SUPPLEMENTARY INFORMATION SECTION

RNA Binding and Inhibition of Primer Extension by a Ru(III)-Pt(II) Metal Complex

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EXPERIMENTAL DETAILS

Metal Complexes

CDDP (cis-diammine-dichloro-platinum *aka* cisplatin) was purchased from Sigma-Aldrich (Cat No. P4394) and used without further purification. The ruthenium (III) complex NAMI-A was prepared according to previously published protocols.[1] AH197 was prepared as recently described.[2] Stock solutions of each metal complex were prepared in deionized water immediately before usage.

Cell Culture Assays

Saccharomyces cerevisiae cells (strain BY4741) from a single colony were grown overnight in YEPD medium at 30°C with mild shaking at 220 rpm. Cells from the overnight culture were diluted 20-fold in Synthetic Complete (SC) medium and grown at 30°C with shaking until OD_{600} was ~ 0.1. SC medium was prepared by dissolving 10 g of glucose, 3.4 g of Yeast Nitrogen base, and 1 g of Dropout mix in 500 mL of deionized water. The solution was autoclaved and stored at 4°C until further use. The Dropout mix contains all 20 amino acids as well as uracil and adenine sulfate. Stock solutions of cisplatin, NAMI-A, and AH197 were freshly prepared in water. Metal complexes were added to the yeast cells at desired final concentrations. The samples were grown in dark with shaking at 30°C for 14 hours. All of the samples including control were run in triplicates. Absorbance at 600 nm was measured after 14-hour incubation period using an Agilent UV/Vis spectrophotometer. Absorbance was used to quantify growth of yeast where A_{600} = 1.0 corresponds to a cell density of 2.0 x 10^7 cells/mL. Each sample was measured against a blank containing the SC medium and the specific metal complex.

RNA Mobility Shift Assays

A 409-bp DNA fragment from the genome of *Tetrahymena thermophila* was PCR amplified using plasmid DNA (courtesy of Prof. Dan Herschlag, Stanford). DNA primers were engineered to contain a T7 promoter region at the 5'-end of the amplified DNA template. The 388-base RNA fragment was synthesized using T7 RNA Polymerase and purified using gel electrophoresis. Purified RNA was dephosphorylated at the 5'-end using Calf Intestinal Phosphatase (CIP) enzyme purchased from Life Technologies. Subsequent to the removal of CIP enzyme, RNA was labeled at the 5'-end using ATP Gamma ^{32}P (Perkin Elmer) and T4 Polynucleotide Kinase enzyme (Life Technologies). After purification, radiolabeled RNA was incubated in 10 mM phosphate buffered saline (pH 7.1) at a final concentration of 1 μM for 1 hr at 25°C in the presence of varying concentrations of cisplatin, NAMI-A, and AH197. RNA samples were resolved on 5% denaturing polyacrylamide gels. After electrophoresis, gels were exposed overnight to a Phosphor screen and visualized using STORM 825 scanner (GE Healthcare) equipped with ImageQuant TL software.

Inhibition of DNA Synthesis

50 picomoles of unlabeled RNA (see above for RNA Mobility Shift Assays) was incubated in 10 mM phosphate buffered saline (pH 7.1) with varying concentrations of cisplatin, NAMI-A, and AH197 for 1 hour at 25°C. A 12-base DNA primer (5'- GCA ATT TGA CGG - 3') was radiolabeled at the 5'-end using ATP Gamma ^{32}P. The DNA primer is complementary to a 12-base region that is 89 bases from the 5'-end of the RNA template. Labeled DNA primer was annealed in a 1:1 ratio (50 picomoles) to the RNA template. Full extension of this primer yields a product that is 89 bases in length. AMV reverse transcription kit (Promega) was used to generate DNA strands by extending the DNA primer on the RNA template. Reverse transcription reactions were run for 15 minutes at 42°C. Samples were resolved on a 10% denaturing polyacrylamide gel. Gels were dried and imaged as described above.

Nucleic Acid Sequences

Primer DNA

5'- GCA ATT TGA CGG - **3'**

Tetrahymena thermophila Group 1 Intron RNA

5' – GGA GGG AAA AGU UAU CAG GCA UGC ACC UGG UAG CUA GUC UUU AAA CCA AUA GAU UGC AUC GGU UUA AAA AGG CAA GAC CGU CAA AUU GCG GGA AAG GGG UCA ACA GCC GUU CAG UAC CAA GUC UCA GGG GAA ACU UUG AGAUGG CCU UGC AAA GGG UAU GGU AAU AAG CUG ACG GAC AUG GUC CUA ACC ACG CAG CCA AGU CCU AAG UCA ACA GAU CUU CUG UUG AUA UGG AUG CAG UUC ACG ACU AAA UGU CGG UCG GGG AAG AUG UAU UCU UCU CAU AAG AUA UAG UCG GAC CUC UCC UUA AUG GGA GCU AGC GGA UGA AGU GAU GCA ACA CUG GAG CCG CUG GGA ACU AAU UUG UAU GCG AAA GUA UAU UGA UUA GUU UUG GAG U – **3'**

* Underlined region is the Primer DNA binding site

REFERENCES

(1) Mestroni, G., Alessio, E., and Sava, G. New salts of anionic complexes of Ru(III), as antimetastatic and antineoplastic agents, World Intellectual Property Organization, I. B., 1998, WO 98/00431.

(2) Herman, A.; Tanski, J. M.; Tibbetts, M. F.; Anderson, C. M. *Inorg. Chem.* **2008**, *47*, 274.

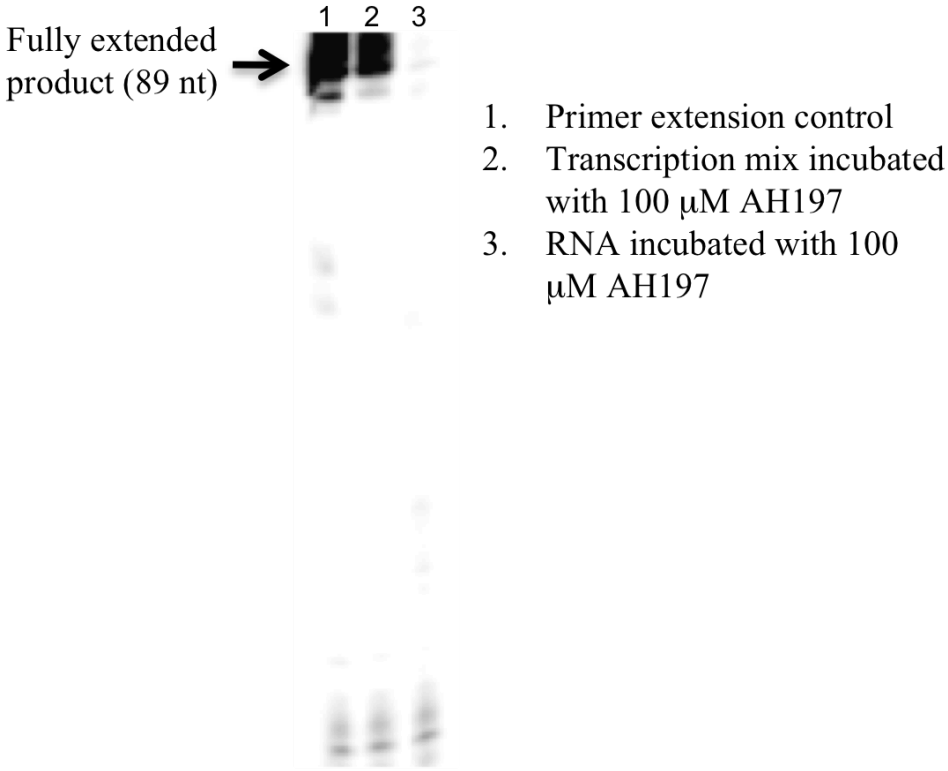
Table S1: RNA band intensity values as a function of AH197 concentration.

[AH197] μM	Counts *	Norm. Counts
0	612833	1.00
50	609738	0.99
100	636510	1.04
250	587004	0.96
500	595012	0.97

* Band intensity counts after blank subtraction

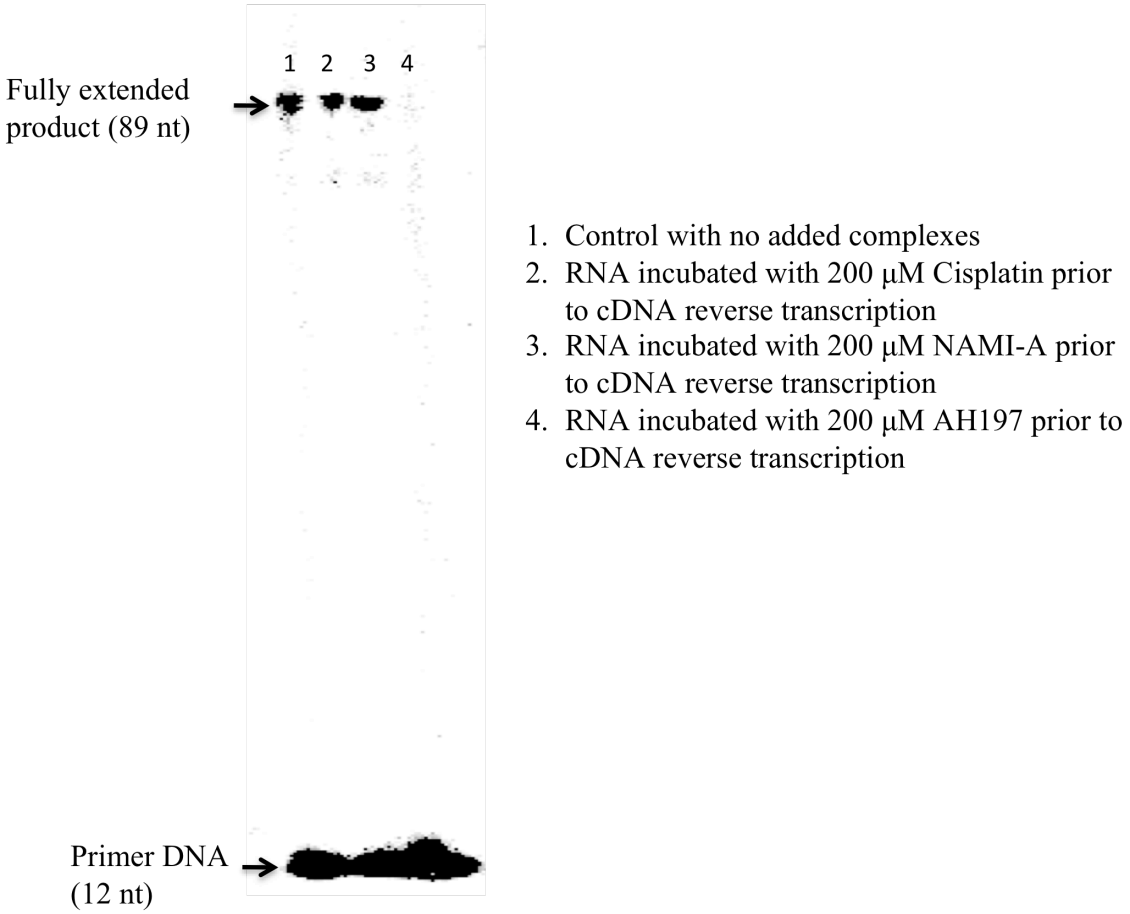
Explanation: Quantified band intensity values from the mobility shift gel in Figure 3 of the manuscript. The slight differences in counts are most likely a result of gel loading variation. These results illustrate that there is no RNA degradation that takes place upon incubation of RNA with AH197 metal complex.

Figure S2: Reverse Transcriptase Primer DNA Extension Controls



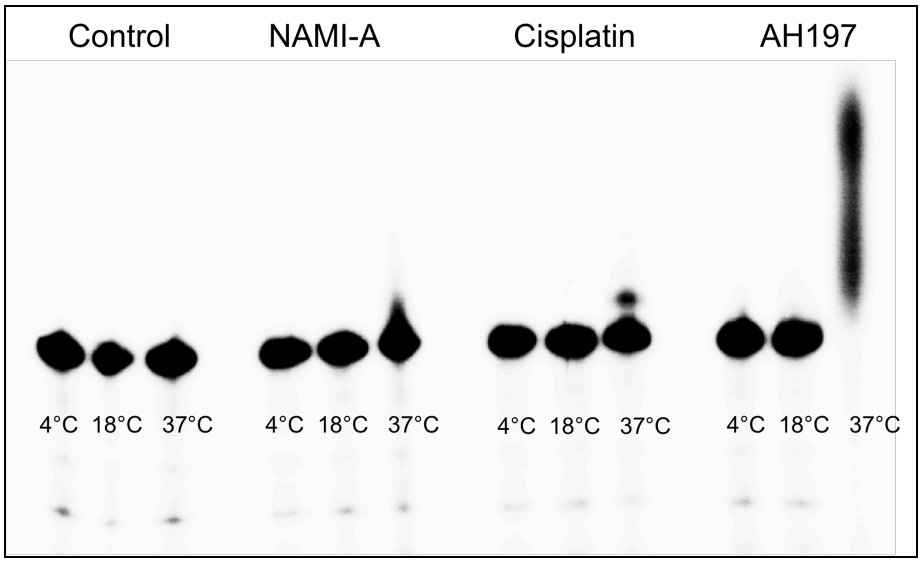
Explanation: Lane 3 shows near complete inhibition of DNA synthesis when AH197 is incubated for 1 hour with the RNA template prior to the DNA synthesis reaction, which is run for 15 minutes. Lane 1 is a control reaction, which shows formation of full length DNA product. Lane 2 is a reaction where AH197 was incubated with **reverse transcriptase enzyme and DNA primer** for 15 minutes prior to incubation with free template RNA during the primer extension reaction. DNA synthesis reaction is run for 15 minutes. Lane 2 shows a slightly lower intensity band (lower amount of product is formed) in comparison to lane 1. This is to be expected because AH197, even during the 15 minutes of incubation that occurs during the extension reaction, has opportunity to bind to RNA and thereby inhibit DNA synthesis when compared to lane 1.

Figure S3: Modified Reverse Transcriptase Assay



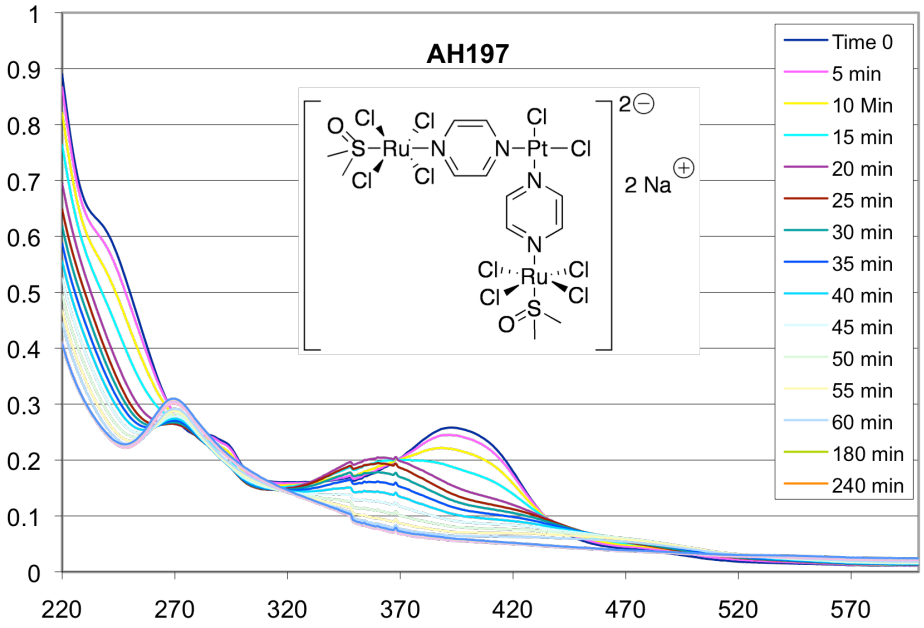
Explanation: RNA samples were incubated with 200 μM metal complex for 1 hr in 10 mM phoshphate buffer saline (pH 7.1). The samples were column purified to remove unbound metal complex and quantified via UV @ 260 nm. Equimolar concentration of RNA was used to carry out the reverse transcription assays with a 12-base radiolabeled DNA primer. Inhibition of DNA synthesis is observed only in the presence of AH197. This result is consistent with the data shown in Figure 4 of the manuscript. Our modified assay ensures that free metal complexes are not available to bind to DNA or the reverse transcriptase enzyme and that the inhibition that is observed is primarily due to AH197 binding to RNA, which acts as a block to primer DNA synthesis.

Figure S4: Metal complex binding to RNA is temperature dependent.



Explanation: 5'- radiolabeled RNA was incubated with 250 μM Cisplatin, NAMI-A, and AH197 for 1 hr in 10 mM phosphate-buffered saline (pH 7.1). The incubation reactions were carried out at 4°C, 18°C, and 37°C (physiological temperature). Control samples depict no change in mobility or degradation of RNA. Our data shows that RNA mobility is dependent on temperature with the most significant gel shift occurring at physiological temperature for each metal complex. Furthermore, AH197 is substantially more effective than Cisplatin and NAMI-A in reducing gel mobility at 37°C.

Figure S5A: UV/vis spectra of AH197 and AH403 in PBS as a function of time (Abs vs. wavelength).



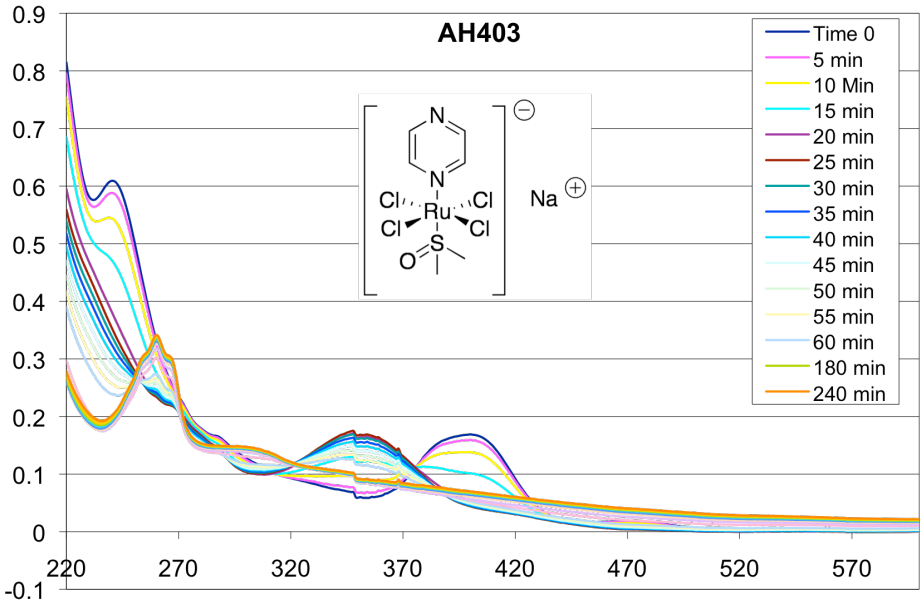
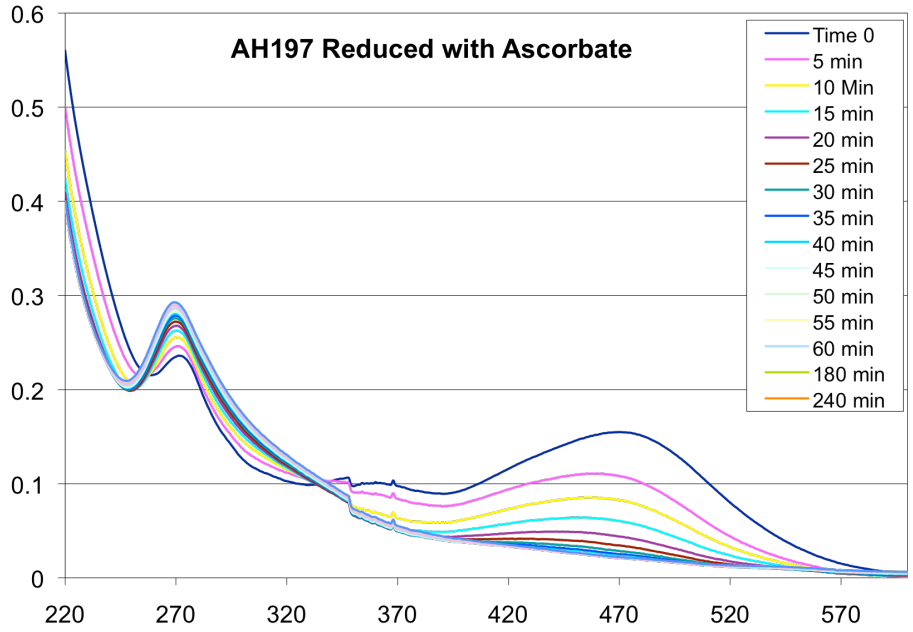


Figure S5B: UV/vis spectra of AH197 and AH403 in PBS as a function of time after reduction with ascorbate (Abs vs. wavelength).



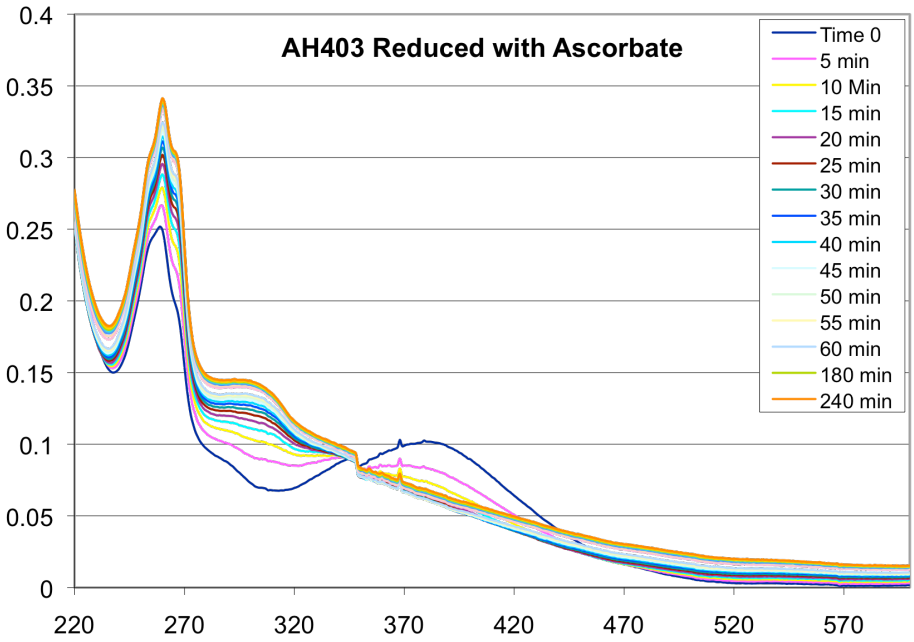
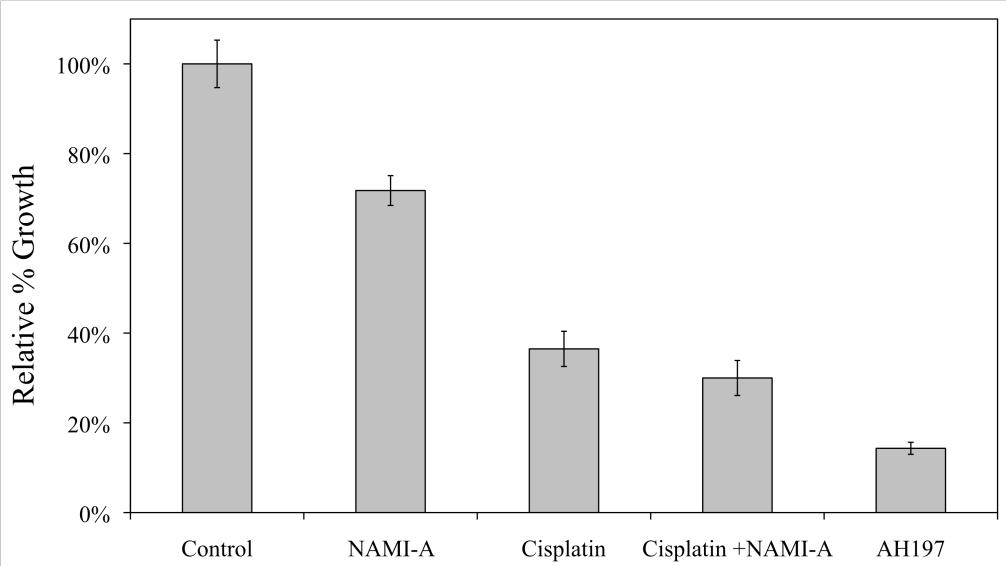


Figure S6: Yeast growth inhibition data in the presence of metal complexes



Explanation: Yeast cells were grown for 14 hrs at 30°C in the presence of 500 μM metal complexes. In the sample labeled Cisplatin + NAMI-A, 500 μM Cisplatin + 1 mM NAMI-A was used. These concentrations ensure that the concentration of metal centers present in this sample is equal to the AH197 sample. Our data shows that this samples has similar inhibition to the sample containing 500 μM Cisplatin alone but the growth is significantly higher than AH197 sample. It is too simplistic to assume that the inhibition observed in case of AH197 is solely due to the present of metal centers and the structure of ligands and the overall complex do not play an important role. If this were the case, the Cisplatin +NAMI-A sample would yield a similar inhibition level as observed for AH197. Furthermore, bioavailability of the metal complexes is an important consideration that cannot be ignored.