Supporting Information

Chemistry and molecular dynamics simulations of heme *b*-HemQ and coproheme-HemQ

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Figure S1. Simulation of the kinetics of coproheme binding to apo-HemQ. Simulation of coproheme binding to (A, B) apo-LmHemQ and (C, D) apo-SaHemQ using the Pro-K software (Applied Photophysics) according to the model: a + b > c; c > d. a - coproheme: black line; c - coproheme-HemQ intermediate: green line; d - coproheme bound to HemQ: red line. Insets depict calculated concentration profiles over time of binding. Lower panels show time traces and simulated double exponential fits at 390 nm.

Figure S2. Coproheme and heme *b* binding to apo-myoglobin. (A) UV-vis absorption spectra of coproheme (black) and after addition of excess apo-myoglobin (red). (B) UV-vis absorption spectra of hemin (black) and after addition of excess apo-myoglobin (red). The inset show time traces of absorbance changes in the Soret region.

Figure S3. Heme transfer from coproheme-HemQ and heme *b*-HemQ to apo-myoglobin. Incubation of (A) 1 μM coproheme-SaHemQ or (B) 1 μM heme *b*-SaHemQ with 10 μM apo-myoglobin. Spectra prior to addition are shown as solid black lines, resulting spectra are shown as solid red lines. Insets show absorbance change in the Soret region. Conditions: 50 mM phosphate buffer, pH 7.0.

Figure S4. Thermal stability of apo-HemQs, heme *b*-HemQs and coproheme-HemQs followed by differential scanning calorimetry. Thermograms (black line) and fits of the endotherms (grey lines) of apo-LmHemQ, coproheme bound LmHemQ, and heme *b*-LmHemQ. Conditions: 50 mM phosphate buffer, pH 7.0.

Figure S5. pH-dependence of UV-vis absorption spectra of HemQ. UV-vis absorption spectra and corresponding 2nd derivatives of (A) LmHemQ and (B) SaHemQ at different pH values. Conditions: 50 mM citrate/phoshpate buffer, pH 5.5 - 7.0; 50 mM phosphate buffer, pH 6.5 - 8.0, 50 mM glycine/NaOH buffer, pH 8.0 - 10.0.

Figure S6. Electronic circular dichroism spectroscopy of coproheme-HemQ and heme *b*-HemQ. (A, C) Far-UV spectra of 5 μM apo-HemQs (pink), coproheme-HemQs (black), and heme *b*-HemQs (green) of (A) LmHemQ and (C) SaHemQ in 5 mM phosphate buffer, pH 7.0; spectra are shifted by -5 mdeg for clarity. Insets depict the ratios of ellipticity minima. (B, D) Near-UV and visible ECD spectra of 10 μM coproheme-HemQs (black), of coproheme-HemQs in presence of 1 mM cyanide (red), heme *b*-HemQs (green), and 10 μM coproheme (blue, dashed) of (B) LmHemQ and (D) SaHemQ in 50 mM phosphate buffer, pH 7.0. The insets show the corresponding UV-vis absorption spectra, recorded simultaneously.

Figure S7. Root-mean-square fluctuations of the protein backbone of apo-LmHemQ, coproheme-LmHemQ and heme *b*-LmHemQ. RMSF analysis of 30 ns MD simulations of (A) apo-LmHemQ and (B) coproheme-LmHemQ and 10 ns of (C) heme *b*-HemQ over the entire protein sequence (i.e. all 5 subunits).

Figure S8. Root-mean-square deviation and fluctuation of the protein backbone of monomeric and pentameric apo-LmHemQ and coproheme-LmHemQ. (A) RMSD calculations with respect to the initial structure for chain A simulated as monomer (apo-LmHemQ in black; coproheme-LmHemQ in red) and in the pentameric form (apo-LmHemQ in green; coproheme-LmHemQ in blue). (B) RMSF analysis of the same simulations. Early onset of unfolding can be observed around residues 125 and 150 – 175 in the apo-LmHemQ simulations.

Figure S9. Accessibilty of the coproheme/substrate binding site in apo-LmHemQ and coproheme-LmHemQ. Surface representations of (A) apo-LmHemQ (pdb-code: 4WWS) and (B) coproheme-LmHemQ (last frame of 30 ns simulation) shown in yellow; surface of backbone atoms of R179 are shown in cyan, nitrogens of R179 in blue, oxygens in red, and hydrogens in grey. Coproheme in (B) is shown as grey sticks.

Fig. S1

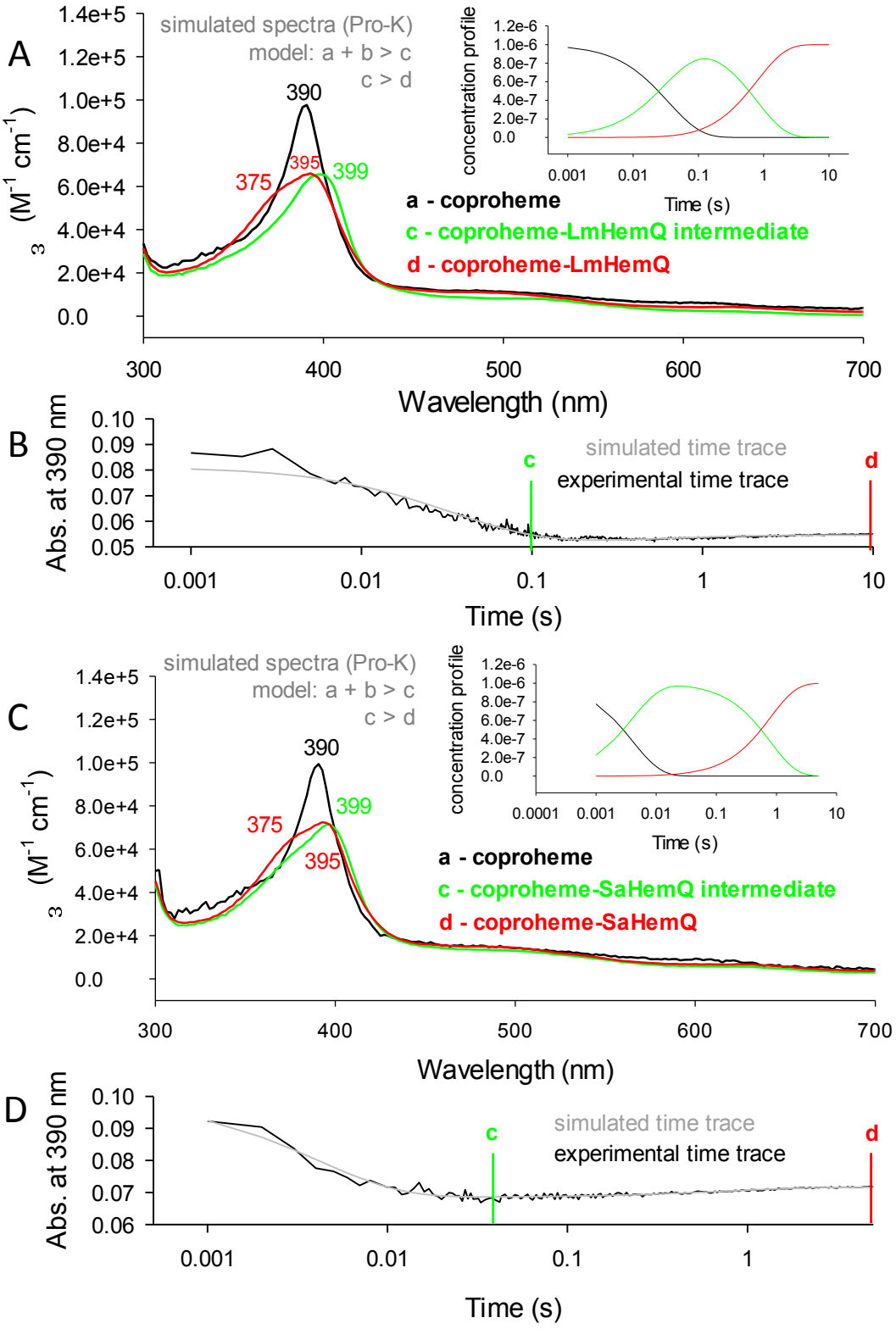

A
simulated spectra (Pro-K)
model: a + b > c
c > d
390
375
395
399
a - coproheme
c - coproheme-LmHemQ intermediate
d - coproheme-LmHemQ
concentration profile
Time (s)
ε (M-1 cm-1)
Wavelength (nm)
B
Abs. at 390 nm
simulated time trace
experimental time trace
c
d
Time (s)
C
simulated spectra (Pro-K)
model: a + b > c
c > d
390
375
395
399
a - coproheme
c - coproheme-SaHemQ intermediate
d - coproheme-SaHemQ
concentration profile
Time (s)
ε (M-1 cm-1)
Wavelength (nm)
D
Abs. at 390 nm
simulated time trace
experimental time trace
c
d
Time (s)

Fig. S2

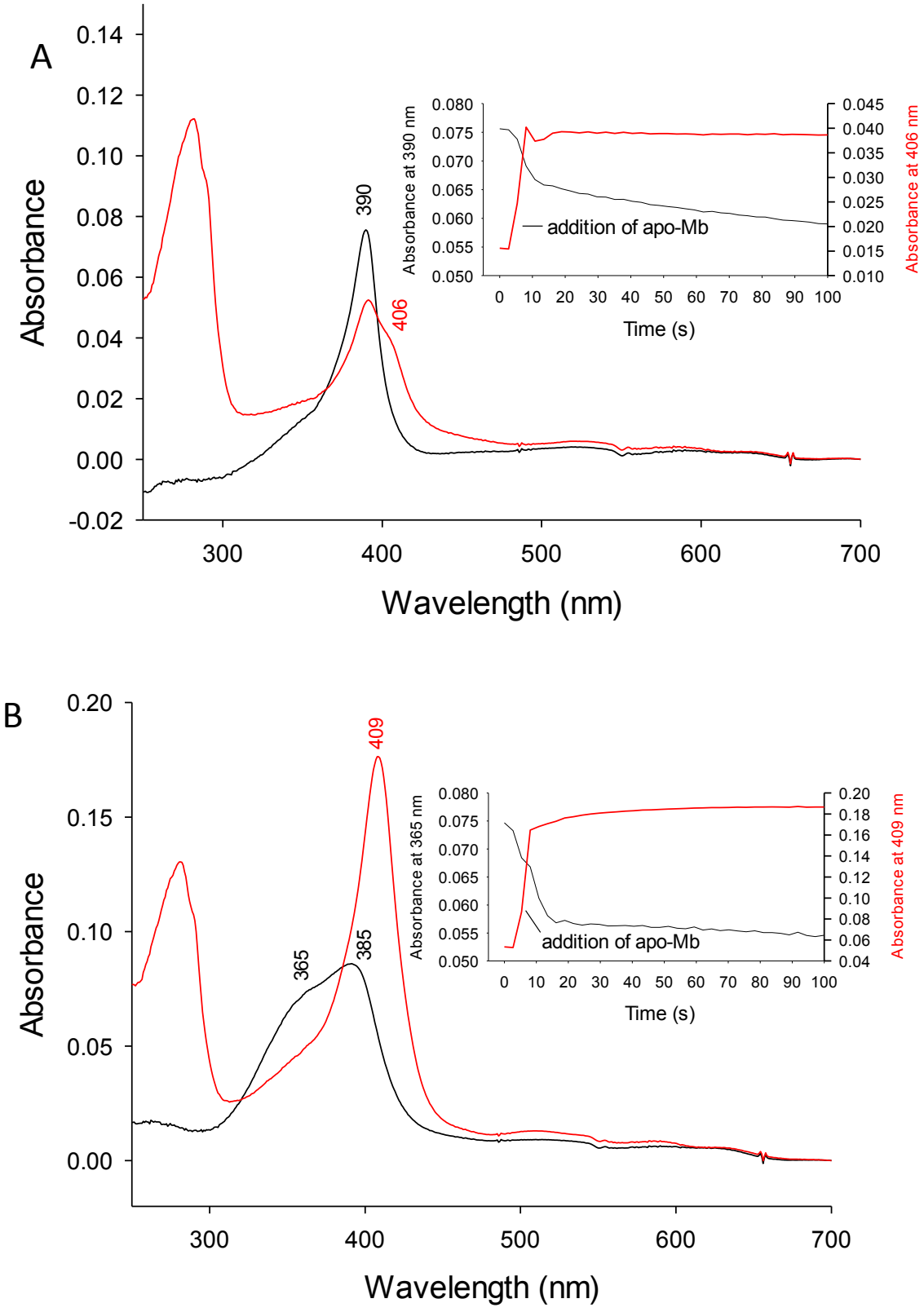
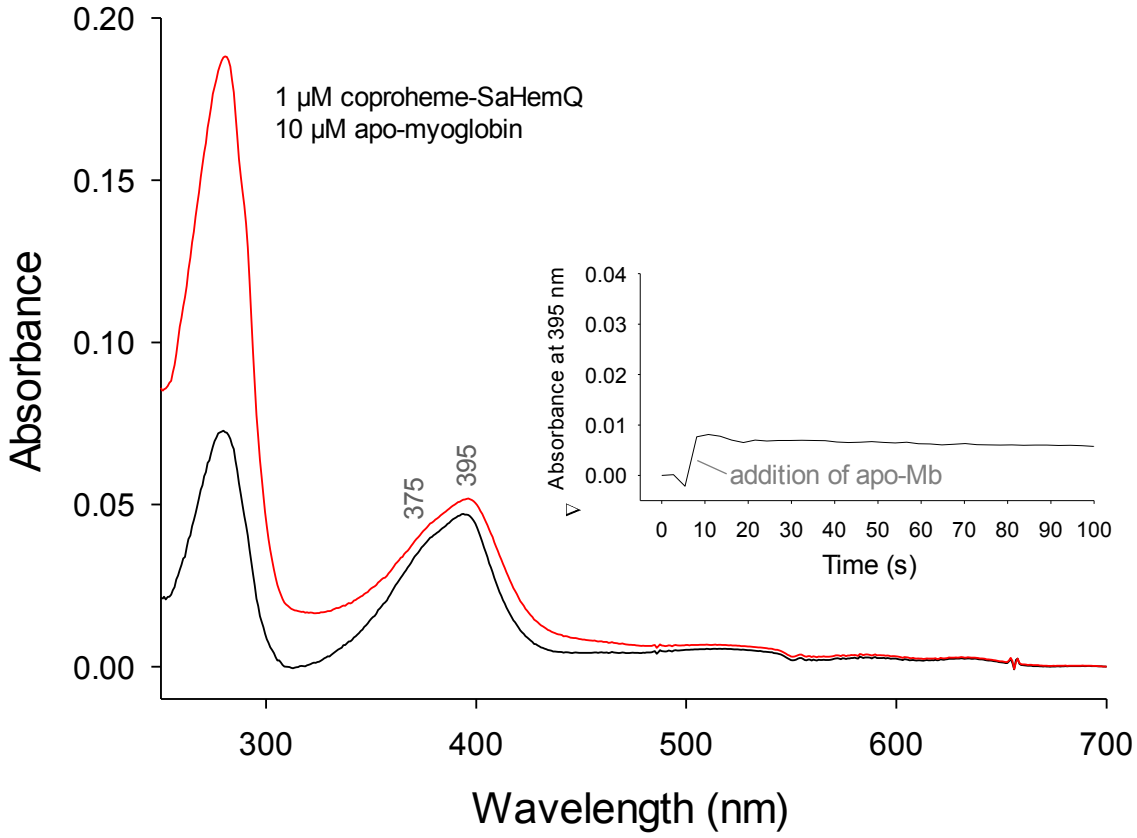

A
Absorbance
Wavelength (nm)
390
406
addition of apo-Mb
Absorbance at 390 nm
Absorbance at 406 nm
Time (s)
B
Absorbance
Wavelength (nm)
365
385
409
addition of apo-Mb
Absorbance at 365 nm
Absorbance at 409 nm
Time (s)

Fig. S3


1 μM coproheme-SaHemQ
10 μM apo-myoglobin
375
395
Absorbance
Wavelength (nm)
Absorbance at 395 nm
addition of apo-Mb
Time (s)

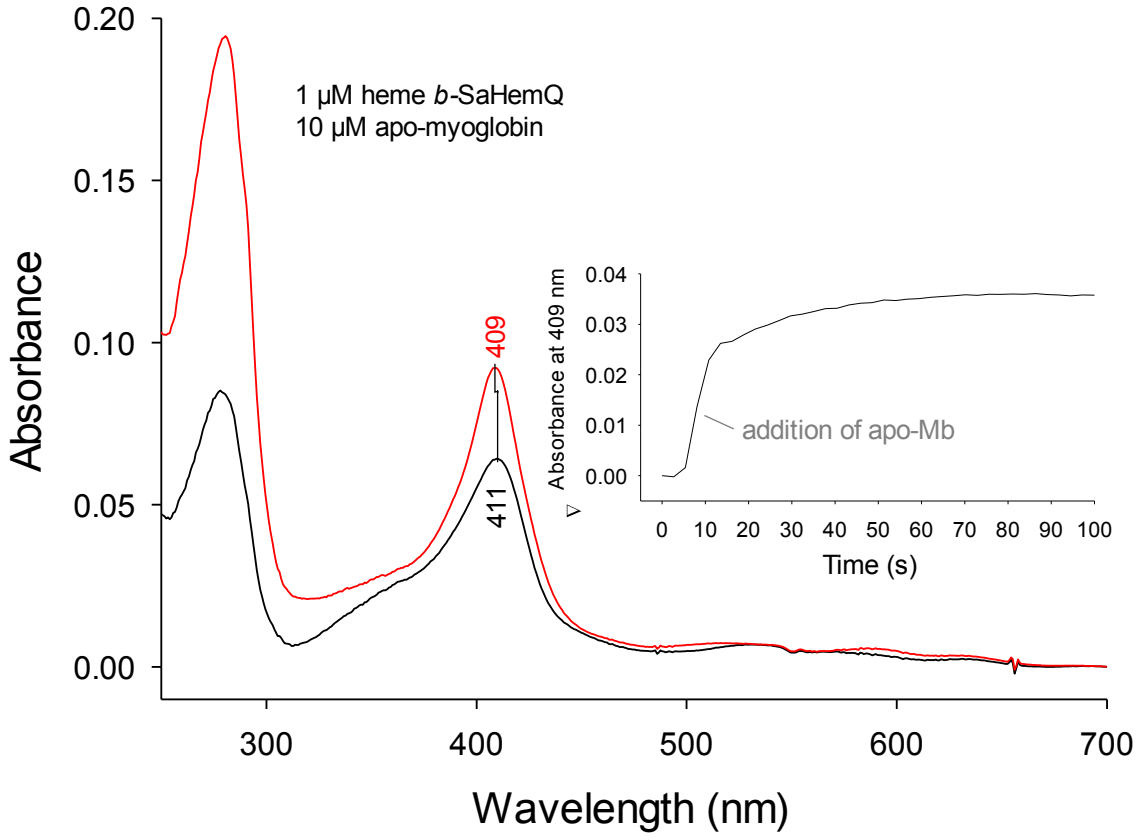

1 μM heme *b*-SaHemQ
10 μM apo-myoglobin
409
411
Absorbance
Wavelength (nm)
Absorbance at 409 nm
addition of apo-Mb
Time (s)

Fig. S4

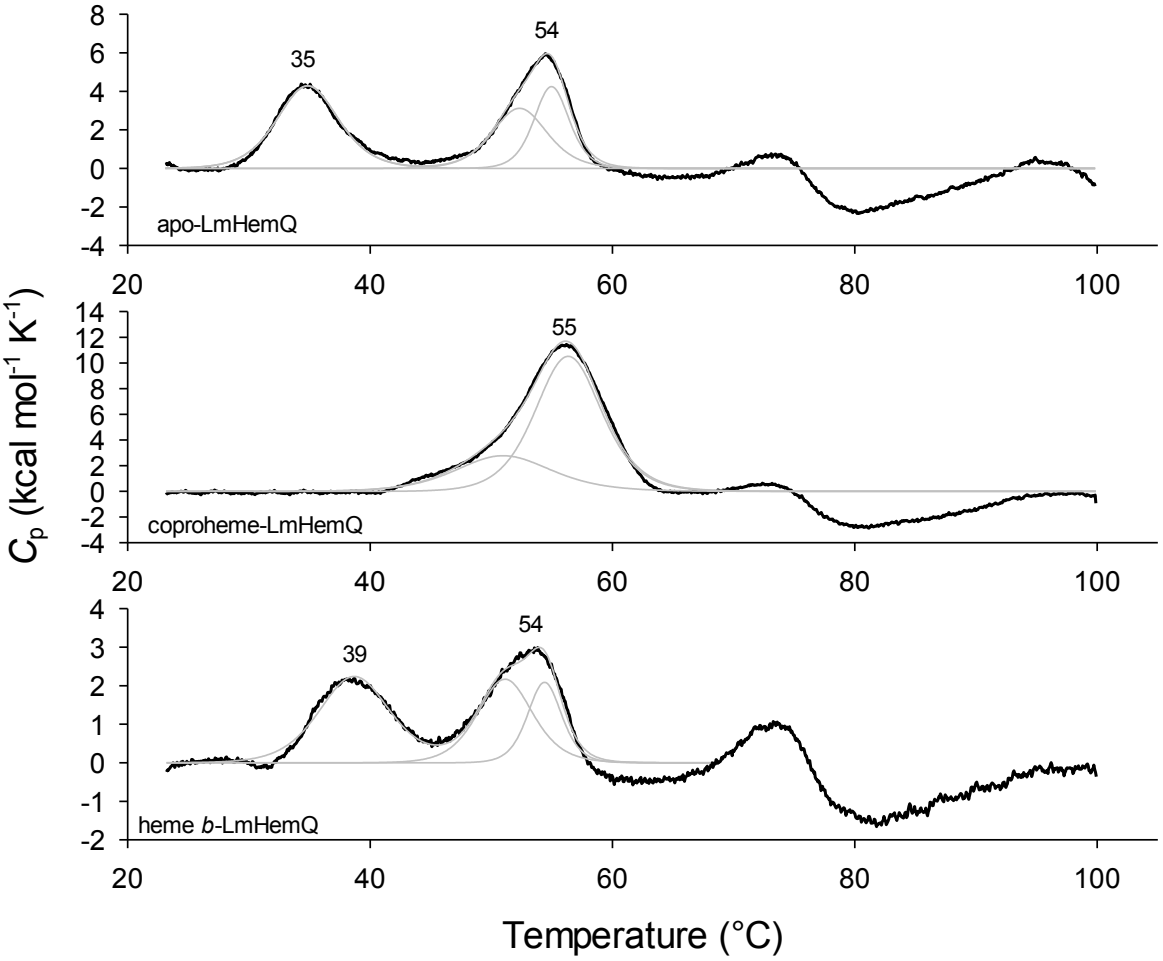

35
54
apo-LmHemQ
55
coproheme-LmHemQ
39
54
heme b-LmHemQ
C_p (kcal mol^{-1} K^{-1})
Temperature (°C)

Fig. S5

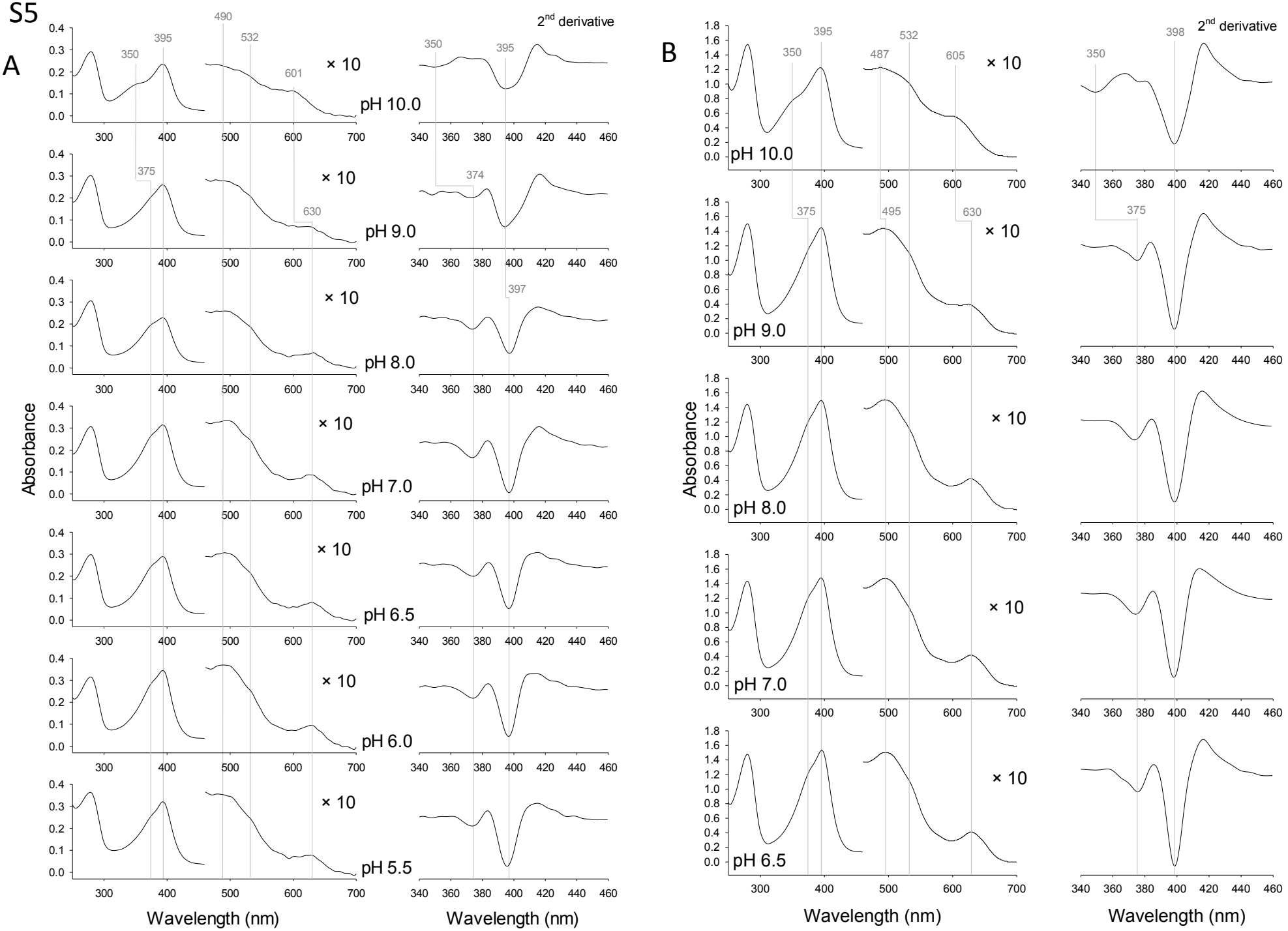

A
B
2nd derivative
× 10
pH 10.0
pH 9.0
pH 8.0
pH 7.0
pH 6.5
pH 6.0
pH 5.5
Absorbance
Wavelength (nm)
350
395
490
532
601
375
630
374
397
487
605
495
398

Fig. S6

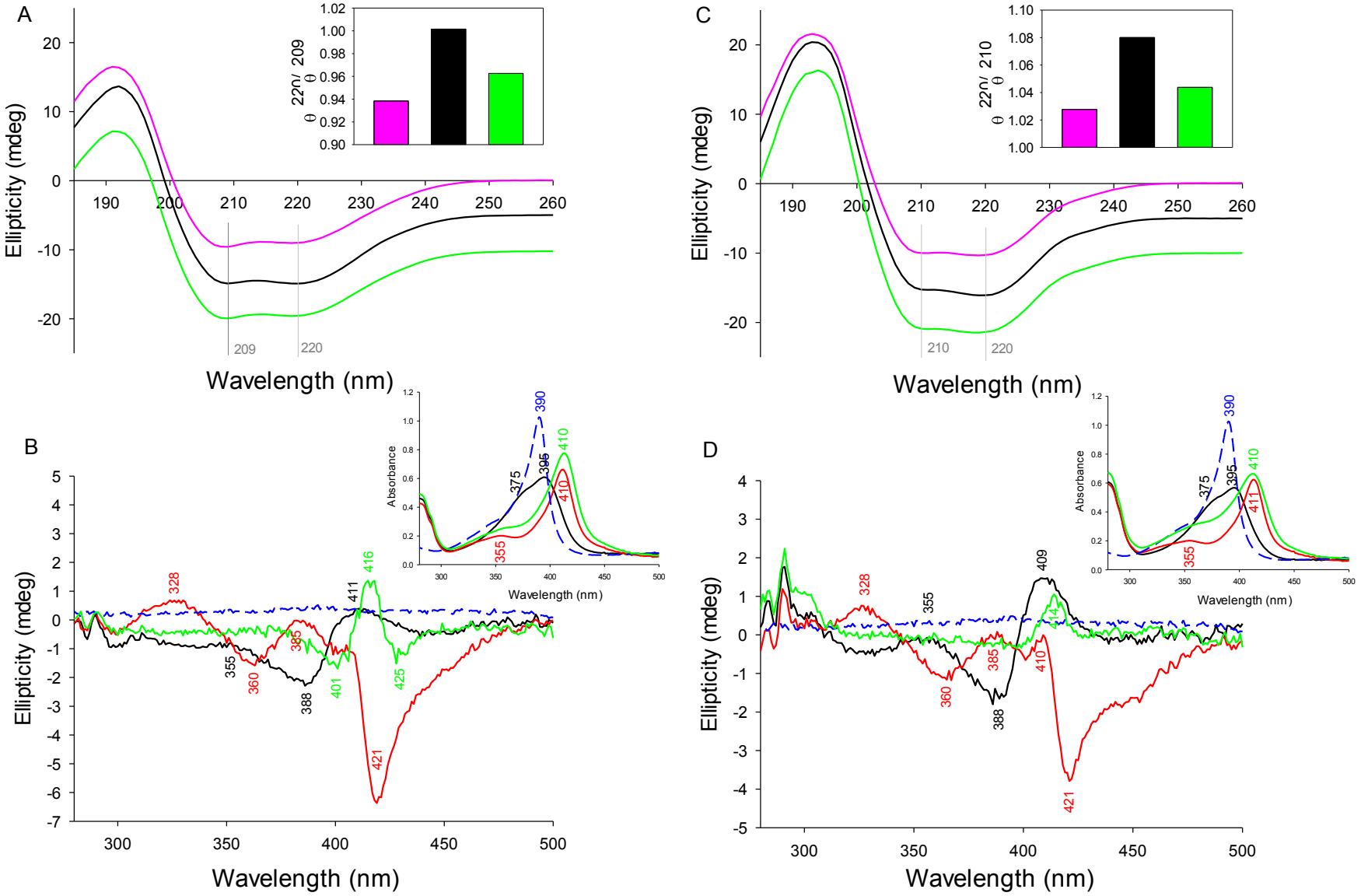

A
Ellipticity (mdeg)
Wavelength (nm)
209
220
θ 220/ θ 209
B
Ellipticity (mdeg)
Wavelength (nm)
Absorbance
C
Ellipticity (mdeg)
Wavelength (nm)
210
220
θ 220/ θ 210
D
Ellipticity (mdeg)
Wavelength (nm)
Absorbance

Fig. S7

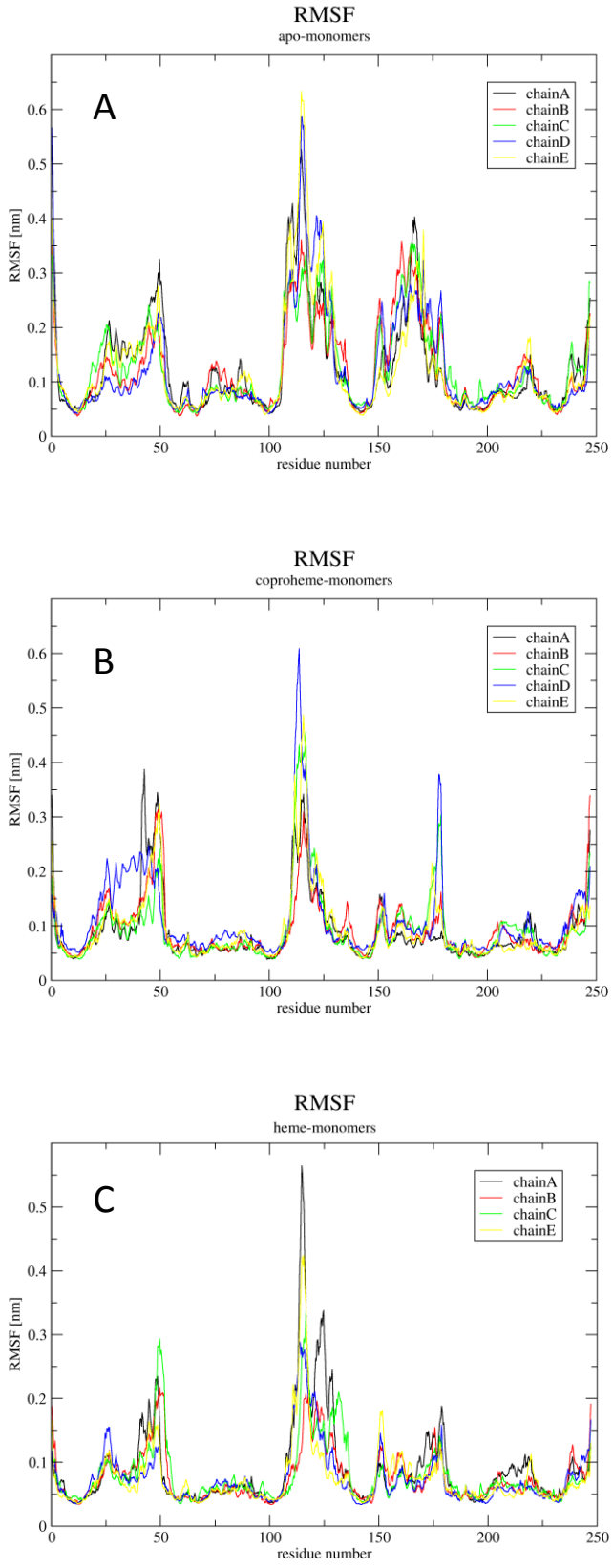
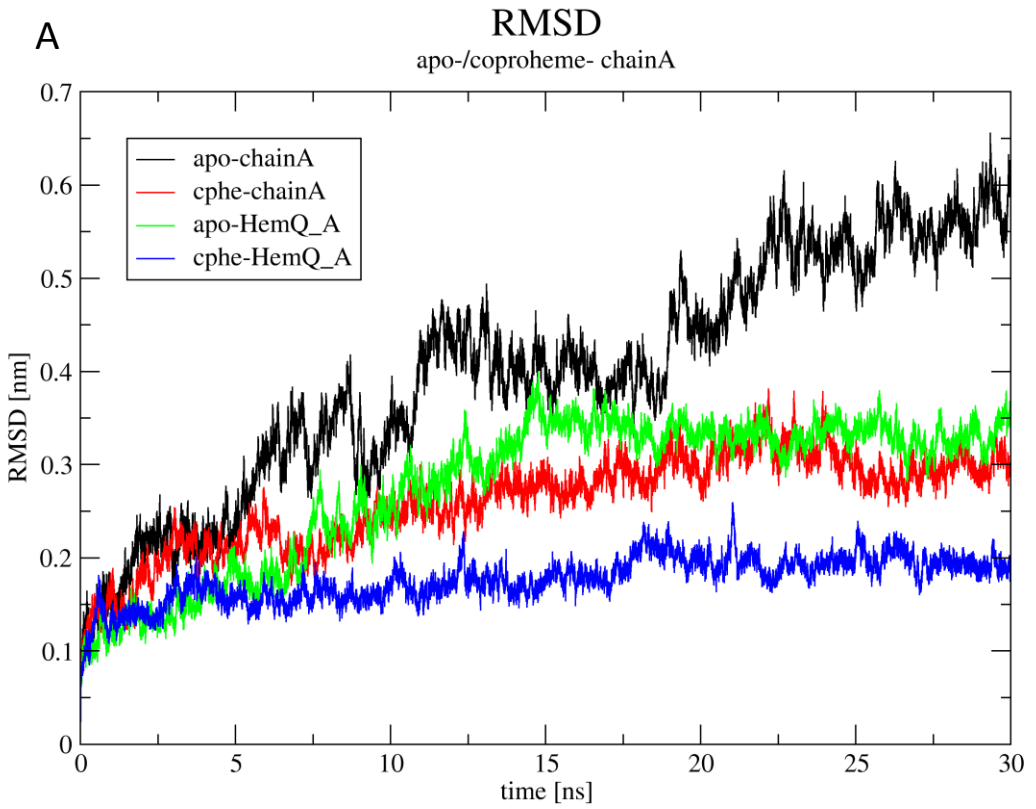

RMSF
apo-monomers
A
chainA
chainB
chainC
chainD
chainE
RMSF [nm]
residue number
RMSF
coproheme-monomers
B
chainA
chainB
chainC
chainD
chainE
RMSF [nm]
residue number
RMSF
heme-monomers
C
chainA
chainB
chainC
chainE
RMSF [nm]
residue number

Fig. S8


A
RMSD
apo-/coproheme- chainA
apo-chainA
cphe-chainA
apo-HemQ_A
cphe-HemQ_A
RMSD [nm]
time [ns]
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
5
10
15
20
25
30

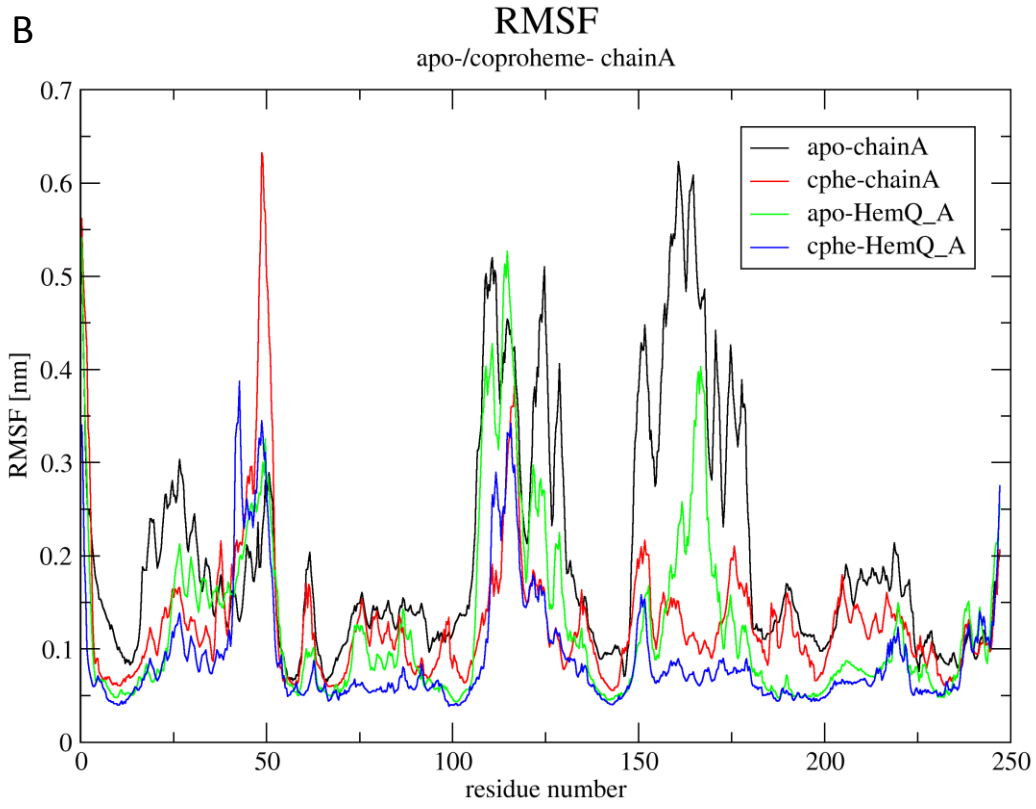

B
RMSF
apo-/coproheme- chainA
apo-chainA
cphe-chainA
apo-HemQ_A
cphe-HemQ_A
RMSF [nm]
residue number
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
50
100
150
200
250

Fig. S9

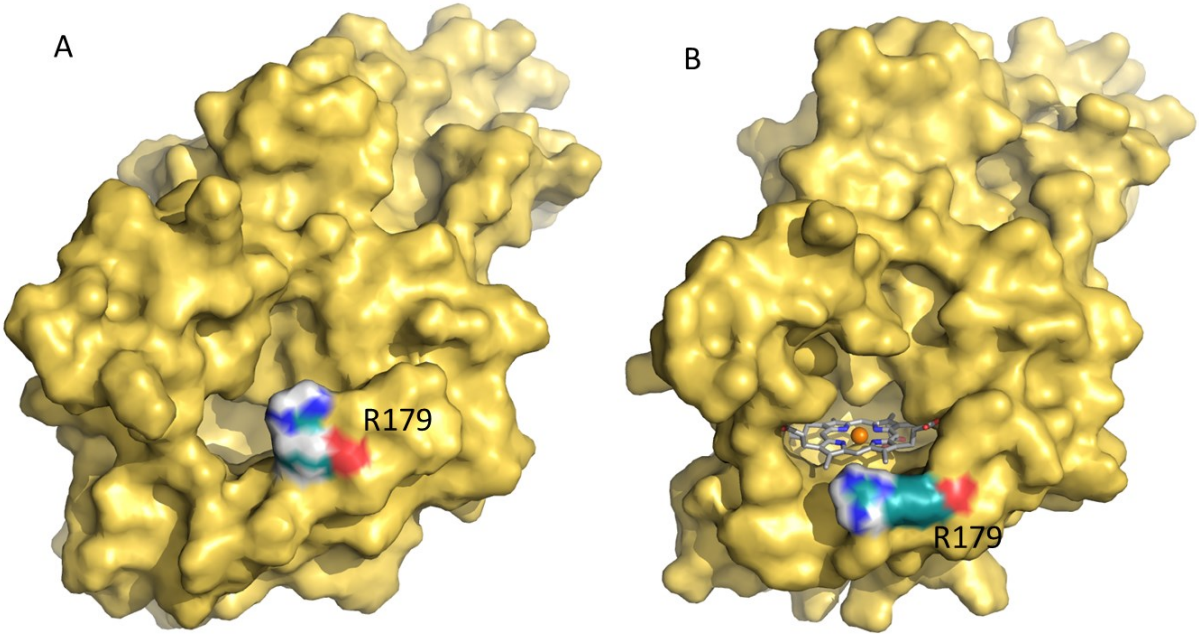


Table S1. H-bond analysis of LmHemQ and coproheme. Hydrogen bonds formed between the coproheme propionates (p2, p4, p6, p7) and amino acids of LmHemQ throughout the 30 ns simulation over all five subunits are represented.

H-bond Analysis: coproheme-HemQ

	# H-bonds	interacting atoms		# H-bonds
Chain A	1	1:111:SER:OG:HG	6:1:CPHE:O1D/O2D:	0.96
	2	1:112:ASN:N:H	6:1:CPHE:O1C:	0.03
	3	1:113:TYR:N:H	6:1:CPHE:O1D/O2D:	0.92
	4	1:113:TYR:N:H	6:1:CPHE:O1C:	0.02
	5	1:113:TYR:OH:HH	6:1:CPHE:O1C/O2C:	0.87
	6	1:114:LEU:N:H	6:1:CPHE:O1D/O2D:	0.94
	7	1:115:ALA:N:H	6:1:CPHE:O1D/O2D:	0.60
	8	1:116:SER:N:H	6:1:CPHE:O1D/O2D:	0.11
	9	1:116:SER:OG:HG	6:1:CPHE:O1D/O2D:	0.07
	10	1:147:TYR:OH:HH	6:1:CPHE:O1B/O2B:	0.98
	11	1:151:LYSH:NZ:HZ1/HZ2/HZ3	6:1:CPHE:O1C/O2C:	0.15
	12	1:159:TRP:NE1:HE1	6:1:CPHE:O1C/O2C:	0.02
	13	1:183:GLY:N:H	6:1:CPHE:O1A/O2A:	1.26
	14	1:187:GLN:NE2:HE21/HE22	6:1:CPHE:O1A/O2A:	0.84
	15	1:220:ARG:NE:HE	6:1:CPHE:O1B/O2B:	0.03
	16	1:220:ARG:NH2:HH22	6:1:CPHE:O1B/O2B:	0.05
Chain B	17	2:111:SER:OG:HG	10:1:CPHE:O1D/O2D:	1.01
	18	2:113:TYR:N:H	10:1:CPHE:O1D/O2D:	0.92
	19	2:114:LEU:N:H	10:1:CPHE:O1D/O2D:	1.10
	20	2:115:ALA:N:H	10:1:CPHE:O1D/O2D:	0.11
	21	2:116:SER:N:H	10:1:CPHE:O1D:	0.85
	22	2:116:SER:OG:HG	10:1:CPHE:O1D:	0.66
	23	2:147:TYR:OH:HH	10:1:CPHE:O1B/O2B:	1.00
	24	2:151:LYSH:NZ:HZ1/HZ2/HZ3	10:1:CPHE:O1C/O2C:	1.06
	25	2:174:HISA:ND1:HD1	10:1:CPHE:O1C/O2C:	0.88
	26	2:183:GLY:N:H	10:1:CPHE:O1A/O2A:	0.69
	27	2:187:GLN:NE2:HE22	10:1:CPHE:O1A/O2A:	0.90
	28	2:225:SER:OG:HG	10:1:CPHE:O1B/O2B:	0.11
Chain C	29	3:111:SER:N:H	8:1:CPHE:O1D/O2D:	0.09
	30	3:111:SER:OG:HG	8:1:CPHE:O1D/O2D:	0.92
	31	3:113:TYR:N:H	8:1:CPHE:O1D/O2D:	0.66
	32	3:113:TYR:OH:HH	8:1:CPHE:O1C/O2C:	0.49
	33	3:114:LEU:N:H	8:1:CPHE:O1D/O2D:	0.98
	34	3:115:ALA:N:H	8:1:CPHE:O1D/O2D:	0.87

	35	3:116:SER:N:H	8:1:CPHE:O1D/O2D:	0.11
	36	3:116:SER:OG:HG	8:1:CPHE:O1D/O2D:	0.05
	37	3:147:TYR:OH:HH	8:1:CPHE:O1B/O2B:	0.99
	38	3:151:LYSH:NZ:HZ1/HZ2/HZ3	8:1:CPHE:O1C/O2C:	0.94
	39	3:159:TRP:NE1:HE1	8:1:CPHE:O1C/O2C:	0.05
	40	3:179:ARG:NE:HE	8:1:CPHE:O1A/O2A:	0.07
	41	3:179:ARG:NH1:HH11/HH12	8:1:CPHE:O1A/O2A:	0.01
	42	3:179:ARG:NH2:HH21/HH22	8:1:CPHE:O1A/O2A:	0.04
	43	3:183:GLY:N:H	8:1:CPHE:O1A/O2A:	0.14
	44	3:187:GLN:NE2:HE21/HE22	8:1:CPHE:O1A/O2A:	0.03
	45	3:220:ARG:NH2:HH22	8:1:CPHE:O1B:	0.02
	46	3:225:SER:OG:HG	8:1:CPHE:O1B/O2B:	0.96
Chain D	47	4:111:SER:N:H	7:1:CPHE:O1D/O2D:	0.91
	48	4:111:SER:OG:HG	7:1:CPHE:O1D/O2D:	0.30
	49	4:113:TYR:OH:HH	7:1:CPHE:O1C/O2C:	0.63
	50	4:133:ARG:NH1:HH11	7:1:CPHE:O1A/O2A:	0.10
	51	4:133:ARG:NH2:HH21/HH22	7:1:CPHE:O1A/O2A:	0.11
	52	4:147:TYR:OH:HH	7:1:CPHE:O1B/O2B:	1.11
	53	4:151:LYSH:NZ:HZ1/HZ2/HZ3	7:1:CPHE:O1C/O2C:	0.41
	54	4:159:TRP:NE1:HE1	7:1:CPHE:O1C/O2C:	0.06
	55	4:186:GLN:NE2:HE22/HE21	7:1:CPHE:O1A/O2A:	0.06
	56	4:187:GLN:N:H	7:1:CPHE:O1A/O2A:	0.86
	57	4:187:GLN:NE2:HE21/HE22	7:1:CPHE:O1B/O2B:	0.57
Chain E	58	5:111:SER:OG:HG	9:1:CPHE:O1D/O2D:	0.85
	59	5:111:SER:OG:HG	9:1:CPHE:O1C/O2C:	0.16
	60	5:112:ASN:N:H	9:1:CPHE:O1C/O2C:	0.11
	61	5:113:TYR:N:H	9:1:CPHE:O1D/O2D:	0.98
	62	5:113:TYR:OH:HH	9:1:CPHE:O1C/O2C:	0.75
	63	5:114:LEU:N:H	9:1:CPHE:O1D/O2D:	0.87
	64	5:115:ALA:N:H	9:1:CPHE:O1D/O2D:	0.76
	65	5:147:TYR:OH:HH	9:1:CPHE:O1B/O2B:	0.97
	66	5:151:LYSH:NZ:HZ1/HZ2/HZ3	9:1:CPHE:O1C/O2C:	0.53
	67	5:159:TRP:NE1:HE1	9:1:CPHE:O1C/O2C:	0.14
	68	5:183:GLY:N:H	9:1:CPHE:O1A/O2A:	0.99
	69	5:187:GLN:NE2:HE21/HE22	9:1:CPHE:O1A/O2A:	0.90
	70	5:200:TRP:NE1:HE1	9:1:CPHE:O1C/O2C:	0.02
	71	5:225:SER:OG:HG	9:1:CPHE:O1B/O2B:	0.97