Supporting Information

Figure SI.1. Spectrum of NR reduction by Fd_{red} in the presence of nitrate with [PSI] = [NR] × 2. A. The spectrum was recorded under conditions similar to those of Figure 4 (1 mM sodium nitrate, differences between signals recorded in two 1-mm cuvettes, averages of 4 signals, time interval of 7 s between two consecutive measurements) except for the protein concentrations. Data from two different sets of measurements are shown together. First set: 0.75 µM PSI, 6 µM Fd and 0.4 µM NR. Second set: 1.56 µM PSI, 8 µM Fd and 0.75 µM NR. The signals were obtained after fitting the data with a constant between 100 and 150 ms after the flash. The standard error of the fit parameter is less than 0.03 $mM^{-1}cm^{-1}$. The vertical scale was converted to millimolar absorption coefficients from the PSI concentration. The black continuous line corresponds to the Fd difference spectrum ($Fd_{ox} – Fd_{red}$) and the gray one corresponds to 85% of the former Fd spectrum. B. An example of absorption change kinetics is shown at 490 nm (1.56 µM PSI).

Figure SI.2: Incomplete electron transfer from Fd_{red} to NR in the absence of nitrate. A. Difference between flash-induced absorption changes at 490 nm recorded in the presence and in the absence of NR, with both 1-cm cuvettes containing 0.186 µM PSI and 2 µM Fd. The lower and upper traces correspond to NR concentrations of 0.5 and 2 µM, respectively. Each trace represents an average of 4 measurements (Δt = 3 or 8 min between two measurements for the upper and lower traces, respectively). With the final amplitude of the lowest trace being 64% that of the upper one, a crude calculation of the equilibrium constant K_{eq} for Reaction (1) ($Fd_{red} + NR_{ox} <-> Fd_{ox} + NR_{red1}$) can be made by assuming that the upper trace corresponds to 100% electron transfer: $K_{eq} = ([Fd_{ox}] \times [NR_{red1}])/([Fd_{red}] \times [NR_{ox}])$ ("final" concentrations , at 70 ms) with $[NR_{red}] = [PSI] \times 0.64$

= 0.119 μM, $[NR_{ox}]$ = 0.5 μM - $[NR_{red}]$ = 0.381, $[Fd_{red}]$ = 0.186 μM - $[NR_{red}]$ = 0.067 and $[Fd_{ox}]$ = 2 μM - $[Fd_{red}]$ = 1.933 μM. One gets K_{eq} = 9, corresponding to a difference of 56 mV between the midpoint potentials (E_m) of Fd and NR. With E_m(Fd) = -412 mV vs NHE (Bottin, H and Lagoutte, B. (1992) *Biochim. Biophys. Acta 1101*, 48-56), one gets $E_m(NR_{\text{first reduction, no nitrate}})$ = -357 mV. Conversely, one can easily calculate the percentage of electron transfer for a given value of $E_m(NR_{\text{first reduction, no nitrate}})$ by solving a quadratic equation in $[NR_{red1}]$. With E_m = -300 mV, one finds that the signal for [NR] = 0.5 μM should be 92% that of the signal with [NR] = 2 μM. Therefore our data suggest that $E_m(NR_{\text{first reduction, no nitrate}})$ is lower than -300 mV. B. Similar experiment as (A) at 580 nm in the presence of 1 mM nitrate. 1-cm cuvettes contained 0.21 μM PSI and 2 μM Fd. As in (A), the lower and upper traces correspond to NR concentrations of 0.5 and 2 μM, respectively. Each trace represents the average of 4 measurements (Δt = 10 seconds between two measurements).

Figure SI3: Spectrum of NR reduction by Fd_{red} with NR in large excess over PSI, in the absence of nitrate and just after a pre-flash. The spectrum was recorded with the concentrations used for measuring the spectrum in the absence of nitrate in Figure 6B, except that the measurement was preceded by an unrecorded preflash given 10 seconds before the measurement. The signals were obtained after fitting the data with a constant between 35 and 45 ms after the flash. The standard error of the fit parameter is 0.03 to 0.04 $mM^{-1}cm^{-1}$. The vertical scale was converted to millimolar absorption coefficients from the PSI concentration.

Figure SI.1

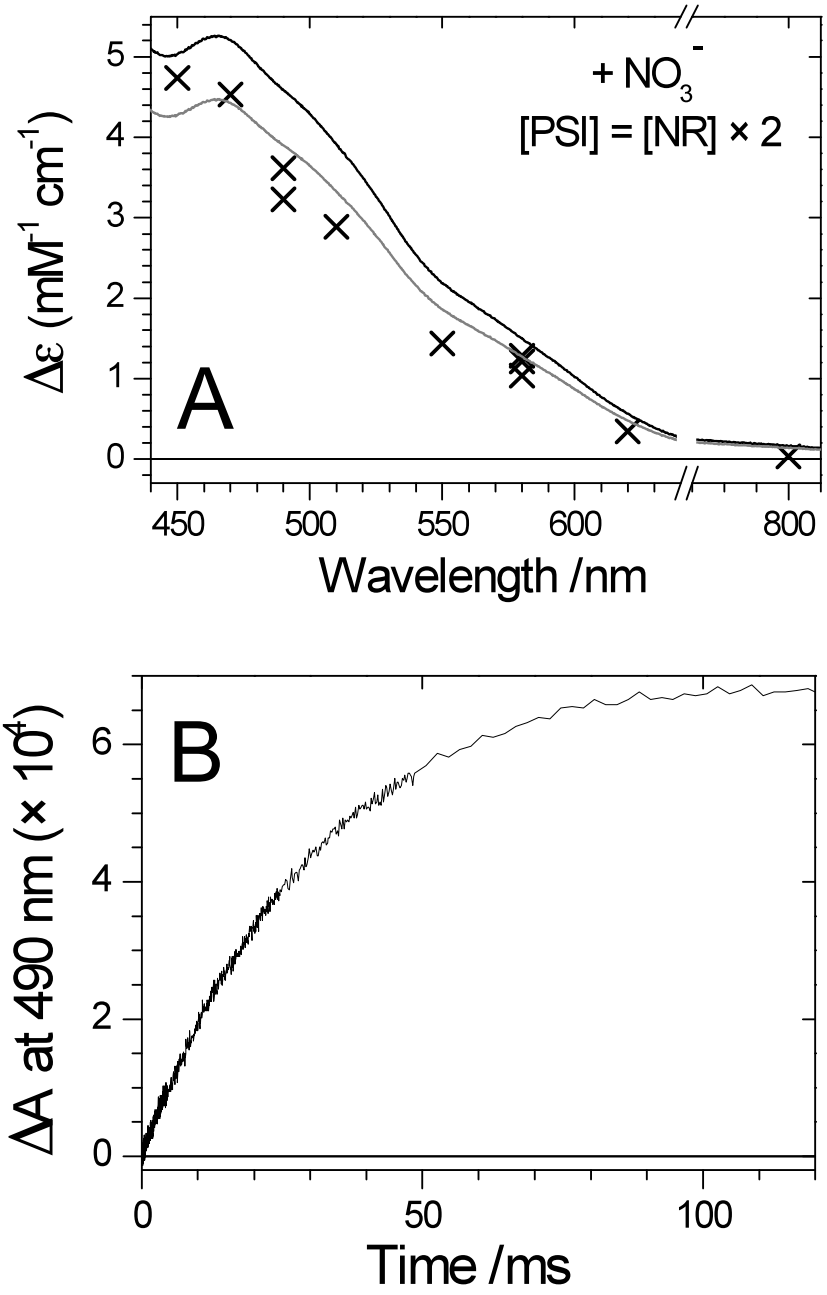


Figure SI.2

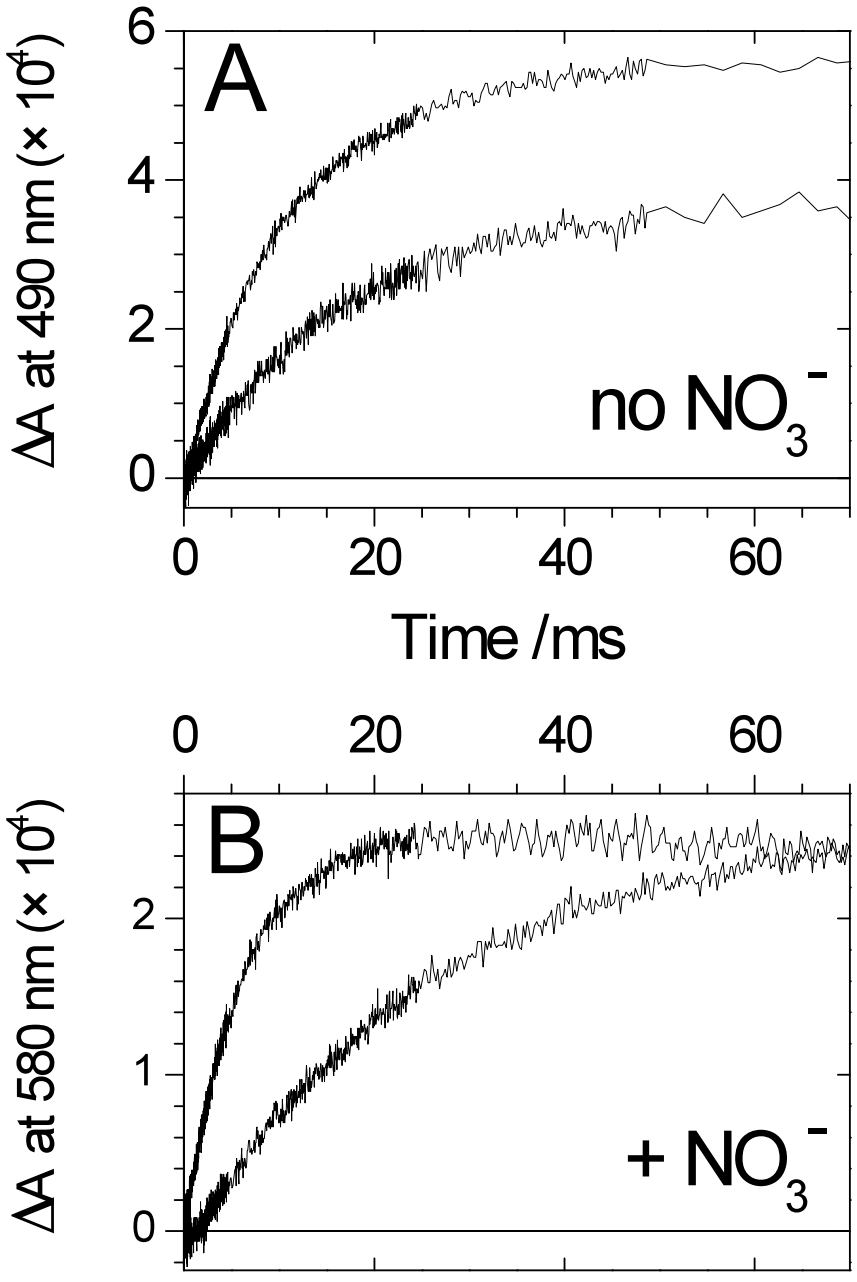
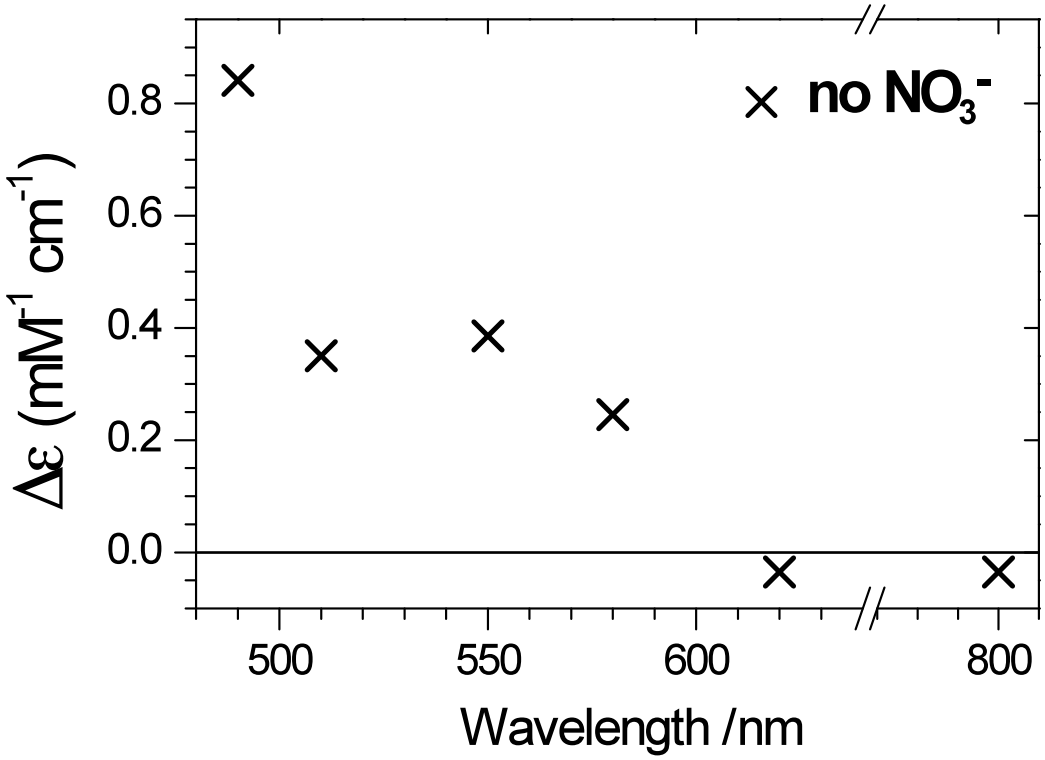

A
ΔA at 490 nm (× 10⁴)
6
4
2
0
no NO3⁻
0
20
40
60
Time /ms
B
ΔA at 580 nm (× 10⁴)
2
1
0
+ NO3⁻

Figure SI.3


no NO_3^-
$\Delta\varepsilon$ (mM^{-1} cm^{-1})
0.0
0.2
0.4
0.6
0.8
500
550
600
800
Wavelength /nm