ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

An Integrated ESI-MS/EPR/Computational Characterization of the Binding of Metal Species to Proteins: Vanadium Drugs–Myoglobin Application †

Giuseppe Sciortino,[a,b] Daniele Sanna,*[c] Valeria Ugone,[a] Jean-Didier Maréchal,*[b] and Eugenio Garribba,*[a]

[a] *Dipartimento di Chimica e Farmacia, Università di Sassari, Via Vienna 2, I-07100 Sassari, Italy. E-mail: garribba@uniss.it; Tel: +39 079 229487.*

[b] *Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallés, Barcelona, Spain. Jeandidier.Marechal@uab.cat; Tel: +34 935814936.*

[c] *Istituto CNR di Chimica Biomolecolare, Trav. La Crucca 3, I-07040 Sassari, Italy. E-mail: daniele.sanna@icb.cnr.it; Tel: +39 079 2841207.*

* Corresponding authors.

Table S1 Species identified in the ESI-MS spectra of the system $V^{IV}O^{2+}$/L-mimosinate.

Ion	Composition	Exptl m/z [a]	Calcd m/z [a]	Error (ppm) [b]
$[VO(mim)_2+H]^-$	$C_{16}H_{17}N_4O_9V$	460.04490	460.04407	1.8
$[VO(mim)_2]^{2-}$	$C_{16}H_{16}N_4O_9V$	229.51825	229.51839	-0.6
$[VO(mim)_2(OH)+2H]^-$	$C_{16}H_{17}N_4O_{10}V$	476.03973	476.03898	1.6
$[VO(mim)_2+2H]^-$	$C_{16}H_{18}N_4O_{10}V$	477.04744	477.04680	1.2

[a] The experimental and calculated m/z values refer to the monoisotopic representative peak. [b] Deviation in ppm from the calculated values, calculated as $10^6 \times$ [(Exptl m/z – Calcd m/z)/Calcd m/z].

Table S2 ^{51}V hyperfine coupling constants calculated at the level of theory BHandHLYP/6-311+g(d) for the possible bis-chelated $V^{IV}O$ complexes formed by L-mimosinate.[a]

Complex	A_x^{calcd}	A_y^{calcd}	A_z^{calcd}	A_z^{exptl}	PD [b]
SPY-5-12	-48.75	-57.32	-158.2	-169.1	-6.4
SPY-5-13	-48.53	-57.59	-158.0	-169.1	-6.5
OC-6-32	-59.17	-63.54	-163.8	-169.1	-3.1
OC-6-23	-57.37	-62.77	-162.9	-169.1	-3.7
***OC*-6-34** [c]	-60.51	-64.32	-164.6	-169.1	-2.6
***OC*-6-24** [c]	-59.40	-63.48	-164.4	-169.1	-2.8

[a] All the A values are reported in 10^{-4} cm^{-1}. [b] Percent deviation (PD) with respect to the absolute experimental value calculated as: $100 \times [(|A_z|^{calcd} - |A_z|^{exptl})/|A_z|^{exptl}]$. [c] With boldface text the most probable isomers are shown.

Table S3 Docking solutions for the interaction of $VO(acac)^+$ with myoglobin.

Site	Residues	V–D [a]	F_{max} [b]	F_{mean} [c]	Pop.[d]
1st	His24; His119	2.108, 2.440	44.8	42.6	92%
2nd	His82; Asp141	2.180, 2.452	34.5	30.7	64%
3rd	Glu83; Asp141	2.181, 2.406	38.7	36.8	94%
4th	His116; Gln124	2.683, 2.174	32.8	31.9	52%

[a] Distance in Å; D = N, O. [b] *Fitness* value for the most stable pose of each cluster (F_{max}). [c] Mean *Fitness* value of the GoldScore scoring function for each cluster (F_{mean}). [d] Percent population of the cluster.

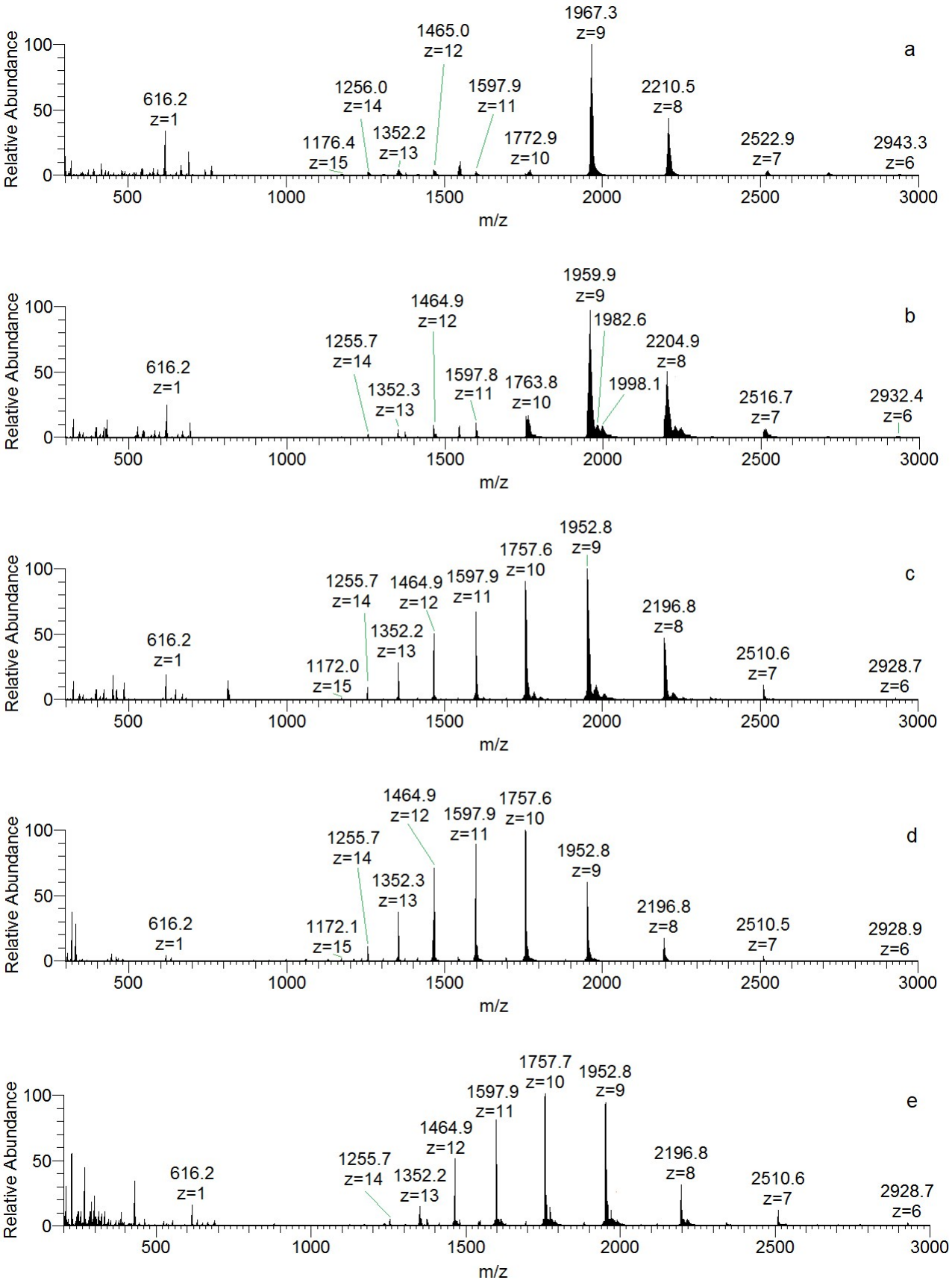


Fig. S1 ESI-MS spectra recorded in the systems: (a) Mb; (b) $[VO(dhp)_2]$/Mb; (c) *cis*-$[VO(mim)_2(H_2O)]^{2-}$/Mb; (d) *cis*-$[VO(ma)_2(H_2O)]$/Mb; (e) $[VO(acac)_2]$/Mb. Mb concentration was 5 μM and molar ratio V/Mb was 3/1.

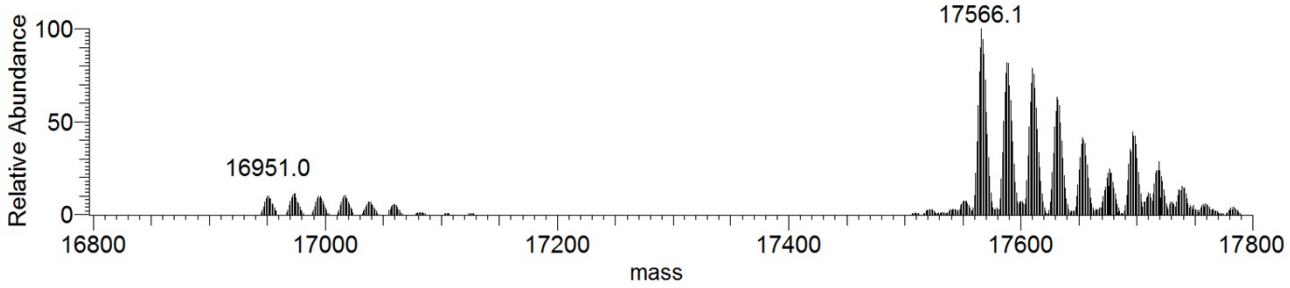


Fig. S2 Deconvoluted ESI-MS spectrum of myoglobin (concentration 5 μM). Mass is expressed in Da.

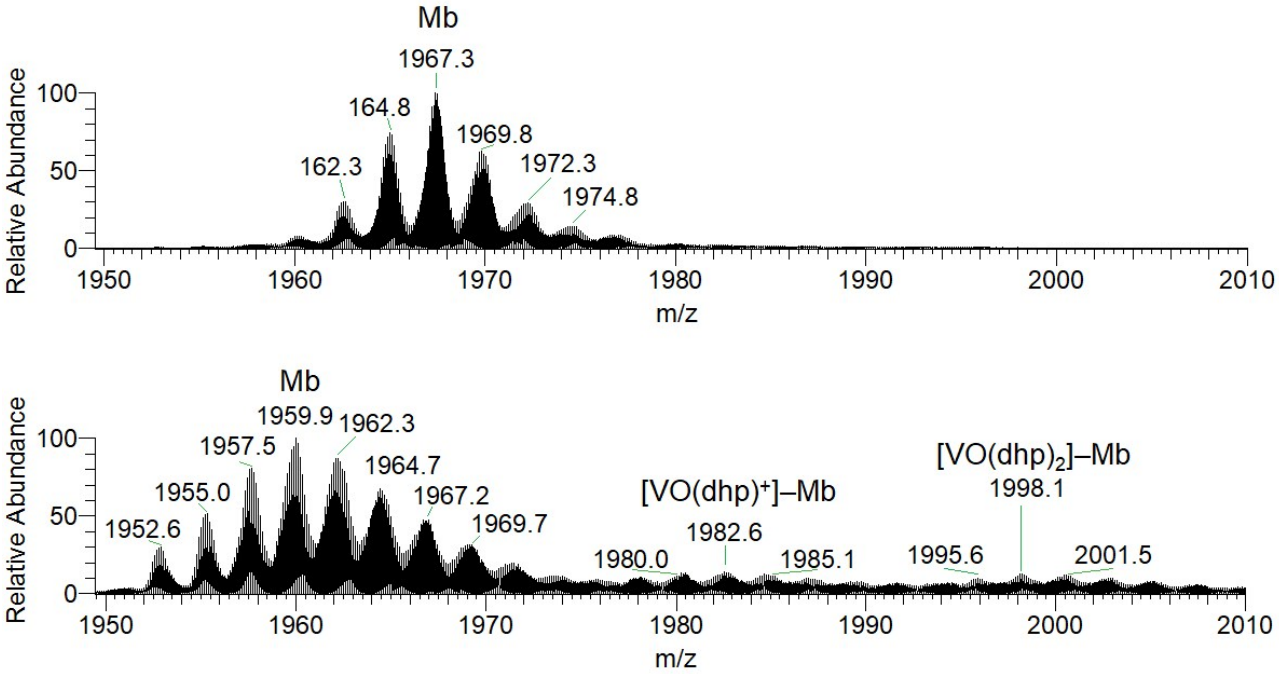


Fig. S3 Zoom of the multipeak with charge +9 of (a) Mb and (b) system $[VO(dhp)_2]$/Mb. Mb concentration was 5 μM and molar ratio V/Mb was 3/1. The most intense peak due to the adducts $[VO(dhp)^+]$–Mb and $[VO(dhp)_2]$–Mb are indicted.

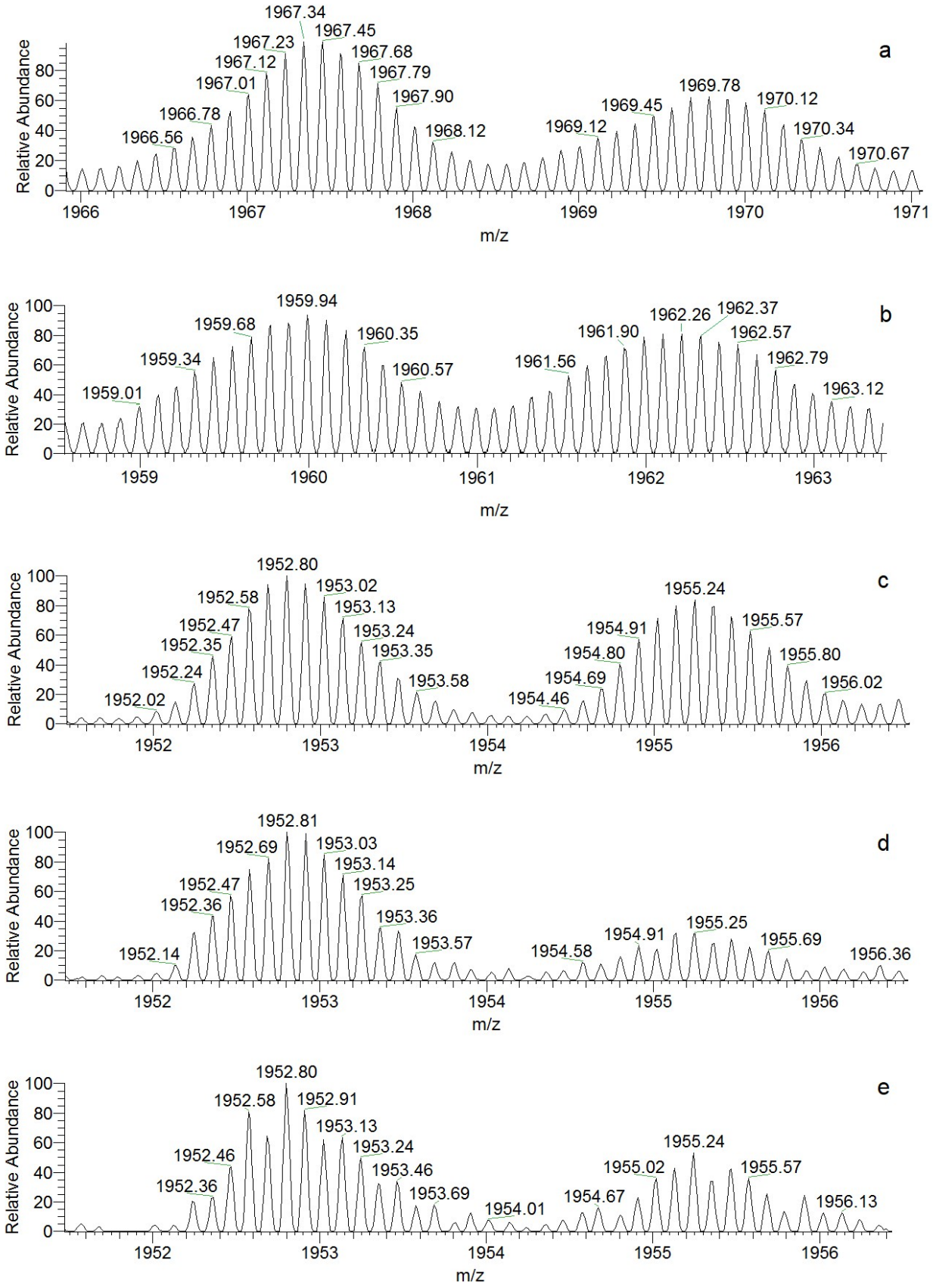


Fig. S4 Ultrazoom of the two most intense peaks with charge +9 detected in the systems: (a) Mb; (b) $[VO(dhp)_2]$/Mb; (c) *cis*-$[VO(mim)_2(H_2O)]^{2-}$/Mb; (d) *cis*-$[VO(ma)_2(H_2O)]$/Mb; (e) $[VO(acac)_2]$/Mb. Mb concentration was 5 μM and molar ratio V/Mb was 3/1.

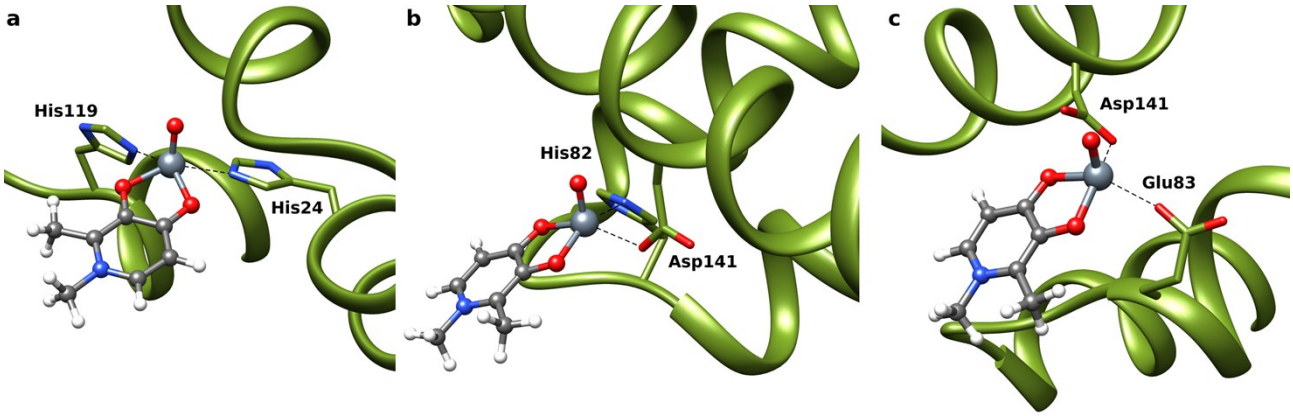


Fig. S5 Most stable adducts predicted by docking methods for the interaction of the $VO(dhp)^{+}$ moiety with myoglobin: a) *SPY*-5-13-A-$VO(dhp)(H_2O)_2$ with His24 and His119; b) *SPY*-5-13-C-$VO(dhp)(H_2O)_2$ with His82 and Asp141 and c) *SPY*-5-13-C-$VO(dhp)(H_2O)_2$ with Glu83 and Asp141.

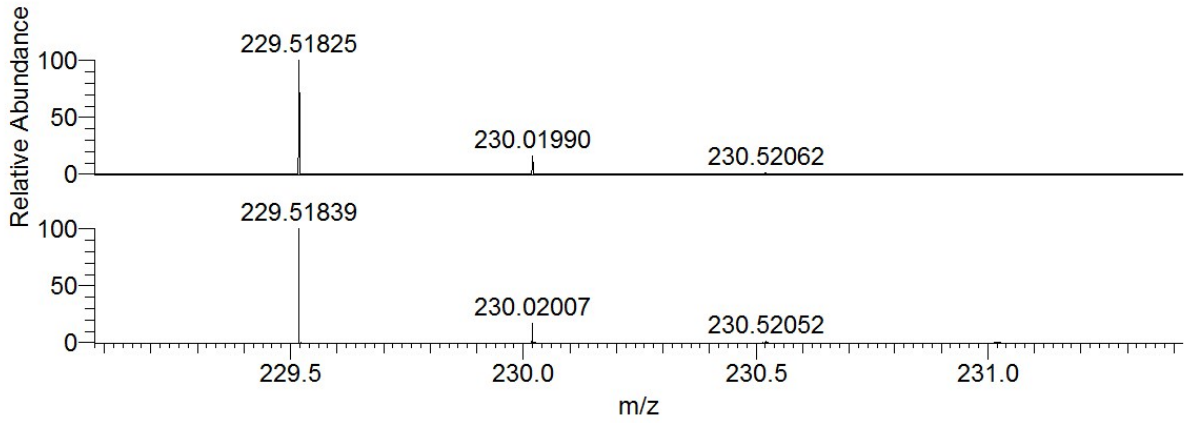


Fig. S6 Experimental (above) and calculated (below) isotopic pattern for the peak of $[VO(mim)_2]^{2-}$ revealed at m/z = 229.5 in the negative ESI-MS spectrum recorded on the system $V^{IV}O^{2+}$/mim 1/2 (V concentration 50 μM).

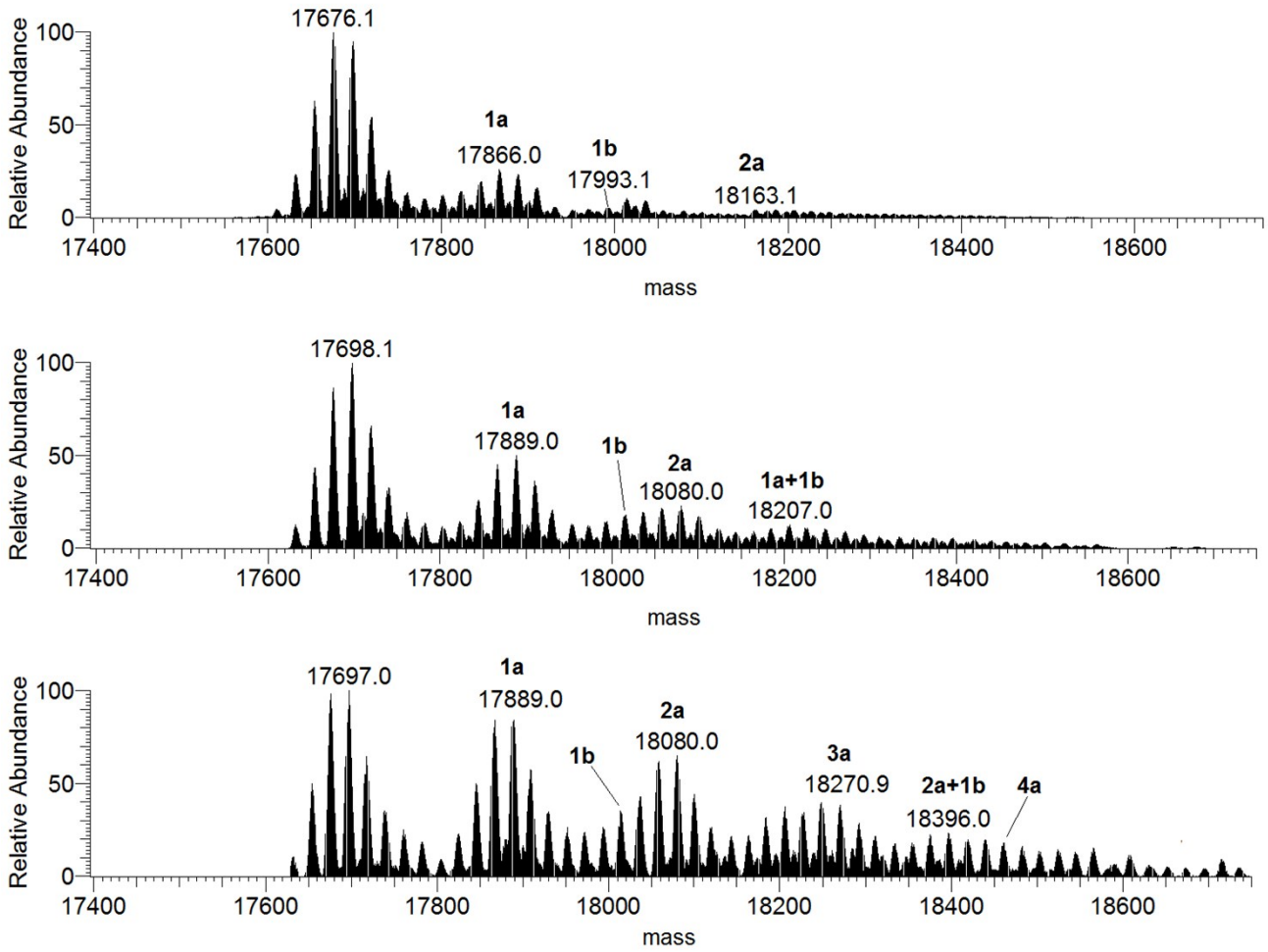


Fig. S7 Deconvoluted ESI-MS spectra recorded on the system containing $VO(ma)_2$ and myoglobin (50 μM): molar ratio 3/1 (top), 5/1 (centre) and 10/1 (bottom). With **a** and **b** the fragments $VO(ma)^+$ and $VO(ma)_2$ are indicated. Mass is expressed in Da.

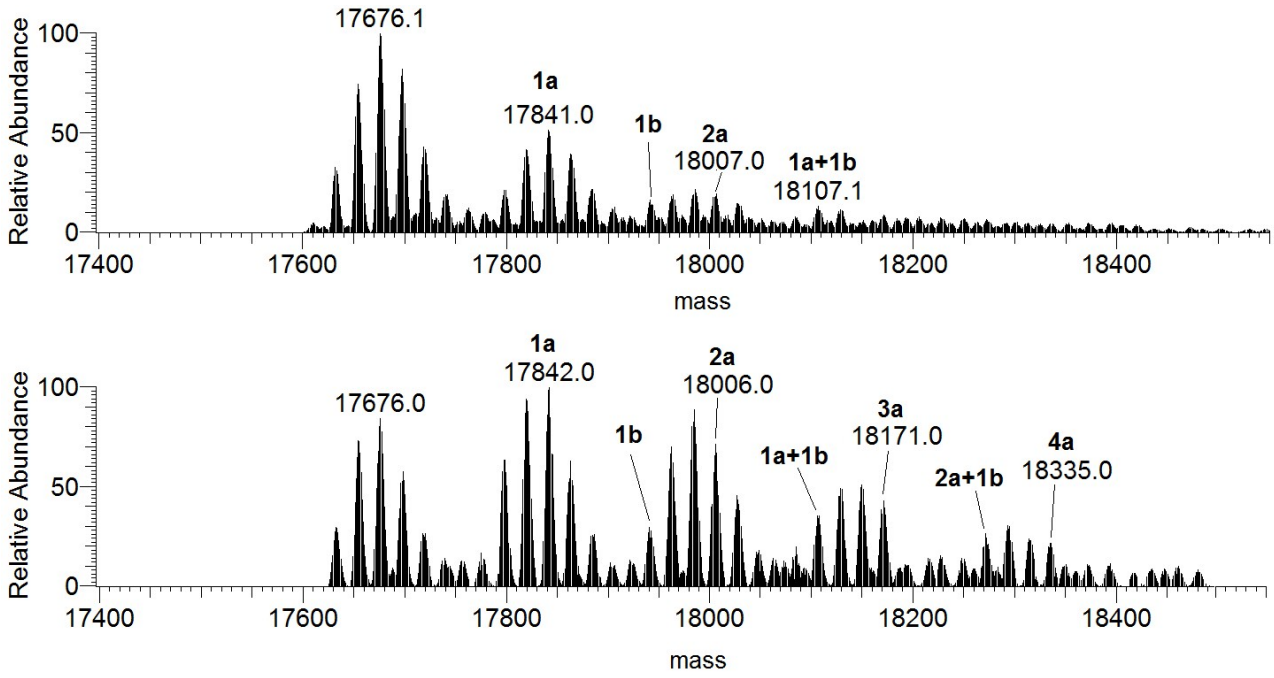


Fig. S8 Deconvoluted ESI-MS spectra recorded on the system containing $[VO(acac)_2]$ and myoglobin (50 μM): molar ratio 3/1 (top) and 5/1 (bottom). With **a** and **b** the fragments $VO(acac)^+$ and $VO(acac)_2$ are indicated. Mass is expressed in Da.

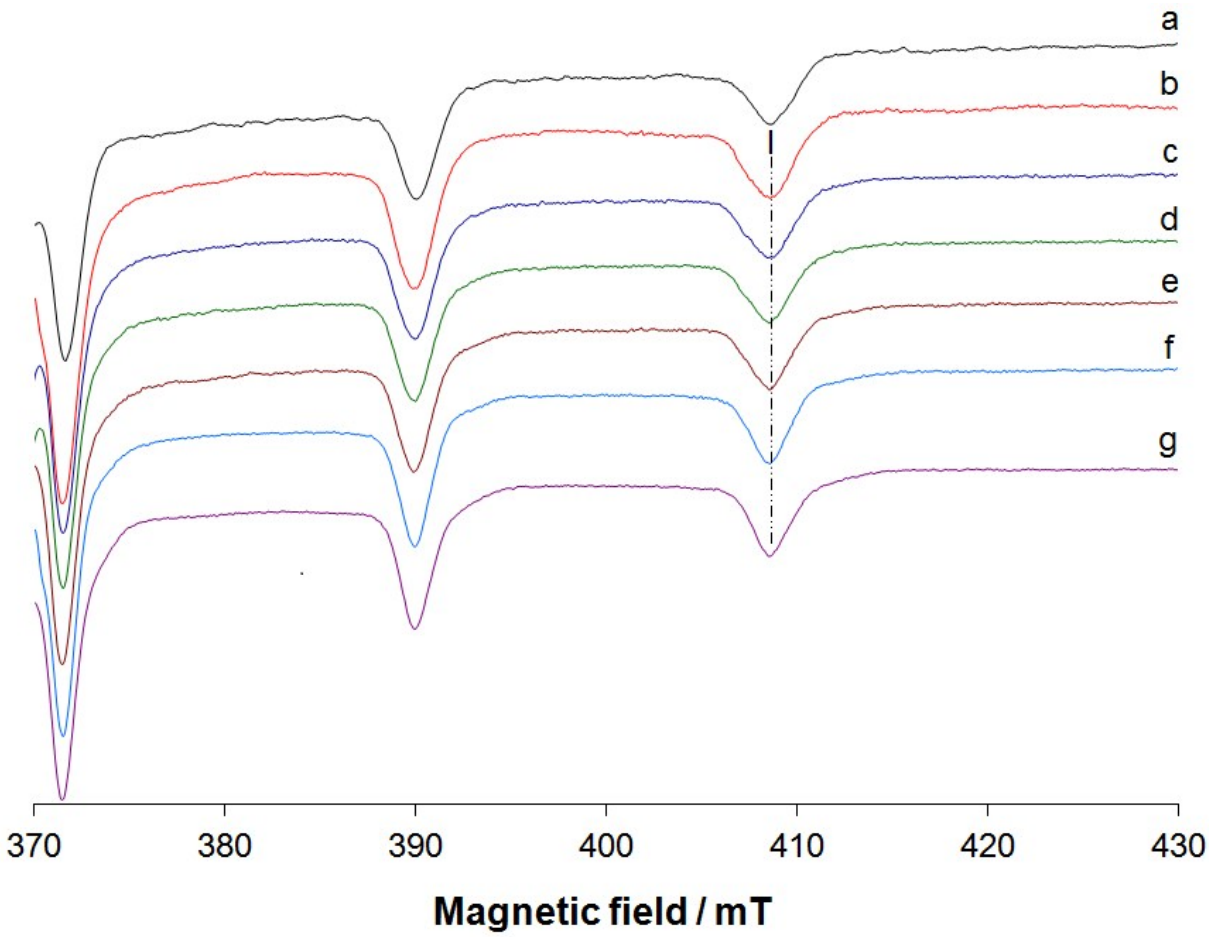


Fig. S9 High field region of the X-band anisotropic EPR spectra recorded on frozen solutions (120 K) containing: a) $V^{IV}O^{2+}$/acac/MeIm 1/2/4; b) $V^{IV}O^{2+}$/acac/Mb 1/2/1; c) $V^{IV}O^{2+}$/acac/Mb 2/4/1; d) $V^{IV}O^{2+}$/acac/Mb 4/8/1; e) $V^{IV}O^{2+}$/acac/Mb 6/12/1; f) $V^{IV}O^{2+}$/acac/Mb 8/16/1; g) $V^{IV}O^{2+}$/acac/Mb 10/20/1 and h) $V^{IV}O^{2+}$/acac 1/2. $V^{IV}O^{2+}$ concentration was 1.0×10^{-3} M. **I** and the dash-dotted lines indicate the $M_I = 7/2$ resonance of the species $[VO(acac)_2]$.

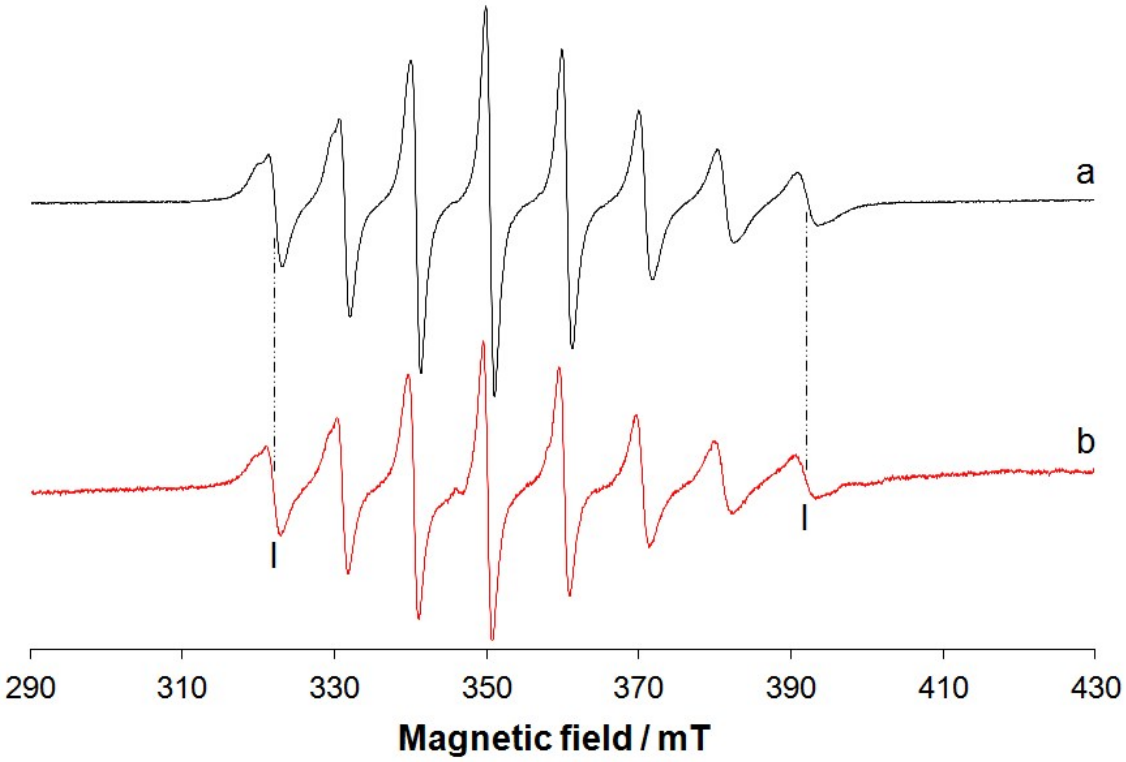


Fig. S10 High field region of the X-band isotropic EPR spectra recorded on aqueous solutions (298 K) containing: a) $V^{IV}O^{2+}$/acac 1/2 and b) $V^{IV}O^{2+}$/acac/Mb 1/2/1. $V^{IV}O^{2+}$ concentration was 1.0×10^{-3} M. **I** and the dash-dotted line indicate the M_I = -7/2 and 7/2 resonances of the species $[VO(acac)_2]$.

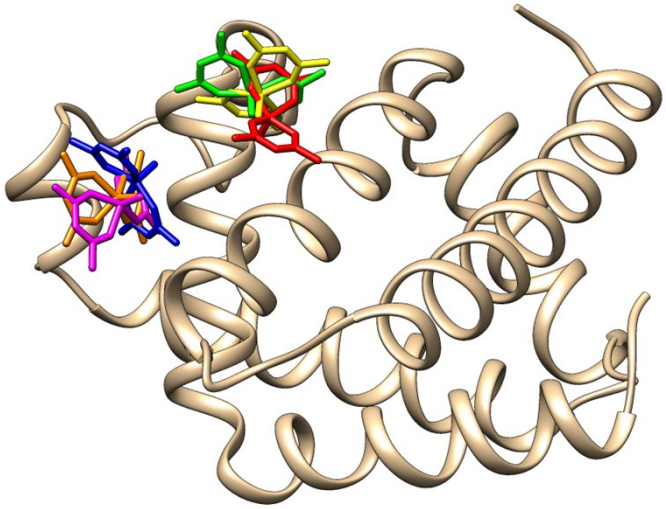
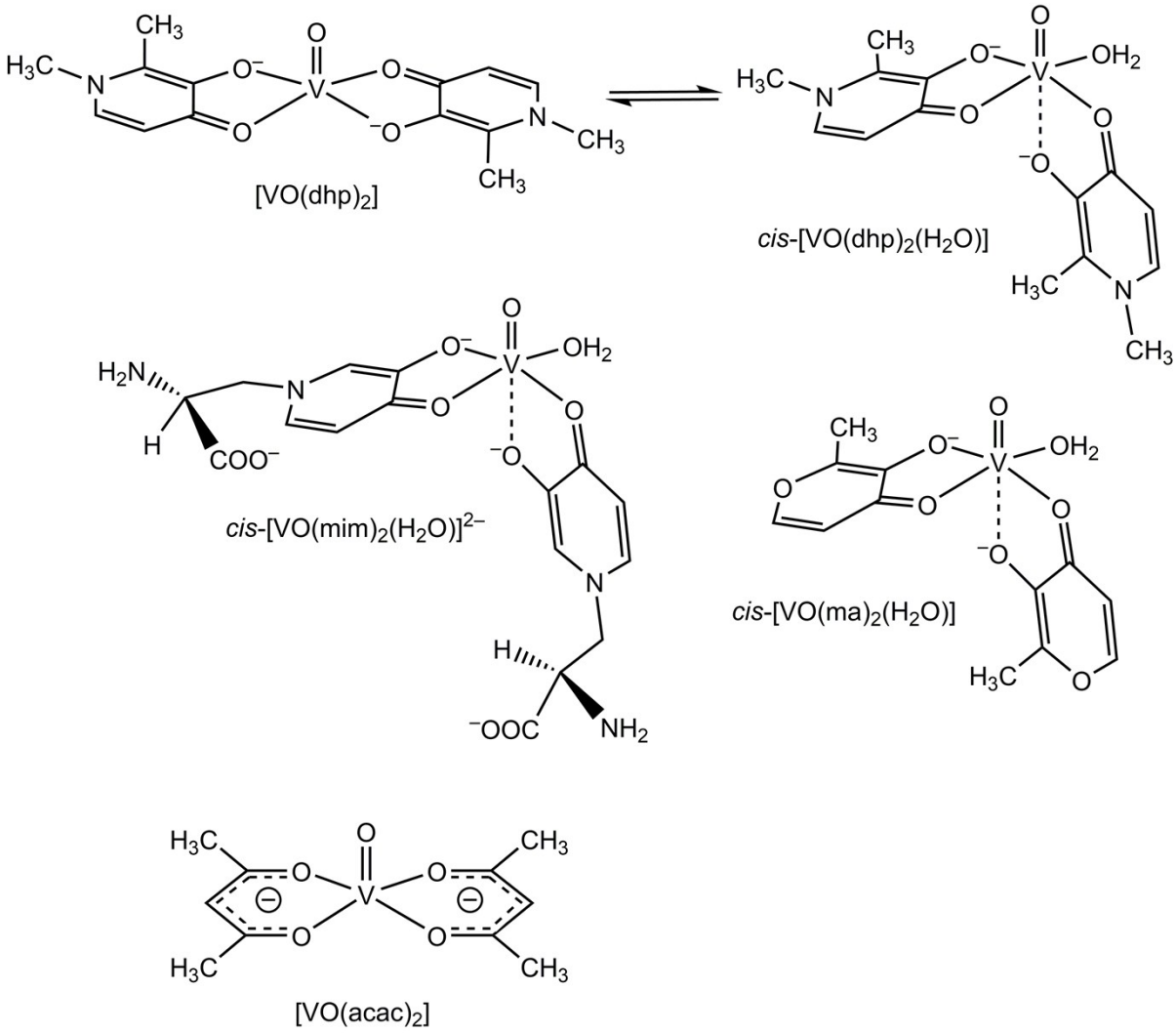
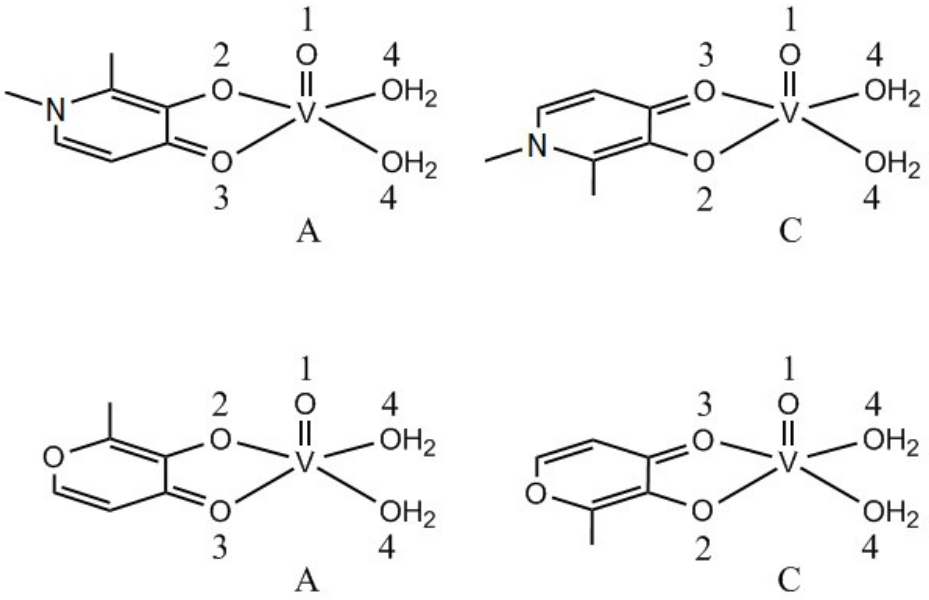


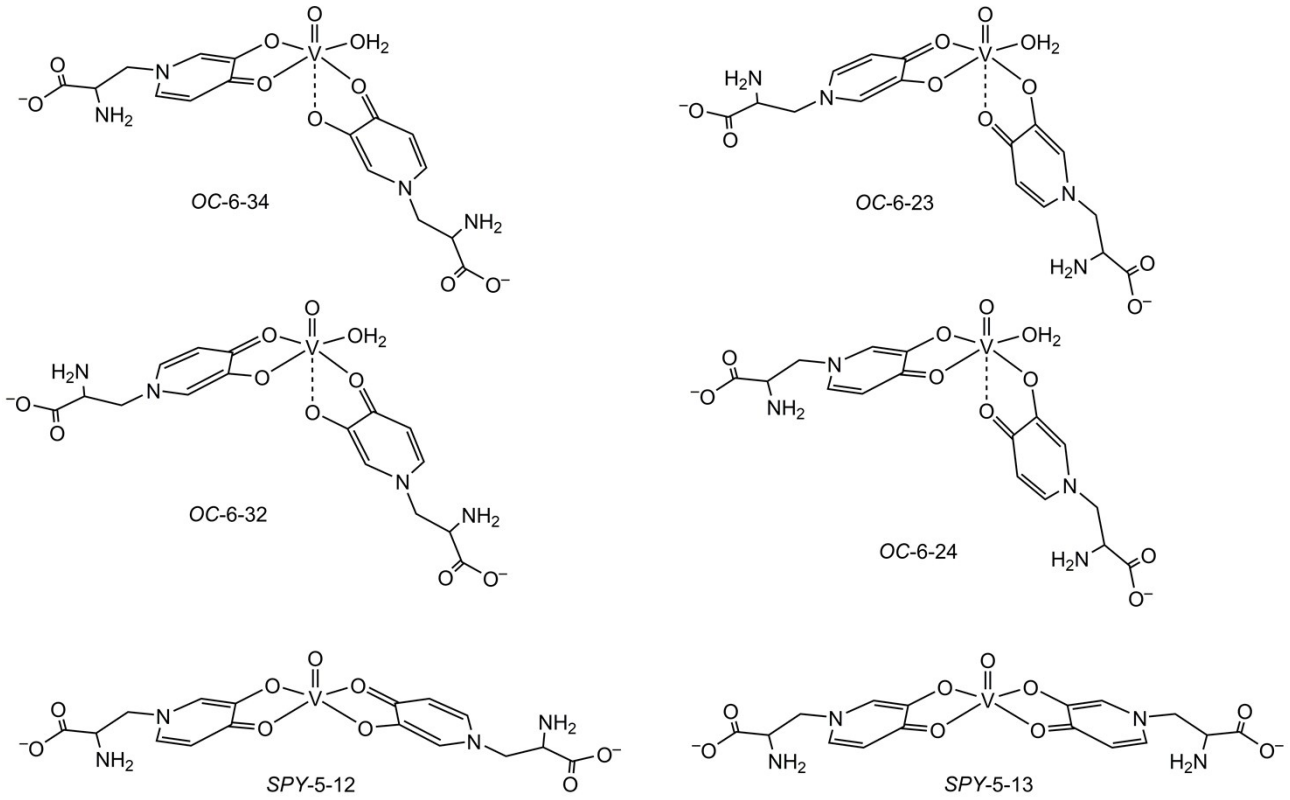
Fig. S11 Cluster distribution for the interaction of $[VO(acac)_2]$ with myoglobin. The six clusters are represented with different colors.



Scheme S1 Structures in aqueous solution and physiological pH of the bis-chelated $V^{IV}O$ complexes formed by 1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinonate, L-mimosinate, maltolate and acetylacetonate.



Scheme S2 Enantiomers of $[VO(dhp)(H_2O)_2]^+$ (above) and $[VO(ma)(H_2O)_2]^+$ (below).



Scheme S3 Possible isomers of the bis-chelated $V^{IV}O$ complex formed by L-mimosinate.