

# IUCrJ

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**Supporting information for article:**

**Cryo-EM structure of *Neurospora crassa* respiratory complex IV**

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***N. crassa* Cytochrome c oxidase subunit 1 (named Cox1), mitochondrially encoded**  
**61.17 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 1 (chain a in PDB 6HU9)**

```

SP|P03945|COX1_NEUCR MSSISIWTERWFLSTNAKDIGVLYLIFALFSGLLGTAFSVLIRMELSGPGVQYIAD-NQL 59
SP|P00401|COX1_YEAST -----MVQRWLYSTNAKDIAVLYFMLAIFSGMAGTAMSLIIRLELAAPGSQYLHGNSQL 54
      .:**: *****:**:~::~:**: **:*::~:**:**:~* ** : . **

SP|P03945|COX1_NEUCR YNAIITAHAILMIFFMVPALIGGFGNFLPLLVGGPDMAFPRLNNISFWLLPPSLLLV 119
SP|P00401|COX1_YEAST FNVLVVGHAVLMIFFLVMPALIGGFGNLYLPLMIGATDTAFPRINNIAFWVLEPMLVCLV 114
      *::~:**:*****:*****~::~:**:~* ** **:*::~:**:**:~* ** : **

SP|P03945|COX1_NEUCR FSACIEGGAGTGWTIYPPLSGVQSHSGPSVDLAIIFALHLSGVSSLLGSINFITIVNMRT 179
SP|P00401|COX1_YEAST TSTLVESGAGTGWTVYPLSSIQAHSGPSVDLAIIFALHLTSSISLLGAINFIVTTLNMRT 174
      *::~:**:*****:*****~::~:**:*****~::~:**:~* ** **:*::~:**:**:~* **

SP|P03945|COX1_NEUCR PGIRLHKLALFGWAVVITAVLLLSLPVLGAIITMLLTDRNFNTSFFETAGGGDPILFQH 239
SP|P00401|COX1_YEAST NGMTMHKLPFLVWSIFTAFLLLSLPVLGAIITMLLTDRNFNTSFFEVSGGGDPILYEH 234
      *::~:**:~* ** *::~:**:*****~::~:**:~* ** **:*::~:**:**:~* **

SP|P03945|COX1_NEUCR LFWFFGHPEVYILIIIPGFGIISTTISAYSNKSVMFYIGMVMYAMMSIGILGFVWSHHMYT 299
SP|P00401|COX1_YEAST LFWFFGHPEVYILIIIPGFGIISHVSTYSKKPVFGEISMVYAMASIGLLGLVWSHHMYI 294
      *****~::~:**:~* ** * **:*::~:**:**:~* **

SP|P03945|COX1_NEUCR VGLDVDTRAYFTAATLIIAVPTGIKIFSWLATCYGGSIRLTPSMLFALGFVEMFTIGGLS 359
SP|P00401|COX1_YEAST VGLDADTRAYFTSATMIIAIPGTGIKIFSWLATIHGGSIRLATPMLYAIAFLEFLFTMGGLT 354
      ***~::~:**:~* **:*::~:**:*****~::~:**:~* ** **:*::~:**:**:~* **

SP|P03945|COX1_NEUCR GVVLANASLDIAFHDTYYVVAHFHYVLSMGAIVFAMFSGWYHWVPKILGLNYNMVLSKAQF 419
SP|P00401|COX1_YEAST GVALANASLDVAFHDTYYVVGHFHYVLSMGAIFSLFAGYYSWSPQILGLNYNEKLAQIQF 414
      *~::~:**:*****~::~:**:*****~::~:**:~* ** **:*::~:**:**:~* **

SP|P03945|COX1_NEUCR WLLFIGVNLTFPPQHFLGLQGMPRRISDYPDAFSGWNLISSFGSIVSVVASWFLYIVYI 479
SP|P00401|COX1_YEAST WLIFIGANVIFPPMHFLGINGMPRRIPDYPDAFAGWNYVASIGSFIATLSLEFLFIYIYLD 474
      **~::~:**:~* ** **:*::~:**:*****~::~:**:~* ** **:*::~:**:**:~* **

SP|P03945|COX1_NEUCR QLVQGEYAGRPWS----IPQFYT-DSLRLALNRSYPSLEWSISSPPKPHSFVSLPLQS 533
SP|P00401|COX1_YEAST QLVNGLNKNVNNKSVIYNKAPDFVESNTIFNLNTVKSSSIEFLLTSPPAVHSFNTPAVQS 534
      ***~::~:**:~* ** **:*::~:**:~* ** **:*::~:**:**:~* **

SP|P03945|COX1_NEUCR SSFFLSFFRLSSYGEQKEISGRQN 557
SP|P00401|COX1_YEAST -----

```

**Figure S1** Sequence alignment of Cox1 from *N. crassa* and *S. cerevisiae* with Clustal Omega (1.2.4). Bold letters show transmembrane helices visible in the map. *N. crassa* has a longer C-terminus than *S. cerevisiae*.







***N. crassa* Cytochrome c oxidase polypeptide 5 (named Cox5a), nuclear-encoded****29.31 % identity with *S. cerevisiae* Cytochrome c oxidase polypeptide 5a (chain e in PDB 6HU9)**

```

SP|P06810|COX5_NEUCR MLRTPTVSALVRNVAVRAA--KPTMAVRAASTMPI SNPTLANIEKRWEQMPMQEQAELWM 58
SP|P00424|COX5A_YEAST -----MLRNTFTFRAGGLSRITSVRFAQTHALSNAAVMDLQSRWENMPSTEQQDIVS 51
      ::* . *. . :** *. * :** :: :*:**:* ** * :

SP|P06810|COX5_NEUCR ALRDRMKGNWADLTLOEKKAAYYIAFGPHGPRALPPP-GEQKKVLAYTVAGVFLSFVIFA 117
SP|P00424|COX5A_YEAST KLSERQKLPWAQLTEPEKQAVVWYISYGEWGPRRPVLNKGDSSFIAGKVAAGLLFSVGLFA 111
      * : * * **:* ** :*.:**:* ** * * * : . . : . **:* : * . : **

SP|P06810|COX5_NEUCR TMRAFAKPPPATMTKEWQEATNEFLKAQKSDPLTGLTSEGYNGKGHVQSPSASA 171
SP|P00424|COX5A_YEAST VVRMAGGQDAKTMNKEWQLKSDEYLSKNANPWGGYSQVQSK----- 153
      . : * . **.* ** :*:**:* : * * : . :

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***N. crassa* Uncharacterized protein (named Cox7), nuclear-encoded****15.493 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 7 (chain g in PDB 6HU9)**

```

TR|Q7S7U5|Q7S7U5_NEUCR MPGLVNAPNHVPEKQRYQQAFKNHTRLWKIGPRSGIIMTTFNIAMWGTFGASMYAMSRK 60
SP|P10174|COX7_YEAST -----MANKVIQLQKIFQS---STKPLWWRHPRSALYLYPFYAIFAVAVVTPLLYIPNA 51
      * : * : * : * . . ** ***.: : * : . : : : .

TR|Q7S7U5|Q7S7U5_NEUCR VLGYNTWFSED 71
SP|P10174|COX7_YEAST IRGIKAKKA-- 60
      : * : :

```

***N. crassa* Cytochrome c oxidase chain VIIIc (named Cox8), nuclear-encoded****16.471 % identity with *S. cerevisiae* Cytochrome c oxidase polypeptide VIII (chain h in PDB 6HU9)**

```

TR|Q1K528|Q1K528_NEUCR MFSRVALRAAPRQQPFSLVARRTFQTTAQLSSPYHYPEGPRSNLFPNPKTRFFWFRY-- 58
SP|P04039|COX8_YEAST MLCQQMIRTTAKRS-----SNIMTRPIIMKRSVHFKDGVIYENIPFKVKGRKTPYALSH 53
      *.: : *.: : . . : * . . * : * . * : * * :

TR|Q1K528|Q1K528_NEUCR LMYCVVGFSGSPVAIAVWQTYRPRS- 82
SP|P04039|COX8_YEAST FGFFAIGFAVPFVACYVQLKKSGAF 78
      : : . : * . * . * : :

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***N. crassa* Cytochrome c oxidase subunit 7A (named Cox7a), nuclear-encoded****32.812 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 7A (chain j in PDB 6HU9)**

```

TR|Q7S5M7|Q7S5M7_NEUCR MAATAVRPITMLRRGLILDIGIALGVGFVMANGYWYGYHMPRTNNARDNYYKKLEERAA 60
SP|P07255|COX9_YEAST ---MTIAPITGTIKRRVIMDIVLGFSLGGVMASYWWWGFHMDKINKREKFYAELAERKKQ 57
      : : * * * : * : * * : . : : * * * . : * : * * : * * : * * :

TR|Q7S5M7|Q7S5M7_NEUCR RMGA 64
SP|P07255|COX9_YEAST EN-- 59

```

**Figure S4** Sequence alignments of *N. crassa* and *S. cerevisiae* subunits Cox5a, Cox7, Cox8 and Cox7a with Clustal Omega (1.2.4). Grey letters indicate residues that are absent in the *S. cerevisiae* model (PDB 6HU9), bold letters mark transmembrane regions. All proteins have one transmembrane helices.

***N. crassa* Encodes anonymous transcript-5 protein (named Cox6a), nuclear-encoded****33.813 % identity with *S. cerevisiae* Cytochrome c oxidase subunit 6A, named Cox13 (chain k in PDB 6HU9)**

```

TR|V5IRD7|V5IRD7_NEUCR MFAQRQMFARLANLRAPAVRQTVQRFFASTPANESGKNAFVREEREAVKQHAAETTTELW 60
SP|P32799|COX13_YEAST MFRQCAKRY---ASSLPPN----ALKP--AFGPPDKVAAQKFKESLMATEKHAKDTSNMW 51
** * : *:. * ::: * * :: . : * .. *.:** :*:.:*

TR|V5IRD7|V5IRD7_NEUCR RKISLYGIPPALALAGYNAYTLYNEHWE---HWSHLPPLEERTEYPYQNIRTRNYPWGDG 117
SP|P32799|COX13_YEAST VKISVWVALPAIALTAVNTYFVEKEHAEHREHLKHVPDSEWPRDYEFMNIRSKPFFWGDG 111
***:. : **:.**:. *:* : :** * * .:* * * : * : ***:. : ****

TR|V5IRD7|V5IRD7_NEUCR DKTLEWNESVNYHNRDKVT 136
SP|P32799|COX13_YEAST DKTLEWNPVVNRHIEHDD- 129
***** ** * ...

```

***S. cerevisiae* Uncharacterized protein YDR119W-A, named Cox26 (chain l in PDB 6HU9) absent in *N. crassa* genome**

```

sp|Q2V2P9|YD19A_YEAST MFFSQVLRSSARAAPIKRYTGGGRIGESWVITEGRRLIPEIFQWSAVLSVCLGWPGAVYFFSKARKA 66

```

**Figure S5** Sequence alignment of *N. crassa* subunit Cox6a and *S. cerevisiae* subunit Cox13 with Clustal Omega (1.2.4), and sequence of *S. cerevisiae* subunit Cox26. Grey letters indicate residues that are absent in the *S. cerevisiae* model (PDB 6HU9), bold letters mark transmembrane regions. *S. cerevisiae* Cox6a has a homologue in *N. crassa* that is not present in the Cryo-EM map. *N. crassa* does not have a Cox26 homologue, and no density is visible in the Cryo-EM map.