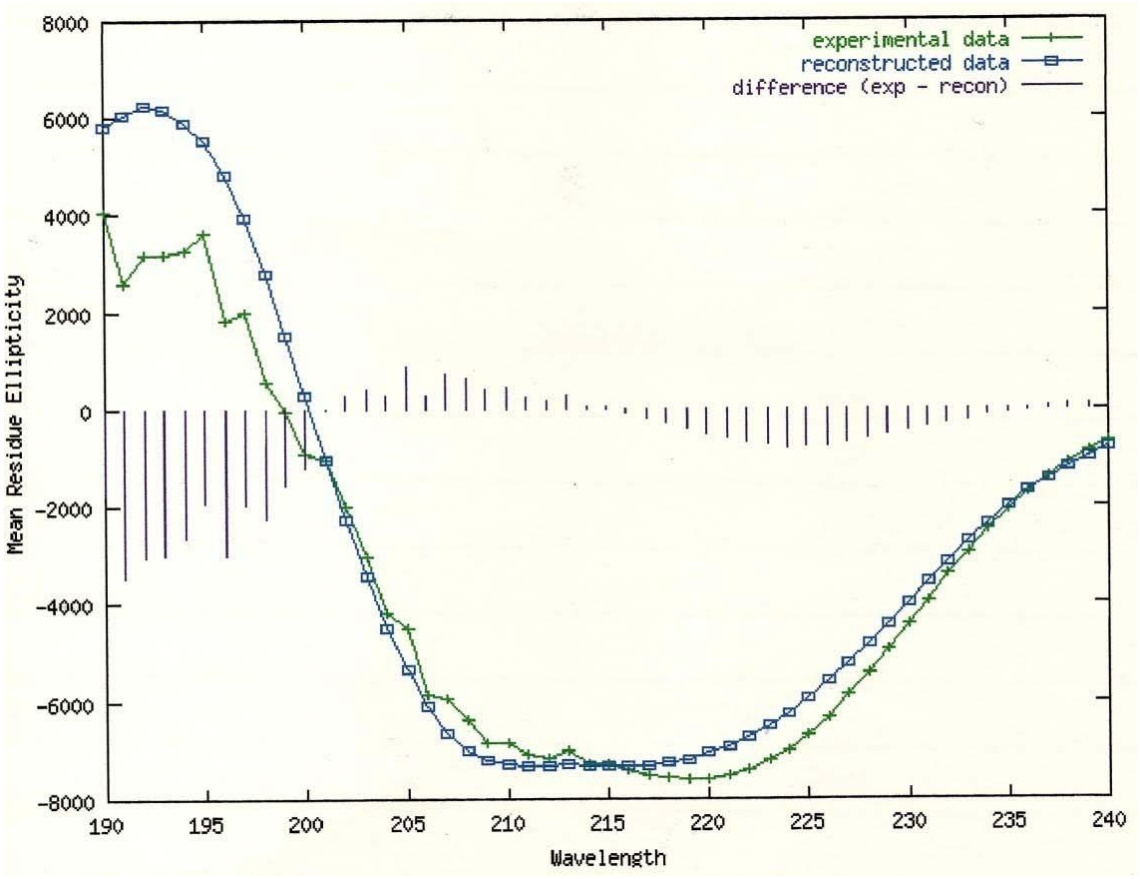
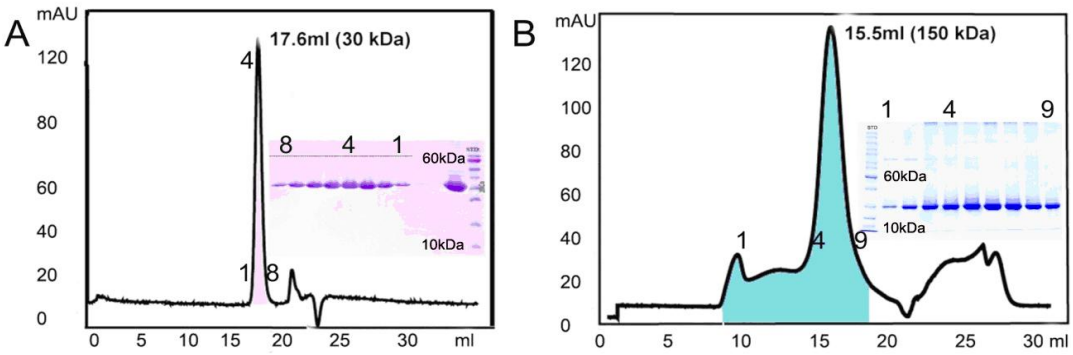
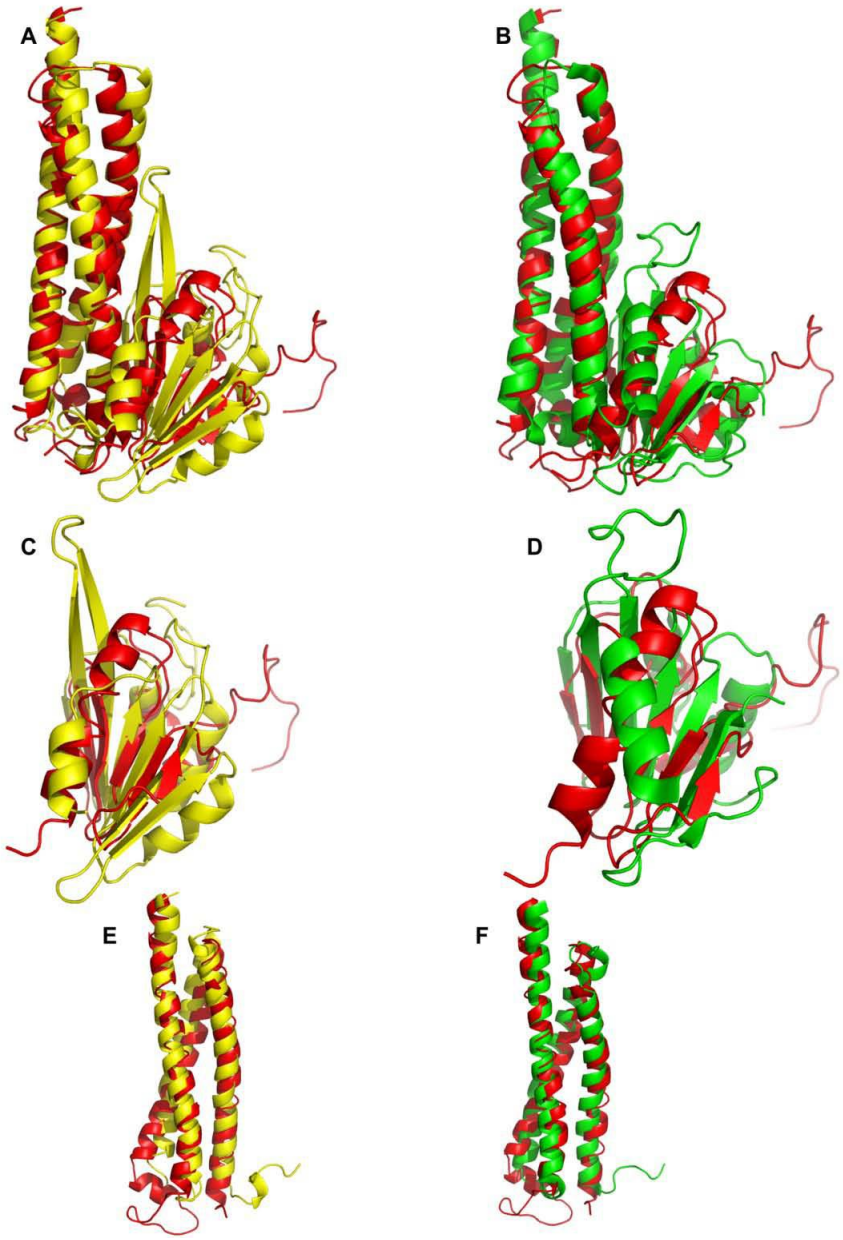
Supplementary Material



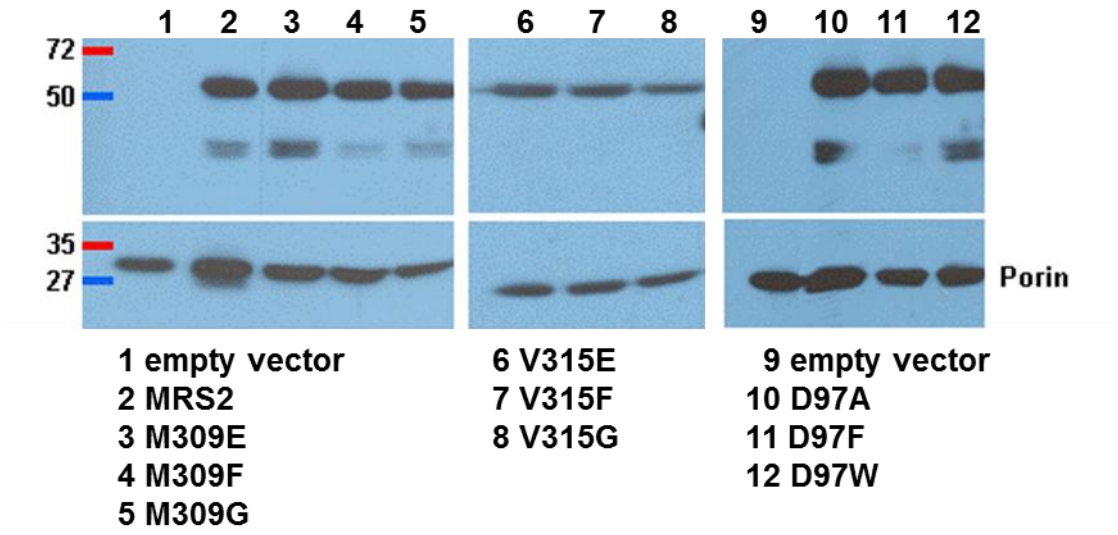
Supplementary Figure S1. **Circular dichroism spectra of $Mrs2_{48-308}$.** The mean residual ellipticity of $Mrs2_{48-308}$ at 1 mg/ml was monitored from 240 to 190 nm. Three scans were performed on individual sample and subsequently averaged. The data were reconstructed and the difference between the reconstructed and the experimental data was determined. The circular dichroism (CD) spectrum of $Mrs2_{48-308}$ showed the minima at 208 and 219 nm, a characteristic for a protein rich in α-helices.



Supplementary Figure S2. **Analytical size exclusion chromatography (ASEC) of $Mrs2_{48-308}$.** (A) SEC studies showed that $Mrs2_{48-308}$ behaves as a monomer in high ionic strength buffer (50 mM Tris-HCl, 300 mM NaCl) and (B) as a homo-pentamer in low ionic strength buffer (10 mM Tris-Hcl, 10 mM NaCl). The corresponding peaks are visualized by SDS-PAGE, 1,4,8 and 9 represent the corresponding fractions. The column was pre-calibrated with standard molecular weight markers.



Supplementary Figure S3. Superposition of Mrs2$_{48-308}$ on the soluble domains of Tm-CorA and ZntB. (A) Superposition of the complete soluble domain of Mrs2$_{48-308}$ (red) on the soluble domain of Tm-CorA (PDB code 2IUB) with an RMSD of 2.85 Å and mscore of 70 %. (B) Superposition of the complete soluble domain of Mrs2$_{48-308}$ (red) on the soluble domain of ZntB (PDB code 3CK6) (green) with an RMSD of 2.61 Å and mscore of 67 %. (C) Superposition of α/β/α subdomains of Mrs2$_{48-308}$ (red) and Tm-CorA (yellow) with an RMSD of 2.96 Å and mscore of 59 %. (D) Superposition of α/β/α subdomains of Mrs2$_{48-308}$ (red) and ZntB (green) with an RMSD of 2.75 Å and mscore of 49 %. (E) Superposition of helical subdomains of Mrs2$_{48-308}$ and Tm-CorA with an RMSD of 2.62 Å and mscore of 85 %. (F) Superposition of helical subdomains of Mrs2$_{48-308}$ and ZntB with an RMSD of 2.71 Å and mscore of 91 %. Superpositions were made with the program SHEBA (Jung & Lee, 2000).



Supplementary Figure S4. Western Blot analysis of the expression levels of *MRS2* and the different *MRS2* mutant variants. Isolated mitochondria of *mrs2*Δ cells transformed with an empty plasmid or high copy number vector Yep351 expressing *MRS2*-HA or the mutant variants were separated by SDS/PAGE and proteins were visualized by immunoblotting with an antiserum against the HA tag. Porin was used as a loading control. The black arrow indicates a non-specific band consistently recognized by the HA-antibody

Detailed structural comparisons of $Mrs2_{48-308}$, Tm-CorA, and Vp-ZntB, supporting Figure 1 and Figure S3

Despite the low sequence homology $Mrs2_{48-308}$, Tm-CorA, and Vp-ZntB are structurally similar with regard to overall architecture and also perform similar functions. Nevertheless, the protomer and pentamer structures cannot be superimposed easily, because the relative orientations of the α/β/α and helical subdomains, as well as the folds, are different. $Mrs2_{48-308}$ and the soluble domain of Tm-CorA (PDB code 2IUB) can be superimposed with an RMSD value of 2.85 Å (for Cα, 179 of 256 residues aligned, with a sequence identity of 16%) (Figure S3A). Separately, the subdomains of $Mrs2_{48-308}$, Vp-ZntB and Tm-CorA (α/β/α and helical) align with different RMSD values.

The α/β/α subdomains of $Mrs2_{48-308}$ and Tm-CorA can be superimposed over the Cα atoms with an RMSD value of 2.96 Å (for Cα, 69 of 117 residues aligned, with a sequence identity of 8%) (Figure S3C). Similarly, the corresponding helical subdomains can be aligned with an RMSD value of 2.62 Å (for Cα, 112 of 131 residues aligned, with a sequence identity of 13%) (Figure S3E).

$Mrs2_{48-308}$ and Vp-ZntB (PDB code 3CK6) can be superimposed with an RMSD value of 2.61 Å (for Cα, 158 of 237 residues aligned, with a sequence identity of 14%) (Figure S3B). Conversely, the α/β/α subdomains of $Mrs2_{48-308}$ and Vp-ZntB can be superimposed with an RMSD value of 2.75 Å (for Cα, 57 of 116 residues aligned, with a sequence identity of 5%) (Figure S3D), and the helical subdomains can be aligned with an RMSD value of 2.22 Å (for Cα, of 110 of 121 residues aligned, with a sequence identity of 8%) (Figure S3F). These changes in orientation between the 2 sub-domains may be due to different levels of funnel opening in the 3 different ion transporters.

The soluble domains of Tm-CorA (PDB code 2IUB) and Vp-ZntB (PDB code 3CK6) can be superimposed with an RMSD value of 1.89 Å (for Cα, 199 of 237 residues aligned, with a sequence identity of 19%). Separately, the soluble domains α/β/α aligned with an RMSD of 1.93 Å (for Cα, 92 of 116 residues aligned, with a sequence identity of 9%), whereas the helical domain was superimposed with an RMSD value of 1.71 Å (for Cα, 112 of 121 residues aligned, with a sequence identity of 19%).

Mutagenic forward and reverse primers, supporting methods paragraph plasmid constructs

The primers for mutagenesis of Met309, Val315, and the putative sensing site Asp97 (changed nucleotides are in bold):

MRS2 M309E fw: 5'-CGCAAATAG**G**AATTCCTTA**GAG**TTGTTGGAGTTGAAAGTTACC-3' **MRS2 M309E rev: 5'-**GGTAACTTTCAACTCCAACAA**CTC**TAAGGAATT**C**CTATTTGCG-3' **MRS2 M309F fw: 5'-**CGCAAATAG**G**AATTCCTTA**TTC**TTGTTGGAGTTGAAAGTTACC-3' **MRS2 M309F rev: 5'-**GGTAACTTTCAACTCCAACAA**GAA**TAAGGAATT**C**CTATTTGCG-3' **MRS2 M309G fw: 5'-**CGCAAATAG**G**AATTCCTTA**GGA**TTGTTGGAGTTGAAAGTTACC-3' **MRS2 M309Grev: 5'-**GGTAACTTTCAACTCCAACAA**TCC**TAAGGAATT**C**CTATTTGCG-3'
MRS2 V315E fw: **5'-**CGCAAATAG**G**AATTCCTTAATGTTGTTGGA**GAG**GAAAGTTACCATCTACACGTTGGG-3' **MRS2 V315E rev:** **5'-**CCCAACGTGTAGATGGTAACTTTC**CTC**TCCAACAACATTAAGGAATT**C**CTATTTGCG-3' **MRS2 V315F fw:** **5'-**CGCAAATAG**G**AATTCCTTAATGTTGTTGGA**TTC**GAAAGTTACCATCTACACGTTGGG-3' **MRS2 V315F rev:** **5'-**CCCAACGTGTAGATGGTAACTTTC**GAA**TCCAACAACATTAAGGAATT**C**CTATTTGCG-3' **MRS2 V315G fw:** **5'-**CGCAAATAG**G**AATTCCTTAATGTTGTTGGA**GGA**GAAAGTTACCATCTACACGTTGGG-3' **MRS2 V315G rev:** **5'-**CCCAACGTGTAGATGGTAACTTTC**TCC**TCCAACAACATTAAGGAATT**C**CTATTTGCG-3'
MRS2 D97A fw: **5'-**CATTCCCTTTTCCCGAGA**GCG**CTGAGGAAAATAGATAACTCC-3' **MRS2 D79A rev**: **5'-** GGAGTTATCTATTTTCCTCAG**CGC**TCTCGGGAAAAGGGAATG-3' **MRS2 D97F fw**: **5'-**CATTCCCTTTTCCCGAGA**TTT**CTGAGGAAAATAGATAACTCC-3' **MRS2 D97F rev**: **5'-**GGAGTTATCTATTTTCCTCAG**AAA**TCTCGGGAAAAGGGAATG-3' **MRS2 D97 Wfw**: **5'-**CATTCCCTTTTCCCGAGA**TGG**CTGAGGAAAATAGATAACTCC-3' **MRS2 D97 Wrev**: **5'-**GGAGTTATCTATTTTCCTCAG**CCA**TCTCGGGAAAAGGGAATG-3'

The above mentioned mutagenic primers were used in combination with the forward primer MRS2Mcsfw: 5'-CGATTAAGTTGGGTAACGCCAGGG-3' and the reverse primer MRS2Mcsrev: 5'-GCACGACAGGTTTCCCGACTGGAAAGC-3.