Supplemental data

Supplemental Figure 1.

(1) $$A \overset{k_1}{\rightarrow} B \overset{k_2}{\rightarrow} C$$

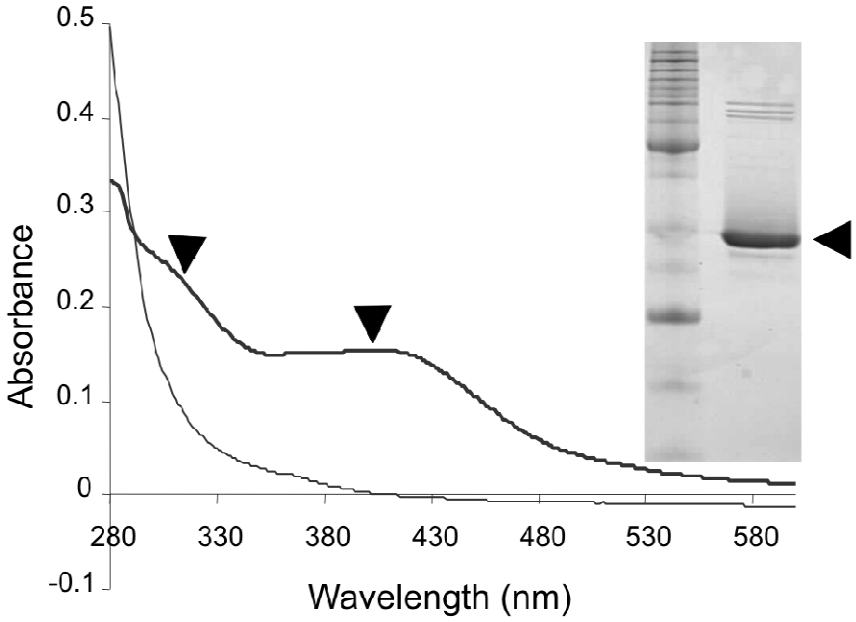
(2) $$\frac{d[A]}{dt} = -k_1[A], \frac{d[B]}{dt} = k_1[A] - k_2[B], \frac{d[C]}{dt} = k_2[B]$$

(3) $$A(t) = A_0 e^{-k_1 t}, B(t) = \frac{A_0 k_1}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right), C(t) = \frac{A_0}{k_2 - k_1}\left(k_1 e^{-k_2 t} - k_2 e^{-k_1 t} + k_2 - k_1\right)$$

(4) $$\alpha(t) = A_0\left[\alpha_C + \frac{(\alpha_A(k_2 - k_1) + \alpha_B k_2 - \alpha_C k_2)}{k_2 - k_1} e^{-k_1 t} + \frac{k_1(\alpha_B - \alpha_C)}{k_2 - k_1} e^{-k_2 t}\right]$$

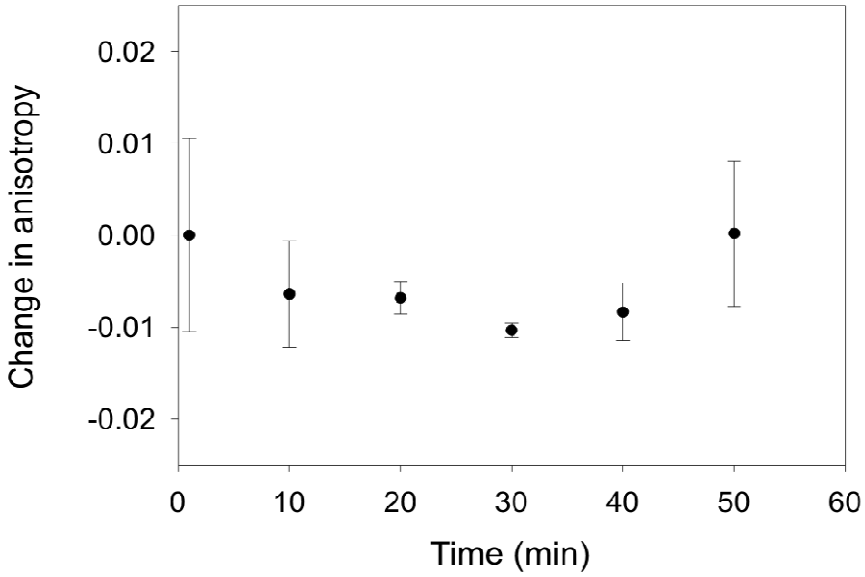
Supplemental Figure 1. To fit the kinetic data we adopted a model consisting of two, sequential reaction steps (1) where, for example, A represents the 4Fe-4S cluster, B represents the 3Fe-4S, and C represents the 2Fe-2S. If we assume these reactions proceed via mass-action kinetics then this model can be equivalently described as a sequence of differential equation for the concentrations of the chemicals (2) where it is the concentration of the intermediate [B] that is of primary interest. Under these assumptions the rate constants, k_1 and k_2, may be also be termed rates as they have the units of time^{-1}. This system of equations has the useful property of being exactly solvable, so that we can determine A(t), B(t) and C(t), given the initial conditions [A](t=0) = A_0, [B](t=0) = 0, [C](t=0) = 0 as shown in (3). To fit this equation to the data we require the absorbance, α, as a function of time. Denoting the absorbance of substance A as α_A and so forth, the equation for the absorbance at time t is given by (4). We can experimentally determine A_0 and the absorbances α_A and α_C can be determined in the limits $t \rightarrow 0$ and $t \rightarrow \infty$, respectively. This exact form can then be fitted to the data using a standard non-linear least square fit implemented, in this case using the R routine nls, to provide fitted values for k_1, k_2 and α_B.

Suppplemental Figure 2.



Supplemental Figure 2. UV/visible spectra of NmFNR. Spectra of NmFNR prepared with incorporated cofactor (thick line) and apo-NmFNR (thin line) are shown. The peak at 405 nm and the shoulder around 310 nm are marked with arrowheads. Inset shows a gel with purified NmFNR (right hand lane, arrowhead marks major band at 28 kDa) and molecular weight standards (Benchmark™, Invitrogen, left hand lane).

Supplemental Figure 3.



Supplemental Figure 3. The NmFNR-DNA complex from Figure 4 was aerated and the fluorescence anisotropy measured over the period of an hour. No significant loss of anisotropy was observed.