

Supporting Information

Roles of Conserved Residues of the NuoC region in Proton-translocating NADH-Quinone Oxidoreductase (NDH-1) from *Escherichia coli*[#].

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Table S1

Forward oligonucleotides used for the cloning and mutagenesis of *E. coli nuoCD* gene. *Italic* bases represent the restriction site for *Bam*HI (primers A and D) and *Hind* III (primers B, C, E and F). From S104A onwards, the sequence represents the primers used for the introduction of a site-specific mutation. The underlined bases represent mutations. The bold bases mean mutant codons.

A	5'-CCG GGATC CTACGCCTGTCCGCCAAGTTTTATCTGG-3'
B	5'-GCAGTAAG CTT CGCGAAATAGATTTAGGAATAAGC-3'
C	5'-CGAAG CTT AGGACTTCATGTTCCTCAACCTCGG-3'
D	5'-GTAG GGATC CGATCCCTTTGGTCGCCTCAATCATCTGG-3'
E	5'-GGGTCAAAG CTT CAGTGGAACGAAAACCTCAC-3'
F	5'-GGAAGATAAG CTT TCTATTTTCAATAGTTACAAATTG-3'
S104A	5'-CCGTTTTCTACCATCTGATT GCT ATCGATCGTAACCGCGAC - 3'
A134S	5'-CACCAAACCTGTTCCCGAACT TCT AACTGGTATGAGCG - 3'
E138A	5'-CCCGAACGCTAACTGGTAT GCG CGTGAAACCTGGGATCTG - 3'
E138D	5'-CCCGAACGCTAACTGGTAT GAT CGTGAAACCTGGGATCTG - 3'
E138Q	5'-CCCGAACGCTAACTGGTAT CAG CGTGAAACCTGGGATCTG - 3'
R139A	5'-CGCTAACTGGTATGAG GCT GAAACCTGGGATCTGTTTGG - 3'
E140A	5'-CGCTAACTGGTATGAGCGT GCG ACCTGGGATCTGTTTGGCATTAC - 3'
E140D	5'-CGCTAACTGGTATGAGCGT GAT ACCTGGGATCTGTTTGGCATTAC - 3'
E140Q	5'-CGCTAACTGGTATGAGCGT CAA ACCTGGGATCTGTTTGGCATTAC - 3'
D143A	5'-CTGGTATGAGCGTGAAACCTGG GCT TCTGTTTGGCATTACTTTCGACGG - 3'
D143E	5'-CTGGTATGAGCGTGAAACCTGG GAA ACTGTTTGGCATTACTTTCGACGG - 3'
D143N	5'-CTGGTATGAGCGTGAAACCTGG AAT TCTGTTTGGCATTACTTTCGACGG - 3'
G146A	5'-CGTGAAACCTGGGATCTGTTT GCC ATTACTTTCGACGGTCACC - 3'
F149A	5'-GGATCTGTTTGGCATTACT GCC GACGGTCACCCGAACCTGCG - 3'
R156A	5'-GTCACCCGAACCTG GCG CGCATCATGATGCCGC - 3'
G166A	5'-GCCGCAAACCTGGAAAG CT CACCCGCTGCGTAAAGATTATCC - 3'
H167A	5'-GCCGCAAACCTGGAAAGGT GCC CCGCTGCGTAAAGATTATCC - 3'
P168A	5'-GCCGCAAACCTGGAAAGGTCAC GCG CTGCGTAAAGATTATCC - 3'
K171A	5'-GAAAGGTCACCCGCTGCGT GCG GATTATCCGGCGCGCGC - 3'
K171R	5'-GAAAGGTCACCCGCTGCGT GCG GATTATCCGGCGCGCGC - 3'
P182A	5'-GCGCGCGCTACCGAATTCTCG GCG TTTGAGCTGACCAAAGCC - 3'


NuoCD_Eco	MVNNMTDLTAQEPAWQTRDHLDD-----	23
Nqo5_Tth	-----	
NuoCD_Rsp	-----MSLDQAIP-----	8
Nqo5_Pde	-----MSEALSDEALLE-----	12
NUGM_Yli	-----MLSRFARIGSMG--IRPVAAARAT--FVTSARAAQAAPSWENIKD	41
30kDa_Ncr	-----MASKLCRSRALASALRSKPSPAIRCLATTSRNLINMEGPNPRQ	45
30kDa_Hsa	-----MAAAVARLWWRGILGASALTRGTGRPSVLLLLPVRRESAGADTRPTVRP	49
SU-9_Aca	-----MDNQFIFK-	8
SU-9_NTa	-----MDNQFIFK-	8
NuoCD_Eco	-----PVIGELRNRFPGPDAFTVQATRTGVPV	49
Nqo5_Tth	-----MRLERVLEEERAKGYPIEDNGLGNLW	26
NuoCD_Rsp	-----EALQALRTRFGAAVRAEQATGEAFPV	34
Nqo5_Pde	-----LAEHIAVRRENDVIS-TQAVGELT	35
NUGM_Yli	IRLDPKVHVDEVYEPVNPADRYLQHVSDLHQYAKYIMAALP-KYIQQFSVWKDELTT--	97
30kDa_Ncr	FPREP--LPGALNAAVNPADKYQSKADNLHKYGSWLMGCLP-KYIQQFSVWKDELTT--	99
30kDa_Hsa	RNDVA-----HKQLSAFGEYVAEILP-KYVQQVQV--SCFNELE	85
SU-9_Aca	-----YSWETLPKKWVKKMER--SEHGNS	31
SU-9_NTa	-----YSWETLPKKWVKKMER--SEHGNS	31
NuoCD_Eco	VWIKREQLLEVGDFL-KKLPKPYVMLFDLHGMDERLRTHREGLPAADFSVFYHLISID--	106
Nqo5_Tth	VVLPRERFKEEMAHYKAMGFNFLADIVGLDYLTYDPDRPE-----RFAVVYELVSLPGW	80
NuoCD_Rsp	LWLDASVWEAAHRFLREEIAAPFPLADLWAIDESLRQHRTGQPASRITLCSHLVSLV--	92
Nqo5_Pde	VNATLSGVIGLIEFLRNDPNCRFSTLIDITAVDNPAPPA-----RFDVVYHLLSMY--	86
NUGM_Yli	LHVAPSAVIPVTTFLRDNTSTQYKSIIDITAVDYPSREN-----RFEVVYNFLSVR--	148
30kDa_Ncr	IYISPAGVIPVFSFLKYNTAAEYTVQVSDITAVDFPTKDQ-----RFEVVYNLLSVR--	150
30kDa_Hsa	VCIHDPGVI PVLTFRLDHTNAQFKSLVDLTAVDVPTRQN-----RFEIVYNLLSLR--	136
SU-9_Aca	D-TNTDYLFQLLCFLKLHTYTRVQVSDICGVDHPSRKR-----RFEVVYNLLSTR--	81
SU-9_NTa	D-TNTDYLFQLLCFLKLHTYTRVQVSDICGVDYPSRQK-----RFEVVYNLLSIR--	81
		
NuoCD_Eco	---RNRDIMLKVALAENDLHVPTFTKLFNPANWYERETWDLFGITFDGHPNLRRIMMPQT	163
Nqo5_Tth	KDGDGSRFFVRVYVPEEDPRLPTVTDLWGSANFLEREVYDLFGIVFEGHPDLRKILTPED	140
NuoCD_Rsp	---RNADRLKLATDG---RAPSIAGVYANADWYEREAHDMFGLDFG--RETRRILMPPT	144
Nqo5_Pde	---QNQRIRVKVQVREDE-LVPSLIGVFPGANWYEREVFDLFGILFSGHSDLRRILTDYG	142
NUGM_Yli	---HNSRIRLKTYATEVT-PVPSITCLYEGANWFEREAYDMYGVFFEGHPDLRRIMTDYG	204
30kDa_Ncr	---HNSRIRVKTYADEVS-PVPSITPLYDGANWYEREVYDLFGVFFTGHPDLRRIMTDYG	206
30kDa_Hsa	---FNSRIRVKTYTDELTP-PIESAVSVFKAANWYEREIWDMFVGFANHPDLRRILTDYG	192
SU-9_Aca	---YNSRIRVQTSADDEVTR-RI SPVVSFLFPSAGRWEREVWDMFGVSPINHPDLRRISTDYG	137
SU-9_NTa	---YNSRIRVQTSADDEVTR-RI SPVVSFLFPSAGRWEREVWDMFGVSSINHPDLRRISTDYG	137
NuoCD_Eco	WKGHPLRKDYPAR-----ATEFSPFELTKAKQDLE	193 → 600
Nqo5_Tth	LEGHPLRKDYPLGETPTLFRE----GRYIIPAEFRAALTGKD	178 → 207
NuoCD_Rsp	WEGHPLLKTHYAR-----ATEKPPFVLTDRLFEEAE	174 → 210
Nqo5_Pde	FRGHPLRKDFPTTG YVEVRWSDIEKRVVYEPVNLVQEYRQFD	184 → 207
NUGM_Yli	FEGHPLRKDFPLTGYTEVRWDEEKRRVYEPLELTQAFRNFS	246 → 281
30kDa_Ncr	FDGHPLRKDFPMTGYTEIRYDEEKKRIVTEPLEMTQAFRNFE	248 → 283
30kDa_Hsa	FEGHPRKDFPLSGYVELRYDDEVKRVVAEPVELAQEFKRFD	234 → 264
SU-9_Aca	FEGHPLRKDLPLSGYVQVRYDDPEKRVVSEPIEMTQEFRYFD	179 → 190
SU-9_NTa	FEGHPLRKDLPLSGYVEVRYDDPEKRVVSEPIEMTQEFRYFD	179 → 190

Figure S1: Sequence alignment of the NuoC segment of *E. coli* NDH-1 with its homologues from other organisms. NuoCD_Eco, *E. coli* NuoCD (NP_416789); Nqo5_Tth, *T. thermophilus* Nqo5 (YP_005887.1); NuoCD_Rsp, *R. sphaeroides* NuoC (YP_3531178.1); Nqo5_Pde, *Paracoccus denitrificans* Nqo5 (YP_916036.1); NUGM_Yli, *Yarrowia lipolytica* NUGM (XP_504891.1);

30kDa_Ncr, *Neurospora crassa* 30kDa (XM_952596.2); 30kDa_Hsa, *Homo sapiens* 30kDa (NP_004542.1); SU-9_Aca, *Acanthamoeba castellanii* SU-9 (NP_042560.1); SU-9_Nta, *Nicotiana tabacum* SU-9 (YP_173479.1). Conserved residues are shown in red. Arrow shows the sequence of a conserved α -helix in the interface of the NuoC and NuoD segments. The NuoD segment sequence of NuoCD is not shown in the alignment.

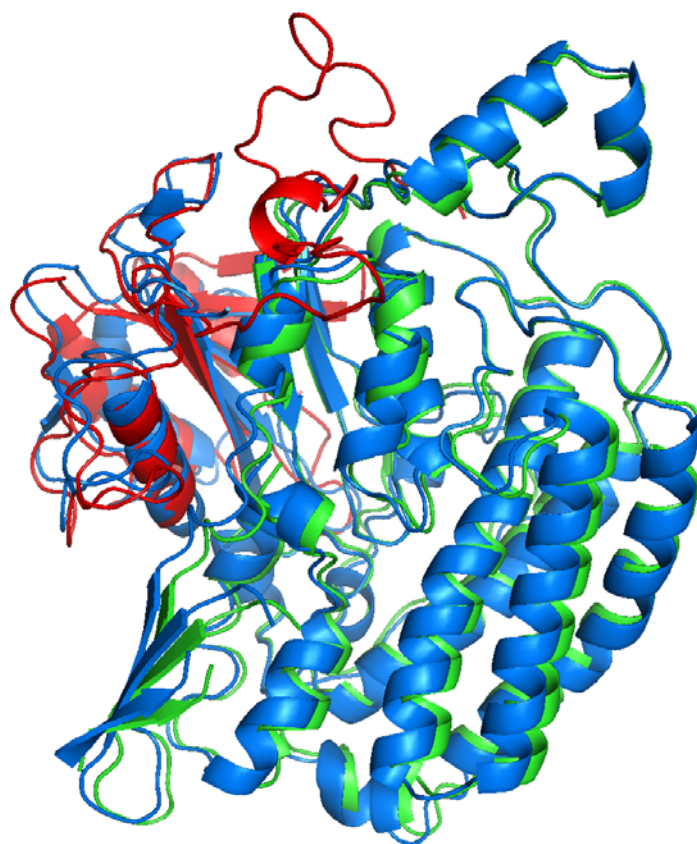


Figure S2: A cartoon representation of the 3-D model of the NuoCD subunit of *E. coli* NDH-1 obtained by homology modeling based on the crystallographic data of the *T. thermophilus* enzyme as detailed in Experimental Procedures. The NuoCD subunit of *E. coli* (blue) was superimposed with subunits Nqo4 (green) and Nqo5 (red) of *T. thermophilus* NDH-1.

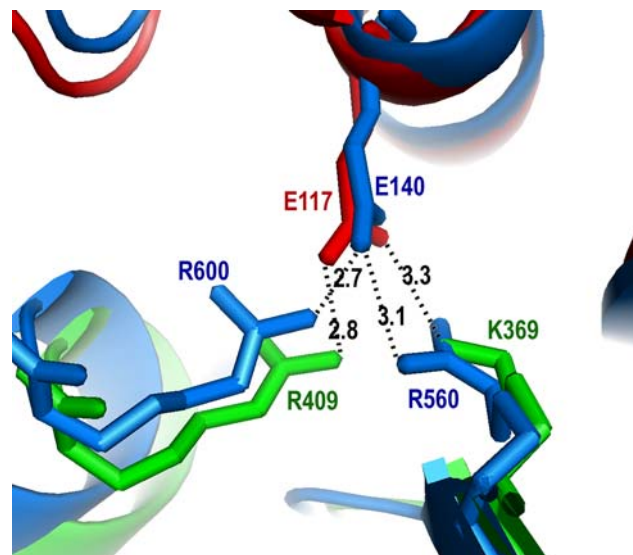


Figure S3: Visualization of the NuoC-NuoD interface in the NuoCD subunit of *E. coli* NDH-1 (blue) showing possible ionic interactions between residues Glu-140 and Arg-560 (3.1 Å) or Glu-140 and Arg-600 (2.7 Å). The *E. coli* structure is superimposed with Nqo4 (green) and Nqo5 (red) of the *T. thermophilus* structure. The corresponding ionic pairs are between Glu-117 and Lys-369 (3.3 Å) or Glu-117 and Arg-409 (2.8 Å). Distance measurements were carried out using the measurement tool available from the Pymol software as described in Experimental Procedures.