Supporting Information

# Silver Metallation of Hen Egg White Lysozyme: X-ray Crystal Structure and NMR Studies

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## X-ray structure details

A single crystal of silver metallated HEWL was harvested directly from the crystallization drop without further cryoprotection. X-ray data were collected at 1.54 Å using a Bruker Kappa APEX II Duo equipped with an ImuS micro-focus source with QUAZAR optics to a resolution of 1.35 Å and a completeness of 99.2%. Data integration and scaling were performed using the APEX II software suite in the tetragonal space group $P4_32_12$.[1] An initial solution was obtained by molecular replacement using phaser and PDB 3KAM as a model.[2] Additional model building and refinement were accomplished using Coot and Refmac5.[3-7] Solvent water atoms were added using the arp_waters feature in Refmac5 up to a B value of 40 $Å^2$.[8] All atoms were refined anisotropically in the final refinement cycles.

A final R-factor of 19.2% and Rfree of 21.5% were obtained for the structure. Initial structure validation was performed using Coot[3,4] and the final structure was validated using Sfcheck[9], Procheck[10] and MolProbity.[11] The model has a clash score of 7.96 putting it in the 67$^{th}$ percentile of structures of comparable resolution. 98.4% of the residues are within the favored regions according to the Ramachandran plot with no Ramachandran outliers. The final model and structure factors have been submitted to the PDB and given the accession code 3RU5.

## NMR spectra

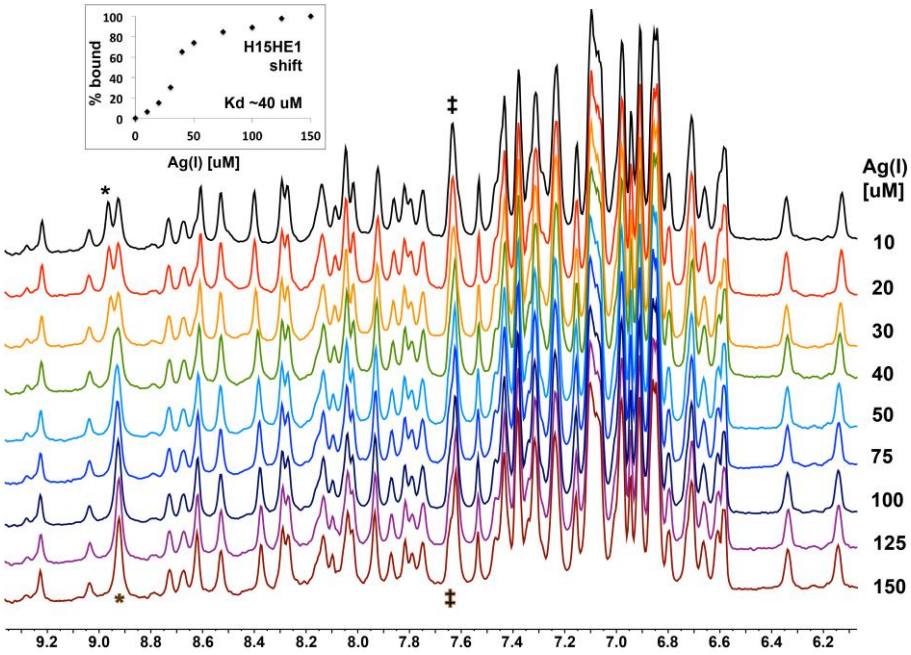


**Figure 1**. Nitrate re-crystalyzed HEWL titration with $AgNO_3$ monitored by 1D NMR. The star indicates histidine 15's HE1 resonance while the double dagger is the HD2 from the same residue. HEWL concentration was 50 µM in 100% $D_2O$ (many residual amides remain un-exchanged in these spectra). HEWL was titrated with small portions of a 1 mM $AgNO_3$ solution in the same buffer. The Kd for silver was estimated to be around 40 µM (inset) by plotting the percent chemical shift change as a function of concentration.

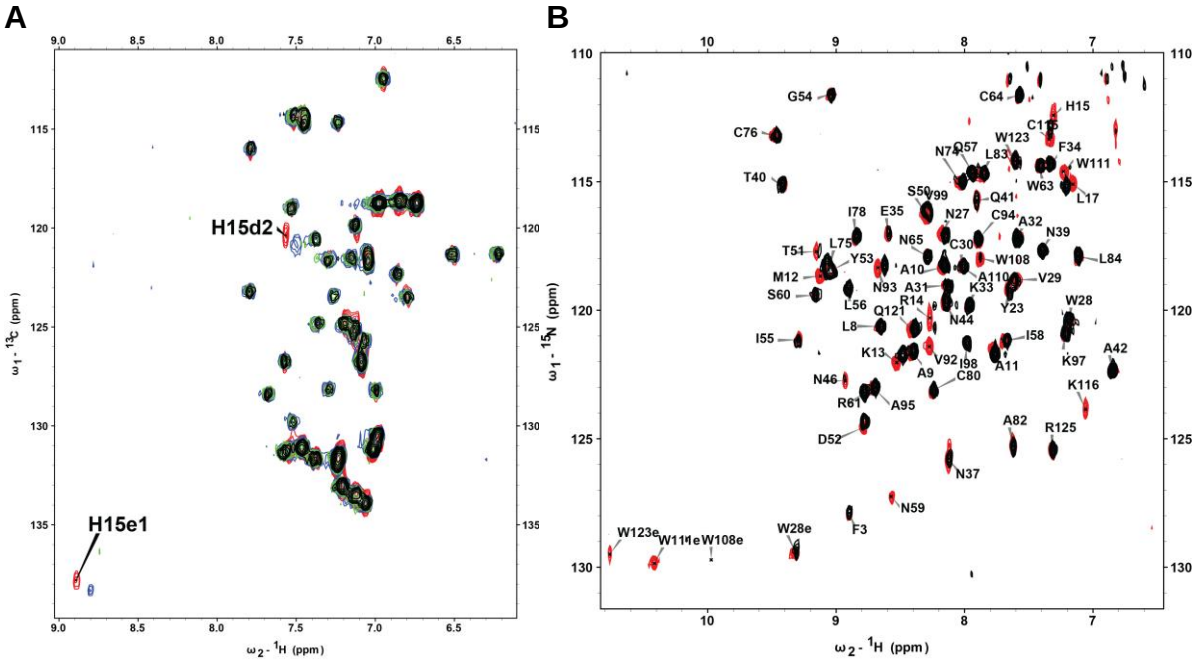

**Figure 2**. HSQC spectra of 10 mM nitrate-HEWL in $D_2O$ with various concentrations of $AgNO_3$. **A**) natural abundance $^{13}C$ HSQC with 0 mM (red), 5 mM (blue), 10 mM (green), 20 mM (black) $AgNO_3$. **B**) natural abundance $^{15}N$ HSQC spectra with 0 (red) or 20 mM (black) $AgNO_3$. The consistent broadening upon metalation in these 2D spectra is consistent with the 40 μM $K_d$ estimated in from the data in Supplementary Figure 1. The disappearance of the exchangeable tryptophan epsilon indole resonances is not surprising due to continuing solvent deuteron exchange during the titration.

## Enzyme activity data

*Lysozyme Assay Procedures*

Three stock samples of HEWL were made at 500U/mL (3.497 μM) and each were given 0.5, 1, or 2 equivalents of silver that were made in 50 mM MES pH 6.5. A commercially available cell wall encapsulated fluorescein release assay (Invitrogen; ENZCheck Lysozyme Assay) was run according to the instructions except for substitution of the supplied buffer with 0.1 M MES pH 6.5. For the assay involving varied silver concentrations at constant protein concentrations the following modifications were used: in each well 50 μL of a 2x stock of the protein was added along with 1 μL of the required stock silver solution to give the desired concentration of silver in a volume of 100 μL, and 50 μL of the fluorescent substrate.

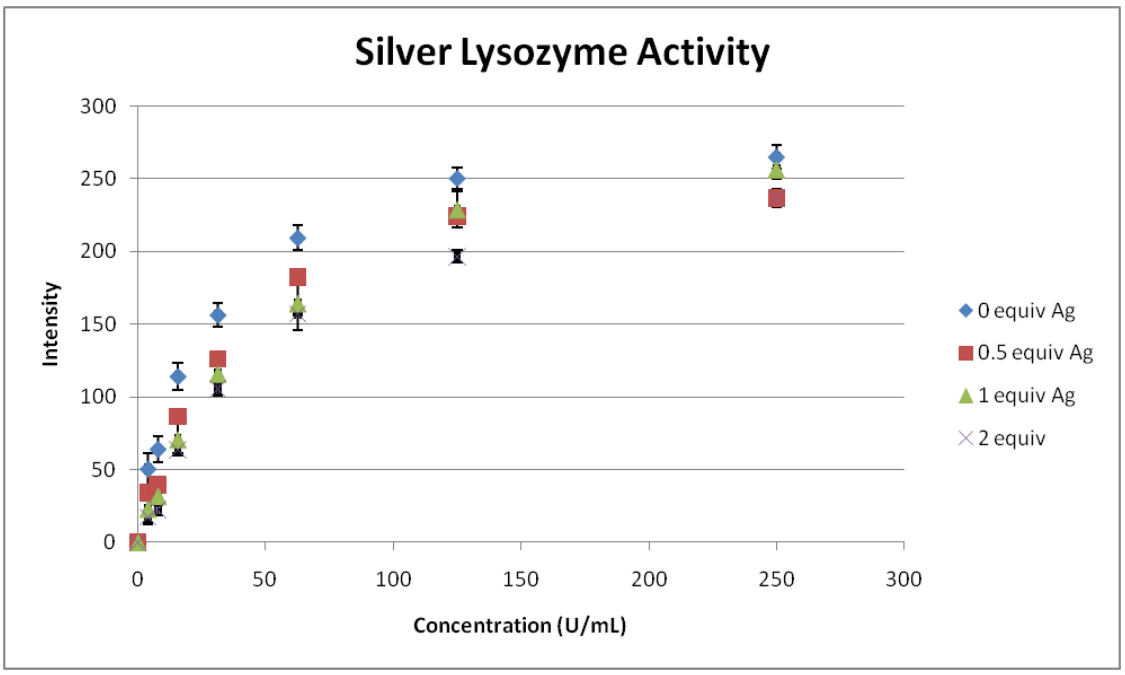


**Figure 3**. **N**itrate-HEWL activity assays versus silver. Nitrate HEWL activity with various equivalents of silver ion. Error bars are based on the standard error calculation for the values.

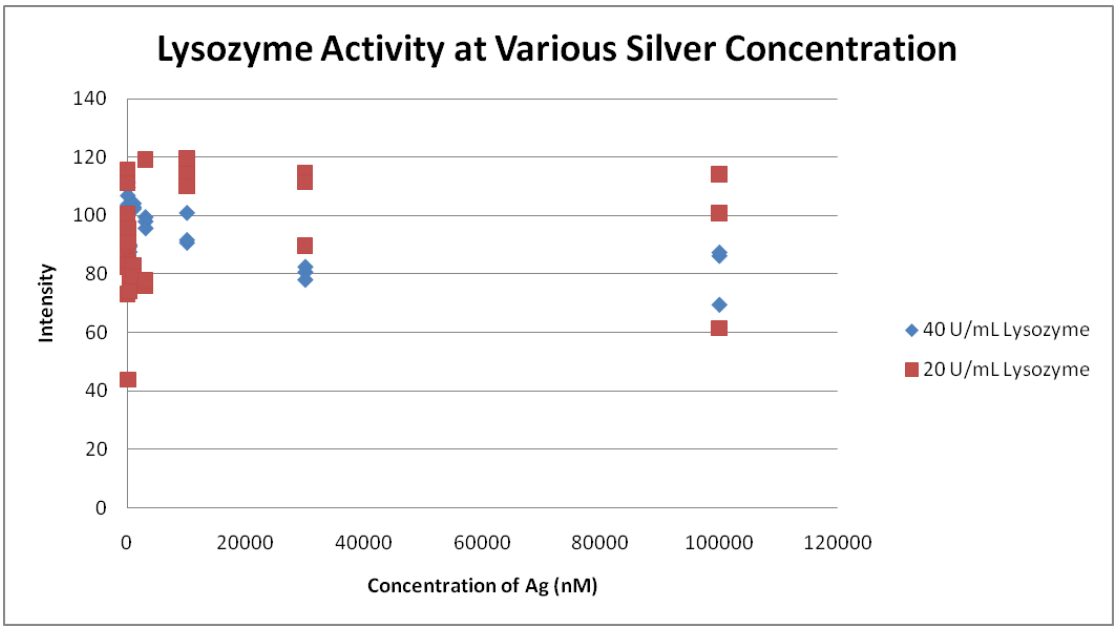


**Figure 4**. Nitrate-HEWL activity assay at differing concentrations of silver and constant protein levels.

References

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