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The key role of glutamate 172 in the mechanism of type II NADH:quinone oxidoreductase of *Staphylococcus aureus*

Filipe M. Sousa[1], Filipa V. Sena[1], Ana P. Batista[2], Diogo Athayde[1], José A. Brito[1], Margarida Archer[1], A. Sofia F. Oliveira[1,3], Cláudio M. Soares[1], Teresa Catarino[1,4] and Manuela M. Pereira[1]*

Supplementary Information

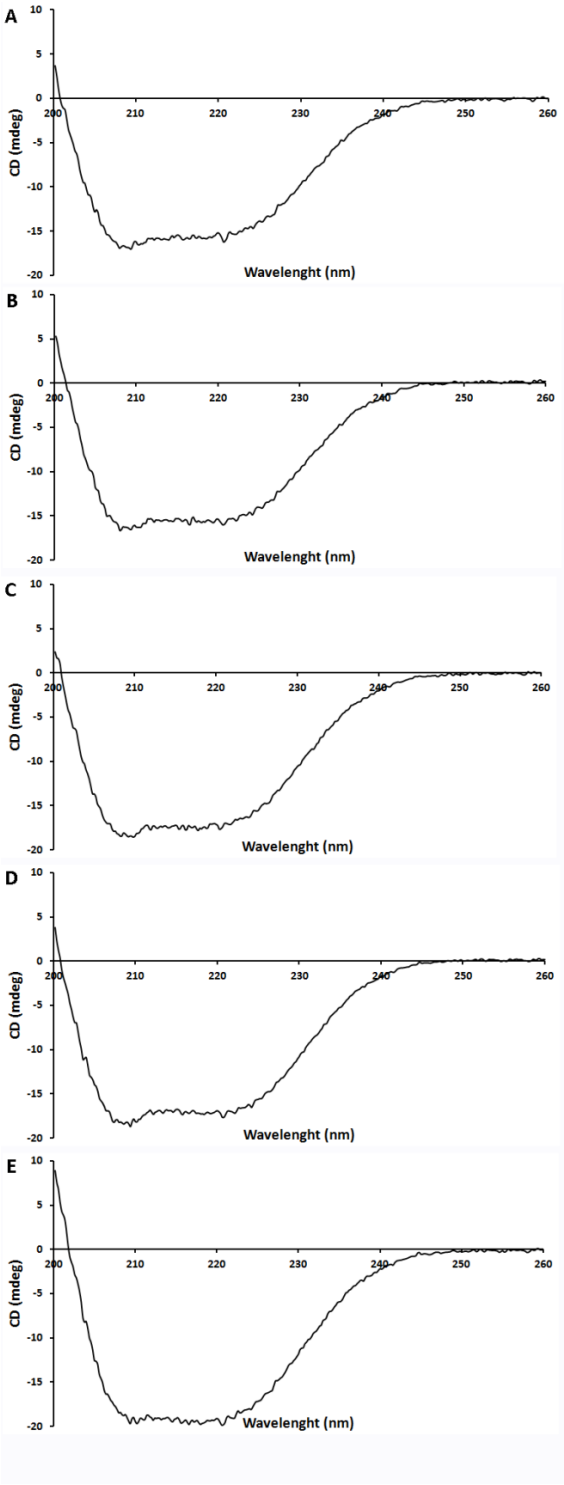

Fig. S1 – Far UV CD spectra of the studied NDH-2. CD spectra ranging from 200 to 260 nm regarding: **A)** WT **B)** E172A **C)** E172Q **D)** E172S **E)** E172D. CD spectra were acquired at 25 ºC using 5 µM [NDH-2]. Proteins show a similar CD spectrum but analyzing their specific $^{208nm}/_{222nm}$ ratio allowed inferring about their relative secondary structure content.

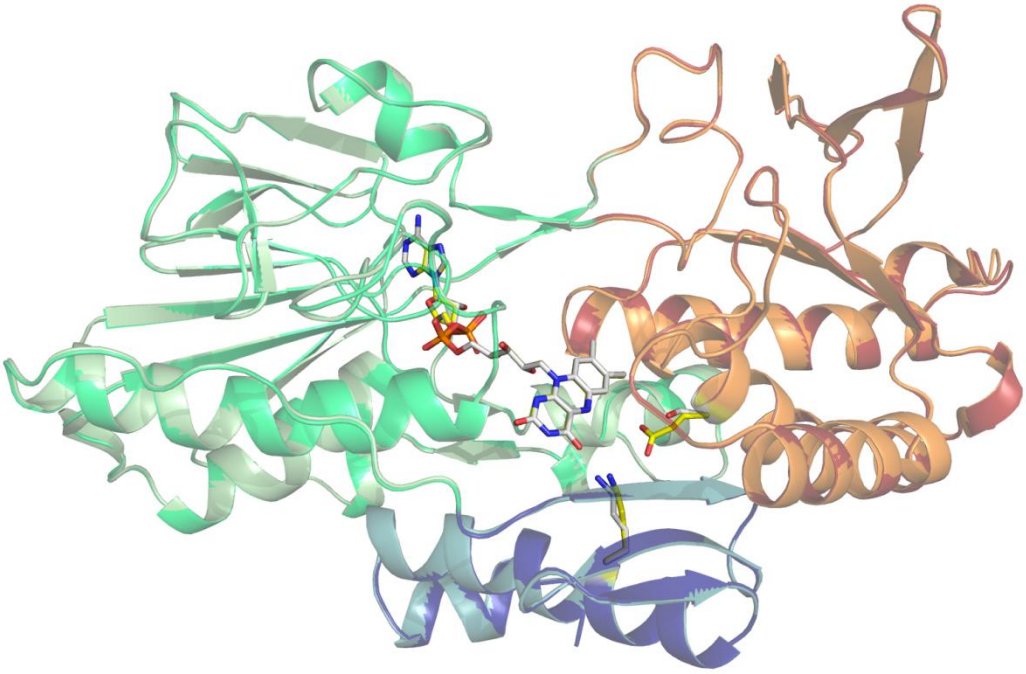

Fig. S2 – Cartoon representing the superimposition of WT and E172S crystallographic structures. The three domains from NDH-2 are displayed as green, orange/red and blue for the FAD binding, NADH binding and C-terminal domains, respectively. FAD cofactor and residues E172 and K379 are shown in sticks with oxygen colored in red, nitrogen in blue, and carbon in yellow for WT and gray for E172S. The overall tertiary structure of the protein was kept unchanged by the mutation.

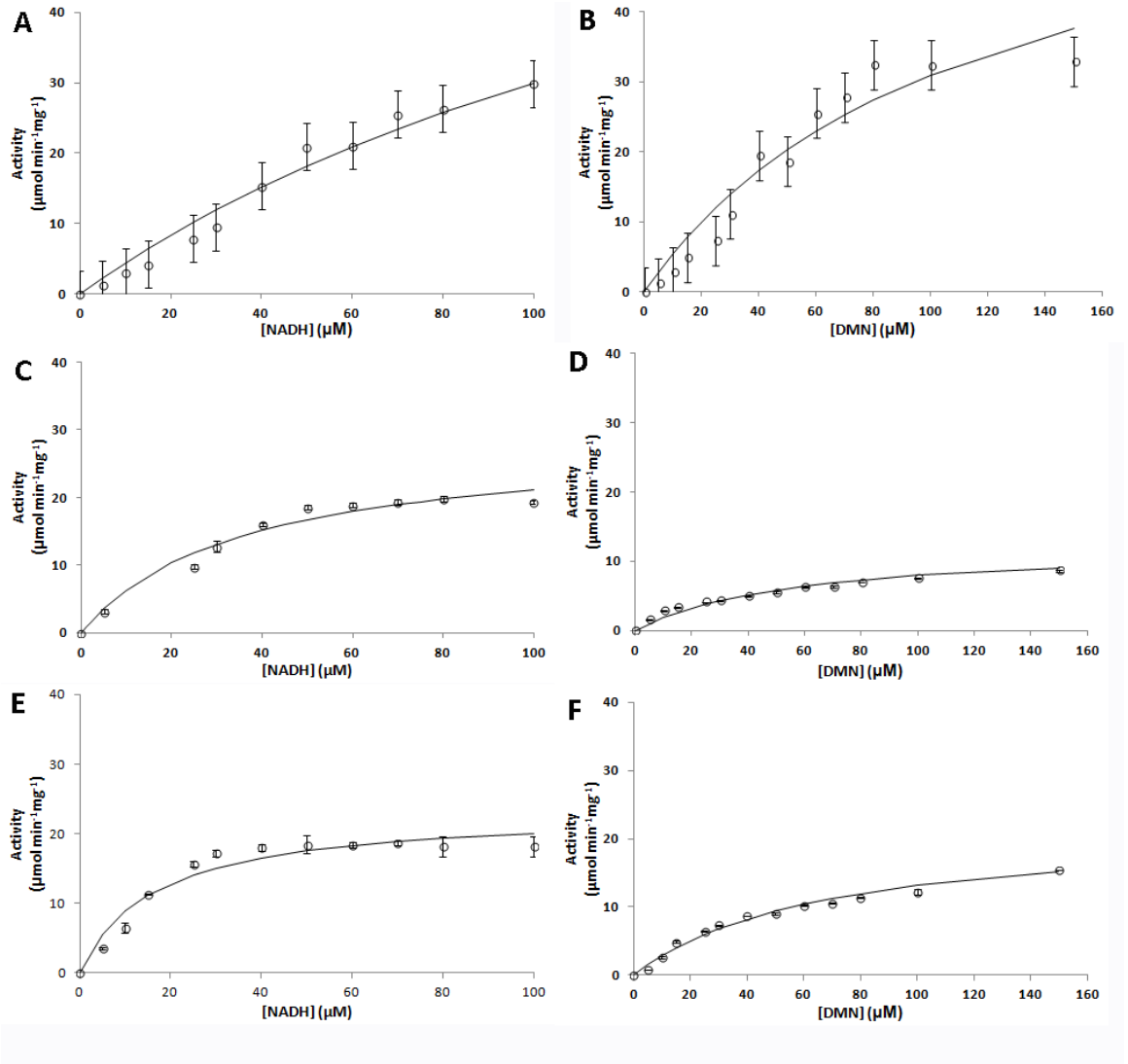

Fig. S3 – Steady state kinetic curves from the studied NDH-2. A) E172A NADH titration **B)** E172A DMN titration **C)** E172Q NADH titration **D)** E172Q DMN titration **E)** E172S NADH titration **F)** E172S DMN titration. In black filled lines is represented the fitting curve obtained using the Michaelis-Menten model equation, maintained as a visual guideline**.** Error bars represent the standard deviation.

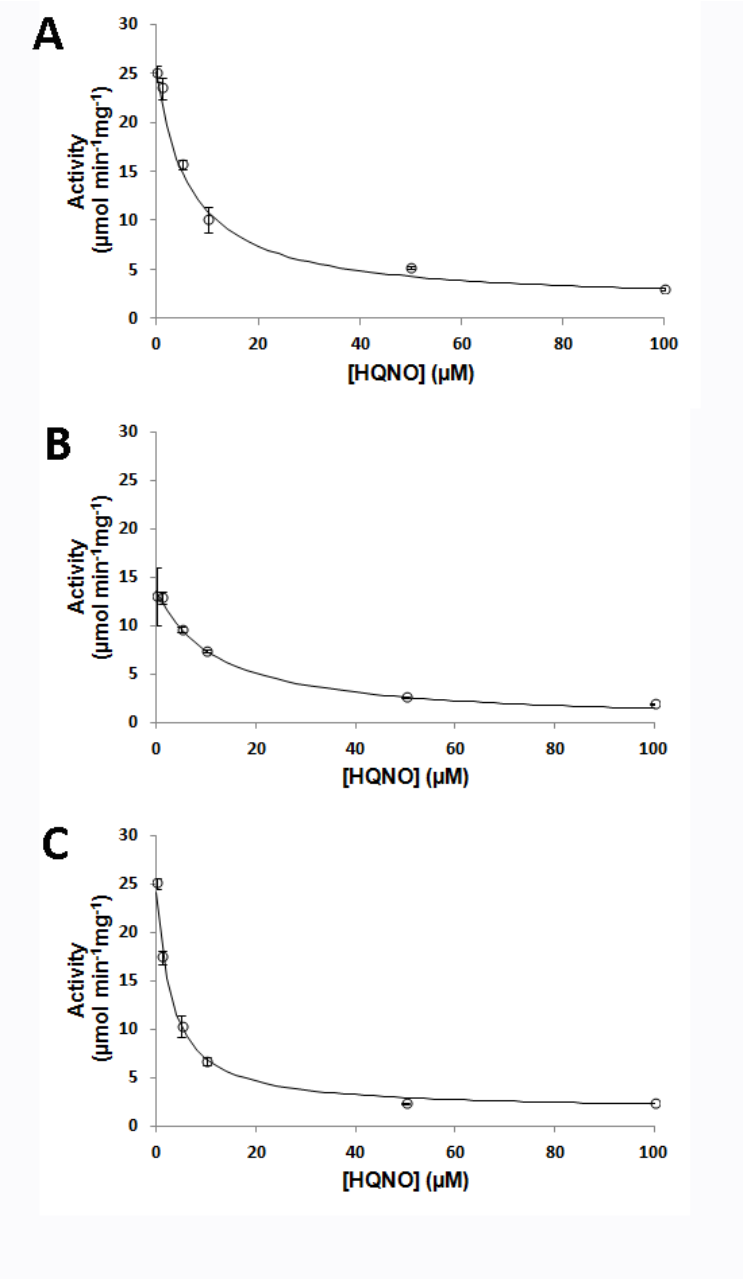

Fig. S4 – Inhibition study of HQNO for the three NDH-2 variants. The inhibition curves of HQNO in the NADH:quinone oxidoreductase activity of **A)** E172A, **B)** E172Q and **C)** E172S; are represented for [HQNO] ranging between 0 and 100 µM. The curves allowed calculating the inhibition coefficient for each variant.

Table S1 – Data collection and refinement statistics for *WT* and E172S variant.

	WT	**E172S**
PDB code	5NA1	5NA4
Data collection		
Synchrotron	Diamond Light Source (UK)	ESRF (France)
Beamline	I04	ID30A-3
Wavelength (Å)	0.9795	0.9677
Space group	$P4_3$ 3 2	$P4_3$ 3 2
Unit cell		
a, *b*, *c* (Å)	150.4, 150.4, 150.4	151.3, 151.3, 151.3
α, *β*, *γ* (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution range[1] (Å)	75.21 – 2.32 (2.33 – 2.32)	67.65 – 2.55 (2.59 – 2.55)
Total no. of reflections	148451 (1562)	153169 (8113)
No. of unique reflections	25557 (251)	19844 (980)
Completeness (%)	98.9 (100.0)	99.8 (100.0)
Multiplicity	5.8 (6.2)	7.7 (8.3)
$\langle I/\sigma(I)\rangle$	13.3 (2.1)	20.0 (2.2)
Wilson B-factor	45.0	66.6
R_{meas}[2] (%)	11.2 (92.1)	8.8 (104.2)
R_{pim}[3] (%)	4.6 (36.7)	3.1 (35.2)
$CC_{1/2}$[4] (%)	99.8 (53.8)	99.9 (73.4)
Refinement		
Resolution range (Å)	27.07 – 2.32 (2.33 – 2.32)	67.65 – 2.55 (2.59 – 2.55)
R_{cryst}[5] (%)	17.90 (26.93)	17.40 (24.79)
R_{free}[6] (%)	21.00 (28.08)	22.40 (28.99)
No. of non-H atoms	3256	3192
Protein	3072	3069
Ligands	72	53
Waters	112	70
r.m.s.d bonds (Å)	0.009	0.014

r.m.s.d. angles (°)	1.05	1.79
Protein residues	Arg5 – Phe402	
Ramachandran plot		
Most favoured (%)	98.7	97.5
Allowed (%)	1.3	2.5
Outliers (%)	0.00	0.00
Rotamer outliers (%)	0.61	1.53
MolProbity score[7]	0.50	1.03
Clashscore	0.00	0.96
B-factors (Å^2)		
Protein	48.20	56.88
Ligands	47.01	43.38
Solvent	45.32	55.26

[1] Information in parenthesis refers to the last resolution shell.

[2] $R_{meas} = \Sigma_h (n_h/n_h - 1)^{1/2} \Sigma_i |<I_h> - I_{h,i}|/\Sigma_h \Sigma_i I_{h,I}$, where n_h denotes multiplicity

[3] $R_{pim} = \Sigma_h [1/(/n_h - 1)]^{1/2} \Sigma_i |<I_h> - I_{h,i}|/\Sigma_h \Sigma_i I_{h,i}$

[4] $CC_{1/2}$ is as described previously (Karplus, P. A., and Diederichs, K. (2012) Linking crystallographic model and data quality. Science 336, 1030 –1033)

[5] $R_{cryst}=\sum_{hkl}||F_{obs(hkl)}|-|F_{calc(hkl)}||/\sum_{hkl}|F_{obs(hkl)}|$, where $F_{obs(hkl)}|-|F_{calc(hkl)}$ are the observed and calculated structure factors for reflection (*hkl*), respectively.

[6] R_{free} was calculated as R_{cryst} but using only 5% of reflections randomly selected and omitted from refinement.

[7] *MolProbity* score provides a single number that represents the central *MolProbity* protein quality statistics; it is a log-weighted combination of the clashscore, Ramachandran not favored and bad side-chain rotamers, giving one number that reflects the crystallographic resolution at which those values would be expected.

Table S2 – Summary table of the main parameters determined for the studied proteins.

Protein	X-ray Structure (Å)	CD $208_{nm}/222_{nm}$ (%)	Molecular mass (KDa)	Kcat (s^{-1}) (NADH:quinone oxidoreductase)	Half reactions rate (s^{-1})		Substrate interaction	
					k1(FAD reduction)	k2 (FAD oxidation)	Kd NAD^+ (µM)	Kd DMN (µM)
WT	2.32	100	90	67.9 ± 2.4*	180 ± 30*	5 ± 0.5*	20.3*	16.3*
E172A	-	97	90	20.6 ± 2.7	115 ± 16	0.98 ± 0.21	35 ± 0.58	30 ± 3.7
E172Q	-	96	90	5.7 ± 1.1	108 ± 12	0.27 ± 0.05	37 ± 14	39 ± 1.9
E172S	2.55	97	90	11.3 ± 1.2	104 ± 3	0.32 ± 0.05	30 ± 2.6	51 ± 15
E172D	-	88	150	-	-	-	-	-

* F. V. Sena, A.P. Batista, T. Catarino, J.A. Brito, M. Archer, M. Viertler, T. Madl, E.J. Cabrita, M.M. Pereira, Type-II NADH: Quinone oxidoreductase from Staphylococcus aureus has two distinct binding sites and is rate limited by quinone reduction, Mol. Microbiol. 98 (2015) 272–288. doi:10.1111/mmi.13120.