu} - 3\mathbf{r}_\mu \mathbf{r}_\nu}{\mathbf{r}^5} \right| \Phi_l \right\rangle \tag{3}$$

$$A^{\mathrm{SO}}_{\mu\nu} = -\frac{1}{2S} g_{\mathrm{e}} g_{\mathrm{N}} \beta_{\mathrm{e}} \beta_{\mathrm{N}} \sum_{k,l} \frac{\partial P^{\alpha-\beta}_{k,l}}{\partial I_\mu} \left\langle \Phi_k \left| h^{\mathrm{SOC}}_\nu \right| \Phi_l \right\rangle \tag{4}$$

g_{e} and g_{N} are the g-factors of the free electron and the nucleus, β_{e} and β_{N} the electron and nuclear magnetons, $\left\langle S_{\mathrm{z}} \right\rangle$ the expectation value of the electronic spin on the z axis, $\rho_{\mathrm{N}}^{\alpha-\beta}$ the spin density at the nucleus, $P^{\alpha-\beta}_{k,l}$ the spin density matrix, $\mathbf{r}$ the distance between the unpaired electron and the nucleus, and h^{SOC}_ν the spatial part of an effective one-electron spin-orbit operator.

The tensor $\boldsymbol{A}^{\mathrm{D}}$ is always traceless: $A^{\mathrm{D}}_{\mathrm{x}}$, $A^{\mathrm{D}}_{\mathrm{y}}$ and $A^{\mathrm{D}}_{\mathrm{z}}$ are the elements of the diagonilized tensor, their sum being zero:

$$A^{\mathrm{D}}_{\mathrm{x}} + A^{\mathrm{D}}_{\mathrm{y}} + A^{\mathrm{D}}_{\mathrm{z}} = 0 \tag{5}$$

The values of the ^{51}V anisotropic hyperfine coupling constants along the x, y and z axes result to be:

$$A_{\mathrm{x}} = A^{\mathrm{FC}} + A^{\mathrm{D}}_{\mathrm{x}} + A^{\mathrm{SO}}_{\mathrm{x}} \tag{6}$$

$$A_{\mathrm{y}} = A^{\mathrm{FC}} + A^{\mathrm{D}}_{\mathrm{y}} + A^{\mathrm{SO}}_{\mathrm{y}} \tag{7}$$

$$A_{\mathrm{z}} = A^{\mathrm{FC}} + A^{\mathrm{D}}_{\mathrm{z}} + A^{\mathrm{SO}}_{\mathrm{z}} \tag{8}$$

From eqs. 5-8, the value of A_{iso} is:

$$A_{iso} = \frac{1}{3}\left(A_x + A_y + A_z\right) = A^{FC} + \frac{1}{3}\left(A_x^{SO} + A_y^{SO} + A_z^{SO}\right) = A^{FC} + A^{PC} \quad (9)$$

The term $\frac{1}{3}\left(A_x^{SO} + A_y^{SO} + A_z^{SO}\right)$ is named isotropic pseudocontact, A^{PC}.[19] With Gaussian 03, that neglects the terms $A_{\mu\nu}^{SO}$, A_{iso} coincides with A^{FC}:

$$A_{iso} = \frac{1}{3}\left(A_x + A_y + A_z\right) = A^{FC} \quad (10)$$

Table S1 EPR parameters of the mixed species formed by BMOV with 1-MeIm, hTf, HSA and IgG.[a]

Species	g_z	A_z	$\lvert A_z\rvert^{calcd\ b}$	Donor set
cis-[VO(ma)$_2$(1-MeIm)]	1.948	164.8	162.6	[(O_{ket}, O^-_{phen}); (O_{ket}, $O^-_{phen}{}^{ax}$); N_{imid}]
cis-VO(ma)$_2$(hTf)	1.944	164.7	163.4	[(O_{ket}, O^-_{phen}); (O_{ket}, $O^-_{phen}{}^{ax}$); N_{His}]
cis-VO(ma)$_2$(HSA)	1.945	165.5	163.4	[(O_{ket}, O^-_{phen}); (O_{ket}, $O^-_{phen}{}^{ax}$); N_{His}]
cis-VO(ma)$_2$(IgG)	1.946	164.6	163.4	[(O_{ket}, O^-_{phen}); (O_{ket}, $O^-_{phen}{}^{ax}$); N_{His}]

[a] Values of A_z measured in 10^{-4} cm^{-1}. [b] Values calculated at the level of theory BHandHLYP/6-311g(d,p), see Table S2.

Table S2 ^{51}V A_z values calculated by DFT methods and Gausssian 03 software for the species formed for reaction of BMOV with the side chains of the most important amino acids.[a]

Complex	A_{iso}	A_x^D	A_y^D	A_z^D	A_x	A_y	A_z	A_z^{exptl} (hTf) [c]	A_z^{exptl} (HSA) [d]	A_z^{exptl} (IgG) [e]	Dev. % $\|A_z\|$ [f]
cis-[VO(ma)$_2$(1-MeIm)]	-95.1	32.3	35.1	-67.4	-62.8	-60.0	-162.6		-164.8 [g]		-1.3
cis-[VO(ma)$_2$(Ac-His-NH$_2$)]	-96.0	32.4	35.0	-67.4	-63.6	-61.0	-163.4	-164.7	-165.5	-164.6	From -0.7 to -1.3
cis-[VO(ma)$_2$(Ac-Tyr-NH$_2$)]	-91.9	31.5	36.3	-67.8	-60.4	-55.6	-159.7	-164.7	-165.5	-164.6	From -3.0 to -3.5
cis-[VO(ma)$_2$(Ac-Ser-NH$_2$)]	-90.1	31.1	36.4	-67.6	-58.9	-53.6	-157.7	-164.7	-165.5	-164.6	From -4.2 to -4.7
cis-[VO(ma)$_2$(Ac-Asp-NH$_2$)]	-102.8	31.2	36.4	-67.7	-71.6	-66.4	-170.5	-164.7	-165.5	-164.6	From +3.0 to +3.6
cis-[VO(ma)$_2$(Ac-Cys-NH$_2$)]	-87.8	32.4	35.0	-67.4	-55.4	-52.8	-155.2	-164.7	-165.5	-164.6	From -5.7 to -6.2

[a] All the values measured in 10^{-4} cm^{-1} units. [b] Value expected on the basis of the "additivity rule". [c] Experimental value for the mixed species *cis*-VO(ma)$_2$(hTf) formed in the ternary system with transferrin. [d] Experimental value for the mixed species the mixed species *cis*-VO(ma)$_2$(HSA) formed in the ternary system with albumin. [e] Experimental value for the mixed species *cis*-VO(ma)$_2$(IgG) formed in the ternary system with immunoglobulin G. [f] Percent deviation from $|A_z|^{exptl}$ calculated as: 100 x $[(|A_z|^{calcd} - |A_z|^{exptl})/|A_z|^{exptl}]$. [g] Experimental value for the mixed species *cis*-[VO(ma)$_2$(1-MeIm)] formed in the ternary system with 1-methylimidazole.

TableS3 ^{51}V A_z values calculated by DFT methods and ORCA software for the species formed for reaction of BMOV with the side chains of the most important amino acids.[a]

Complex	A^{FC}	A_x^D	A_y^D	A_z^D	A_x^{SO}	A_y^{SO}	A_z^{SO}	A_x	A_y	A_z	A_z^{exptl} (hTf) [c]	A_z^{exptl} (HSA) [d]	A_z^{exptl} (IgG) [e]	Dev. % $\|A_z\|$ [f]
cis-[VO(ma)$_2$(1-MeIm)]	-87.6	33.3	30.0	-63.3	-3.5	-4.1	-10.3	-57.8	-61.6	-161.2		-164.8 [g]		-2.2
cis-[VO(ma)$_2$(Ac-His-NH$_2$)]	-88.9	33.1	30.2	-63.2	-3.5	-4.0	-10.3	-59.3	-62.8	-162.4	-164.7	-165.5	-164.6	From -1.3 to -1.9
cis-[VO(ma)$_2$(Ac-Tyr-NH$_2$)]	-83.7	34.4	29.2	-63.6	-3.9	-4.5	-10.8	-53.3	-59.1	-158.1	-164.7	-165.5	-164.6	From -3.9 to -4.5
cis-[VO(ma)$_2$(Ac-Ser-NH$_2$)]	-81.6	35.0	28.8	-63.8	-3.7	-4.3	-11.2	-50.3	-57.1	-156.6	-164.7	-165.5	-164.6	From -4.8 to -5.4
cis-[VO(ma)$_2$(Ac-Asp-NH$_2$)]	-95.1	33.5	30.4	-63.9	-3.7	-4.1	-10.9	-65.3	-68.8	-169.9	-164.7	-165.5	-164.6	From +2.7 to +3.2
cis-[VO(ma)$_2$(Ac-Cys-NH$_2$)]	-80.1	33.1	30.0	-63.1	-3.9	-4.3	-9.9	-50.8	-54.4	-153.2	-164.7	-165.5	-164.6	From -6.9 to -7.5

[a] All the values measured in 10^{-4} cm^{-1} units. [b] Value expected on the basis of the "additivity rule". [c] Experimental value for the mixed species *cis*-VO(ma)$_2$(hTf) formed in the ternary system with transferrin. [d] Experimental value for the mixed species the mixed species *cis*-VO(ma)$_2$(HSA) formed in the ternary system with albumin. [e] Experimental value for the mixed species *cis*-VO(ma)$_2$(IgG) formed in the ternary system with immunoglobulin G. [f] Percent deviation from $|A_z|^{exptl}$ calculated as: 100 x $[(|A_z|^{calcd} - |A_z|^{exptl})/|A_z|^{exptl}]$. [g] Experimental value for the mixed species *cis*-[VO(ma)$_2$(1-MeIm)] formed in the ternary system with 1-methylimidazole.

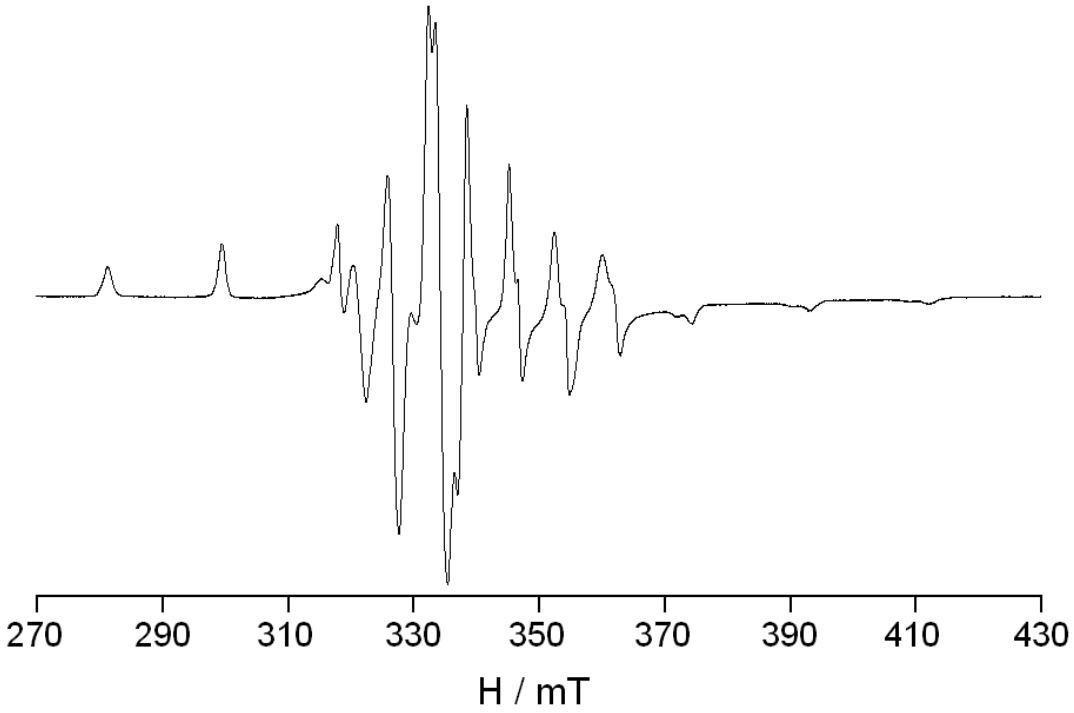

Figure S1 X-band anisotropic EPR spectrum recorded at pH 7.4 on aqueous solution containing VO^{2+}/hTf/Hma 2/1/4 (VO^{2+} $5.0x10^{-4}$ M). HEPES, NaCl and $NaHCO_3$ were $1.0x10^{-1}$, $1.5x10^{-1}$ and $2.5x10^{-2}$ M, respectively.

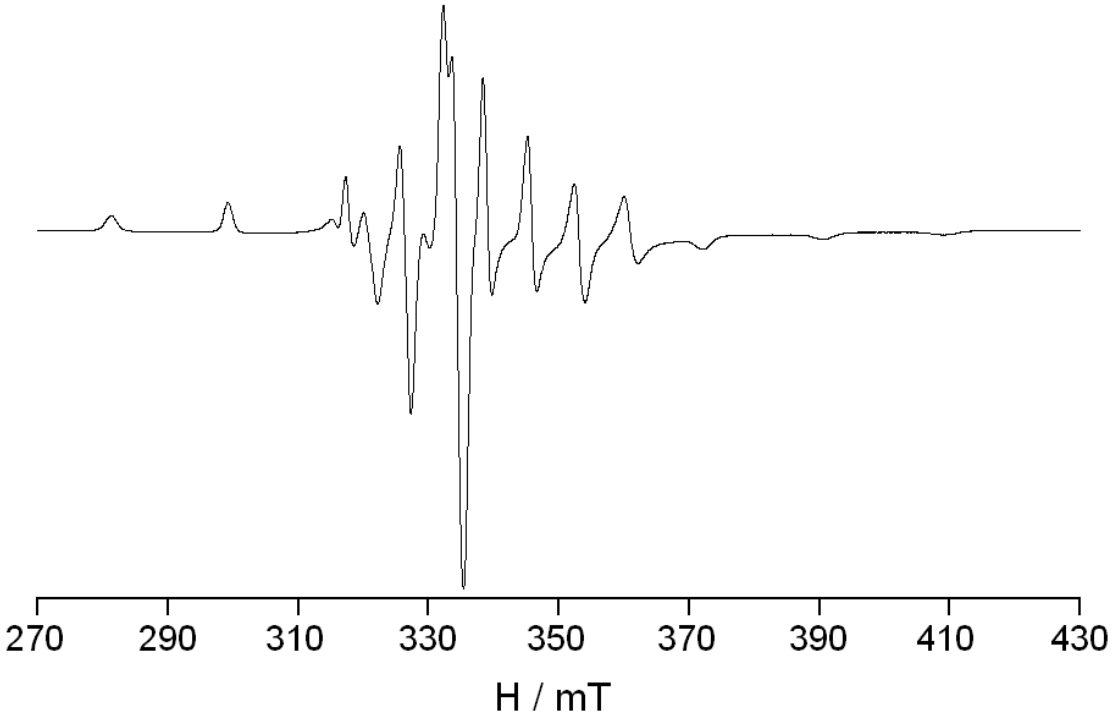

Figure S2 X-band anisotropic EPR spectrum recorded at pH 7.4 on aqueous solution containing VO^{2+}/HSA/Hma 4/1/8 (VO^{2+} $1.0x10^{-3}$ M). HEPES, NaCl and $NaHCO_3$ were $1.0x10^{-1}$, $1.5x10^{-1}$ and $2.5x10^{-2}$ M, respectively.

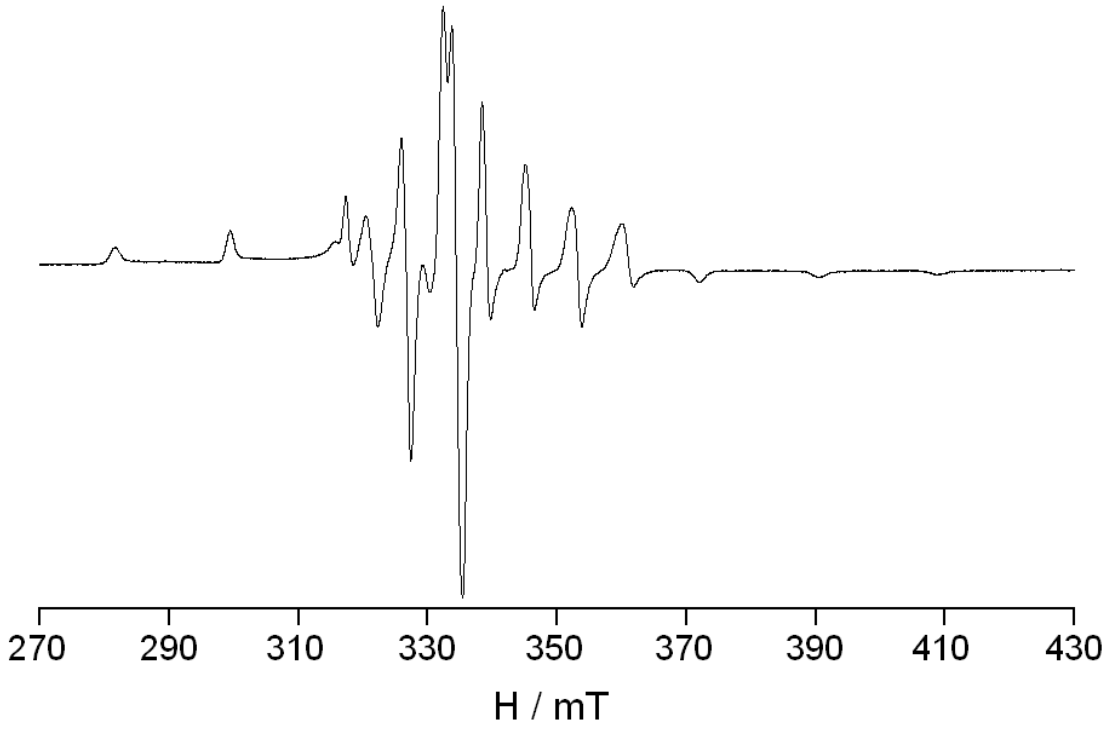

Figure S3 X-band anisotropic EPR spectrum recorded at pH 7.4 on aqueous solution containing VO^{2+}/IgG/Hma 1/1/8 (VO^{2+} $3.0x10^{-4}$ M). HEPES, NaCl and $NaHCO_3$ were $1.0x10^{-1}$, $1.5x10^{-1}$ and $2.5x10^{-2}$ M, respectively.

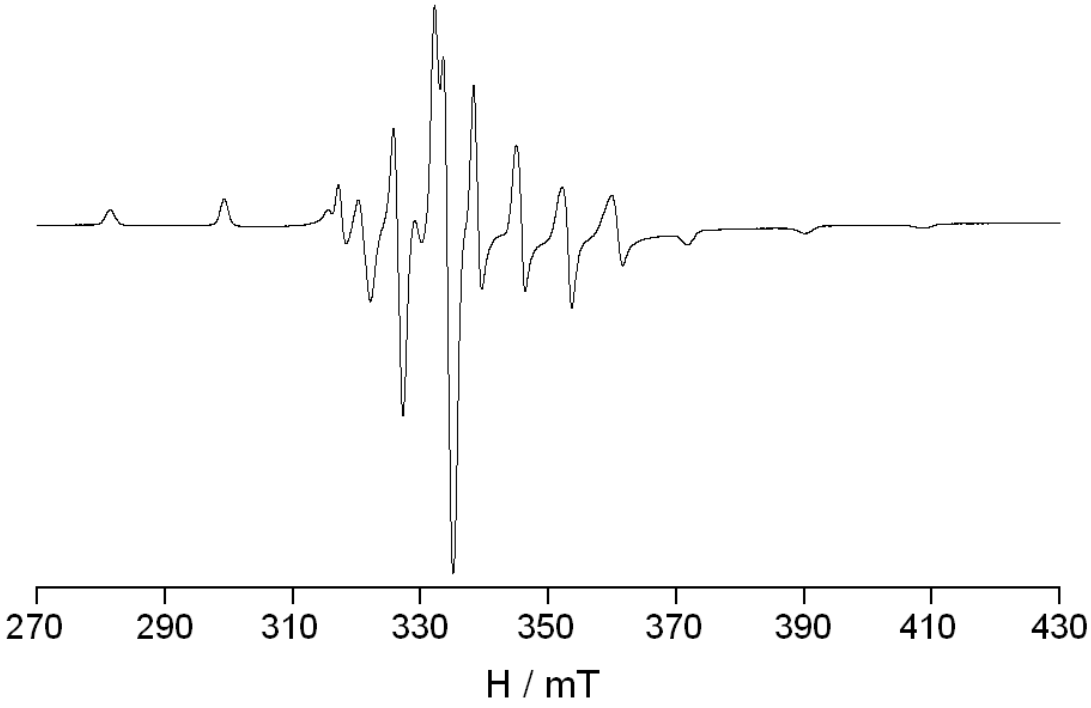

Figure S4 X-band anisotropic EPR spectrum recorded at pH 7.4 on aqueous solution containing VO^{2+}/Hma/1-MeIm 1/2/4 (VO^{2+} $1.0x10^{-3}$ M). HEPES, NaCl and $NaHCO_3$ were $1.0x10^{-1}$, $1.5x10^{-1}$ and $2.5x10^{-2}$ M, respectively.

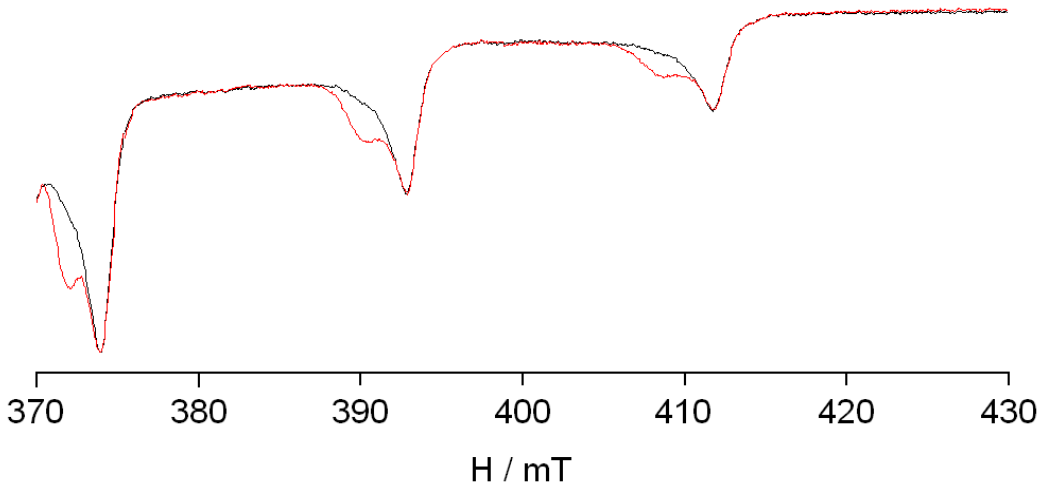

Figure S5 Superimposition of the high field X-band anisotropic EPR spectrum recorded at pH 7.4 on aqueous solution containing VO^{2+}/hTf 2/1 (VO^{2+} 5.0x10^{-4} M, in black) and VO^{2+}/hTf/Hma 2/1/4 (VO^{2+} 5.0x10^{-4} M, in red).

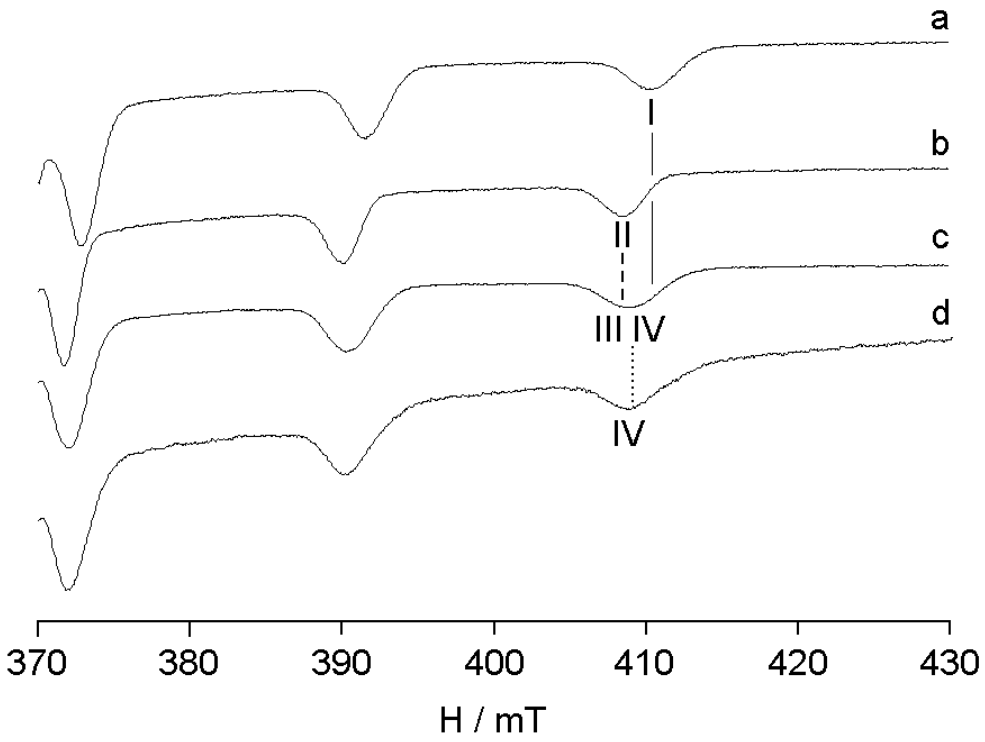

Figure S6 High-field region of anisotropic X-band EPR spectra recorded at pH 7.4 on aqueous solution containing: a) VO^{2+}/Hma 1/2 (VO^{2+} 1.0x10^{-3} M); b) VO^{2+}/Hma/1-MeIm 1/2/4 (VO^{2+} 1.0x10^{-3} M); c) VO^{2+}/HSA/Hma 4/1/8 (VO^{2+} 1.0x10^{-3} M) and d) VO^{2+}/HSA 4/1 (VO^{2+} 1.0x10^{-3} M). The M_I = 7/2 resonances of *cis*-[VO(ma)$_2$(1-MeIm)] and *cis*-VO(ma)$_2$(HSA) (broken line), of (VO)$_x^m$HSA (dotted line), and of *cis*-[VO(ma)$_2$(H_2O)] (full line) are indicated; **I**, **II**, **III** and **IV** denote the species *cis*-[VO(ma)$_2$(H_2O)], *cis*-[VO(ma)$_2$(1-MeIm)], *cis*-VO(ma)$_2$(HSA) and (VO)$_x^m$HSA, respectively.

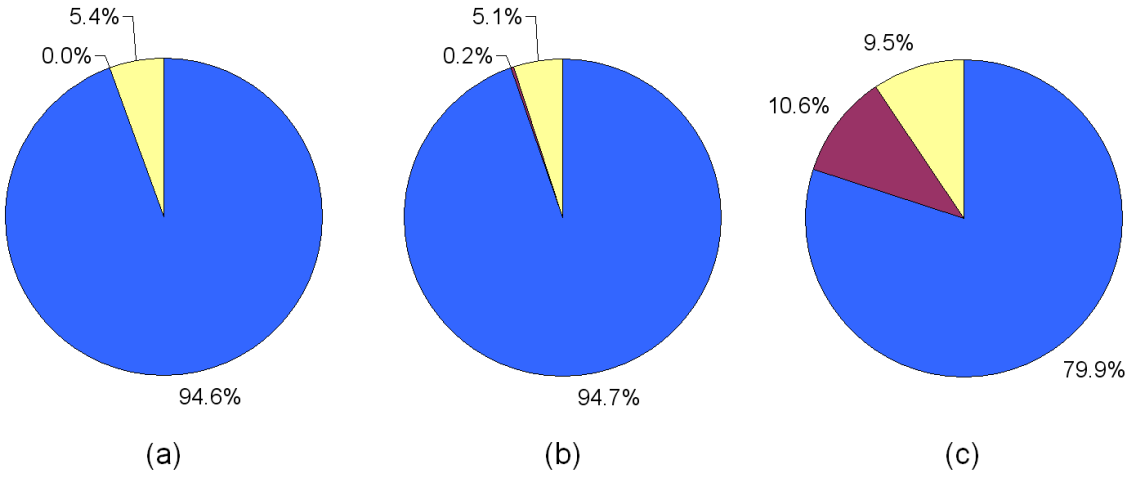

Figure S7 Percent distribution of VO^{2+} ion between the components of the blood serum at pH 7.4 when the metal ion concentration is: (a) 1 µM; (b) 10 µM and (c) 50 µM. The sum of the binary species of hTf ((VO)hTf and $(VO)_2$hTf) is represented in light blue, of the mixed species *cis*-$VO(ma)_2$(Protein) (where Protein indicates hTf, HSA or IgG) in purple and of all the other species in yellow.

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