Supplemental data

for

Cisplatin Inhibits Protein Splicing Suggesting Inteins as Therapeutic Targets in Mycobacteria

Liyun Zhang,[†] Yuchuan Zheng,[†] Brian Callahan,[§] Marlene Belfort,[§] Yangzhong Liu[*†]

[†]Department of Chemistry, CAS Key Laboratory of Soft Matter Chemistry, University of Science and Technology of China, Hefei 230026, China
[§]Wadsworth Center, New York State Department of Health, Center for Medical Science, Albany, New York, USA

Additional experimental details.

Materials- Cisplatin was purchased from Sigma. Carboplatin and oxaliplatin were purchased from Shandong Boyuan Chemical Company. Other platinum(II) complexes and ^{15}N labeled cisplatin were synthesized according to literature methods (E. Wong and C. M. Giandomenico, ***Chem Rev,*** 1999, 99, 2451-2466)

In vitro splicing assay- The splicing assay was performed on a GFP protein with the RecA intein inserted before residue 129 of GFP, in *E. coli* strain BL21 (DE3). Various concentrations of platinum(II) complexes were added to the renatured protein, then 5 mM EDTA and 2 mM TCEP were added to the solution to trigger the protein splicing. The solution was incubated for 18 hours at 25°C. Fluorescence was measured with a Shimadzu RF-5301PC spectrofluorometer using a path length of 10 mm and excitation at 395 nm by scanning the emission spectra between 450 and 600 nm.

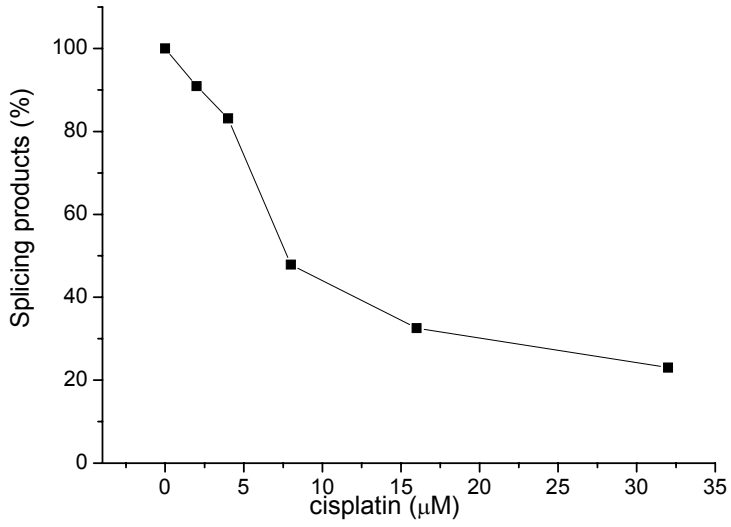

Figure S1. Quantification of the splice products measured on SDS-PAGE gel from Figure 2C. Data were processed by the Multi Gauge V3.0 software on a LAS 4000 mini Luminescent Image Analyzer. The sum of two splicing products is taken into account due to the partial overlap of two bands.

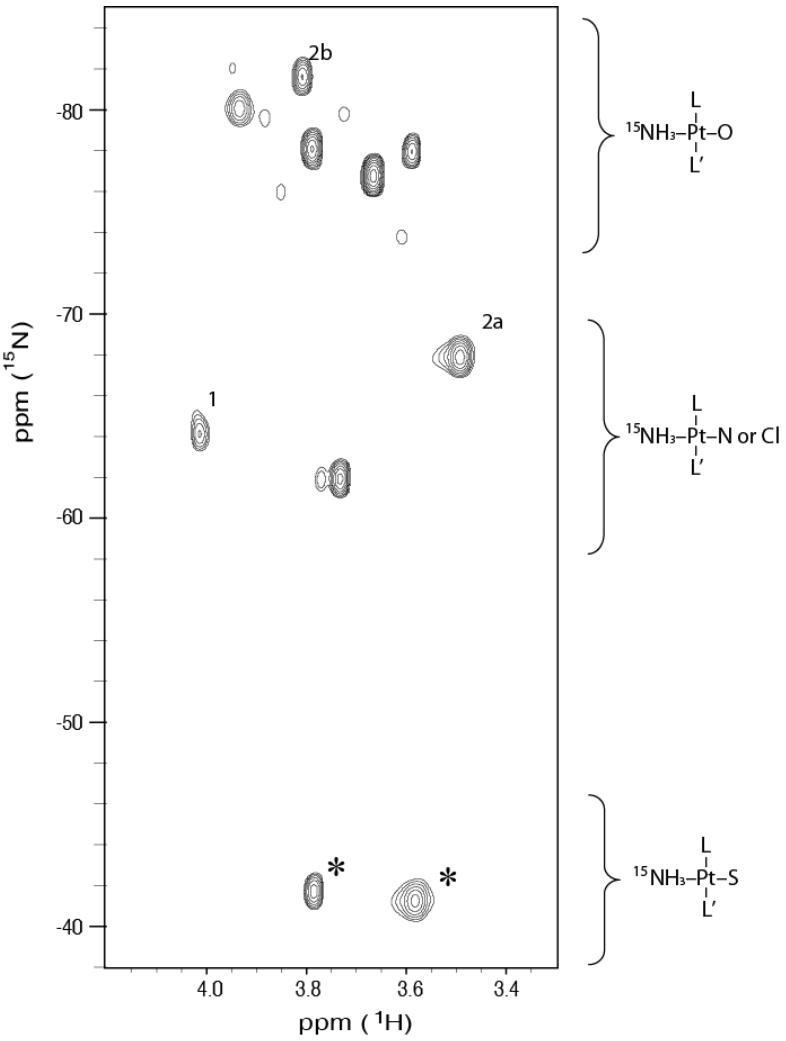

Figure S2. 2D [^{1}H ^{15}N] HSQC NMR spectra of 1.5 mM ^{15}N isotope labeled *cis*-[Pt(^{15}NH$_3$)$_2$Cl$_2$] interaction with RecA Intein ($\Delta\Delta I_{hh}$-CM [1]). Reaction was carried out in 100 mM $NaNO_3$, 20 mM PBS (pH 7.0) at 37°C for 24 hours. As the chemical shifts of $^{15}NH_3$ bound to platinum are dependent on the atom at the trans position, the coordination residue can be deduced.[2] The two peaks labeled with asterisks correspond to sulfur coordination trans to $^{15}NH_3$. Cys1 is the only cysteine in the intein. Salt coordination to platinum, such as phosphate and nitrate used in the sample, exhibits several peaks at -75 – -85 ppm on ^{15}N dimension.[3]

References.

[1] Hiraga, K.; Derbyshire, V.; Dansereau, J. T.; Van Roey, P.; Belfort, M. *Journal of Molecular Biology* **2005**, 354, 916-926.

[2] Susan J. Berners-Price, Luca Ronconi, Peter J. Sadler, *Progress in Nuclear Magnetic Resonance Spectroscopy* **2006**, 49, 65 – 98

[3] Murray S. Davies, Susan J. Berners-Price, and Trevor W. Hambley, *Inorg. Chem.* 2000, 39, 5603-5613

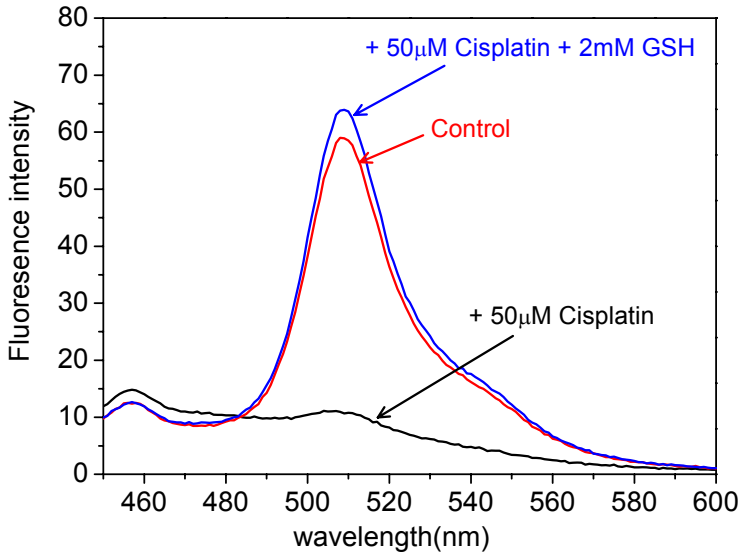

Figure S3. Cisplatin inhibition assay in the absence and presence of glutathione (GSH). The GFP-based intein splicing assay was performed in buffer (20 mM sodium phosphate, pH 7.0, 0.5 M NaCl, 5 mM EDTA and 0.5 M arginine) with 2 mM TCEP (red line). Inhibition was carried out by adding 50 μM cisplatin (black line). The presence of 2 mM GSH prevented the inhibition of cisplatin (blue line). Adding 2 mM GSH into the cisplatin pre-treated sample did not restore the splicing, suggesting the irreversible inhibition by cisplatin (data not shown).

Table S1. Selected peaks in the ESI-MS spectrum of intein ΔI-SM interaction with cisplatin for 24 hours

	Charge	m/z	
		Calculated	Observed
ΔI-SM	0	18608.10	-
	23+	810.04784	810.16686
	24+	776.3375	776.416685
ΔI-SM + $[Pt(NH_3)_2]$	0	18837.254	-
	23+	820.01104	819.916687
	24+	785.88558	785.916686
ΔI-SM + $[PtCl(NH_3)_2]$	0	18872.411	-
	23+	821.55265	821.666687
	24+	787.36296	787.416685

Table S2. *In vivo* inhibition efficiency of platinum complexes based on the TS reporter system.

	complexes	Class	IC_{50} (μM)
1	cisplatin	*cis*	7.8
2	$Pt(Phen)Cl_2$	*cis*	>250
3	oxaliplatin	*cis*	84
4	carboplatin	*cis*	>200
5	*c*DPCP	mono	>250
5	$Pt(NH_3)_2(im)Cl$	mono	>250
7	$Pt(NH_3)_2(3\text{-}py\text{-}CH_2OH)Cl$	mono	>250
8	*trans*-DDP	*trans*	> 200
9	*trans-EE*	*trans*	> 200