Journal Name

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Supplementary information

Ru-based CO releasing molecules with azole ligands: interaction with proteins and CO release mechanism disclosed by X-ray crystallography

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b00000

Table S1. Data collection and refinement statistics for HEWL-Ru^{II}IM, HEWL-Ru^{II}MIM, RNase A-Ru^{II}IM, RNase A-Ru^{II}MIM and RNase A-Ru^{II}MBI structures

	HEWL-Ru^{II}IM First structure	HEWL-Ru^{II}IM Second structure	HEWL-Ru^{II}MIM Crystals grown using NaCl	HEWL-Ru^{II}MIM Crystals grown using ethylene glycol	RNase A-Ru^{II}IM	RNase A-Ru^{II}MIM	RNase A-Ru^{II}MBI
PDB code							
Crystallization conditions	1.1M NaCl, 0.1 M sodium acetate pH 4.4	1.1M NaCl, 0.1 M sodium acetate pH 4.0	1.1M NaCl, 0.1 M sodium acetate pH 4.0	20% Ethylene glycol, 0.1 M sodium acetate pH 4.0, 0.6 M sodium nitrate	18% PEG4K, 10 mM sodium citrate pH 5.1 Soaking: 4 days Ligand concentration: 5 mM	22% PEG4K, 10 mM sodium citrate pH 5.1 Soaking: 14 days Ligand concentration: 5 mM	22% PEG4K, 10 mM sodium citrate pH 5.1 Soaking: 3 days Ligand concentration: 5 mM
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$	C2	C2	C2
Unit cell parameters							
a,b,c (Å)	79.78, 79.78, 36.64	78.97, 78.97, 36.60	78.57, 78.57, 36.66	77.74, 77.74, 37.17	100.55, 32.67, 74.69	99.63, 32.77, 72.25	100.65, 32.98, 73.05
β (°)	90.0	90.0	90.0	90.0	90.5	90.0	90.2
Molecules per asymmetric unit	1	1	1	1	2	2	2
Observed reflections	51100	20497	46898	18791	46153	36034	50056
Unique reflections	9931	6694	9488	4846	14981	15576	21285
Resolution	56.41-1.88	55.84-2.10	55.56-1.85	54.97-2.38	74.68-2.07	72.25-1.98	73.05-1.82

(Å)	(1.91-1.88)	(2.14-2.10)	(1.88-1.85)	(2.42-2.38)	(2.11-2.07)	(2.01-1.98)	(1.85-1.82)
Completeness (%)	98.2 (100)	93.3 (93.3)	92.3 (86.3)	98.1 (97.9)	99.0 (94.2)	92.2 (81.9)	97.6 (95.4)
Rmerge†	0.091 (0.597)	0.057 (0.296)	0.048 (0.603)	0.099 (0.691)	0.085 (0.548)	0.103 (0.448)	0.072 (0.384)
I/σ(I)	13.3 (2.7)	11.4 (4.2)	36.8 (2.3)	15.6 (2.3)	9.5 (2.0)	8.2 (2.2)	6.3 (1.7)
Multiplicity	5.1 (3.2)	3.1 (2.8)	4.9 (3.0)	3.9 (3.6)	3.1 (2.5)	2.3 (1.7)	2.4 (1.4)
Refinement							
Resolution (Å)	56.41-1.88	55.84-2.10	55.56-1.85	54.97-2.38	74.68-2.07	72.251.98	73.051.82
number of reflections in working set	9428	6307	8981	4597	14224	14790	20184
number of reflections in test set	477	310	457	228	755	785	1091
R factor/Rfree (%)	17.5/24.2	15.3/23.4	16.0/23.2	18.8/30.8	18.1/27.2	17.6/27.9	16.5/23.6
Number of non-H atoms	1256	1234	1201	1147	2139	2385	2423
Number of Ru containing fragments	5	3	2	2	3	2	2
Occupancy of Ru atoms	0.8/0.5/0.3-0.3/0.3/0.5/0.3	0.8/0.5/0.3	0.8/0.4	0.4/0.6	0.4/0.4/0.6	0.7/0.7	0.6/0.5
B-factor of Ru atoms	38.6/54.8/52.1-52.5/58.5/50.0	44.2/51.0/46.4	43.4/56.7	55.1/49.1	54.4/52.7/52.8	51.5/61.8	44.0/58.8
Overall B-factor	32.20	33.79	33.26	40.28	42.10	30.53	30.82
Ramachandran values (%)							
Preferred/ Allowed/Outl	97.2/2.8/0.0	97.2/2.8/0.0	97.5/2.5/0.0	96.1/3.9/0.0	95.8/3.4/0.8	92.3/5.0/2.7	95.1/2.2/2.7

iers							
R.m.s.d. bonds(Å)	0.021	0.016	0.023	0.013	0.014	0.015	0.016
R.m.s.d. angles(°)	1.87	1.674	1.913	1.626	1.726	1.626	1.771

Table S2. Details of the structures of RuCORMs-HEWL adducts.

	HEWL-$[Ru(CO)_3Cl_2]_2$ Crosslinked crystals	HEWL-$[Ru(CO)_3Cl_2]_2$ Cross-linked crystals -followed by the reaction in deoxy-myoglobin solution	HEWL-ruthenium based CORM with a methione oxide ligand	HEWL-ruthenium based CORM with a pyridine ligand (7)	HEWL-ruthenium based CORM with a pyridine ligand (8)	HEWL-$[Ru(CO)_3Cl_2H_2NCH_2CO_2]$	**HEWL-Ru^{II}IM** **First structure**	**HEWL-Ru^{II}IM** **Second structure**	**HEWL-Ru^{II}MIM** **Crystals grown using NaCl**	**HEWL-Ru^{II}MIM** **Crystals grown using ethylene glycol**	HEWL-Ru^{II}MBI
PDB code	4W94	4W96	4UWN	4UWU	4UWV	2XJW					5E9R
Reference	Reference 13 of the main text	Reference 13 of the main text	Reference 15 of the main text	Reference 15 of the main text	Reference 15 of the main text	Reference 15 of the main text	This work	This work	This work	This work	Reference 18 of the main text
Resolution (Å)	1.55	1.50	1.67	1.78	1.77	1.67	1.88	2.10	1.85	2.38	2.25
Rfactor/ Rfree(%)	18.2/22.2	19.5/22.7	16.6/21.2	16.1/19.5	18.1/22.2	16.9/20.8	17.5/24.2	15.4/23.2	16.0/22.9	18.8/30.8	23.7/15.2
	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å²)) Ru ligands **Axial** Equat.
Lys13	Ru (0.30; 29.3)										
His15	Ru (0.75; 39.4) **CO** CO Wat Cl Cl	Ru (0.70; 38.2) **CO** CO Wat Cl Cl	Ru (1.00; 38.2) **Wat** CO Wat Wat Wat	Ru (0.80; 27.3) **Wat** CO COOH Wat Wat	Ru (0.70; 49.5) **Wat** CO Wat Wat Wat		Ru (0.8; 38.6) **CO** CO CO Wat Wat	Ru (0.8; 44.3) **CO** CO CO Cl Wat	Ru (0.8; 43.4) **CO** CO CO Wat Cl	Ru (0.5; 55.1) **Wat** CO CO Wat x	Ru (0.8; 46.2) **CO** CO Cl Wat Wat
							Anomalous peak:	Anomalous peak:	Anomalous peak:	Anomalous peak:	

							rmsd, e⁻/Å³ 10.8; 0.24	rmsd, e⁻/Å³ 5.4; 0.27	rmsd, e⁻/Å³ 4.2; 0.20	rmsd, e⁻/Å³ 3.1; 0.13	
Asp18	Ru (0.5; 25.7) **CO** CO Cl Cl Ru (0.5; 27.9) **CO** CO Wat Cl	Ru (0.5; 29.5) Ru (0.5; 28.1) Cl	Ru (0.65; 32.6) **Wat** CO Wat Wat	Ru (0.50; 23.1) **Wat** Wat Wat Ru (0.50; 25.4) **Wat** Wat Wat	Ru (0.50; 41.7) **Wat** CO CO Wat Wat		Ru (0.5; 54.8) **Wat** CO Wat Wat Wat	Ru (0.5; 51.0) **Wat** CO CO Wat Wat	Ru (0.4; 56.7) **Wat** CO Wat Wat Wat		
							Anomalous peak: rmsd, e⁻/Å³ 3.7; 0.08	Anomalous peak: rmsd, e⁻/Å³ 2.9; 0.14	Anomalous peak: rmsd, e⁻/Å³ 2.6; 0.13		
Asp52	Ru (0.35; 29.4) **Wat** Wat Wat	Ru (0.3; 22.5) **x** Wat Wat Wat	Ru (0.5; 32.2) **Wat** Wat Wat	Ru (0.40; 42.1) **x** CO Wat Wat	Ru (0.40; 32.7) **Wat** CO CO Wat Wat	Ru (0.40; 32.7) **Wat** CO CO Wat Wat	Ru A/B (0.3/0.3; 52.1/52.5) **Wat**				
							Anomalous peak: rmsd, e⁻/Å³ 3.1; 0.07				
Asp101	Ru (0.10; 33.7) Ru (0.10; 26.9)	Ru (0.1; 25.5) Ru (0.1; 45.5)		Ru (0.70; 22.5) **x** Wat Wat	Ru (0.65; 56.9) **Wat** Wat Wat						

				Wat Ru (0.70; 21.8) **Wat** Wat Wat Wat	Wat Ru (0.65; 81.0) Wat						
Asp119	Ru (0.5; 42.4)	Ru (0.3; 31.8) Cl	Ru (0.5; 51.3) **Wat** Wat Wat	Ru (0.70; 23.1) **Wat** CO Wat Wat Wat			Ru (0.5; 58.5) **CO** CO	Ru (0.3; 42.3) Wat		Ru (0.6;49.1) **CO** CO CO Wat OE1 Gln121	
							Anomalous peak: rmsd, $e^-/Å^3$ 3.5; 0.08	Anomalous peak: rmsd, $e^-/Å^3$ -		Anomalous peak: rmsd, $e^-/Å^3$ 4.8; 0.20	
Leu129	Ru (0.5; 43.7)	Ru (0.3; 27.4) Ru (0.5; 29.9)					Ru (0.3; 58.5) Wat Wat Wat				
							Anomalous peak: rmsd, $e^-/Å^3$ 4.0; 0.09				

Table S3. Root Mean Square Deviations values (Å) computed on CA atoms among HEWL adducts formed with $Ru^{II}IM$, $Ru^{II}MIM$ and $Ru^{II}MBI$.

	HEWL-$Ru^{II}IM$ First structure	HEWL-$Ru^{II}IM$ Second structure	HEWL-$Ru^{II}MIM$ Crystals grown using NaCl	HEWL-$Ru^{II}MIM$ Crystals grown using ethylene glycol
HEWL-$Ru^{II}IM$ First structure	-	0.12	0.18	0.30
HEWL-$Ru^{II}IM$ Second structure		-	0.14	0.28
HEWL-$Ru^{II}MIM$ Crystals grown using NaCl			-	0.28
HEWL-$Ru^{II}MIM$ Crystals grown using ethylene glycol				-

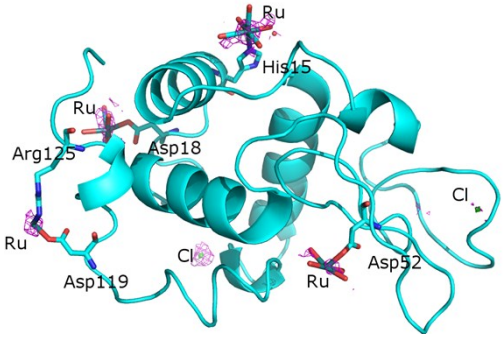
Table S4. Root Mean Square Deviations values (Å) computed on CA atoms among RNase A adducts formed with Ru^{II}IM Ru^{II}MIM and Ru^{II}MBI.data were compared also with the two RNase A molecules in the structure of the ligand-free RNase A solved from isomorphous crystals.

	RNase A-Ru^{II}IM Chain A	RNase A-Ru^{II}IM Chain B	RNase A-Ru^{II}MIM Chain A	RNase A-Ru^{II}MIM Chain B	RNase A-Ru^{II}MBI Chain A	RNase A-Ru^{II}MBI Chain B	Ligand-free RNase A Chain A (1JVT)	Ligand-free RNase A Chain B (1JVT)*
RNase A-Ru^{II}IM Chain A	-	0.47	0.37	1.18	0.38	0.59	0.48	0.49
RNase A-Ru^{II}IM Chain B		-	0.60	1.04	0.52	0.36	0.53	0.29
RNase A-Ru^{II}MIM Chain A			-	1.22	0.22	0.66	0.42	0.54
RNase A-Ru^{II}MIM Chain B				-	1.18	1.00	1.19	1.12
RNase A-Ru^{II}MBI Chain A					-	0.58	0.34	0.44
RNase A-Ru^{II}MBI Chain B						-	0.61	0.44
Ligand-free RNase A Chain A							-	0.48
Ligand-free RNase A Chain B								-

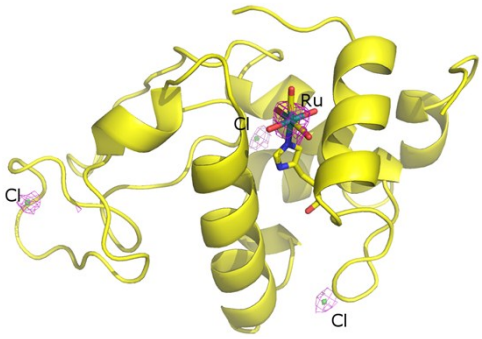
*from reference 36 of the main text.

Table S5. Details of the structures of RuCORMs-RNase A adducts.

	RNase A-Ru^{II}IM	RNase A-Ru^{II}MIM	RNase A-Ru^{II}MBI
Resolution (Å)	2.07	1.98	1.82
Rfactor/Rfree(%)	18.1/27.2	17.6/27.9	16.5/23.6
	Ru (Occupancy; B-factor (Å^2)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å^2)) Ru ligands **Axial** Equat.	Ru (Occupancy; B-factor (Å^2)) Ru ligands **Axial** Equat.
His105 mol A	Ru (0.7, 61.8) **x** CO CO Wat x	Ru (0.7, 61.8) **x** CO CO Wat x	Ru (0.5, 58.8) **x** CO Wat Wat x
	Anomalous peak: rmsd, e^-/Å^3 4.8; 0.20	Anomalous peak: rmsd, e^-/Å^3 .	Anomalous peak: rmsd, e^-/Å^3 2.4; 0.28
His119 mol B	Ru (0.6, 52.8) **wat** CO Wat Wat x	Ru (0.7, 51.5) **CO** CO CO Wat x	Ru (0.6, 44.0) **CO** CO CO Wat x
	Anomalous peak: rmsd, e^-/Å^3 3.5; 0.14	Anomalous peak: rmsd, e^-/Å^3 -	Anomalous peak: rmsd, e^-/Å^3 5.8; 0.68
His105 mol B	Ru (0.4, 52.7) **x** Wat Wat Wat x		
	Anomalous peak: rmsd, e^-/Å^3 3.2; 0.13		



A



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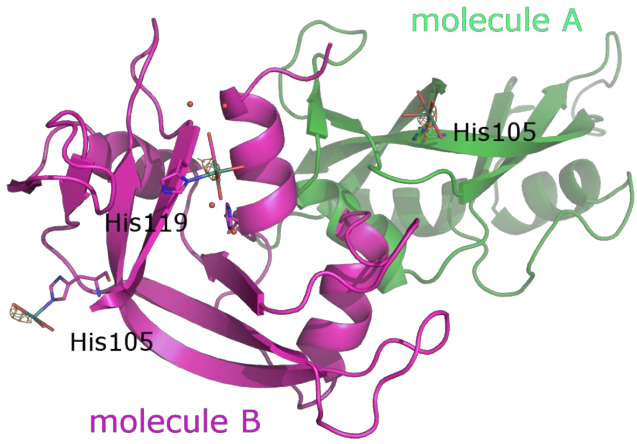

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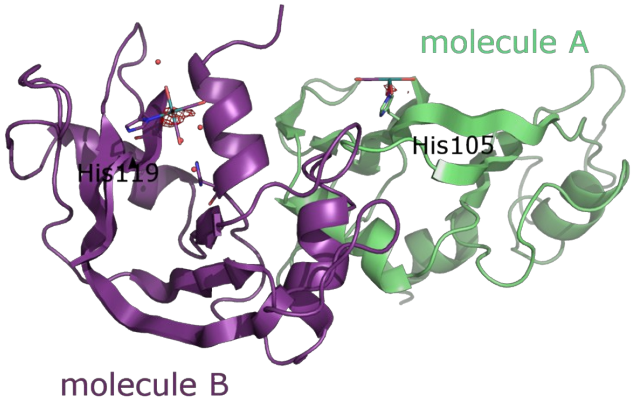

D

Figure S1. Anomalous maps for HEWL-Ru^{II}IM (A, 3.0σ level), HEWL Ru^{II}MIM (B, 3.0σ level), RNase A-Ru^{II}IM (C, 2.5σ level), RNase A-Ru^{II}MBI (D, 2.5σ level),