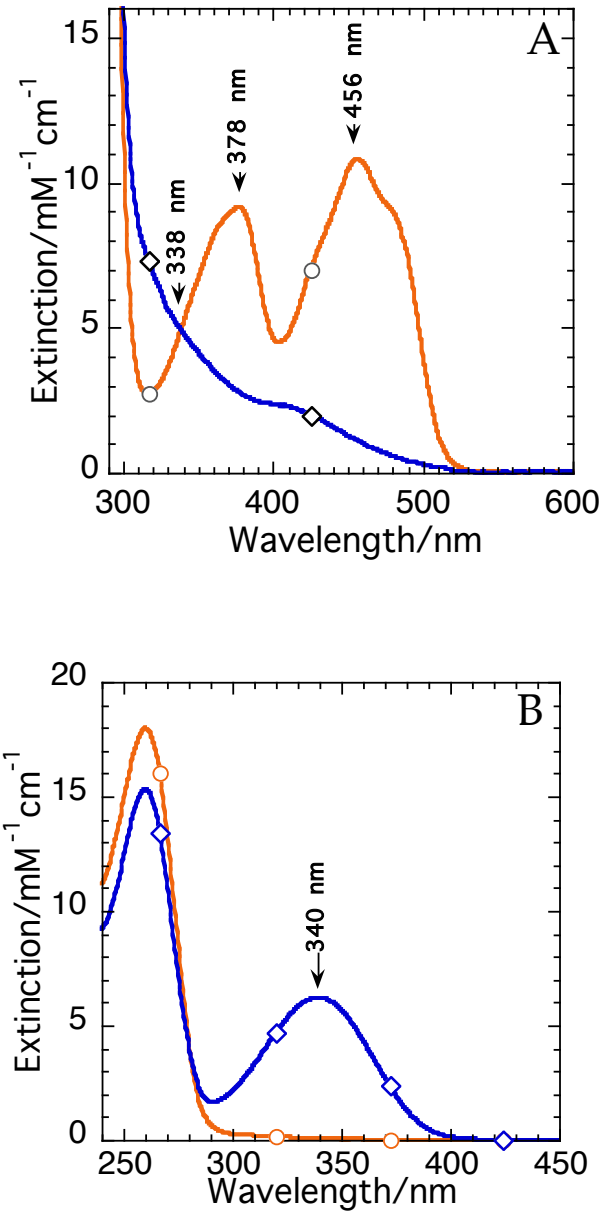

Figure S1. Spectra of Oxidized and Reduced SMOB and NADH. Spectra of oxidized (orange) and reduced (blue) SMOB (Panel A) and NADH (Panel B). Absorbance maxima and the isosbestic point of SMOB are indicated.

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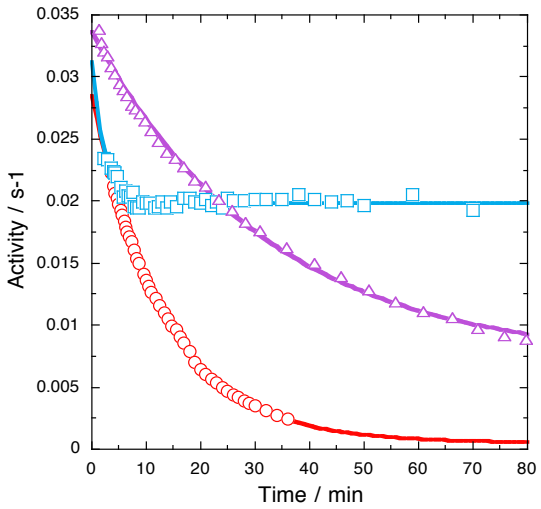

Figure S2. Stabilizing effect of oxidized FAD and the NSMOA FAD C(4a)–hydroperoxide intermediate on SMOB. Shown are activity measurements of 600 nM SMOB-FAD_{ox} (purple), 600 nM apoSMOB generated by activated carbon treatment (red) and 600 nM SMOB-FAD_{ox} together with 15 µM NSMOA and 100 µM NADH (blue), all incubated at 15 ˚C over a period of 35–80 minutes. Activity measurements were taken by mixing incubated protein with 100 µM NADH and 20 µM FAD and periodically observing absorbance at 340 nm on the stopped-flow instrument. Activity was determined by measuring the slope during steady-state turnover of NADH.

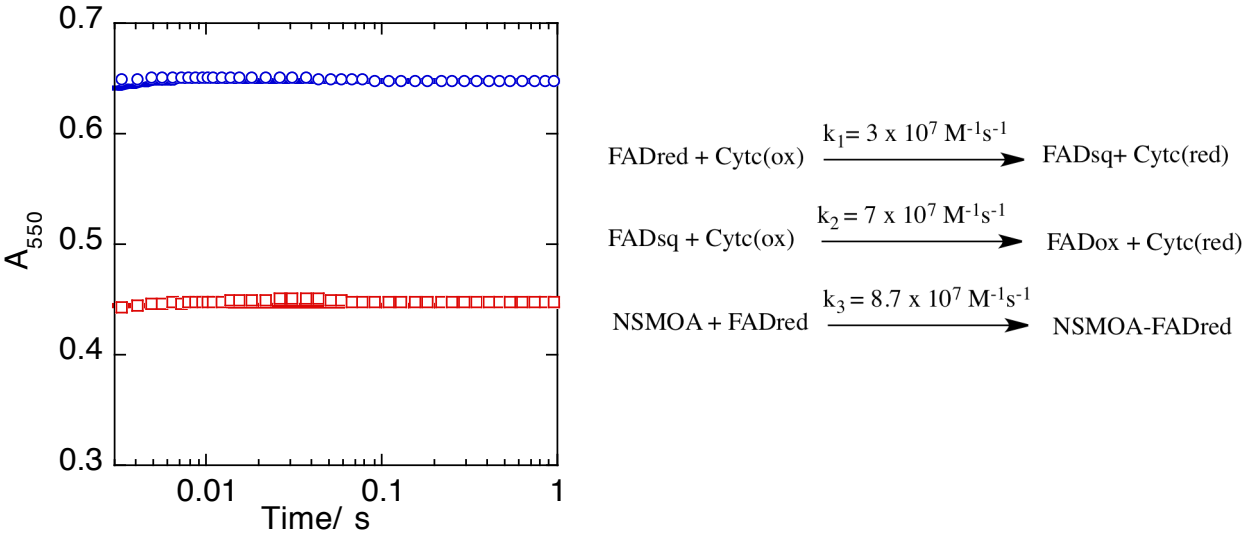

Figure S3. Estimation of the Second-Order Rate Constant for the Reaction of NSMOA with FAD_{red}.

25 µM of reduced FAD was reacted with 30 µM cytochrome c under anaerobic conditions in 20 mM MOPSO buffer pH 7 at 15° C in the presence (red) and absence (green) of 30 µM NSMOA. The final concentrations of reduced and oxidized cytochrome c were calculated by using the final reaction absorbance and the reduced and oxidized cytochrome c extinction coefficients (corrected for the spectral resolution of the stopped flow instrument) of 21,600 M^{-1} cm^{-1} and 8,400 M^{-1} cm^{-1}, respectively. The second order rate constant k_3 for the reaction of NSMOA with reduced FAD was estimated by using the program Berkeley Madonna to simulate the model shown above and to the right. Values of k_1 and k_2 used in this calculation are those previously reported in the literature for the reaction of FMN with cytochrome c (1). The curves passing through the data represent the results of simulation the model below using the value of $k_3 = 8.7 \times 10^7$ M^{-1} s^{-1}.

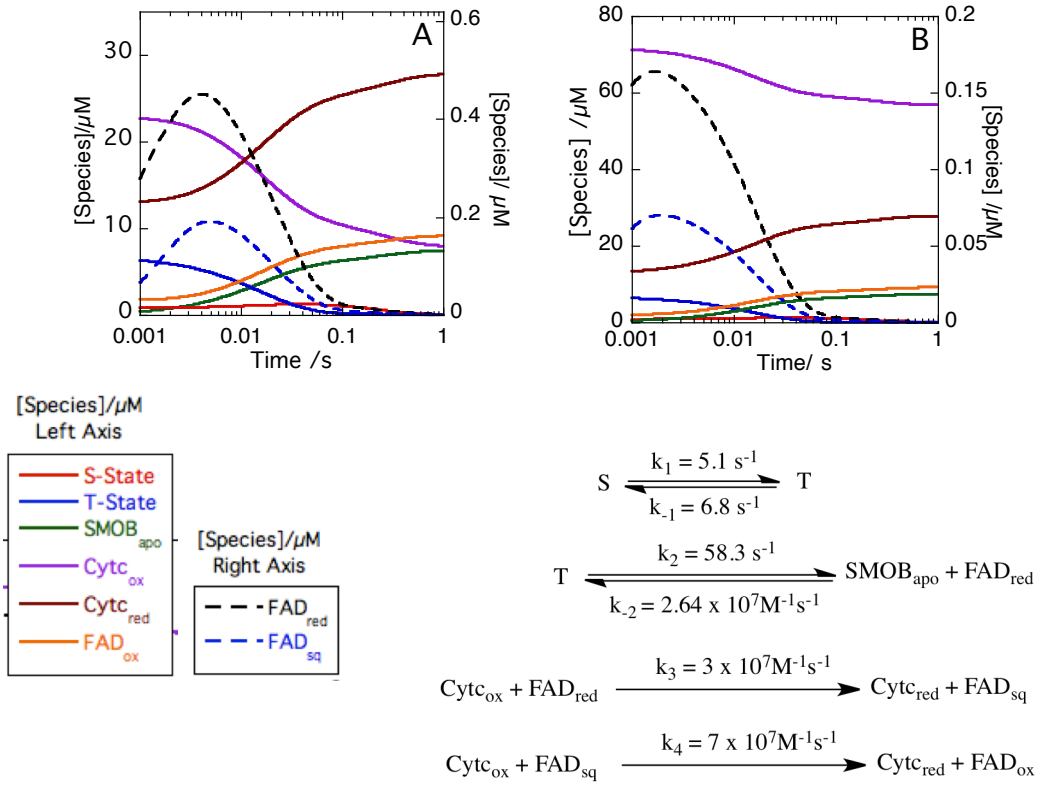

Figure S4. Modeling Electron-Transfer from SMOB to cytochrome c.

The kinetics of electron-transfer from reduced SMOB to oxidized cytochrome c were simulated according to the model shown above using the Berkeley Madonna program. The changes in reactant, intermediate, and product species concentrations are plotted as a function of time as computed by this model for two different initial conditions are shown above in Panels A and B; the identity of the species represented in these plots is given in the legend.

Since oxidized cytochrome c reacts rapidly with free-reduced FAD in the mixing time of the stopped flow, the initial speciation of reactants observed at the completion of these rapid reactions includes only SMOB-bound states of reduced FAD and oxidized

cytochrome c. In simulating the reaction of 13.3 μM SMOB (reduced by titration with dithionite) with 22.8 μM (Plot A) or 84.5 μM (Plot B) oxidized cytochrome c, starting concentrations of free reduced FAD (5.8 μM) and SMOB-bound reduced FAD (7.4 μM) were computed by using the dissociation equilibrium constant for reduced FAD and SMOB (K_d = 2.5 μM). The concentrations of oxidized cytochrome c (13.4 μM) and reduced cytochrome c (9.4 μM) present at the end of the stopped flow mixing time were computed by using the starting absorbance at 550 nm (Figure 7) and the extinction coefficients for oxidized and reduced FAD (as described in the legend of Figure S3).

The initial values of parameter estimates for k_1 and k_2 came from biexponential fits of the 550 nm absorbance data. Values of k_3 and k_4 are those reported for similar reactions of reduced FMN with oxidized cytochrome c (1). The value of k_{-2} was computed by using the value of k_2 estimated from exponential fitting and the K_d of reduced FAD computed in this work. The value of k_{-1} was manually adjusted until good agreement between the data and simulation were obtained (See traces passing though data in Figure 7).

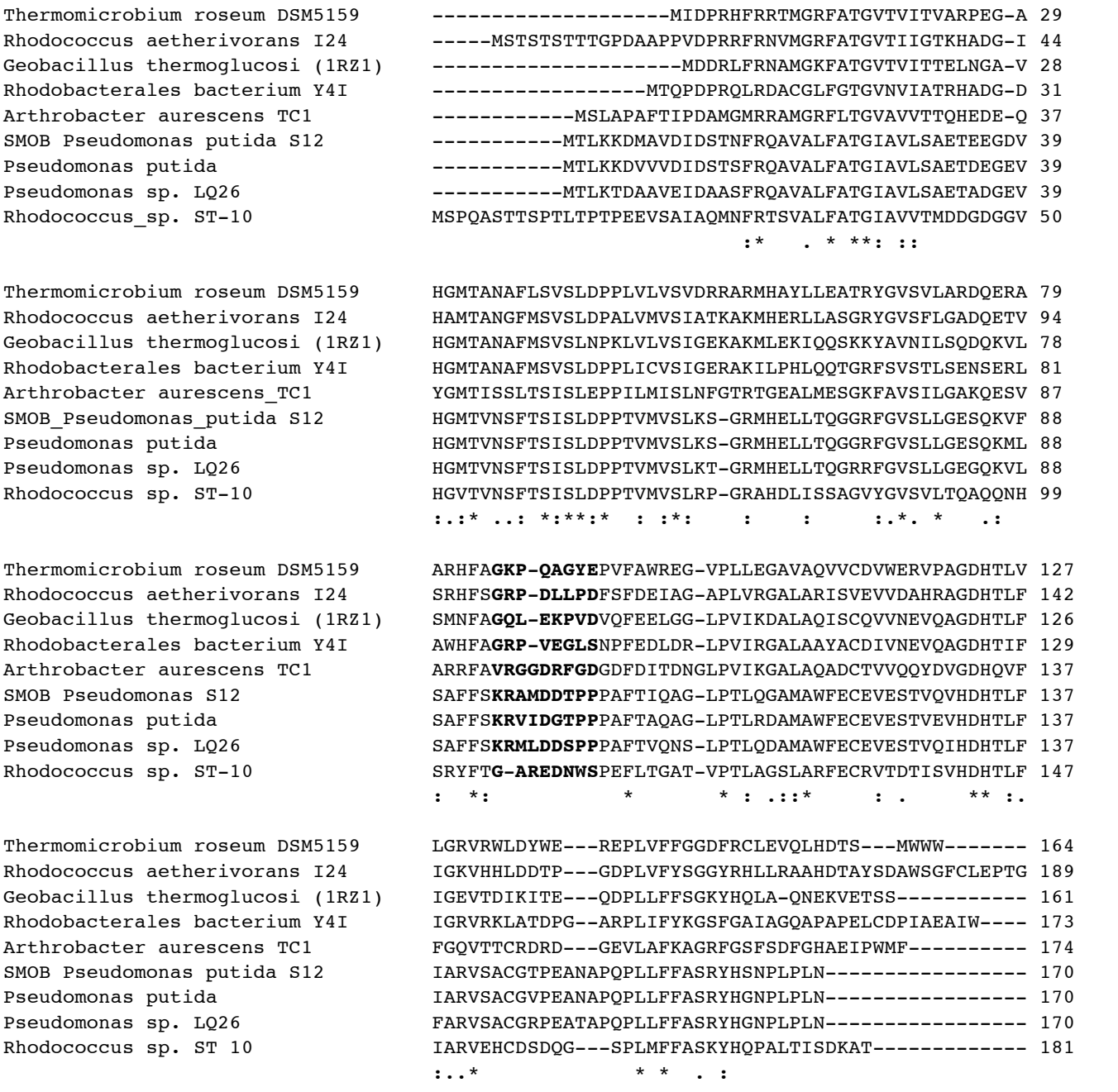

Figure S5. Sequence alignment of bacterial flavin reductases from multicomponent systems. The flexible loop responsible for binding the ADP moiety of FAD in 1RZ1 is highlighted in yellow. Percent identity of each protein to SMOB in in parenthesis next to the accession number. *Thermomicrobium roseum* DSM5159, YP_002522596.1 (37%); *Rhodococcus aetherivorans* I24, AAL61657.1 (39%) Geobacillus thermoglucosi, 1RZ1_A (38%); *Rhodobacterales bacterium* Y4I, ZP_05081154.1 (38%); *Arthrobacter aurescens* TC1, YP_946422.1 (37%); *Pseudomonas putida* S12, CAA04001.1 (100%);

Pseudomonas putida, ABB03728.1 (91%); *Pseudomonas* sp. LQ26, ADE62391.1 (85%); *Rhodococcus* sp. ST 10, BAL04130.1 (53%).

References:

(1) Ahmad, I., Cusanovich, M. A., and Tollin, G. (1981) Laser flash photolysis studies of electron transfer between semiquinone and fully reduced free flavins and horse heart cytochrome c. *Proceedings of the National Academy of Sciences of the United States of America* 78, 67246728.