Supplementary information for:

Delineating redox cooperativity in water-soluble and membrane multiheme cytochromes through protein design

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Figure S1. Structure predictions of m2-4D2 with ESMfold and AF2.

Figure S2. Comparison of helical packing order in predicted structures from AF2 vs. the theoretical Rosetta-modelled structure of m2-4D2.

Figure S3. Comparison of ESMfold and AF2 predicted structures vs the crystal structure of 4D2.

Figure S4. Structure predictions of m1-4D2 with ESMfold and AF2.

Figure S5. Overlay of ESMfold predicted structures vs. target structure of diheme and monoheme proteins.

Figure S6. ESMfold structure prediction of CytbX containing the nine core packing mutations from m4D2.

Figure S7. Score and sequence similarity analysis for the top 10 Rosetta-designed sequences for m1-CytbX and m2-CytbX.

Figure S8. ESMfold structure predictions of m1-CytbX and m2-CytbX.

Figure S9. AlphaFold2 structure predictions of m1-CytbX and m2-CytbX.

Figure S10. DeepTMHMM predicted transmembrane topologies of designed membrane protein sequences.

Figure S11. Size-exclusion chromatography elution traces.

Figure S12. Spectral evidence for ZnPPIX binding to m2-CytbX

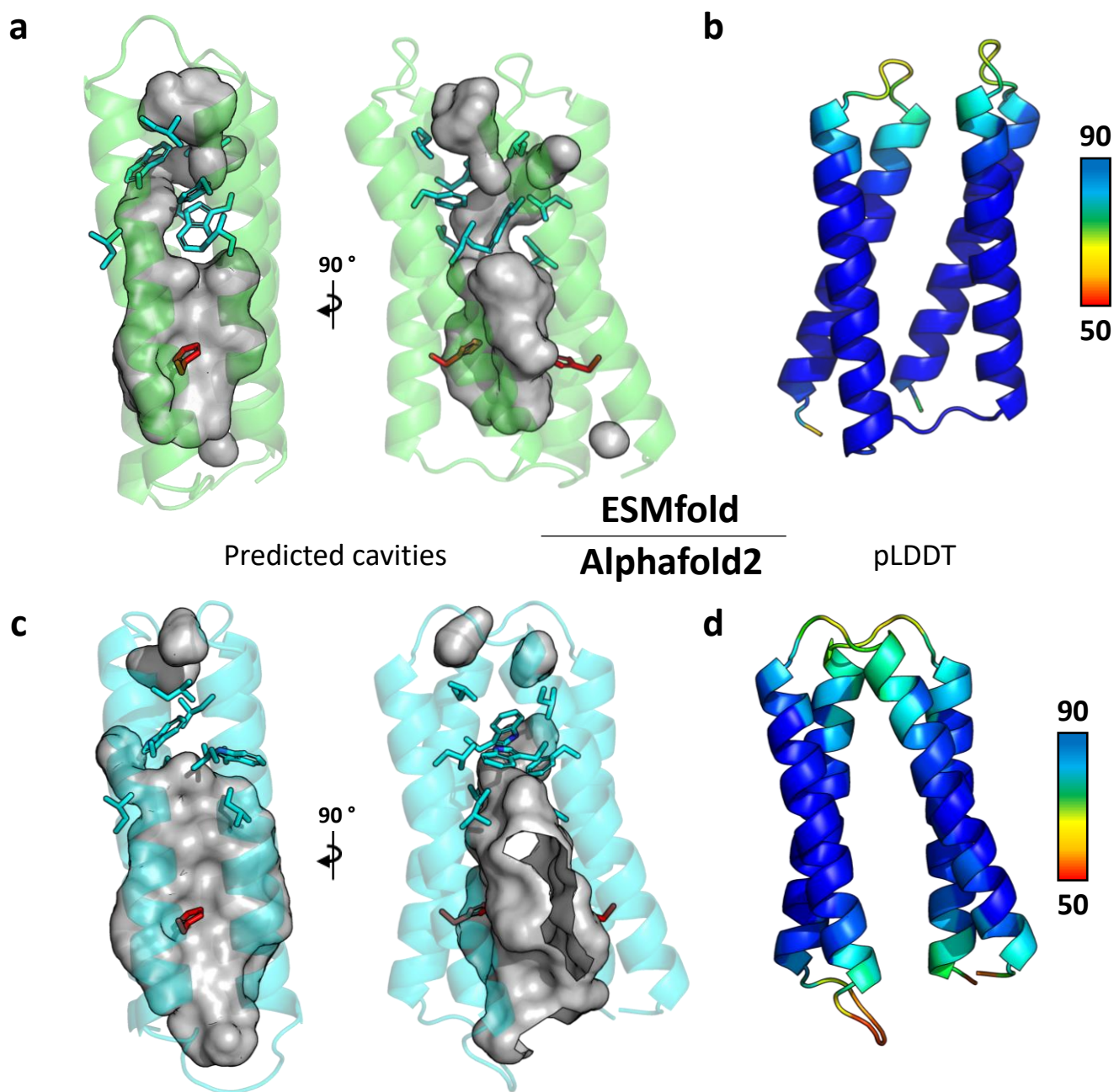**Figure S13.** Native mass spectrometry of m1-CytbX, m2-CytbX and m2-4D2.

Figure S1. Structure predictions of m2-4D2 from ESMfold (top) and AlphaFold2 (bottom). (a,c) Depictions of predicted cavities, highlighting heme binding and core packing regions and (b, d) predicted structure coloured by confidence (pLDDT), as a rainbow from red to blue scaled from 50 to 90. Cavities were generated with PyMol. Histidine residues and core packing residues are shown as sticks, coloured red and cyan respectively consistent with Figure 1.

Rosetta Model

AlphaFold2
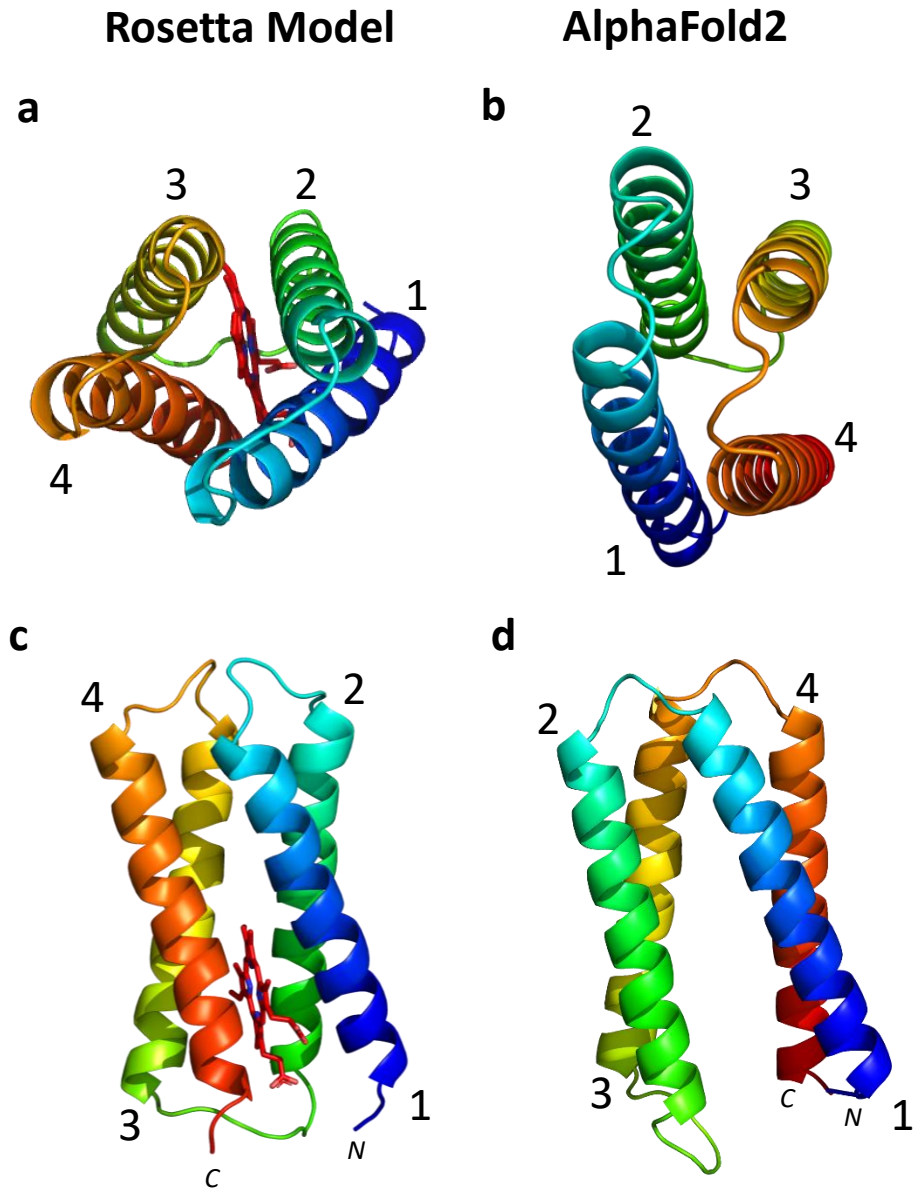

Figure S2. Comparison of helical packing order in predicted structures from AF2 vs. the theoretical Rosetta-modelled structure of m2-4D2. (a-b) Top views and (c-d) side view of helical packing. Models are coloured from N to C terminus as a rainbow from blue to red. The number of each helix in the protein is labelled. AlphaFold2 predicts a mirrored packing order to the theoretical structure.
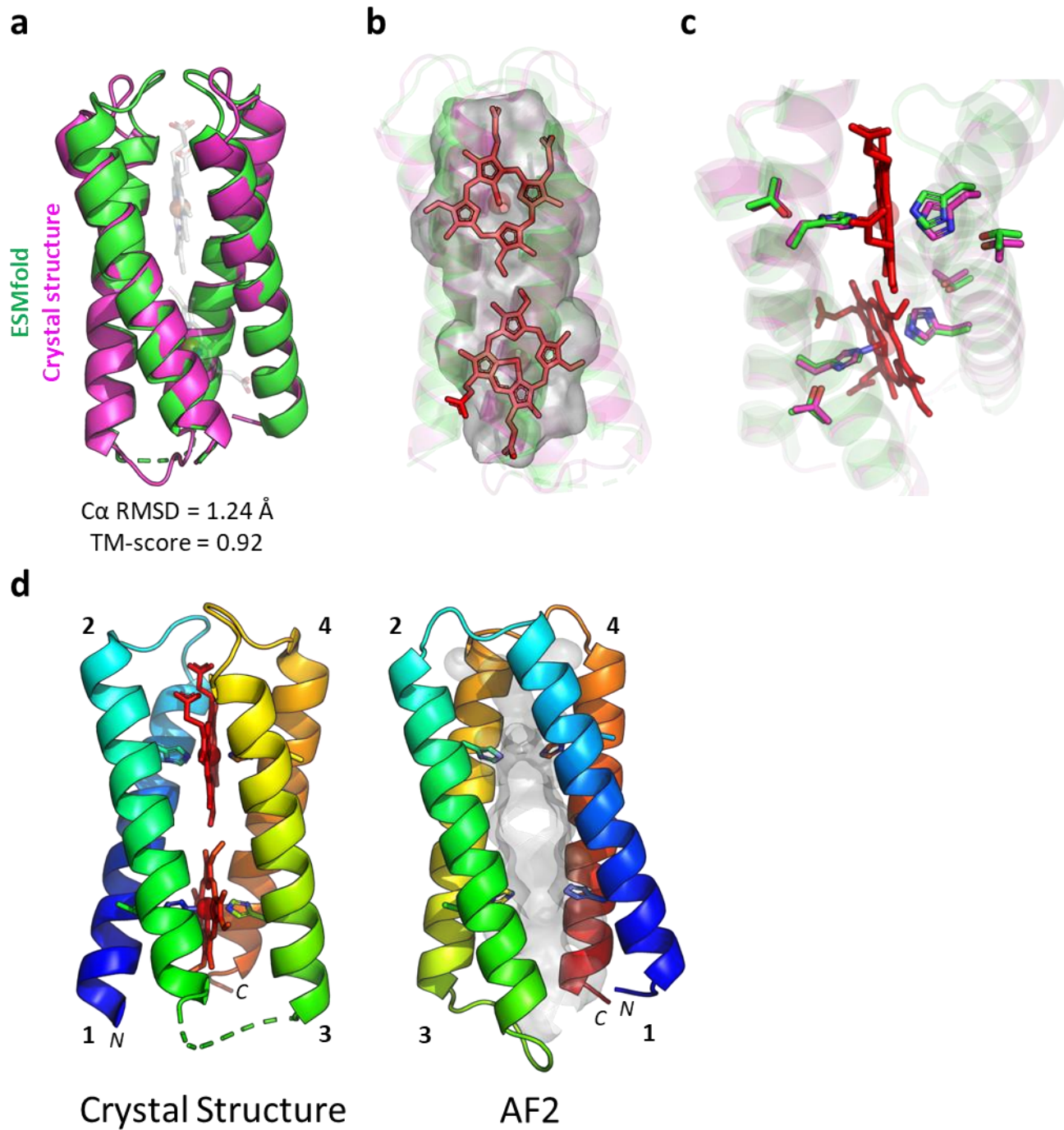
Figure S3. Comparison of ESMfold and AF2 predicted structures vs the crystal structure of 4D2 (PDB ID: 7AH0). (a) Alignment of ESMfold model (green) vs 4D2 crystal structure (magenta). (b) Heme-shaped cavities within the ESMfold predicted structure, showing the hemes of 4D2. (c) The heme-coordinating histidine-threonine residue pairs align almost exactly between the 4D2 crystal structure and ESMfold model. (d) The crystal structure and AF2 model coloured as a rainbow from N to C terminus, with hemes and internal cavities shown. The helix numbers are labelled, showing that AF2 packs the 4D2 helical bundle in a mirrored image to the crystal structure.
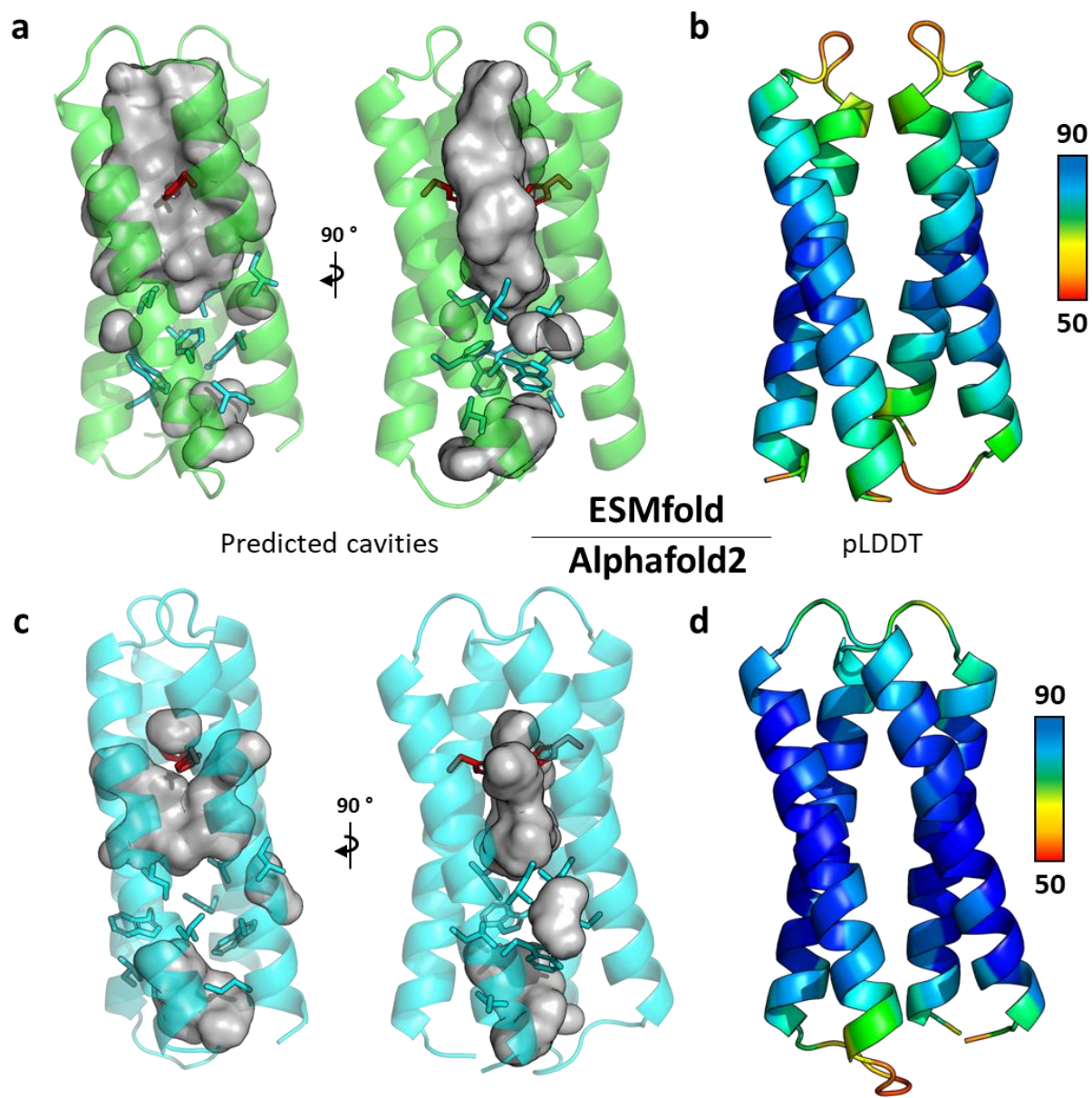
Figure S4. Structure predictions of m1-4D2 from ESMfold (top) and AlphaFold2 (bottom). (**a,c**) Depictions of predicted cavities, highlighting heme binding and core packing regions and (b, d) predicted structures coloured by confidence (pLDDT), as a rainbow from red to blue scaled from 50 to 90. Cavities were visualised with PyMol. Histidine residues and core packing residues are shown as sticks, coloured red and cyan respectively consistent with Figure 1.
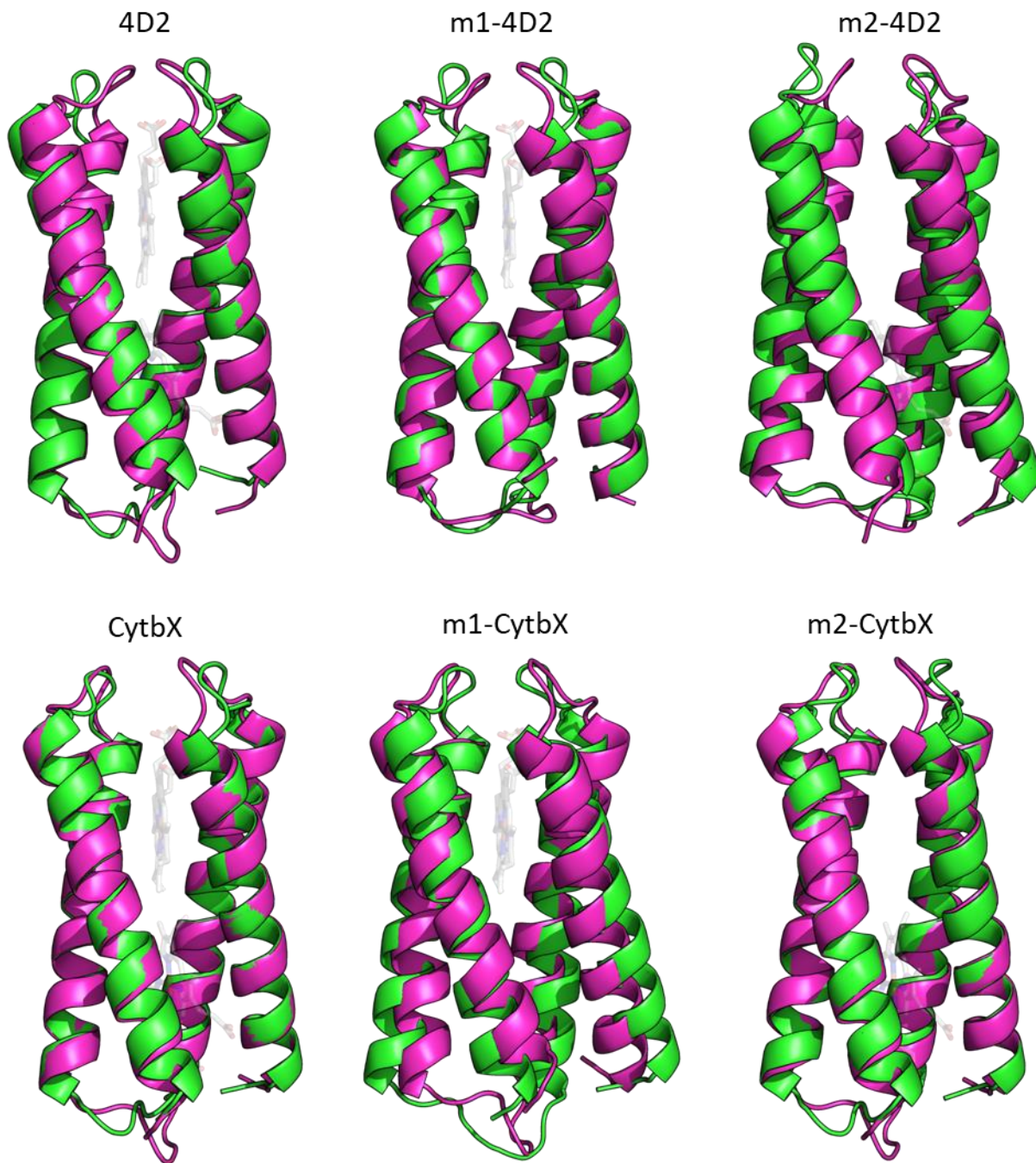
Figure S5. Overlay of ESMfold predicted structures vs. crystal structure (4D2) or modelled theoretical structures of the designed heme proteins. Green: ESMfold structure, magenta: Crystal structure (4D2) or Rosetta structure. Hemes are shown as semi-transparent sticks and are absent in predicted structures. For RMSD and TM metrics for structural alignments see supplementary table 2.

Protein	ESMfold		AlphaFold2	
	pTM	Mean pLDDT	pTM	Mean pLDDT
CytbX	0.835	88.6	0.60	79.5
m1-CytbX (0016)	0.852	89.7	0.73	86.0
m1-CytbX (0151)	0.823	86.8	0.81	90.8
m2-CytbX (0320)	0.836	86.6	0.52	77.7
m2-CytbX (0152)	0.811	86.6	0.55	78.3
CytbX m4D2 copy	0.811	86.3	0.64	83.1
4D2	0.814	85.3	0.56	79.9
m1-4D2	0.665	77.7	0.66	82.8
m2-4D2	0.810	87.0	0.57	82.3

Table S1. Structure prediction metrics for designed heme proteins. ESMfold was run using default settings with 3 recycles. AlphaFold2 was run in single sequence mode with 6 recycles, and metrics are reported for the top ranked model. Mean pLDDT reports the average pLDDT across all residues.

Protein	ESMfold		AlphaFold2	
	Ca RMSD (Å)	TM	Ca RMSD (Å)	TM
4D2*	1.24	0.92	11.41	0.42
m1-4D2	1.38	0.93	11.04	0.46
m2-4D2	2.63	0.73	11.58	0.40
CytbX	1.29	0.93	10.35	0.43
CytbX (m4D2 mutations)	2.22	0.80	10.33	0.43
m1-CytbX	2.14	0.82	10.46	0.42
m2-CytbX	1.45	0.91	10.63	0.46

Table S2. Structural deviations of ESMfold predicted structures vs target structures for the final six soluble and membrane heme proteins. *Calculated vs the crystal structure of 4D2 (PDB ID: 7AH0). All others were calculated vs. the Rosetta model of each protein. RMSD and TM were calculated using the TM-score web server. The top ranked model was used from the AF2 output.
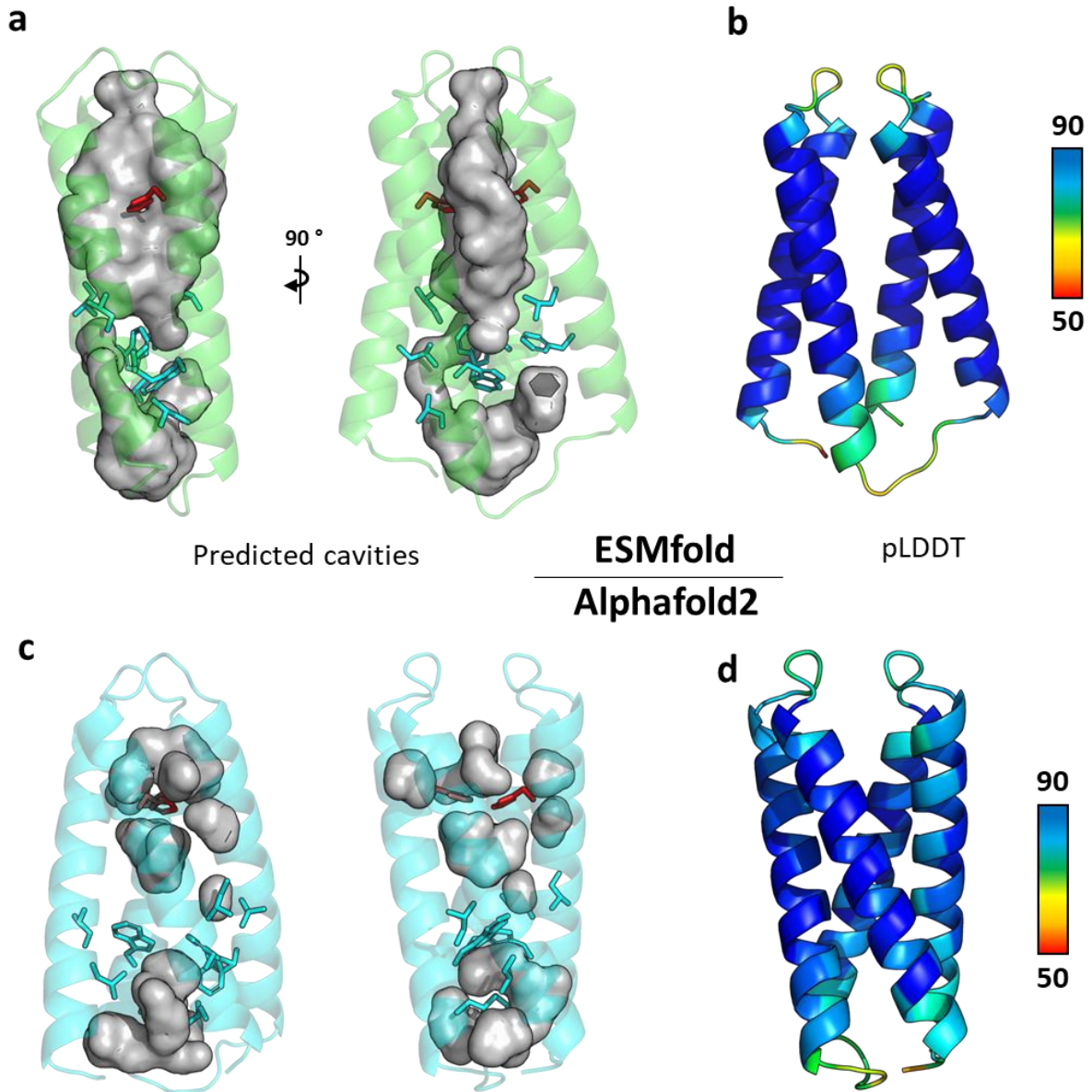
Figure S6. ESMfold structure prediction of CytbX containing the nine core packing mutations from m4D2. (a) Representation of the cavities within the ESMfold predicted structure. A heme-shaped hole can be seen in the binding site with histidine side chains pointing towards a central hole in the cavity reminiscent of bis-histidine heme ligation. A cavity can also be seen in the other end of the bundle showing that the m1-4D2 mutations are not predicted to form a well packed core. Red: histidines, cyan: m1-4D2 mutations. (b) ESMfold model coloured by pLDDT (low to high, red to blue). Respective (c) cavity and (d) pLDDT representations of the AlphaFold2 predicted structure. AlphaFold2 predicts a lower-confidence, incorrectly packed structure.

a

m1-CytbX

Rosetta score (REU)	Cα RMSD vs. CytbX (Å)	pStat	Rosetta decoy name
-472.843	0.787	0.500	10914940CytbX_Rosetta_Monoheme23_flexV1KIH_0151*
-472.819	0.771	0.529	10914953CytbX_Rosetta_Monoheme26_flexV1KIH_0049
-472.697	0.775	0.557	10914885CytbX_Rosetta_Monoheme10_flexV1KIH_0400
-472.539	0.675	0.574	10914932CytbX_Rosetta_Monoheme19_flexV1KIH_0371
-472.257	0.807	0.550	10914940CytbX_Rosetta_Monoheme23_flexV1KIH_0065
-471.664	0.764	0.545	10914938CytbX_Rosetta_Monoheme21_flexV1KIH_0492
-471.618	0.785	0.516	10914881CytbX_Rosetta_Monoheme6_flexV1KIH_0014
-471.473	1.017	**0.606**	10914881CytbX_Rosetta_Monoheme6_flexV1KIH_0016**
-471.343	0.677	0.547	10914887CytbX_Rosetta_Monoheme12_flexV1KIH_0274
-471.325	0.677	0.570	10914879CytbX_Rosetta_Monoheme4_flexV1KIH_0433

b


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0049 0.205915
0065 0.1875
0151 0.1875
0274 0.208984
0400 0.198661
0016 0.196429
0014 0.169643
0492 0.169643
0433 0.1875
0371 0.1875
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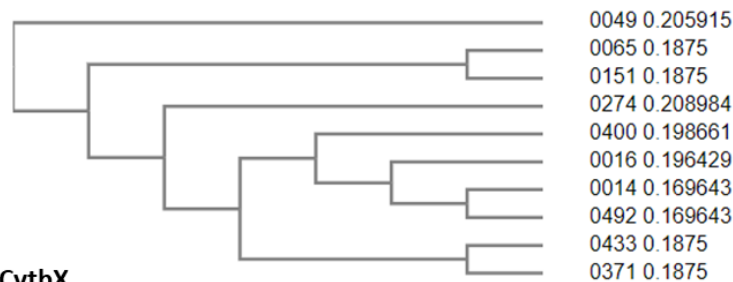
c

m2-CytbX

Rosetta score (REU)	Cα RMSD vs. CytbX (Å)	pStat	Rosetta decoy name
-477.497	1.023	0.587	10918652CytbX_Rosetta_Monoheme27_flexV1KIH_0152*
-477.045	1.065	0.546	10938018CytbX_Rosetta_Monoheme213_flexV1KIH_0256
-475.719	0.960	0.597	10936424CytbX_Rosetta_Monoheme228_flexV1KIH_0114
-475.660	0.960	0.614	10938017CytbX_Rosetta_Monoheme212_flexV1KIH_0493
-475.584	0.953	0.615	10938032CytbX_Rosetta_Monoheme227_flexV1KIH_0280
-475.374	0.959	**0.630**	10938027CytbX_Rosetta_Monoheme222_flexV1KIH_0320**
-475.136	1.020	0.594	10938018CytbX_Rosetta_Monoheme213_flexV1KIH_0235
-474.967	0.936	0.583	10936424CytbX_Rosetta_Monoheme228_flexV1KIH_0366
-474.814	0.950	0.613	10938016CytbX_Rosetta_Monoheme211_flexV1KIH_0155
-474.723	1.061	0.560	10938008CytbX_Rosetta_Monoheme23_flexV1KIH_0325

d


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0256 0.21596
0325 0.19029
0114 0.15625
0493 0.15625
0155 0.15625
0235 0.15625
0152 0.165179
0320 0.15625
0366 0.15625
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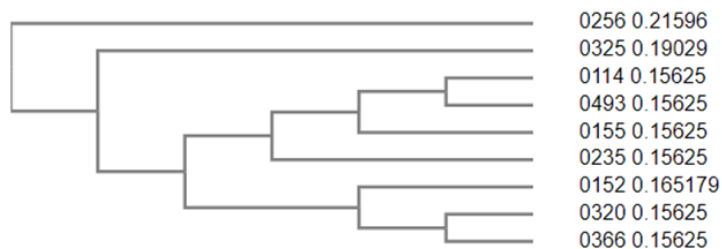
Figure S7. Score and sequence similarity analysis for the top 10 Rosetta-designed sequences for m1-CytbX and m2-CytbX. (a, c) Rosetta score, RMSD and Packstat scores for the top 10 Mono1 and Mono2 sequences, ranked by Rosetta score. Sequences were aligned in ClustalOmega, and are coloured by unique sequence identity, as identified by the guide tree. (b, d) Guide trees (as cladograms) generated by ClustalOmega showing sequence similarity of the top decoys. *Highest ranking decoy. **Highest Packstat within the top 10 decoys.
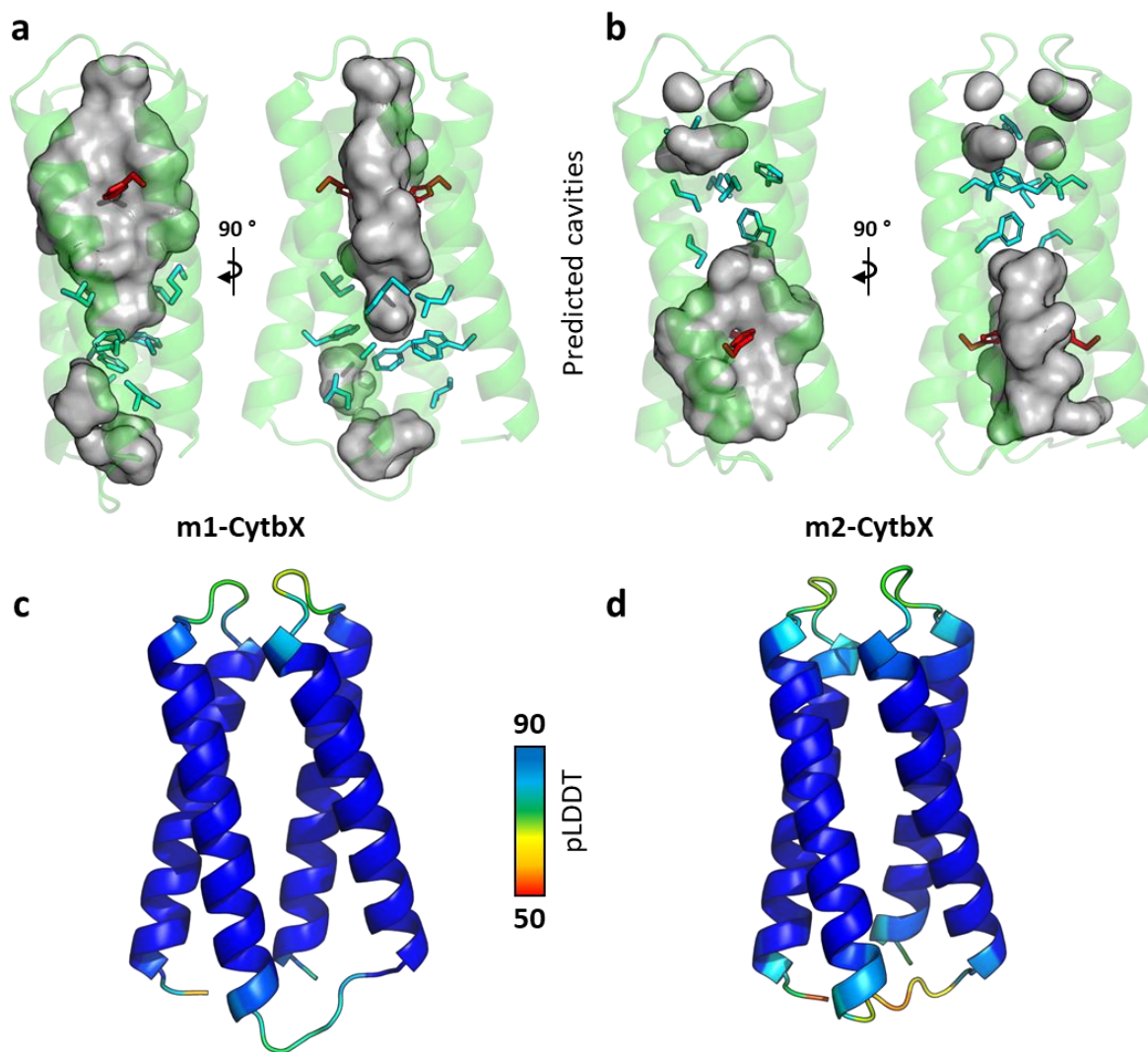
Figure S8. ESMfold structure predictions of m1-CytbX and m2-CytbX. (a-b) Depictions of predicted heme-shaped cavities within both proteins, and compact designed cores. Cavities were as calculated by PyMol. Histidine residues are shown as red sticks and designed core packing residues are shown as cyan sticks. (c-d) Predicted models coloured by confidence (pLDDT), as a rainbow from red to blue scaled from 50 to 90.

Figure S9. AlphaFold2 structure predictions of m1-CytbX and m2-CytbX. (a-b) Depictions of predicted heme-shaped cavities within both proteins, and compact designed cores. Cavities were as calculated by PyMol. Histidine residues are shown as red sticks and designed core packing residues are shown as cyan sticks. (c-d) Predicted models coloured by confidence (pLDDT), as a rainbow from red to blue scaled from 50 to 90. AlphaFold2 was run in Single Sequence mode.
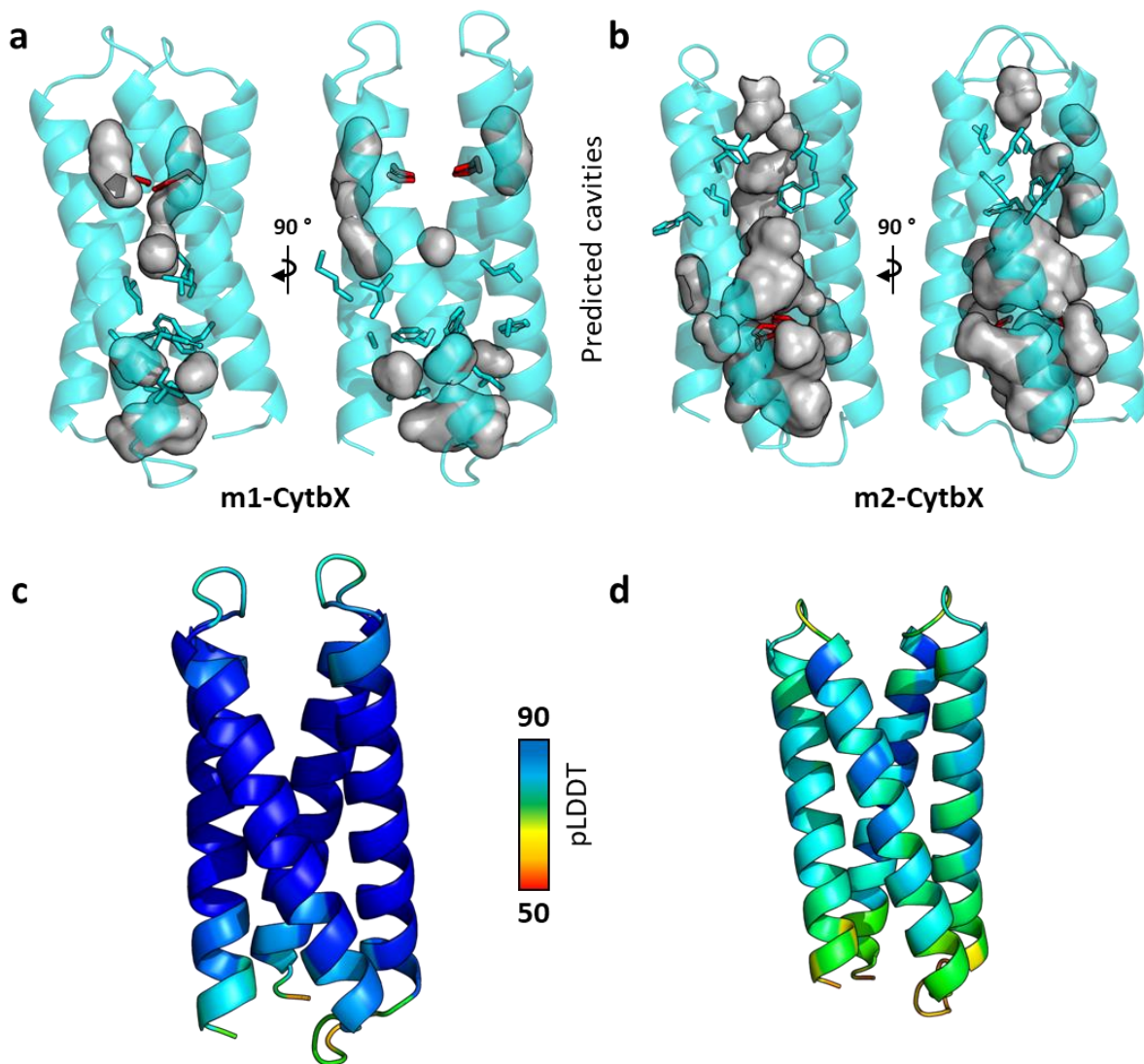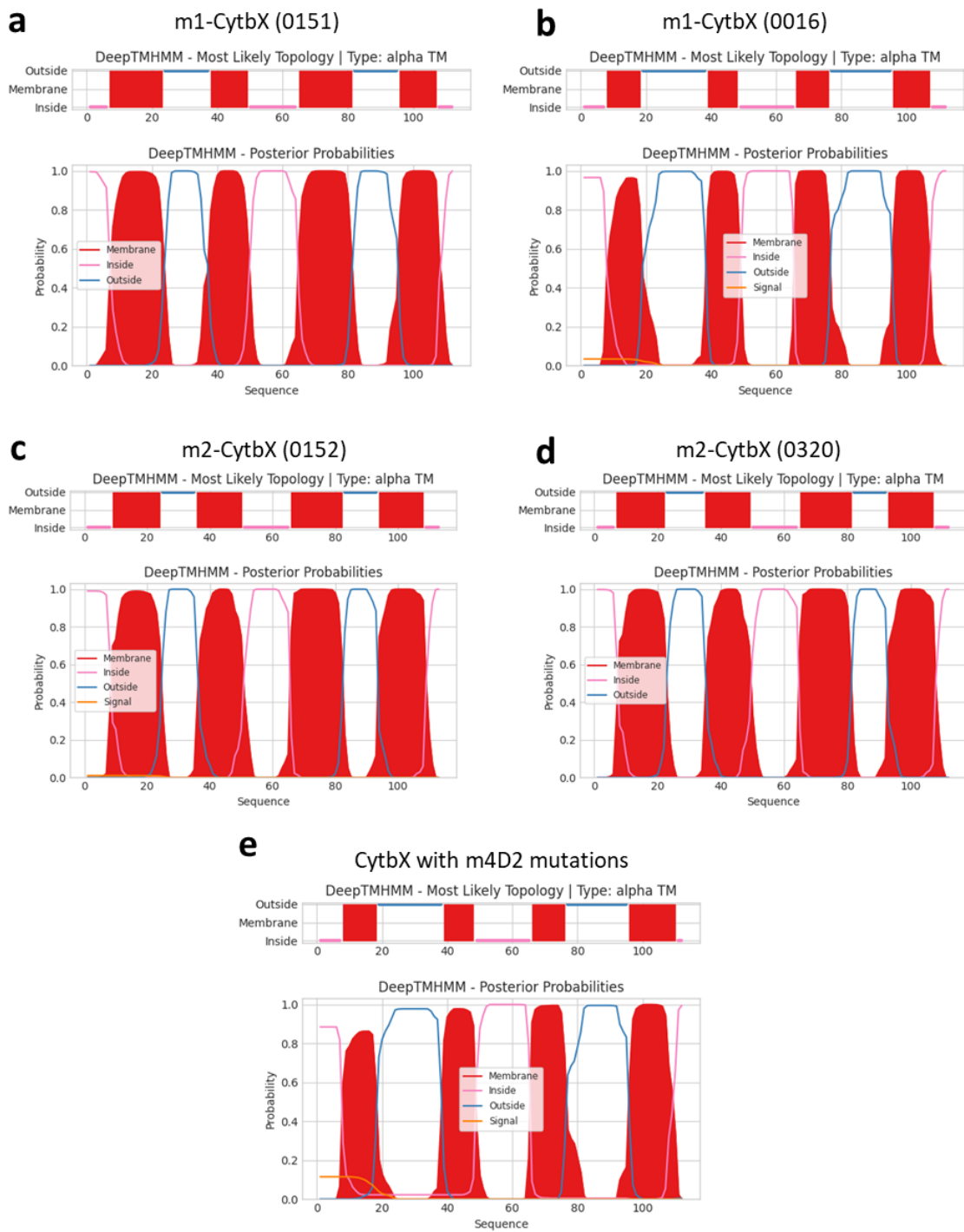
Figure S10. DeepTMHMM predicted transmembrane topologies of designed membrane protein sequences. (a-d) The four selected Rosetta-designed CytbX-Mono sequences and (e) the sequence of CytbX containing the m4D2 core mutations.
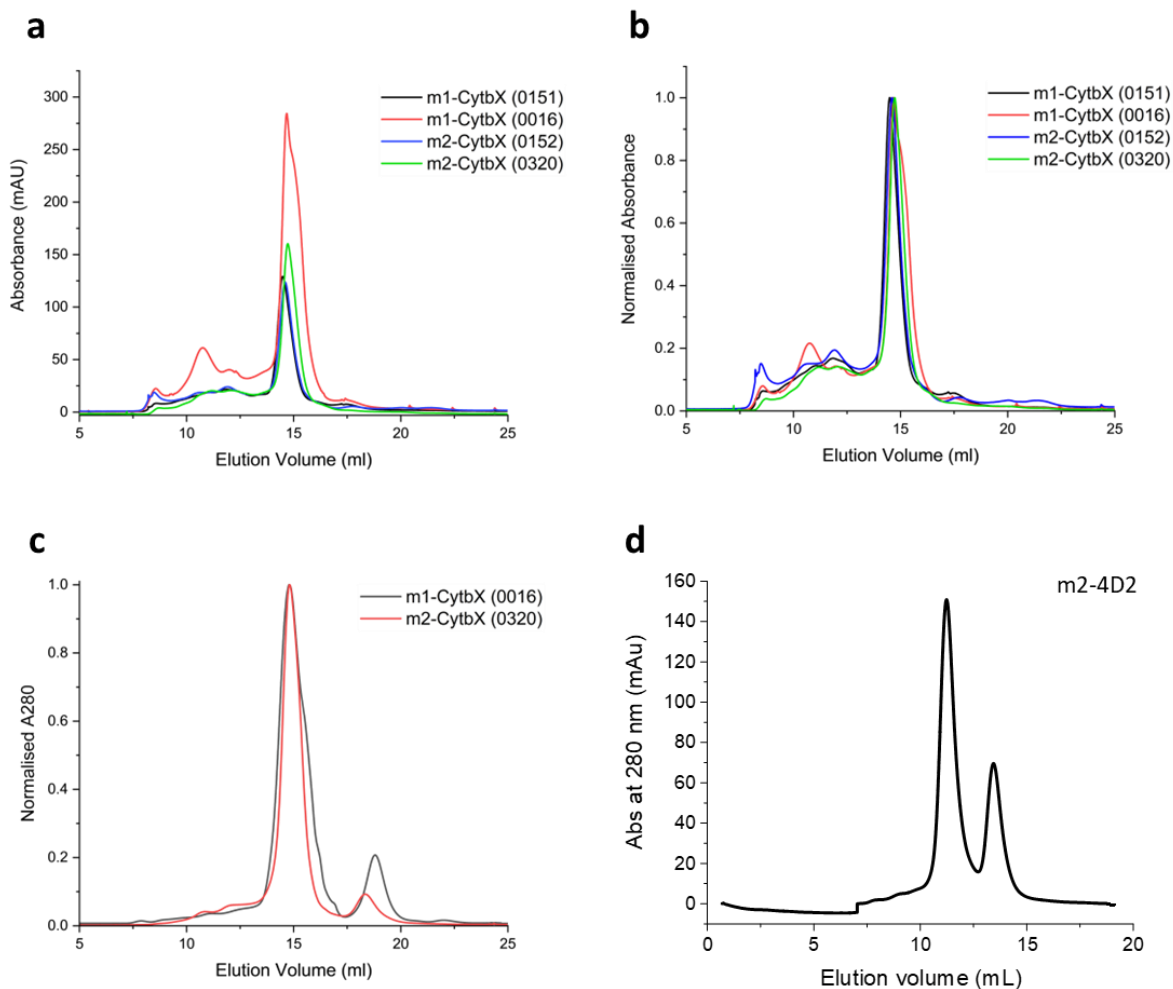
Figure S11. Size-exclusion chromatography elution traces of designed hemoproteins. (A) Raw and (b) normalised elution traces for the four monoheme CytbX sequences purified with Cymal-5, expressed with 10xHis tags. As can be seen, m1-CytbX 0016 and m2-CytbX 0320 are the best expressing of each pair and are therefore discussed in detail in the accompanying paper. (c) Normalised elution traces of m1-CytbX 0016 and m2-CytbX 0320 with triple-Strep(II) tags removed. (d) Raw elution trace for m2-4D2 after heme loading. Absorbance was measured at 280 nm.
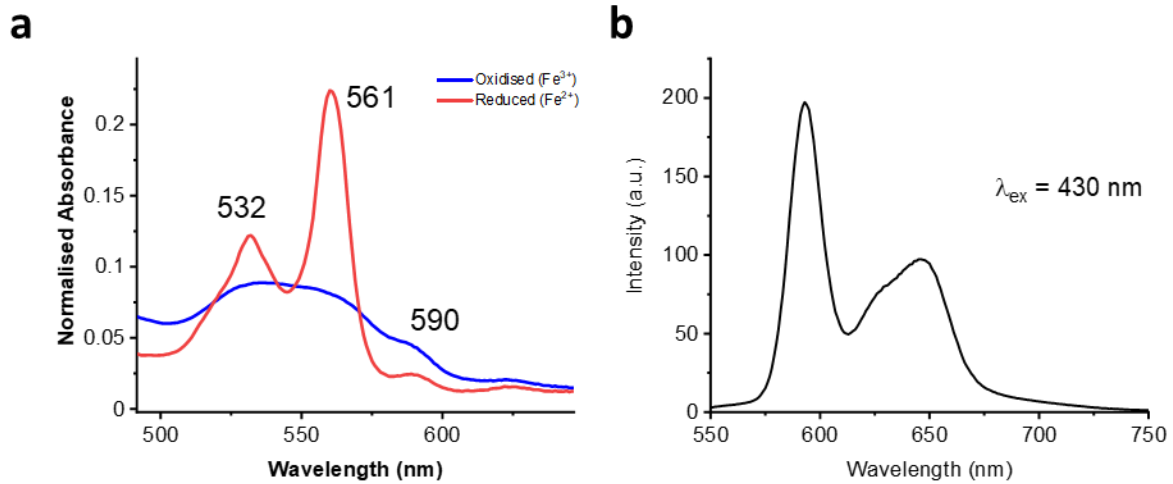
Figure S12. Spectral evidence for ZnPPIX binding to m2-CytbX. (a) UV-Vis absorbance spectrum of purified m2-CytbX with purification tags cleaved, zoomed into the region of the Q-bands. The peak at 590 nm is suggestive of ZnPPIX binding, whilst the 532 nm and 561 nm peaks are characteristic of heme *b*. (b) Fluorescence spectrum of purified m2-CytbX, excited at 430 nm. Peaks at 593 nm and 647 nm are characteristic of ZnPPIX. Slit width = 10 nm. Spectra are shown for the protein with the triple-StrepII tag removed.
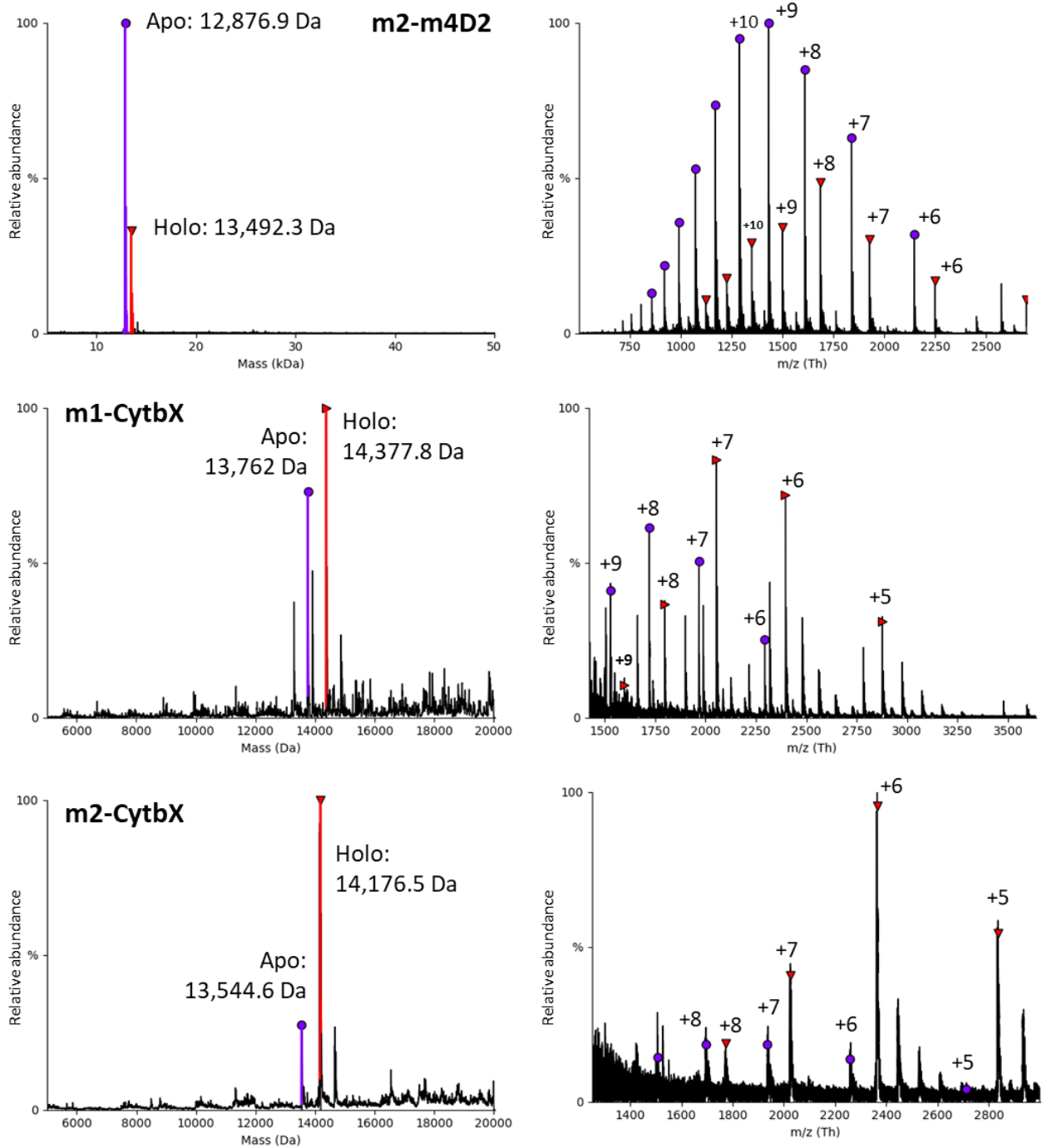
Figure S13. Native mass spectrometry of the three novel monoheme proteins. Deconvoluted (left) and raw (right) data are shown for purified proteins with purification tags removed. Purple peaks and circles represent apo-protein species and red peaks and triangles represent protein bound to a single heme (holo). Major charge state series are labelled on the m/z plots. Deconvolution of raw data was performed using UniDec, sampling masses at every 0.1 Da.

Protein	Expected Mass Apo (Da)	Observed Mass Apo (Da)	Expected Mass Holo (Da)	Observed Mass Holo (Da)	Apo vs Holo observed mass shift (Da)
m1-CytbX	13,735.02	13,762.0	14,351.52	14,377.8	615.8
m2-CytbX	13,513.83	13,544.6	14,130.33	14,176.5	631.9
m2-4D2	12,874.49	12,876.9	13,490.98	13,492.3	615.4

Table S3. Expected and observed masses from native mass spectrometry of the three designed monoheme proteins. Expected molecular weights are reported for all proteins with purification tags removed.
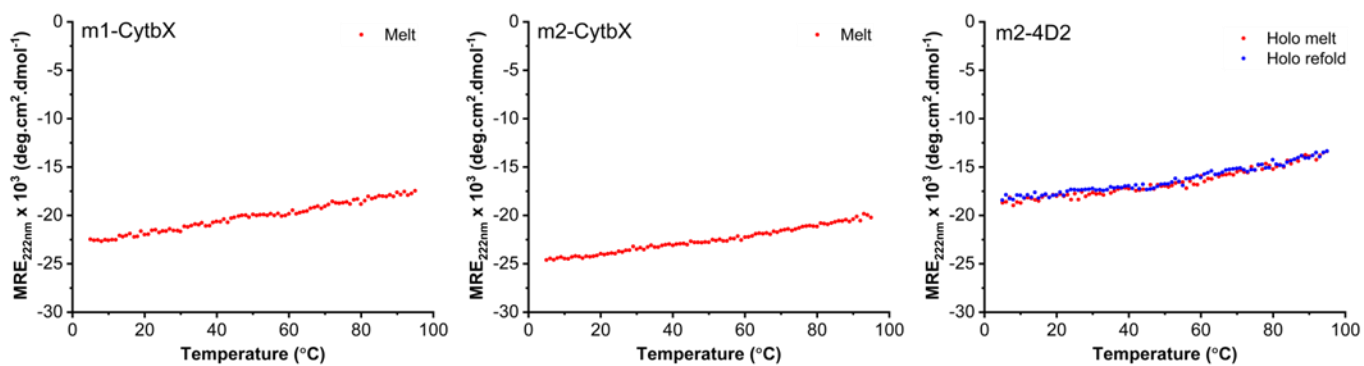
Figure S14. Thermal unfolding traces of m1-CytbX, m2-CytbX and m2-4D2. The mean residue ellipticity at 222 nm as a proxy for helicity is plotted vs temperature. All proteins show a roughly linear decrease in helicity with no major unfolding events. For m2-4D2 the trace when cooled from 95 °C to 5 °C is also overlayed in blue.
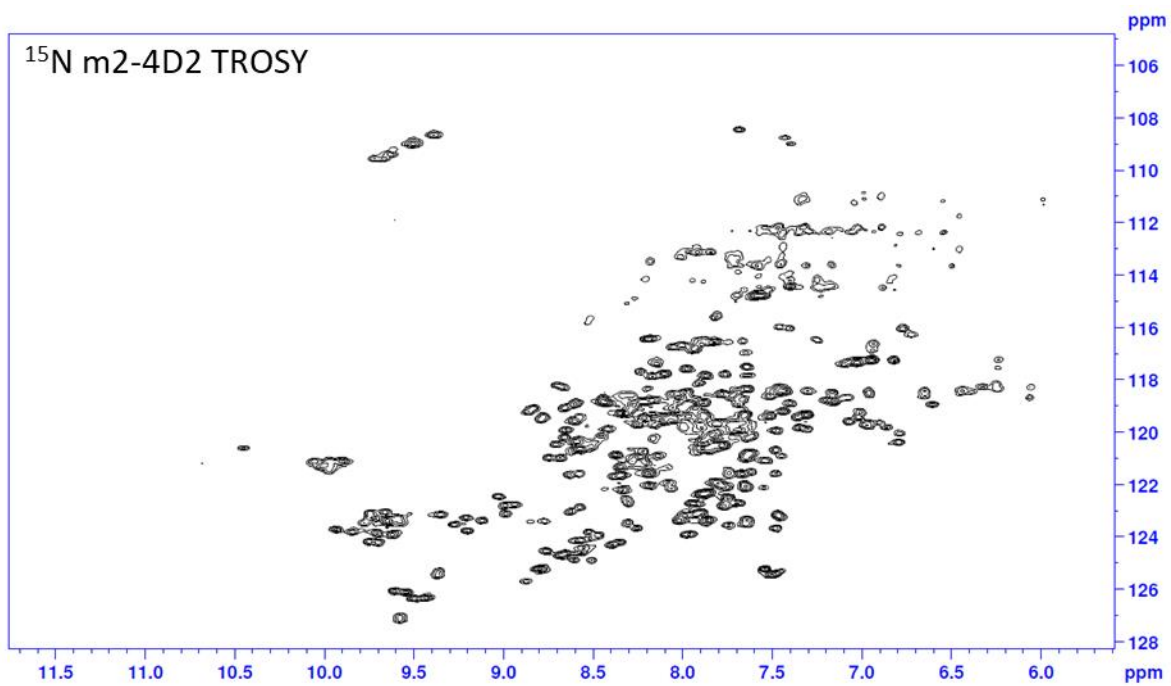
Figure S15. Nuclear magnetic resonance (NMR) spectroscopy of m2-4D2. TROSY spectrum of ^{15}N-labelled m2-4D2 (pH 6.4, 298 K, 10% D_2O).

Protein	Measured E_m (mV)	Observed ΔE_m (mV)	Predicted ΔE_m (mV) (PB-MC)	Predicted ΔE_m (mV) (BioDC)
4D2 (Heme 1)	-104†	0	n/a	0
4D2 (Heme 2)	-167†	-63	n/a	-51
m1-4D2	-117	-13 (0)*	0	16 (0)*
m2-4D2	-125 ± 2	-19 (-6)*	-5	18 (+2)*
CytbX (Heme 2)	-10	0	n/a	0
CytbX (Heme 1)	-121	-111	n/a	-126
m1-CytbX	-66 ± 2	-46 (0)*	0	-35 (0)*
m2-CytbX	-82 ± 1	-62 (-16)*	+2	-65 (-30)*

Table S4. Comparison of experimental and calculated reduction potentials of hemes. ΔE_m values are expressed vs. standard hydrogen electrode (SHE) relative to Heme 1 of 4D2 or Heme 2 of CytbX for soluble and membrane proteins respectively. Values in brackets (*) are expressed relative to m1-4D2 or m1-CytbX respectively. †Previously reported values.

Protein		ϵ_{int}	ϵ_{mem}	ϵ_{ext}	Charge Set	ΔE_{ox} (eV)
4D2						
	Site #1	6.776		78.2	RESP	-0.369
	Site #2	6.776		78.2	RESP	-0.420
	Site #1	6.776		78.2	CM5	0.006
	Site #2	6.776		78.2	CM5	-0.044
m1-4D2						
	Site #1	6.776		78.2	RESP	-0.353
	Site #1	6.776		78.2	CM5	0.018
m2-4D2						
	Site #1	6.776		78.2	RESP	-0.351
	Site #1	6.776		78.2	CM5	0.018
CytbX						
	Site #1	6.445		78.2	RESP	-0.307
	Site #2	6.445		78.2	RESP	-0.233
	Site #1	6.445	6.445	78.2	RESP	-0.325
	Site #2	6.445	6.445	78.2	RESP	-0.199
	Site #1	6.445	6.445	78.2	CM5	0.002
	Site #2	6.445	6.445	78.2	CM5	0.130
m1-CytbX						
	Site #1	6.445	6.445	78.2	RESP	-0.225
	Site #1	6.445	6.445	78.2	CM5	0.097
m2-CytbX						
	Site #1	6.445	6.445	78.2	RESP	-0.262
	Site #1	6.445	6.445	78.2	CM5	0.065

Table S5. Oxidation energies computed with using BioDC with RESP and CM5 charge-fitting schemes for the heme group in the oxidised and reduced states. ϵ_{int}, ϵ_{mem}, and ϵ_{ext} are respectively the dielectric constants assigned to the protein interior, implicit membrane slab if present, and exterior solvent.

a 4D2 H H

b m1-4D2 H P

c m2-4D2 P H

d CytbX H H N_{in} C_{in}

e m1-CytbX H P
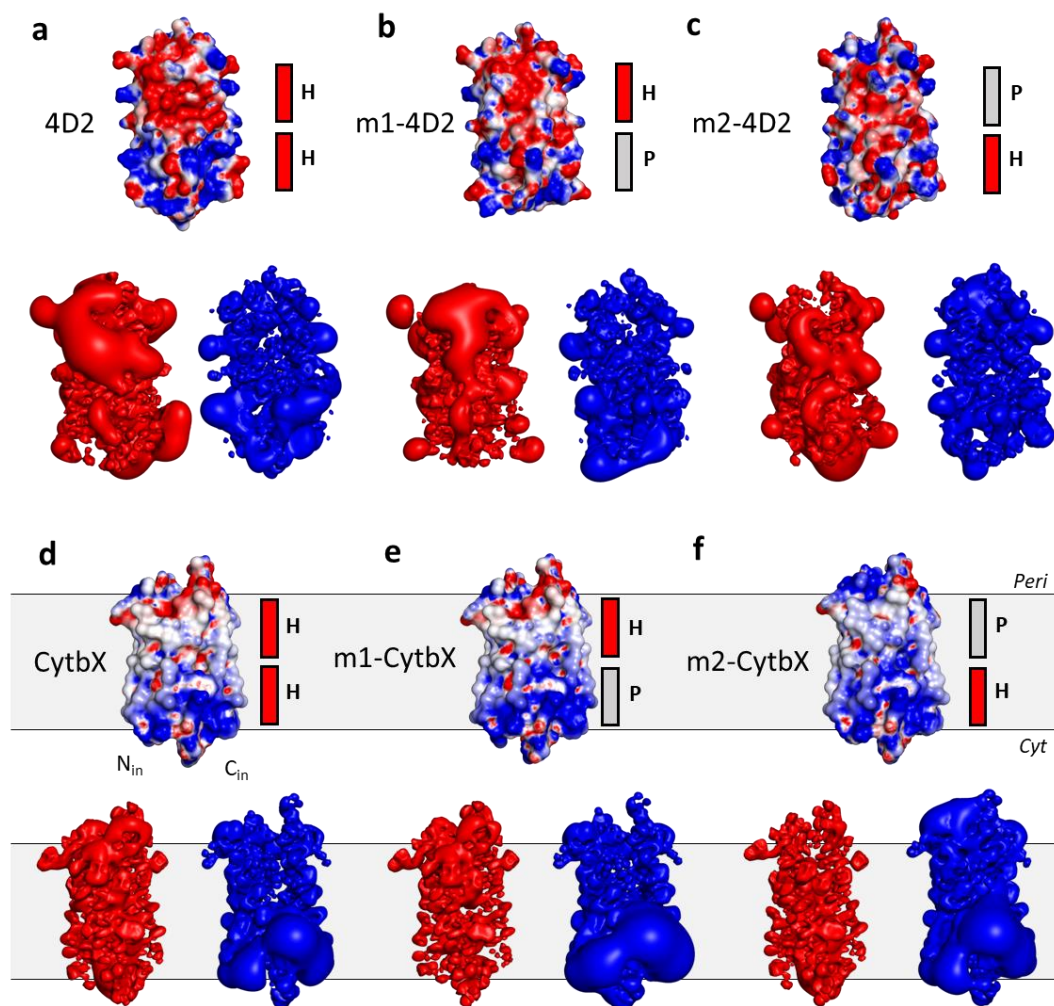
f m2-CytbX Peri P H Cyt

Figure S16. Electrostatic maps and isosurfaces of the two diheme and four monoheme proteins. For each protein, top: electrostatic surface map, bottom: negative (red) and positive (blue) charge isosurfaces. Red and grey boxes beside the electrostatic maps denote the positions of heme-binding (**H**) and packing (**P**) modules respectively. In all cases, proteins are positioned with N and C termini facing downwards. The position of the membrane is approximate.

Figure S17. Amino acid and DNA sequences.

All water-soluble constructs have an upstream **His tag**, **V5 epitope** and a **TEV cleavage site**:
Amino acid (AA) sequence: MHHHHHHGKPIPNPLLGLDSTENLYFQ
DNA sequence: ATGCATCATCACCATCACCATGGTAAGCCTATCCCTAACCCTCTCCTCG
GTCTCGATTCTACGGAAAACCTGTATTTTCAG

>Inv-m4D2
Vector: pET-21(+)
AA sequence (112 aa):
GSPELREKHRALAEQVYATWQELLKNTSNSPELREKLRALIEQVYATGQEMLKNGSVSPSPELREKHRALAEQVIATWQ
ELLKNTSNSPELREKFRALLEQVYATGQEMLKN
DNA sequence:

GGGAGTCCGGAACTACGTGAAAAACATCGGGCTTTGGCAGAGCAGGTTTATGCCACTTGGCAGGAACTTCTTAAG
AATACATCCAATAGCCCTGAGCTGCGTGAAAAATTACGTGCACTGATCGAACAAGTGTACGCCACGGGTCAAGAA
ATGTTGAAAAATGGCTCTGTAAGCCCCTCACCGGAACTGCGCGAAAAACACCGCGCCTTAGCGGAGCAGGTCATT
GCTACCTGGCAAGAACTGCTGAAAAACACCAGTAACTCGCCAGAACTCCGAGAGAAGTTTCGCGCGCTGCTGGAG
CAGGTGTATGCGACGGGCCAGGAAATGCTGAAAAAC

>m4D2
Vector: pET151
AA sequence (112 aa):
GSPELREKLRALIEQVYATGQEMLKNTSNSPELREKHRALAEQVYATWQELLKNGSVSPSPELREKFRALLEQVYATGQE
MLKNTSNSPELREKHRALAEQVIATWQELLKN
DNA sequence:
GGAAGTCCGGAACTTCGTGAAAAACTGCGTGCACTGATTGAACAGGTTTATGCAACCGGTCAGGAAATGCTGAAA
AATACGAGCAATAGCCCTGAGCTGCGCGAGAAACATCGCGCCCTGGCAGAGCAAGTCTACGCCACGTGGCAAGA
ACTGTTAAAGAACGGTAGCGTTTCTCCGTCACCAGAATTACGCGAAAAATTTCGGGCGCTTCTGGAACAAGTGTAT
GCCACAGGCCAAGAGATGCTTAAAAACACCTCGAACTCTCCTGAGCTGCGGGAAAAGCACCGTGCATTAGCCGAG
CAGGTTATTGCGACTTGGCAGGAATTACTGAAGAATTGA

>4D2
Vector: pET151
AA sequence (112 aa):
GSPELREKHRALAEQVYATGQEMLKNTSNSPELREKHRALAEQVYATGQEMLKNGSVSPSPELREKHRALAEQVYATG
QEMLKNTSNSPELREKHRALAEQVYATGQEMLKN
DNA sequence:
GGATCGCCAGAACTGCGCGAGAAACACCGTGCGTTAGCCGAACAAGTGTACGCCACAGGCCAAGAAATGCTGAA
GAACACGAGCAATTCGCCGGAACTTCGCGAGAAACATCGTGCTCTGGCAGAACAGGTGTATGCGACTGGCCAGG
AAATGCTGAAAAACGGGTCTGTAAGTCCGTCACCTGAACTGCGGGAGAAACACCGCGCTTTGGCCGAACAGGTTT
ACGCAACCGGTCAGGAGATGCTCAAGAACACCTCCAATAGCCCGGAACTGCGTGAGAAACATCGCGCATTAGCGG
AACAAGTCTATGCGACCGGTCAGGAAATGTTGAAAAAT

Where mentioned in the text, membrane protein designs were purified either with 10xHis or triple-StrepII tags at their C-termini, encoded by the DNA sequences below:

10xHis C-terminal tag, including a **triple alanine spacer**, **V5 epitope** and **10x His tag**
AA sequence:
AAAGKPIPNPLLGLDST**HHHHHHHHHH**
DNA sequence:
GCGGCGGCTGGTAAACCGATCCCAAACCCTCTGCTTGGATTGGATTCCACACACCACCATCATCATCATCATCACCATCAT

Strep3 C-terminal tag, including a **triple alanine spacer**, **thrombin cleavage site** and **triple Strep-II tag**
AA sequence:
AAALE**LVPRGS**GGGSGGGSGGGSWSHPQFEKGGGSGGGSGGGSWSHPQFEKGGGSGGGSGGGSWSHPQFEK
DNA sequence:
GCGGCCGCACTCGAGCTGGTTCCGCGTGGATCCGGTGGTGGGTCTGGTGGTGGGAGCGGTGGAGGCAGCTGGTCGCATCCGCAGTTTGAGAAGGGCGGCGGATCAGGCGGCGGATCCGGCGGTGGCTCGTGGTCCCATCCGCAATTCGAGAAGGGTGGCGGCAGTGGTGGCGGCTCTGGCGGTGGGTCGTGGAGCCACCCACAGTTCGAAAAG

>CytbX
Vector: pET-29
AA sequence (113 aa):
MGSPILRIIHLILALLVLITGLIMLLNTSNSPYLRLIHFLLALLVLITGWLMLKNGSKSPSPILRLIHIILAILVFITGIIMLLNTSNSPFLRILHFILALLVFITGFLMLNQ
DNA sequence:
ATGGGCTCTCCTATTCTGCGCATCATTCACCTGATTTTGGCCTTGCTGGTTCTGATTACCGGACTTATCATGCTGCTGAATACGTCAAATAGCCCCTATCTTCGCCTCATTCATTTTTTACTGGCACTGCTCGTGCTGATTACCGGTTGGCTGATGCTAAAAAACGGTAGTAAGAGTCCGAGCCCGATCCTCCGTTTAATCCACATAATTCTGGCAATACTGGTATTTATTACTGGCATCATTATGTTACTGAACACATCGAACAGCCCATTCCTGCGGATTTTGCATTTCATCCTTGCGTTATTGGTCTTTATCACGGGCTTCCTTATGCTGAACCAG

>m1-CytbX (Rosetta decoy ID: 0016)
Vector: pET-29
AA sequence (113 aa):
MGSPILRIIWLILLLLVLITGLIMLLNTSNSPYLRLIHFLLALLVLITFWLILKNGSKSPSPILRLIFIILIILVFITGIIMLLNTSNSPFLRILHFILALLVMITAFLLLNQ
DNA sequence:
ATGGGCTCACCGATTCTGCGCATTATCTGGCTCATACTGCTTCTTCTGGTACTAATCACTGGCCTGATCATGCTTCTGAATACCTCGAACAGCCCGTATCTGCGTCTGATTCACTTTCTGCTGGCATTATTAGTTCTGATTACCTTTTGGCTAATTCTGAAAAATGGCTCTAAGAGCCCGTCGCCGATCCTGCGTTTGATTTTCATCATTCTCATTATTTTGGTCTTTATTACGGGTATCATTATGCTGCTCAATACCAGTAACAGCCCCCTTCCTGCGCATTCTGCACTTTATCCTCGCCTTGTTAGTGATGATTACGGCCTTCTTATTACTGAACCAG

>m1-CytbX (Rosetta decoy ID: 0151)
Vector: pET-29
AA sequence (113 aa):
MGSPILRIILLILFLLVLITGLIMLLNTSNSPYLRLIHFLLALLVLITLWLWLKNGSKSPSPILRLILIILLILVFITGIIMLLNTSNSPF
LRILHFILALLVLITFFLVLNQ
DNA sequence:
ATGGGCTCTCCCATTCTGCGCATAATTCTACTCATCTTGTTTCTTCTGGTATTAATTACCGGCCTGATTATGCTGTTA
AATACCTCAAACTCCCCGTATCTCCGTCTAATTCACTTTTTACTTGCACTGCTGGTGCTCATTACGCTGTGGCTGTGG
TTAAAAAATGGTAGCAAGTCGCCTTCGCCGATTCTGCGTTTGATCTTAATCATTCTGCTGATTTTGGTGTTCATCACT
GGCATCATTATGCTTCTGAACACCAGCAATAGTCCATTTTTGCGCATTCTCCACTTTATCCTGGCTCTTCTGGTCCTG
ATCACATTCTTCCTGGTTCTTAACCAG

>m2-CytbX (Rosetta decoy ID: 0320)
Vector: pET-29
AA sequence (113 aa):
MGSPILRIIHLILALLVLITFLIMLLNTSNSPYLRLILFLLMLLVLITGWLMLKNGSKSPSPILRLIHIILAILVFITIIILLLNTSNSPF
LRILLFILFLLVFITGFLMLNQ
DNA sequence:
ATGGGCTCCCCGATCCTGCGTATAATTCACCTCATCTTAGCGCTGTTGGTCCTGATTACATTTCTTATTATGTTGCTT
AACACGAGCAATTCTCCATATCTGCGTCTGATTCTTTTTCTGTTAATGCTGCTCGTACTGATTACCGGCTGGCTCATG
CTTAAGAATGGAAGCAAAAGCCCGAGTCCGATTCTGCGCCTAATTCATATTATCCTGGCGATTTTGGTGTTCATCAC
GATTATAATCCTGCTCTTGAATACCTCGAACTCGCCGTTTCTGCGCATCCTACTGTTCATTCTGTTCTTACTGGTTTTT
ATCACCGGTTTTCTGATGTTAAACCAG

>m2-CytbX (Rosetta decoy ID: 0152)
Vector: pET-29
AA sequence (113 aa):
MGSPILRIIHLILALLVLITFLIILLNTSNSPYLRLILFLLMLLVLITGWLMLKNGSKSPSPILRLIHIILAILVFITIIILLLNTSNSPFL
RILLFILFLLVFITGFLMLNQ
DNA sequence:
ATGGGGTTCGCCTATCTTACGCATTATTCATCTTATCTTGGCACTGCTCGTGCTGATCACTTTTTTAATTATTCTGCTGA
ATACCTCCAATTCGCCGTATCTTCGTCTCATCTTATTCCTGCTGATGCTGTTGGTTTTGATCACCGGTTGGTTAATGC
TTAAAAACGGATCAAAAAGCCCGTCTCCGATTCTACGTCTGATACACATCATCCTAGCCATTCTGGTCTTTATTACC
ATTATTATTCTGCTGTTAAATACGAGCAACAGCCCGTTTTTACGCATTCTGCTGTTCATCCTCTTTCTTCTGGTATTTA
TTACAGGCTTCCTGATGCTCAACCAG

>CytbX with m4D2 core packing mutations
AA sequence:
MGSPILRIILLILILLVLITGLIMLLNTSNSPYLRLIHFLLALLVLITWWLLLKNGSKSPSPILRLIFIILLILVFITGIIMLLNTSNSPF
LRILHFILALLVIITWILLLNQ
No DNA sequence was ordered