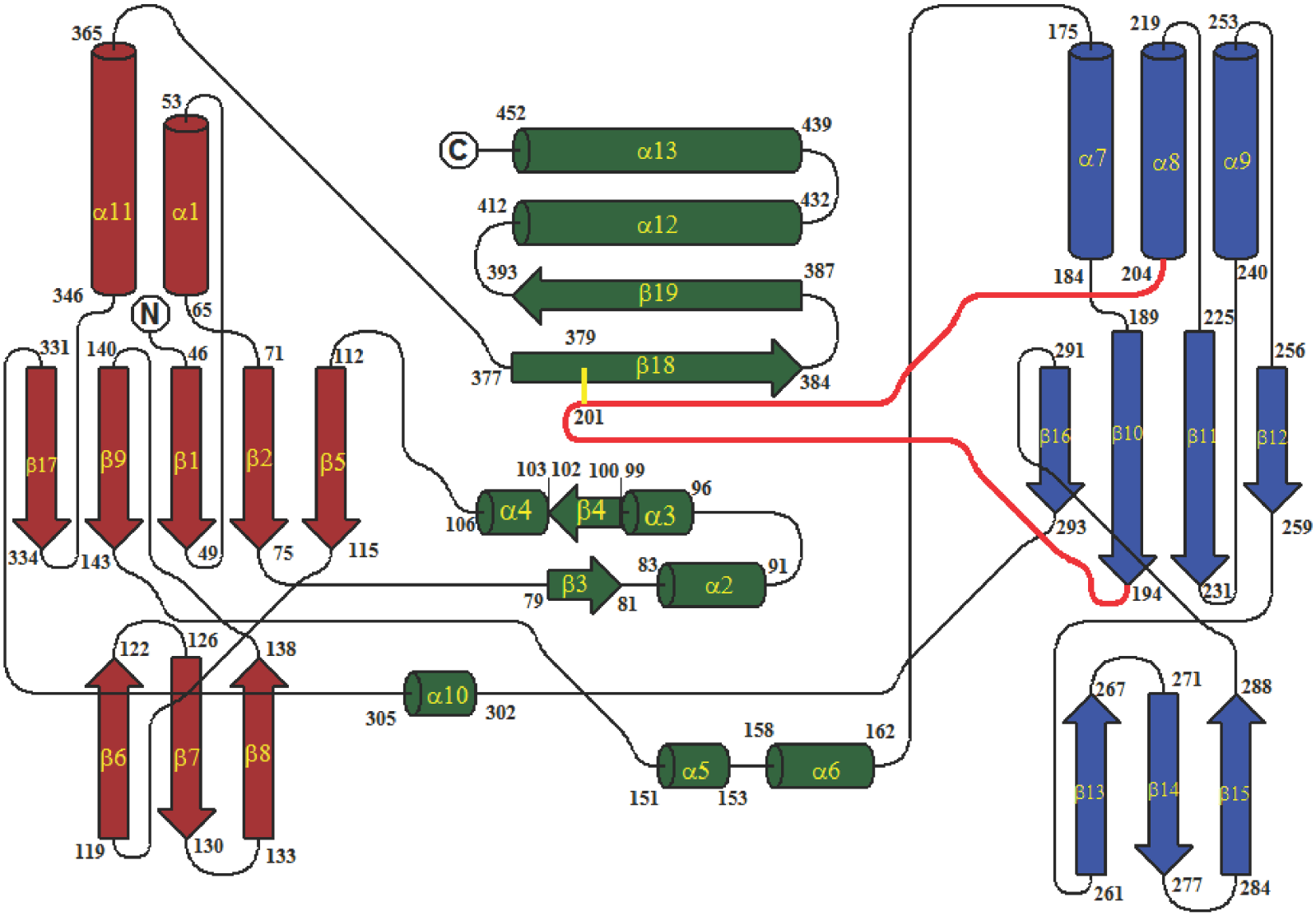

Figure S1, Related to Figures 1A, 1B, 2B and 6A: A topology diagram of the human SQOR structure. β strands are shown as arrows and α helices are shown as cylinders. β strands and α helices in the first (N-terminal) and second Rossmann fold domains are colored red and blue, respectively. All other β strands and α helices are colored green. The loop (Pro195 to Gly203) that connects the C-terminal end of β10 to the N-terminal end of α8 is colored red. All other loop regions are colored black. The figure was generated using TopDraw, a program within the CCP4 suite.

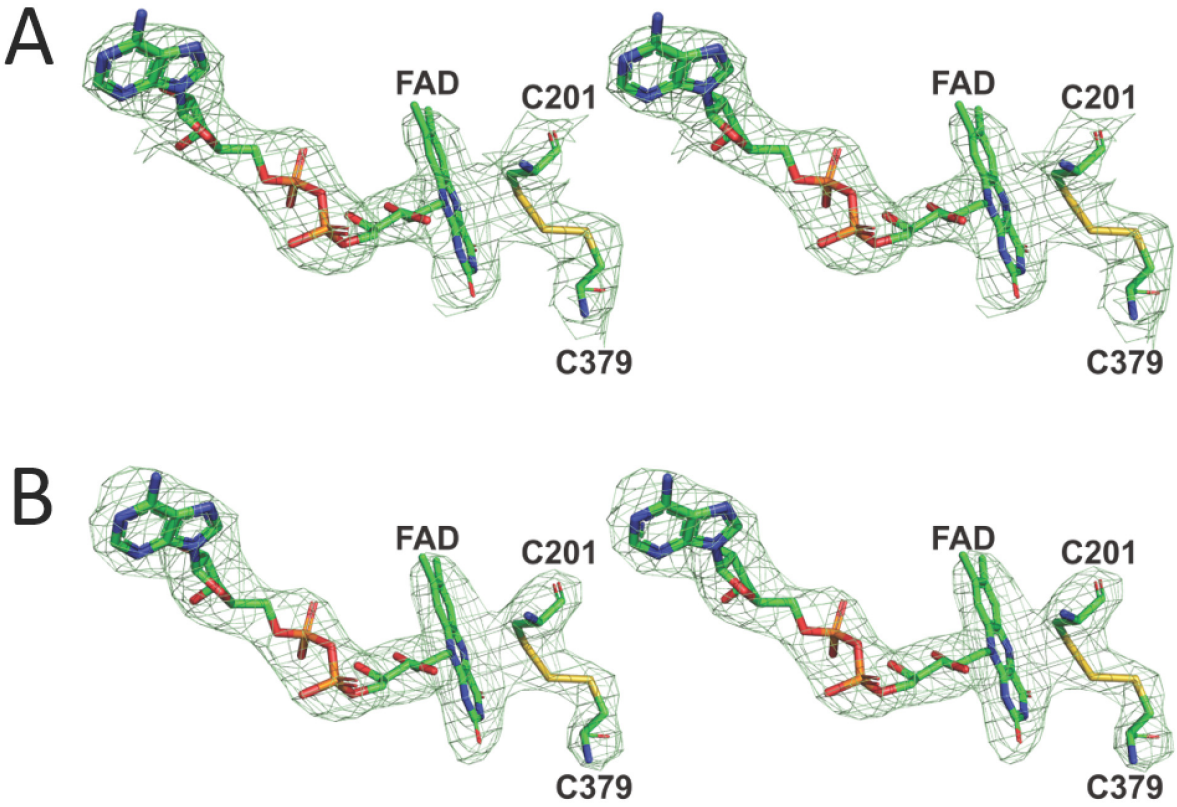

Figure S2, Related to Figure 2A: Stereoviews (wall-eye) of the redox-active site in native SQOR. FAD and the active site cysteine residues (Cys201, Cys379) are shown as sticks with carbons colored green. Panel A shows the $2F_o$ - F_c electron density omit map, drawn at 1.0 σ. Panel B shows the polder (F_o - F_c) electron density omit map, drawn at 3.0 σ. In each case, the electron density around FAD and the active site cysteines was carved using a 2 Å carving radius. The $2F_o$ - F_c omit map and polder maps show electron density connecting Cys201:S with FAD:C4a. This density is observed for chains C (shown) and D, but is much less apparent for chains A and B. We initially tried to model and refine the structure of native SQOR with a covalent link in chains C and D between flavin C(4a) and Cys201:S, a modification that introduces a pucker in the otherwise planar ring characteristic of oxidized FAD. However, refinement statistics and final electron density for this model were poorer than those obtained for the final model in which all chains contain oxidized FAD and thiocystine (Cys_{201}-S-S-S-Cys_{379}).

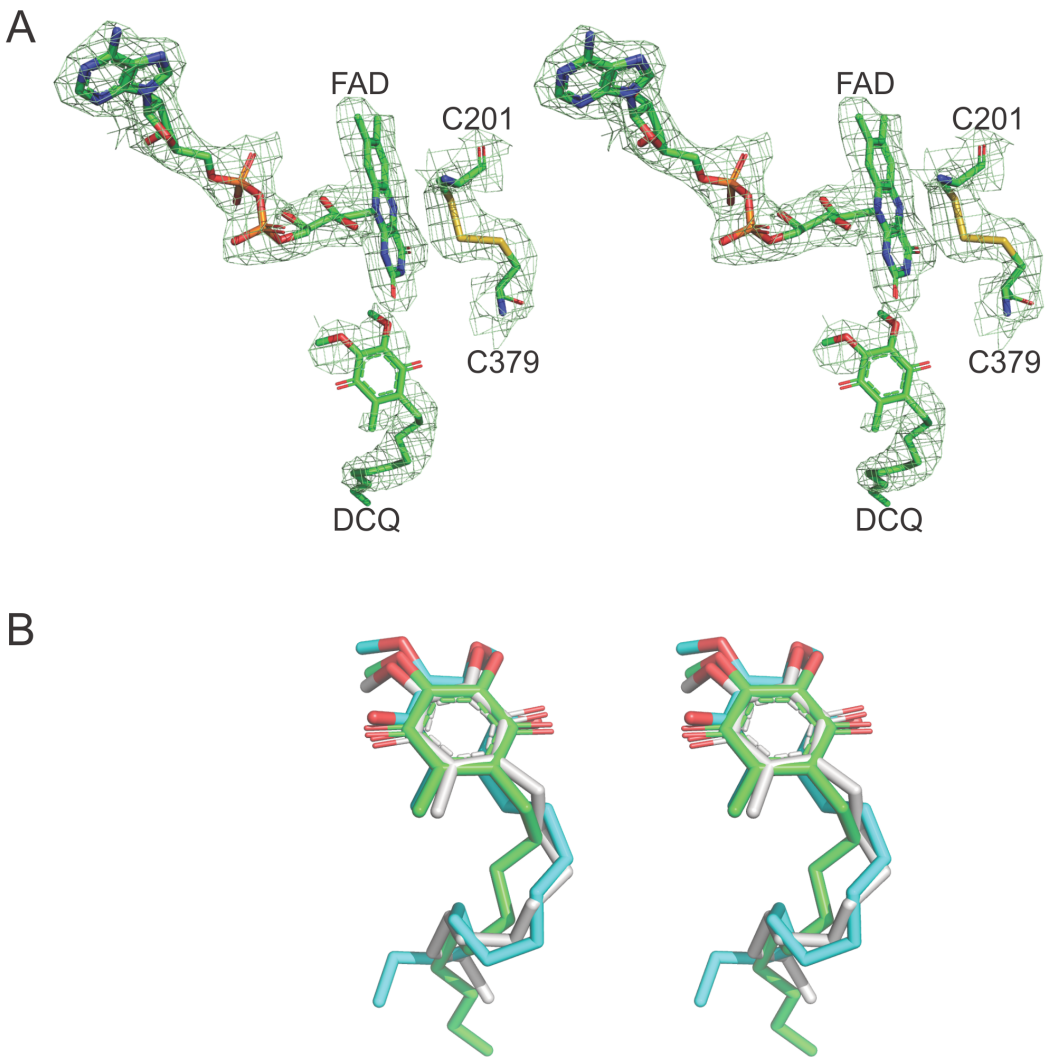

Figure S3, Related to Figure 2A: Stereoview (wall-eye) comparison of a model of DCQ fitted to the electron density with DCQ docked into the CoQ binding cavity of SeMet-substituted SQOR. A) FAD, active site cysteine residues (Cys201, Cys379), and an initial DCQ model are shown as sticks with carbons colored green. The $2F_o$ - F_c electron density omit map is drawn at 1.0 σ. The electron density is consistent with the decyl tail in the DCQ model. However, the model was rejected, in part, because the density for the quinone ring is poor. B) The DCQ model is shown as sticks with carbons colored green. DCQ docked using Flare 1.0 (Cresset) or dockingserver.com is shown as sticks with carbons colored white or cyan, respectively.

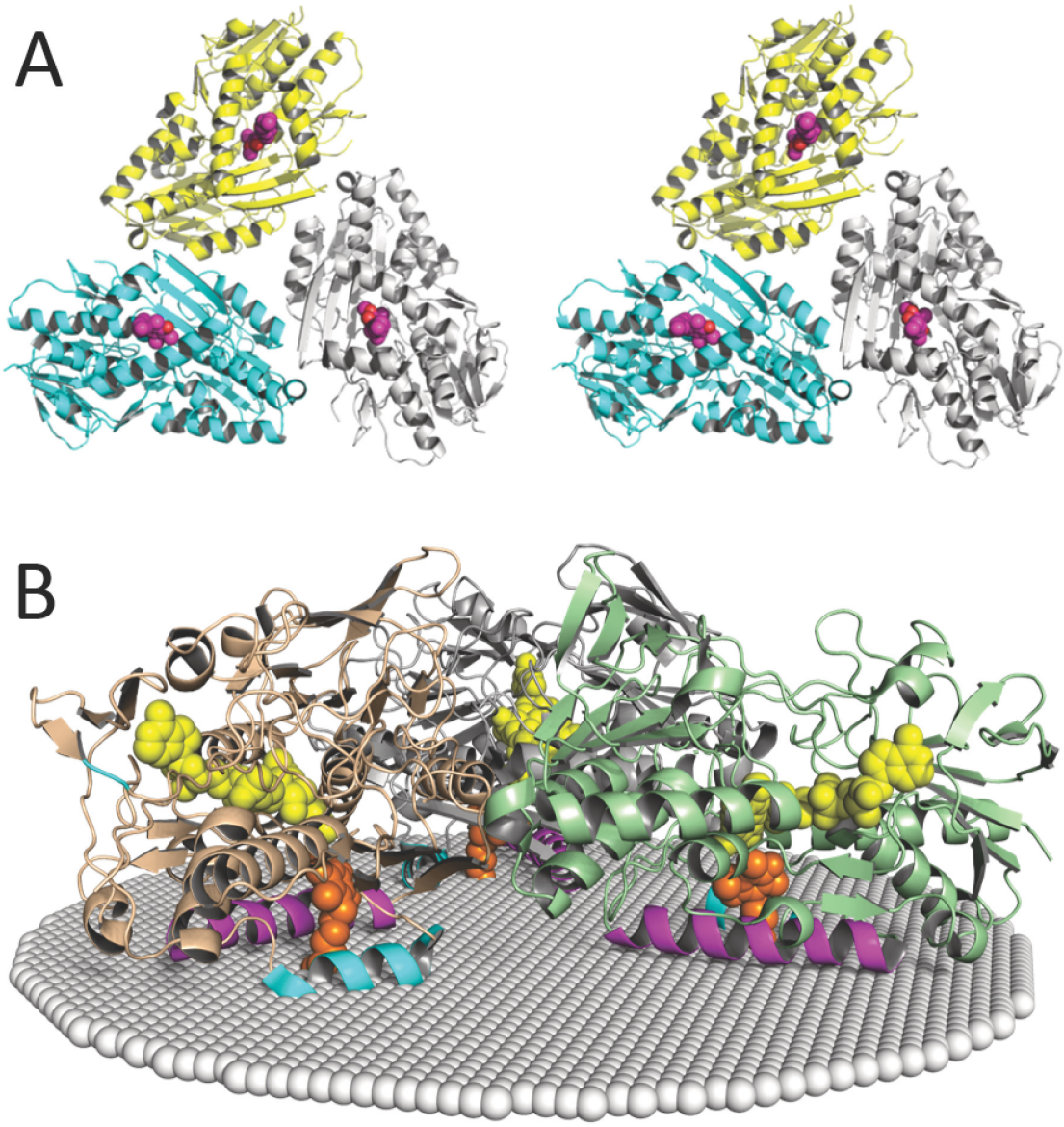

Figure S4, Related to Figure 4: Stereoview (wall-eye) cartoon diagram showing the membrane-facing side of one of the two trimers in the asymmetric unit of *A. aeolicus* SQOR (3HYW.pdb) (A) and the spatial position of the trimer in the membrane (white spheres) (B), as calculated using the PPM server (http://opm.phar.umich.edu/server.php). Panel A: The cartoons for chains B, D and F are colored cyan, yellow and white, respectively. DCQ is shown in spacefill with carbons colored magenta. The two trimers in the asymmetric unit are arranged with the membrane faces stacked against each other. Panel B: The C-terminal and penultimate C-terminal helices in each chain are colored cyan and magenta, respectively; all other regions in the three chains are colored pale green, tan, or grey. FAD and DCQ are shown as spheres, colored yellow and gold, respectively.

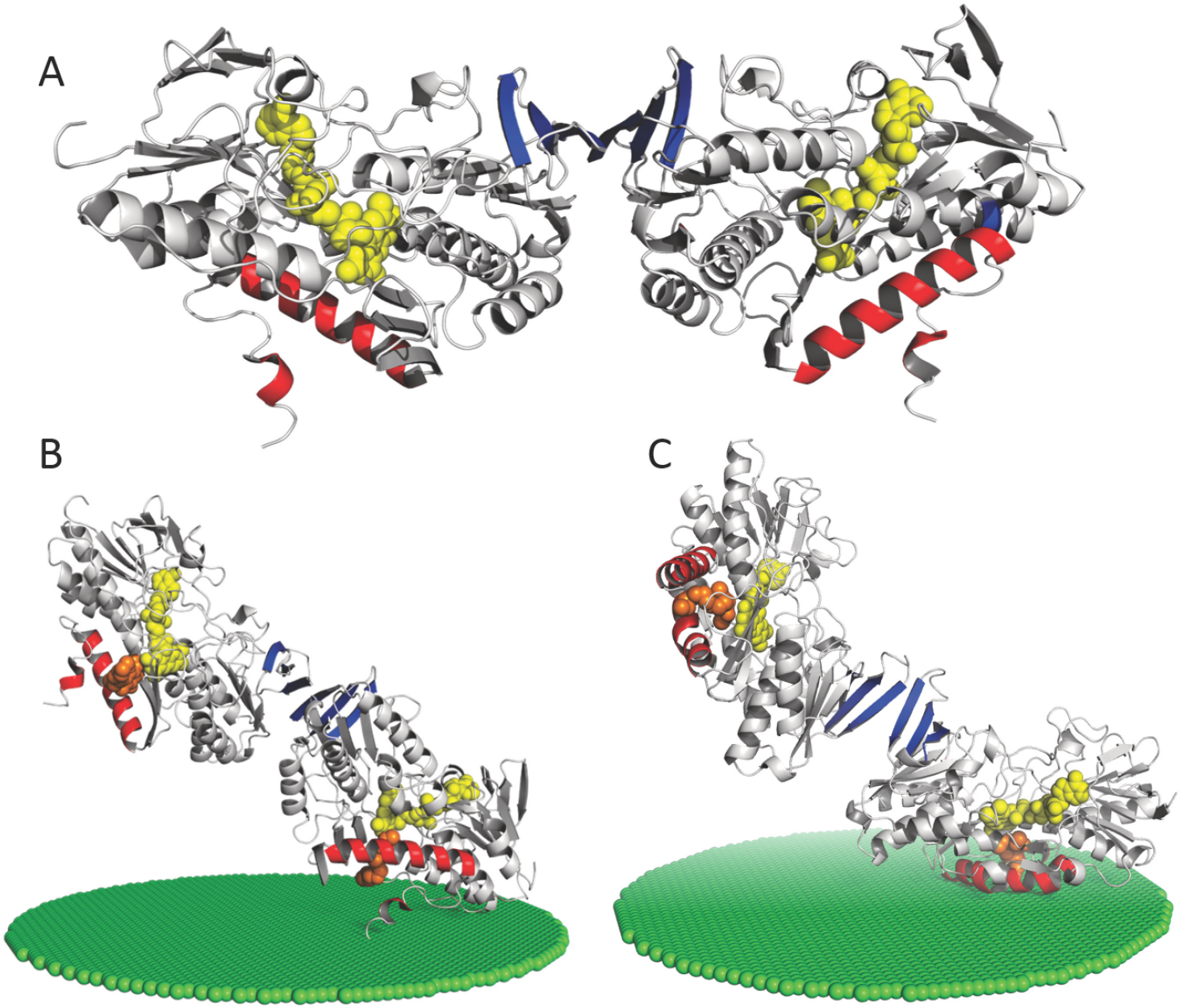

Figure S5, Related to Figure 4: Cartoon diagram (wall-eye stereo) of the proposed biological dimer of *A. ferrooxidans* SQOR (3T31.pdb) (A) and cartoon diagrams of the spatial position of the *A. ferrooxidans* SQOR dimer (B) and a hypothetical human SQOR dimer (C) in the membrane (green spheres), as calculated using the PPM server (http://opm.phar.umich.edu/server.php). The C-terminal and penultimate C-terminal helices in each cartoon diagram are colored red. The β-strands forming the extended β-sheet at the dimer interface are colored blue. All other regions are colored white. FAD and DCQ are shown as spheres, colored yellow and gold, respectively.

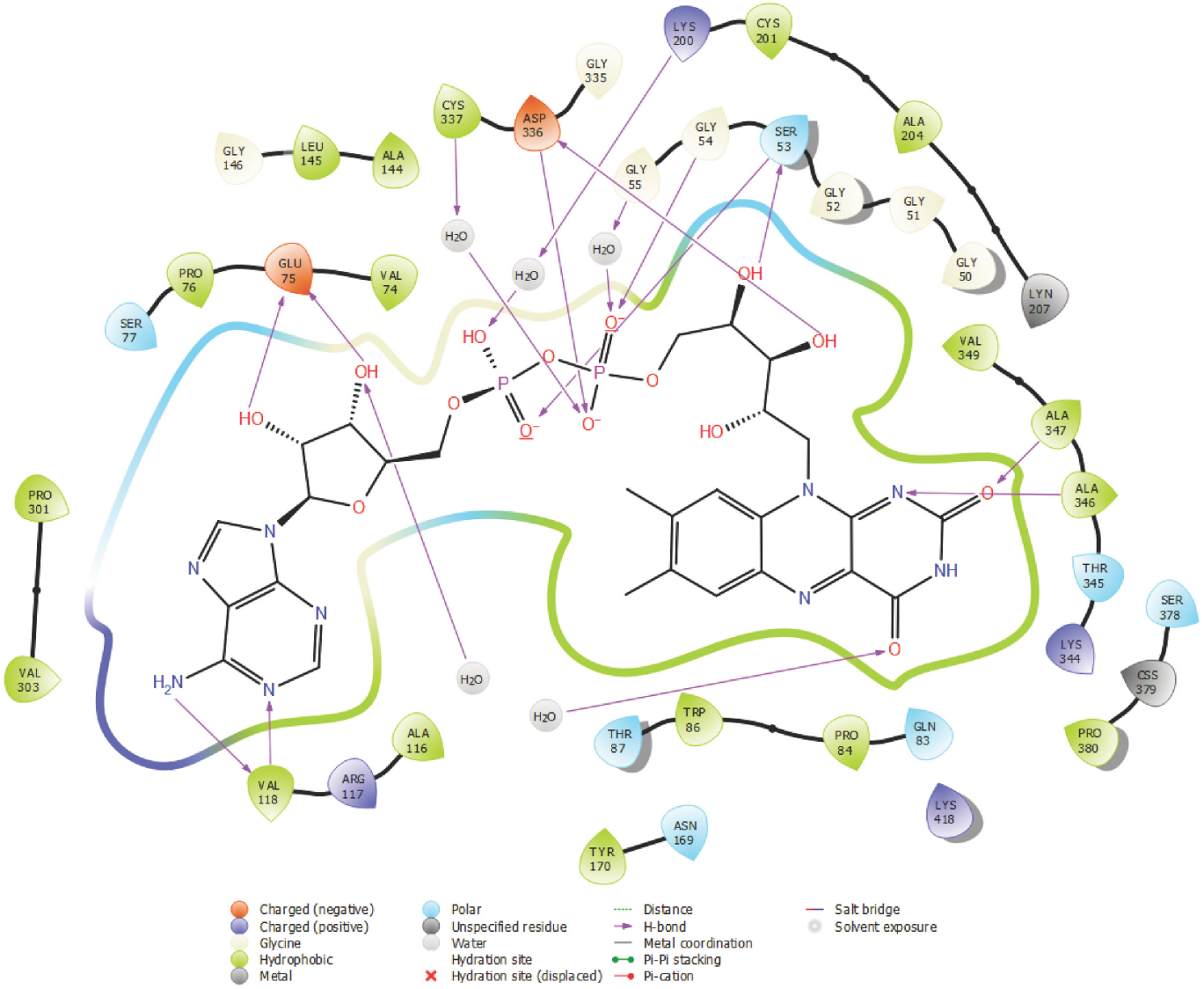

Figure S6, Related to Figure 2B: A schematic representation of protein and solvent interactions with FAD in the structure of SeMet-substituted SQOR. The diagram does not include two helices (α1, α11) that form electrostatic interactions with FAD. The N-terminus of helix α1 points toward the pyrophosphate group of FAD; the first two residues in α1 (Ser53, Gly54) are hydrogen bonded to the pyrophosphate. The N-terminus of helix α11 points toward the N(1)-O(2) position of the FAD ring; the first two residues in α11 (Ala346, Ala347) are hydrogen bonded to FAD:N1 and FAD:O2, respectively (see **Figure S7**). The diagram was prepared using Maestro 11.5 (Schrödinger).

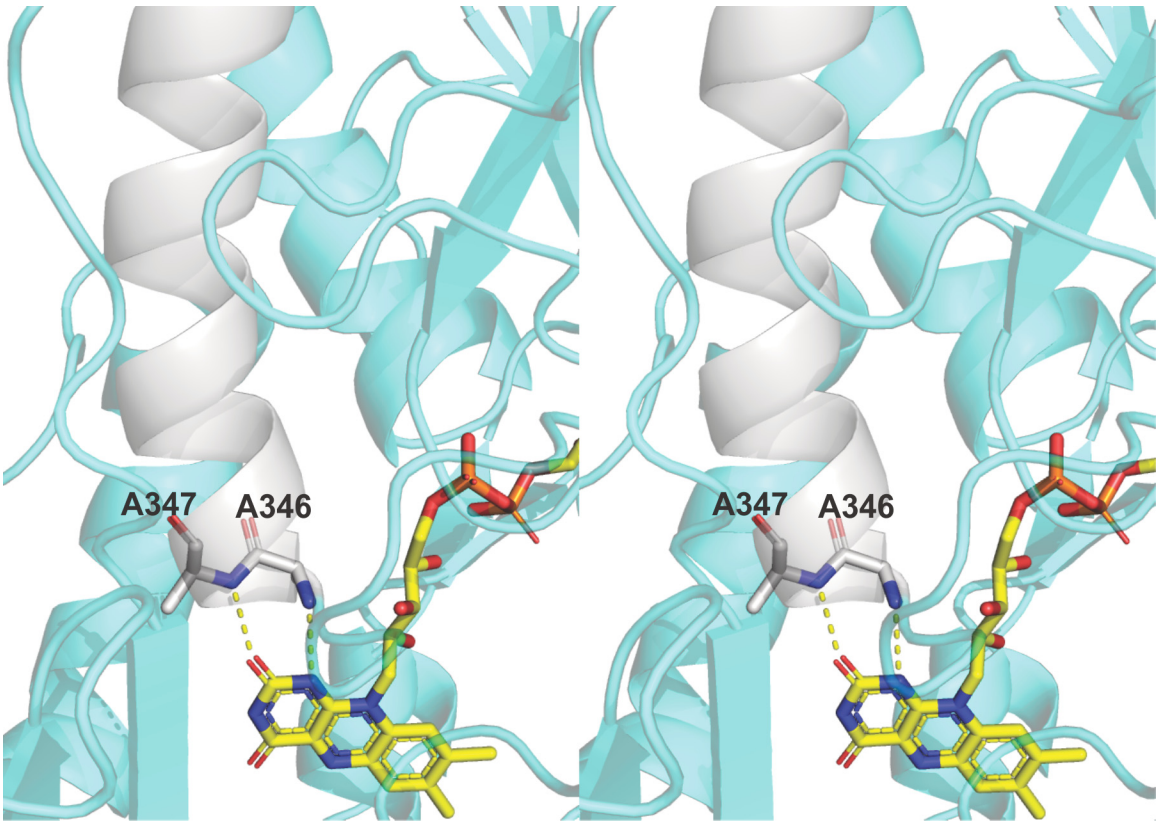

Figure S7, Related to Figure 2B: Stereoview (wall-eye) of the electrostatic interaction of the N-terminus of helix α11 with FAD. Helix α11 in the cartoon diagram is colored white; other regions are colored cyan. The first two residues of helix α11 (Ala346, Ala347) and FAD are shown as sticks with carbon atoms colored white and yellow, respectively. Hydrogen bonds are indicated by dashed yellow lines.

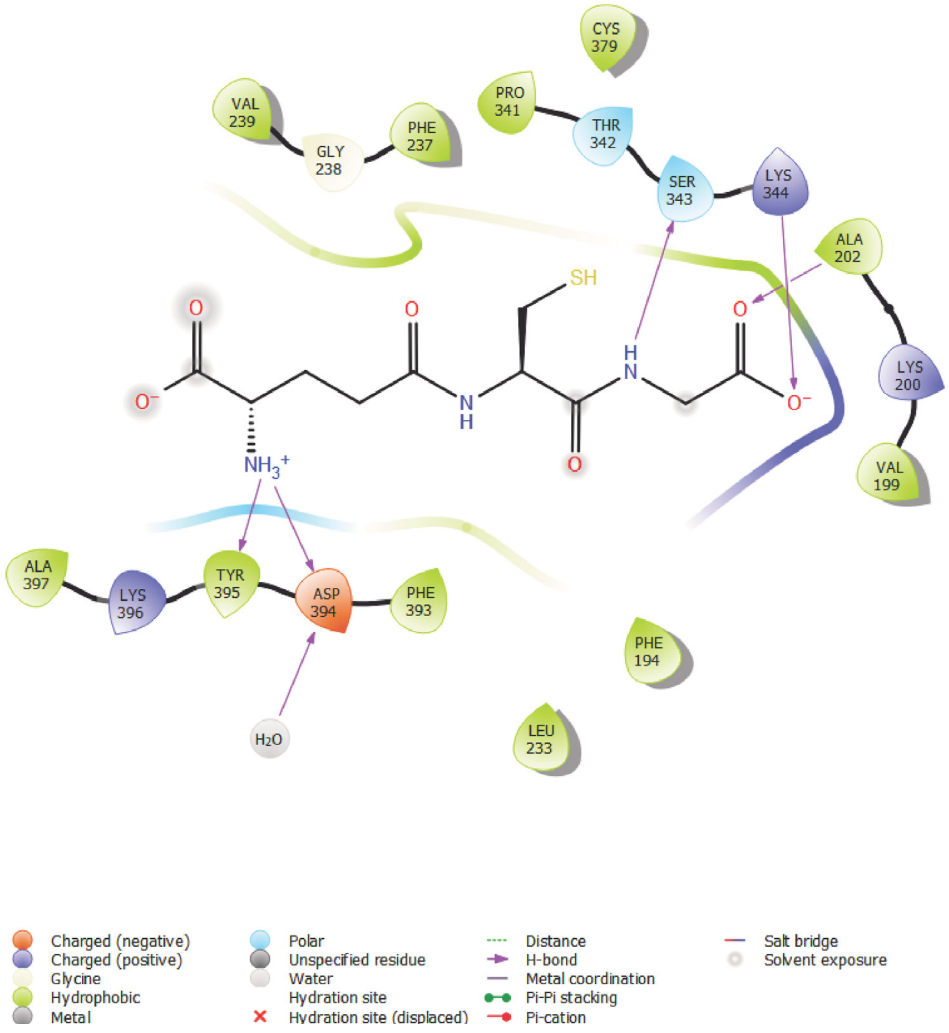

Figure S8, Related to Figure 6C: A schematic representation of interactions of docked glutathione with the protein and solvent in the structure of SeMet-substituted SQOR. The diagram was prepared using Maestro 11.5 (Schrödinger) and the top pose obtained using Glide (Schrödinger Release 2018-2) in XP-mode.