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Supporting info

Reactivity of a fluorine-containing dirhodium tetracarboxylate compound with proteins

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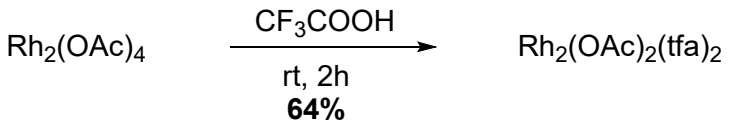
Synthesis and characterization of [cis-$Rh_2(OAc)_2(tfa)_2$]

[*cis*-$Rh_2(OAc)_2(tfa)_2$] was prepared as described in Scheme S1 as previously reported.[1]



Scheme S1. Synthesis of [cis-$Rh_2(OAc)_2(tfa)_2$]

$Rh_2(OAc)_4$ (15.2 mg, 0.034 mmol) was dissolved in an excess of trifluoroacetic acid, (2 mL). The resulting teal-blue solution was stirred at room temperature for 2h. Afterwards, the mixture was concentrated under reduced pressure. Chromatography of the crude residue over silica gel (toluene/CH_3CN = 8:2) afforded the pure [*cis*-$Rh_2(OAc)_2(tfa)_2$] (11.6 mg, 64% yield) as purple powder along with a small amount of [$Rh_2(OAc)(tfa)_3$] (2.1 mg). Analytical data including NMR spectra were consistent with those reported elsewhere [1].

^{19}F NMR spectra

^{19}F NMR spectra were recorded at 25 °C using Bruker AVANCE spectrometer (Billerica, Massachusetts, US) operating at 376 MHz with TOPSPIN using autolocking and auto shimming. Chemical shifts (δ) are reported in parts per million (ppm). Spectra were acquired in 10 mM sodium citrate pH 5.1 and in 5 mM HEPES pH 7.5 (10% D_2O) using 0.5 mM of the metal complex in the absence and in the presence of RNase A and HEWL, respectively (protein to metal molar ratio 1:1). The spectra were referenced with pure trifluoracetic acid (TFA) in the same buffer solutions (10 mM sodium citrate 10% D_2O at pH 5.1 and 5 mM HEPES 10% D_2O at pH 7.5).

^{19}F {^1H dec} NMR data in 10 mM sodium citrate buffer 10% D_2O at pH 5.1.
[*cis*-$Rh_2(OAc)_2(tfa)_2$] t = 5': δ = -75.5, -74.8, -74.7 ppm.
[*cis*-$Rh_2(OAc)_2(tfa)_2$] t = 4 h: δ = -75.5, 74.7 ppm.
[*cis*-$Rh_2(OAc)_2(tfa)_2$] t = 24 h: δ = -75.5 ppm.
[*cis*-$Rh_2(OAc)_2(tfa)_2$] with RNase A t = 5' δ = -75.5 ppm.
Trifluoroacetic Acid (TFA): δ = -75.5 ppm.

^{19}F {^1H dec} NMR data in 5 mM HEPES buffer 10% D_2O at pH 7.5.
[*cis*-$Rh_2(OAc)_2(tfa)_2$] t = 5': δ = -74.8 ppm.
[*cis*-$Rh_2(OAc)_2(tfa)_2$] t = 4 h: δ = -74.8, -75.1, -75.4 ppm.
[*cis*-$Rh_2(OAc)_2(tfa)_2$] t = 24 h: δ = 75.1, -75.4 ppm.
[*cis*-$Rh_2(OAc)_2(tfa)_2$] with HEWL: t = 5' δ = -74.8, -75.4 ppm.
[*cis*-$Rh_2(OAc)_2(tfa)_2$] with HEWL: t = 2 h δ = -75.4 ppm.
Trifluoroacetic Acid (TFA): δ = -75.4 ppm.

[1] Y. Lou, T.P. Remarchuk, E.J. Corey. Catalysis of Enantioselective [2+1]-Cycloaddition Reactions of Ethyl Diazoacetate and Terminal Acetylenes Using Mixed Ligand Complexes of the Series Rh2(RCO2)n (L*4-n). Stereochemical Heuristics for Ligand Exchange and Design for Catalyst Synthesis.*J. Am. Chem. Soc*. 2005, 127, 14223–14230.

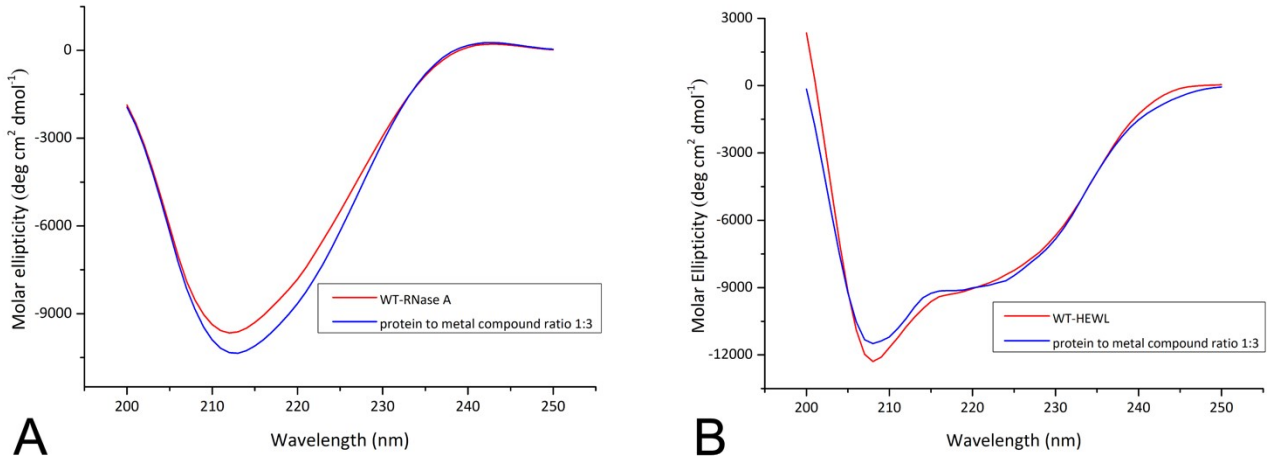


Figure S1. (A) CD spectra of RNase A (red line) and of RNase A incubated for 24 h in the presence of [*cis*-$Rh_2(OAc)_2(TFA)_2$] (blue line) in 10 mM sodium citrate buffer pH 5.1. (B) CD spectra of HEWL (red line) and of HEWL incubated for 24 h in the presence of [*cis*-$Rh_2(OAc)_2(TFA)_2$] (blue line) in 5 mM HEPES buffer pH 7.5.

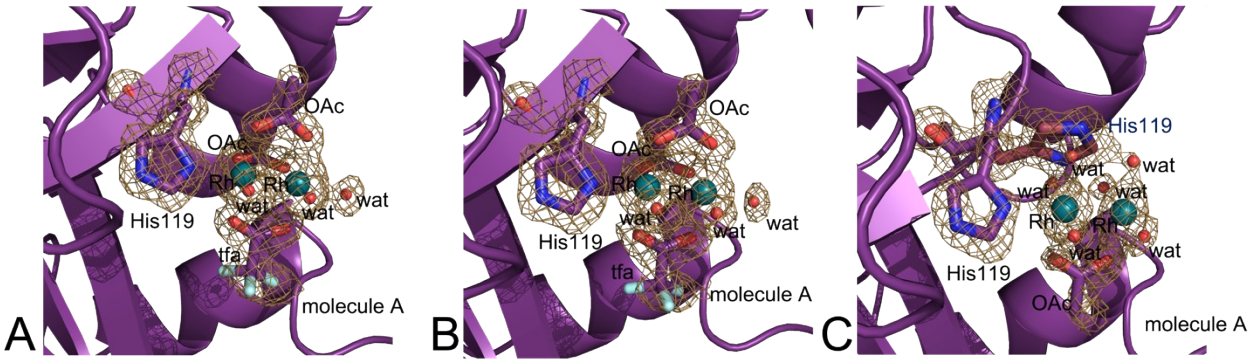


Figure S2. Details of the Rh binding site close to His119 of molecule A in crystal **3** (panel A), **4** (panel B) and **2** (panel C). 2Fo-Fc electron density maps are contoured at 1.0 σ in panels A and B and at 0.7 σ in panel C. In panel C, the two conformations of His119 are shown.

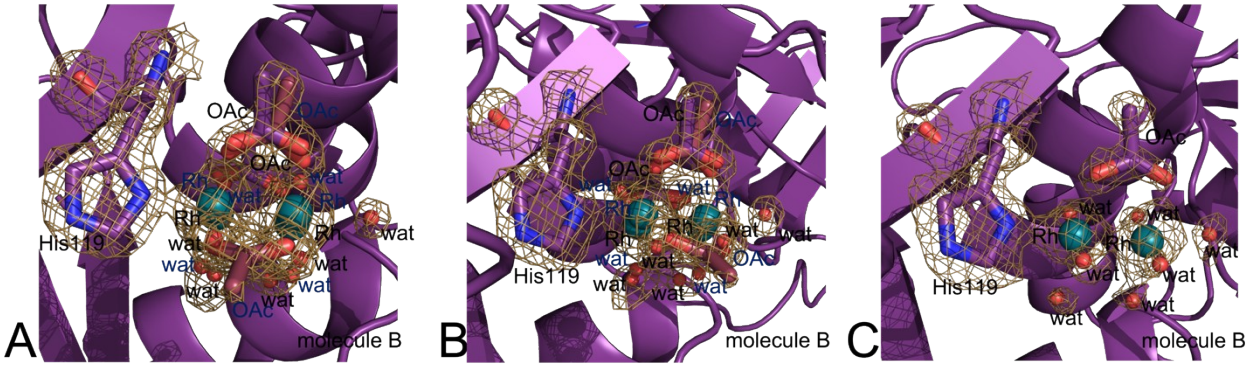


Figure S3. Details of the Rh binding site close to His119 of molecule B in crystal **3** (panel A), **4** (panel B) and **2** (panel C). 2Fo-Fc electron density maps are contoured at 1.0 σ in panels A and B and at 0.7 σ in panel C. In panels A and B the two conformations of the dirhodium-containing fragment bound to the His side chain are shown.

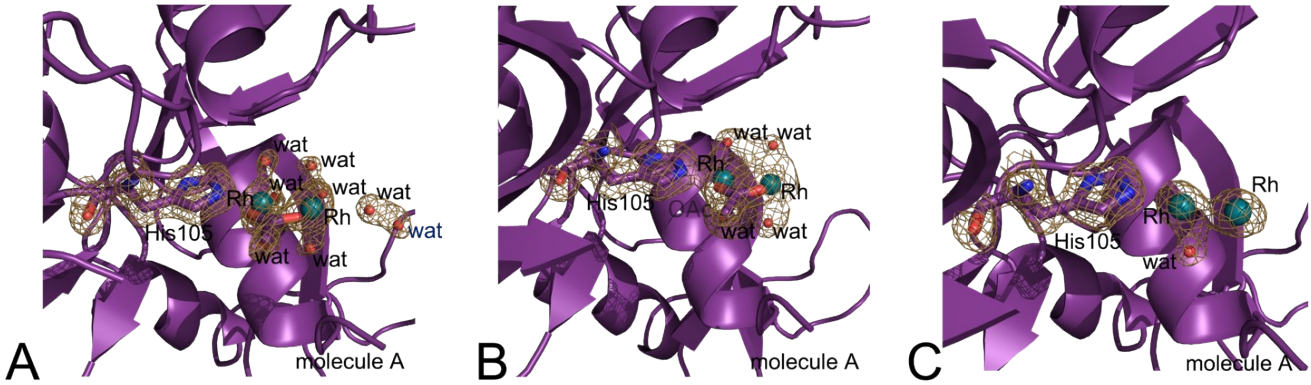


Figure S4. Details of the Rh binding site close to His105 of molecule A in crystal **1** (panel A), **3** (panel B) and **2** (panel C). 2Fo-Fc electron density maps are contoured at 1.0 σ in panels B and C, at 0.7 σ in panel A.

Table S1		
Crystal	Comments on the 10 highest difference Fourier (Fo-Fc) electron-density peaks (> 5 σ)	PDB Validation Report assessment (clashscores)
Crystal 1	The peak at 12.21σ is too close to a water molecule (distance 0.70Å). The peak at 9.61σ is too close to a water molecule. The peak at 9.15σ is between two water molecules that are at 3.44Å from each other. The peak at 8.33σ is too close to three water molecules. The peak at 7.94σ is between two water molecules that are at 2.61Å from each other. The peak at 7.44σ is found close to the carbonyl group of Ser22 (molecule A). This is a very flexible and disordered region of the protein. The peak at 7.42σ is between the carbonyl group of Thr99 and OG1 atom of Thr100 side chain, which are at 3.44Å of distance each other (molecule A). The peak at 7.27σ is too close to a water molecule. The peak at 7.19σ is between a water molecule and OG atom of Ser59 side chain (molecule A), that are at 3.22Å distance from each other. The peak at 7.18σ is too close to a water molecule.	Clashscore 17.
Crystal 2	The peaks at 8.62σ and -5.29σ are too close to the metal complex.	Clashscore 5.

	The peak at 7.61σ is between two water molecules that are at 3.46Å from each other. The peak at 7.60σ is too close to CD2 atom of His119 side chain (molecule A). The peak at 6.35σ is 1.22Å apart from a disulfide bond formed by Cys65 and Cys72 (molecule B). The peak at 5.82σ is between two water molecules that are at 2.63Å from each other. The peak at 5.68σ is found close to the carbonyl group of Gln28 (molecule B). A negative peak at 5.19σ is close to the disulphide bond formed by Cys26 and Cys64. This peak could arise from X-ray radiation damage. The peak at 5.13σ is too close to a peptide bond formed by Asn27 and Gln28 of molecule B. The peak at 5.04σ is too close to a water molecule.	
Crystal 3	The peaks at 14.63σ, 10.61σ, -9.62σ, 8.36σ, -7.30σ, 7.08σ, 6.86σ, 6,75σ are too close to the metal complex. The peak at 8.61σ is too close to the OG atom of Ser32 side chain (molecule A). The peak at 7.51σ is not far from a water molecule (distance 1.75Å).	Clashscore 5.
Crystal 4	The main 10 peaks found in this structure have a sigma ranging from 13.42σ to 6.96σ. The peaks at 13.42σ, 10.77σ, 9.42σ, 8.60σ, 8.02σ, 7.95σ, 6.96σ are too close to the metal complex.	Clashscore 7.

	The peaks at 7.07σ and 7.04σ are found in molecule B and are too close to OG and NE atoms of the side chains of Ser32 and Arg33, respectively.	
HEWL	There are 7 peaks above ±5.00σ. The peak at 7.22σ is too close to OD1 atom of Asp18 side chain. The peak at 7.00σ is 1.33Å apart from a disulfide bond formed by Cys6 and Cys127. The peak at 6.80σ is between two water molecules that are at 2.72Å from each other. A negative peak at 5.98σ is present in proximity of sulphate moiety of a HEPES molecule. The peak at 5.91σ is too close to a water molecule. The peak at 5.24σ is in proximity of the NE atom of Arg21 side chain. The peak at 5.16σ is at only 1.32 Å from a water molecule.	Clashscore 8.

Table S2. Rmsd of Cα among the structures of the adducts obtained upon reaction of [*cis*-$Rh_2(OAc)_2(tfa)_2$] with RNase A. Rmsd with the metal-free protein (PDB CODE 1JVT)** and with the adduct of the protein with dirhodium tetraacetate (PDB code 6XVX)* are also reported for comparison. Values are in Å.

	Crystal 1		Crystal 2		Crystal 3		Crystal 4		$[Rh_2(OAc)_4]$/RNase A adduct		Metal-free RNase A	
PDB code	7QPW		7QQ0		7QPY		7QPZ		6XVX*		1JVT**	
	Molecule A	Molecule B	Molecule A	Molecule B	Molecule A	Molecule B	Molecule A	Molecule B	Molecule A	Molecule B	Molecule A	Molecule B
Crystal 1												
Molecule A	0	0.354	0.117	0.397	0.081	0.398	0.063	0.361	0.397	0.086	0.207	0.383
Molecule B		-	0.431	0.062	0.383	0.110	0.322	0.063	0.105	0.379	0.353	0.211
Crystal 2												
Molecule A			-	0.431	0.110	0.438	0.151	0.410	0.449	0.113	0.273	0.446
Molecule B				-	0.391	0.093	0.342	0.069	0.102	0.393	0.374	0.208
Crystal 3												
Molecule A					-	0.406	0.082	0.385	0.412	0.077	0.233	0.390
Molecule B						-	0.306	0.077	0.077	0.397	0.390	0.187
Crystal 4												
Molecule A							-	0.358	0.377	0.070	0.220	0.382
Molecule B								-	0.090	0.377	0.367	0.209
6XVX												
Molecule A									-	0.405	0.365	0.194
Molecule B										-	0.214	0.361
1JVT												
Molecule A											-	0.345
Molecule B												-

***** G. Ferraro, A. Pratesi, L. Messori, A. Merlino. Protein Interactions of Dirhodium Tetraacetate: a Structural Study. *Dalton Trans.* 2020, 49 (8), 2412-2416

****** L. Vitagliano, A. Merlino, A. Zagari, L. Mazzarella. Reversible Substrate-Induced Domain Motions in Ribonuclease A. *Proteins*, 46**,** 97-104

Table S3. Rh-containing fragments found in the five structures of Rh/protein adducts obtained upon reaction of [*cis*-$Rh_2(OAc)_2(TFA)_2$] with RNase A or HEWL. The Rh ligands identified in each binding site are described. Values in parentheses refer to the occupancy of metal and ligands.

Rh/RNase A adduct binding site	**Metal and Ligands in structure 1**	**Metal and Ligands in structure 2**	**Metal and Ligands in structure 3**	**Metal and Ligands in structure 4**
PDB code	7QPW	7QQ0	7QPY	7QPZ
His119 of molecule A (His in double conformation in structures 1 and 2)	Rh (0.55) Rh (0.55) OAc (0.55) TFA (0.55) H_2O (0.55) H_2O (0.55) H_2O_{ax} (0.55)	Rh (0.40) Rh (0.40) OAc (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40)	Rh (0.80) Rh (0.80) OAc (0.80) TFA (0.80) H_2O (0.80) H_2O (0.80) H_2O_{ax} (0.80)	Rh (0.75) Rh (0.75) OAc (0.75) TFA (0.75) H_2O (0.75) H_2O (0.75) H_2O_{ax} (0.75)
His119 of molecule B (His in double conformation in structure 2)	Rh (0.20/0.20) (d.c.) Rh (0.20/0.20) (d.c.) OAc (0.20/0.20) (d.c.) OAc (0.20/0.20) (d.c.) H_2O (0.20/0.20) (d.c.) H_2O (0.20/0.20) (d.c.) H_2O (0.20/0.20) (d.c.) H_2O (0.20/0.20) (d.c.) H_2O_{ax} (0.40)	Rh (0.40) Rh (0.40) OAc (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O_{ax} (0.40)	Rh (0.30/0.30) (d.c.) Rh (0.30/0.30) (d.c.) OAc (0.30/0.30) (d.c.) OAc (0.30/0.30) (d.c.) H_2O (0.30/0.30) (d.c.) H_2O (0.30/0.30) (d.c.) H_2O (0.30/0.30) (d.c.) H_2O (0.30/0.30) (d.c.) H_2O_{ax} (0.60)	Rh (0.30/0.30) (d.c.) Rh (0.30/0.30) (d.c.) OAc (0.30/0.30) (d.c.) OAc (0.30/0.30) (d.c.) H_2O (0.30/0.30) (d.c.) H_2O (0.30/0.30) (d.c.) H_2O (0.30/0.30) (d.c.) H_2O (0.30/0.30) (d.c.) H_2O_{ax} (0.60)
His105 of molecule A	Rh (0.40) Rh (0.40) OAc (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O_{ax} (0.20) (d.c.)	Rh (0.40) Rh (0.40) H_2O (0.40)	Rh (0.70) Rh (0.70) OAc (0.70) H_2O (0.70) H_2O (0.70) H_2O (0.70) H_2O (0.70) H_2O (0.70) H_2O (0.70) H_2O_{ax} (0.70)	Rh (0.55) Rh (0.55) OAc (0.55) OAc (0.55) H_2O (0.55) H_2O (0.55) H_2O (0.55) H_2O (0.55)
His105 of molecule B	/	/	Rh (0.40) Rh (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40) H_2O (0.40)	/

	H_2O (0.40) H_2O (0.40)
Rh/HEWL adduct binding site	**Metal and Ligands in the structure**
PDB code	**7QQ1**
His15	Rh (0.50) Rh (0.50) H_2O (0.50)
Asp101	Rh (0.30) H_2O (0.30)

d.c. = double conformation, ax=axial

Table S4. Geometric parameters of the Rh-containing fragments in the structures of Rh/RNase A adducts here refined.

			Binding sites in the Rh/RNase A adducts obtained upon reaction of the protein with [*cis*-$Rh_2(OAc)_2(tfa)_2$]			
PDB code	Crystal		His119 Molecule A	His119 Molecule B	His105 Molecule A	His105 Molecule B
7QPW	1	Rh—N (Å)	2.25	2.13	2.17	/
		Rh—Rh (Å)	2.38	2.41	2.50	
		Rh—O_{OAc}[a] (Å)	2.09	2.10	2.13	
		Rh—O_{TFA}[a] (Å)	2.09	/	/	
		Rh—O_{wat}[a] (Å)	2.08	2.10	2.08	
		O_{OAc}—Rh—N[a] (°)	92.6	89.7	89.5	
		O_{TFA}—Rh— N[a] (°)	87.2	/	/	
		N—Rh—Rh (°)	178.7	170.7	169.2	
		O_{wat}—Rh—N[a] (°)	86.6	90.9	91.7	
7QQ0	2	Rh—N (Å)	2.27	2.16	2.18	/
		Rh—Rh (Å)	2.39	2.38	2.27	
		Rh—O_{OAc}[a] (Å)	2.11	2.12	/	
		Rh—O_{TFA}[a] (Å)	/	/	/	
		Rh—O_{wat}[a] (Å)	2.11	2.10	1.98	
		O_{OAc}—Rh—N[a] (°)	82.1	96.7	/	
		O_{TFA}—Rh— N[a] (°)	/	/	/	
		N—Rh—Rh (°)	175.0	172.45	164.1	
		O_{wat}—Rh—N[a] (°)	91.2	89.3	107,2	
7QPY	3	Rh—N (Å)	2.25	2.16	2.11	2.23
		Rh—Rh (Å)	2.35	2.47	2.43	2.48
		Rh—O_{OAc}[a] (Å)	2.10	2.10	2.13	/
		Rh—O_{TFA}[a] (Å)	2.10	/	/	/
		Rh—O_{wat}[a] (Å)	2.10	2.10	2.09	2.10
		O_{OAc}—Rh—N[a] (°)	90.4	89.5	90.9	/
		O_{TFA}—Rh— N[a] (°)	90.2	/	/	/
		N—Rh—Rh (°)	179.0	159.5	170.0	176.6
		O_{wat}—Rh—N[a] (°)	87.8	89.9	91.1	89.7
7QPZ	4	Rh—N (Å)	2.27	2.30	2.20	/
		Rh—Rh (Å)	2.36	2.39	2.42	
		Rh—O_{OAc}[a] (Å)	2.10	2.10	2.10	
		Rh—O_{TFA}[a] (Å)	2.12	/	/	
		Rh—O_{wat}[a] (Å)	2.11	2.11	2.10	
		O_{OAc}—Rh—N[a] (°)	90.8	89.9	91.7	
		O_{TFA}—Rh— N[a] (°)	87.5	/	/	
		N—Rh—Rh (°)	179.4	159.0	175.6	
		O_{wat}—Rh—N[a] (°)	89.5	92.6	90.4	

		Rh—N (Å)	2.16	2.27	2.20	2.18
		Rh—Rh (Å)	2.34	2.37	2.42	2.42
		Rh—O_{OAc}[a] (Å)	2.09	2.11	2.06	2.13
		Rh—O_{TFA}[a] (Å)	/	/	/	/
6XVX*	Structure of the $[Rh_2(OAc)_4)]$/RNase A adduct	Rh—O_{wat}[a] (Å)	2.24	2.28	2.29	2.28
		O_{OAc}—Rh—N[a] (°)	93.7	93.9	94.4	92.5
		O_{TFA}—Rh— N[a] (°)	90.9	91.9	91.1	93.3
		N—Rh—Rh (°)	177.1	175.4	176.3	176.6
		O_{wat}—Rh—Rh[a] (°)	90.1	88.0	88.9	87.3

[a] Average values. Standard deviations for the distances are in the range of 0.00-0.04 Å. Standard deviations for the angles are in the range of 1.4-7.8 °. 'wat' in the table refers only to water coordinating Rh atoms at equatorial positions.

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