Distinct flavin properties underlie flavin-based electron bifurcation in a novel electron transfer flavoprotein FixAB from *Rhodopseudomonas palustris*

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Running title: FixAB flavin properties underlying electron bifurcation.

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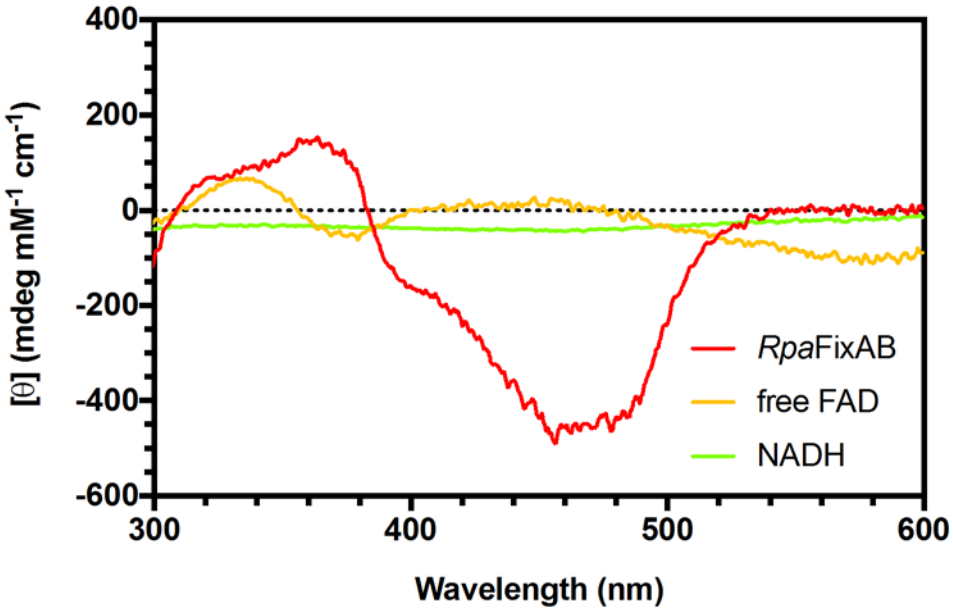

Figure S1. Comparison of near UV/Visible CD spectra of 65 µM *Rpa*FixAB, 130 µM free FAD and 130 µM NADH in 20 mM bis-Tris propane, pH 9.0, 200 mM KCl, 10% glycerol (w/v) and 1 mM TCEP at 4 °C.

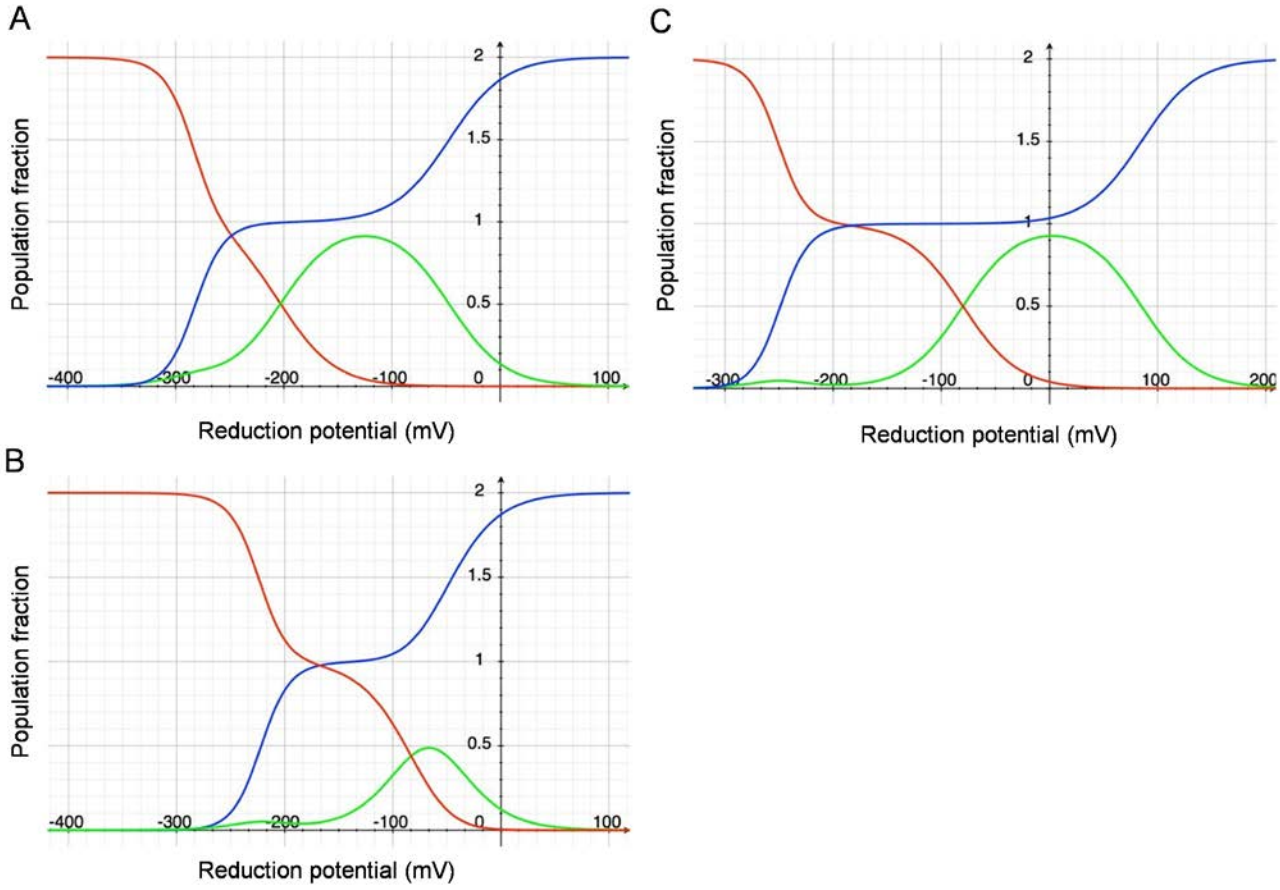

Figure S2. Populations of the different flavin oxidation states present in bifurcating ETF as a function of reduction potential. E° values for both flavins were combined to calculate the total population of OX (blue curves), ASQ (green curves) or HQ (red curves) for each of *Rpa*FixAB at pH 9.0 (A), *Rpa*FixAB at pH 7.0 (B) for comparison and *Mel*ETF at pH 6.0 (C), as in data shown by Sato et al (1). E° values were from the text for *Rpa*FixAB at pH 9.0 (A), from Table 1 for *Rpa*FixAB at pH 7.0 (B) and +85 mV, -80 mV and -250 mV for *Mel*ETF at pH 6.0 from Sato et al (1). Consistent with these simulations, considerably less ASQ accumulates when *Rpa*FixAB is titrated with NADH at pH 7.8 than at pH 9.0. The equations used were the following, where the E° superscripts identify the flavin to which they apply and the subscripts identify the couple to which they apply with the generalization that SQ indicates semiquinone, which in this case is ASQ. The simulations assume that for the Bf-FAD, the two one-electron couples are crossed by 120 mV, which is to say that $E_{OX/SQ}$ is 120 mV more negative than $E_{SQ/HQ}$.

$$HQ = \left(1 + 10^{\left(x - E^{Bf}_{SQ/HQ}\right)/59} + 10^{\left(2x - E^{Bf}_{SQ/HQ} - E^{Bf}_{OX/SQ}\right)/59}\right)^{-1} + \left(1 + 10^{\left(x - E^{ET}_{SQ/HQ}\right)/59} + 10^{\left(2x - E^{ET}_{SQ/HQ} - E^{ET}_{OX/SQ}\right)/59}\right)^{-1}$$

$$ASQ = \left(10^{\left(x - E^{Bf}_{SQ/HQ}\right)/59}\right)\left(1 + 10^{\left(x - E^{Bf}_{SQ/HQ}\right)/59} + 10^{\left(2x - E^{Bf}_{SQ/HQ} - E^{Bf}_{OX/SQ}\right)/59}\right)^{-1} + \left(10^{\left(x - E^{EF}_{SQ/HQ}\right)/59}\right)\left(1 + 10^{\left(x - E^{ET}_{SQ/HQ}\right)/59} + 10^{\left(2x - E^{ET}_{SQ/HQ} - E^{ET}_{OX/SQ}\right)/59}\right)^{-1}$$

$$OX = \left(10^{\left(2x - E^{Bf}_{SQ/HQ} - E^{Bf}_{OX/SQ}\right)/59}\right)\left(1 + 10^{\left(x - E^{Bf}_{SQ/HQ}\right)/59} + 10^{\left(2x - E^{Bf}_{SQ/HQ} - E^{Bf}_{OX/SQ}\right)/59}\right)^{-1} + \left(10^{\left(2x - E^{ET}_{SQ/HQ} - E^{ET}_{OX/SQ}\right)/59}\right)\left(1 + 10^{\left(x - E^{ET}_{SQ/HQ}\right)/59} + 10^{\left(2x - E^{ET}_{SQ/HQ} - E^{ET}_{OX/SQ}\right)/59}\right)^{-1}$$

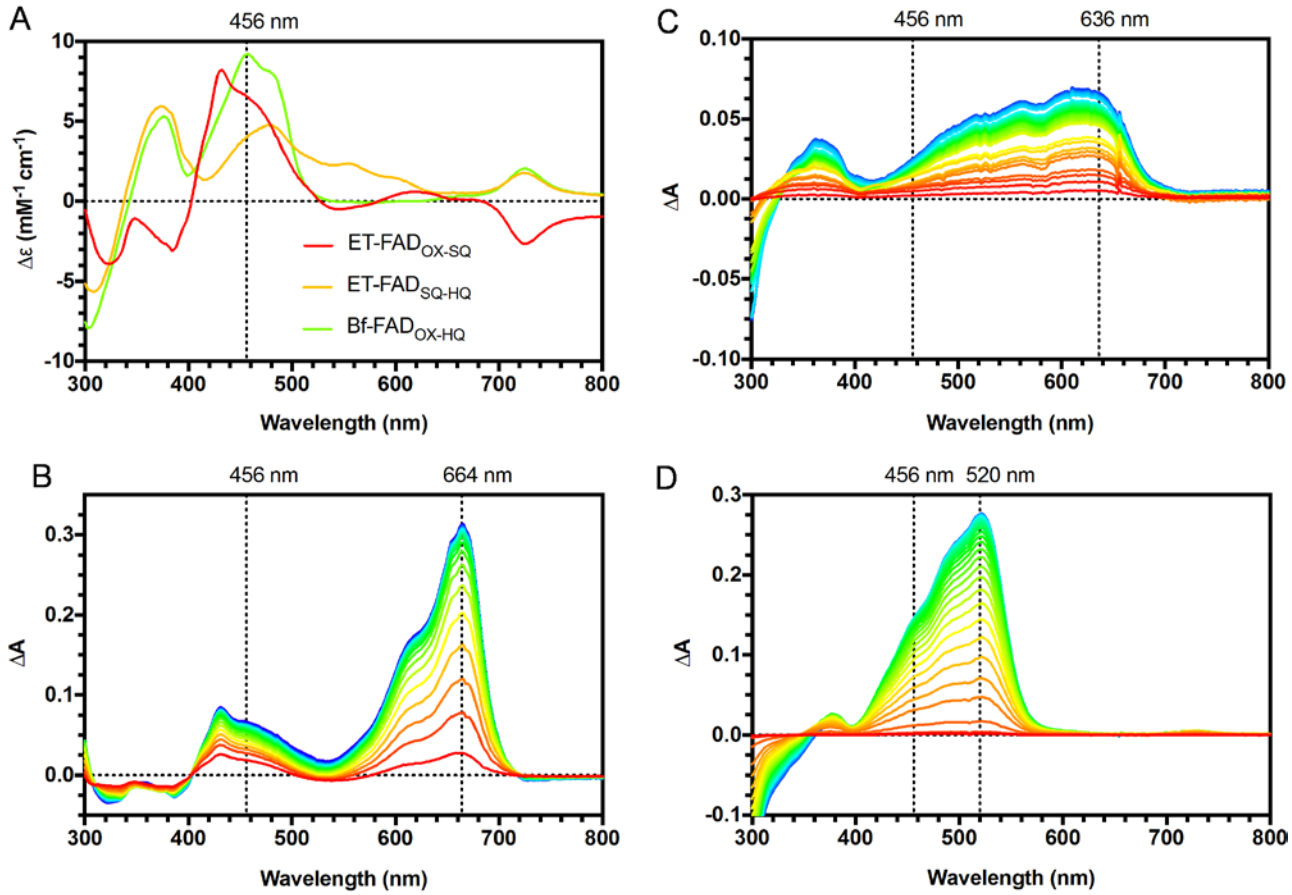

Figure S3. (A) Difference spectra obtained from extrema spectra for the three phases. Data were derived from Figure 4C. By doing so, the absorbance contribution from the flavin that does not change in each phase is eliminated. Difference spectra used for calculation of midpoint potentials associated with the three phases using methylene blue (B), Nile blue (C) and phenosafranin (D) as the reference dye, respectively. The rainbows indicate the increase of the poplulation of the reduced species at the wavelength monitored. Reduction of FAD was monitored at 456 nm, reduction of methylene blue at 664 nm, reduction of Nile blue at 636 nm and reduction of phenosafranin at 520 nm.

References

1. Sato, K., Nishina, Y., and Shiga, K. (2013) Interaction between NADH and electron-transferring flavoprotein from Megasphaera elsdenii. *Journal of biochemistry* **153**, 565-572