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**Electronic Supplementary Information**

# Deconstruction of a Metallodrug: Mechanism of Drug Resistance by the 9 Cysteines in β-Metallothionein rh1a

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## Experimental Procedure

### Preparation of recombinant human Metallothionein1a

Preparation of isolated MT domain fragments followed previously reported methods. [1] The amino acid sequence for the isolated domain used in this study was based on the recombinant human MT1a 38-residue β-MT domain fragment sequence (MGKAAAACSC ATGGSCTCTG SCKCKECKCN SCKKAAAA). Each of the corresponding DNA sequences was inserted as an N-terminal S-tag fusion protein into pET29a plasmids and individually expressed in Escherichia coli BL21- (DE3) with cadmium-supplemented growth medium. The protein was expressed and purified as the cadmium saturated form for stability. All purified protein solutions were evacuated and saturated with argon to impede cysteine oxidation.

### Preparation of apo-β-Metallothionein

Cadmium was removed from the purified, isolated MT domains by acidifying the protein solutions to pH 2.7. The protein was desalted and buffer exchanged with argon-saturated deionized water, returned to neutral pH, and concentrated using Millipore Amicon Ultra-4 centrifuge filter units (3 kDa MWCO). Protein concentrations of the final, pH-adjusted apo-β-MT solutions were determined by cadmium remetalation of small fractions of each protein monitored using UV–visible absorption (Cary 50, Varian Canada): $\varepsilon_{250}$ values of $Cd_3$-β-MT is 36000 $M^{-1}$ $cm^{-1}$. Protein solutions were diluted to a final concentration of 10 μM.

### Preparation of $Rh_2(OAc)_4$ solutions

Stock solutions of 500 μM dirhodium(II) tetraacetate (Sigma-Aldrich) were freshly prepared in deionized water, evacuated and argon saturated. The MT cysteine concentration was determined and two-fold excess of $Rh_2(OAc)_4$ was added to the MT solution and mixed immediately prior to MS acquisition. The room-temperature UV-visible and circular dichroism (CD) spectra of the solutions were also measured following $Rh_2(OAc)_4$ saturation.

**Electrospray Ionization Mass Spectrometry, UV- Visible and Circular Dichroism Absorption Spectroscopy Parameters**

A Bruker Micro-TOF II instrument (Bruker Daltonics, Toronto, ON) operated in positive ion mode was used to collect the data. NaI was used as an external calibrant. The following settings were used: scan, m/z 500−3000; rolling average, 2; nebulizer, 2 bar; dry gas, 80°C at a rate of 8.0 L/min; capillary, 4000 V; end plate offset, −500 V; capillary exit, 175 V; skimmer 1, 30.0 V; skimmer 2, 23.5 V; hexapole RF, 800 V. The spectra were collected continuously over 1 hr and time slices were deconvoluted using the Maximum Entropy algorithm of the Bruker Compass DataAnalysis software package. A Jasco J810 spectropolarimeter was used to collect CD spectral data. The following scan parameters were used: step scan; range, 800−200 nm; data pitch, 1 nm; bandwidth, 0.5 nm; response, 1 s.

**Molecular Dynamics Calculations**

A minimized structure of the 38-residue $Cd_3$-β-MT1a domain fragment was used (Scigress MM3/MD method with augmented force field; Fujitsu Poland) [2] to test if the $Rh_2^{4+}$ core could be reasonably coordinated by 8 of the 9 cys of the β-MT1a. The three $Cd^{2+}$ ions were deleted from the original structure and the $Rh_2^{4+}$ core inserted using cys thiolate connections that were in close proximity to the 8 x/y ligand positions on the $Rh_2^{4+}$ core. The alignment of the core was locked to stop rotation about the Rh-Rh bond. The molecular dynamics calculation was carried out for 200 ps at an average temperature of 355 K with 2 fs steps using a dielectric of 78.5. The initial structure relaxed immediately then was stable.

**References**


1 T. B. J. Pinter, G. W. Irvine, and M. J. Stillman, *Biochemistry*. 2015, **54**, 5006.
2 K. E. Rigby, J. Chan, J. Mackie, and M. J. Stillman, *Proteins: Struct., Funct., Bioinf.* 2006, **62**, 159.