X-ray Structure of a Hydroxylase-Regulatory Protein Complex from a Hydrocarbon-Oxidizing Multicomponent Monoxygenase, *Pseudomonas stutzeri* OX1 Phenol Hydroxylase[†,‡]

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Running Title: Structure of a Phenol Hydroxylase-Regulatory Protein Complex

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[‡]The coordinates and structure factors for the PHH-PHM complex have been deposited in the Protein Data Bank (entries XXXX and XXXX) for the native and SeMet enzyme, respectively.

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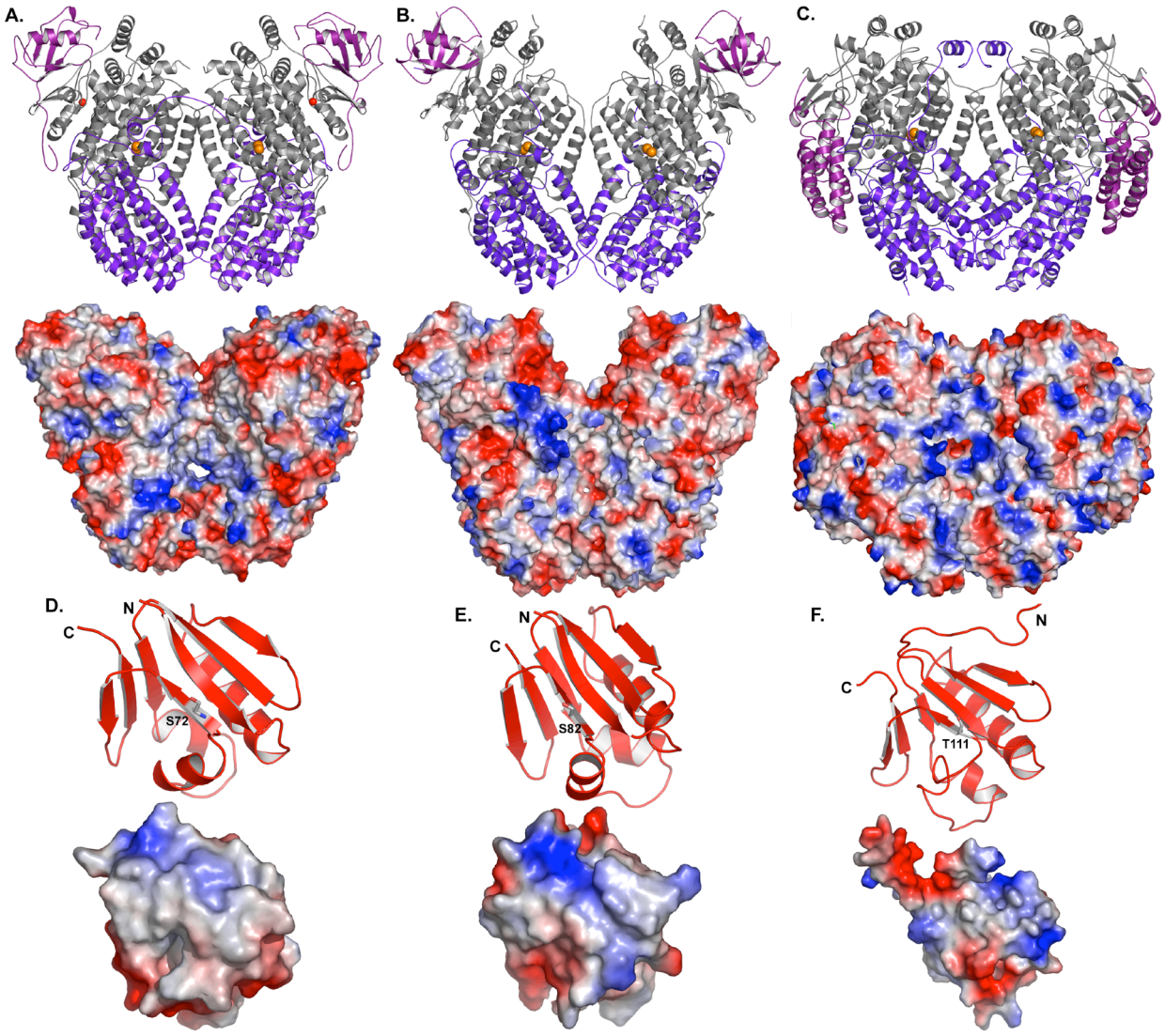

Figure S1. Structure and electrostatic surfaces of (A) PHH, (B) ToMOH, and (C) MMOH. The hydroxylase α, β, and γ subunits are colored gray, blue, and purple, respectively. Protein and electrostatic surfaces of (D) PHM, and of those predicted by homology for (E) T4MOD and (F) MMOB, that pack against the hydroxylase are also shown. Electrostatic surfaces were generated by using APBS in PyMol. Red surfaces are negatively charged and blue surfaces are positively charged.

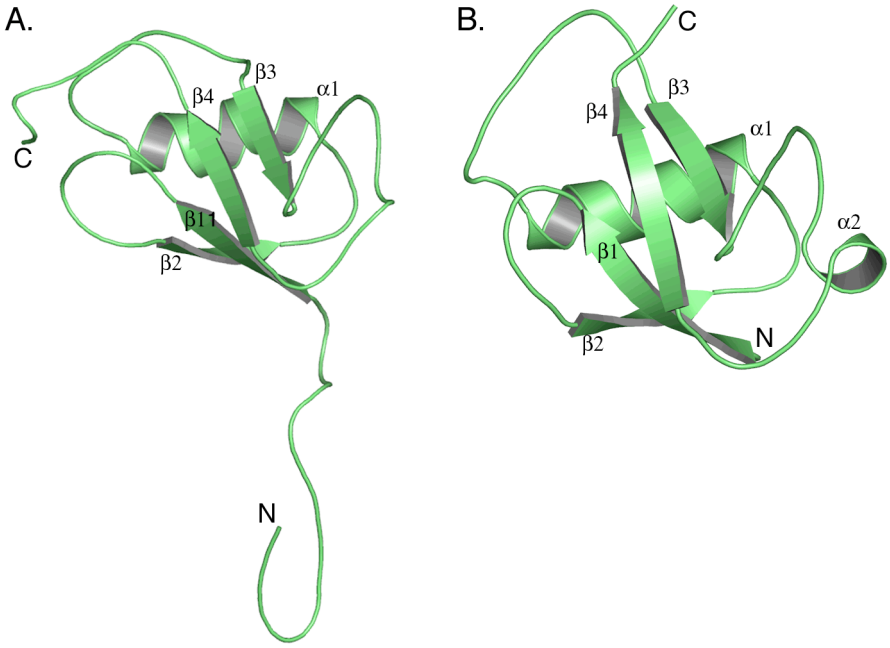

Figure S2. Structures of the (A) PHH and (B) ToMOH γ-subunits. The α-helices and β-strands are labeled accordingly.

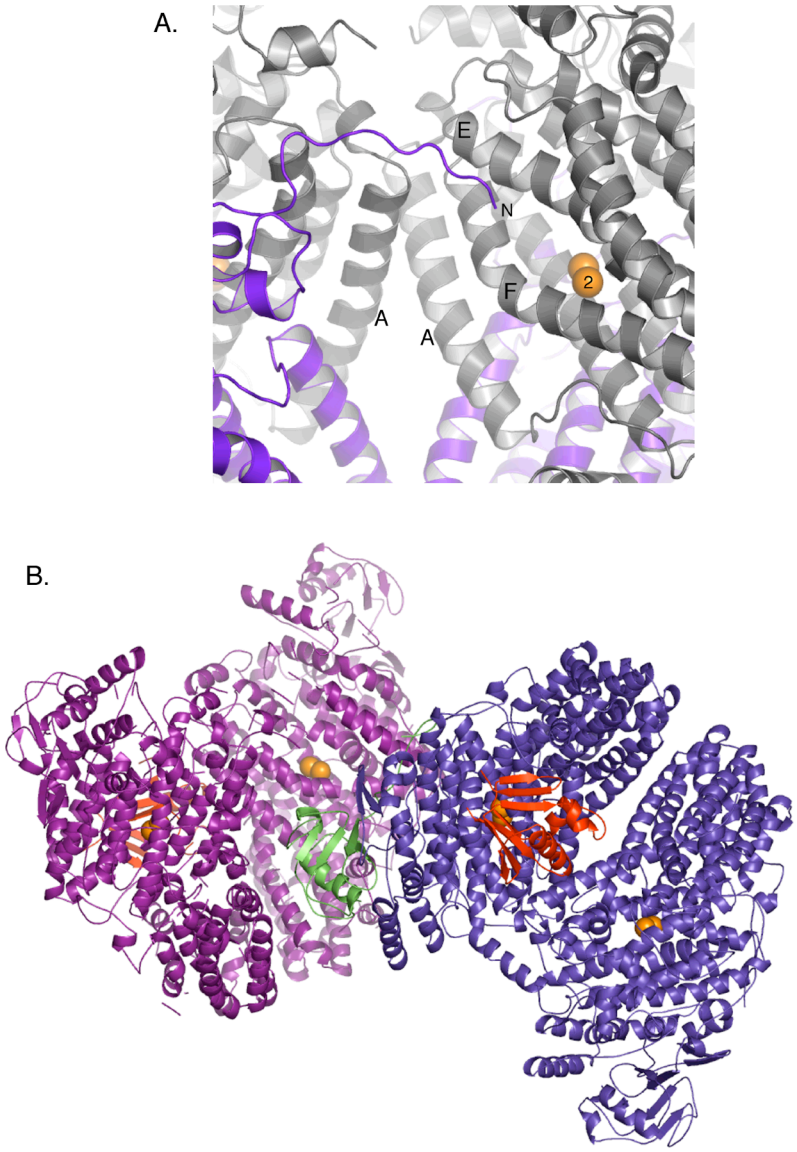

Figure S3. (A) Interaction of the PHH β-subunit N-terminus (purple) with α-subunit helices E and F (gray) on the hydroxylase surface in which PHM is not bound. (B) Packing interaction of PHH and PHM molecules in the crystal lattice. The γ-subunit (green) of one PHH molecule (blue) packs against the canyon region of an adjacent PHH molecule (purple). PHM is colored red.

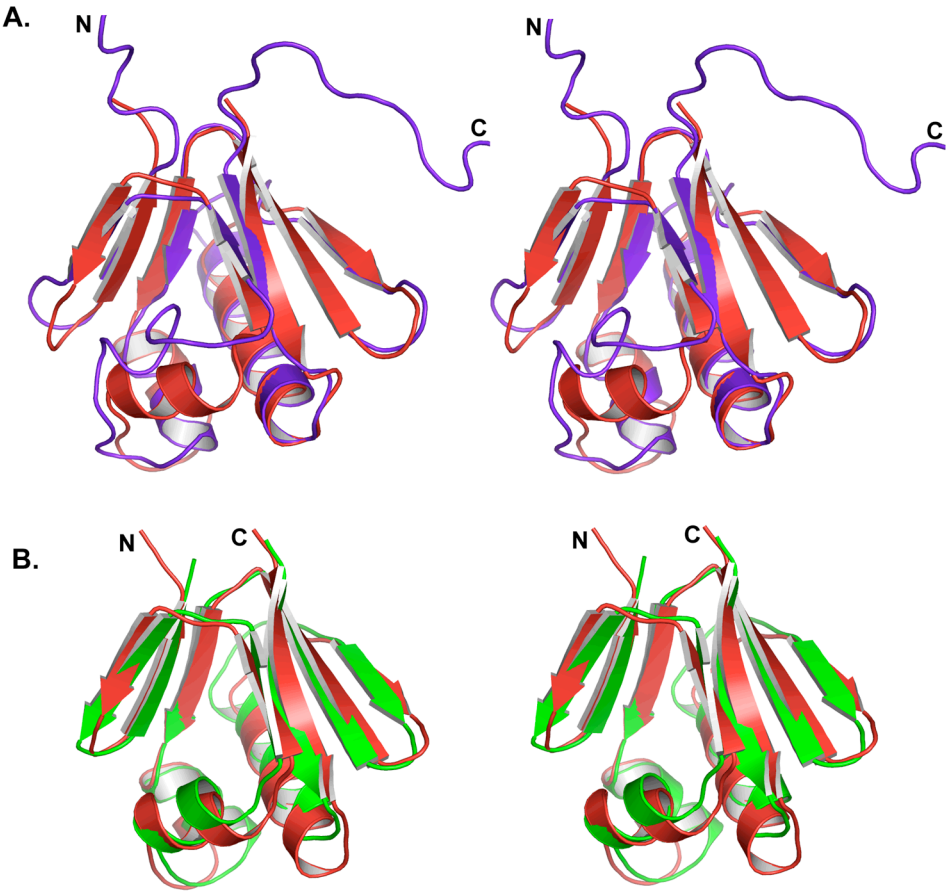

Figure S4. Structural comparisons between BMM regulatory proteins. Alignments of (A) PHM (red) and MMOH (purple) and (B) PHM and T4MOD (green) in stereo.

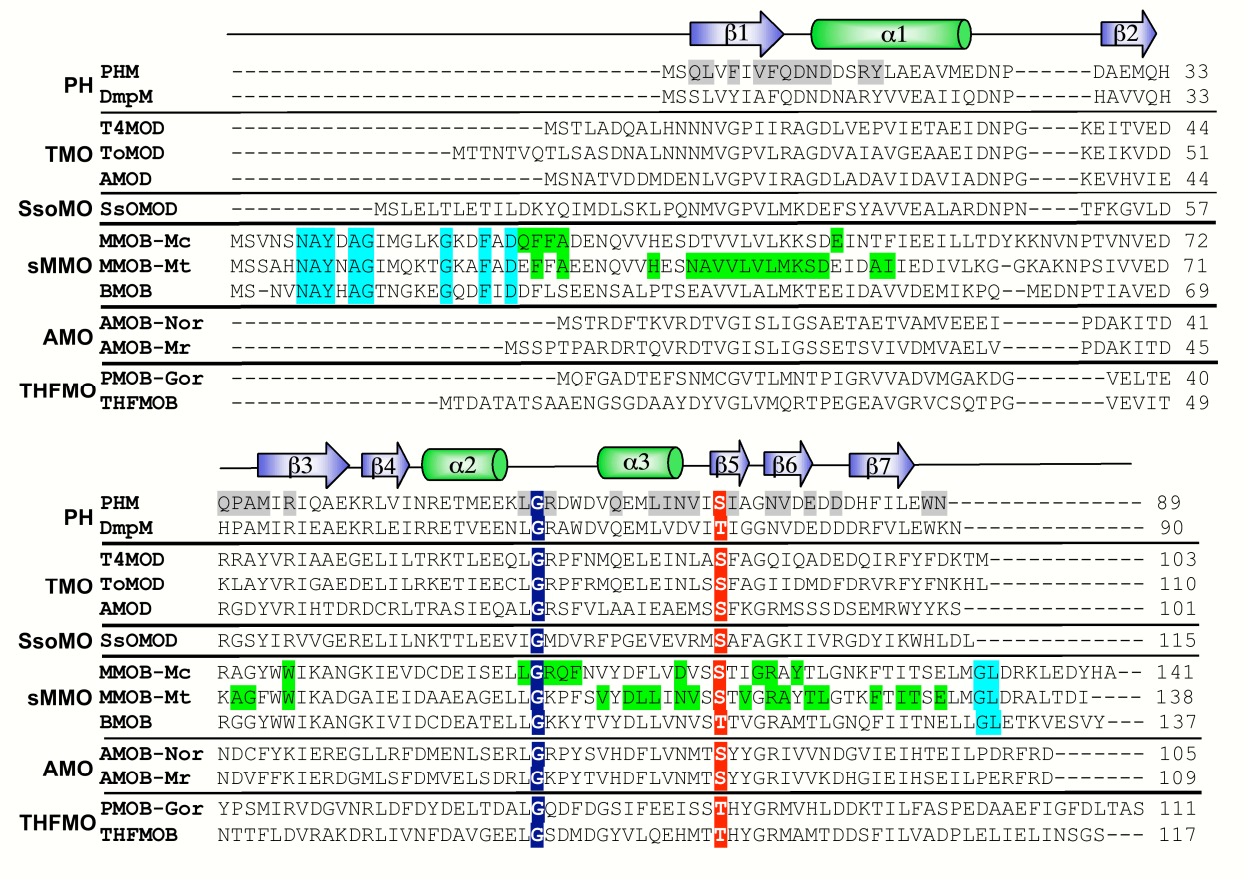

Figure S5. Sequence alignment of BMM regulatory proteins within their specific sub-families. PHM residues highlighted in gray form contacts with the PHH surface. Residues in green mark MMOB positions that experience greater than average NMR line broadening in the presence of MMOH. Teal shading denotes highly conserved sMMOH residues in the N- and C-termini that may be important for function. Blue shading denotes a strictly conserved residue while red shading marks a conserved residue having important catalytic function. The regulatory protein sequences and accession numbers used for the alignment are: PHM, *Pseudomonas stutzeri* OX1 (AAO47357), DmpM *Pseudomonas* sp. strain CF600 (P19731); T4MOD, *Pseudomonas mendocina* (2BF2_B); ToMOD, *Pseudomonas stutzeri* OX1 (AAT40434); AMOD,

Xanthobacter autotrophicus Py2 (CAA09914); SsoMOD, *Sulfolobus solfataricus* P2 (NP_342687); MMOB-Mc, *Methylococcus capsulatus* Bath (P18797); MMOB-Mt, *Methylosinus trichosporium* OB3b (P27356); BMOB, *Pseudomonas butanovora* (AAM19729); AMOB-Nor, *Nocardioides* sp. JS614 (AAV52083); AMOB-Mr, *Mycobacterium rhodesiae* (AAO48575); PMOB, *Gordonia* sp. TY-5 (BAD03959), THFMOB, *Pseudonocardia* sp. K1 (CAC10510).

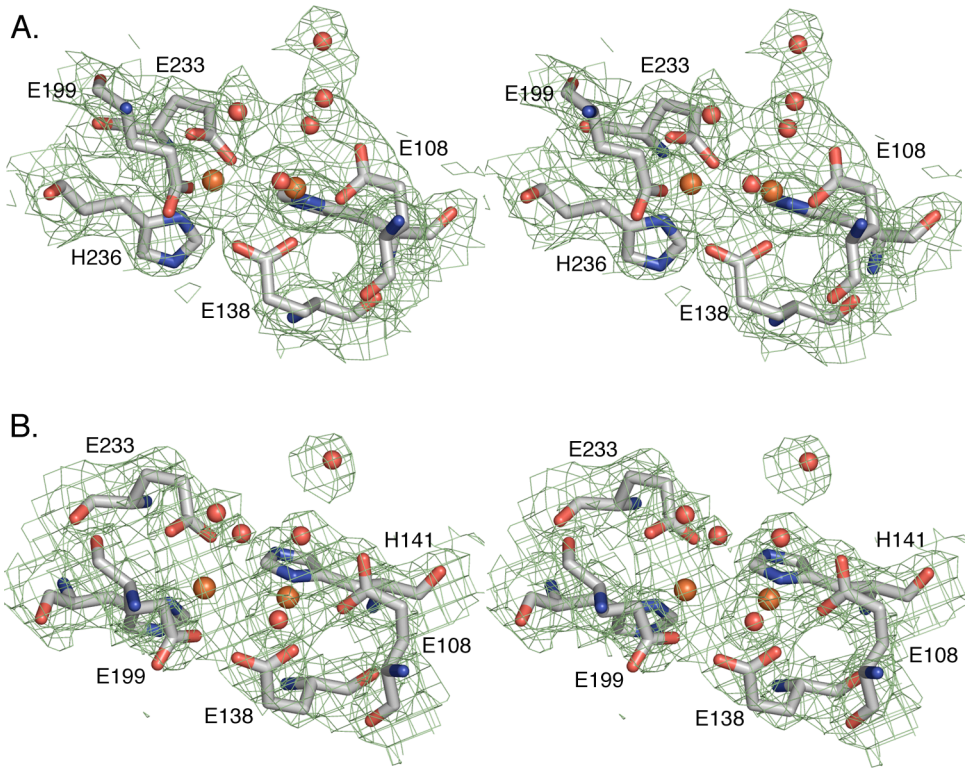

Figure S6. Stereo view of the $|2F_o|-|F_c|$ simulated-annealing omit electron density maps (green) surrounding the native PHH diiron active sites from (A) chain A and (B) PHM-bound chain B contoured to 1.2 σ.

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sMMO IYIDRVSQVP-FCPSLAKGASTLRVHE--YNGQMHTFSDQWGERM 456
AMO  P-MCQVCQVPCVMPRLDMNAA--RIIE--FEGQKIALCSEPCQRI 430
PhN  PQLCQVCQIPTIFTEKD-APTMLSHRQIEHEGERYHFCSDGCCDI 439
MopN PQLCQVCQIPMTFFEMDGDPTLFSYRDSIYKDERYHTCSDGCHDI 447
PhhN PHLCQVCQVPAIFFEPD-DPTKLSLRSLVHEGERYHFCSDGCCDI 439
PhlD PHLCQVCQVPAIFFEPD-DPTKLSLRSLVHEGERYHFCSDGCCDI 439
DmpN PHLCQVCQLPVIFFEPD-DPTKLSLRSLVHEGERYQFCSDGCCDI 440
PhcN PMLCTTCQIPMGFFEP-GDATKICYRESDYEGSKYHFCSDGCKHV 441
TbmD PMLCTTCQIPMGFFEP-GDATKIAYRESDYFGMKYHFCSDHCKHI 440
TomA PMLCTTCQIPMIFFEP-GDATKICYRESAYLGDKYHFCSDHCKEI 441
PoxD PMLCTTCQIPMIFFEPD-DPTQTCYRESSYHGMKFHFCSDGCKDI 438
ToMO PTICNMCNLPIAHT--PGNKWNVKDYQLEYEGRLYHFGSEADRWC 435
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Figure S7. Sequence alignment of the C-terminal portion of the PH hydroxylase α-subunit depicting the conserved cysteines contributing to the zinc binding site in PHH. The alignment was adapted from Leahy, et al. (3). Phenol hydroxylase α-subunit sequences are from *Pseudomonas stutzeri* OX1 (PhN), *Acinetobacter calcoaceticus* NCIB8250 (MopN), *Pseudomonas putida* P35X (PhhN), *Pseudomonas putida* H (PhlD), *Pseudomonas* sp. Strain CF600 (DmpN), *Comanonas testosteroni* R5 (PhcN), *Burkholderia cepacia* JS150 (TbmD), *Burkholderia cepacia* G4 (TomA3), and *Ralstonia eutropha* E2 (PoxD).

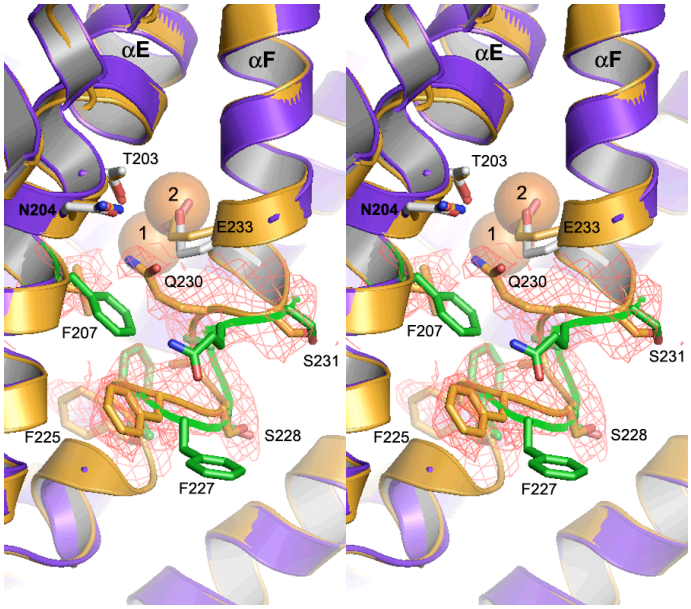

Figure S8. Structural changes in helix F. A $|2F_o|$ - $|F_c|$ simulated annealing electron density omit map (red) contoured at 1.0 σ around α-subunit helices E and F from the SeMet PHH-PHM structure (orange), depicts structural changes in stereo. Residues in green depict the unaltered conformation of helix E and F residues in the other PHH α-subunits.