

Supporting information

Regulation of nitrite reductase and lipid binding properties of Cytochrome b5 by surface and distal histidine mutations

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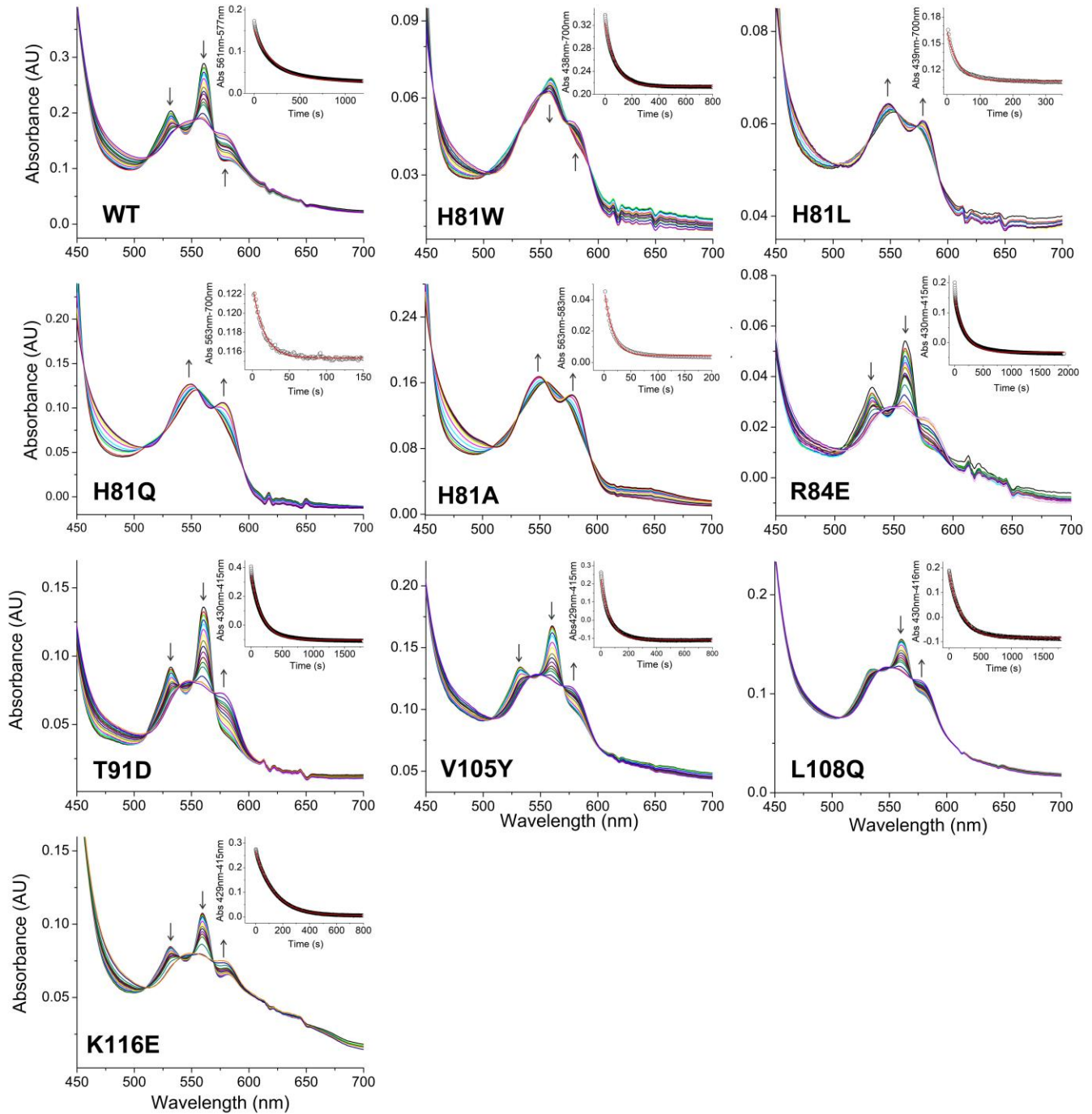
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Supplemental Figure 1. Reaction of wild type Cytochrome b5 and mutants with nitrite.

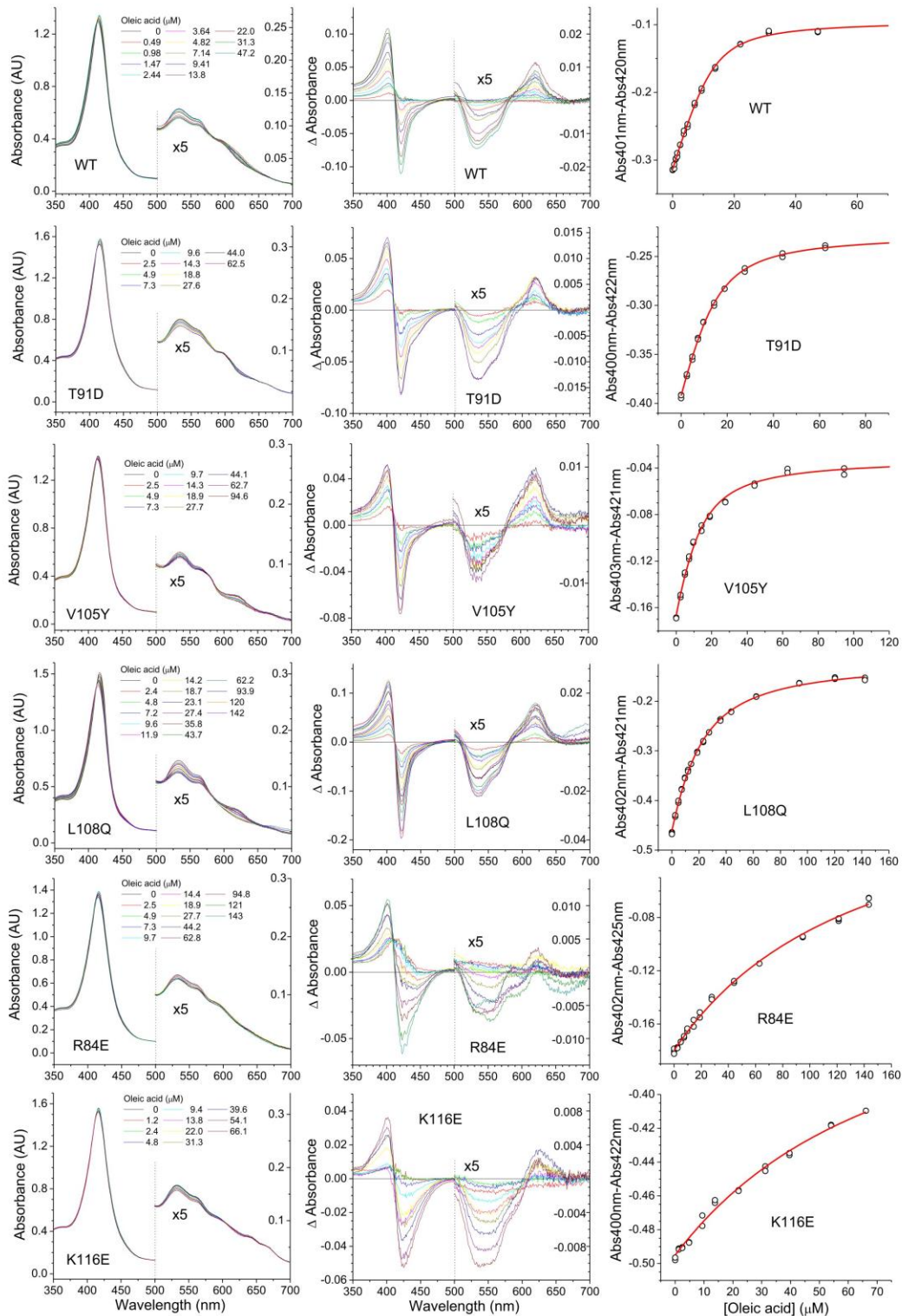
Supplemental Figure 2. Oleate binding to wild type Cytochrome b5 and mutants determined by differential spectroscopy.

Supplemental Figure 3. Octanoate binding to wild type Cytochrome b5 and mutants determined by differential spectroscopy.

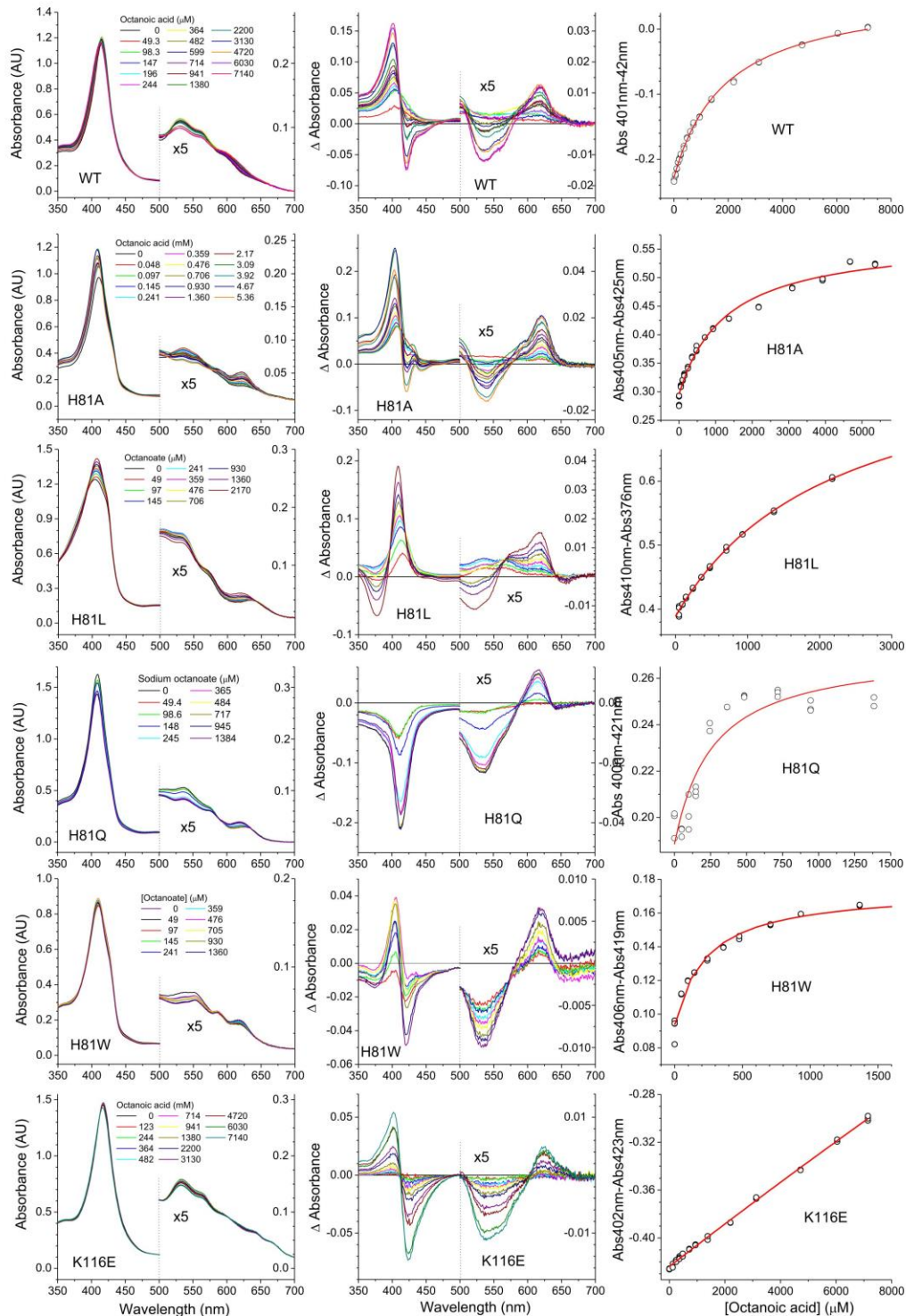
Supplemental Figure 4. Imidazole binding to wild type Cytochrome b5 and mutants determined by differential spectroscopy.



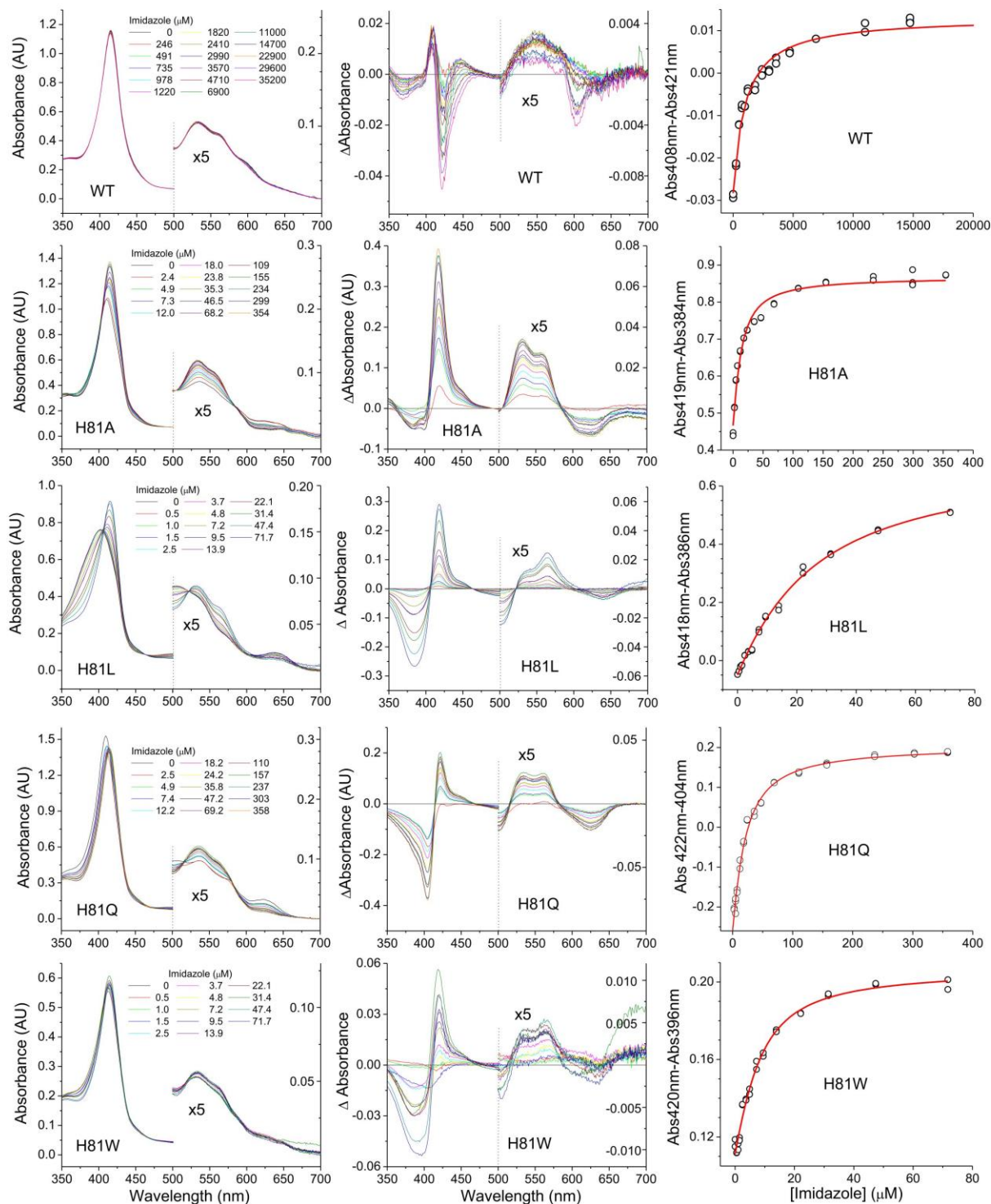
Supplemental Figure 1. Reaction of wild type Cytochrome b5 and mutants with nitrite. Panels show the reaction of 2-10 μM Cytochrome b5 with Sodium nitrite. Nitrite concentrations for each panel were as follows: WT, 5 mM; H81W, 1 mM; H81L, 500 μM ; H81Q, 250 μM ; H81A, 50 μM ; R84E, 10 mM; T91D, 5 mM; V105Y, 5 mM; L108Q, 10 mM; K116E, 10 mM. The main panels show the spectral changes during the reaction; the insets show the absorbance changes at selected wavelengths. The arrows in the main panels indicate the direction of the spectral changes. Red traces in the insets indicate the fit of the experimental data to a single exponential equation. The reactions were followed at 37 $^{\circ}\text{C}$ in 100 mM sodium phosphate, pH 7.4.



Supplemental Figure 2. Oleate binding to wild type Cytochrome b5 and mutants determined by differential spectroscopy. Left panels indicate the absorbance changes upon addition of increasing amounts of oleate. The concentrations of oleate are indicated in each panel. Middle panels show the difference spectra -subtracting the initial spectrum from each spectrum. Right panels show the fit to determine the apparent K_D values by fitting the absorbance differences at the wavelengths showing the largest absorbance change *versus* the concentration of oleate. The red line denotes the fit of the data to Equation 1. The 500nm-700nm range in the left and middle panels is enlarged to show the spectral changes in detail.



Supplemental Figure 3. Octanoate binding to wild type Cytochrome c and mutants determined by differential spectroscopy. Left panels indicate the absorbance changes upon addition of increasing amounts of sodium octanoate. The concentrations of octanoate are indicated in each panel. Middle panels show the difference spectra -subtracting the initial spectrum from each spectrum. Right panels show the fit to determine the apparent K_D values by fitting the absorbance differences at the wavelengths showing the largest absorbance change *versus* the concentration of octanoate. The red line denotes the fit of the data to Equation 1. The 500nm-700nm range in the left and middle panels is enlarged to show the spectral changes in detail.



Supplemental Figure 4. Imidazole binding to wild type Cytochrome c and mutants determined by differential spectroscopy. Left panels indicate the absorbance changes upon addition of increasing amounts of imidazole. The concentrations of imidazole are indicated in each panel. Middle panels show the difference spectra -subtracting the initial spectrum from each spectrum. Right panels show the fit to determine the apparent K_D values by fitting the absorbance differences at the wavelengths showing the largest absorbance change *versus* the concentration of imidazole. The red line denotes the fit of the data to Equation 1. The 500nm-700nm range in the left and middle panels is enlarged to show the spectral changes in detail.