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Response from Boal and Rosenzweig to *Crystallography and chemistry should always go together: a cautionary tale of protein complexes with cisplatin and carboplatin*

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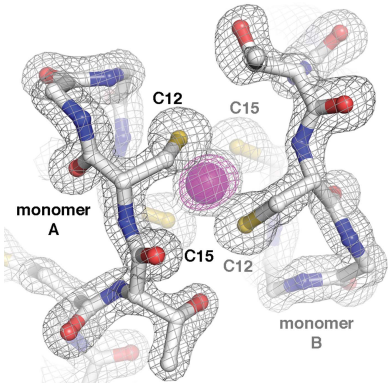
In this issue of *Acta Cryst. D*, Shabalin *et al.* (2015) reinterpret diffraction data for a series of protein complexes with cisplatin (cis-Pt) and related drugs, an attractive target for study because of the possibility of drawing upon the extensive knowledge of the chemical and biological properties of platinum-based anticancer therapeutics (Berners-Price, 2000; Arnesano *et al.*, 2009). cis-Pt and associated drugs bind avidly to duplex DNA inside the cell to form intrastrand crosslinks, driven in large part by the bonding preferences of Pt^{II}, and typically distort the structure of the biomolecule significantly (Johnstone *et al.*, 2015). The clinical importance of cisplatin interaction with proteins, however, is far less well understood. Association of Pt^{II} compounds with these targets can be slow and more non-specific than corresponding interactions with nucleic acids (Peleg-Shulman *et al.*, 2002). These properties are perhaps reflected in the findings of the study here in which low occupancies and heterogeneity in the structure and location of cis-Pt binding sites give rise to unique challenges in crystallographic modeling.

The authors examined two structures we solved in 2009 of cis-Pt adducts with a human copper chaperone, Atox1 (Boal & Rosenzweig, 2009*b*). Of the structures discussed in the paper, these are perhaps the most biologically relevant owing to the implication of human copper transport proteins in clinical platinum resistance (Howell *et al.*, 2010), although it remains unknown whether this correlation is a direct consequence of Pt^{II}-protein association *in vivo* or simply an indirect effect. Our crystallographic study showed that cis-Pt is capable of binding to a Cys-XX-Cys site normally occupied by copper in these proteins, suggesting they are at least capable of trafficking or inactivating platinum-based drugs. In one of these structures, in which cis-Pt is likely first modified by a phosphine reductant (TCEP) included to reduce the Cys ligands, Pt^{II} interacts with a single Atox1 monomer and maintains a square planar coordination environment that distorts the structure of the Cys-XX-Cys motif.

In a second structure obtained afterwards in which the reductant was removed under anaerobic conditions prior to Pt^{II} exposure, the metal binds at the same location but instead bridges an Atox1 dimer, similar to what is observed upon interaction with the native substrate Cu^{I} (Boal & Rosenzweig, 2009*a*). Interestingly, Pt^{II} exerts different structural effects on the protein in the absence of a ligand with a strong *trans* effect



such as TCEP. Here Pt^{II} interaction fails to alter the protein structure, and the Cys ligands remain in the tetrahedral configuration observed in all other metal-bound dimer structures of Atox1, a coordination environment implicated in specific recognition of Cu^{I} and in hand-off of the substrate to protein partners of similar fold. The observation is surprising when viewed from the perspective of Pt^{II} coordination geometry preferences, as noted by Shabalin *et al.* (2015), and was part of their impetus to reassign the identity of the metal in this structure to Cu^{I}. Our interpretation is different and informed both by knowledge of the difficulties associated with *in vitro* formation of Cu^{I} complexes in these proteins and by additional characterization of the cis-Pt Atox1 sample used for the structure determination by mass spectrometry, which indicates clear formation of Pt^{II}-Atox1 adducts. Persistent difference map peaks in the crystallographic data, coupled with information from MS analysis consistent with retention of the ammine ligands, suggested that the metal might still bear these substituents and use them to complete the square planar arrangement preferred by Pt^{II}. Subsequent analyses performed in other laboratories of these and similar Atox1-Pt^{II} complexes using NMR and MS methods are consistent with cis-Pt interaction at the Cys-XX-Cys site accompanied by retention of the NH_2 ligands (Arnesano *et al.*, 2011).

However, the analysis by Shabalin *et al.* (2015) is accurate in that it reveals steric clashes between these ligands and the surrounding protein backbone. Subsequent unpublished structures from our laboratory obtained using altered crystallization conditions, including several in which the crystals were given weeks to mature instead of only a few days, reveal more complete loss of the density modeled as the original ammine ligands and full tetrahedral coordination of the Pt^{II} ion by the Cys-XX-Cys motif (Fig. 1). The differences in the structures probably reflect extremely slow dissociation of the labile ammines accompanied by additional Cys coordination, consistent with kinetic analysis (Arnesano *et al.*, 2011). We are confident in our assignment of the metal ion in this binding site as Pt^{II}, given the validation from other experimental work and the observation of strong anomalous Fourier density at this site (18σ for the original structure with modeled ammine ligands and 33σ for the unpublished data set). In addition, there are absolutely no other peaks in the anomalous Fourier map indicative of an additional Pt binding site, as suggested by Shabalin *et al.* (2015).

We propose that the unexpected coordination geometry is a result of the rigid nature of the metal binding site in these proteins. This property likely serves an important functional role in Atox1, perhaps as a selectivity filter to promote binding of Cu^{I} over Cu^{II}, which is similar to Pt^{II} in its preference for a square-planar ligand environment. Although previously not observed for cis-Pt adducts with biological targets, in which dramatic contortion of the macromolecule to conform to the coordination chemistry preferences of Pt^{II} is in fact believed to be linked to the anticancer activity of these compounds, the cis-Pt/Atox1 dimer structure is reminiscent of an entatic state (Williams, 1995), in which a particularly rigid protein scaffold can alter the properties of a ligand to impart a specific function. One classic illustration of this concept also involves Cu^{I}-binding proteins, but those implicated in electron-transfer pathways instead of metal homeostasis, in which an entatic state tunes the reduction potential of the metal (Gray *et al.*, 2000). Shabalin *et al.* (2015) state in their manuscript that *crystallography has been a committed and faithful companion to chemistry, even if certain initial results were at first surprising to some chemists* ... The unexpected observations in the cis-Pt/Atox1 crystal structures, rather than being a consequence of misassignment of the metal ion involved, are perhaps instead a reflection of the very idea that inspired this study.

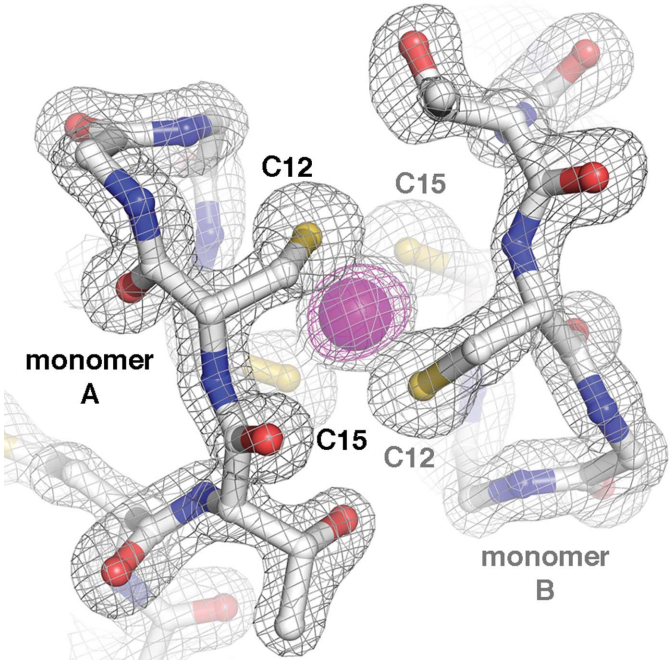


Figure 1
An unpublished X-ray crystal structure of Pt^{II}-Atox1 solved to 1.65 Å resolution ($2F_o - F_c$ electron-density map shown in gray mesh, contoured at 1.5σ) exhibiting complete loss of electron density for the original ammine ligands of the cis-Pt complex. The thiolate ligands contributed by the Cys-XX-Cys motif retain a tetrahedral configuration about the Pt^{II} ion. The identity of the metal ion was assigned as Pt^{II} based on strong anomalous Fourier density (purple mesh, 9.0σ) using diffraction data sets collected above 11.6 keV near the Pt *L* X-ray absorption edge.

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