SUPPORTING INFORMATION

Use of Tissue Metabolite Analysis and Enzyme Kinetics to Discriminate Between Alternate Pathways for Hydrogen Sulfide Metabolism

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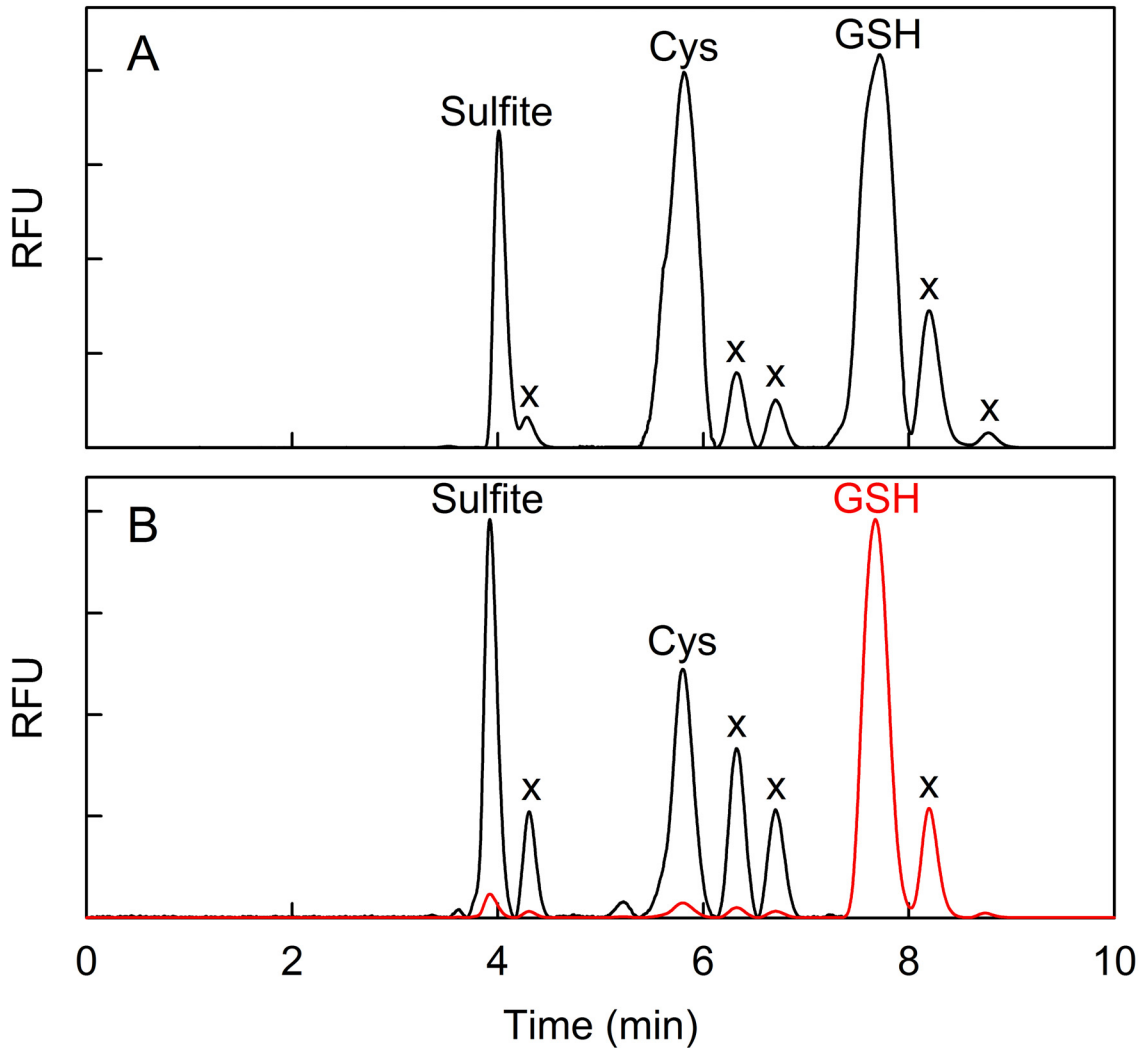


Figure S1 HPLC elution profiles of various bimane derivatives. Panel A shows the elution profile obtained for bimane standards, prepared by reaction of a mixture of sulfite, cysteine, and glutathione with monobromobimane. For comparison, panel B shows the elution profile of bimane derivatives obtained with a heart extract and is identical to the profile shown in Figure 1D. RFU, relative fluorescence units; x, reagent-derived peaks.

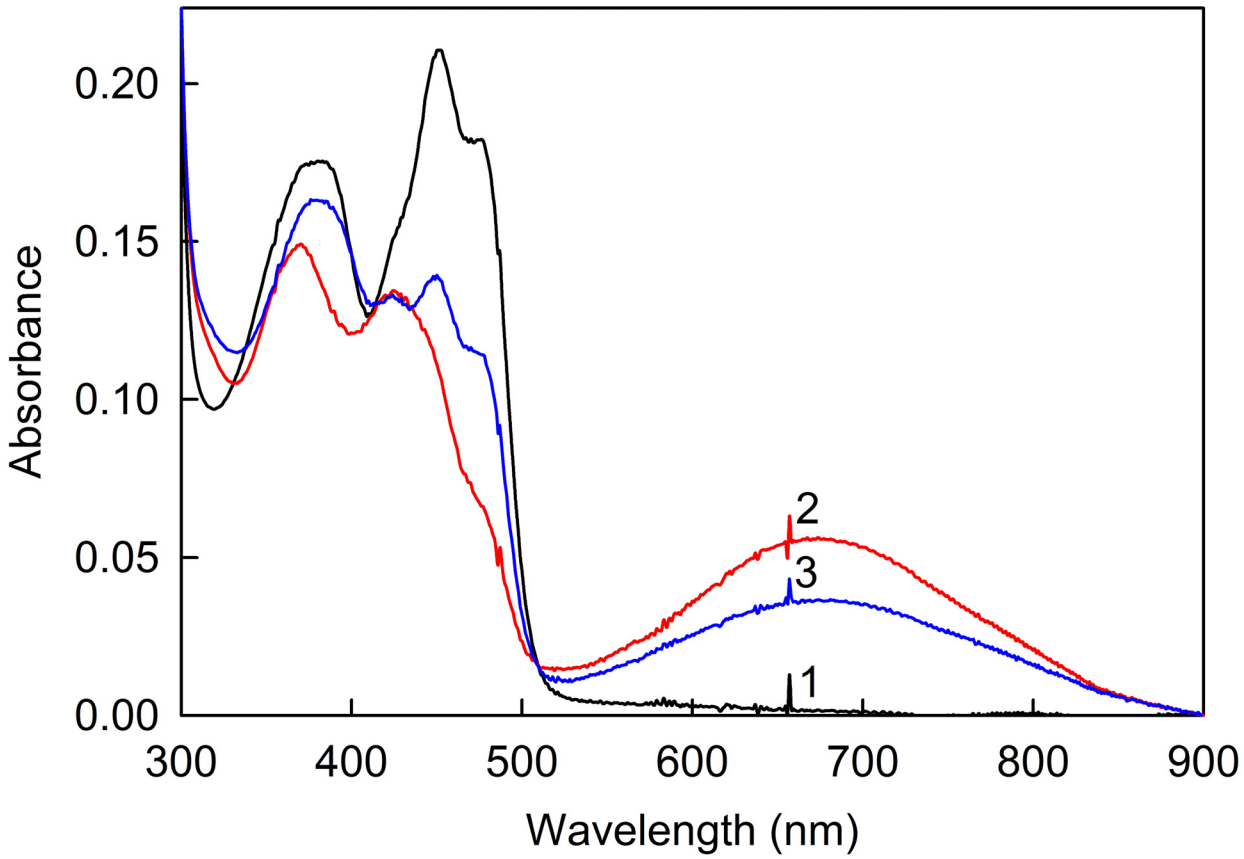
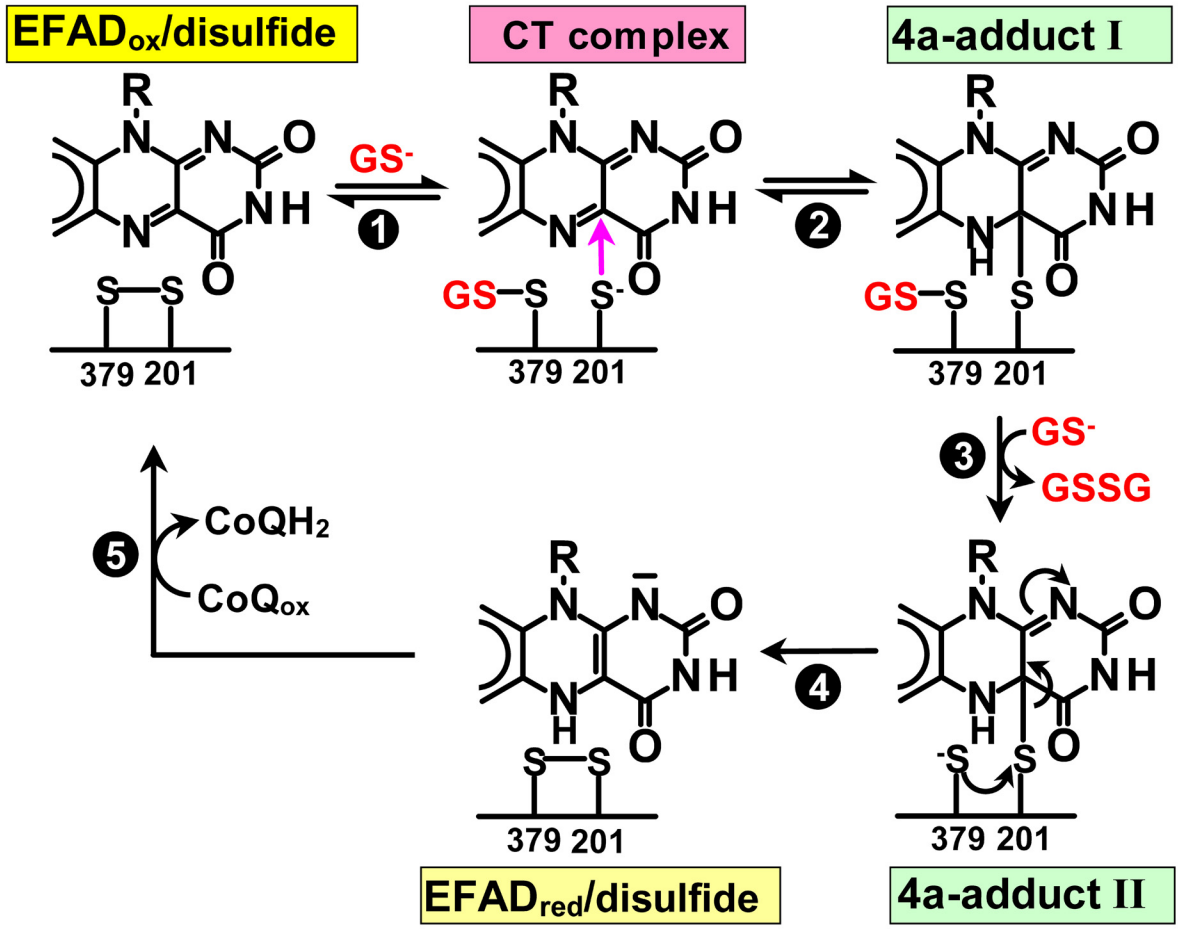


Figure S2 Comparison of the spectral properties of long-wavelength absorbing intermediates produced by anaerobic reaction of oxidized SQOR (curve 1) for 310 s with 8.26 mM glutathione (curve 2) or observed immediately after reaction with 1 or 2 equivalents of H_2S (curve 3), as previously reported[27] but normalized to the same initial oxidized enzyme concentration.



Scheme S1 Proposed mechanism for the SQOR-catalyzed glutathione:CoQ reductase reaction. Human SQOR contains two highly conserved cysteine residues (Cys201 and Cys379) that form a redox-active disulfide located ~3.5 Å above the *re*-face of the flavin

ring in an homology model of human SQOR (M. M. White and M. S. Jorns, unpublished observations). The "proximal" cysteine, Cys201, is closer to the C(4a) position of FAD. Nucleophilic attack of glutathione thiolate (GS^-) at the distal cysteine, Cys379, produces a long-wavelength absorbing charge-transfer (CT) complex of FAD with $Cys_{201}S^-$ (step 1). Nucleophilic attack of $Cys_{201}S^-$ at the C(4a) position of the flavin ring produces a covalent flavin adduct, 4a-adduct I (step 2). Reaction of 4a-adduct I with a second molecule of GS^- generates oxidized glutathione (GSSG) and 4a-adduct II, an intermediate that contains the thiolate form of Cys379 (step 3). Nucleophilic attack of $Cys_{379}S^-$ at the sulfur atom in 4a-adduct II produces an intermediate containing 1,5-dihydroFAD plus the original disulfide ($EFAD_{red}$/disulfide) (step 4). The catalytic cycle is completed upon transfer of electrons from $EFAD_{red}$/disulfide to CoQ (step 5).