Chemoselective Tryptophan Labeling with Rhodium Carbenoids at Mild pH

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Supporting Information

Procedures

Modification of 3-Methylindole. In a 0.6 mL microcentrifuge tube was combined 1.0 μL of a *t*BuOH solution of 3-methylindole (200 mM, 0.20 μmol), 4.0 μL of a *t*BuOH solution of **1** (1.0 M, 4.0 μmol), 5.0 μL of an aqueous solution of $Rh_2(OAc)_4$ (4.0 mM, 20 nmol), 150 μL of an aqueous solution of *t*BuNHOH (pH 6.0, 100 mM, 15 μmol), and 40 μL of ddH_2O. The reaction mixture was mixed gently at room temperature for 18 h, then diluted with 200 μL of MeCN and analyzed by ESI-MS.

Determination of pKa for H_2NOH and *t*BuNHOH. Solutions of H_2NOH•HCl or *t*BuNHOH•HCl (~100 mM) were prepared in ddH_2O. These solutions were then titrated with known volumes of 1.0 M NaOH and the resulting pH was measured using an accumet® Basic AB15 pH meter (Fisher Scientific, USA). The pH was then plotted as a function of the volume of 1.0 M NaOH added, and the resulting titration curves were used to estimate the pKa.

pH Profiles for Catalytic Degradation of Diazo Compound 1 with tBuNHOH and H_2NOH. Buffered solutions containing 100 mM H_2NOH•HCl or 100 mM *t*BuNHOH•HCl were prepared and adjusted to the pH values depicted in Figure S4. To a 0.6 mL microcentrifuge tube was then added 150 μL of the appropriate buffer, 5.0 μL of an aqueous solution of $Rh_2(OAc)_4$ (1.8 mg/mL, 20 nmol), 5.0 μL of a 400 mM *t*BuOH solution of **1** (134 mg/mL, 2.0 μmol), and 40.0 μL of ddH_2O. The reactions were then incubated on a laboratory vortexer for 10 h at room temperature and then diluted with 150 μL of MeCN. The diluted samples were then analyzed by reversed-phase HPLC (monitoring at 280 nm). The extent of diazo compound degradation was determined by comparing the observed peak area for **1** to standard samples of **1** that had been incubated for an identical period of time in pH 3.0 buffer in the absence of $Rh_2(OAc)_4$.

MS/MS Sequencing of Modified Melittin. ~20.0 μL of a crude melittin reaction mixture (modified at pH 6.0 using *t*BuNHOH) was desalted using a μC18 ZipTip® pipet tip. Modified and unmodified peptides were eluted in MeCN/ddH2O (60:40, 0.1% TFA). The desalted peptide mixture was then analyzed by MALDI-TOF/TOF MS/MS (CHCA matrix).

Supporting Figures

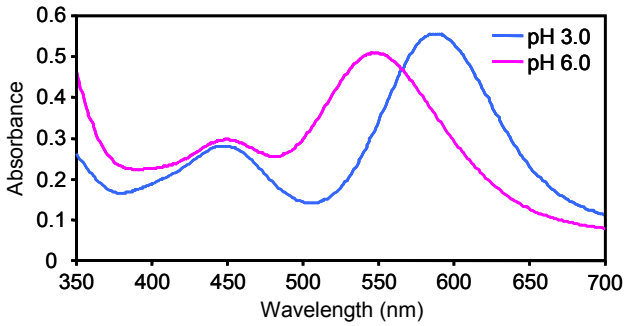

Figure S1. UV-vis spectrum of 1 mM $Rh_2(OAc)_4$ in 80% water / 20% ethylene glycol in the presence of 75 mM H_2NOH at pH 3.0 and pH 6.0.

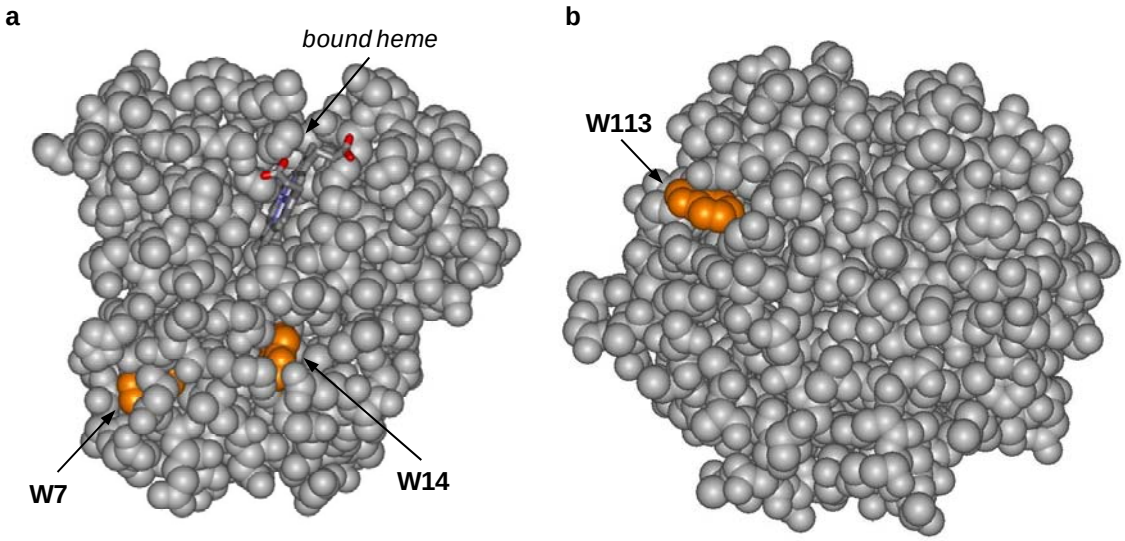

Figure S2. Space-filling models highlighting the positions of tryptophan residues (indole side chain shown in orange) in (a) horse heart myoglobin (PDB ID: 1WLA) and (b) subtilisin Carlsberg (PDB ID: 1SBC).

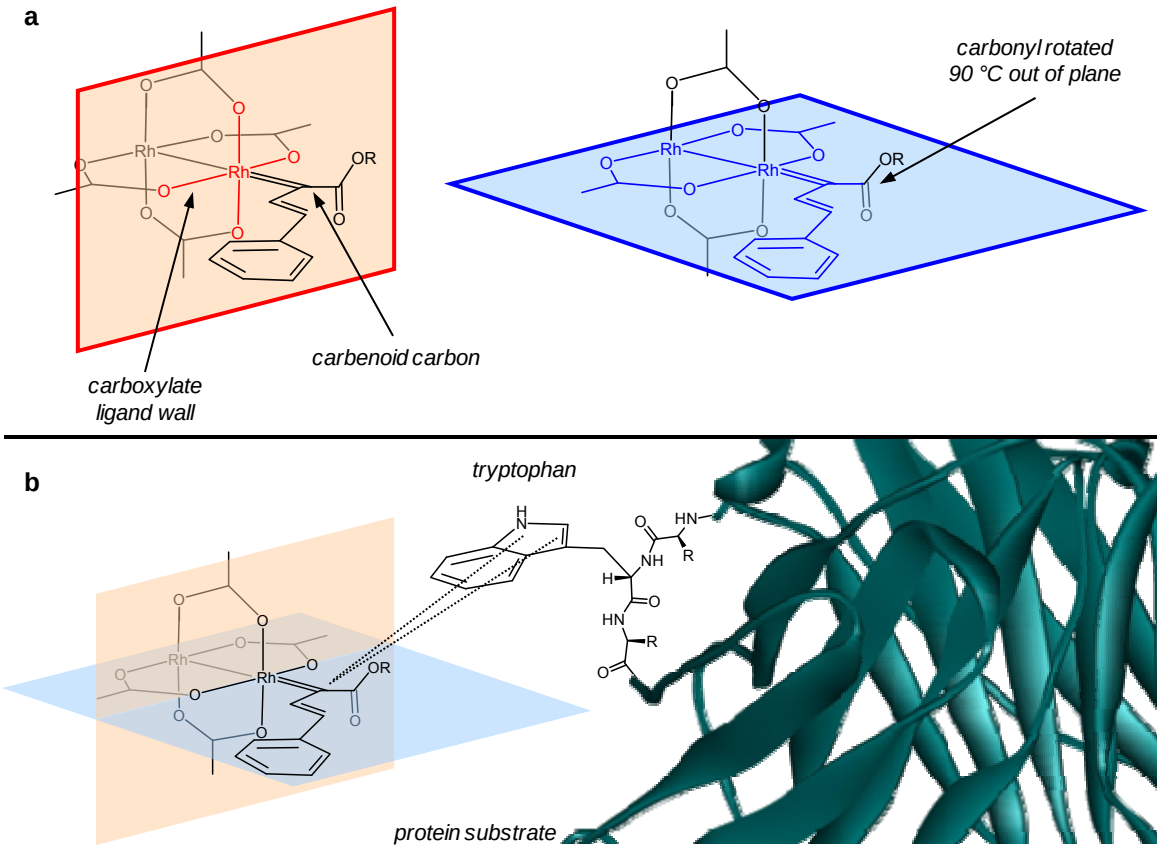

Figure S3. Steric considerations for tryptophan modification with rhodium carbenoid intermediates. (a) The carbenoid carbon lies in close proximity to the carboxylate ligands of the rhodium dimer and the ester functional group, resulting in a highly congested reactive center. (b) A high degree of solvent accessibility is required for tryptophan residues to approach the sterically congested carbenoid carbon successfully .

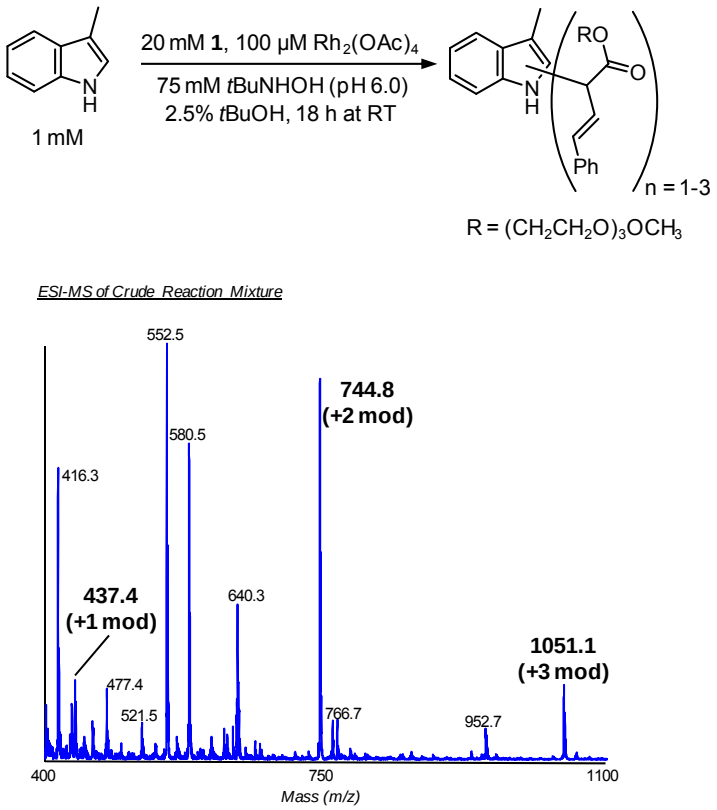

Figure S4. A 1 mM solution of 3-methylindole was treated with 20 mM **1** and 100 µM $Rh_2(OAc)_4$ in the presence of 75 mM *t*BuNHOH (pH 6.0), resulting in up to three modifications as indicated by ESI-MS. Calculated masses $[M+H]^+$ for +1 mod = 438.2, +2 mod = 744.4, +3 mod = 1050.5.

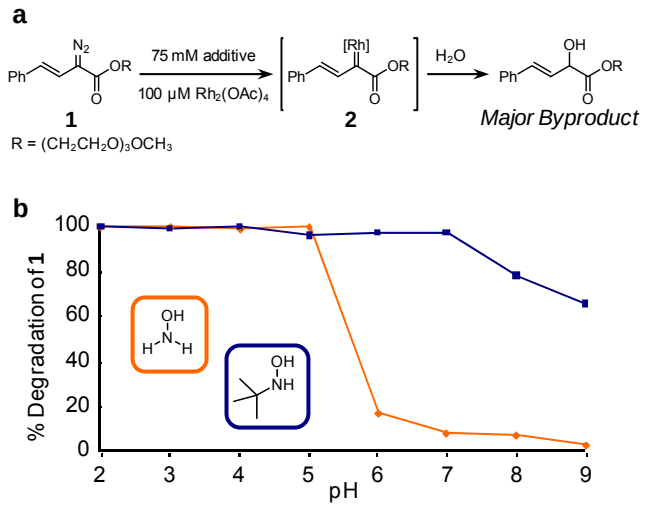

Figure S5. (a) Catalytic degradation of **1** by $Rh_2(OAc)_4$ in the presence of H_2NOH or *t*BuNHOH. (b) The consumption of **1** was determined by reversed-phase HPLC. Note: Reactions contain 2.5% *t*BuOH (v/v).

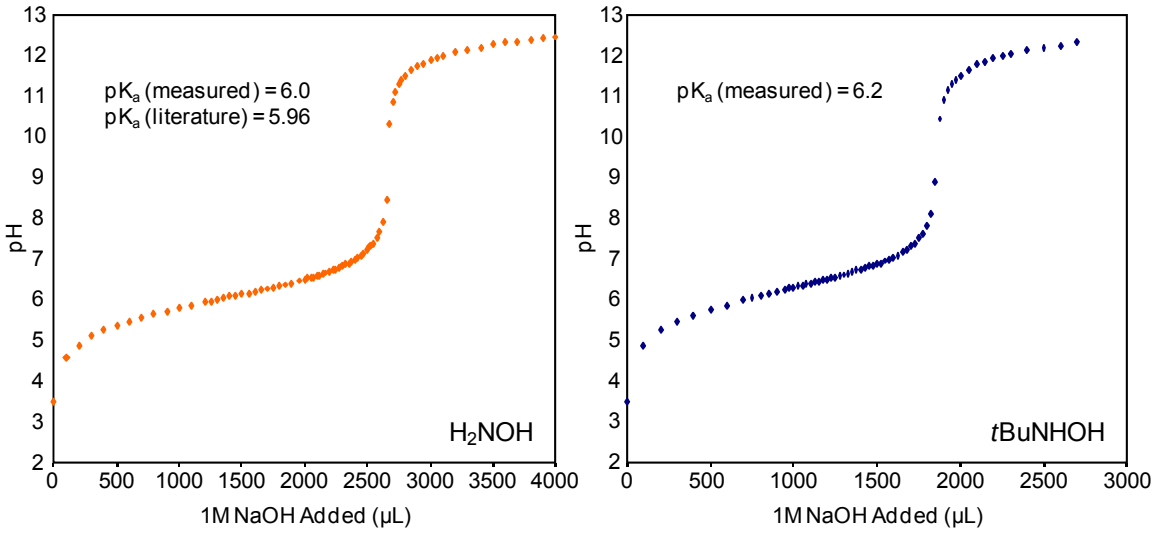

Figure S6. Titration curves for the determination of pK_a for H_2NOH and *t*BuNHOH.

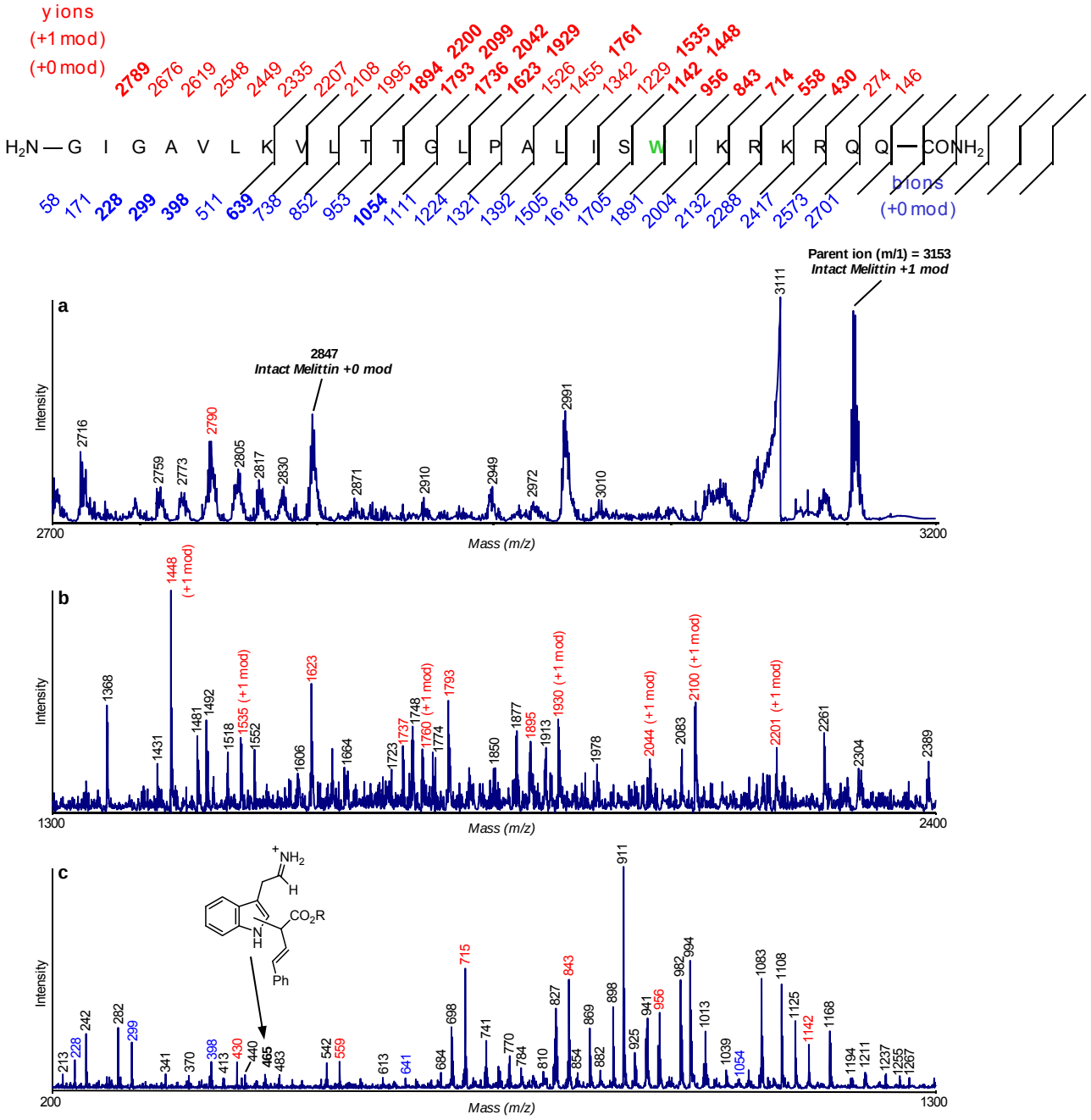

Figure S7. MALDI-TOF/TOF analysis of singly modified melittin. (a) Mass range m/z = 2700-3200. (b) Mass range m/z = 1300-2400. (c) Mass range m/z = 200-1300. Expected m/z = 465 for the immonium ion.

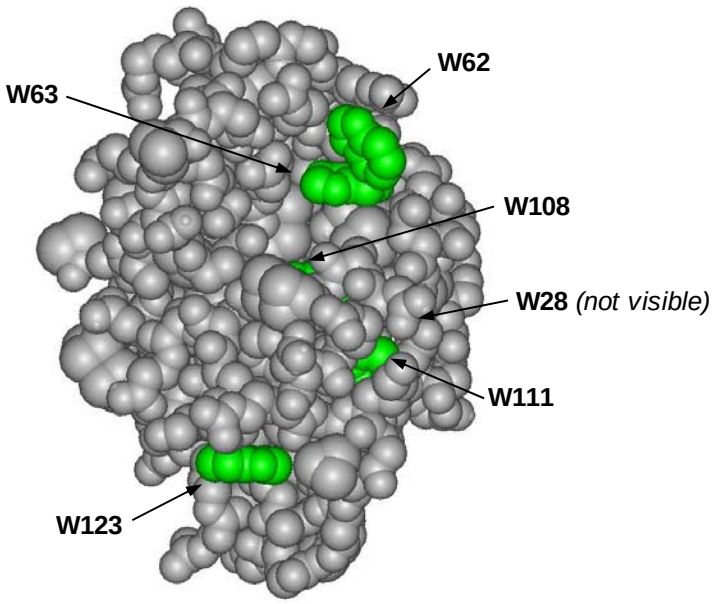

Figure S8. Space-filling model highlighting the positions of tryptophan residues (indole side chain shown in green) in hen egg white lysozyme (PDB ID: 2VB1). W28, W63, W108, and W111 are largely buried while W123 and W62 show a modest and significant degree of surface accessibility, respectively. The view of W28, W108, and W111 is obscured in most orientations of the space-filling model.

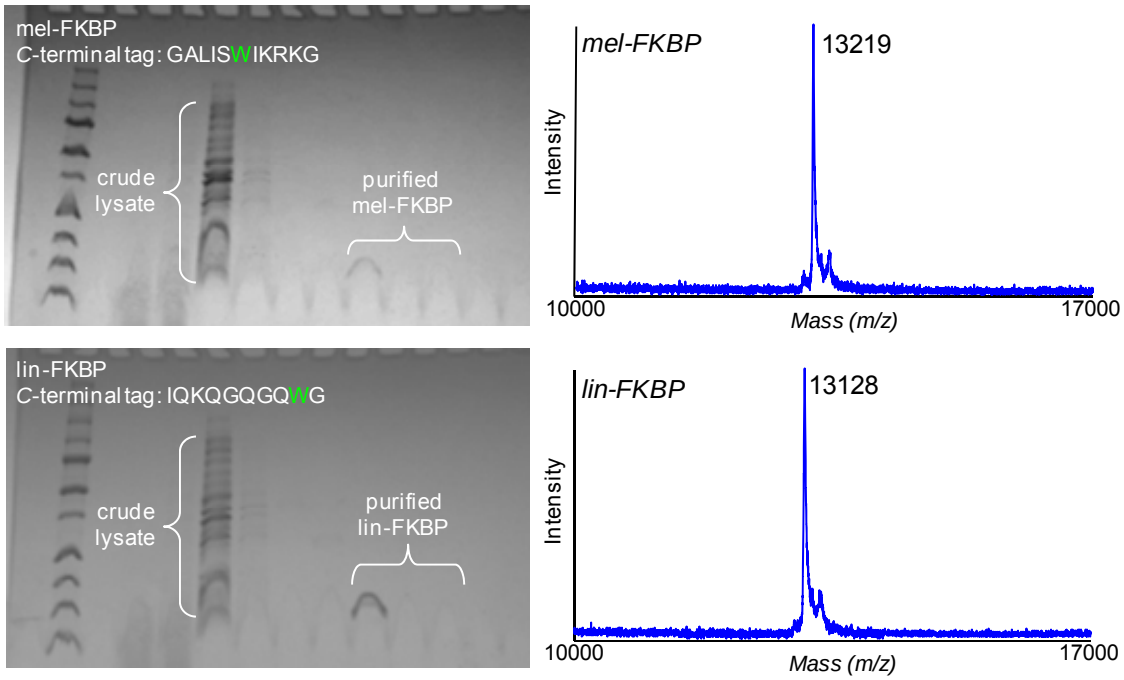

Figure S9. Two FKBP mutants were expressed with *C*-terminal peptide tags bearing tryptophan residues. The proteins were readily isolated by chitin affinity chromatography followed by intein cleavage. Protein purity was confirmed by SDS-PAGE. MALDI-TOF-MS (sinapinic acid matrix) revealed appropriate molecular weights for each mutant.