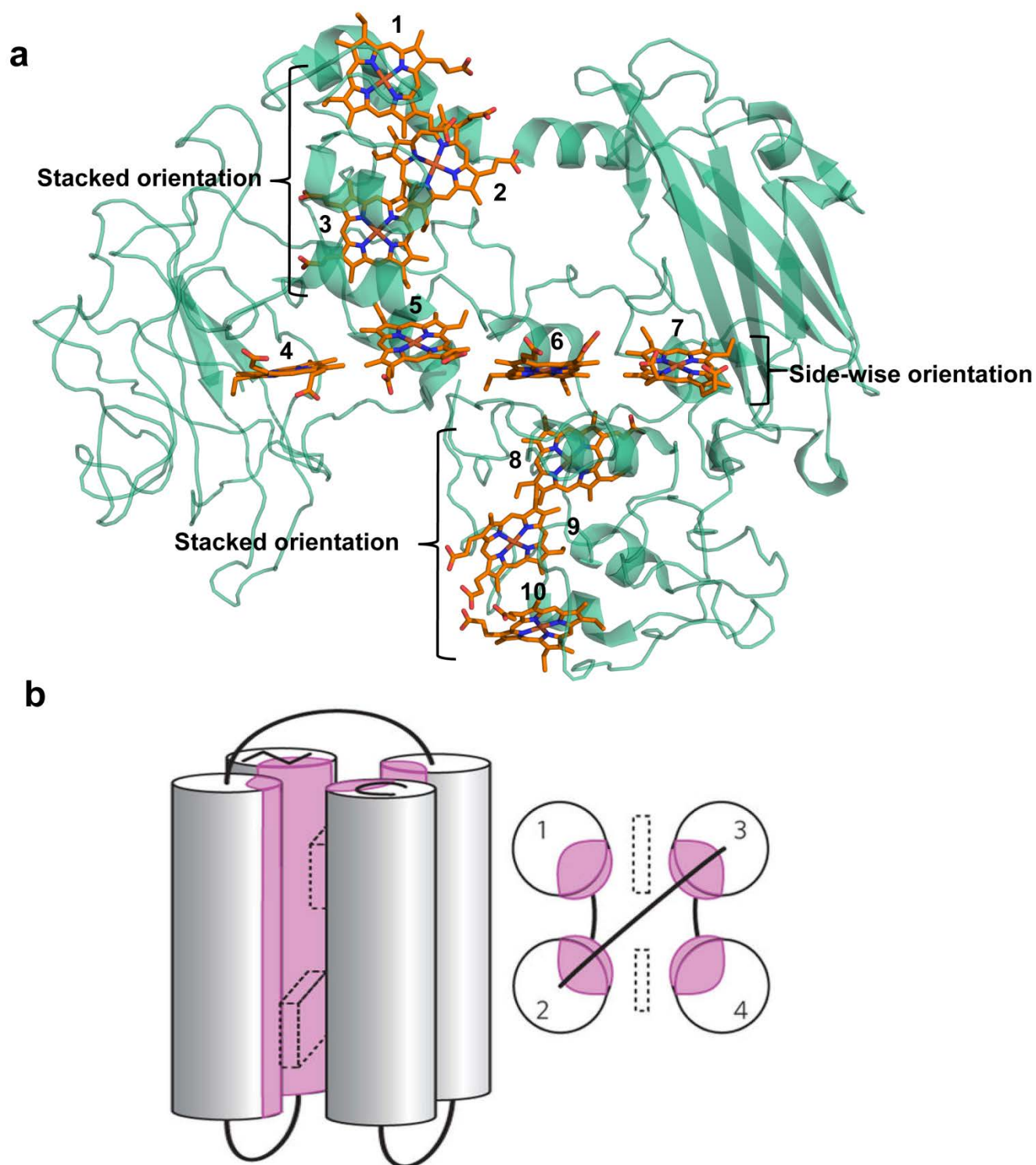
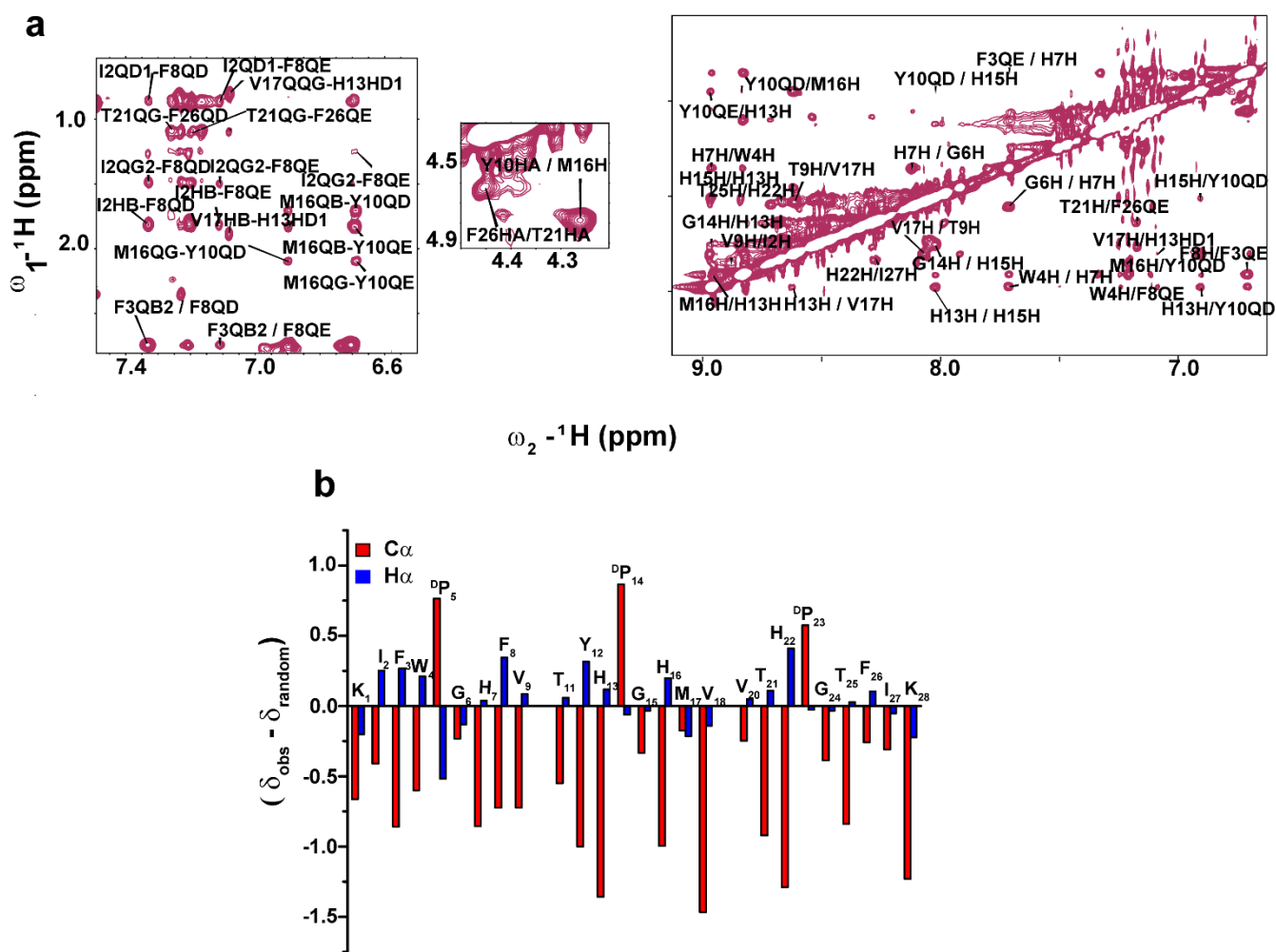
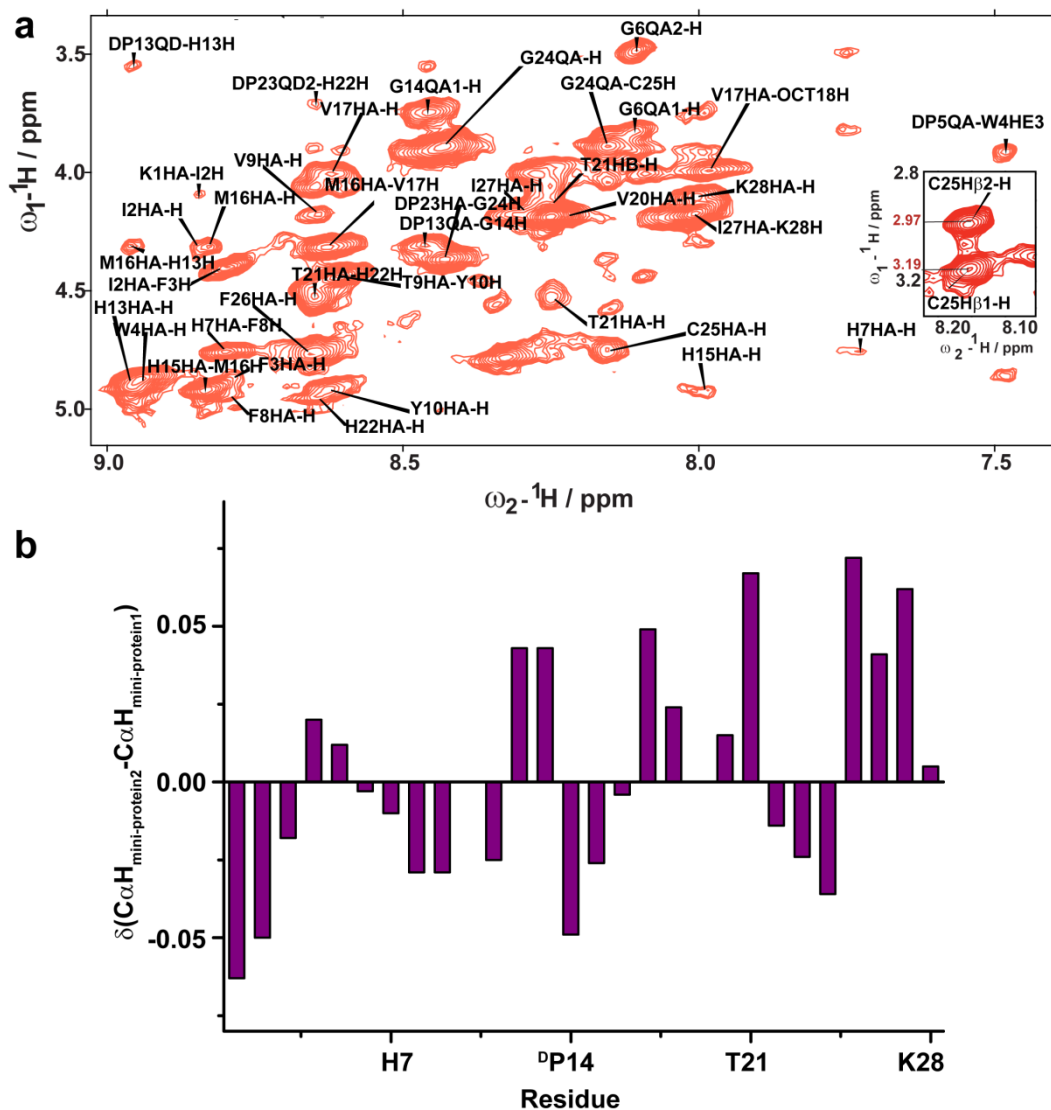




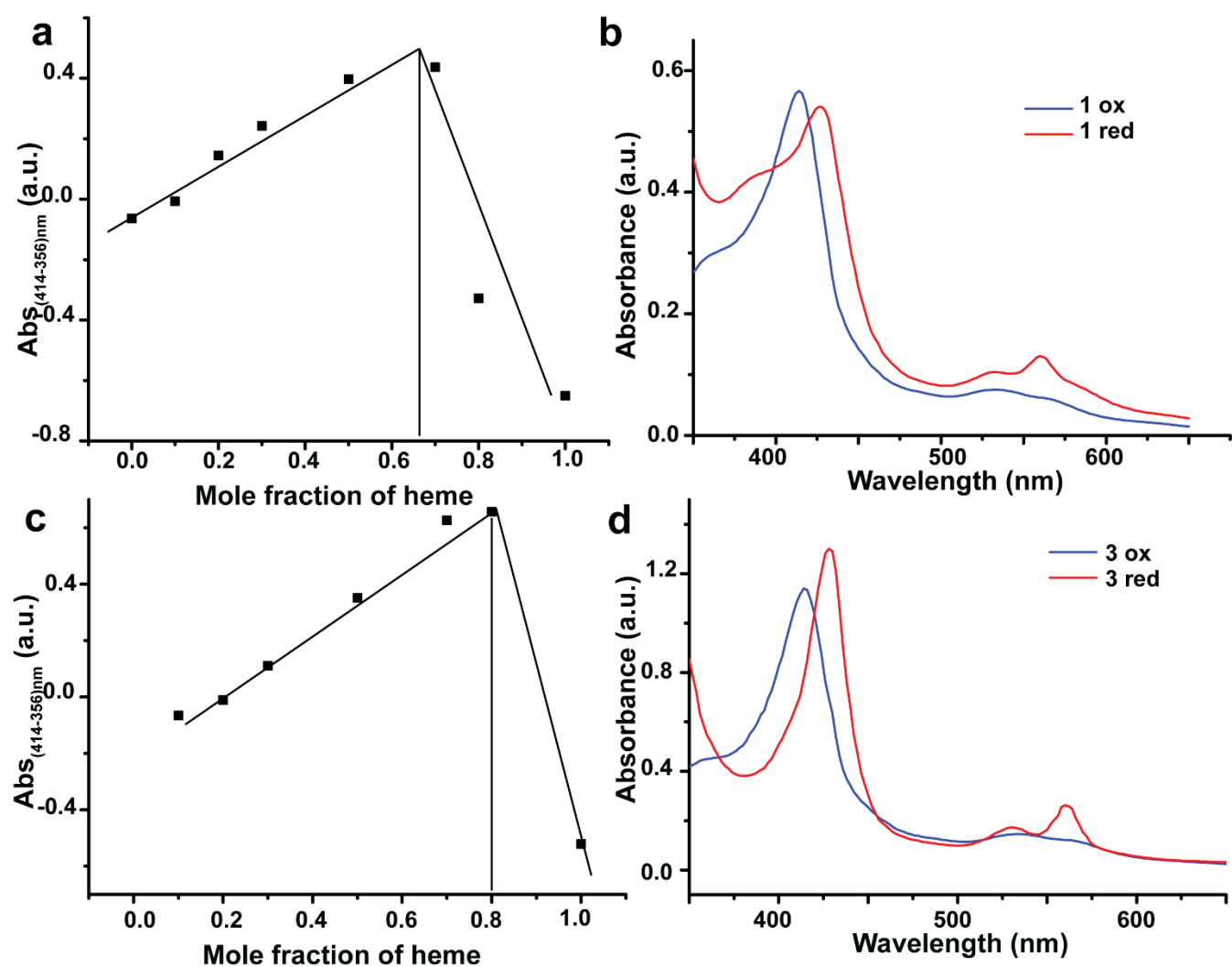
Supplementary Figure 1: (a) 3-D structure of a decaheme conduit: cytochrome MtrF (PDB:3PMQ) where hemes are arranged in a sidewise and stacked orientation. (b) Cartoon showing di-heme binding sites, in a stacking orientation, engineered in designed helices.



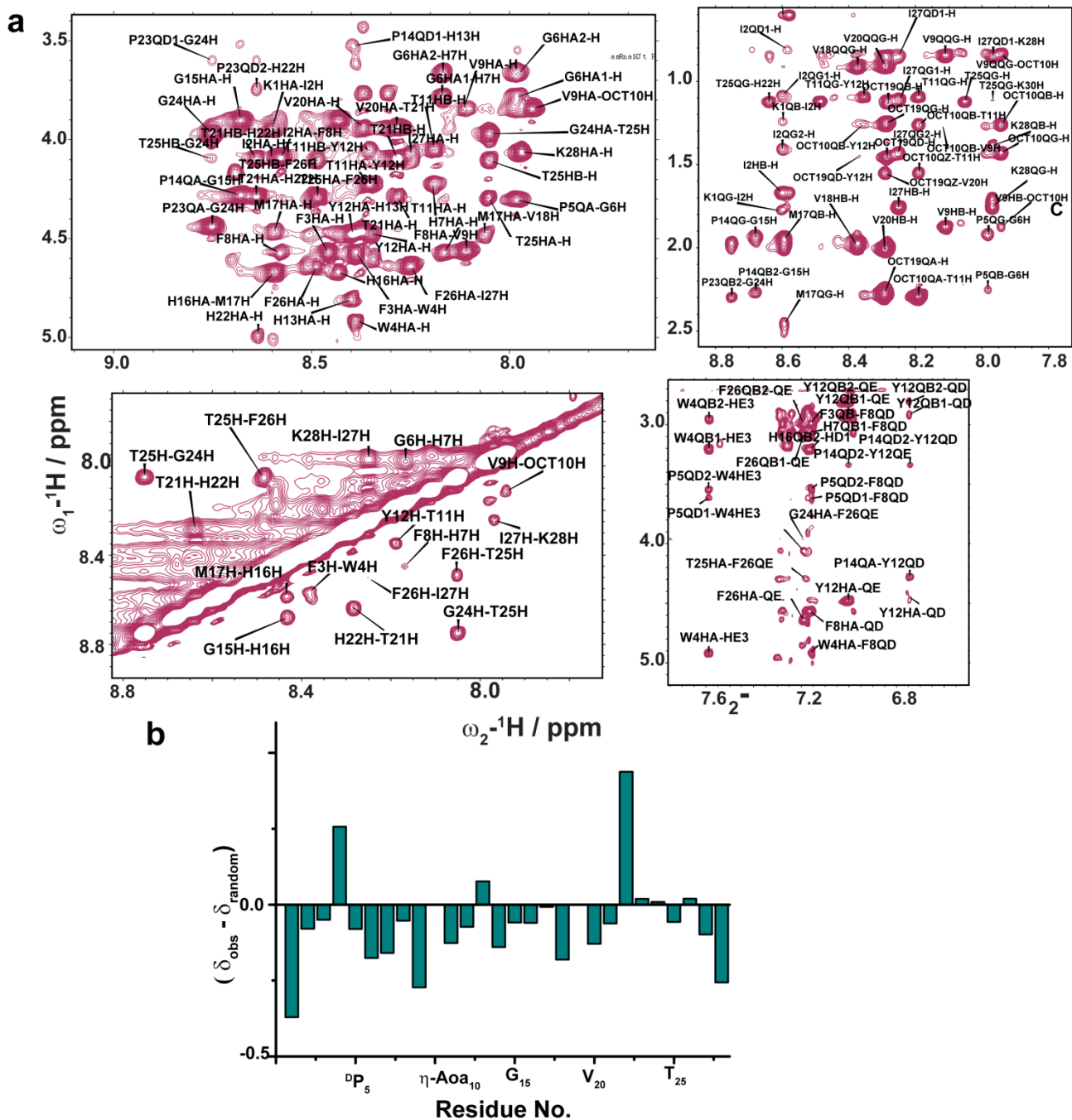
Supplementary Figure 2: (a) Sections of ^1H - ^1H NOESY spectra of the mini-protein1 showing NOEs between amide/amide, amide/sidechain and sidechain/sidechain protons diagnostic to β -sheets. (b) $^1\text{H}\alpha$ and $^{13}\text{C}\alpha$ chemical shift deviation from random coil values of mini-protein1. Negative deviation in $^{13}\text{C}\alpha$ chemical shift can be observed for all residues except turn-residue $^{\text{D}}\text{P}$. Positive deviation in $^1\text{H}\alpha$ chemical shift can be seen for all residues except turn-residue G and end lysine residues indicative of a six stranded β -sheet conformation.



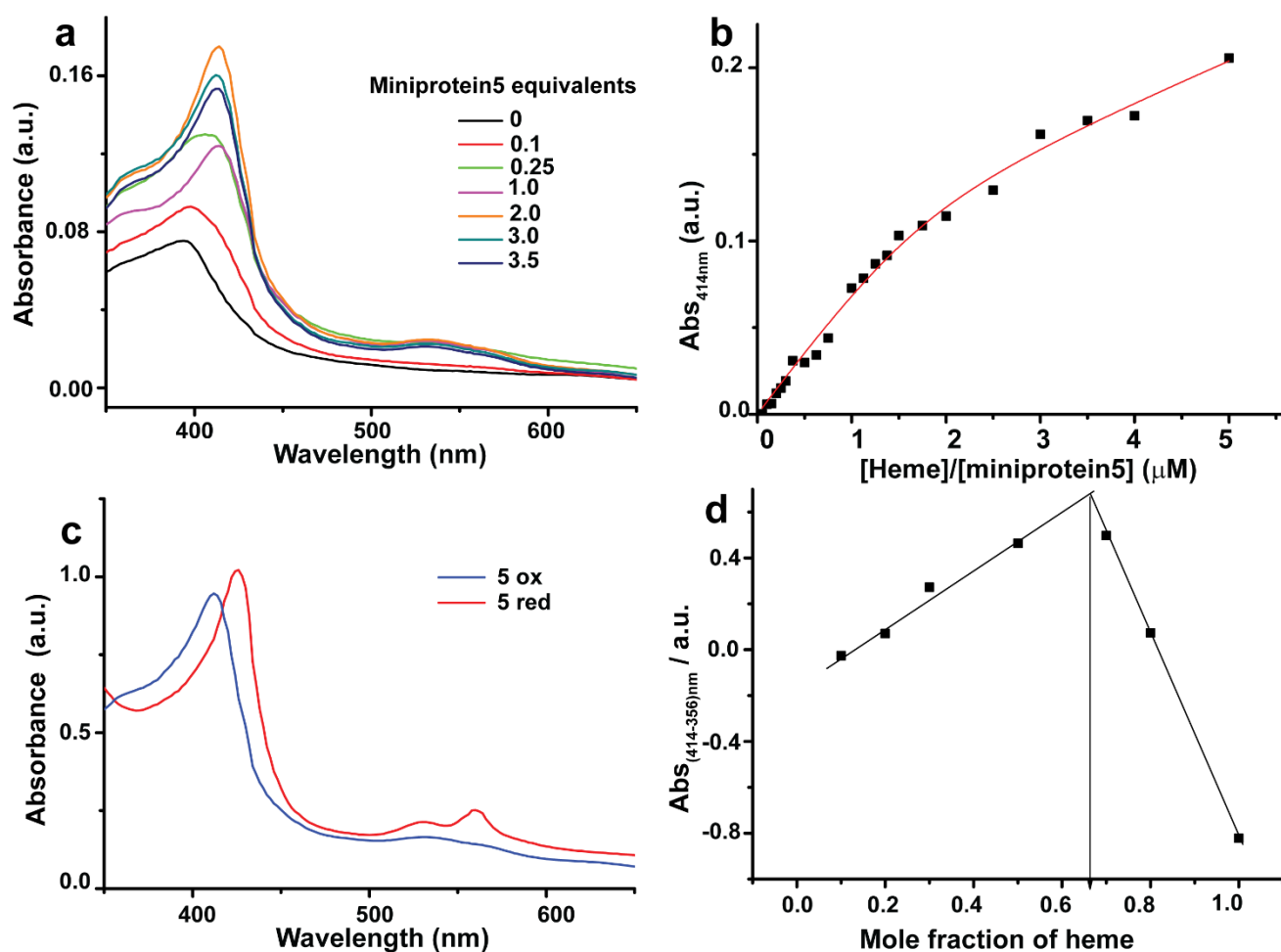
Supplementary Figure 3: (a) The fingerprint region of ^1H - ^1H NOESY spectrum of the mini-protein3 in oxidized S-S bonded form. Only one set of signal can be seen indicating a symmetric homo-dimer. The two cross-peaks for C^βH s of residue Cys 25 resonating at downfield shifted 3.19 and 2.97 ppm supported disulfide bond formation. (b) Bar diagram showing $^1\text{H}\alpha$ chemical shift difference of each amino acids of mini-protein3 and mini-protein1. Only limited chemical shift differences were observed indicating similar β -sheet structures of each subunit of the symmetric S-S bonded homo-dimer.



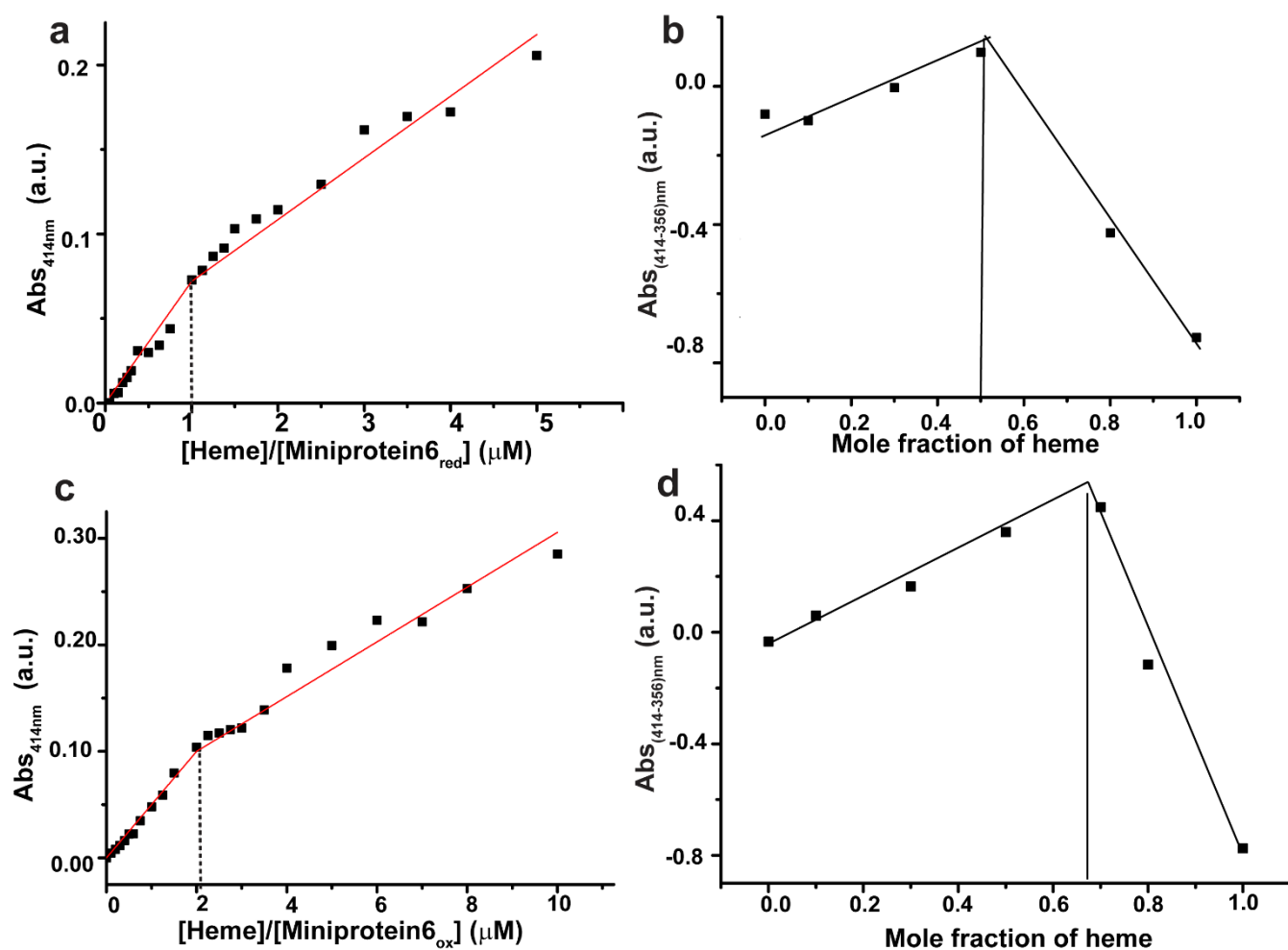
Supplementary Figure 4: (a) Jobs plot of mini-protein1 (b) Absorption spectra of mini-protein1-heme oxidized (blue) and mini-protein1-heme reduced (red) by addition of sodium dithionite. (c) Jobs plot of mini-protein3 (d) Absorption spectra of mini-protein3-heme oxidized (blue) and mini-protein1-heme reduced (red) by addition of sodium dithionite.



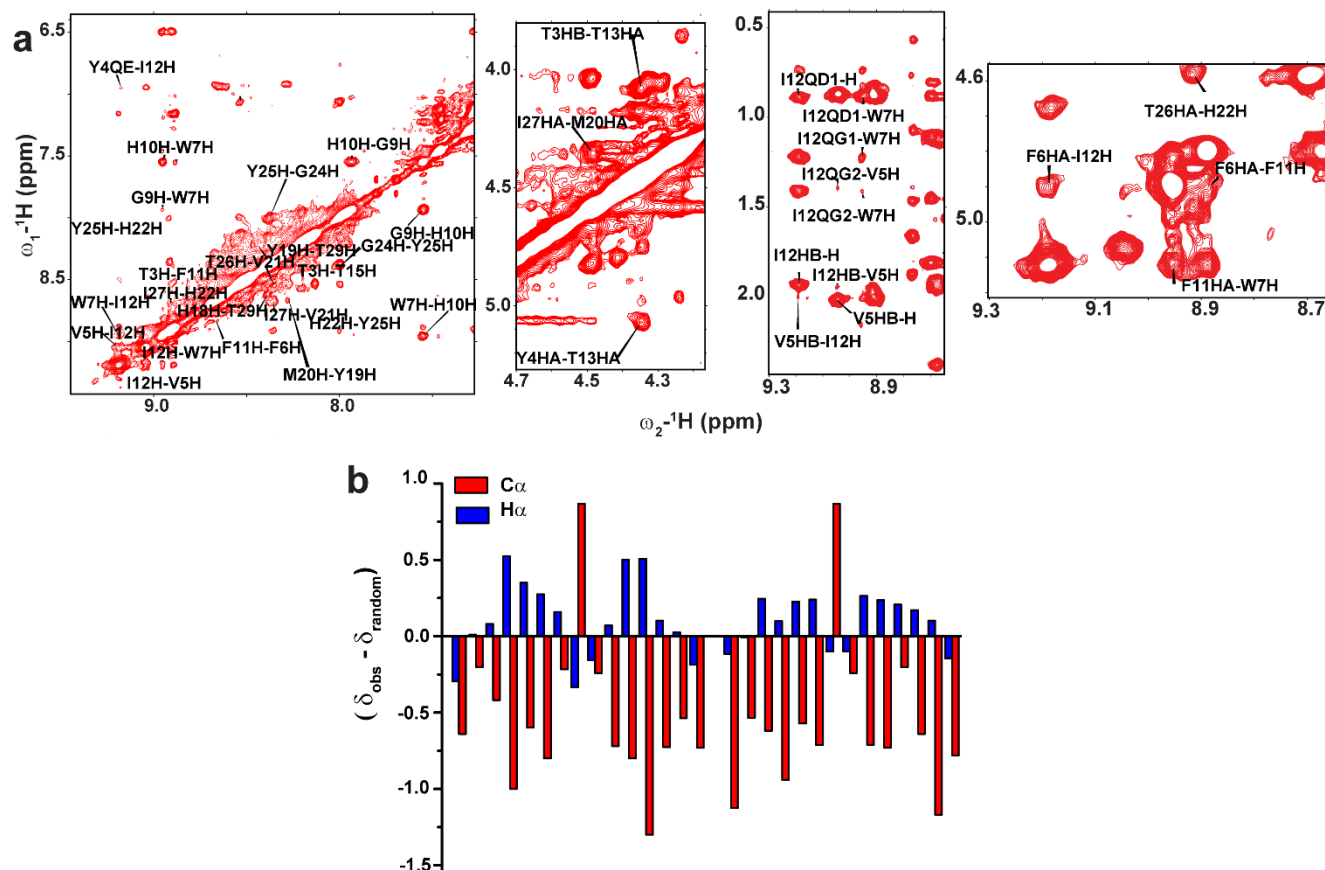
Supplementary Figure 5: Summary of NMR analyses of mini-protein5. (a) Sections of ^1H - ^1H NOESY spectra showing sequential NOEs between amide/amide, amide/sidechain and sidechain/sidechain protons (b) $^1\text{H}\alpha$ chemical shift deviation from random coil values of mini-protein5. NOESY spectra and chemical shift deviations are indicative of extended conformations of the mini-protein5.



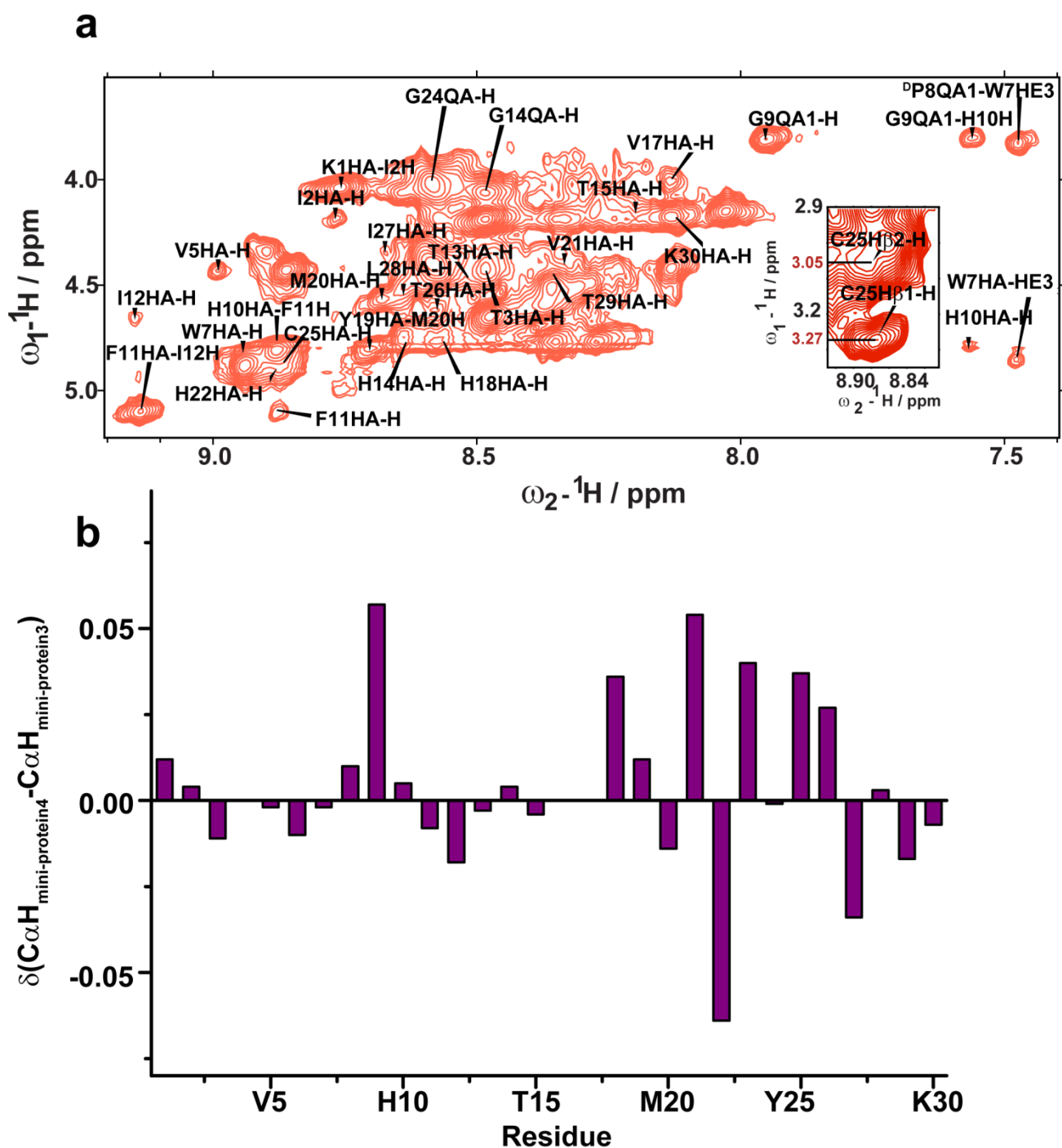
Supplementary Figure 6: (a) Absorption changes of heme (5 μM) on titrating mini-protein5 (0-3.5 equivalents). (b) Heme binding isotherm of mini-protein5. (c) Absorption spectra of mini-protein5-heme oxidized (blue) and mini-protein5-heme reduced (red). (d) Jobs plot of mini-protein5 shows protein:heme stoichiometry 1:2.



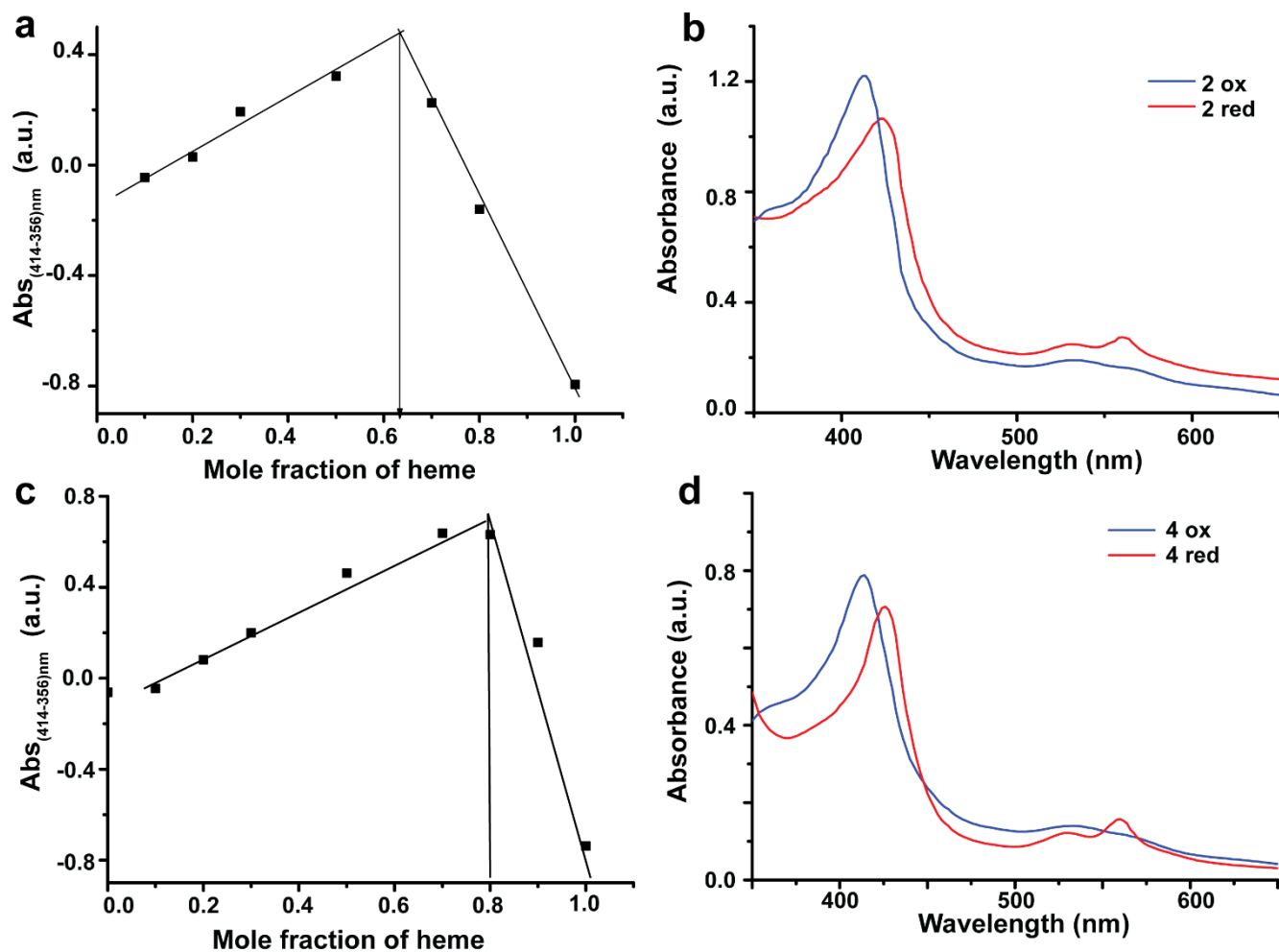
Supplementary Figure 7: (a) Heme binding isotherm of mini-protein 6_{red} (b) Jobs Plot of mini-protein 6_{red} shows protein:heme stoichiometry 1:1. (c) Heme binding isotherm of mini-protein 6_{ox} (d) Jobs Plot of mini-protein 6_{ox} shows protein:heme stoichiometry 1:2.



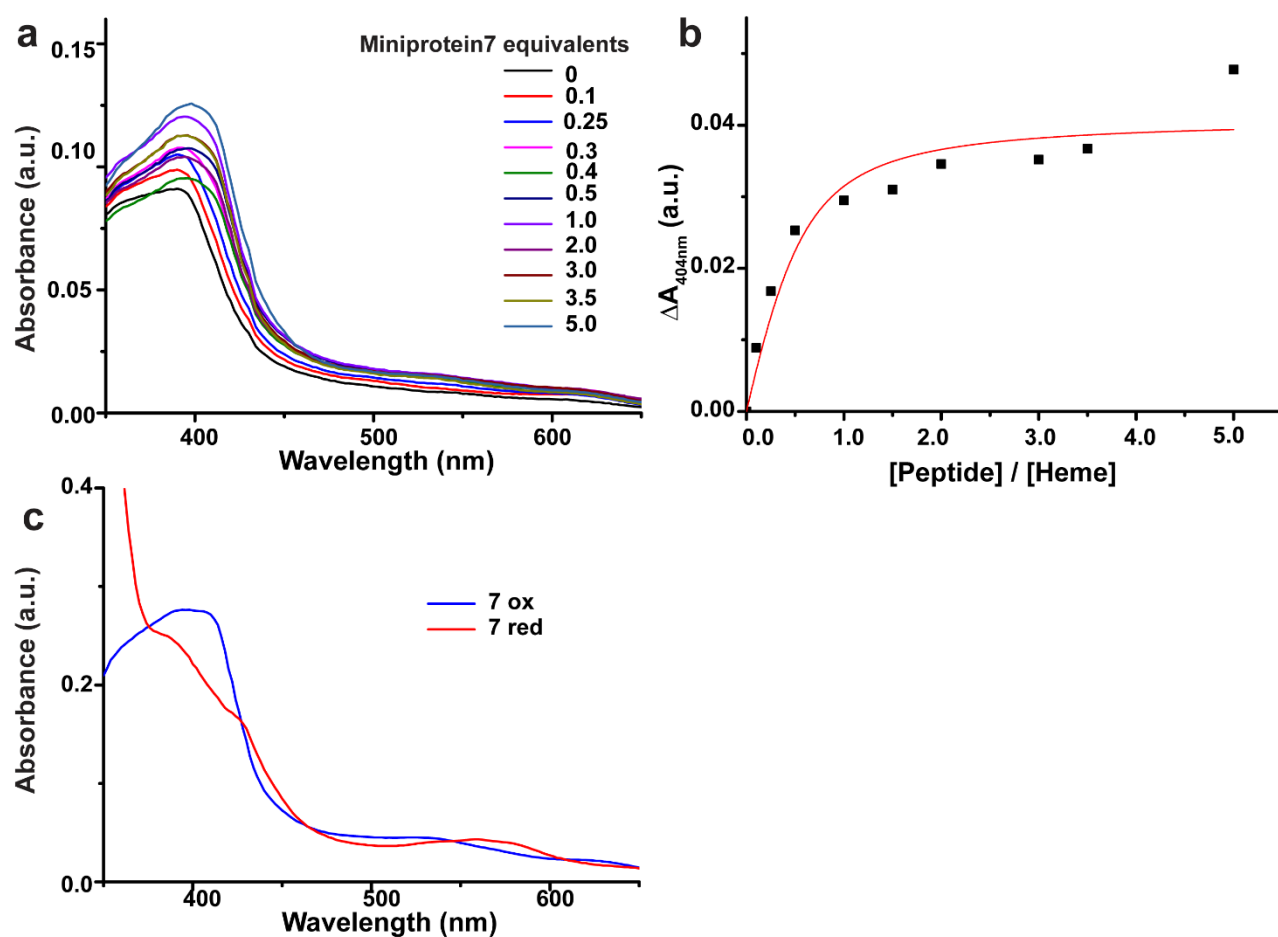
Supplementary Figure 8: (a) Sections of ^1H - ^1H NOESY spectrum of the mini-protein2 showing NOEs between amide/amide, amide/sidechain and sidechain/sidechain protons diagnostic to β -sheets. (b) $^1\text{H}\alpha$ and $^{13}\text{C}\alpha$ chemical shift deviation from random coil values of mini-protein2. Negative deviation in $^{13}\text{C}\alpha$ chemical shift can be observed for all residues except turn-residue $^{\text{D}}\text{P}$. Positive deviation in $^1\text{H}\alpha$ chemical shift can be seen for all residues except turn-residues $^{\text{D}}\text{P}$ -G and end lysine residues indicative of a four stranded β -sheet conformation.



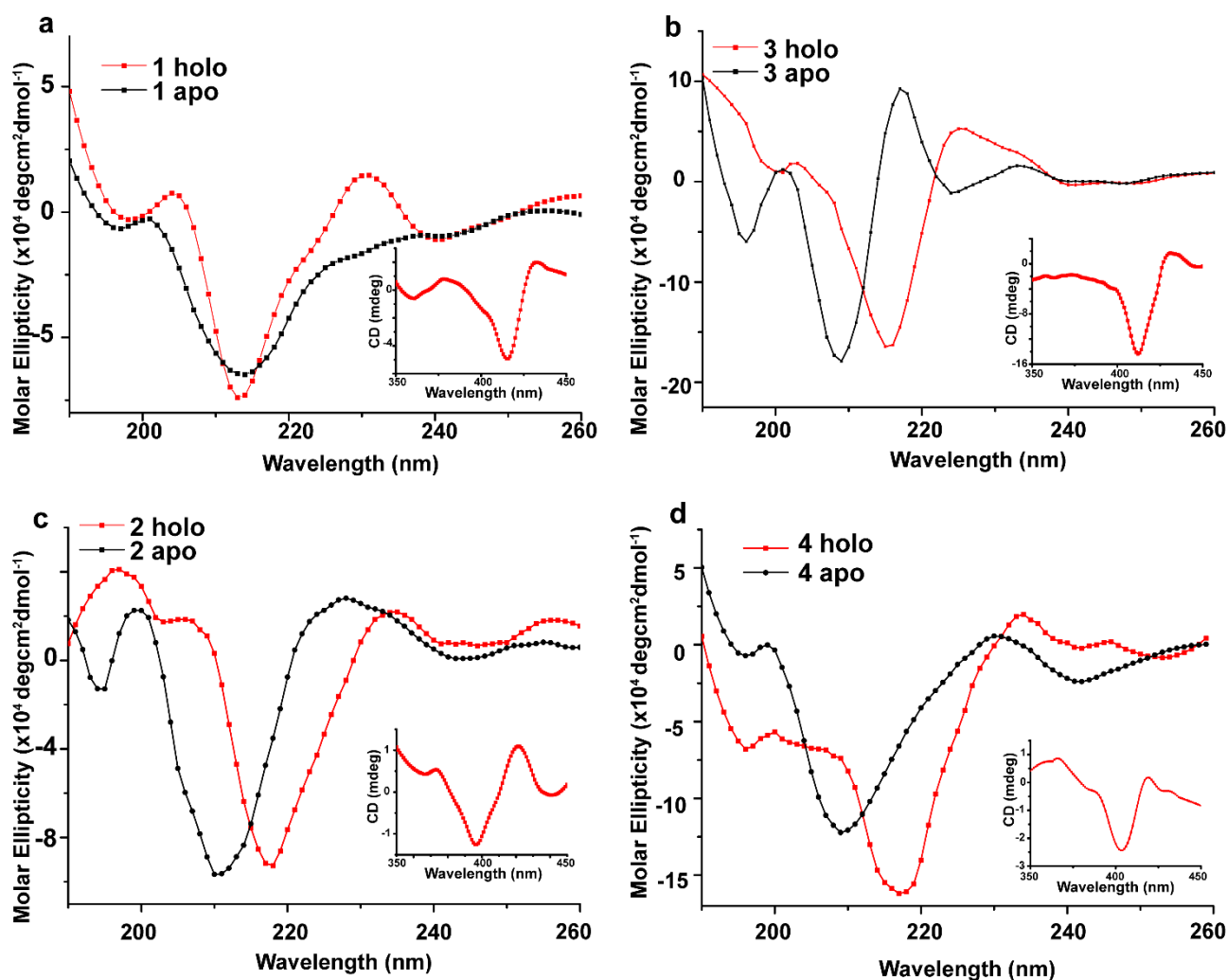
Supplementary Figure 9: (a) The fingerprint region of ${}^1\text{H}$ - ${}^1\text{H}$ NOESY spectrum of the mini-protein4 in oxidized S-S bonded form. Only one set of signal can be seen indicating a symmetric homo-dimer. The two cross-peaks for C^βH s of residue Cys 25 resonating at downfield shifted 3.27 and 3.05 ppm supported disulfide bond formation. (b) Bar diagram showing ${}^1\text{H}\alpha$ chemical shift difference of each amino acids of mini-protein3 and mini-protein1. Only limited chemical shift differences were observed indicating similar β -sheet structures of each subunit of the symmetric S-S bonded homo-dimer.



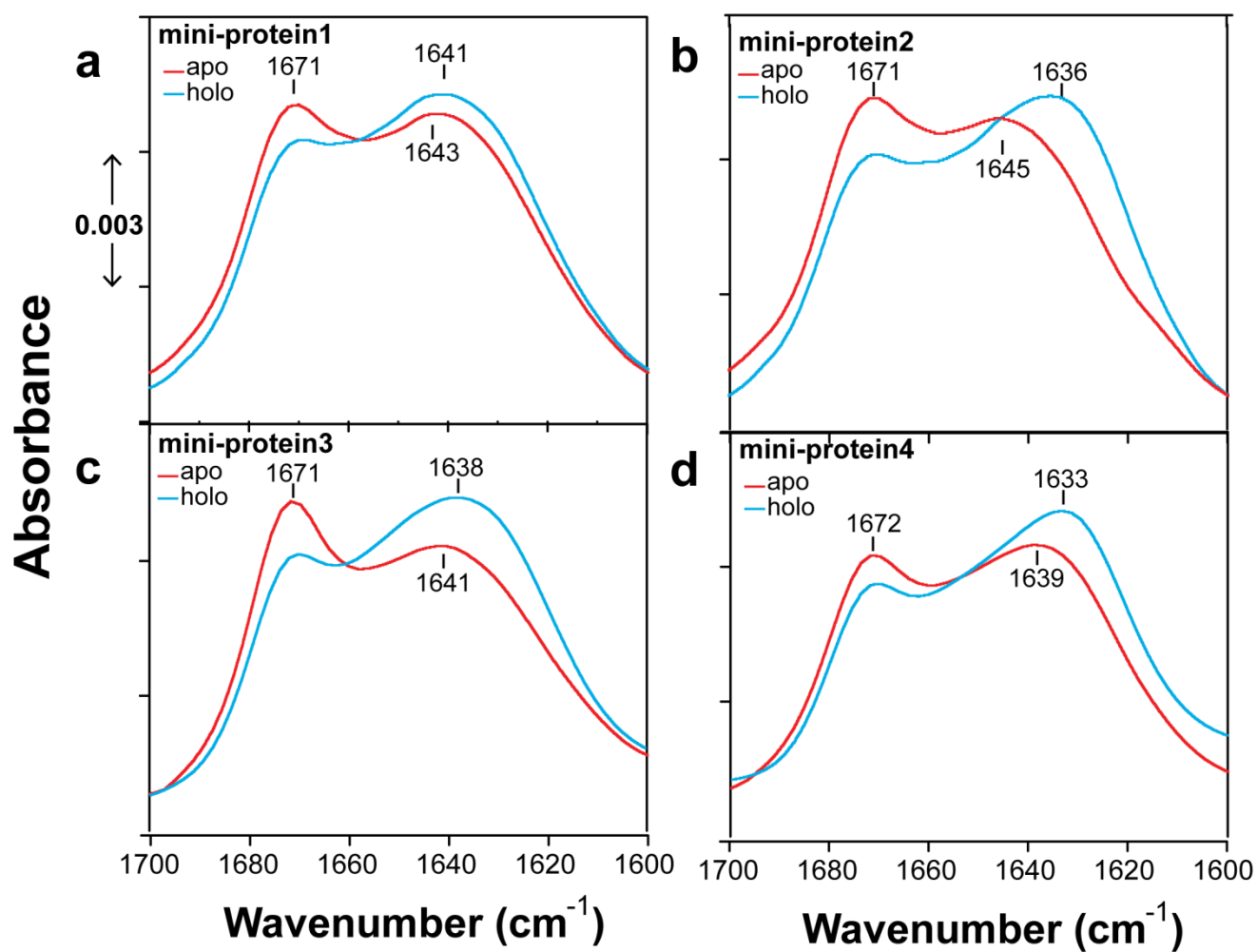
Supplementary Figure 10: (a) Jobs plot of mini-protein2 shows protein:heme stoichiometry 1:2. (b) Absorption spectra of mini-protein2-heme oxidized (blue) and mini-protein2-heme reduced (red). (c) Jobs plot of mini-protein4 shows protein:heme stoichiometry 1:4. (d) Absorption spectra of mini-protein4-heme oxidized (blue) and mini-protein4-heme reduced (red).



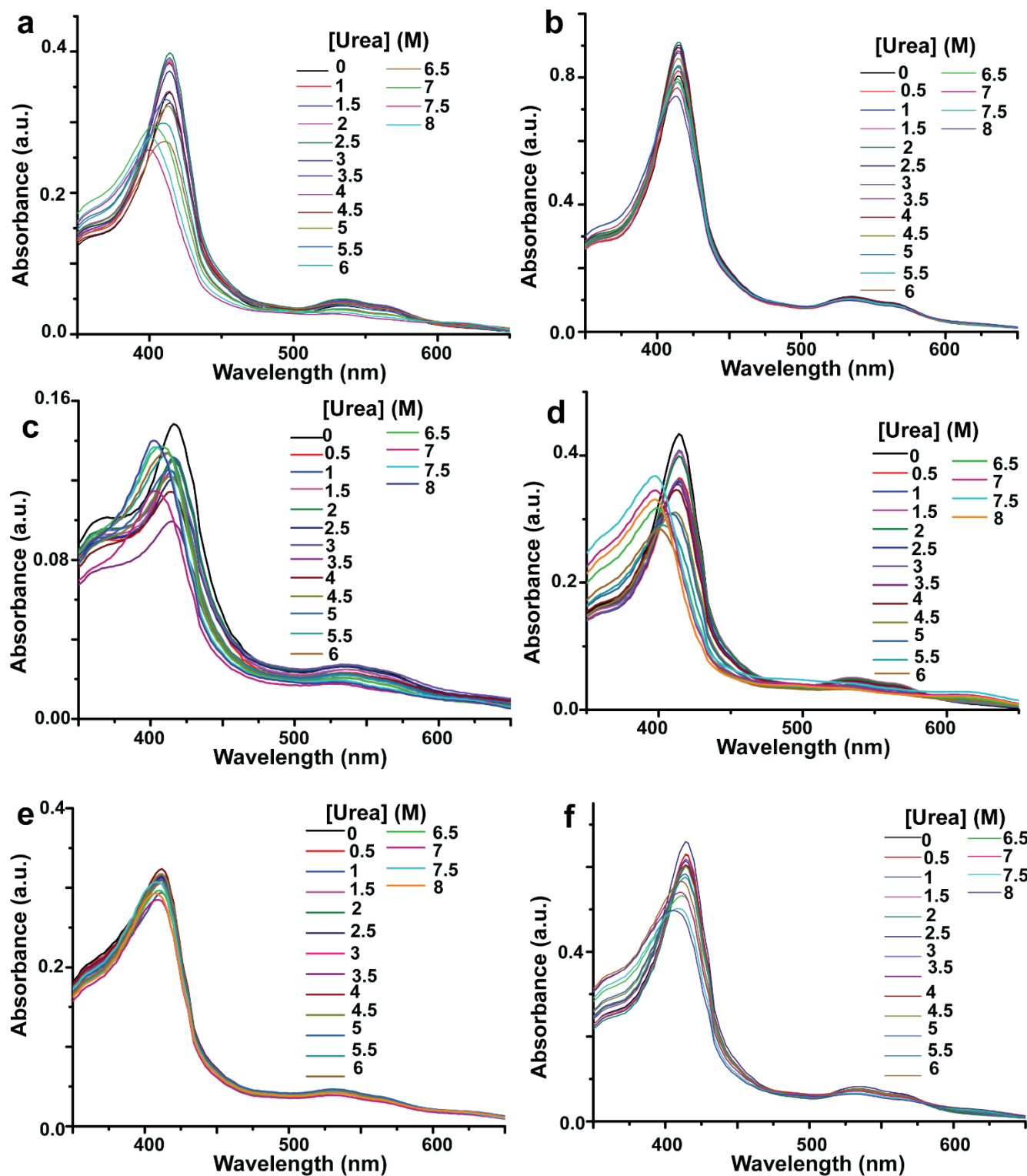
Supplementary Figure 11: (a) Absorption changes of heme (10 μ M) on titrating mini-protein7 (0-5 equivalents). (b) Heme binding isotherm of mini-protein7. (c) Absorption spectra of mini-protein5-heme oxidized (blue) and mini-protein5-heme reduced (red).



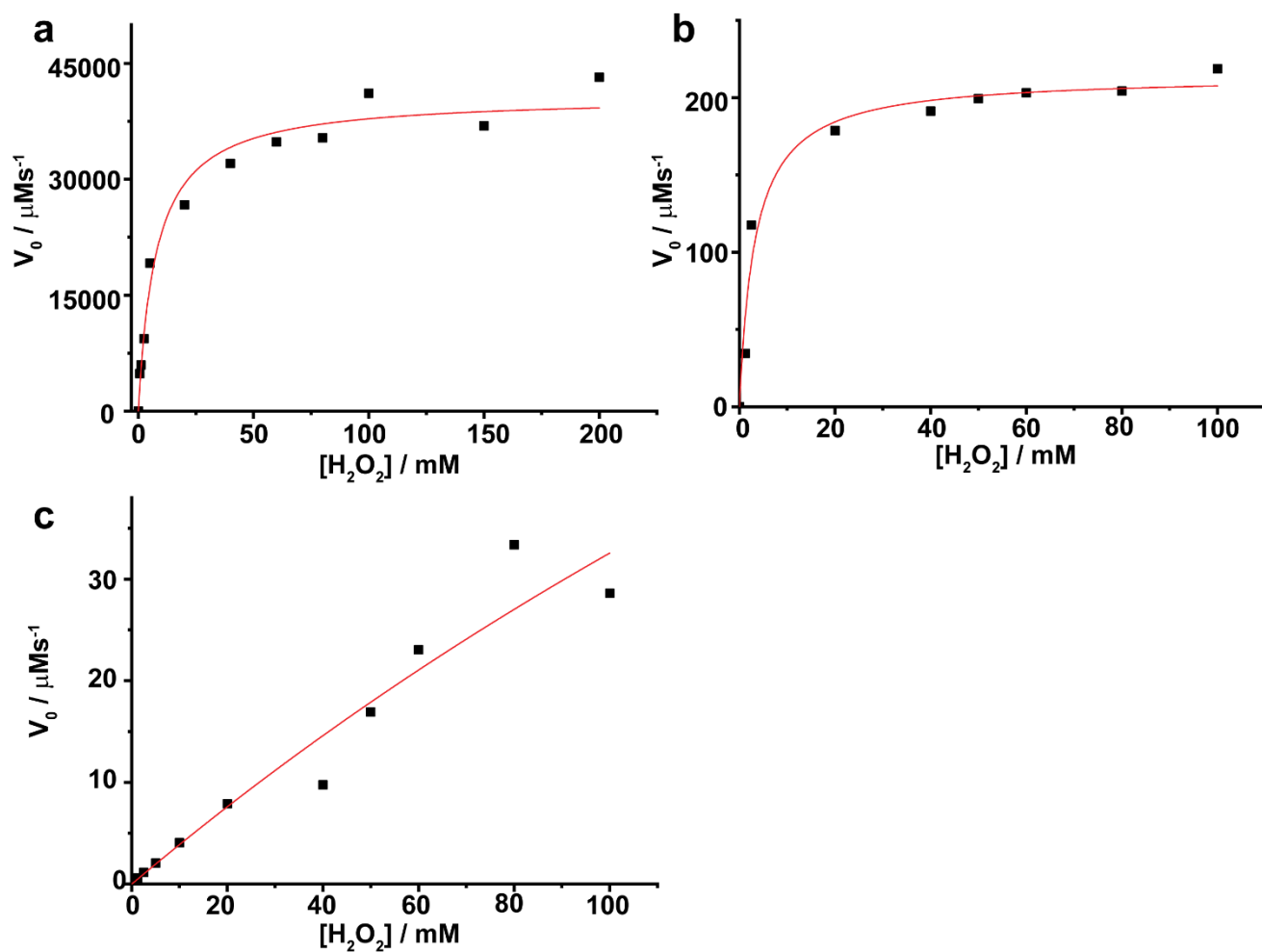
Supplementary Figure 12: Far-UV CD spectra of mini-proteins (panel a: mini-protein1, panel b: mini-protein3, panel c: mini-protein2 and panel d: mini-protein4) alone (black) and in heme-bound states (red). 10 μM of each mini-protein along with stoichiometric amount of heme dissolved in 5 mM Tris buffer pH 9.0 was used for the experiment. CD studies were performed using a 1cm path length cuvette with a 1nm bandwidth and a step size of 0.5 nm at 0.5 seconds per data set. (inset, panels a to d) Near-UV CD spectra of heme in heme/mini-proteins complexes.



Supplementary Figure 13: The amide I region of the FT-IR spectra of mini-proteins alone (red) and in complex with heme (blue).



Supplementary Figure 14: Spectral changes of heme (a) mini-protein1 (b) mini-protein2 (c) mini-protein3 (d) mini-protein4 (e) mini-protein5 and (f) mini-protein6_{ox} on titration with urea. Concentration of peptide and heme was 50 μ M in 5 mM Tris buffer, pH 9.0. The Soret maximum of the peptide5-heme complex at 414 nm is blue shifted to 395 nm (free heme Soret maximum) upon titration with increasing concentrations of urea.



Supplementary Figure 15: Michaelis-Menton plots of (a) Microperoxidase-8 (MP-8), (b) Myoglobin and (c) Cytochrome C (Cyt C). Concentration of proteins and heme used were 8 μM and 0.5 μM , respectively and dissolved in 5 mM Tris buffer, pH 9.0.

Supplementary Table 1: Translation diffusion coefficients and molecular weight, estimated from PFG-NMR, of mini-proteins 1, 2,3,4 using DSS as a reference molecule

Peptide	D($10^{-10}\text{m}^2/\text{s}$)	Experimental MW (Da)	Molecular Weight (Da)
DSS	2.75		218.31
1	1.1	3411.08	3320.5
3	0.91	6192.6	6643.2
2	1.14	3064.47	3631.1
4	0.85	7392.8	7140.2

Supplementary Table 2: Summary of NMR structural statistics of the designed mini-proteins

	1	2
Distance constraints:		
Sequential [$ i-j = 1$]	352	387
Medium range [$1 < i-j < 4$]	26	34
Long range [$ i-j \geq 4$]	74	51
Total	452	472
Dihedral-angle constraints	48	50
Constraints violations		
Minimum NOE violation (Å)	0.19	0.15
Maximum NOE violation (Å)	0.49	0.5
Minimum dihedral angle violation (°)	3.09	3.19
Maximum dihedral angle violation (°)	20.7	19.03
Deviation from mean structure (Å)		
All backbone atoms	0.28	0.34
All heavy atoms	0.66	0.68
Ramachandran Plot		
Most favoured region	90	88.5
Additionally allowed region	10	11.5
Generously allowed region	0	0
Disallowed region	0	0

Supplementary Table 3: UV-vis characteristics of heme/mini-protein complexes and known bis-His heme ligating proteins

Protein	Oxidized (nm)			Reduced (nm)		
	Ligation	Soret (γ)	α - β	Soret (γ)	α	β
1	His/His	414	530-560	428	532	560
2	His/His	412	530-560	426	532	560
3	His/His	414	530-560	428	532	560
4	His/His	412	530-560	426	532	560
5	His/His	414	530-560	428	532	560
6	His/His	414	530-560	428	532	560
7	His	402	-	-	-	-
Cyt b5 microsome	His/His	410	530	423	527	555
Cyt b5 mitochondria	His/His	412	530	423	525	556