

Supporting Information

Exploring Interactions between the 49 kDa and ND1 Subunits in Mitochondrial NADH-Ubiquinone Oxidoreductase (Complex I) by Photoaffinity Labeling

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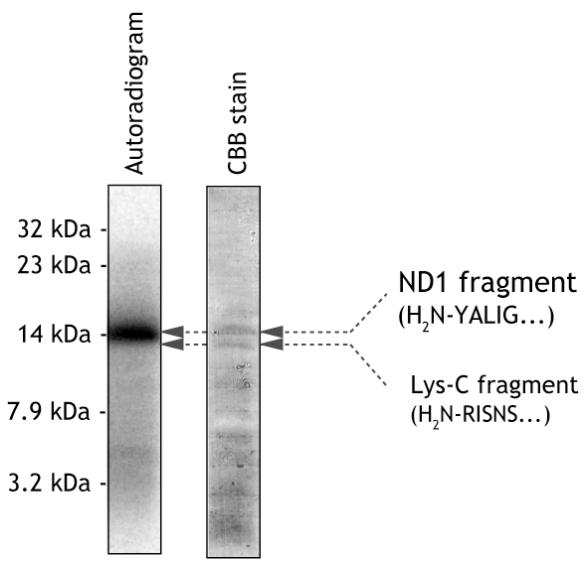


Figure S1. Lys-C digestion of the [¹²⁵I]AzQ-labeled ND1 subunit. The labeled ND1 subunit was purified by SDS-PAGE and electroelution according to Experimental Procedures. Then the ND1 subunit (equivalent to 180 μ g of complex I) was digested by Lys-C (0.6 μ g), followed by tricine/SDS-PAGE (16% T, 6% C) and transfer onto the PVDF membrane. The membrane was subjected to autoradiography or CBB staining. Bands were excised and their N-terminal amino-acid residues were determined by Edman degradation as described in Experimental Procedures. A protein band below the ND1 fragment was identified to be a Lys-C fragment resulting from self-digestion.

<i>B. taur</i> -49kDa	-----ARQ--WQPDVEWAEQ- 13
<i>Y. lipo</i> -49kDa	-----MLRSAARAVRAVRPLSARYMATTALPQDPIPSGALGQ--KVPHVDESHQD 50
<i>P. deni</i> -Nqo4	-----
<i>T. ther</i> -Nqo4	-----
<i>E. coli</i> -NuoC/D	DLHVPTFTKLFPNANWYERETWDLFGITFDGHPNLRRIMMPQTWKGHPLRKDYPARATEF 180
<i>B. taur</i> -49kDa	--YGGAVMYPTKETAHKPPPWNDVDPKDTLVSNLTLNFGPOHPOAAHGVLRLVMELSGE 71
<i>Y. lipo</i> -49kDa	LLFRSHMVEDLETYDEDSP-INTSDAN--TRIRRAFTINFGPOHPOAAHGVLRLILELSGE 107
<i>P. deni</i> -Nqo4	-----MDGDIRKNSLDDGMSDALTGE--OSIRNFNINFGPOHPOAAHGLLRMVLELDGE 51
<i>T. ther</i> -Nqo4	-----MREEFLEEIPLDAPPPEAKELR---TEVMTLNVGPOHPSTHGVLRLMVTLSGE 50
<i>E. coli</i> -NuoC/D	SPFELTKAKQDLEMEALTFKPEEWGMKRGTENEDFMFLNLGPNHPSAHGAFRIVLQLDGE 240
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<i>B. taur</i> -49kDa	MVRKCDPHIGLLHRGTEKLIEYKTYLQALPYFDRLDYVSMCNEQAYSLAVEKLLNIQPP 131
<i>Y. lipo</i> -49kDa	EIIRSDPHVGLLHRGTEKLIEYKTYMQALPYFDRLDYVSMMTNEQVFSLAVEKLLNVEVP 167
<i>P. deni</i> -Nqo4	IVERADPHIGLLHRGTEKLMESRTYLQNLPLYLDRLDYVAPMNQEHAWCIAIERLTGTVIP 111
<i>T. ther</i> -Nqo4	EVLEVVPHIGYLHTGFEEKTMERTYLNQNTYTPRMDYLHSFAHDLAYALAVEKLLGAVVP 110
<i>E. coli</i> -NuoC/D	EIVDCVPDIGYHHRGAEMGERQSWSHYIPYTDRIEYLGGCVNEMPYVLAVEKLAGITVP 300
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<i>B. taur</i> -49kDa	PRAQWIRVLFGEITRLLNHIMAVTHALDIGAMTPFFWMFEEREKMFYERVSGARMHA 191
<i>Y. lipo</i> -49kDa	LRGKYIARTMFGEITRVLNHLMSVCASHMDVGALTTPFLWGFEEREKLMFYERVSGARLHA 227
<i>P. deni</i> -Nqo4	RRASLIRVLYSEIGRILNHLMGVTGAMDVGALTTPLGFEARELMIYERACGARLHA 171
<i>T. ther</i> -Nqo4	PRAETIRVILNELSRLASHLVFLGTGLLDLGALTPFFYAFRERETILDLFEWVTGQRFH 170
<i>E. coli</i> -NuoC/D	DRVNVIRVMLSELFRLNLLYISTFIQDVGAMTPVFFAFTDRQKIYDLVEAITGFRMH 360
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<i>B. taur</i> -49kDa	AYVRPGGVHQDLPLGLMDDIYEFSKNFSLRIDELEEMLTNNRIWRNRTVDIGIVTAEDAL 251
<i>Y. lipo</i> -49kDa	AYVRPGGSVQDLPAGLDDIYMWATQFGDRLEIEELLTDNRWIKLRTVNIGTVTAQDAL 287
<i>P. deni</i> -Nqo4	AYFRPGGVHQDLPPDLDDIEEWCEFPKLVDDLTLLTENRIFQQLRVLDIGIVTEADAL 231
<i>T. ther</i> -Nqo4	NYIRIGGVKEDLPEEFVPELKKLLEVLPHRIDEYEALFAESPIFYERARGVGVIPPEAI 230
<i>E. coli</i> -NuoC/D	AWFRIGGVAHDLPRGDWDRLLREFLDWMPKRLASYEKAALQNTILKGRSQGVAYAKEAL 420
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<i>B. taur</i> -49kDa	NYGFSGVMLRGSGIQWDLRKTPQYDQVEFDVPIGS-RGDCYDRYLCRVEEMRQSIRI 310
<i>Y. lipo</i> -49kDa	NLGLSGPMLRGSGIPFDIRKNAPYDAYDKVDFDVPVGM-NGDCYDRYLIRMAEFRQSLRI 346
<i>P. deni</i> -Nqo4	DWGYTGVMRGSGLAWDLRRSQPYECYDEFDFQIPVGR-NGDCYDRYLCRMAEMRESCKI 290
<i>T. ther</i> -Nqo4	DLGLTGGSLRASGVNYDVRKAYPYSGYETYTFDVPVGE-RGDFVDRMLVRIREMRESVKI 289
<i>E. coli</i> -NuoC/D	EWGTTGAGLRATGIDFDVRKARPYSGYENFDFEIPVGGVSDCYTRVMLKVEELRQSLRI 480
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<i>B. taur</i> -49kDa	ISQCLNKM---PPGEIKVDDAKVSPPKRAEMKTSMESLIHHFKLYTEGYQVPPGATYTAI 367
<i>Y. lipo</i> -49kDa	IEQCCNDM---PAGAVKVEDFKINSPPRNLMKEDMEALIHHFLYTKGYSVPPGETYTAI 403
<i>P. deni</i> -Nqo4	MQQAVQKLRAEPAGDVLRAG-KLTPPRAEMKRDMESLIHHFKLYTEGFKVPAGEVYAAV 349
<i>T. ther</i> -Nqo4	IKQALERLE---PGPVRDPNPQITPPPRHLLETSMEAIVYHFHYTEGFHPPKGEVYVPT 346
<i>E. coli</i> -NuoC/D	LEQCLNNMP---EGPFKAHDPLTTPPKERTLQHIETLITHFLQSVWGPVMPANESFQMI 537
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<i>B. taur</i> -49kDa	EAPKGEFGVYLVDGSSRPYRCKIKAPGFAHLAGLDKMSKGMLADVVAIIGTQDIVFGE 427
<i>Y. lipo</i> -49kDa	EAPKGEMGVVVSDGSERPYKCKIRAPGFAHLGAFDHIARGHFLPDAVVAIIGTMDLVFGE 463
<i>P. deni</i> -Nqo4	EGPKGEFGVYLADGTONKPWRALKRAPGFAHLQSIDWMSRGHMLADVPAIIATLDIVFGE 409
<i>T. ther</i> -Nqo4	ESARGELGYIVSDGGSMPYRVKVRAPSFVNQLQSLPYACKGEQVPMVMAIASLDPVMGD 406
<i>E. coli</i> -NuoC/D	EATKGINSYYLTSGSTMSYRTRVRTPSFAHLQQIPAAIRGSLVSDLIVYLGSIIDFVMSD 597
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<i>B. taur</i> -49kDa	VDR 430
<i>Y. lipo</i> -49kDa	VDR 466
<i>P. deni</i> -Nqo4	VDR 412
<i>T. ther</i> -Nqo4	VDR 409
<i>E. coli</i> -NuoC/D	VDR 600

Figure S2. Multiple sequence alignment of 49 kDa subunit from various organisms. The alignment was performed with Clustal W (Thompson, J. D. *et al.* (1994) *Nucl. Acids Res.* 22, 4673-4680), using amino acid sequences as follows: *B. taurus* (bovine) 49 kDa (P17694), *Y. lipolytica* 49 kDa (XP_505527), *P. denitrificans* Nqo4 (P29916), *T. thermophilus* Nqo4 (Q56220), and *E. coli* NuoC/D (P33599). In the bovine sequence, the residue numbers refer to the mature sequence after the removal of the mitochondrial target peptide. The sequences corresponding to the [^{125}I]AzQ binding-site in bovine 49 kDa (Asp41-Arg63, in *red*) are surrounded by a *dark rectangle*. In this region of *T. thermophilus* Nqo4, only Met26-Gly31 and Gly39-Arg42 (in *green*) are resolved in the crystal structure (PDB entry: 3M9S).