The binding of the platinum hexahalides (Cl, Br and I) to hen egg-white lysozyme and the chemical transformation of the PtI₆ octahedral complex to a PtI₃ moiety bound to His15

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S1. HEWL+K₂PtBr₆

After soaking HEWL crystals with K₂PtBr₆ for 24hrs, an octahedral PtBr₆ molecule is bound to site 1 (using the naming convention of Helliwell et al 2010) which is at a special position between two Arg-14 residues in symmetry related molecules with a two-fold axis passing through the platinum atom and two bromines (Figure S1). Also, an octahedral complex is bound in site two near to Ser-86, Lys-1 and Gln-41 of chain A in a crevice next to Pro-79, Asn-65 and Asn-74 in a symmetry related molecule (Figure S2), similar to the 3hr soak study (Helliwell et al, 2010). However, no PtBr₃ moiety is bound to the Nδ atom of His-15.

A large anomalous difference electron density peak of 8σ is observed 2.4Å away from the sulphur atom of Cys-6, which is disulphide bonded to Cys-127. A platinum atom is assumed bound due to the distance from the S atom. Binding of platinum to disulphide bonded Cys residues has been previously described by Moreno-Gordaliza et al, (2009; 2010) using mass spectrometry to study the interaction of cisplatin with insulin.

Figure S1  PtBr₆ binding in a special position between two Arg-14 residues in symmetry related molecules. The 2Fo-Fc electron density map (blue) and the anomalous difference electron density map (orange) are shown. The platinum atom is in grey and bromine atoms in red.
Figure S2  PtBr$_6$ molecule bound near Ser-86, Lys-1 and Gln-41 of chain A in a crevice next to Pro-79, Asn-65 and Asn-74 in a symmetry related molecule. The 2Fo-Fc electron density map (blue) and the anomalous difference electron density map (orange) are shown. The platinum atom is in grey and bromine atoms in red.

S2. HEWL+K$_2$PtCl$_6$

After soaking HEWL crystals with K$_2$PtCl$_6$ for 24hrs, an octahedral PtCl$_6$ molecule is seen bound to site 1, i.e. a special position between two Arg-14 residues in symmetry related molecules with a two-fold axis passing through the platinum atom and three chlorines (Figure S3). Also, an octahedral complex is bound in site two near Pro-79, Asn-65 and Asn-74 of chain A in a crevice next to Ser-86, Lys-1 and Gln-41 in a symmetry related molecule (Figure S2), similar to the 10min soak study (Sun et al., 2002). However, no PtCl$_3$ moiety is seen bound to the Nδ atom of His-15.
**Figure S3**  PtCl₆ binding in a special position between two Arg-14 residues in symmetry related molecules. The 2Fo-Fc electron density map (blue) and the anomalous difference electron density map (orange) are shown. The platinum atom is grey and the chlorine atoms are in yellow.

**Figure S4**  PtCl₆ molecule bound near Pro-79, Asn-65 and Asn-74 of chain A in a crevice next to Ser-86, Lys-1 and Gln-41 in a symmetry related molecule. The 2Fo-Fc electron density map (blue) and the anomalous difference electron density map (orange) are shown. The platinum atom is in grey and chlorine atoms are in yellow.
Table S1  X-ray crystallographic data and final protein model refinement statistics for HEWL crystals soaked in K₂PtBr₆ and K₂PtCl₆.

|                     | HEWL+K₂PtBr₆ | HEWL+K₂PtCl₆ |
|---------------------|--------------|--------------|
| **PDB id**          | 4OWH         | 4OWE         |
| **Data collection temperature (K)** | 100          | 100          |
| **Data reduction**  |              |              |
| Space group         | P422         | P422         |
| Unit cell parameters (Å) | a=b= 78.66  | a=b= 78.75  |
|                      | c= 36.84     | c= 37.16     |
| Detector to crystal distance (mm) | 50           | 50           |
| Observed reflections | 370257       | 306554       |
| Unique reflections  | 17449        | 21867        |
| Resolution (Å) (last shell) | 55.62 – 1.48 | 55.68-1.41 (1.48-1.41) |
| Completeness (%)    | 94.4 (30.7%) | 99.8 (99.9) |
| Rmerge (%)          | 0.0585 (0.2073) | 0.0941 (0.422) |
| <I/sigma(I)>        | 30.8 (1.9)   | 5.2 (2.6)    |
| Multiplicity        | 18.7 (0.4)   | 5.3 (5.3)    |
| Cruickshank DPI (Å) | 0.08         | 0.07         |
| Number of protein atoms | 1001        | 1001         |
| Average B factor (Å²) for protein atoms | 20.5         | 20.3         |
| Number of water molecules | 119         | 124          |
| Average B factor (Å²) for water molecules | 28.7         | 28.1         |
| Number of Pt and halogen atoms | 23          | 19           |
| Average B factor (Å²) for Pt and halogen atoms | 24.9         | 21.9         |
| Number of other bound molecules or ions* | 9           | 13           |
| Average B factor (Å²) for other bound molecules or ions* | 34.0         | 37.0         |
| **Refinement**      |              |              |
| R factor/ R free    | 18.6/22.1    | 18.5/22.3    |
| RMSD bonds (Å)/ Angles (°) | 0.02/1.85   | 0.02/2.09    |
| **Ramachandran values (%)** |              |              |
| Most favoured       | 96.1         | 94.3         |
| Additional allowed  | 3.9          | 5.7          |
| Disallowed          | 0            | 0            |

* The completeness for this dataset at a diffraction resolution of 1.52 Å is 74%.

* The other bound atoms to the protein include acetate molecules and sodium ions.
S3. Refinement of heavy atom occupancies and B factors

Refinement of heavy atom occupancies and B factors; given the different mathematical functional forms of these two parameters, provided the diffraction resolution is adequate, these two parameters can be refined simultaneously. Furthermore monitoring of how physically sensible the values obtained are form a check on the adequacy of the diffraction resolution and data quality.

Table S2  Occupancy values and anomalous difference electron density peak heights ($\sigma$) for the Pt and I atoms bound to the protein.

| Atom | Nearby residues | Anom peak height ($\sigma$) | Occupancy (%) |
|------|-----------------|-----------------------------|---------------|
|      |                 | His-15 bound to N$\delta$ atom |               |
| Pt1  |                 | 29.6                        | 82            |
| I1   |                 | 20.6                        | 66            |
| I2   |                 | 24.0                        | 77            |
| I3   |                 | 25.7                        | 81            |
|      | Arg-14 in a crevice between 2 symmetry related molecules |               |
| Pt2  |                 | 27.2                        | 44            |
| I4   |                 | 20.1                        | 41            |
| I5   |                 | 16.5                        | 58            |
| I6   |                 | 22.0                        | 32            |
| I7   |                 | 15.5                        | 65            |
| PtI$_3$ bound at His-15 refined as a whole molecule* | 78 |
| PtI$_6$ bound at Arg-14 refined as a whole molecule* | 47 |

* The PDB files contain the refined individual occupancies as, when treated as a group, some residual electron density is observed in the Fo-Fc maps.
Table S3  Occupancy values and anomalous difference electron density peak heights ($\sigma$) for the Pt and Br atoms bound to the protein.

| Atom | Nearby residues | Anom peak height ($\sigma$) | Occupancy (%) |
|------|-----------------|----------------------------|---------------|
| Pt1  | Arg-14 in a crevice | 61.3                      | 46            |
| Br1  |                 | 8.8                       | 40            |
| Br3  |                 | 4.4                       | 46            |
| Br4  |                 | 7.1                       | 49            |
| Br6  |                 | 6.1                       | 41            |
| Pt2  | Gln-41, Ser-86 and Lys-1 in a crevice | 45.6                  | 54            |
| Br1N |                 | 6.3                       | 55            |
| Br1I |                 | 5.3                       | 60            |
| Br1J |                 | 4.3                       | 38            |
| Br1M |                 | 4.3                       | 58            |
| Br1K |                 | 4.0                       | 48            |
| Br1L |                 | 2.9                       | 48            |
| PtBr$_6$ bound at Arg-14 refined as a whole molecule* | 47 |
| PtBr$_6$ bound at Gln-41 refined as a whole molecule* | 52 |

* The PDB files contain the refined individual occupancies as, when treated as a group, some residual electron density is observed in the Fo-Fc maps.

The letters next to the bromine atoms are arbitrary and denote the different atoms, similar to the different numbers.
### Table S4

Occupancy values and anomalous difference electron density peak heights (σ) for the Pt and Cl atoms bound to the protein.

| Atom  | Nearby residues | Anom peak height (σ) | Occupancy (%) |
|-------|-----------------|----------------------|---------------|
|       | Asn-65, Asn-74 and Pro-79 in a crevice |                       |               |
| Pt1   |                 | 21.3                 | 33            |
| Cl1   |                 | 3.1                  | 79            |
| Cl2   |                 | 3.8                  | 39            |
| Cl3   |                 | 3.8                  | 37            |
| Cl4   |                 | 2.6                  | 57            |
| Cl5   |                 | 2.5                  | 19            |
| Cl6   |                 | 3.7                  | 55            |
|       | Arg-14 in a crevice |                       |               |
| Pt2   |                 | 17.4                 | 19            |
| Cl8   |                 | 0                    | 64            |
| Cl9   |                 | 2.6                  | 49            |
| Cl10  |                 | 3.2                  | 42            |
| PtCl<sub>6</sub> bound at Asn-65 refined as a whole molecule* | | 36 |
| PtCl<sub>6</sub> bound at Arg-14 refined as a whole molecule* | | 22 |

* The PDB files contain the refined individual occupancies as, when treated as a group, some residual electron density is observed in the Fo-Fc maps.

### S4. Other remarks on binding sites

In Helliwell et al (2010) a minor occupancy PtBr<sub>6</sub> site (‘site 3’) at the 20% level was seen and specifically whose occupancy did not increase with soak time up to 3 hours. In the 24hr soaking experiment reported in this new study this site 3 is not occupied at all for any of the Pt hexahalide...
cases. In another case, the Cys-6-Cys-127 disulphide bond in the HEWL K₂PtBr₆ 3hr study shows anomalous difference electron density, proximal to the nearest sulphur atom and therefore possibly a platinum atom. In this 24hr K₂PtBr₆ soaking experiment, there is some similar density but even less clear than the 3hr soak study. Previous mass spectrometry studies of cisplatin binding to insulin have reported cisplatin binding to Cys residues partaking in disulphide bond formation (Moreno-Gordaliza et al, 2009;2010). The reason for these differences between the 24hr and 3hr K₂PtBr₆ soaking studies with HEWL are not known.

Besides soaking times being different, the Helliwell et al, 2010 studies used glycerol as the cryoprotectant rather than paratone as reported in these current studies. However this particular difference seems unlikely to be relevant. In both the PtCl₆ and PtI₆ cases, no anomalous difference electron density is observed near the Cys-6-Cys-127 disulphide bond.

The occupancy values for the Pt atom in PtBr₆ (Table S3) at sites one and two are 46% and 54%. These have stayed at a similar occupancy as the 3hr study (Helliwell et al, 2010), thus increasing the soaking times from 3hr to 24hrs has not improved the binding occupancy at these two sites.

Helliwell, J.R, BelI, T.A.M, Bryant, P, Fisher, S.J, Habash, G, HelliwellII, M, Margiolaki, I, Kaenket S, Watier, Y, Wright, J, and Yalamanchilli, S. (2010) Z. Kristallogr. 225 570–575
Moreno-Gordaliza, E., Canas, B., Palacios, M.A., Gomez-Gomez, M.M. (2009) Anal. Chem 81: 3507-3516
Moreno-Gordaliza, E., Canas, B., Palacios, M.A., Gomez-Gomez, M.M. (2010). Analyst 135: 1288-1298
Sun, P.D, Radaev, S and Kattah, M (2002) Acta Cryst D58, 1092-1098