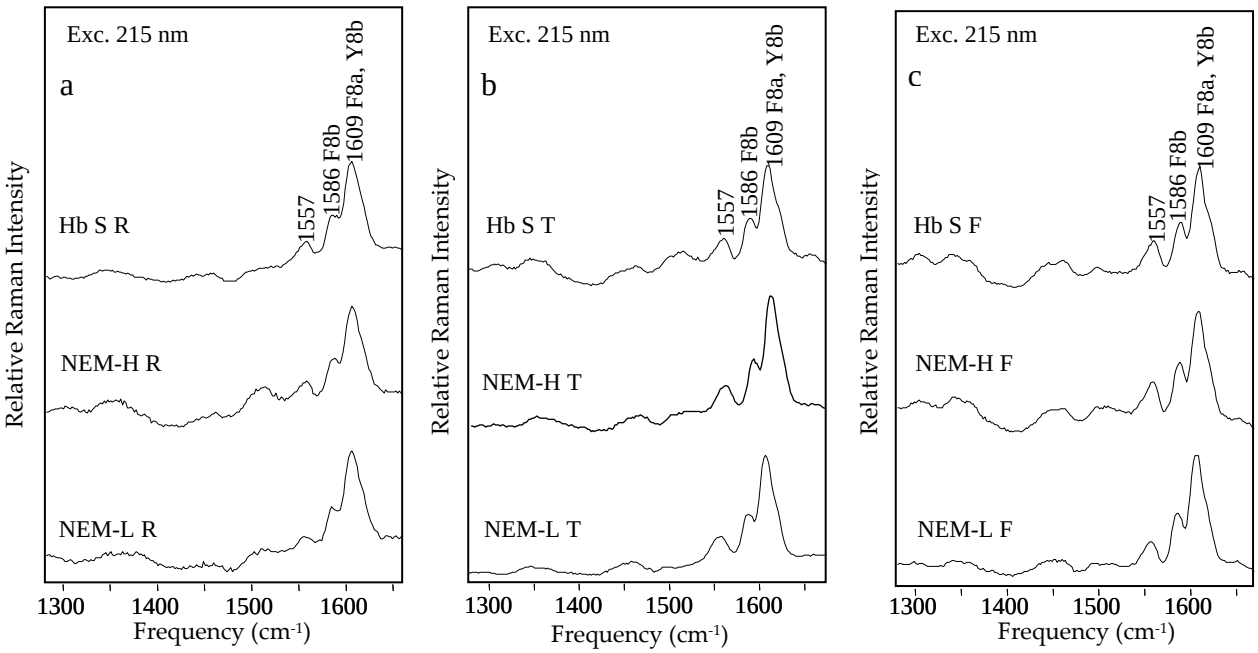
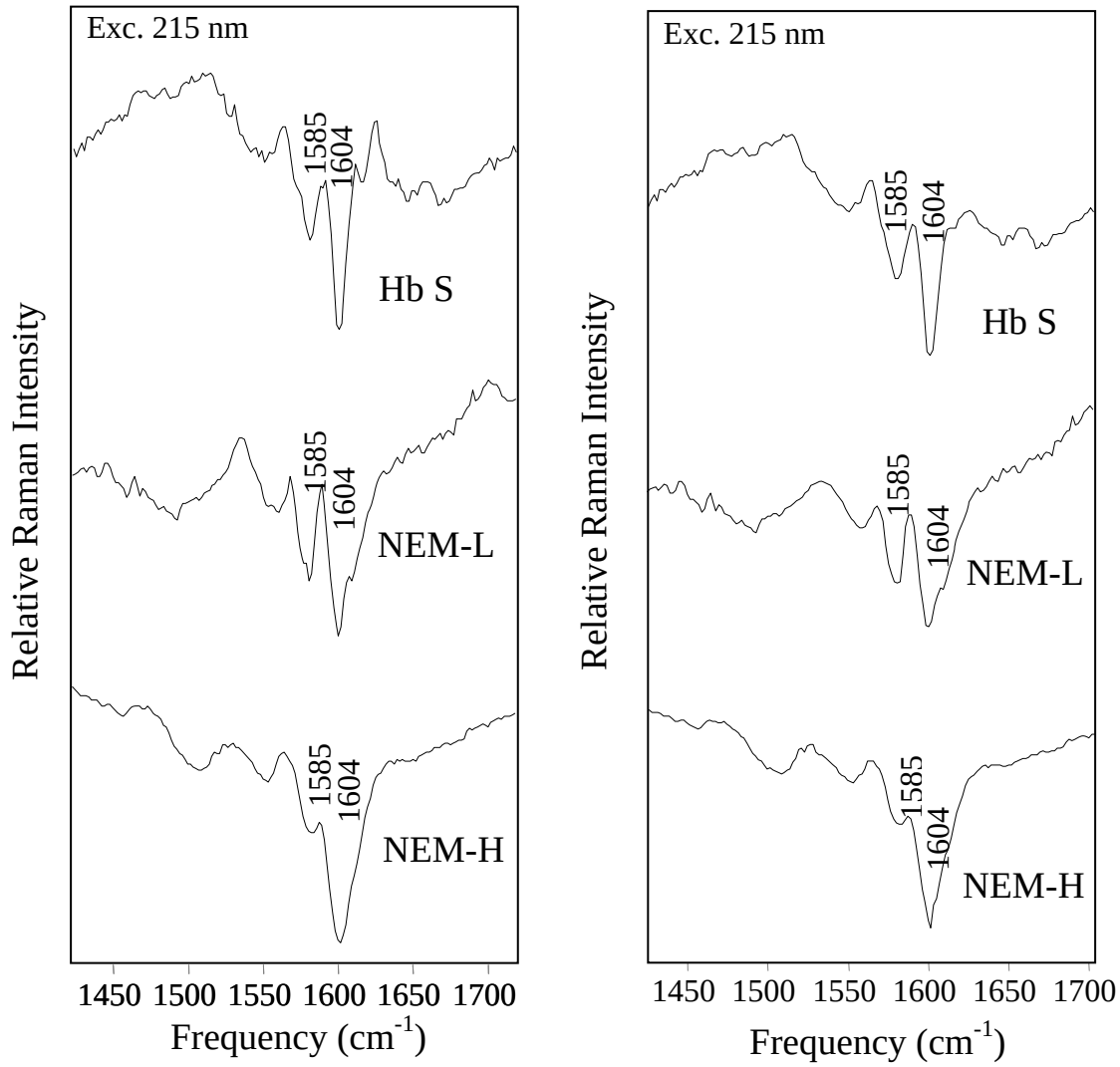
Supplementary Figures



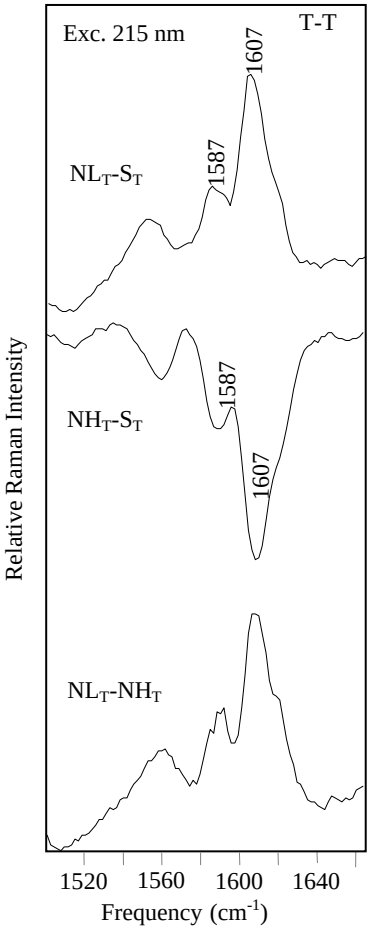

Supplementary Figure 1

Supplementary Figure 1: 215 nm-excited UVRR spectra. R- (a), T- (b), and Fiber-state (c), spectra of Hb S (top), NEM-H Hb (center) and NEM-L Hb (bottom) obtained with 215 nm excitation. Samples were prepared as described in figures 5 and 6. Depicted spectra result from 3 hours of data acquisition.



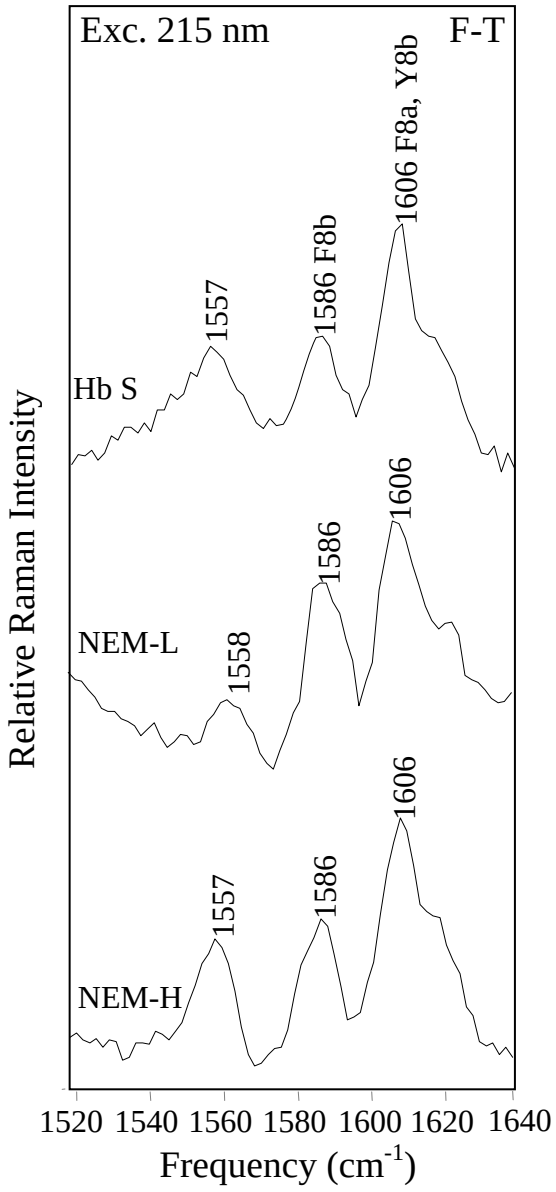

Supplementary Figure 2.

Supplementary Figure 2: T-R difference spectra of Hb S (top), NEM-H Hb (center) and NEM-L Hb (bottom), obtained with 215 nm excitation, in high (left) and low (right) potassium phosphate buffer.. Low phosphate samples were 0.25 mM Hb, in a 0.1 potassium phosphate buffer, pH 7.1, and high phosphate samples were 1.0 mM Hb in a 1.0 mM potassium phosphate buffer, pH 7.1. Samples were prepared as described in Materials and Methods.



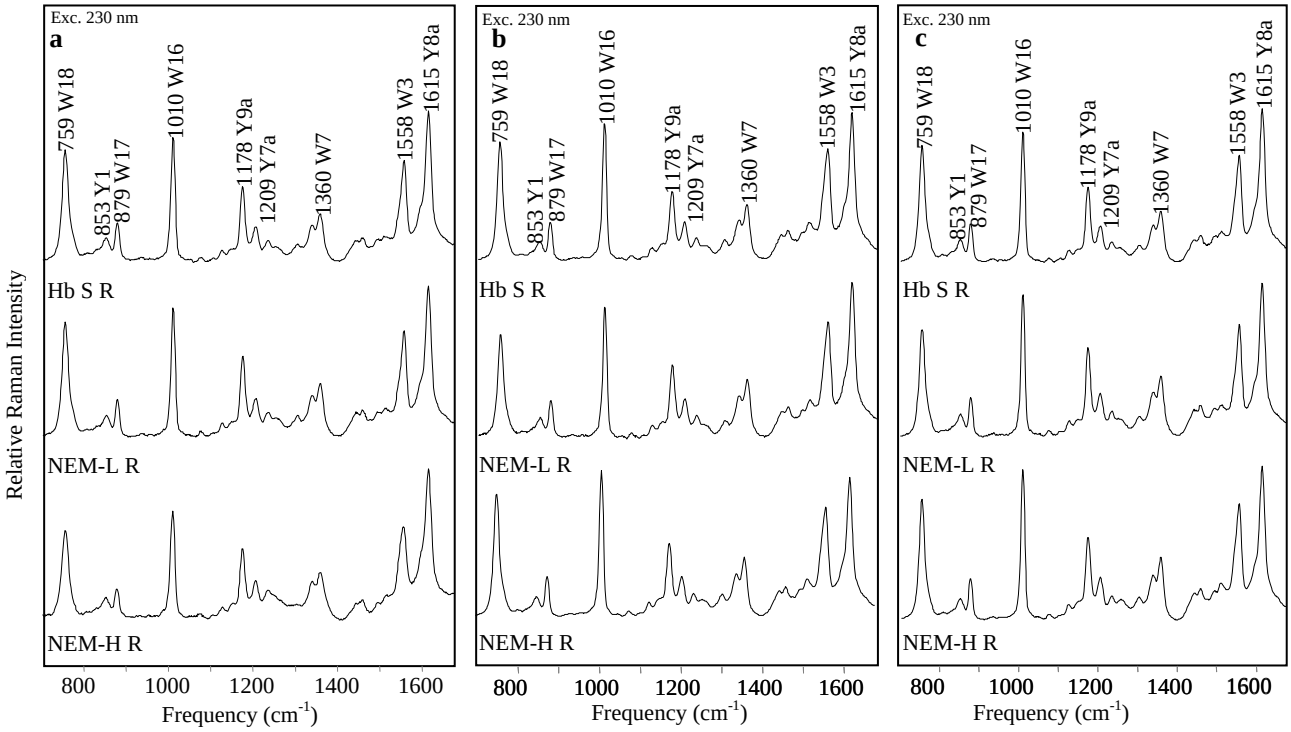

Supplementary Figure 3

Supplementary Figure 3: T-T difference spectra of Hb S (top), NEM-H Hb (center) and NEM-L Hb (bottom), obtained with 215 nm excitation. Samples were 0.25 mM Hb, in a 0.1 potassium phosphate buffer, pH 7.1. Samples were prepared as described in Materials and Methods.



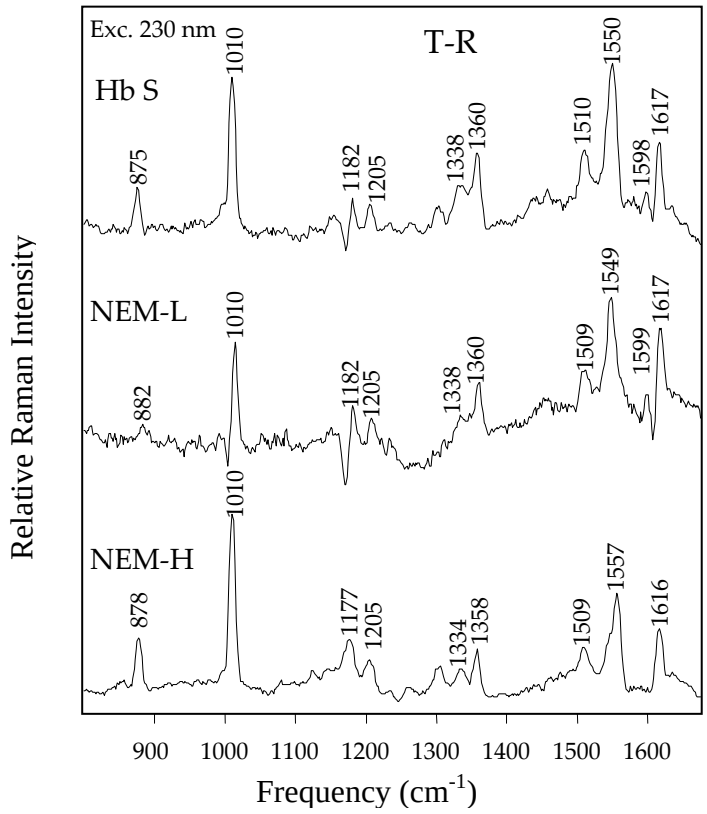

Supplementary Figure 4

Supplementary Figure 4: F-T difference spectra of Hb S (top), NEM-H Hb (center) and NEM-L Hb (bottom), obtained with 215 nm excitation. T-state samples were 0.25 mM Hb, in a 0.1 potassium phosphate buffer, pH 7.1, and fiber samples were 3.0 mM Hb in a 0.1 M potassium phosphate buffer, pH 7.1. Samples were prepared as described in Materials and Methods.



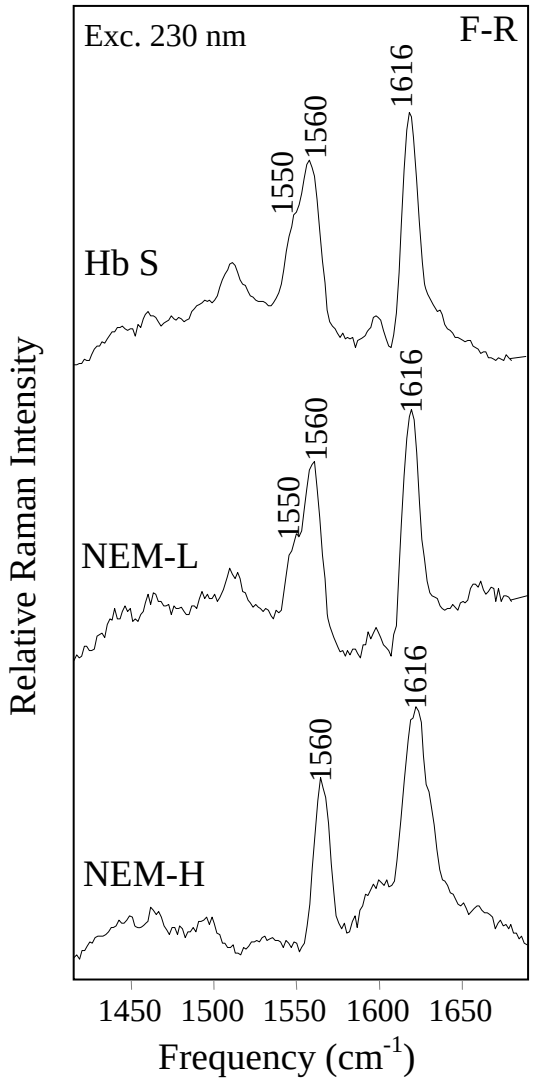

Supplementary Figure 5

Supplementary Figure 5: 230 nm excited UVRR spectra. R- (a), T- (b), and Fiber-state (c) spectra of Hb S (top), NEM-H Hb (center) and NEM-L Hb (bottom) obtained with 230 nm excitation. Samples were prepared as described in figures 5 and 6. Depicted spectra result from 3 hours of data acquisition.




Supplementary Figure 6

Supplementary Figure 6: T-R difference spectra of Hb S (top), NEM-L (center) and NEM-H (bottom) obtained with 230 nm excitation. Samples were 0.25 mM Hb in a 0.1 M potassium phosphate buffer, pH 7.1.




Supplementary Figure 7

Supplementary Figure 7: F-R difference spectra of Hb S (top), NEM-L (center) and NEM-H (bottom) obtained with 230 nm excitation. R-state samples were 0.25 mM Hb in a 0.1 M potassium phosphate buffer, pH 7.1, and fiber samples were 3.0 mM Hb in a 0.1 M potassium phosphate buffer, pH 7.1.

Supplementary Table 1

Supplementary Table 1: List of sequence of all Cys containing labeled peptides associated with the β93 and α104 Cys residues, observed by reverse phase LC mass spectrometry.

α104

VNFKLLSHCLLV
VNFKLLSHCLLVT
VNFKLLSHCLLVTLA
VNFKLLSHCLLVTLAA
VNFKLLSHCLLVTLAAHLPAE
AHKLRVDPVNFKLLSHCLL
FKLLSHCLL
FKLLSHCLLV
FKLLSHCLLVTLA
FKLLSHCLLVTLAA
FKLLSHCLLVTLAAH
FKLLSHCLLVTLAAHLPAEFT
FKLLSHCLLVTLAAHLPAEFTPAV
KLLSHCLL
KLLSHCLLVTLA
KLLSHCLLVTLAA
KLLSHCLLVTLAAH
KLLSHCLLVTLAAHLPAEFT
KLLSHCLLVT
KLRVDPVNFKLLSHCLL
KLRVDPVNFKLLSHCLLV
KLRVDPVNFKLLSHCLLVT
KLRVDPVNFKLLSHCLLVTLA
LHAHKLRVDPVNFKLLSHCLL
LLSHCLL
LLSHCLLV
LLSHCLLVT
LLSHCLLVTLA
LLSHCLLVTLAAH
LLSHCLLVTLAAHLPAE
LLSHCLLVTLAAHLPAEFT
LLSHCLLVTLAAHLPAEFTPAV
LRVDPVNFKLLSHCLL
LRVDPVNFKLLSHCLLV
LRVDPVNFKLLSHCLLVT
LSHCLLVTLAAH
RVDPVNFKLLSHCLLV
RVDPVNFKLLSHCLLVT

RVDPVNFKLLSHCLLVTLA
RVDPVNFKLLSHCLLVTLAAHLPAE
RVDPVNFKLLSHCLL
RVDPVNFKLLSHCLLV
RVDPVNFKLLSHCLLVT
RVDPVNFKLLSHCLLVTLAA
RVDPVNFKLLSHCLLVTLAAH
VDPVNFKLLSHCLL
VDPVNFKLLSHCLLV
VDPVNFKLLSHCLLVT
VDPVNFKLLSHCLLVTLA
VDPVNFKLLSHCLLVTLAA
VNFKLLSHCLLV
VNFKLLSHCLLVT
VNFKLLSHCLLVTLA
VNFKLLSHCLLVTLAA
VNFKLLSHCLLVTLAAHLPAE

## β93

AFSDGLAHLDNLKGTFATLSELHCDKL
AHLDNLKGTFATLSELHCDKLH
AHLDNLKGTFATLSELHCDKLHVDPENFRL
AHLDNLKGTFATLSELHCDKLHVDPENFRLL
ATLSELHCDKLH
ATLSELHCDKLHVD
ATLSELHCDKLHVDPENFRLL
ATLSELHCDKLHVDPENFRLLGNVL
ATLSELHCDKL
ATLSELHCDKLHVDPENFR
ATLSELHCDKLHVDPENFRLLGNVLV
ATLSELHCDKLHVDPENFRLLGNVLVCVL
FATLSELHCDKL
FATLSELHCDKLH
FATLSELHCDKLHVD
FATLSELHCDKLHVDPENFR
FATLSELHCDKLHVDPENFRL
FATLSELHCDKLHVDPENFRLLG
KGTFATLSELHCDKL
KGTFATLSELHCDKLH
KGTFATLSELHCDKLHVD
KGTFATLSELHCDKLHVDPENFRL
KGTFATLSELHCDKLHVDPENFRLLGNVLV
LDNLKGTFATLSELHCDKLHVDPENFR
LDNLKGTFATLSELHCDKL
LGAFSDGLAHLDNLKGTFATLSELHCDKL
LHCDKLH

LHCDKLHVD
LHCDKLHVDPENFRL
LHCDKLHVDPENFRLL
LHCDKLHVDPENFRLLG
LHCDKLHVDPENFRLLGNVLV
LHCDKLHVDPENFRLLGNVLVCVL
LHCDKLHVDPENFR
LKGTFATLSELHCDKL
LKGTFATLSELHCDKLH
LKGTFATLSELHCDKLHVD
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