SUPPLEMENTARY MATERIAL

A NEW CATALYTIC MECHANISM OF BACTERIAL FERREDOXIN-NADP$^+$ REDUCTASES DUE TO A PARTICULAR NADP$^+$ BINDING MODE.

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Table S1. Determination of the content of $NADP^+$ bound to purified FNR enzymes from different organisms by HPLC.

Enzyme	$NADP^+$/FNR (mol/mol)
EcFPR	0.85 ± 0.15
PcFPR	0.58 ± 0.11
BaFPR	0.30 ± 0.03
LepFNR	0.17 ± 0.01
PeaFNR	0.11 ± 0.03

C18 column with a mobile phase composed of 0.2 M ammonium phosphate, pH 5.1 and a gradient with methanol at a final concentration of 30%. Values are mean ± S.D. of at least three independent measurements.

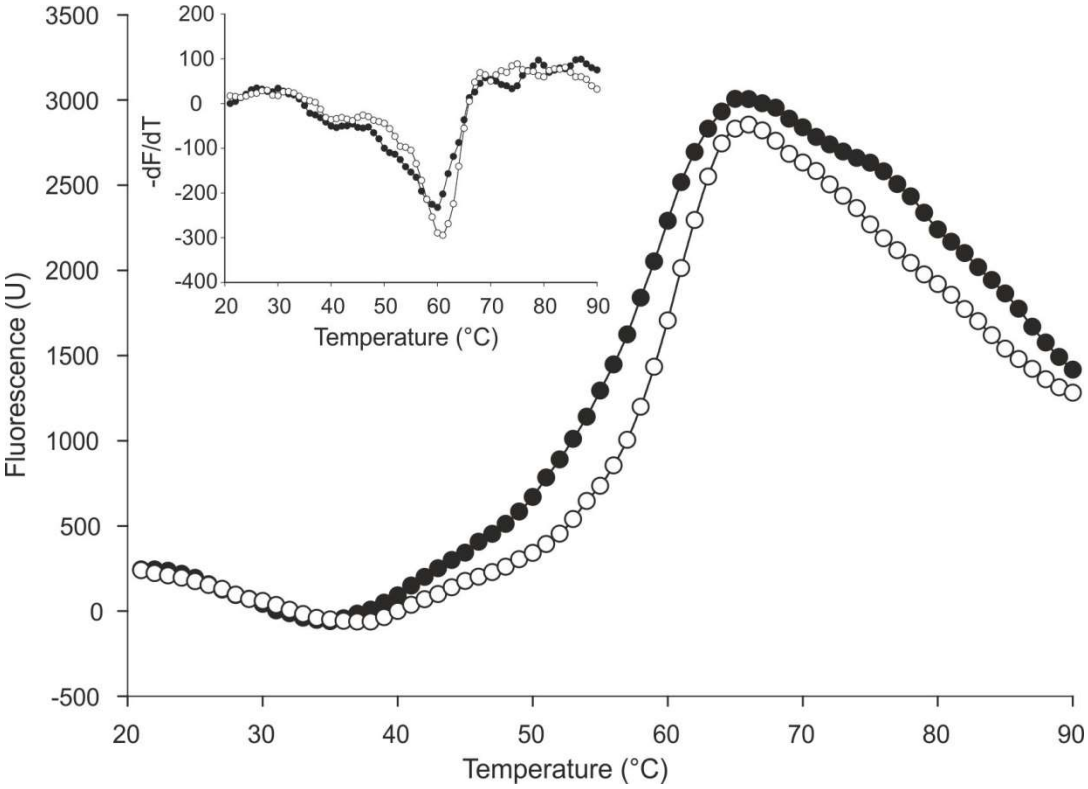


Figure S1: Thermal stability of EcFPR* in the presence or absence of $NADP^+$. The fluorescence emitted was plotted as a function of temperature. The inner graph shows the first derivative of fluorescence emission (-dF / dT) as a function of the temperature, where the minimum value of the curve corresponds to T_m. *EcFPR eluted from the Cibacron Blue column.

Table S2. Melting points of thermal unfolding transitions of the FNRs.

Enzyme	T_m (°C)	
	FNR	**FNR***
EcFPR	57.4 ± 0.7	55.3 ± 0.9
PcFPR	54 ± 1	52.5 ± 0.7
BaFPR	57.6 ± 0.8	58 ± 1
LepFNR	60 ± 1	59.4 ± 0.6
PeaFNR	62.3 ± 0.7	61 ± 1

Values are mean ± S.D. of at least three independent measurements. *FNR eluted from the Cibacron Blue column.

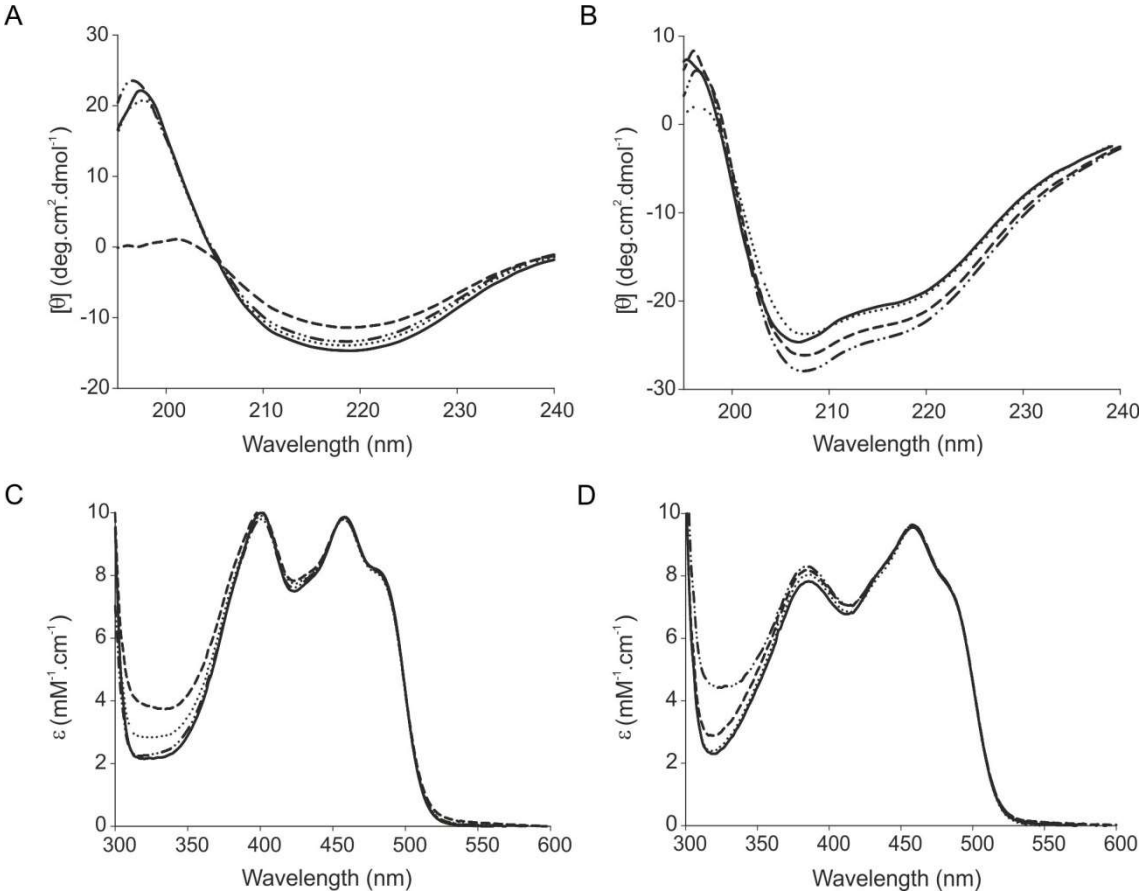


Figure S2: Spectroscopic characterization of wild type and mutant EcFPR and PeaFNR. A) and B) Circular dichroism (CD) spectra in the far UV region of EcFPR and PeaFNR, respectively. C) and D) Absorption spectra of EcFPR and PeaFNR, respectively. . Solid line, wild type EcFPR and PeaFNR; dotted line, EcFPR_R144P and PeaFNR_P199R; dash line, EcFPR_R184Y and PeaFNR_Y240R; dash and dots line, EcFPR_R144P_R184Y and PeaFNR_P199R_Y240R.

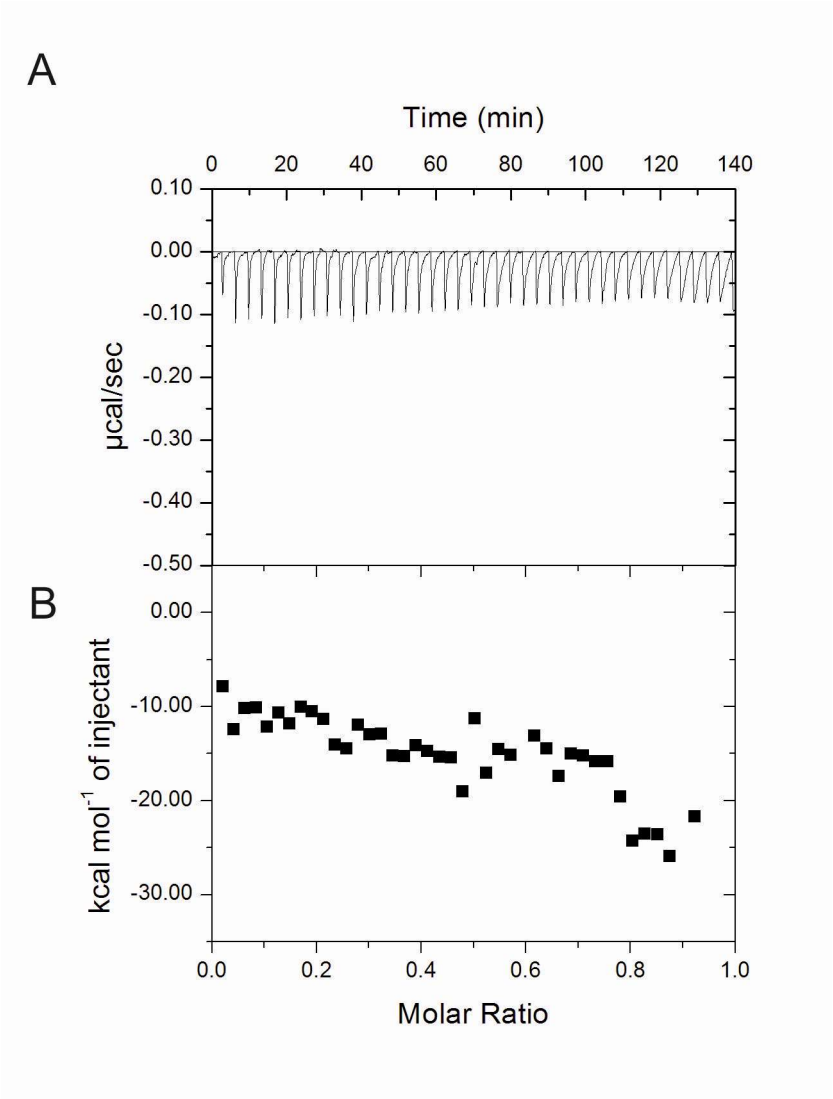
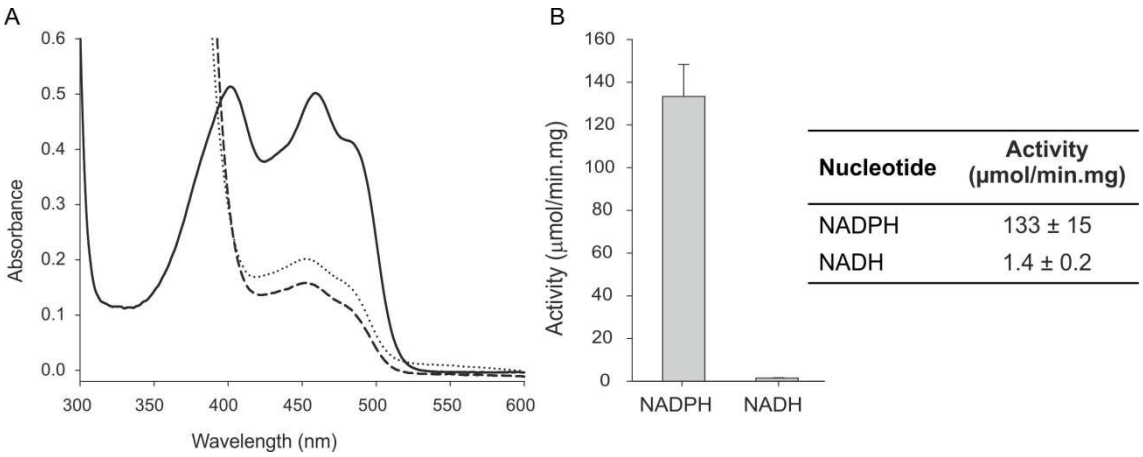


Figure S3: Isothermal titration calorimetry (ITC) for the binding of $NADP^+$ to EcFPR. A) Primary raw data. B) Binding curve derived from the raw data. All the $NADP^+$ added in substoichiometric quantities formed a complex, showing a constant thermal evolution consistent with the addition of $NADP^+$.

Table S3. Melting points of thermal unfolding transitions of wild type and mutant EcFPR and PeaFNR.

Enzyme	T_m (°C)	
	FNR	**FNR***
EcFPR	58.1 ± 0.6	55.3 ± 0.9
EcFPR_R144P	59 ± 1	59.8 ± 0.8
EcFPR_R184Y	57.6 ± 0.6	58.2 ± 0.7
EcFPR_R144P_R184Y	59.4 ± 0.8	59.7 ± 0.6
PeaFNR	60.3 ± 0.8	60.7 ± 0.8
PeaFNR_P199R	58.2 ± 0.4	58.7 ± 0.6
PeaFNR_Y240R	58 ± 1	58.5 ± 0.4
PeaFNR_P199_Y240R	57 ± 1	56.4 ± 0.7

Values are mean ± S.D. of at least three independent measurements. *FNR eluted from the Cibacron Blue column.



Nucleotide	Activity (µmol/min.mg)
NADPH	133 ± 15
NADH	1.4 ± 0.2

Figure S4: Electron transfer between EcFPR and NADPH or NADH. A) Reduction of EcFPR flavin by NADPH or NADH. The spectral changes produced by the addition of the nucleotide substrates are observed in the FAD absorption peak (456 nm). Solid line, EcFPR; dotted line, EcFPR + NADPH; dash line, EcFPR + NADH. B) Determination of NADPH-ferricyanide diaphorase activity of EcFPR.