Supporting Information for:

Dynamic and structural differences between heme oxygenase-1 and -2 are due to differences in the C-terminal regions

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Running title: *C-terminus controls heme oxygenase dynamics*

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Supporting Excel Legend

Excel S1. Excel workbook containing all the measured relative deuterium values for all of the experiments. In each tab, the differences between the two states is shown at the right and corresponds to the difference map in the Figure indicated in the name of the tab.

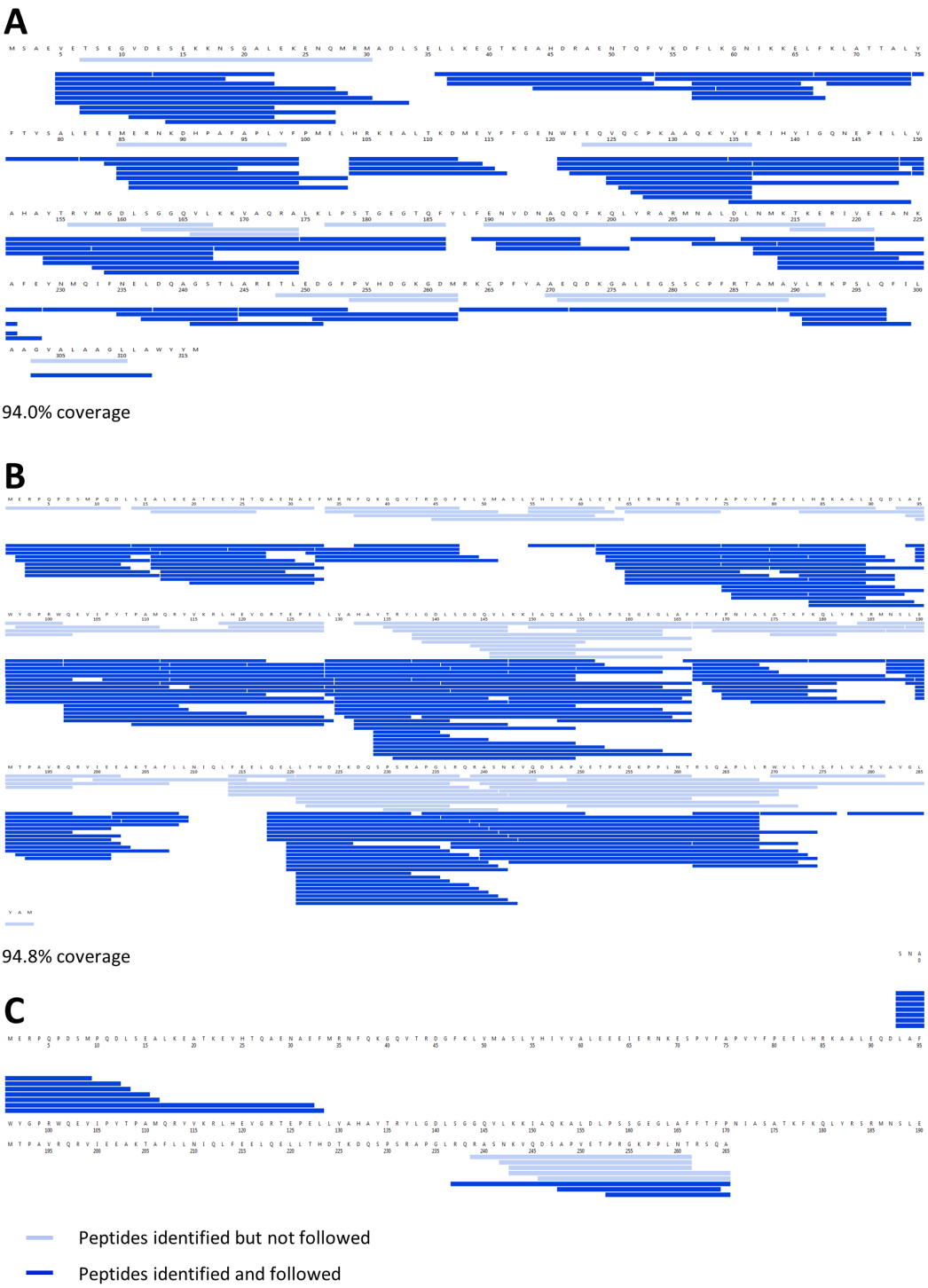

Figure S1. Coverage maps from HDX-MS experiments for HO1 and HO2. Dark blue bars represent peptides that were identified and followed via HDX-MS. Light blue bars represent peptides that were identified but could not be followed via HDX-MS. (A) Coverage map of full-length HO2. 88 peptides were followed covering 94% of the protein. (B) Coverage map of full-length HO1, which contains a mutation (R254K) for purification purposes. 200 peptides were followed covering 94.8% of the protein. (C) HO1 peptides that contain the wild type residue (R254) and the different N-terminal purification tag found in the 1-265 construct.

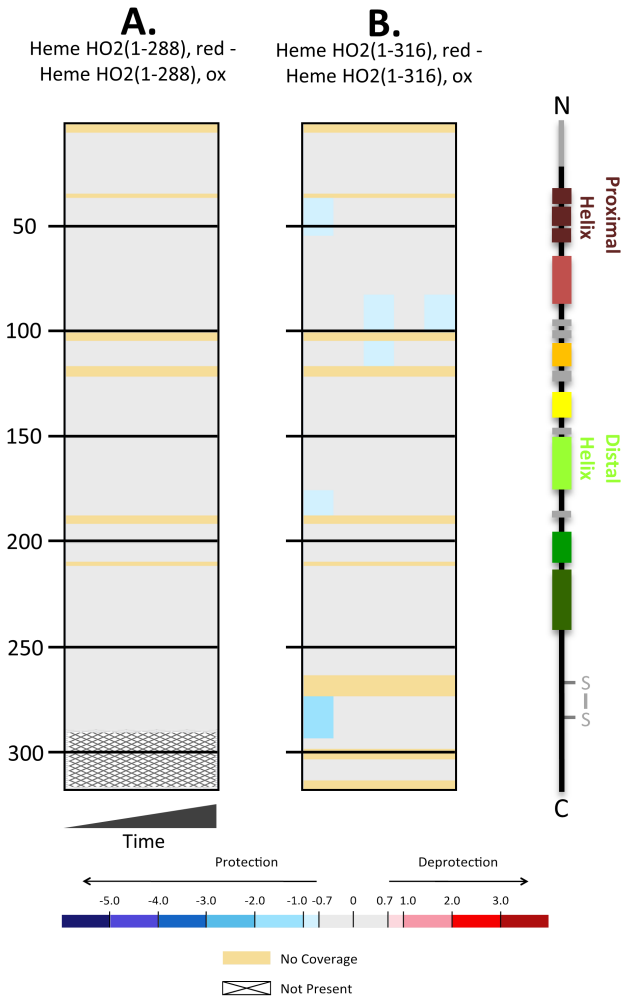

Figure S2. HDX-MS comparisons examining the impact of the HRM status (heme-bound versus disulfide bond) on HO2(1-288). (A) Heme HO2(1-288), ox is Apo HO2(1-288) incubated with heme; heme binds to the core of the protein while the disulfide bond between Cys265 and Cys282 remains intact. Heme HO2(1-288), red is protein treated with TCEP prior to incubation with heme; heme binds to the core as well as Cys265 and Cys282. Deuterium differences were calculated from $D_{\text{heme HO2(1-288), red}} - D_{\text{heme HO2(1-288), ox}}$ and colored according to the scale shown. (B) Heme HO2(1-316), ox is Apo HO2(1-288) incubated with heme; heme binds to the core of the protein while the disulfide bond between Cys265 and Cys282 remains intact. Heme HO2(1-316), red is protein treated with TCEP prior to incubation with heme; heme binds to the core as well as Cys265 and Cys282. Deuterium differences were calculated from $D_{\text{heme HO2(1-316), red}} - D_{\text{heme HO2(1-316), ox}}$ and colored according to the scale shown. All data used to create this figure can be found in the supplemental material (Excel S1).

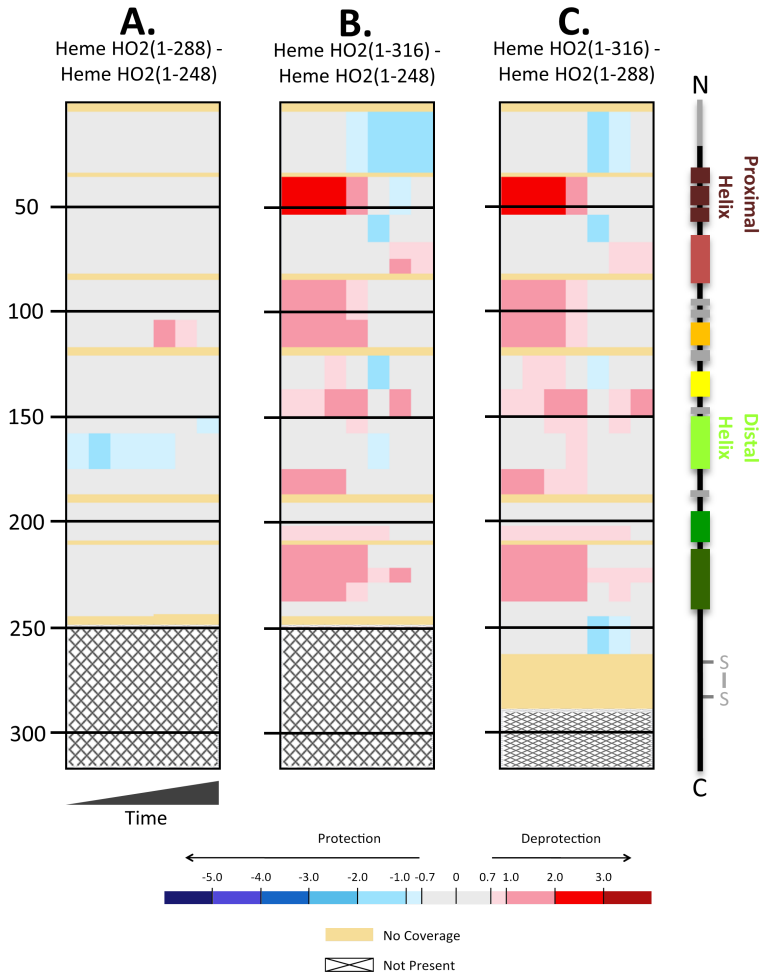

Figure S3. HDX-MS comparisons of the heme-bound states of full-length and truncated HO2 constructs. (A) The impact of residues 249-288 on the heme-bound state of HO2. Deuterium differences were calculated from $D_{\text{heme HO2(1-288)}} - D_{\text{heme HO2(1-248)}}$ and colored according to the scale shown. (B) The impact of residues 249-316 on the heme-bound state of HO2. Deuterium differences were calculated from $D_{\text{heme HO2(1-316)}} - D_{\text{heme HO2(1-248)}}$ and colored according to the scale shown. (C) The impact of residues 289-316 on the heme-bound state of HO2. Deuterium differences were calculated from $D_{\text{heme HO2(1-316)}} - D_{\text{heme HO2(1-288)}}$ and colored according to the scale shown. All data used to create this figure can be found in the supplemental material (Excel S1).

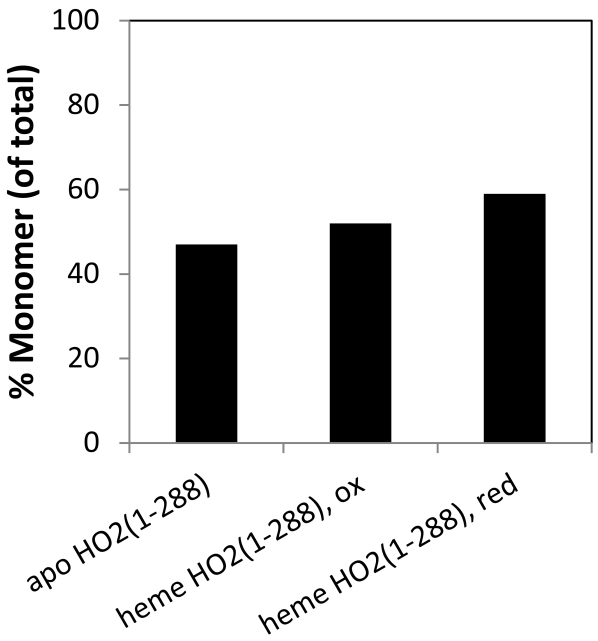

Figure S4. Analysis of HO2(1-288) in various states by size exclusion chromatography. Apo HO2(1-288) is protein as purified in the apo form with a disulfide bond between Cys265 and Cys282. Heme HO2(1-288), ox is Apo HO2(1-288) incubated with heme; heme binds to the core of the protein while the disulfide bond between Cys265 and Cys282 remains intact. Heme HO2(1-288), red is protein treated with TCEP prior to incubation with heme; heme binds to the core as well as Cys265 and Cys282. 25 μM protein was loaded onto a Superdex 75 Increase 10/300 GL column at 0.8 mL min^{-1} in 50 mM Tris buffer (pH 8.0) containing 50 mM KCl. The area of the monomer peak as a percent of the total area of all peaks was quantified and plotted.

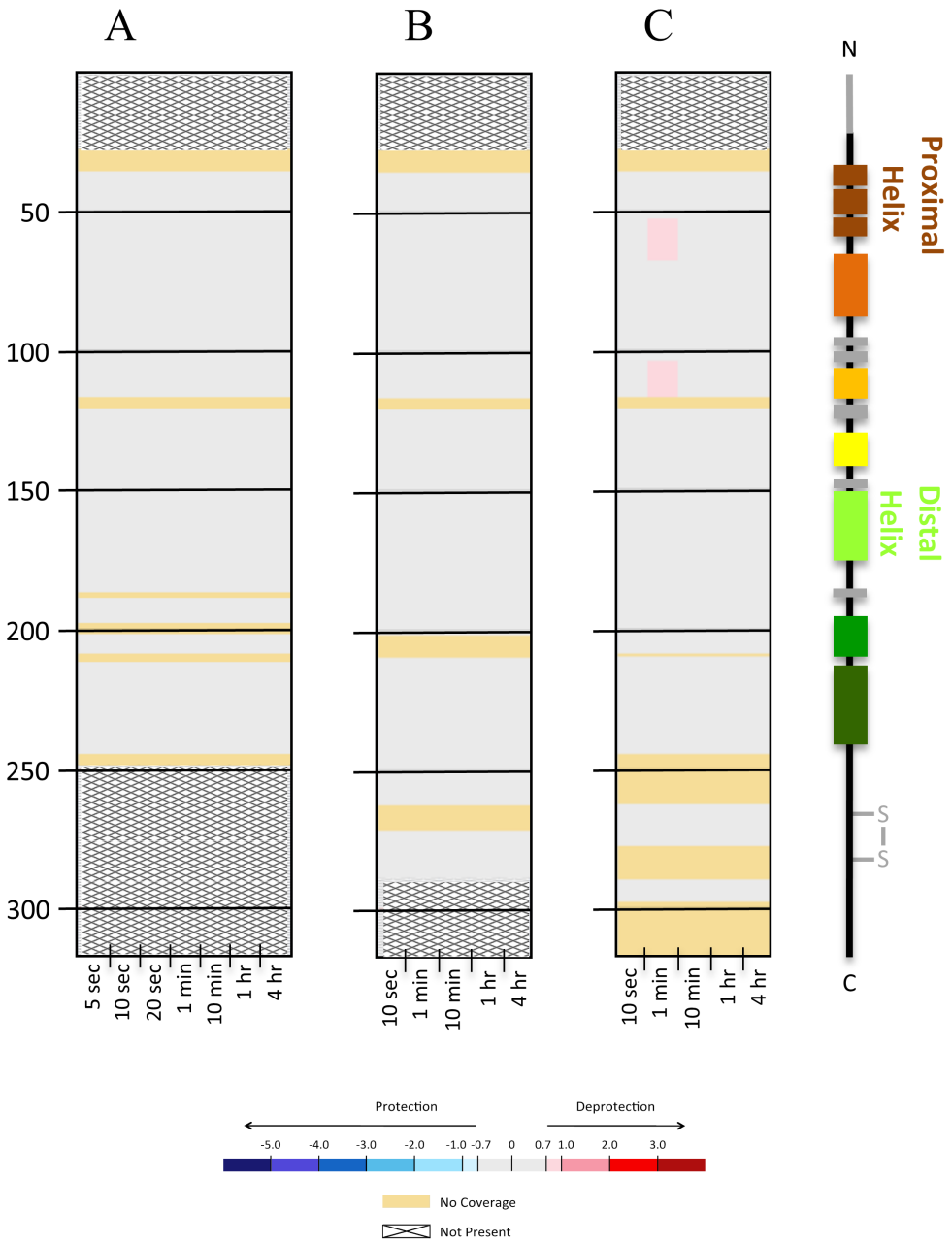

Figure S5. HDX-MS comparisons examining the impact of the N-terminal residues (1-27) on HO2 of various C-terminal length. (A) The impact of residues 1-27 on the apo state of HO2 1-248. Deuterium differences were calculated from $D_{apo\ HO2(1-248)} - D_{apo\ HO2(28-248)}$ and colored according to the scale. (B) The impact of residues 1-27 on the apo state of HO2 1-288. Deuterium differences were calculated from $D_{apo\ HO2(1-288)} - D_{apo\ HO2(28-288)}$, (C). The impact of residues 1-27 on the apo state of HO2 1-316. Deuterium differences were calculated from $D_{apo\ HO2(1-316)} - D_{apo\ HO2(28-316)}$.

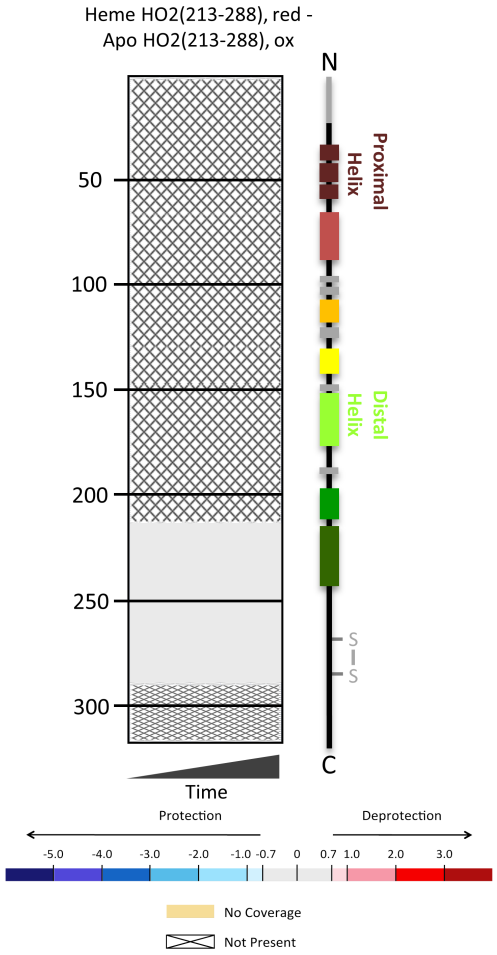

Figure S6. HDX-MS comparisons examining the impact of the HRM status (heme-bound versus disulfide bond) on HO2(213-288). Heme HO2(213-288), red is protein treated with TCEP prior to incubation with heme; heme binds to Cys265 and Cys282. Apo HO2(213-288), ox is protein as purified in the apo form with a disulfide bond between Cys265 and Cys282. Deuterium differences were calculated from $D_{\text{heme HO2(1-316), red}} - D_{\text{heme HO2(1-316), ox}}$ and colored according to the scale shown. All data used to create this figure can be found in the supplemental material (Excel S1).

Excel S1. Excel workbook of deuterium incorporation of the various constructs. The data that were summarized in each figure are show in tabs labeled with the name of each figure. Within each tab, the peptides are listed in the left-hand column, with the time points running across the top. The measured deuterium values for each form of the protein in each tab are shown to the right of the peptides. To the far right are the differences, with the cells colored according to the same scale used in the figure named in each tab.