Supporting info

Digging into protein metalation differences triggered by fluorine containing-dirhodium tetracarboxylate analogues

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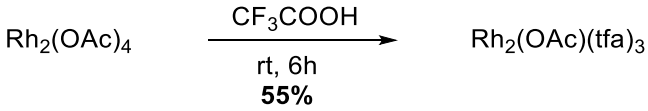
Synthesis and characterization of [$Rh_2(OAc)(tfa)_3$]

[$Rh_2(OAc)(tfa)_3$] was prepared as described in Scheme S1 as previously reported [1,2].

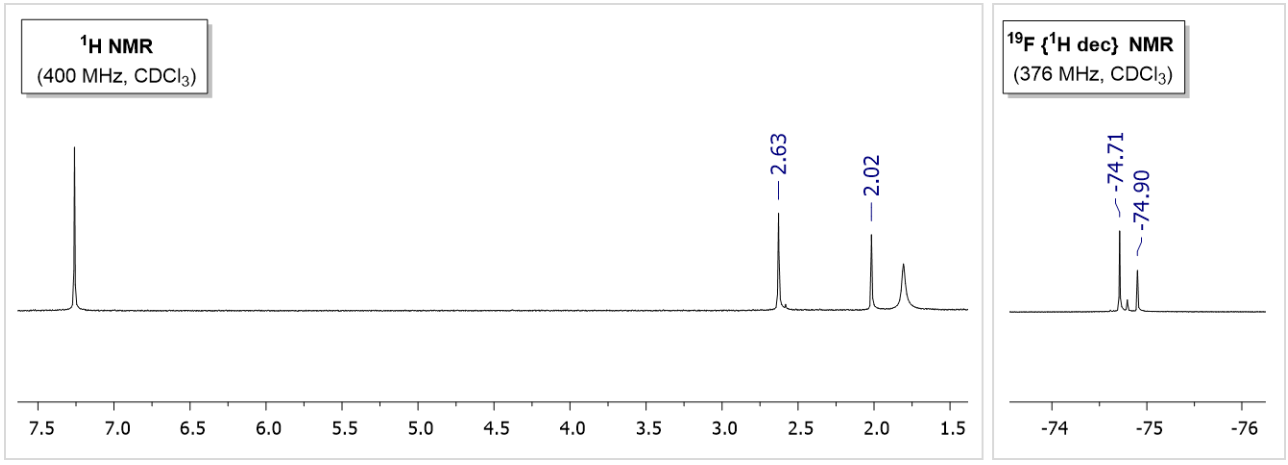


Scheme S1. Synthesis of [$Rh_2(OAc)(tfa)_3$]

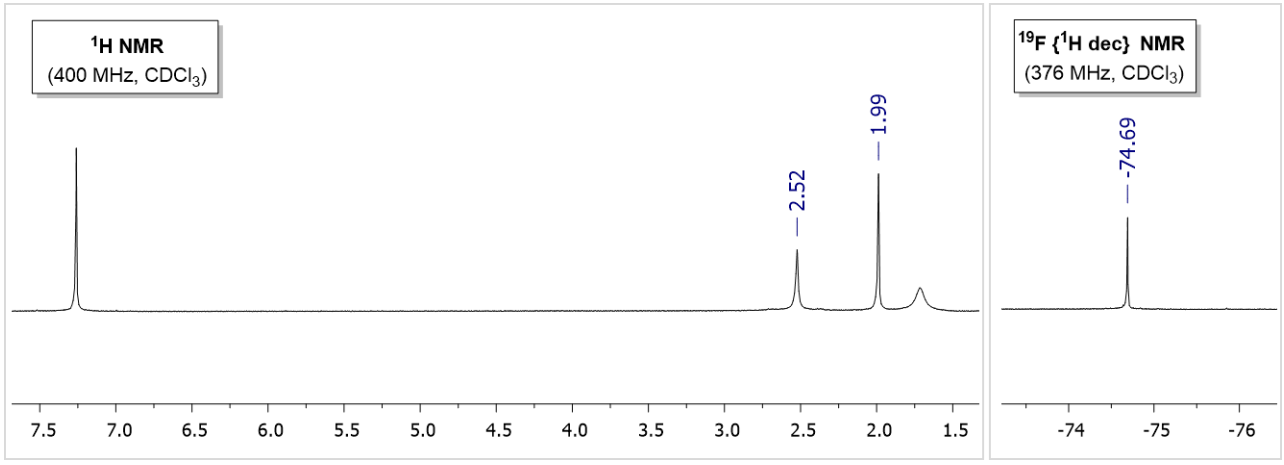
$Rh_2(OAc)_4$ (17.5 mg, 0.039 mmol) was dissolved in an excess of trifluoroacetic acid, (2 mL). The resulting teal-blue solution was stirred at room temperature for 6h. Afterwards, the mixture was concentrated under reduced pressure. Chromatography of the crude residue over silica gel (toluene/CH_3CN = 99:1) afforded the pure [$Rh_2(OAc)(tfa)_3$] (13.1 mg, 55% yield) as blue powder along with the expected [*cis*-$Rh_2(OAc)_2(tfa)_2$]. Analytical data including NMR spectra were consistent with those reported elsewhere.[1]

Data for [$Rh_2(OAc)(tfa)_3$]: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.63 (s, coordinated CH_3CN), 2.02 (s, 3H). ^{19}F {^{1}H dec} NMR (376 MHz, $CDCl_3$): δ = -74.7, -74,9 (Figure S1A).

Data for [*cis*-$Rh_2(OAc)_2(tfa)_2$]: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.52 (s, coordinated CH_3CN), 1.99 (s, 6H). ^{19}F {^{1}H dec} NMR (376 MHz, $CDCl_3$): δ = -74.7 (Fighure S1B).



A



B

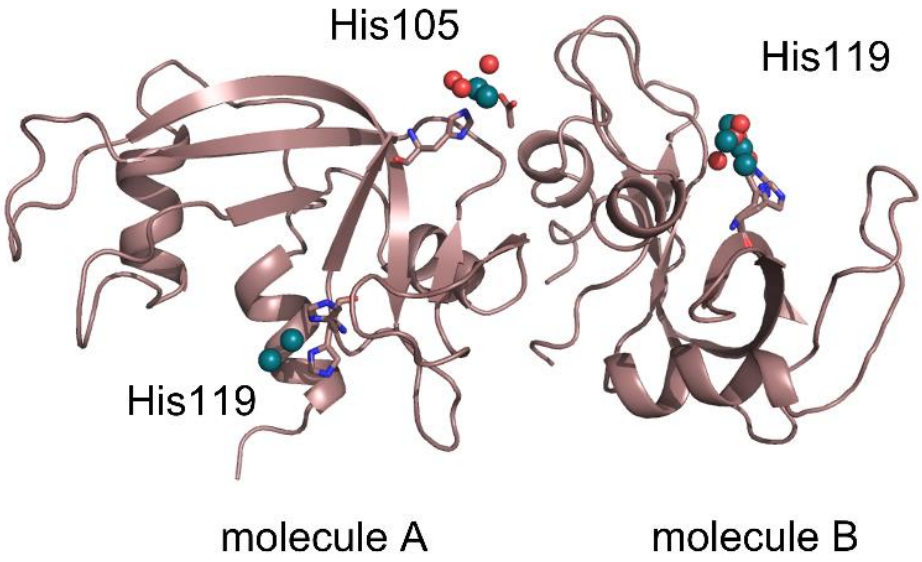
Figure S1 Copies of ^{1}H and ^{19}F NMR spectra for [$Rh_2(OAc)(tfa)_3$] (panel A) and [*cis*-$Rh_2(OAc)_2(tfa)_2$] (panel B).

^{19}F NMR spectra

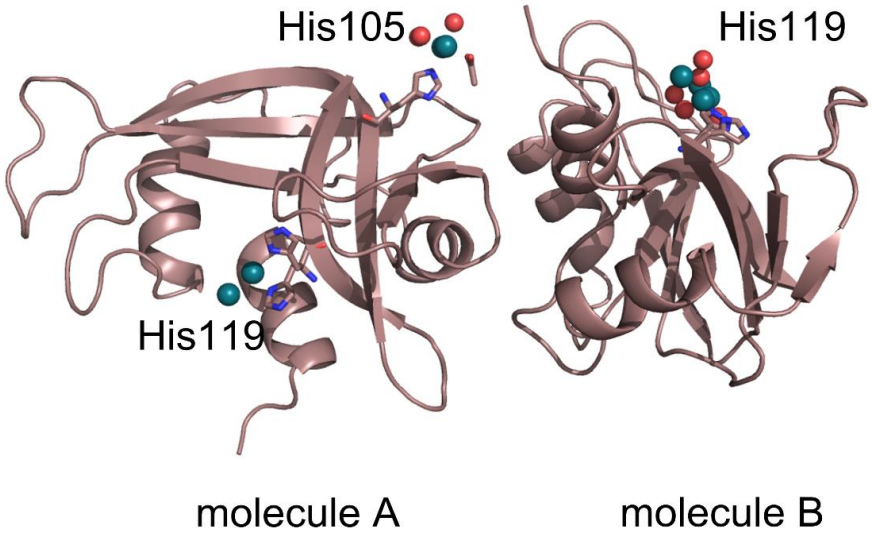
^{19}F NMR spectra were recorded at 25 °C using Bruker AVANCE spectrometer (Billerica, Massachusetts, US) operating at 376 MHz with TOPSPIN using autolocking and auto shimming. Chemical shifts (δ) are reported in parts per million (ppm). Spectra were acquired in 10.0 mM sodium citrate pH 5.1 and in 5.0 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 (10.0 mM sodium citrate 10% D_2O at pH 5.1 and 5.0 mM HEPES 10% D_2O at pH 7.5).

^{19}F {^{1}H dec} NMR data in10 mM sodium citrate buffer 10% D_2O at pH 5.1.
$[Rh_2(OAc)(tfa)_3]$ t = 5': δ = -75.5, -74.8, -74.7, -74.6 ppm.
$[Rh_2(OAc)(tfa)_3]$ t = 4 h: δ = -75.5, -74.6 ppm.
$[Rh_2(OAc)(tfa)_3]$ t = 24 h: δ = -75.5, -74.6 ppm.
$[Rh_2(OAc)(tfa)_3]$ with RNase At = 5'δ = -75.5 ppm.
Trifluoroacetic Acid (TFA): δ = -75.5 ppm.

^{19}F {^{1}H dec} NMR data in5 mM HEPES buffer 10% D_2O at pH 7.5.
$[Rh_2(OAc)(tfa)_3]$ t = 5': δ = -75.5, -74.8, -74.7 ppm.
$[Rh_2(OAc)(tfa)_3]$ t = 4 h: δ= -75.5, -75.1, -74.8, -74.7ppm.
$[Rh_2(OAc)(tfa)_3]$ t = 24 h: δ = 75.5, -75.1, -74.7 ppm.
$[Rh_2(OAc)(tfa)_3]$ with HEWL:t = 5' δ = -74.7, -75.5 ppm.
$[Rh_2(OAc)(tfa)_3]$ with HEWL:t = 2 hδ = -75.5 ppm.
Trifluoroacetic Acid (TFA): δ = -75.5 ppm.

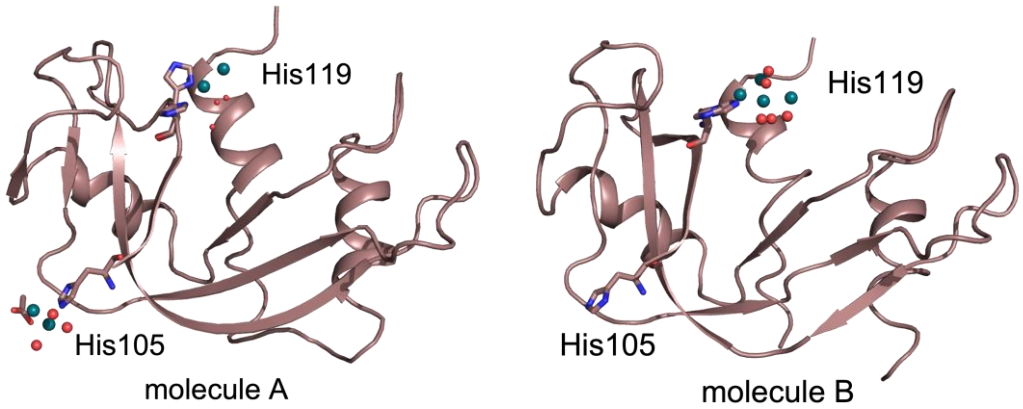


A



B

Figure S2. Overall structure of the two independent RNase A molecules (molecules A and B) in the asymmetric unit of the monoclinic crystal of the adduct formed in the reaction of the protein with $[Rh_2(OAc)(tfa)_3]$ (Crystal **1** in panel A and Crystal **2** in panel B). Rh atoms are in dark green.



A **B**

Figure S3. Overall structure of the two molecules in the asymmetric unit of the monoclinic crystal of the Rh/RNase A adduct formed upon reaction of the protein with $[Rh_2(OAc)(tfa)_3]$ (Crystal **1**). The two molecules are shown with the same spatial orientation. Rh atoms are in dark green. In molecule B, residues 18-21 are not included in the model due to conformational disorder.

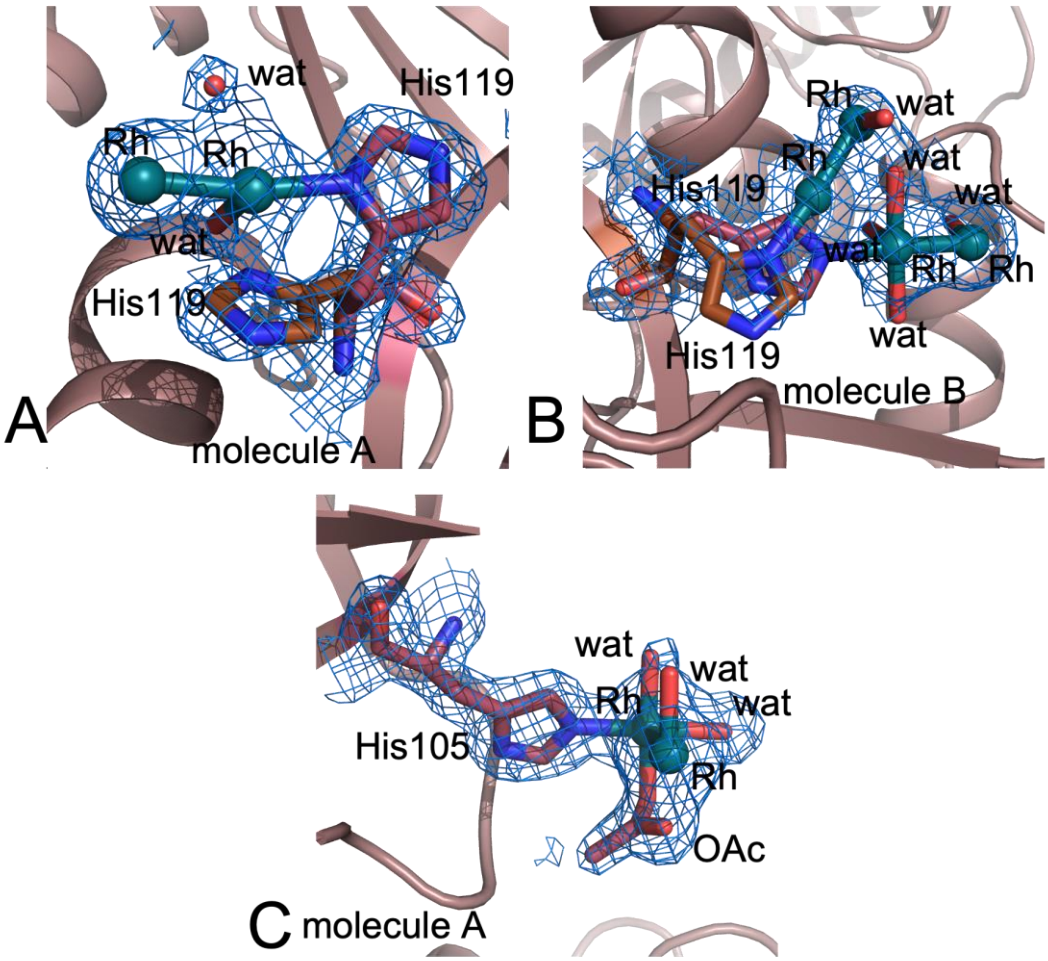


Figure S4. Dirhodium core binding site close to (A) His119 of molecule A, (B) His119 of molecule B. (C) His105 of molecule A in the structure of the adduct formed upon reaction of RNase A with $[Rh_2(OAc)(tfa)_3]$ (Crystal 2). 2Fo-Fc electron density maps are contoured at 1.0 σ.

Table S1.

Crystal	Comments on the highest difference Fourier (Fo-Fc) electron-density peaks	PDB Validation Report assessment (clashscores)
RNase A Crystal 1	There are 7 peaks above ±5.00σ. The first peak, at 5.94σ, is found between two water molecules which are at 3.11 Å distance. The second peak, at 5.76σ, is too close to a water molecule. The peak at 5.30σ is too close to the metal complex in proximity of His119 side chain of molecule A. The peak at 5.25σ is too close to S atom of Cys26 of molecule B, and could be due to X-ray radiation damage. There are two peaks at 5.01σ which are both too close to the metal complex but in two different regions of the protein: a peak is close to His119 side chain of the molecule A while the other lies in proximity of His105 side chain in the molecule A. Finally, there is a peak at 5.00σ, that is close to O atom of Ala20 of molecule A, i.e. close to a residue located in a very flexible region of the protein.	Clashscore 7.
RNase A Crystal 2	There are 6 peaks above ±5.00σ. They range from 10.82σ to 5.36σ. Four peaks, at 10.82σ, 5.96σ, 5.63σ and 5.66σ, are all too close to ligands of the metal complex, in particular: - 10.82σ. Close to His105 side chain of the molecule A; - 5.96σ and 5.63σ. Close to His119 side chain of	Clashscore 4.

	the molecule A; - 5.36σ. Close to His119 side chain of the molecule B. The peak at 5.93σ is found too close to the O atom of Ser21 of molecule A. This is a very flexible region of the protein. Finally, there are two peaks at 5.36σ, that are both too close to water molecules.	
HEWL	There are 4 peaks above ±5.00σ. The first two peaks, at 7.41σ and 6.30σ, are too close to water molecules. A negative peak at5.51σ is present in proximity of sulphate moiety of the HEPES molecule. The last peak at 5.43σ lies between the O atoms of C-terminal tails of the protein and one of its symmetry mates.	Clashscore 9.

Table S2. Rmsd obtained by superimposition of Ca of $[Rh_2(OAc)(tfa)_3]$/RNase A structures each other and with the structures of [*cis*-$Rh_2(OAc)_2(tfa)_2$]/RNase A adduct, $[Rh_2(OAc)_4]$/RNase A adduct and the metal-free protein.

	Crystal 1		Crystal 2		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	Molecule A	Molecule B	Molecule A	Molecule B
Crystal 1																
Molecule A	0	0.269	0.170	0.332	0.134	0.295	0.243	0.293	0.210	0.329	0.120	0.298	0.338	0.157	0.221	0.330
Molecule B		0	0.392	0.153	0.348	0.114	0.429	0.147	0.357	0.174	0.324	0.120	0.139	0.349	0.318	0.210
Crystal 2																
Molecule A			0	0.429	0.117	0.420	0.102	0.438	0.118	0.409	0.146	0.383	0.443	0.109	0.237	0.388
Molecule B				0	0.417	0.130	0.448	0.123	0.421	0.088	0.377	0.108	0.099	0.404	0.377	0.187

Table S3. Rh-containing fragments found in the three structures of Rh/protein adducts obtained upon reaction of $[Rh_2(OAc)(TFA)_3]$ 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.

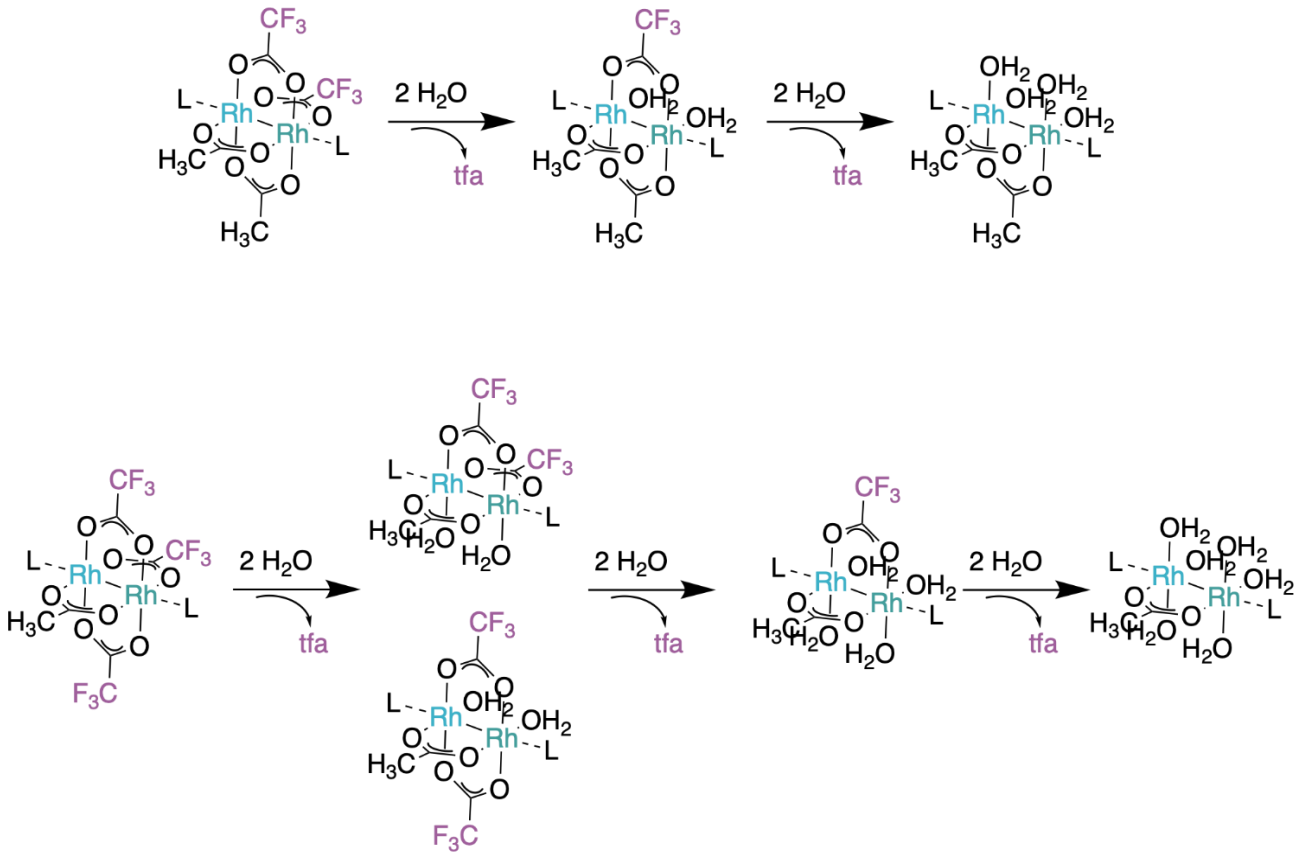
Rh/RNase A adduct binding site	Metal and Ligands in structure 1	Metal and Ligands in structure 2
His119 of molecule A(His in double conformation in structures 1 and 2)	Rh (0.40) Rh (0.40)	Rh (0.55) Rh (0.55)
His119 of molecule B (His in double conformation in structure 1 and 2)	Rh (0.20) (d.c.) Rh (0.20) (d.c.) Rh (0.40) (d.c.) Rh (0.40) (d.c.) H_2O (0.20) (d.c.) H_2O (0.40) (d.c.) H_2O (0.40) (d.c.) H_2O (0.40) (d.c.) H_2O (0.40) (d.c.)	Rh (0.55) (d.c.) Rh (0.55) (d.c.) Rh (0.30) (d.c.) Rh (0.30) (d.c.) H_2O (0.55) (d.c.) H_2O (0.55) (d.c.) H_2O (0.55) (d.c.) H_2O (0.55) (d.c.) H_2O (0.30) (d.c.)
His105 of molecule A	Rh (0.50) Rh (0.50) OAc (0.50) H_2O (0.50) H_2O (0.50) H_2O (0.50)	Rh (0.70) Rh (0.70) OAc (0.70) H_2O (0.70) H_2O (0.70) H_2O (0.70)
His105 of molecule B	/	/
Rh/HEWLadduct binding site	**Metal and Ligands in the structure**	
His15	Rh (0.25) Rh (0.25)	
Lys33	Rh (0.30) H_2O (0.30)	
Asp101	Rh (0.25) H_2O (0.25)	
Leu129	Rh (0.30) Rh*(0.30)	

d.c.=double conformation

Table S3. Geometric parameters of the Rh-containing fragments in the structures of Rh/RNase A adducts here refined.

		Rh/RNase A adducts			
Crystal		His119A	His119B	His105A	His105B
1	Rh—N_{ax}[a] (Å)	2.28	2.23	/	
	Rh—N_{eq} (Å)	2.19	/	2.03	
	Rh—Rh[a] (Å)	2.28	2.45	2.37	
	Rh—O_{OAc} (Å)	/	/	2.10	
	Rh—O_{wat}[a] (Å)	/	2.10	2.11	/
	O_{OAc}—Rh—N (°)	/	/	94.3	
	N_{ax}—Rh—Rh[a](°)	174.3	172.5	/	
	N_{eq}—Rh—Rh (°)	94.7	/	99.8	
	O_{wat}—Rh—Rh[a](°)	/	88.8	86.8	
2	Rh—N_{ax}[a] (Å)	2.31	2.16	/	
	Rh—N_{eq} (Å)	2.01	/	2.15	
	Rh—Rh[a] (Å)	2.53	2.47	2.29	
	Rh—O_{OAc} (Å)	/	/	2.13	
	Rh—O_{wat}[a] (Å)	/	2.10	2.12	/
	O_{OAc}—Rh—N (°)	/	/	89.6	
	N_{ax}—Rh—Rh[a](°)	170.7	171.4	/	
	N_{eq}—Rh—Rh (°)	92.5	/	100.4	
	O_{wat}—Rh—Rh[a](°)	/	90.6	83.9	
Structure of the $[Rh_2(OAc)_4)]$/RNase A adduct	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)	/	/	/	/
	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 0.01-0.08Å. Standard deviations for the angles are in the range of 1.2-8.8 °. 'wat' in the table refers only to water coordinating Rh atoms at equatorial positions.



Scheme S2. Hydrolytic pathway of [*cis*-$Rh_2(OAc)_2(tfa)_2$] and [$Rh_2(OAc)(tfa)_3$].

[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.
[2] Loreto, D.; Esposito, A.; Demitri, N.; Guaragna, A.; Merlino, A. Reactivity of a Fluorine-Containing Dirhodium Tetracarboxylate Compound with Proteins. *Dalton Trans.***2022**. **5**1,3695-3705.