Supporting Information
For

Palladium(II) and platinum(II) bind strongly to an engineered blue copper protein

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General Considerations
Since the H117G mutant was sensitive to oxygen exposure,[1] as evidenced by reduced copper loading after prolonged storage in air, after purification the protein was stored and handled using standard Schlenk or glovebox techniques under a dinitrogen atmosphere. Unless otherwise noted, solutions were made with water purified with a Barnstead NANOpure® DIamond™ Life Sciences (UV/UF) ultrapure water system. Purified water and protein solutions were deoxygenated under vacuum for 12 hours and sparged with nitrogen for 15 minutes before use. Glassware was soaked in 1 M nitric acid and plastics were soaked in 1 M EDTA for 24 hours before use to avoid Zn^{2+} contamination of the apo-protein. Buffer solutions with pH > 4 were stirred with Chelex 100 sodium form (Sigma-Aldrich) overnight to remove trace metals. UV-Vis measurements were taken with either a Cary 50 Bio UV/Vis spectrometer or a HP 8452A Diode Array Spectrophotometer. All buffers were the highest grade available on a per metals basis and purchased from EMD Biosciences, except the acetate buffers which were made with sodium hydroxide, ammonium acetate, and acetic acid purchased from Sigma-Aldrich.

Site-Directed Mutagenesis. The pET9a(Kan^r) plasmid containing the azurin gene was used as a template for site directed mutagenesis. His117Gly was prepared using the QuickChange II method (Stratagene, La Jolla, CA) using the following primer in which the mutated site is underlined.
5′-CTGCACTTTCCCGGGTGGCTCCGCACTGATGAAAGG-3′
The parental DNA was digested with Dpn1 at 37 °C for 2 h and the final product was transformed into XL1-Blue supercompetent cells (Stratagene, La Jolla, CA). DNA sequencing confirmed the incorporation of the mutations into the plasmids. The plasmids were transformed into BL21*(DE3) *E. coli* (Invitrogen) for expression and the protein was overexpressed and purified using methods described below.

Preparation and Purification of H117G Apo-azurin.
Pseudomonas aeruginosa H117G apo-azurin was overexpressed and purified using methods analogous to a previous protocol.[2] *P. aeruginosa* azurin in a Pet9a vector, obtained from Professor John H. Richards, was expressed at 25 °C in BL21*(DE3) *E. coli* (Invitrogen) in 6 L of 2xYT media (16 hours, 25 °C). The overexpression of protein was induced with 450 mg of Isopropyl-beta-D thiogalactopyranoside (IPTG) and shaken for an additional 4 hours at 25 °C. The cells were collected by centrifugation (9000 rpm, 10 min) followed by resuspension in a buffer (20% sucrose (w/v), 1 mM EDTA, 30 mM TrisHCl, pH = 8.0, 1 L) and shaken at 4 °C for 40 minutes. The cells were collected by centrifugation and the supernatant was decanted. The cells were resuspended in a solution composed of 4 mM NaCl and 1 mM DTT (1 L) and shaken vigorously at 4 °C for 10 minutes. The insoluble cell fragments were collected by centrifugation and the protein was decanted into a cool 2 L Erlenmeyer flask. The pH of the solution was reduced by the dropwise addition of 100 mL of 500 mM acetate buffer at pH = 4.1, The precipitated protein was removed by centrifugation and the supernatant was mixed with SP Sepharose fast flow beads (GE Healthcare) and stirred at 4 °C for 5 minutes. The beads were collected on a frit and washed with buffer (50 mM acetate buffer, pH = 4.1) until the UV detector had a flat baseline. The contaminating zinc azurin was removed by washing the column with 192 mL of pH = 5.34 acetate buffer. Apo-azurin was eluted with 448 mL of

pH = 6.01 acetate buffer. The protein was pure by gel electrophoresis. When titrated with copper(II) sulfate and methyl imidazole, it showed the expected 625/280 nm absorbance ratio of 0.48.[3] The buffer was exchanged into 50 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS)) buffer (pH = 7.0) using a PD10 desalting column (GE Healthcare, Sephadex G-25 Medium, volume 13.5 mL). The protein concentration was estimated using the 280 nm absorbance assuming an extinction coefficient of 9000 $M^{-1}cm^{-1}$.[4]

Preparation of Pd(II) Azurin.
$PdCl_2$ was dissolved in 1 M NaCl (10 mg in 1.4 mL, 4.0×10^{-2} M). A solution of apoprotein was diluted to 500 μL with water to give a 0.44 mM solution. The Pd(II) metalloderivatives were prepared by the addition of 5.5 μL (2.2×10^{-7} mol, 1 equiv.) of the $PdCl_2$ solution to the 500 μL solution of apoprotein. Mixtures were left for at least 1 h before purification to allow complete loading, after which point there was no additional change in the UV-Vis spectra. Excess Pd^{2+} was removed by washing the protein with buffer on a Centricon concentrator (GE Healthcare, 3 kDa filter). Loading was monitored by observing the UV-Vis spectrum: the Pd(II) protein showed a broad shoulder above 280 nm with a representative extinction coefficient determined at 324 nm ($\varepsilon = 2.1(5)\times10^4$ $M^{-1}cm^{-1}$). The extinction coefficients were determined by relating the absorbance to the estimated azurin extinction coefficient of 9000 $M^{-1}cm^{-1}$ at 280 nm.[4]

Preparation of Pt(II) Azurin.
K_2PtCl_4 was dissolved in H_2O (11 mg in 1 mL, 0.12 M). A solution of apoprotein was diluted to 500 μL with water to give a 0.44 mM solution. The Pt(II) metalloderivatives were prepared by the addition of 8.2 μL (2.2×10^{-7} mol, 1 equiv) of the $PdCl_2$ solution to the 500 μL solution of apoprotein. Mixtures were left for at least 12 h at 40 °C before purification to allow complete loading. Excess Pt^{2+} was removed by washing the protein with buffer on a Centricon concentrator (GE Healthcare, 3 kDa filter). Loading was monitored by observing the UV-Vis spectrum: the Pt(II) protein showed a broad shoulder above 280 nm with a representative extinction coefficient determined at 330 nm ($\varepsilon = 1.2(7)\times10^5$ $M^{-1}cm^{-1}$). The extinction coefficients were determined by relating the absorbance to the estimated Pd-azurin extinction coefficient of 9000 $M^{-1}cm^{-1}$ at 280 nm.

Quantification of Pd and Pt by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
Sample Preparation: All sample preparation was conducted in a Class 100 trace metal free clean laboratory in the Environmental Earth and Ocean Sciences Department at the University of Massachusetts-Boston. Before preparation, samples were agitated with a GlobalSpec (Troy, NY USA) Laboratory shaker for 30 minutes followed by 1 minute of vortex shaking to re-homogenize the fluid. Class A polypropylene test tubes were pre-washed using a 5% nitric acid bath to remove potential metal contamination. Approximately 0.1 mL of each sample was volumetrically pipetted into the corresponding pre-labeled analytical vials and verified gravimetrically to ±0.001 mg. Dilutions were prepared volumetrically (and validated gravimetrically) by adding water purified to 18.2 MΩ cm resistance (by a Milli-Q water purification system, Millipore, Bedford, Mass., USA) to achieve sample dilution of ~500x and then acidified using trace metal free concentrated (12.4 mol L^{-1}) ultra-pure hydrochloric acid (HCl) (obtained from

Fisher Scientific, Mass, USA). Internal standards consisting of known quantities of indium (In), bismuth (Bi), and iridium (Ir) were added to the samples to correct for instrumental drift. Sample dilution resulted in a final concentration of 2% hydrochloric acid (by volume), and 10 ng/g, 1 ng/g, and 1 ng/g of the internal standards, respectively. All analytical standards, procedural blanks, and interference check standards (e.g. Zr and Hf) were prepared in an analogous fashion.

Sample Analysis: The [Pd] and [Pt] were measured using a Perkin Elmer Axiel Field Technology DRC II inductively coupled plasma mass spectrometer (ICP-MS). Prior to sample analysis, the ICP-MS was optimized for sensitivity, stability, and to reduce the formation of doubly charged species and oxide interferences using a multi-element tuning solution containing In, Ba, Ce, Zr, Hf, Hg, Bi, Ir, and Pt. Optimization continued until CeO^+/Ce^+ and Ba^{++}/Ba^+ was simultaneously less than 2% with ZrO^+/Zr^+ and HfO^+/Hf^+ below 1% to produce the conditions used in Table 1. These interferences were quantified to correct for instrumental and procedural backgrounds and isobaric interferences, respectively. During sample analysis sample lines were rinsed to reduce memory effects by washing sequentially with water purified to 18.2 MΩ cm resistance (by a Milli-Q water purification system, Millipore, Bedford, Mass., USA) for 120 seconds and a 2% hydrochloric acid solution for an additional 120 seconds between analyses.
ICP-MS analyses were conducted with modifications to the EPA 6020A methodologies[5,6], similar to previously reported methods.[7] Pd and Pt detection were performed by monitoring ^{106}Pd, ^{108}Pd, and ^{110}Pd and ^{194}Pt, ^{195}Pt, and ^{196}Pt, respectively. Isobaric corrections for ZrO^+ and HfO^+ interference on Pd and Pt was performed on-line using ICP-MS software. Throughout the analysis, ZrO^+ and HfO^+ isobaric interferences were <1%. [Pd] and [Pt] quantification was obtained using a 9-point external calibration curve spiked with known quantities of Pd and Pt in a linear range from 0.050 ng/g to 100 ng/g. Known aliquots of Pd and Pt spikes were analyzed as unknowns to determine external precision as 2.4 and 2.7%, respectively. Five duplicate analyses (n=5) were performed for all analytes for each sample solution. Limits of detection (LOD) (Pd= 6.8 pg/g and Pt = 4.3 pg/g) and limits of quantification (LOQ) (Pd= 12.3pg/g and Pt =10.7 pg/g) were calculated according to Long and Winefordner[8], while method detection limits (MDL) (Pd=19.7 pg/g and Pt=16.4 pg/g) were calculated according to the two-step approach using the $t_{99}S_{LLMV}$ method[9] at 99% CI (t=3.71).

Table 1: Working conditions for solution based ICP-MS instrument operating conditions.

Perkin Elmer DRC II ICP-MS	
Spray Chamber	Cyclonic; Glass Expansion (Room Temperature)
Cones	Nickel
Plasma Gas	15 L/min
Nebulizer	Glass Expansion Micromist (100µL/min)
Nebulizer Flow Rate	0.87 L/min
Lens Voltage	6.25V
RF Power	1050 W (Cool Plasma optimized for Pd and Pt)
Dwell Time	30 ms
Sweeps	10
Replicates	10
Scan Mode	Peak Hop

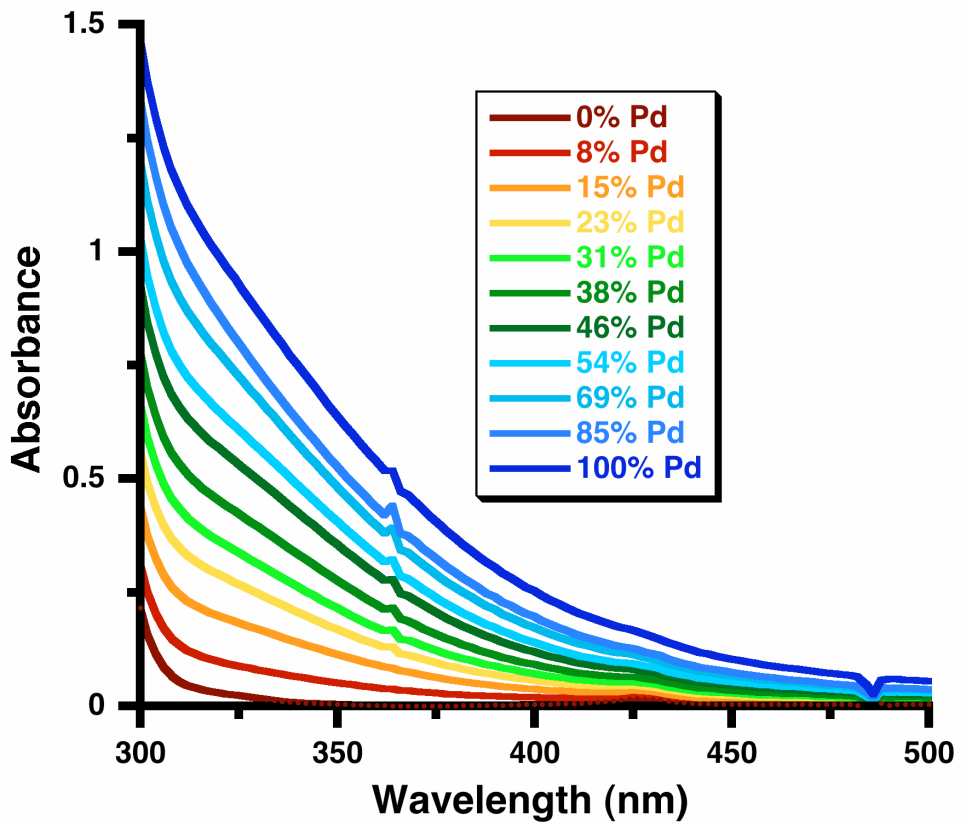

Figure S1. UV-Vis spectra of 480 μM apo H117G azurin in 5 mM MOPS buffer, pH = 7.0 at various concentrations of Pd^{2+} (shown as percentage of protein concentration).

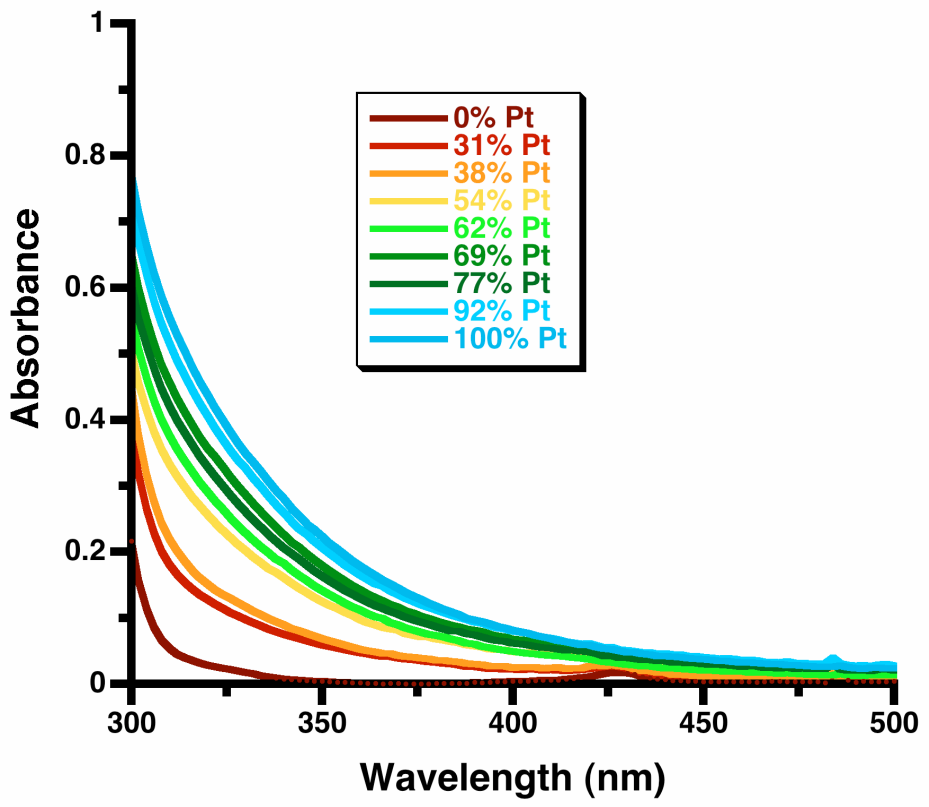

Figure S2. UV-Vis spectra of 480 μM apo H117G azurin in 5 mM MOPS buffer, pH = 7.0 at various concentrations of Pt^{2+} (shown as percentage of protein concentration).

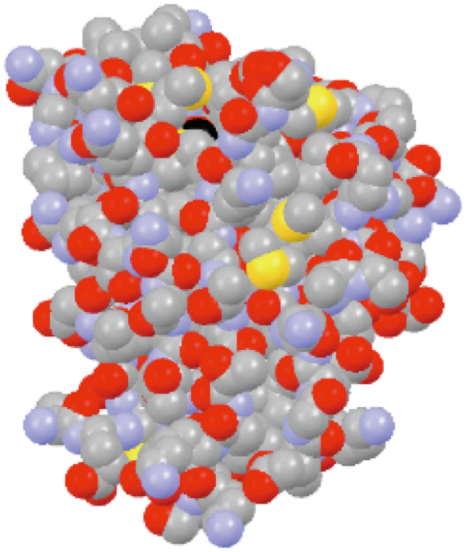

Figure S3. Space-filling diagram of a possible structure of His117Gly azurin-Pd^{2+} complex, derived from Pd addition to the known structure of His117Gly azurin-Zn^{2+}. The palladium atom is shown in black.

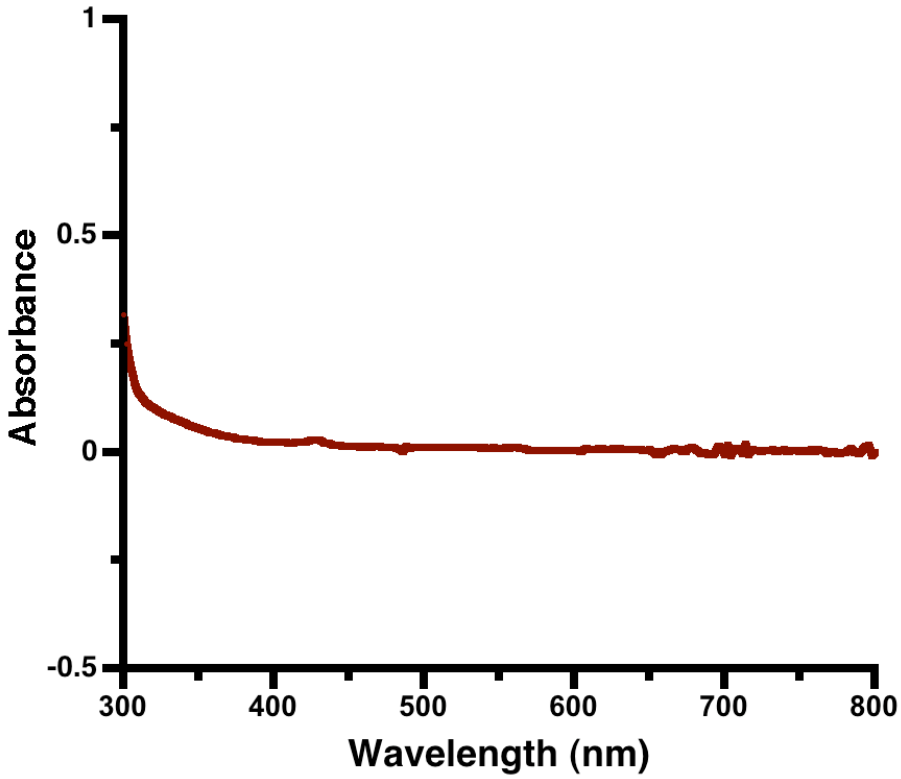

Figure S4. UV-Vis spectra of 480 μM apo wild type azurin in 5 mM MOPS buffer, pH = 7.0 with 480 μM Pd^{2+}.

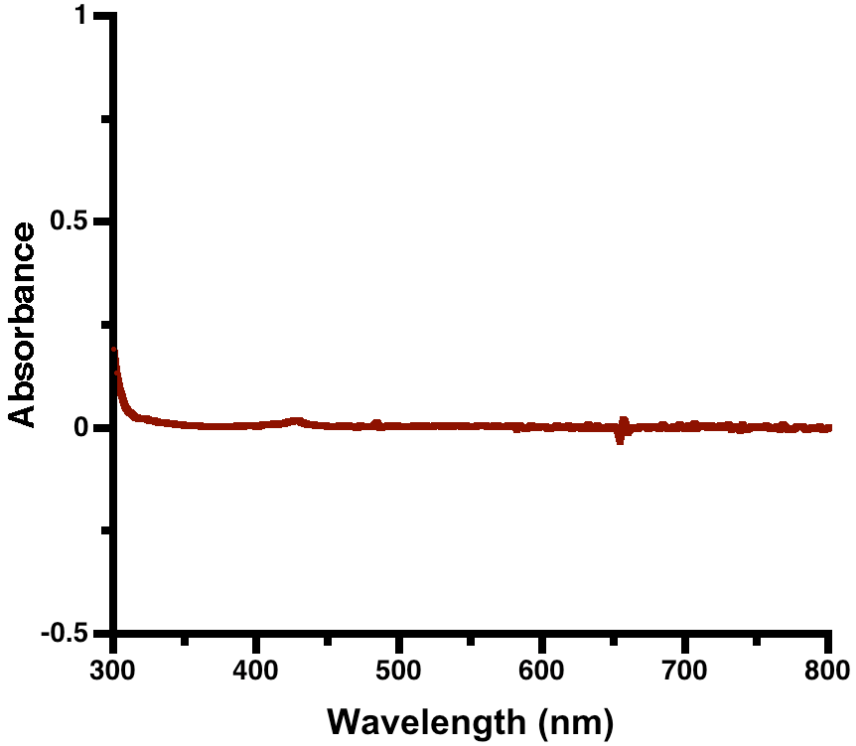

Figure S5. UV-Vis spectra of 480 μM apo wild type azurin in 5 mM MOPS buffer, pH = 7.0 with 480 μM Pt^{2+}.

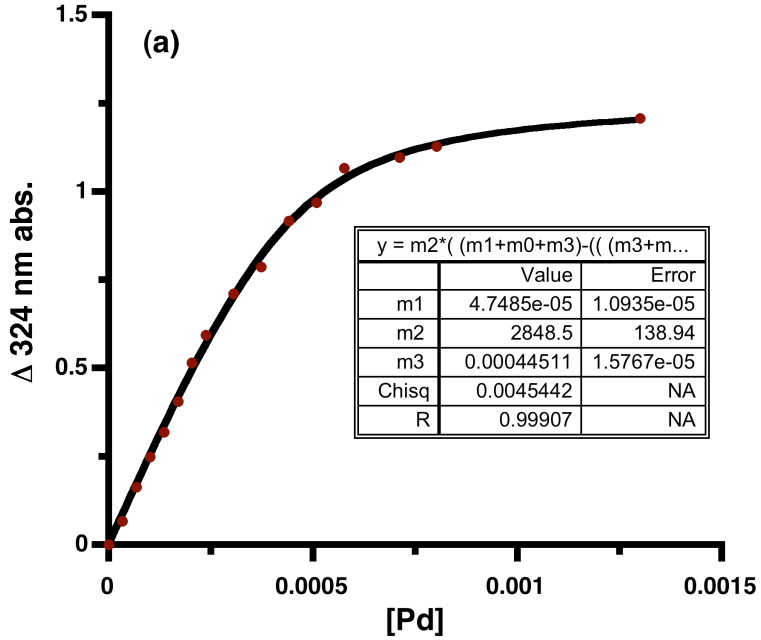

Figure S6. Fit from Figure 2a, with fit parameters. m1 is $1/K_{eq}$; m2 is a scale factor; m3 is the fit value of protein concentration (~ 0.48 mM, the value calculated from the extinction coefficient of the apo-protein).

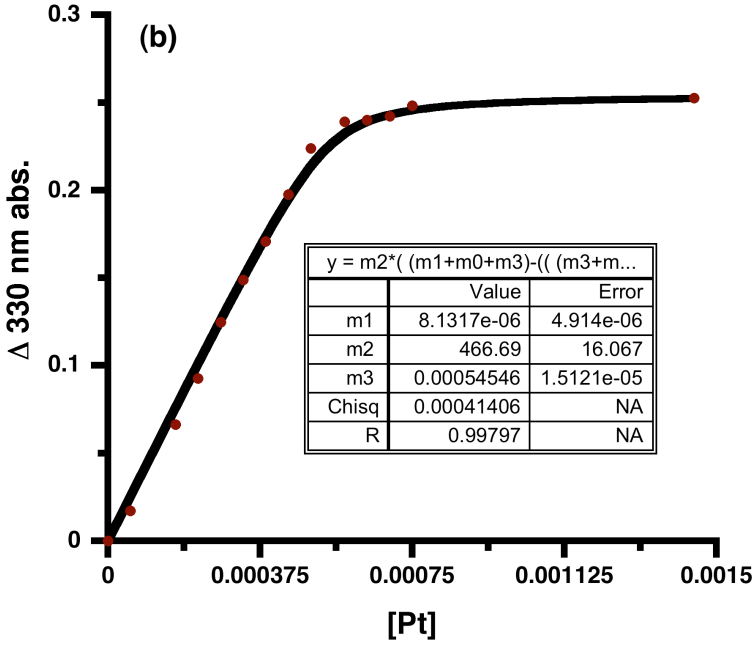

Figure S7. Fit from Figure 2b, with fit parameters. m1 is $1/K_{eq}$; m2 is a scale factor; m3 is the fit value of protein concentration (~ 0.48 mM, the value calculated from the extinction coefficient of the apo-protein).

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