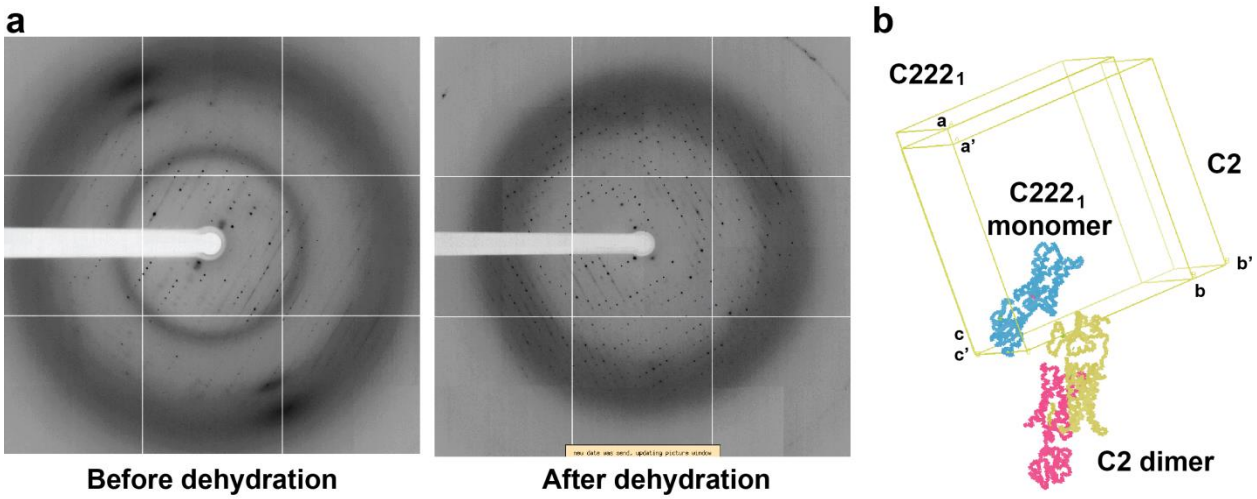
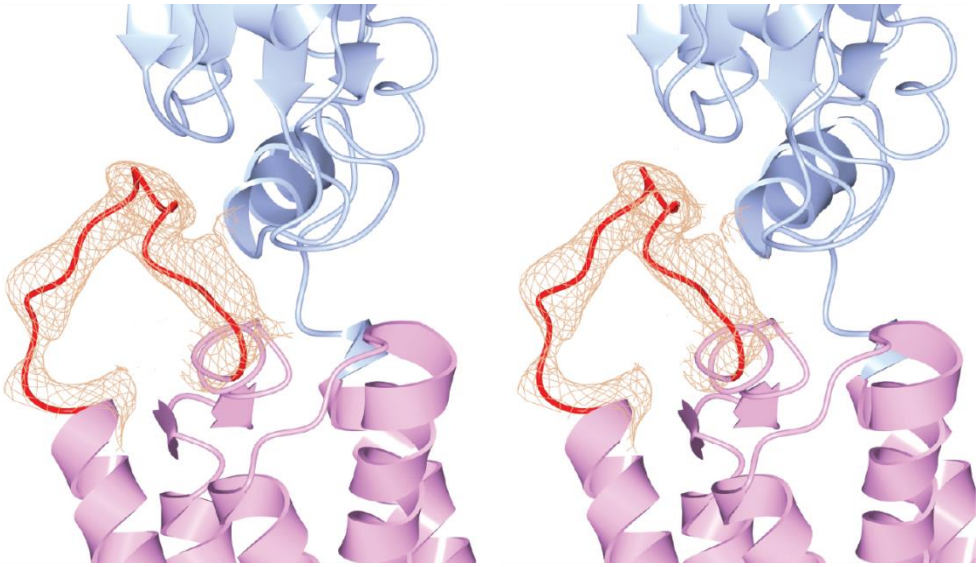
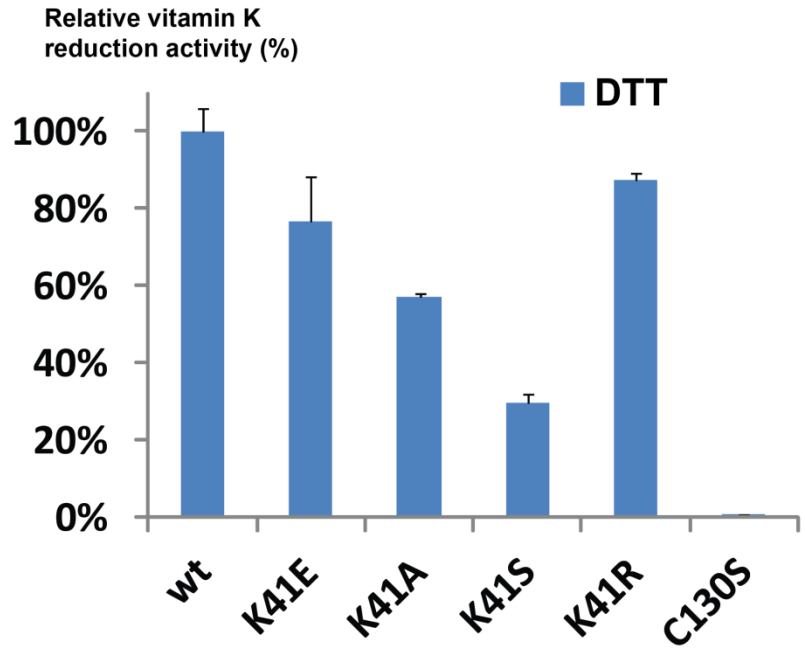
Supplementary Information



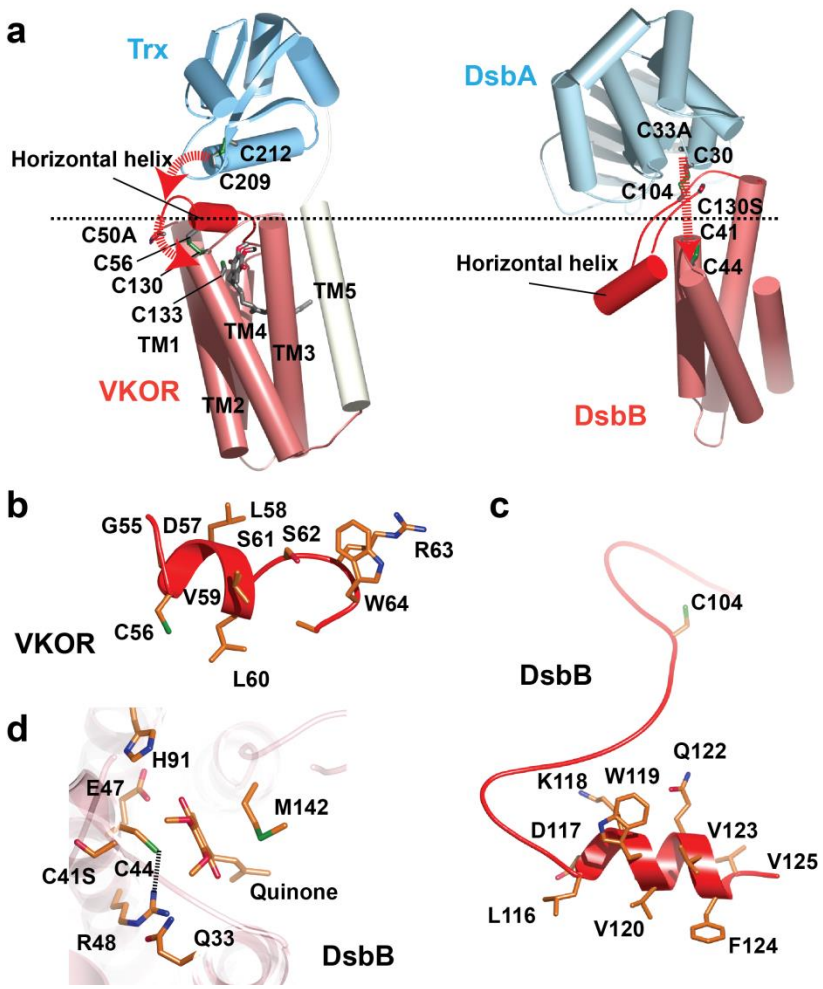
Supplementary Figure 1. Dehydration of the VKOR Cys50Ala crystals. a. Dehydration of the crystals improved the diffraction from 3.6Å (left) to 2.8Å (right). **b**, Changes in the crystal after dehydration. Before dehydration, the crystals are in a $C222_1$ space group and the unit cell dimensions are a=130.8Å, b=142.5Å, c=69.0Å. After dehydration, the crystals changes to a C2 space group and the unit cell dimensions changes to a'=140.8Å, b'=118.8Å, c'=68.0Å. Note that the a and b dimensions are switched and the longest dimension has shrinked from 140.8 Å to 118.8 Å. Due to the break of a 2-fold crystal symmetry, the $C222_1$ space group contains one molecule in the asymmetric unit and the C2 space group contains two molecules.



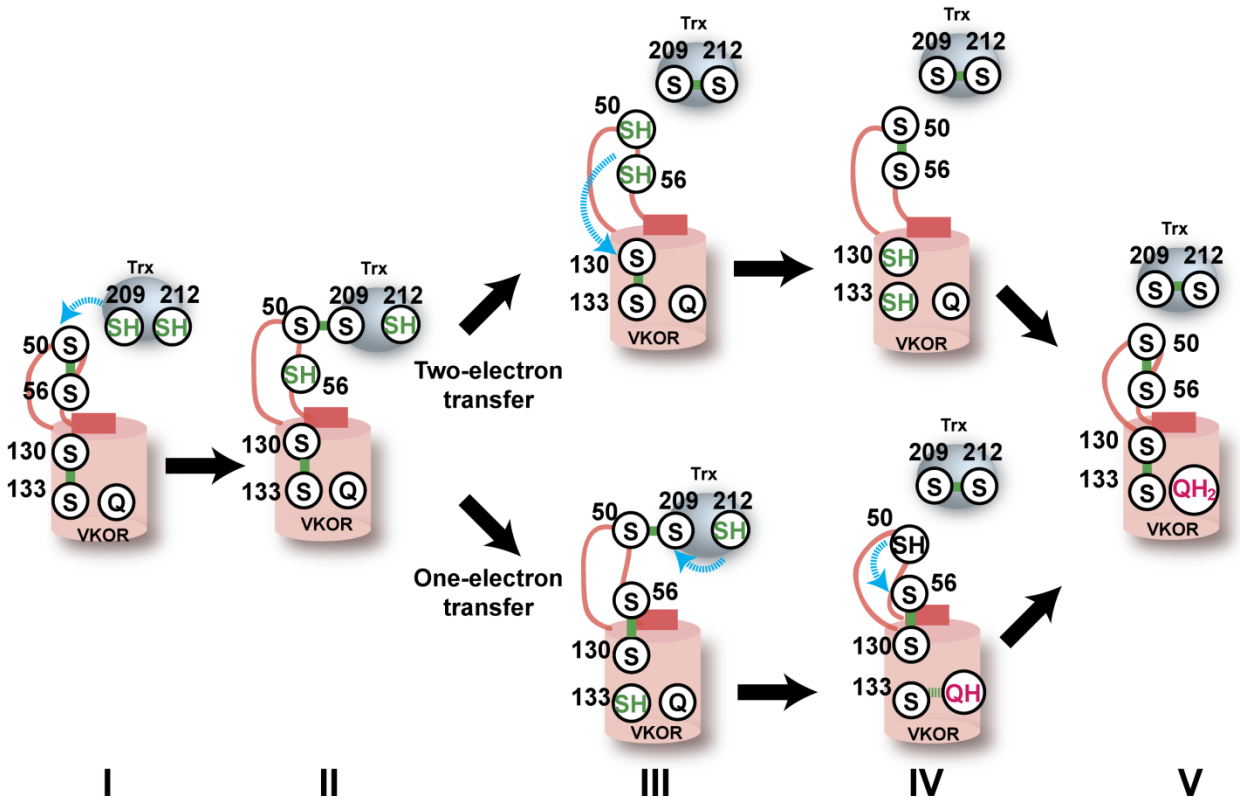
Supplementary Figure 2. The 4.2 Å omit map of the C212A structure in stereo view. Regenerated electron density is contoured at 1σ to show the changed conformation of the electron-transfer loop, which is deleted from the input model.



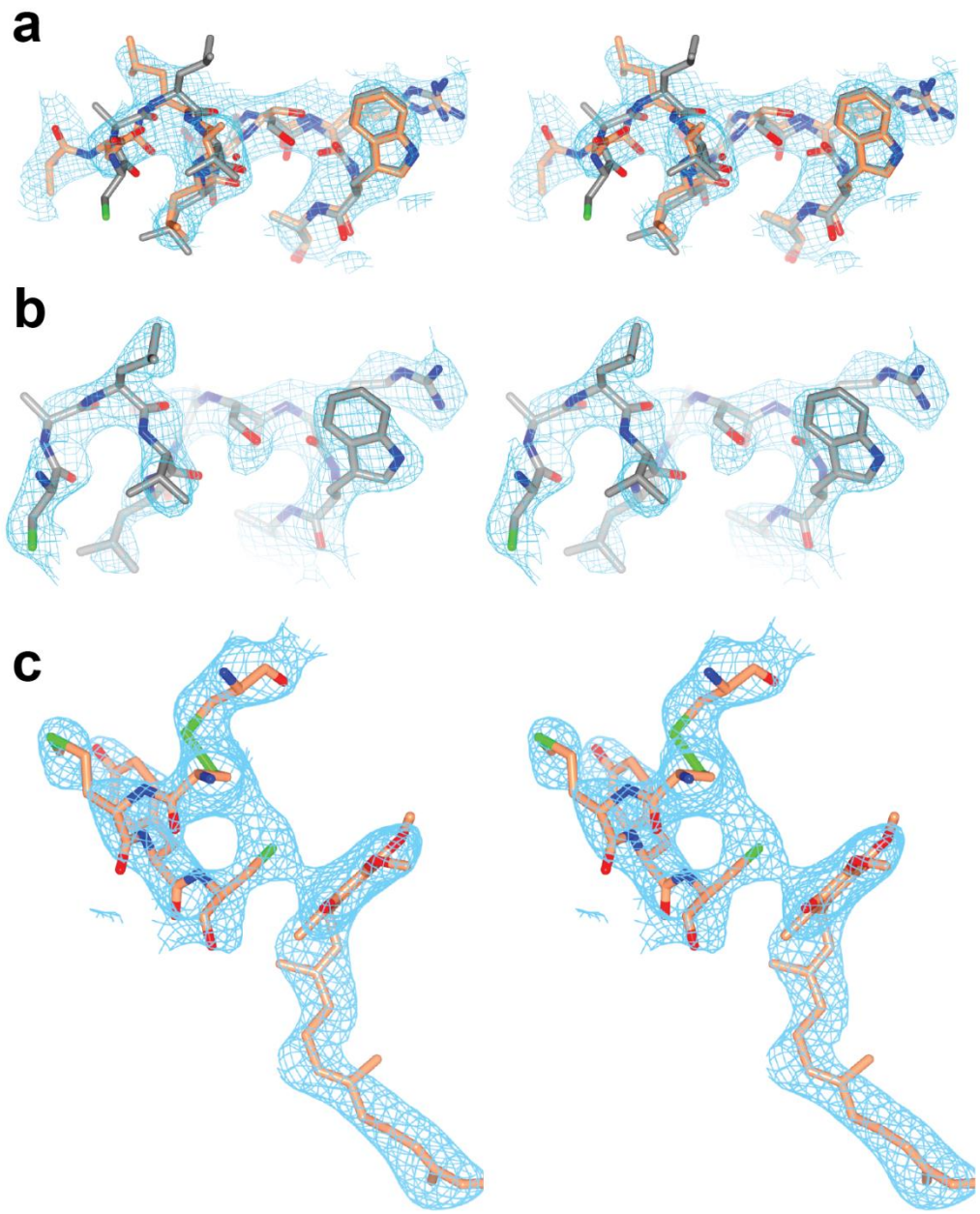
Supplementary Figure 3. Enzymatic activity of lysine mutant proteins. Mutation of Cys130Ser at the active site serves as a negative control. Error bars represent standard error of three duplicated measurements.



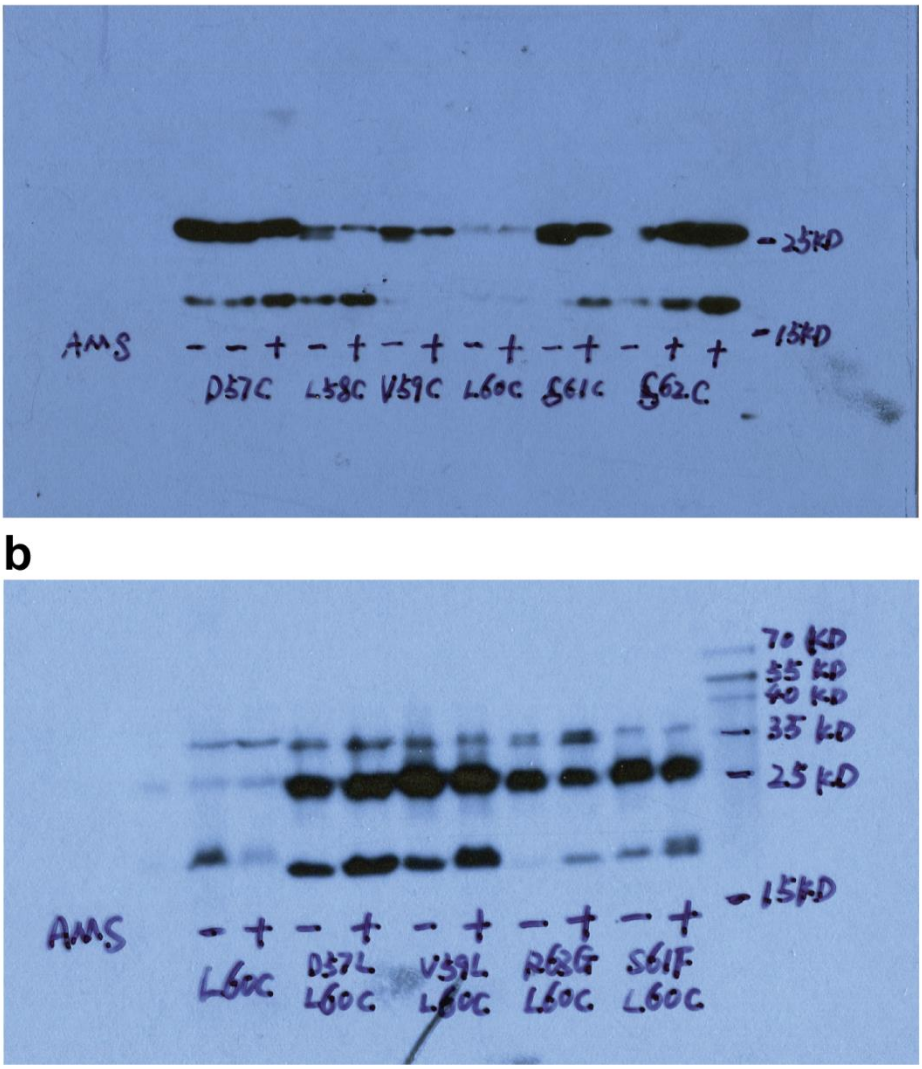
Supplementary Figure 4. Comparison of VKOR-Trx and DsbB-DsbA. a, Different location of the horizontal helix in the VKOR and DsbB. Note the three pairs of cysteines in the DsbB-DsbA complex can be spatially aligned to allow a direct, concerted electron transfer (green arrow in dashed line). This concerted electron transfer is unlikely to happen in the VKOR-Trx system, because movement of the Trx domain is restricted. The horizontal helix in the VKOR is shorter and presumably less buried in membrane (the dotted lines indicate the membrane boundary). **b,** A zoomed view of the horizontal helix in VKOR. **c,** Horizontal helix in DsbB. **d,** Hydrophilic pocket of DsbB showing the conserved Arg48. The coordinates of the DsbB structures shown here are from PDB 2ZUP.



Supplementary Figure 5. Comparison of the one-electron and two-electron transfer pathways. The pathways can be better understood by following changes of the sulfhydryls (colored in green). Top: The Cys50 and Cys56 are both reduced and they move together in the two-electron transfer process. Bottom: The reduced Cys56 is separated from Cys50 to transfer one electron (blue arrow) first. Cys50 is subsequently reduced to transfer the second electron. The Cys212Ala structure supports this one-electron transfer process.



Supplementary Figure 6. Electron density maps in stereo view. a, Omit map (contoured at 1σ) of Cys56Ala horizontal helix (same as in Figure 3b, left panel). **b,** Omit map (contoured at 1σ) of Cys50Ala horizontal helix (same as in Figure 3b, right panel). **c,** Omit map (contoured at 1σ) of Cys50Ala active site (same as in Figure 6a).



Supplementary Figure 7. The full western blots. **a,** Same blot as in Figure 5a. D57C and S62C were loaded twice to avoid edge effect of the western blot. **b,** Same blot as in Figure 5c. A minor band above 35KDa is probably nonspecific due to higher exposure in this blot.