Electronic Supporting Information (ESI)

Interaction of $V^{IV}O$–8-hydroxyquinoline species with RNase A: The effect of metal ligands in the protein adduct stabilization

Giarita Ferraro,[a] Luigi Vitale,[a] Giuseppe Sciortino,[b] Federico Pisanu,[c] Eugenio Garribba,*[c] Antonello Merlino*[a]

[a] *Department of Chemical Sciences, University of Naples Federico II, I-80126 Napoli, Italy. E-mail: antonello.merlino@unina.it*

[b] *Institute of Chemical Research of Catalonia (ICIQ), The Barcelona Institute of Science and Technology, 43007 Tarragona, Spain*

[c] *Dipartimento di Medicina, Chirurgia e Farmacia, Università di Sassari, Viale San Pietro, I-07100 Sassari, Italy. E-mail: garribba@uniss.it*

Table S1. Data collection and refinement statistics.

Data collection	*Parameter*
Space group	*C2*
a (Å)	100.74
b (Å)	32.47
c (Å)	72.86
α/β/γ (°)	90.000/90.511/90.000
Resolution range (Å)	50.37-1.57 (1.60-1.57)
Observations	198236 (6896)
Unique reflections	33104 (1547)
Completeness (%)	99.0 (92.9)
Redundancy	6.0 (4.5)
Rmerge (%)	0.040 (0.725)
Average I/σ(I)	21.6 (2.2)
$CC_{1/2}$	0.999 (0.645)
Anom. completeness (%)	98.7 (90.7)
Anom. Multiplicity	3.1 (2.3)
Refinement	
Resolution (Å)	50.37-1.57
Number of reflections	31551
Number of reflections in test set	1553
R factor/Rfree (%)	0.174 (0.222)
Wilson B-factor ($Å^2$)	23.3
Non-H atoms used in the refinement	2213
Average B-factor ($Å^2$)	30.52
RMS deviations for bond lengths (Å)	0.011
RMS deviations for angles (°)	1.74
Ramachandran values (%)	
Preferred regions/allowed regions (%)	201/9 (94.8/4.2%)
Outliers	2 (0.4%)

Table S2. Distribution (%) of the most important species formed in the systems $V^{IV}O^{2+}$:L 1:2 (L = pic and 8-HQ) at pH 5.1 with varying the vanadium concentration.[a]

Ligand	V conc.	$V^{IV}O^{2+}$	$[V^{IV}OL]^{+}$	$[V^{IV}OL_2]$	$[V^{IV}OL_2H_{-1}]^{-}$
pic	1 mM	0.0	7.8	89.7	1.2
	500 μM	0.1	11.0	86.5	1.1
	100 μM	0.5	23.2	74.2	1.0
	10 μM	0.9	30.9	60.2	0.9
8-HQ	1 mM	0.0	4.5	95.4	0.0
	500 μM	0.0	6.3	93.6	0.0
	100 μM	0.1	13.5	86.3	0.0
	10 μM	0.3	18.4	81.3	0.0

[a] Percentages calculated using the stability data for $V^{IV}O$ complexes reported in refs. 1 and 2.

Table S3. Docking solutions for the binding of isomers of $V^{IV}O^{2+}$–8-HQ fragments with RNasi A.[a]

pH	Isomer [b]	Interactions	F_{max} [c]	F_{mean} [d]	Pop.[e]
5.1	*OC*-6-42	Glu111–OE1⋯V Asn71–NH_2⋯O–8-HQ_{ax} Gln69–π⋯π–8-HQ_{ax} His119–π⋯π–8-HQ_{eq}	44.10	39.20	100%
	OC-6-44	Glu111–OE1⋯V Asn71–NH_2⋯O–8-HQ_{ax} Gln69–π⋯π–8-HQ_{ax} His119–π⋯π–8-HQ_{eq}	49.84	46.37	100%
	OC-6-24	Glu111–OE1⋯V Asn71–NH_2⋯O–8-HQ_{ax} Gln69–π⋯π–8-HQ_{eq}	40.78	37.99	26%
	OC-6-23	Glu111–OE1⋯V Asn71–π⋯π–8-HQ_{ax} Gln69–NH_2⋯O_{oxido}	41.79	37.94	58%
7.4[f]	*SPY*-5-13	His119–N⋯V	43.67	42.20	99%
	SPY-5-13	His105–N⋯V	28.78	24.59	78%
	SPY-5-14	His119–N⋯V His14–NH⋯O–8-HQ	45.59	42.98	32%
	SPY-5-14	His105–N⋯V	36.27	35.77	100%

[a] Clustering was carried out depending on *Fitness*, with a RMSD cutoff of 2.5. Only the clusters with highest *Fitness* are displayed for each isomer. [b] *OC*-6 are octahedral isomers and *SPY*-5 are square pyramidal isomers (see ref. 3). [c] Cluster highest *Fitness* score. [d] Cluster mean *Fitness* score. [e] Cluster population: percentage of number of solutions in the clusters over the total number of solutions. [f] In this set of results, the best poses with different coordinating His residues are presented for each isomer. The names are given to the isomers upon the formation of the adduct with His residue.

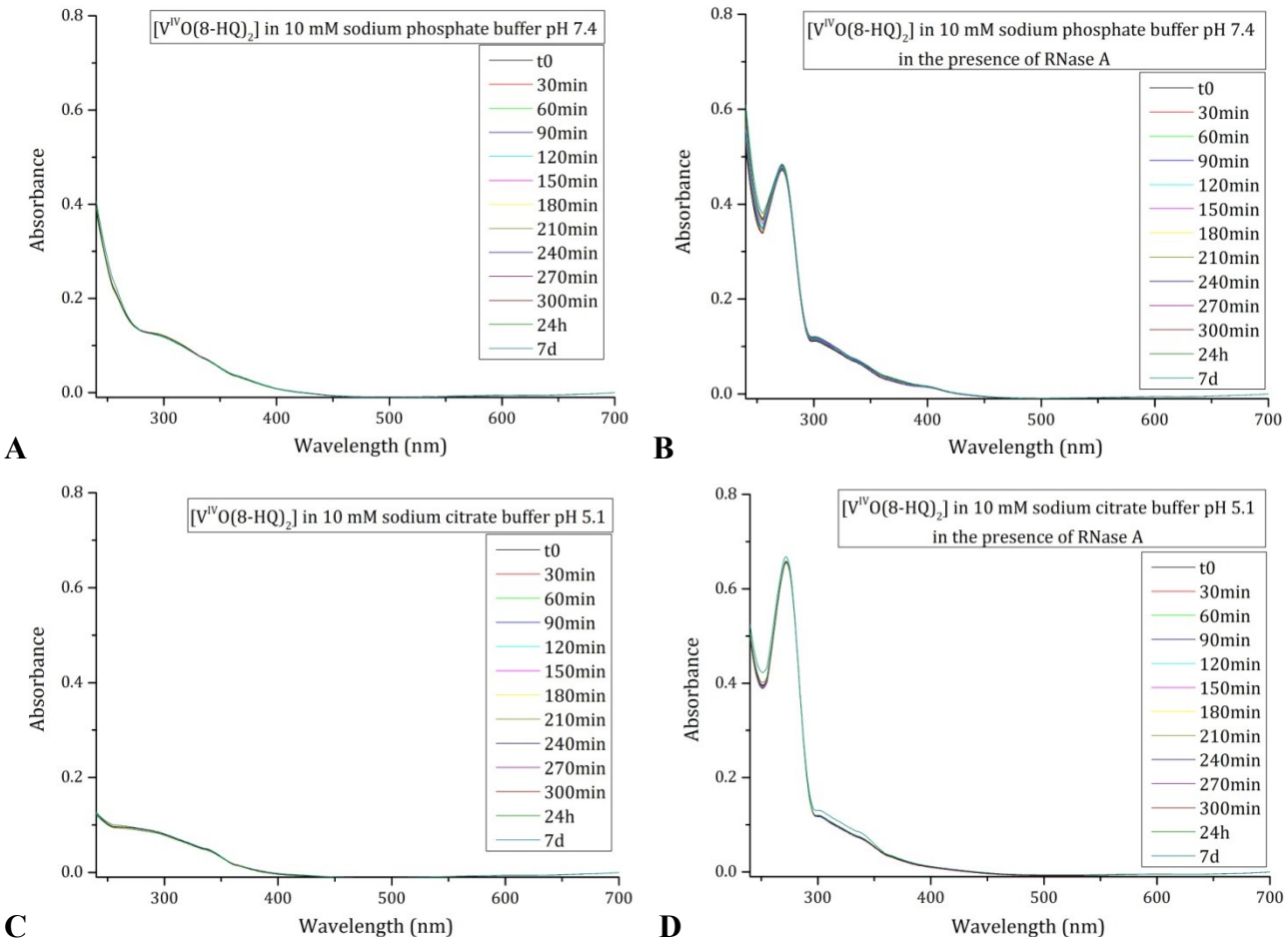


Figure S1. UV-vis spectra overtime of $[V^{IV}O(8\text{-}HQ)_2]$ in 10 mM sodium phosphate buffer at pH 7.4 (A-B) and in 10 mM sodium citrate buffer at pH 5.1 (C-D). The spectra in the presence of RNase A are shown in panels B and D and were recorded with a protein to VC molar ratio of 1:3. Protein and VC concentrations were 16.7 and 50 μM.

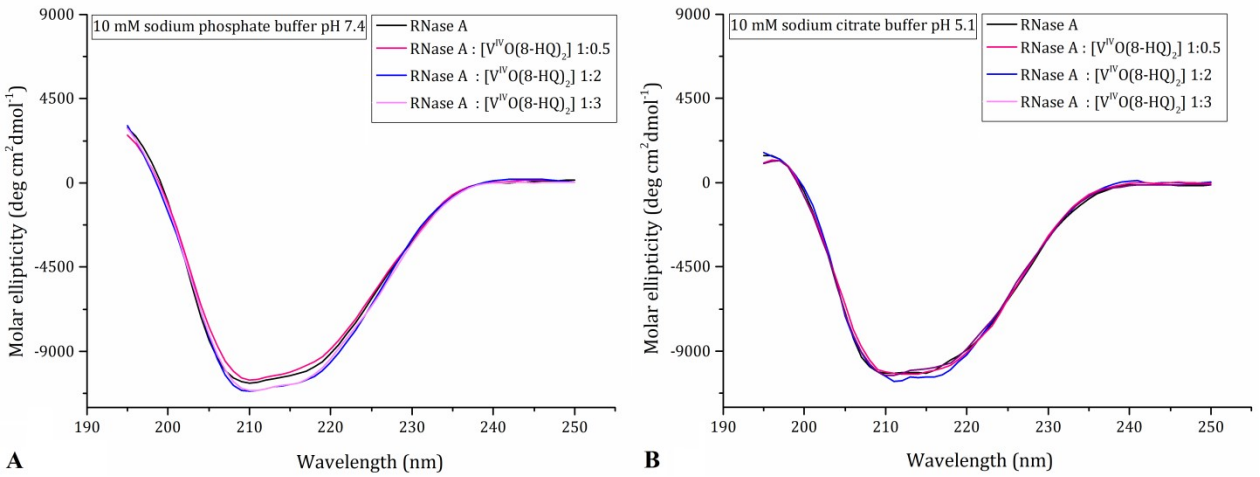


Figure S2. Far-UV CD spectra of RNase A in 10 mM sodium phosphate buffer at pH 7.4 (A) and in 10 mM sodium citrate buffer at pH 5.1 (B). Protein was incubated for 24 h with $[V^{IV}O(8\text{-}HQ)_2]$ with a molar ratio of 1:0.5, 1:2 and 1:3. Protein concentration was 7.3 µM.

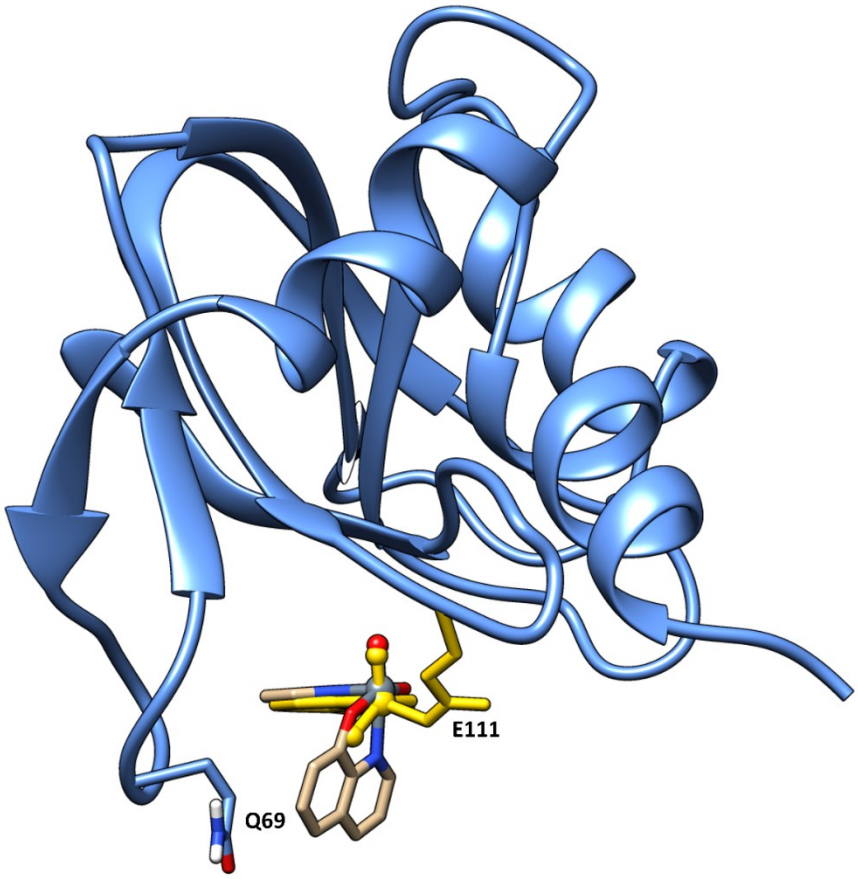


Figure S3. Superimposition of the docking-predicted adduct for *OC*-6-42 isomer of *cis*-$V^{IV}O(8\text{-}HQ)_2$ (tan) and the crystallographic structure (gold).

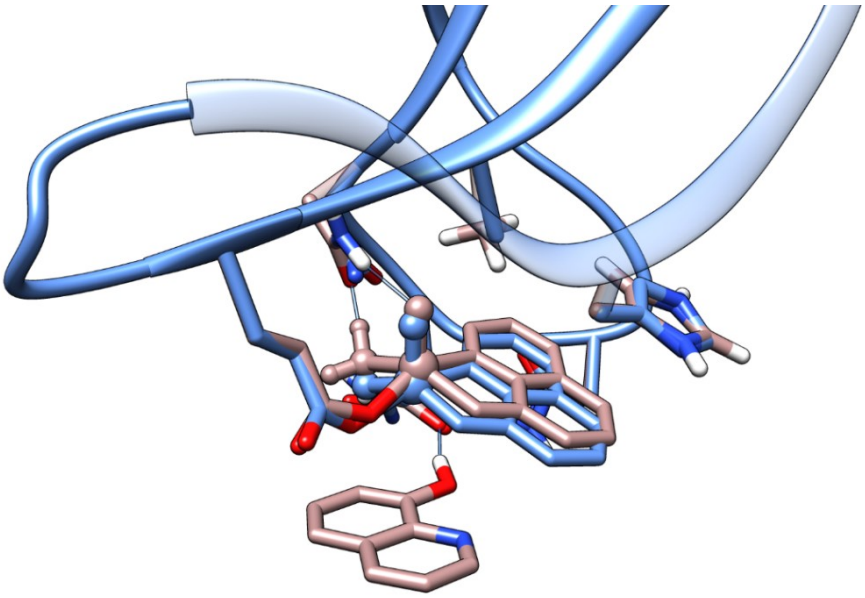


Figure S4. Superimposition of the DFT-predicted adduct (rose) and the crystallographic structure (azure) for the binding of $[V^{IV}O(8\text{-HQ})(H_2O)]^+$ to RNase A.

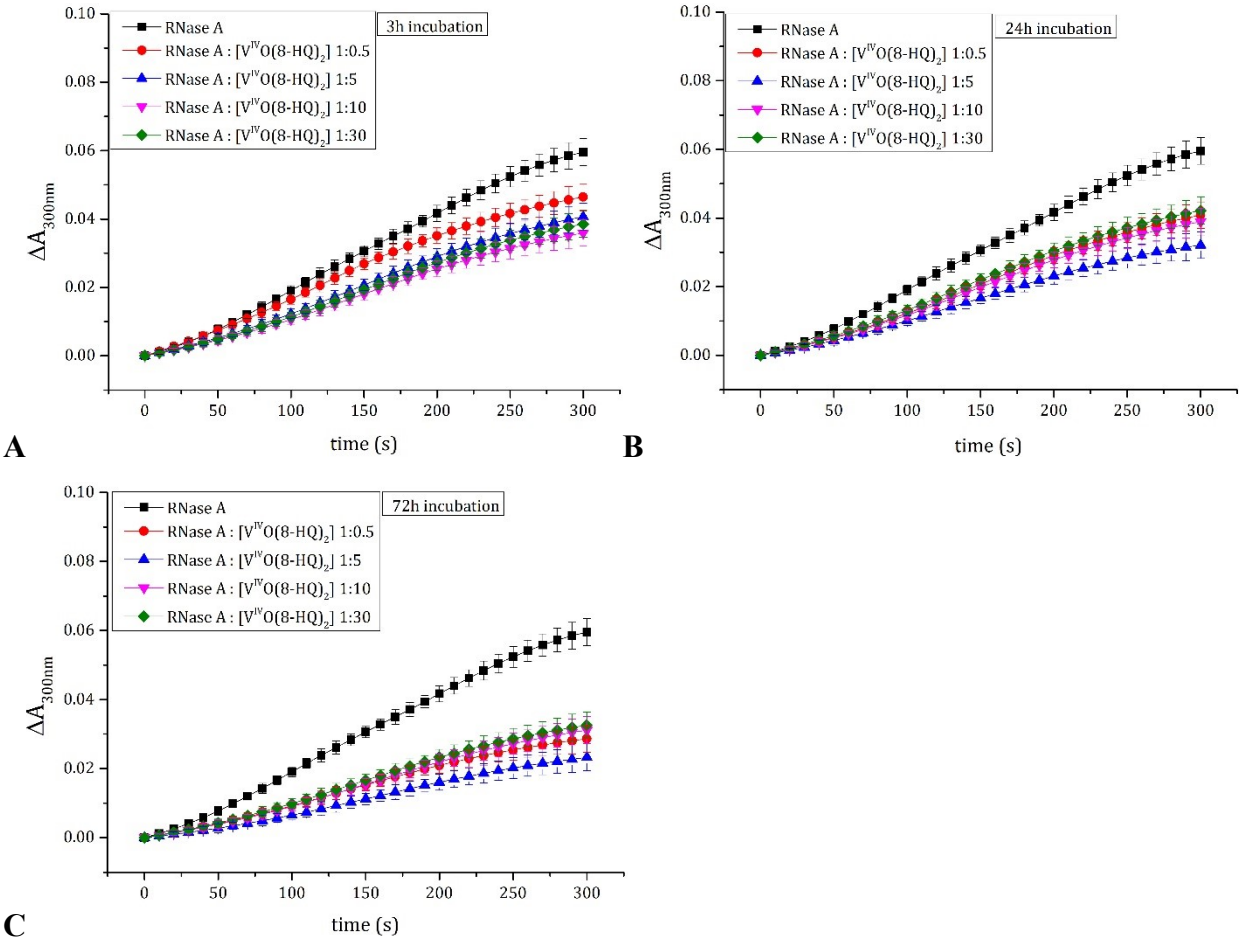
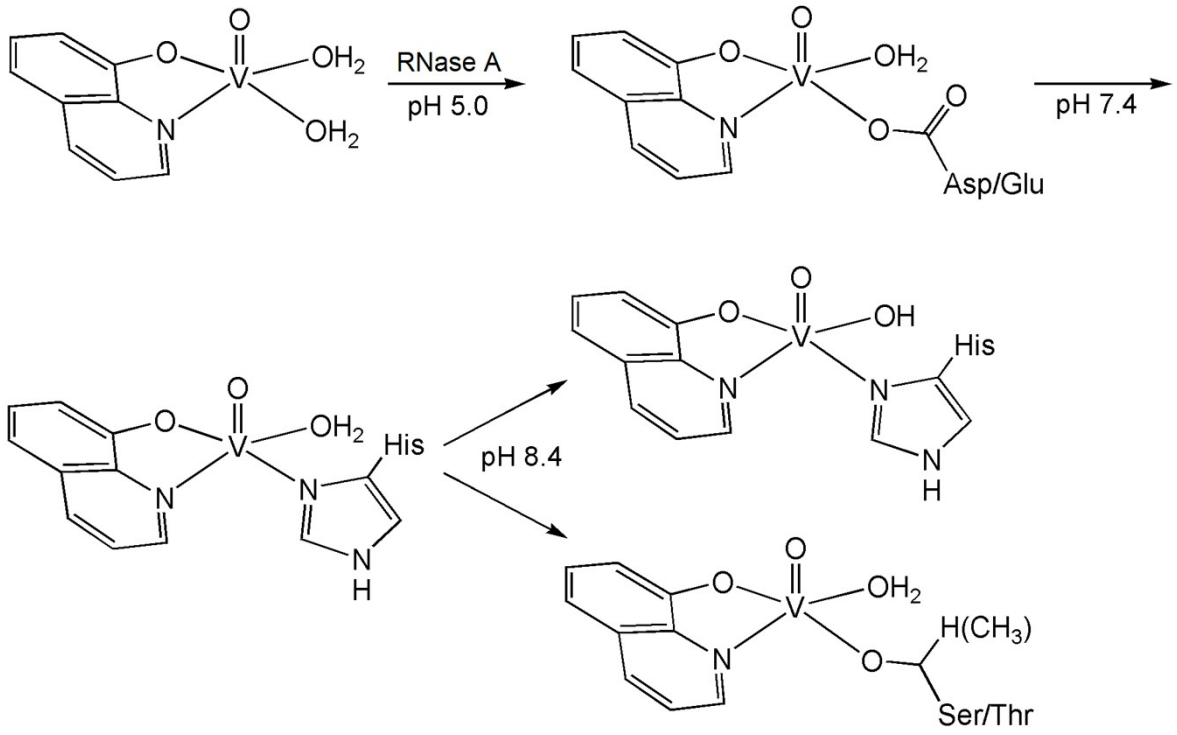
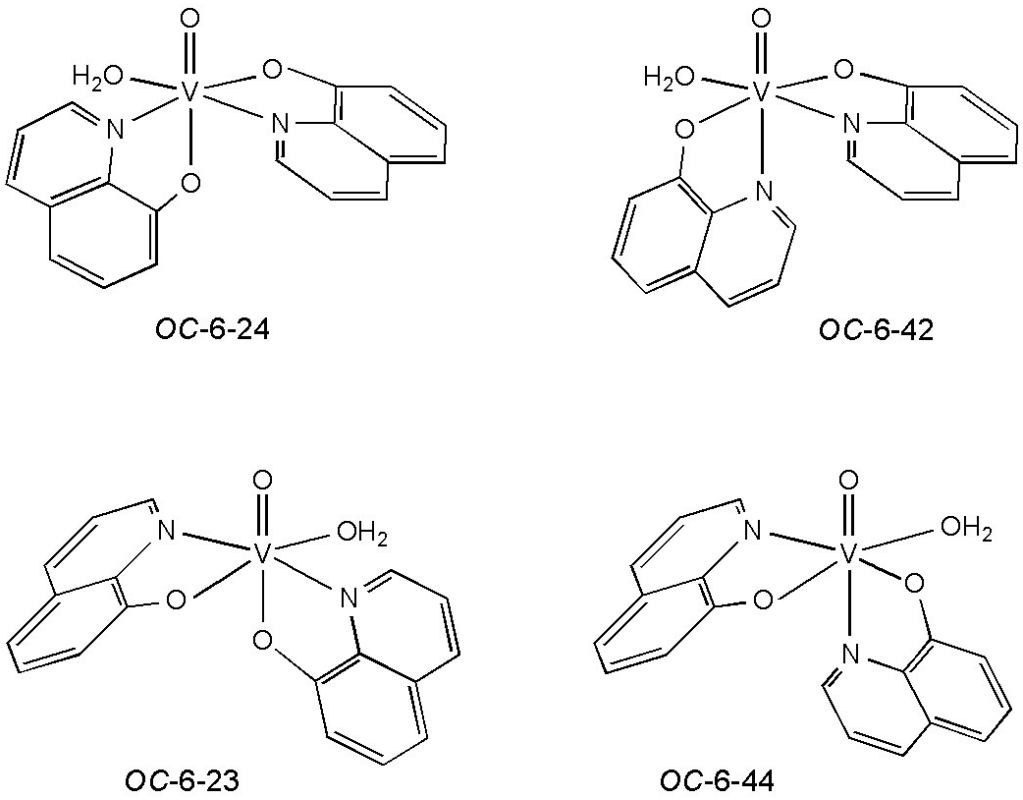


Figure S5. Hydrolysis of yeast RNA in 50 mM sodium acetate buffer at pH 5.0, according to the Kunitz method (ref. 4). The variation of absorbance at 300 nm was followed as function of time upon addition of the protein to the RNA. Four different protein to $[V^{IV}O(8\text{-}HQ)_2]$ molar ratios were tested: 1:0.5, 1:5, 1:10 and 1:30. RNase A was incubated in the presence of VC for 3 h (A), 24 h (B) and 72 h (C). Protein concentration was 7.3 μM. Experiments were performed in triplicate and curves were obtained by averaging the three measurements.



Scheme S1. Possible complexation scheme of the moiety $[V^{IV}O(8\text{-HQ})(H_2O)_2]^+$ as a function of pH in the system $V^{IV}O^{2+}$:8-HQ:RNase A 1:1:1, as determined by EPR spectroscopy.



Scheme S2. Isomers of *cis*-[$V^{IV}O(8\text{-}HQ)_2(H_2O)$] complex formed after the dissolution of [$V^{IV}O(8\text{-}HQ)_2$] in an aqueous solution.

References

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