Electronic Supporting Information

Ruthenium metallation of proteins: X-ray structure and Raman microspectroscopy of the complex between RNase A and AziRu

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Crystallization procedures

RNase A crystals were obtained at 20 °C as previously described [1]. Briefly, crystals were grown by the hanging drop vapor diffusion method by mixing 1μL of 20 mg/mL unbuffered protein with 1μL of reservoir solution containing 20% PEG4K and 20 mM sodium citrate buffer pH 5.5. Crystals appeared within 5-7 days. Crystals were soaked in a saturated solution of AziRu containing 22% PEG4K in the same buffer. After a few hours, crystals changed their colour to yellow and then to brown. The soaking produced cracks on the crystal surface that did not prevent data collection (Figure S1).

Data collections, structure solution and refinements

1.65 Å resolution X-ray diffraction data were collected from ruthenated RNase A crystals at the CNR Institute of Biostructure and Bioimages, Naples, Italy, using a Saturn944 CCD detector equipped with CuKα X-ray radiation from a Rigaku Micromax 007 HF generator. Data were collected on one single crystal flash-frozen in a supercooled nitrogen gas produced by an Oxford Cryosystem Cryostream and maintained at 100 K during the data collection. 1 μl of 50% glycerol solution was added as cryoprotectant to the drop the crystal was grown in. Data were processed and scaled using HKL2000 [2]. Details of data collection statistics are reported in Table S2. Crystals contain two molecules in the asymmetric unit. For reference, the two molecules in the asymmetric unit are designated A and B.

The structure was analyzed by the difference Fourier technique, using the PDB file 1JVT [3] as the starting model. The refinement was carried out using CNS [4]; model building and map inspections were manually performed using O [5]. Several rounds of refinement, energy minimization, individual temperature factor refinement and manual model building were performed. Water molecules, AziRu moieties and multiple conformations of the protein residue side chains were inserted into the model at positions corresponding to peaks in the Fo–Fc electron density maps. Refinement statistics were reported in Table S2. The r.m.s. deviations of the bond distances and angles from the target values are 0.005 Å and 1.86°, respectively. 100% of protein residues fall into core or allowed regions of the Ramachandran plot as defined by Procheck [6]. Coordinates and structure factors have been deposited in the Protein Data Bank (PDB code 4L55).

Raman microscopy

Raman microscopy spectra on RNase A crystals were collected at the Department of Chemical Sciences of University of Naples 'Federico II', using an apparatus described elsewhere [7]. Raman spectra of AziRu powder were collected as reference as well. The excitation line was 645 nm, with a power at the sample of 2 mW. Spectra resolution was 4 cm^{-1}, exposure time 100s.

RNase catalytic assay

The activity of RNase A and of its complex with an excess of AziRu towards yeast RNA was determined by the spectrophotometric assay of Kunitz [8], as previously done in other works [9-10], using 0.5 mg/ml of RNA in 0.050 M sodium acetate/acetic acid buffer, pH 5.2, at 25°C. Enzyme concentration was 0.5 μg/ml. Specific activity values were calculated as Kunitz units/mg protein. Spectrophotometric measurements were performed with a Jasco spectrophotometer. After 24 h of incubation with a large excess of AziRu, the protein is almost completely inactivated.

Table S1. X-ray structure of ruthenated-proteins deposited in the Protein Data Bank*

PDB code	Protein	Ru containing ligand	Resolution (Å)	Ruthenium coordination	Coordinating Atoms	Reference
2XJW	Lysozyme	*fac*-Ru(CO)3Cl	1.67	octahedral	NE2(His15), 2CO, $3H_2O$	[11]
1T3P	Lysozyme	$RuCl_2$(cymene)	1.60	tetrahedral	NE2(His15), $2Cl^-$, C(arene)	[12]
3MNN	Histone H4	Ru ion	2.50	tetrahedral	OE2(Glu63), NZ(Lys59), C(arene), P	[13]
1JZE	Azurin	$Ru(bpy)_2$(im)	1.60	octahedral	NE2(His83), 5N	[14]
1JZF	Azurin	Ru(tpy)(phen)	1.50	octahedral	NE2(His83), 5N	[14]
1JZG	Azurin	Ru(tpy)(phen)	1.40	octahedral	NE2(His83), 5N	[14]
1JZH	Azurin	Ru(tpy)(bpy)	1.70	octahedral	NE2(His83), 5N	[14]
3FXS	Thermolysin	Ru ion	1.55	octahedral	NE2(His142), OE2(Glu143), OE2(Glu166), $3H_2O$	[to be published]
3M1J	Carbonic anhydrase 2	Ru ion	1.80	octahedral	ND1(His64), O(Asn62), $4H_2O$	[15]
3DD3	Glutathione S-transferase P	Ru(eta6-benzene)	2.25	tetrahedral	SG(Cys101A), SG(Cys101B), C (arene), H_2O	[16]
3M0B	Methyl-accepting chemotaxis protein	Ru (mesoporphyrin IX)	2.00	octahedral	NE2(His102), 4N, CO	[17]

3O7R	Ferritin light chain	Ru ion	1.90	octahedral	NE2(His114), OE2(Glu130), SG(Cys126), $3H_2O$	[18]
3O7S	Ferritin light chain	Ru ion	1.73	octahedral	NE2(His114), SG(Cys126), $4H_2O$	[18]
1BEX	Azurin	$Ru(bpy)_2(im)$	2.30	octahedral	NE2(His83), 5N	[19]
4J1A	Lysozyme	sodium trans-(dimethylsulfoxide)-pyridine tetrachlorido-ruthenate(III)	1.79	octahedral	ND1(His15), OD1(Asp17), $3H_2O$, Cl	[20]
4J1B	Lysozyme	sodium trans-(dimethylsulfoxide)-pyridine tetrachlorido-ruthenate(III)	1.66	octahedral	ND1(His15), OD1(Asp17), $4H_2O$	[20]

*This represents the entire dataset of solved protein structures with Ru bound to the protein *via* a coordinative bond with at least one residue side chain.

Table S2. Data collection and refinement statistics for all analyzed crystals.

PDB code	4L55
Crystal colour	Yellow/brown
Data-collection temperature (K)	100
Data reduction	
Space group	C2
Unit-cell parameters a (Å), b (Å), c (Å); β (°)	100.10, 32.53, 72.48; 90.41
Molecules per asymmetric unit	2
Observed reflections	78519
Unique reflections	27646
Resolution (Å)	50.0-1.65 (1.68-1.65)
Completeness (%)	96.4 (78.8)
Rmerge†	0.099 (0.401)
I/σ(I)	52.3 (14.7)
Multiplicity	3.9 (1.9)
Refinement	
Number of reflection used (I>2σ)	24260
Number of reflection in the working set	23075
Number of reflection in the test set	1185
R factor/Rfree (%)	0.203/0.245
Number of protein atoms	1988
Number of Aziru moieties	2
Number of water molecules	232
Occupancy of AziRu moieties	0.5
Average B-factor for main chain atoms	25.89
Average B-factor for side chain atoms	27.15
Average B-factor for Ru atoms	39.53
Average B-factor for solvent atoms	32.35
Ramachandran values (%)	

Most favoured/ Allowed Generously allowed/ Disallowed	86.4/13.6 0/0
R.m.s.d. bonds(Å)	0.005
R.m.s.d. angles (°)	1.86

Values in parenthesis correspond to the last resolution shell.

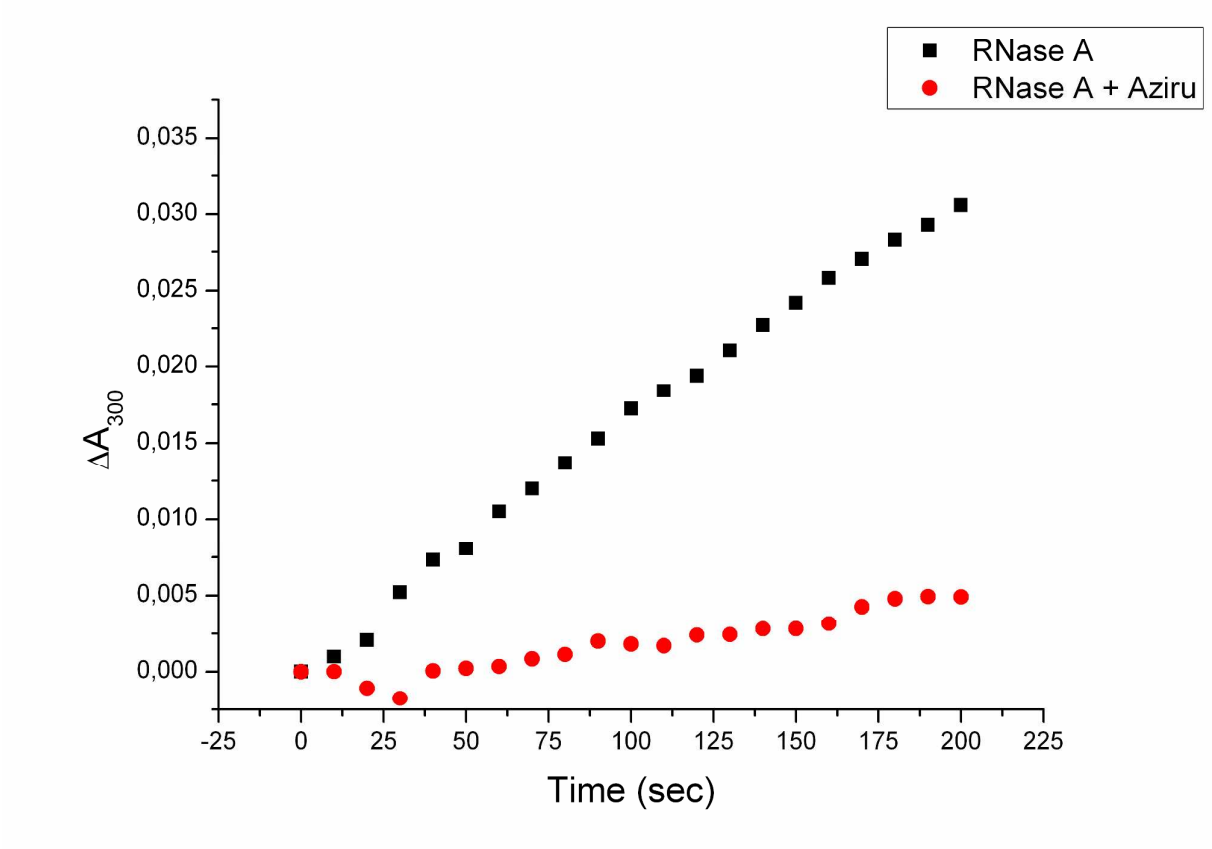

Figure S1. Hydrolysis of yeast RNA by RNase A in the absence (■) and in the presence (●) of 10 mM AziRu

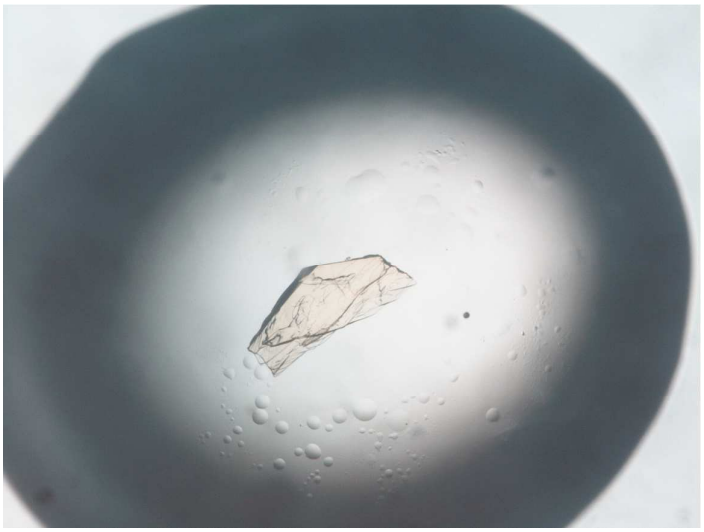

Figure S2 Crystal of RNase A upon soaking in saturated solutions of AziRu. The crystals changed from colourless to yellow/brown in 2-5 days.

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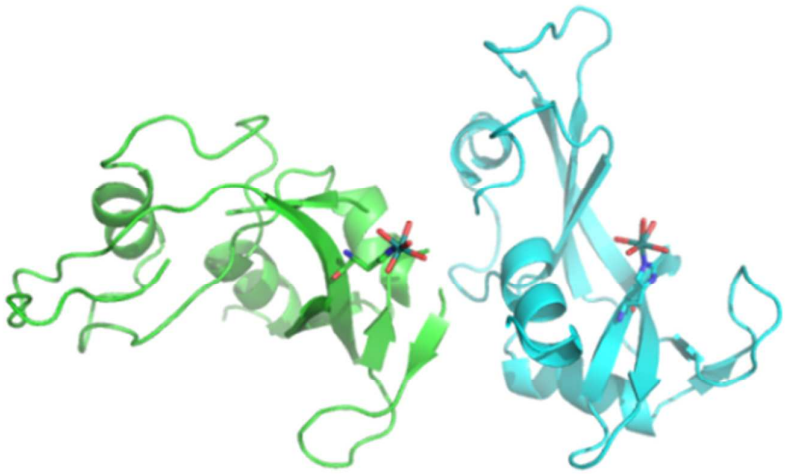
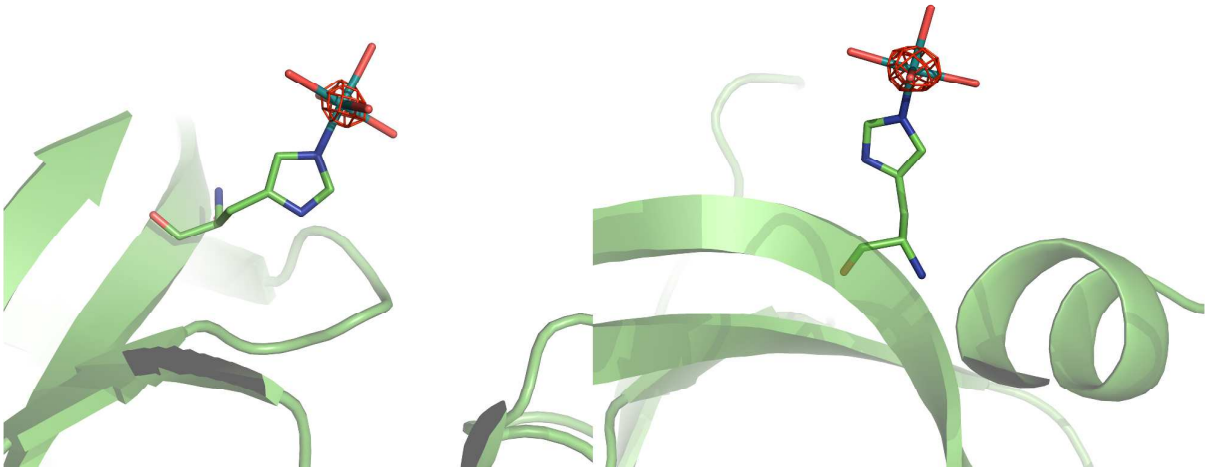


Figure S3. Full view of the asymmetric unit of Ru-metallated RNase A crystals



A **B**

Figure S4. Anomalous electron density maps contoured at 3.0 σ in molecule A (A) and molecule B (B)

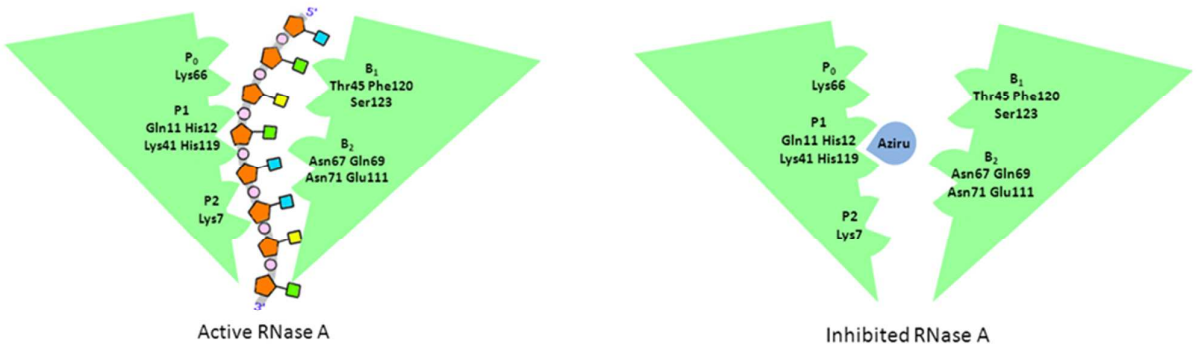


Figure S5. Schematic representation of RNase A inhibition by AziRu. The active site of RNase A is constituted by multiple subsites that bind the phosphate (pink circles), base (green, cyan, yellow squares), and ribose (orange pentagons) components of RNA. Subsites of the ribonucleolytic center of RNase A are represented in the figure: B_1 and B_2 are base-binding sites, P_0, P_1 and P_2 are phosphate-binding sites. The major residues that constitute each subsite are also indicated. AziRu binds in the P1 subsite.

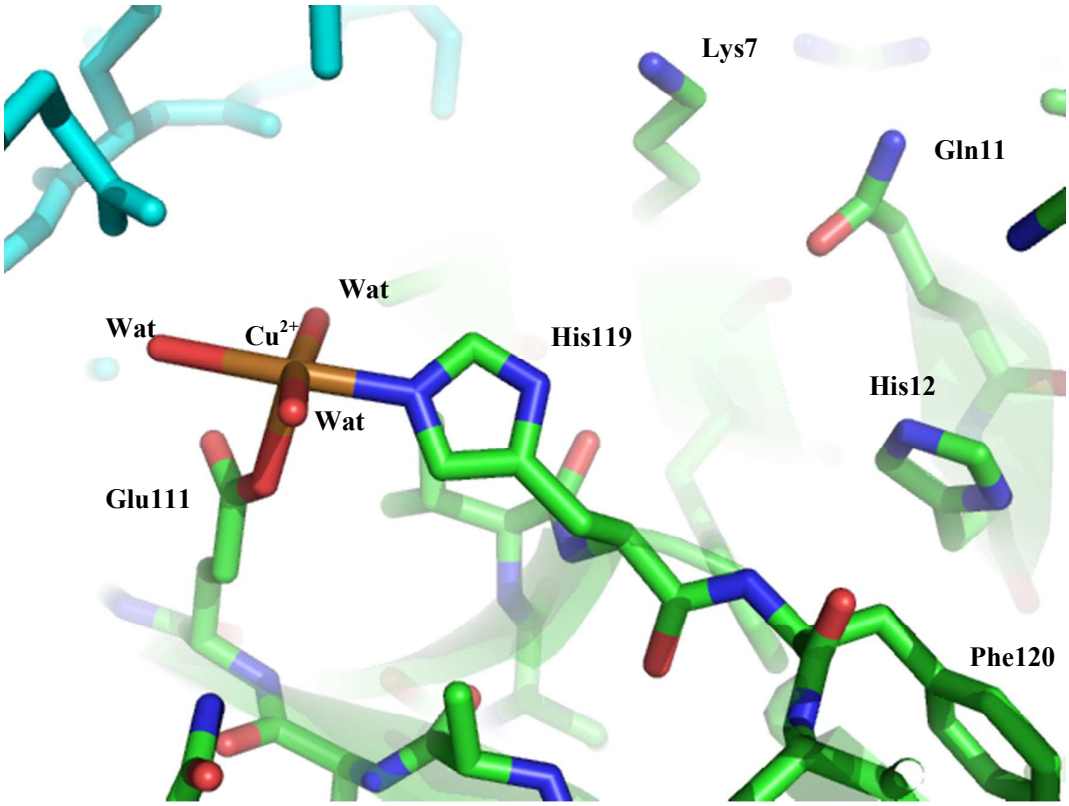


Figure S6. Cu^{2+} binding site in the active site of RNase A (PDB code 1AQP) [21]. Residues belonging to a symmetry related molecule are depicted in cyan.

Supplementary material references

[1] L. Vitagliano, A. Merlino, A. Zagari, L. Mazzarella. Protein Sci. 2000, 9, 1217
[2] Z. Otwinowski, W. Minor, Methods Enzymol.1997, 276, 307.
[3] L. Vitagliano, A. Merlino, A. Zagari, L. Mazzarella. Proteins. 2002, 46, 97.
[4] A.T. Brunger, P.D. Adams, G.M. Clore, P. Gros, R.W. Grosse-Kunstleve, J.S. Jiang, J. Kuszewski, N. Nilges, N.S. Pannu, R.J. Read, L.M. Rice, T. Simonson, G.L. Warren, Acta Cryst. D, 1998, 54, 905.
[5] T.A. Jones, J.Y. Zou, S.W. Cowan, M. Kjedgaard, ActaCryst. D, 1991, 56, 714.
[6] R.A. Laskowski, M.W. Macarthur, D.S. Moss, J.M. Thornton, J. Appl. Cryst.,1993, 26, 283.
[7] A. Vergara, A. Merlino, E. Pizzo, G. D'Alessio, L. Mazzarella L, Acta Cryst D, 2008, 64, 167.
[8] M. Kunitz, J. Biol. Chem., 1946, 164, 563-568.
[9] G. Gotte, L. Testolin, C. Costanzo, S. Sorrentino, U. Armato,M. Libonati. FEBS Letters 415 (1997) 308-312
[10] A. Merlino, D. Picone, C. Ercole, A. Balsamo, F. Sica. Biochimie., 2012, 94, 1108-18.
[11] T. Santos-Silva, A. Mukhopadhyay, J. D. Seixas, G. J. L. Bernardes, C. C. Romao, M. J. Romao, J. Am. Chem. Soc., 2011, 133, 1192.
[12] I. W. McNae, K. Fishburne, A. Habtemariam, T. M. Hunter, M. Melchart, F. Wang, M. D. Walkinshaw, P. J. Sadler, Chem. Commun. (Camb), 2004, 16, 1786.
[13] B. Wu, M. S. Ong, M. Groessl, Z. Adhireksan, C. G. Hartinger, P. J. Dyson, C. A. Davey, Chem. Eur. J, 2011, 17, 3562.
[14] B. R. Crane, A. J. Di Bilio, J. R. Winkler, H. B. Gray, J. Am. Chem. Soc., 2001, 123, 11623.
[15] A. Casini, C. Temperini, C. Gabbiani, C. T. Supuran, L. Messori, ChemMedChem, 2010, 5, 1989.
[16] W. H. Ang, L. J. Parker, A. De Luca, L. Juillerat-Jeanneret, C. J. Morton, M. Lo Bello, M. W. Parker, P. J. Dyson, Angew. Chem. Int. Ed. Engl., 2009, 48, 3854.
[17] M. B. Winter, E. J. McLaurin, S. Y. Reece, C. Olea, D. G. Nocera, M. A. Marletta, J. Am. Chem. Soc., 2010, 132, 5582.
[18] Y. Takezawa, P. Bockmann, N. Sugi, Z. Wang, S. Abe, T. Murakami, T. Hikage, G. Erker, Y. Watanabe, S. Kitagawa, T. Ueno, Dalton Trans., 40, 2190.
[19] S. Faham, M. W. Day, W. B. Connick, B. R. Crane, A. J. Di Bilio, W. P. Schaefer, D. C. Rees, H. B. Gray, Acta Crystallogr. Sect. D, 55, 379.
[20] A. Vergara, G. D'Errico, D. Montesarchio, G. Mangiapia, L. Paduano, A. Merlino, Inorg Chem 2013, 52,4157-9.
[21] R. Balakrishnan, N. Ramasubbu, K.I. Varughese, R. Parthasarathy. Proc Natl Acad Sci USA 1997, 94, 9620.