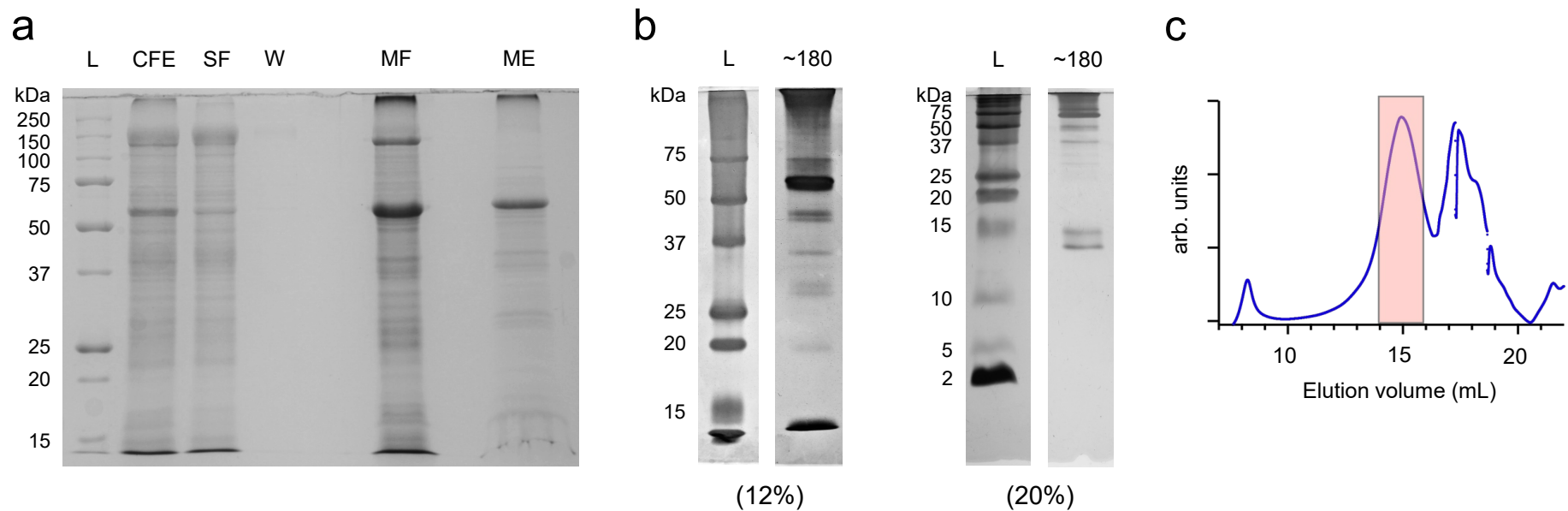




Supplementary Figure 1 – Extraction of the RDase complex from *D. hafniense* strain TCE1. SDS-PAGE of **a**, Representative protein samples obtained during the extraction the complex (out of 4 experiments with similar results), and of **b**, 2D-electrophoresis of the ~180 kDa band in 12% and 20% acrylamide gels. **c**, Elution profile from the size-exclusion chromatographic analysis of the membrane extract (the red box indicates the fractions collected for cryo-EM analysis). Legend: L: protein ladder; CFE: cell-free extract; SF: soluble fraction; W: wash sample; MF: membrane fraction; ME: membrane extract. Source data are provided as a Source Data file.

a

	Total spectrum count (% coverage)
PceA	2935 (84)
PceB	22 (41)

b

PceA

```

MGEINRRNFL KVSILGAAAA AVASASAVKG MVSPLVADAA
DIVAPITETS EFPYKVD AKY QRYNSLKNFF EKTFDPEANK
TPIKFHYDDV SKITGKKDTG KDLPTLNAER LGIKGRPATH
TETSILFHTQ HLGAMLTQRH NETGWTGLDE ALNAGAWAVE
FDYSGFNATG GPGGSVIPLY PINPMTNEIA NEPVMPGLY
NWDNIDVESV RQGGQGWKFE SKEEASKIVK KATRLLGADL
VGIAPYDERW TYSTWGRKIY KPCKMPNGRT KYLPWDLPKM
LSGGGVEVFG HAKFEPDWEK YAGFKPKSVI VVLEEDYEA
IRTSPSVISS ATVGKSYSNM AEVAYKIAVF LRKLGYAAP
CGNDTGISVP MAVQAGLGEA GRNGLLITQK FGPRHRIAKV
YTDLELAPDK PRKFGVREFC RLCKKCADAC PAQAISHEKD
PKVLQPEDCE VAENPYTEKW HLD SNRCGSF WAYNGSPCSN
CVAVCSWNKV ETWNHDVARI ATQIPLLQDA ARKFDEWFGY
NGPVNPDERL ESGYVQNMVK DFWNNPESIK Q

```

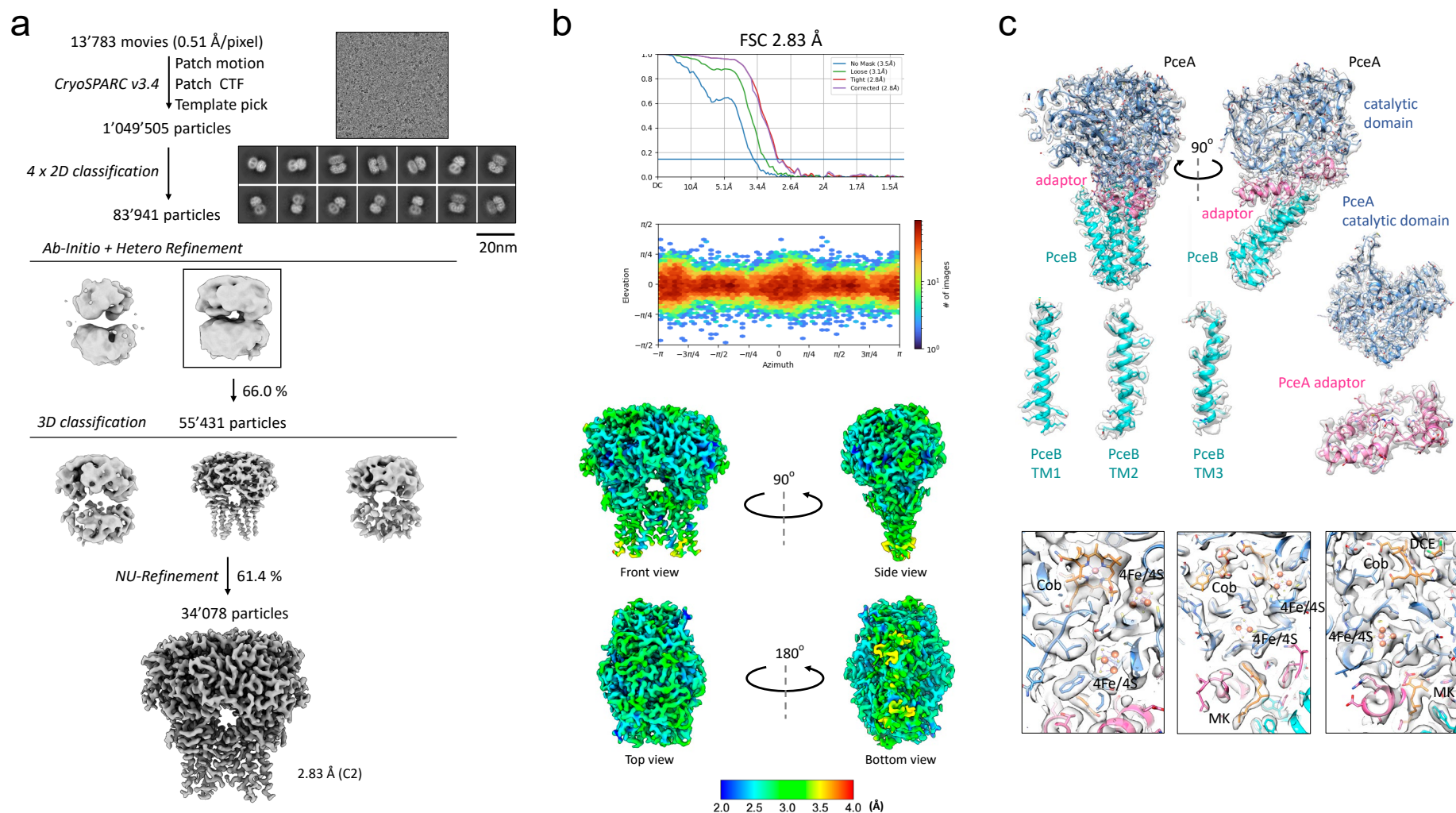
PceB

```

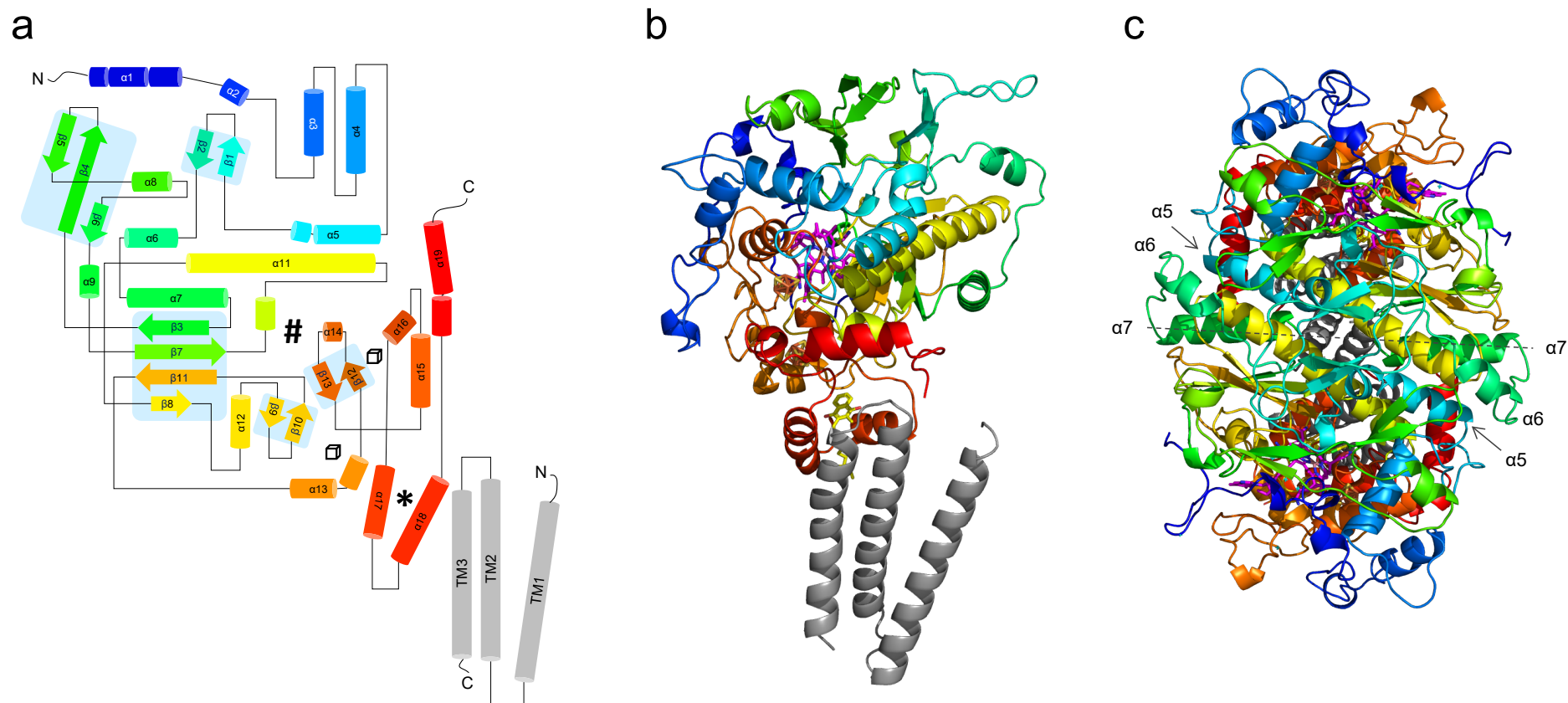
MNIYDVLIWM ALGMTALLIQ YGIWRYLK GKDTIPLQIC
GFLANFFFI F ALAWGYSSFS EREYQAIGMG FIFFGGTALI
PAIITYRLAN HPAKKIRESS DSISA

```

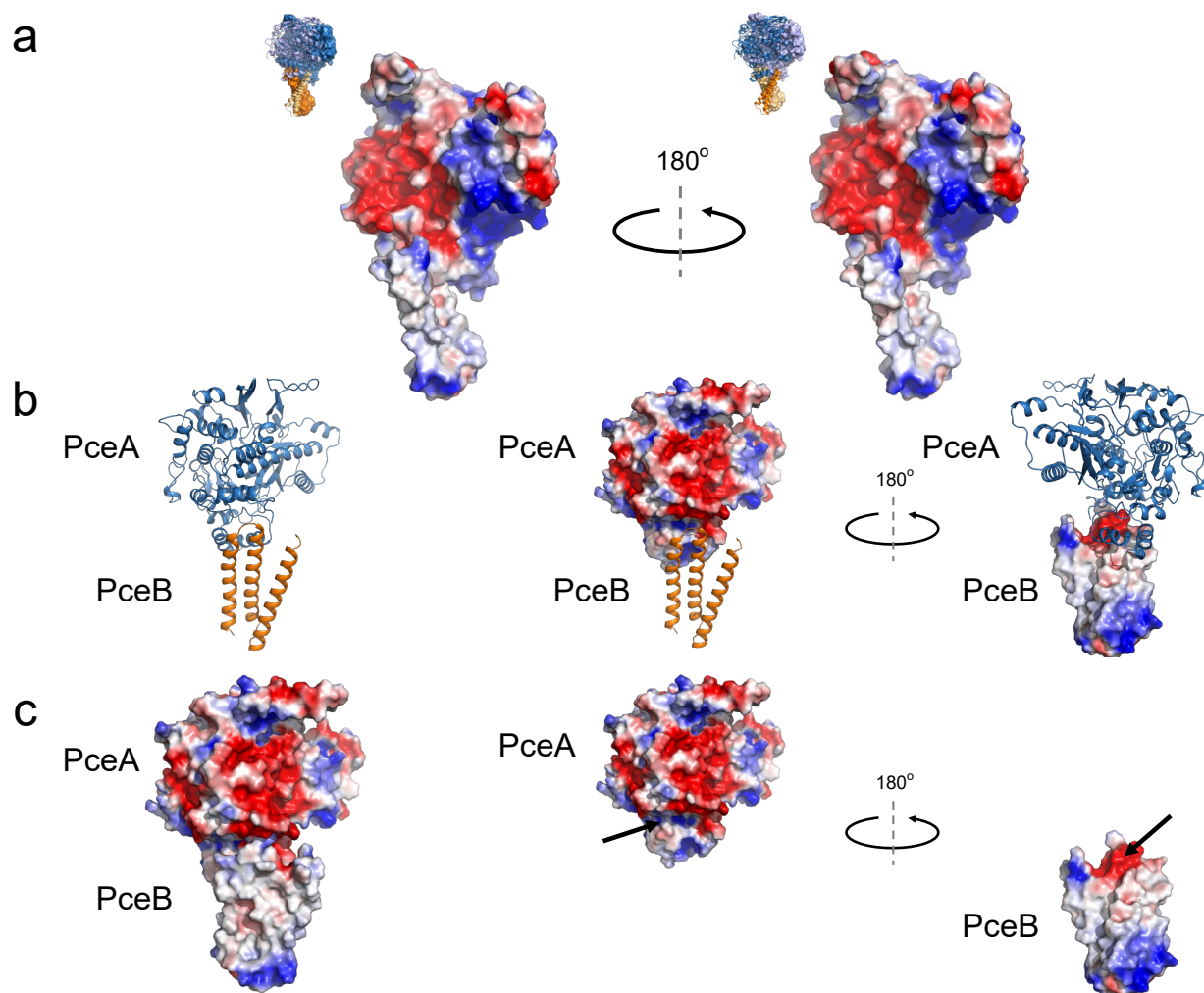
Supplementary Figure 2 - LC-MS/MS analysis of the ~180 kDa CN-PAGE band. **a**, Total spectrum count of PceA and PceB peptides. **b**, Representative coverage of PceA and PceB proteins highlighted in colour. Green residues indicate amino acids that were found post-translationally modified for a certain number of the detected peptides (oxidation for Met; phosphorylation for Ser, Thr and Tyr).



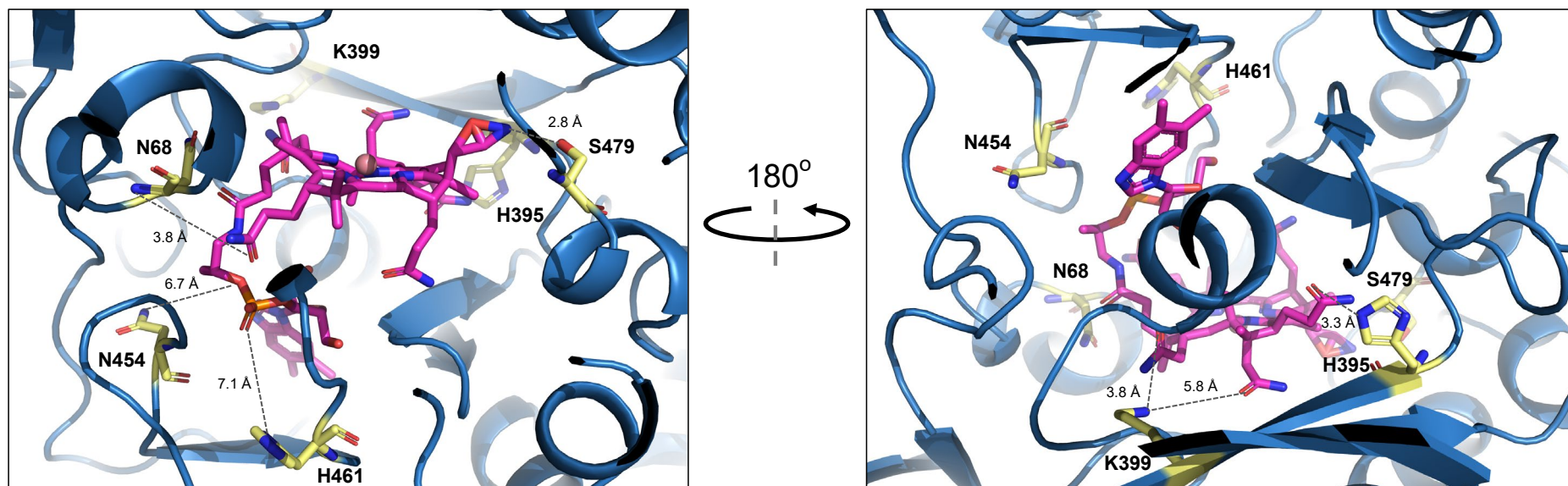
Supplementary Figure 3 - Cryo-EM analysis of the PceA₂B₂ complex. **a**, Flow chart of the cryo-EM data analysis. **b**, Fourier-Shell Correlation (FSC) curve indicating an overall resolution of 2.83 Å (FSC 0.143), direction distribution plot and global refined map coloured by local resolution are given in different views. **c**, Density maps and structure interpretation of PceA and PceB proteins, and of the cofactors.



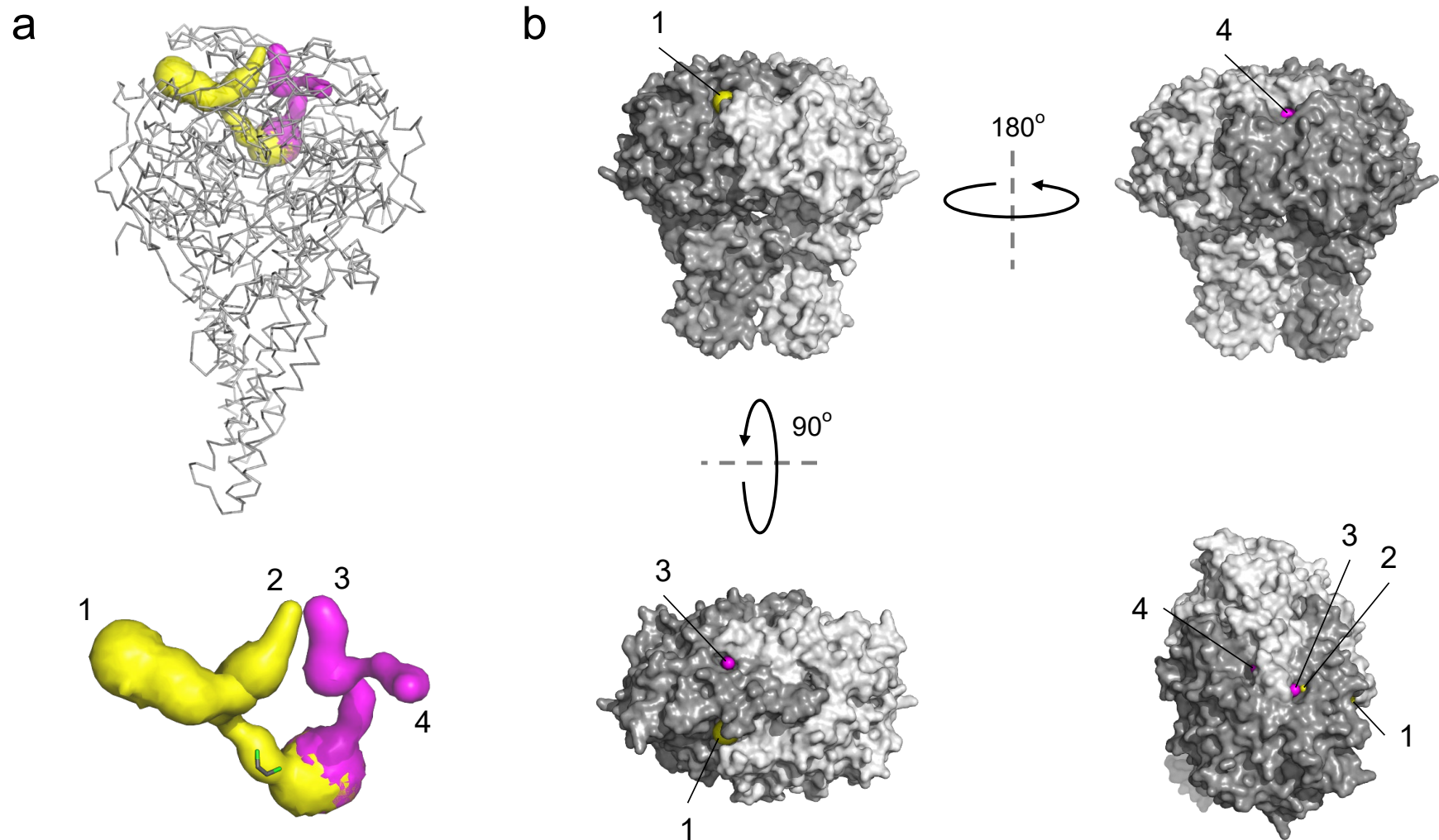
Supplementary Figure 4 - Topology analysis of DhPceA₂B₂ secondary structures. **a**, Topology diagram of the DhPceAB heterodimer depicted in rainbow colour mode. The hash sign, the cubes and the asterisk indicate the position of the cofactors in the PceAB dimer: the cobalamin, both [4Fe-4S] clusters and the menaquinone, respectively. **b**, Front view of the corresponding cartoon structure. **c**, Top view of the DhPceA₂ cartoon structure.



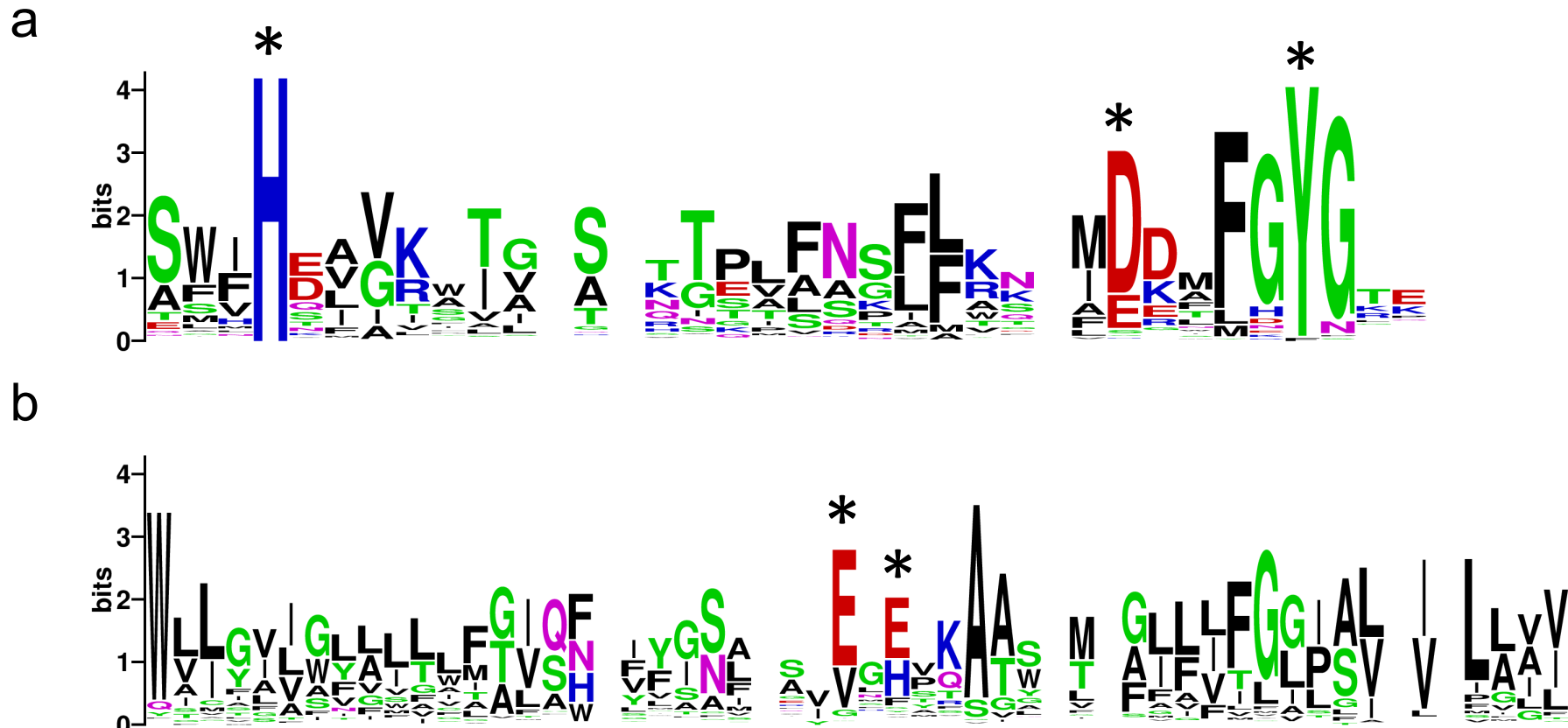
Supplementary Figure 6 - Surface interactions within the PceA₂B₂ complex. **a**, Electrostatic interactions between both PceA monomers highlighting the opposite overall charges in the contact area, and hydrophobic interactions between both PceB monomers. **b** and **c**, Electrostatic interactions between PceA and PceB subunits in each PceAB heterodimer.



Supplementary Figure 7 - Structural insights in the cobalamin binding site of DhPceA showing amino acids at H-bond distance to the cobalamin cofactor.



Supplementary Figure 8 - Channel prediction in DhPceA. **a**, Overview of both channels connecting the active site to the outside solution (see also Figure 4 in the article). **b**, Position of the channel exits at the surface of the DhPceA₂B₂ complex. For clarity, the channel exits are only indicated for one PceA subunit (depicted in dark grey).



Supplementary Figure 9 - Key conserved residues in the menaquinone-binding pocket of RdhAB heterodimers. Weblogo representation of selected regions of **a**, RdhA and **b**, RdhB sequence alignments. Stars indicate in the C-terminal region of RdhA sequences a fully conserved histidine residue (H495 in DhPceA) and an almost fully conserved tyrosine (Y520), as well as a highly conserved negatively charged residue (D515). The loop connecting transmembrane helices 2 and 3 in RdhB sequences harbours highly conserved glutamate residues (E61 and E63 in DhPceB). Legend: Dh, *Desulfitobacterium hafniense*.

Supplementary Table 1 - Cryo-EM data collection, refinement and validation statistics.

	MOOR complex (PceA ₂ B ₂) (EMDB-18148) (PDB 8Q4H) (EMPIAR-11719)
Data collection and processing	
Magnification	155kx (normal)
Voltage (kV)	300
Electron exposure (e ⁻ /Å ²)	60
Defocus range (μm)	1.0 -2.2
Pixel size (Å)	0.51
Symmetry imposed	C2
Initial particle images (no.)	1 049 505
Final particle images (no.)	34 078
Map resolution (Å)	2.83
FSC threshold	0.143
Map resolution range (Å)	30-2.0
Refinement	
Initial model used (PDB code)	n/a
Model resolution (Å)	3.0
FSC threshold	0.143
Model resolution range (Å)	30-3.0
Map sharpening <i>B</i> factor (Å ²)	-63
Model composition	
Non-hydrogen atoms	9510
Protein residues	1184
Ligands	DCE: 2 SF4: 4 MQ7: 2 COB: 2
<i>B</i> factors (Å ²)	
Protein	14.07/88.61/35.26
Ligand	22.0/30.78/27.39
R.m.s. deviations	
Bond lengths (Å)	0.004 (4)
Bond angles (°)	0.574 (6)
Validation	
MolProbity score	1.32
Clashscore	5.82
Poor rotamers (%)	0.00
Ramachandran plot	
Favored (%)	98.38
Allowed (%)	1.45
Disallowed (%)	0.17