Supplementary Materials

Probing the reactivity of [4Fe-4S] FNR with O_2 and NO: increased O_2 resistance and relative specificity for NO of the [4Fe-4S] L28H FNR cluster

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Supplementary Figures

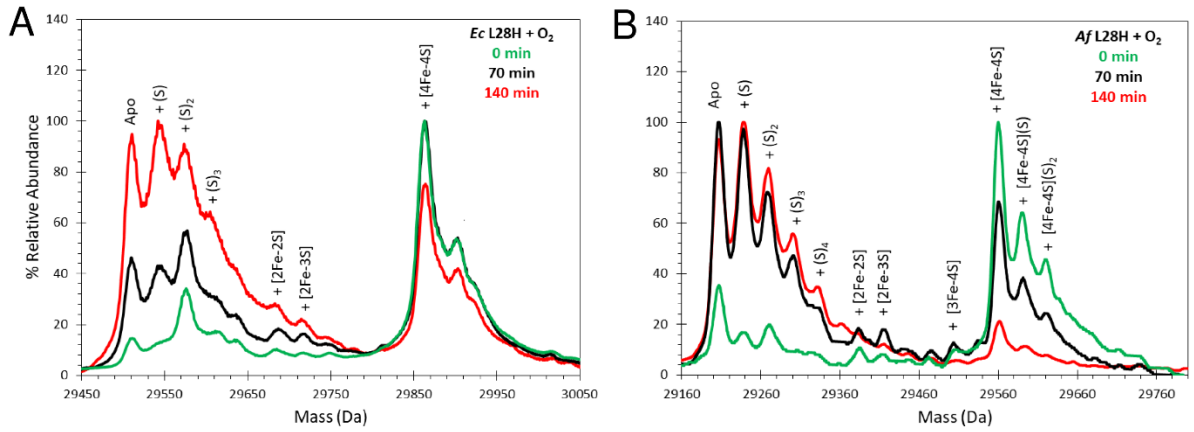

Figure S1. Reaction of L28H-*Ec*FNR and L28H-*Af*FNR with O_2 monitored by native MS. Deconvoluted mass spectrum of (**A**) L28H-*Ec*FNR and (**B**) L28H-*Af*FNR before (green line) and after (70 min, black line; 140 min, red line) exposure to O_2. Persulfide adducts of the apo proteins are the major products observed.

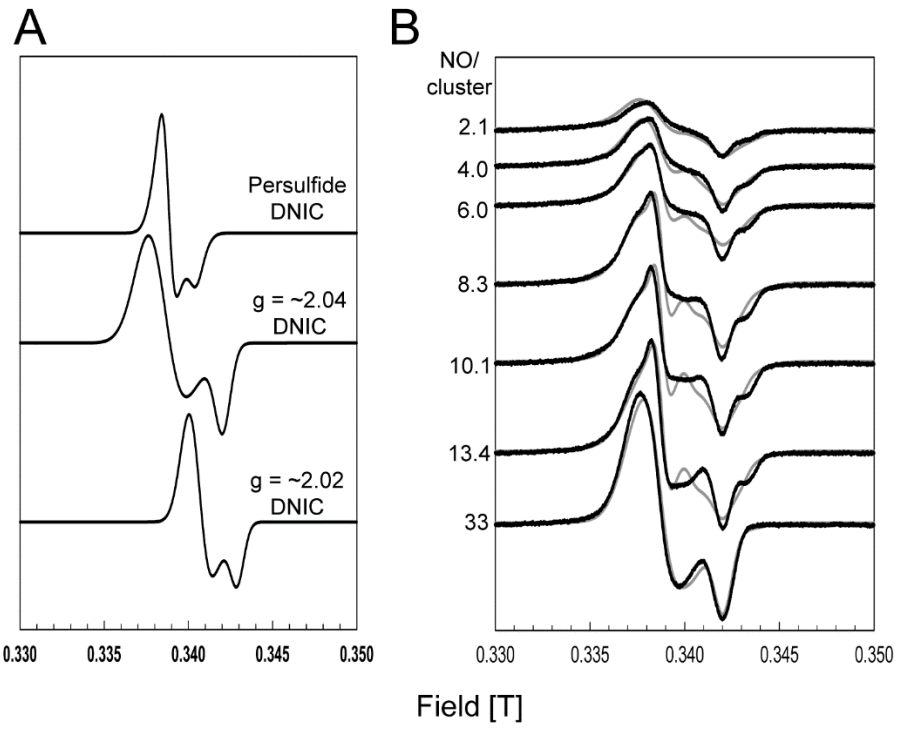

Figure S2. Deconvolution of EPR spectra resulting from nitrosylation of [4Fe-4S] L28H-*Ec*FNR. (**A**), Simulated EPR spectra of persulfide-ligated DNIC ($g_{\perp}$ = 2.044, $g_{=}$ = 2.032) [50,60], and two thiol-coordinated DNICs at g ≈ 2.04 ($g_{\perp}$ = 2.045, $g_{=}$ = 2.023) [61,62], and g ≈ 2.02 ($g_{\perp}$ = 2.023, $g_{=}$ = 2.016) [28,63] that were used to deconvolute the recorded (74 K) EPR spectra in (B). (**B**), EPR spectra for nitrosylated L28H-*Ec*FNR (data, black lines), together with simulations of the experimental data (R^2 ≈0.90) (gray lines) obtained by the combination of varying amounts of the spectra shown in *A*.

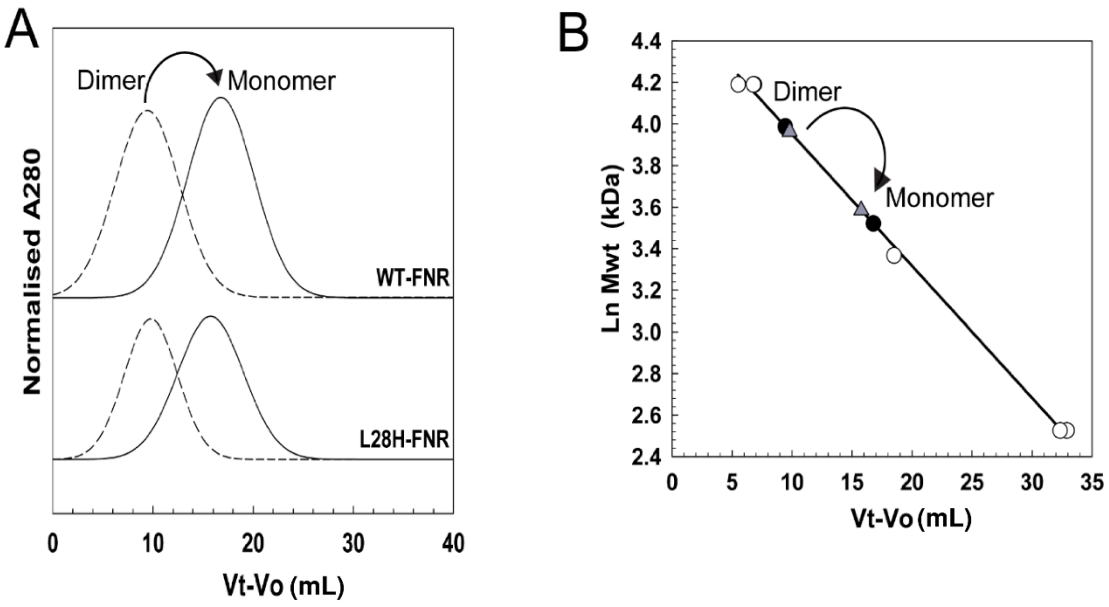

Figure S3. Gel filtration of [4Fe-4S]- L28H-*Ec*FNR before and after nitrosylation. (**A**) Chromatogram for L28H-*Ec*FNR pre- (dashed line) and post-NO exposure (solid black line). (**B**) Standard calibration curve for Sephacryl S100HR column. White circles correspond to standard proteins (BSA, carbonic anhydrase, cytochrome *c*). Grey triangles correspond to L28H-*Ec*FNR (pre-NO, 53 kDa; post-NO, 37 kDa), respectively. Black circles correspond to wt-*Ec*FNR (see [24] for further details). The data indicate that nitrosylation of [4Fe-4S]-L28H-*Ec*FNR results in an iron-nitrosyl bound FNR monomer. Vo is the void volume of the column; Vt is the total elution volume, including void volume.

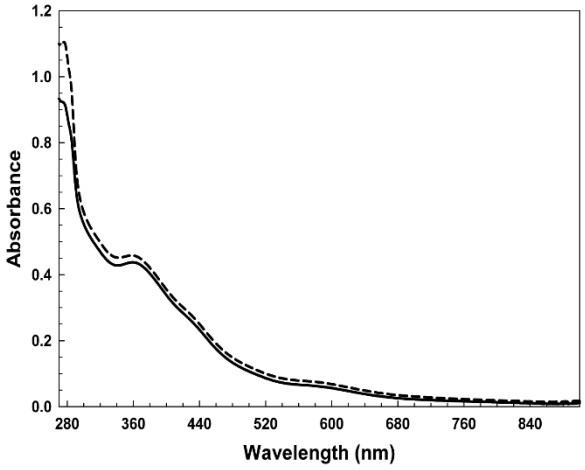

Figure S4. UV-visible properties of nitrosylated [4Fe-4S] L28H-*Ec*FNR. Absorbance spectrum of nitrosylated L28H-*Ec*FNR (dashed black line) after rapid gel filtration (PD10, Cytiva). The samples contained ~1.4 (54 μM) RRE per protein, based on a $\varepsilon_{362\ nm}$ value of 8530 M^{-1} cm^{-1} [61]. The L28H sample corresponds to sample 3 in Table 1. The previously published [24] spectrum of nitrosylated wt FNR (solid black line) is shown for comparison.

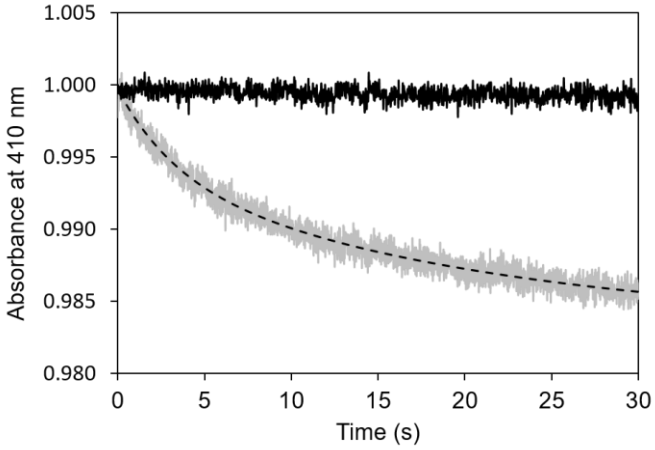

Figure S5. Reaction of [4Fe-4S]-*Af*FNR with O_2. Stopped-flow measurements for the wild-type (gray) and the L28H-FNR variant (black). Curves are translated to $Abs_{410\ nm}$=1 at time 0. The dashed line represents the fit of the wild-type curve to a two-phase exponential decay equation.

Supplementary Tables

Table S1. X-ray data collection and refinement statistics for [4Fe-4S] L28H-*Af*FNR.

X-ray data		**Refinement**	
Space group	I422	Resolution (Å)	47.77-2.40
Cell dimensions		Reflections (work / free)	12080 / 568
a=b, c (Å)	75.20 217.47	R_{work} / R_{free}	19.8 / 22.2
α=β=γ (°)	90	Number of atoms	
Resolution (Å)[a]	47.8-2.4 (2.48-2.4)	Protein	1839
R_{merge} (%)	7.1 (340.8)	[4Fe-4S] cluster	8
mean(I/σ(I))	18.6 (0.7)	Solvent (MPD and water)	115
CC(1/2)	1.000 (0.449)	Average B factors (Å^2)	
Total number of observations	147841 (14671)	Protein	88.2
Total number unique	12709 (1205)	[4Fe-4S] cluster	91.0
Completeness (%)	99.9 (99.7)	Solvent	86.6
Redundancy	11.6 (12.2)	R.m.s. deviations	
Anomalous completeness (%)	99.8 (99.4)	Bond lengths (Å)	0.003
Anomalous redundancy	6.2 (6.4)	Bond angles (°)	0.53
		Ramachandran plot outliers[b]	0
		Rotamer outliers (%)[b]	1.5
		All atom clash score[b]	2.7

[a] Numbers within brackets denote the highest resolution shell.

[b] Molprobity statistics [64].

Table S2. Rate constants for the nitrosylation of [4Fe-4S] L28H-*Ec*FNR with NO and comparison to wild-type FNR and Wbl proteins.

		Rate constants/M^{-1} s^{-1} [a]			
Phase	**Step**	**WT FNR**	**L28H FNR**	**WhiD**	**WhiB1**
1	A→B	2.81 ± 0.10 x10^5	1.33 ± 0.02 x10^5	6.50 x10^5	4.40 x10^5
2	B→C	1.89 ± 0.10 x10^4	1.59 ± 0.07 x10^4	2.13 x10^4	1.38 x10^4
3	C→D	4.61 ± 0. 25 x10^3	9.51 ± 0.49 x10^3	5.36 x10^3	8.34 x10^3
4	D→E	0.75 ± 0.03 x10^3	1.29 ± 0.09 x10^3	1.00 x10^3	0.90 x10^3

[a] Derived from linear fits of the data at low NO concentrations in Fig. 5. Rate constants for the nitrosylation of wild type FNR, *S. coelicolor* WhiD and *M. tuberculosis* WhiB1 [24, 28], are shown for comparison.