# Evidence of negative cooperativity and half-site reactivity within an $F_{420}$ -dependent enzyme: A kinetic analysis of $F_{420}H_2$ :NADP<sup>+</sup> oxidoreductase

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1 CGT GTG GCT CTG CTG GGC GGT ACG GGC AAC CTG GGC AAA GGT CTG GCA CTG CGT CTG 57 1 R V A L L G G T G N L G K G L A L R L 19 58 GCA ACC CTG GGT CAT GAA ATC GTG GTC GGC TCA CGT CGC GAA GAA AAA GCG GAA GCC 114 20 A T L G H E I V V G S R R E E K A E A 38 115 AAA GCG GCC GAA TAT CGT CGC ATT GCA GGC GAT GCT TCG ATC ACC GGT ATG AAA AAC 171 39 K A A E Y R R I A G D A S I T G M K N 57 172 GAA GAC GCA GCT GAA GCG TGC GAT ATT GCC GTG CTG ACC ATC CCG TGG GAA CAT GCA 228 58 E D A A E A C D IAVLT I P WEHA 229 ATT GAC ACG GCT CGT GAT CTG AAA AAT ATT CTG CGC GAA AAA ATC GTT GTT AGT CCG 285 76 I D T A R D L K N I L R E K I V V S P 93 286 CTG GTG CCG GTT TCC CGT GGT GCC AAA GGT TTT ACC TAC AGC TCT GAA CGC TCA GCG 342 94 L V P V S R G A K G F T Y S S E R S A 111 343 GCC GAA ATT GTT GCC GAA GTC CTG GAA AGC GAA AAA GTC GTG TCT GCC CTG CAC ACG 399 112 A E I V A E V L E S E K V VS ALH T 130 400 ATC CCG GCA GCT CGT TTT GCA AAC CTG GAT GAA AAA TTC GAC TGG GAT GTC CCG GTG 456 131 I P A A R F A N L D E K F D H D V P V 149 457 TGT GGC GAT GAC GAT GAA AGC AAA AAA GTT GTC ATG TCA CTG ATT TCG GAA ATT GAT 513 150 C G D D D E S K K V V MSLISE I D 167 514 GGT CTG CGT CCG CTG GAT GCC GGT CCG CTG AGT AAT TCC CGC CTG GTT GAA TCT CTG 570 168 G L R P L D A G P L S N S R L V E S L 185 571 ACG CCG CTG ATT CTG AAC ATT ATG CGT TTT AAC GGT ATG GGC GAA CTG GGT ATC AAA 627 T P L I L N I M R F N G M G E L G I K 628 TTT CTG TGA FL .

#### SI Figure 1:

wtFno sequence synthesized by Genescript (8).



SI Figure 2:

SDS Page gel of Fno purification protocol (lower figure): lane 1, Marker; lane 2, Fno sample after sonication; lane 3, after 90 °C heat precipitation; lane 4, following 40-70 %  $(NH_4)_2SO_4$  precipitation at 4 °C; lane 5, sample after DEAE Cellulose anion exchange column; and lane 6, purified Fno after size exclusion chromatography (8).



#### SI Figure 3

Chromatogram after size exclusion column (Sephacryl S-200). Fno collected from fractions 7-12 (8).



	Coefficient	Std. Error	t	Р
F <sub>max</sub>	0.6505	0.2833	2.2960	0.0553
n	0.9206	1.2898	0.7137	0.4985
$K_d$	2.4955	3.3482	0.7453	0.4803

## SI Figure 4

FO binding experiment in the presence of Tryptophan (no Fno). There is no good fit with the Adair equation nor the Hill equation.



#### Upper Figure

	Coefficient	Std. Error	t	Р
F <sub>max</sub>	0.7767	0.1740	4.4643	0.0010
n	0.9097	0.7598	1.1973	0.2564
$K_d$	1.0342	0.9106	1.1357	0.2802

## Lower Figure

	Coefficient	Std. Error	t	Р
F <sub>max</sub>	21.4657	916.6599	0.0234	0.9818
n	0.3039	0.4070	0.7467	0.4743
$K_d$	587391.6912	95732477.2105	0.0062	0.9952

#### SI Figure 5

Upper panel is NADPH binding in the presence of Tryptophan. There is no good fit, when using all data points (which is what we do with Fno). When fit after removing the highest NADPH concentrations, the  $K_d$  changes significantly and is no longer in the nM range.



#### SI Figure 6

Panel shows that there is no change in emission at 340 nm with tryptophan (red bars). There is a clear change in emission at 340 nm in the presence of Fno (I135G) shown in blue.



SI Figure 7

The steady-state kinetics of reduction of FO by Fno is shown for varying NADPH concentrations. The reaction is carried out in 50 mM MES/NaOH (pH 6.5) buffer at 22 °C in a stopped-flow spectrophotometer. A constant FO concentration of 25  $\mu$ M was used. The plot of NADPH concentration vs rate constant during the steady state kinetics shows non-Michaelis-Menten kinetic behavior at NADPH concentrations >100  $\mu$ M. The kinetic parameters are shown in Table 1.

#### Excitation 290 nm Emission 340 nm

Coefficient	Std.	Error	t	Р
F <sub>max</sub>	0.9951	0.0281	35.3731	< 0.0001
Hill	0.6230	0.0494	12.6096	< 0.0001
$K_d$	14.9621	2.1251	7.0406	< 0.0001

#### SI Table 1

Binding data of Fno for varying concentrations of FO (0-2100 nM FO in the presence of 200 nM NADP<sup>+</sup>

#### Excitation 290 nm Emission 340 nm

Coefficient	Std.	Error	t	Р
F <sub>max</sub>	1.1597	0.0373	31.1277	< 0.0001
Hill	0.6058	0.0301	20.136	< 0.0001
$K_d$	3.6035	0.4677	7.7054	< 0.0001

#### SI Table 2

Binding data of Fno in the presence of varying concentrations of NADP<sup>+</sup>.