**SUPPLEMENTAL DATA**

**for**

# Engineering the respiratory complex I to an energy-converting NADPH:ubiquinone oxidoreductase

by

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**Supplementary Tables:**



## Supplementary Figures:

**Table S1:** Primers for site directed mutagenesis. The mutations introduced are marked in bold. The new restriction site for *Vsp*I is underlined.

| **Primer** | **Sequence** |
|---|---|
| *nuoF* E183D_fwd | 5´-CTGCGGGGAA**GAT**ACAGCATTAATCAACTCCCTGG-3´ |
| *nuoF* E183D_rev | 5´-CCAGGGAGTTGATTAATGCTGT**ATC**TTCCCCGCAG -3´ |
| *nuoF* E183H_fwd | 5´-CTGCGGGGAA**CAC**ACAGCATTAATCAACTCCCTGG-3´ |
| *nuoF* E183H_rev | 5´-CCAGGGAGTTGATTAATGCTGT**GTG**TTCCCCGCAG -3´ |
| *nuoF* E183Q_fwd | 5´-CTGCGGGGAA**CAG**ACAGCATTAATCAACTCCCTGG-3´ |
| *nuoF* E183Q_rev | 5´-CCAGGGAGTTGATTAATGCTGT**CTG**TTCCCCGCAG -3´ |
| *nuoF* E183N_fwd | 5´-CTGCGGGGAA**AAC**ACAGCATTAATCAACTCCCTGG-3´ |
| *nuoF* E183N_rev | 5´-CCAGGGAGTTGATTAATGCTGT**GTT**TTCCCCGCAG -3´ |

**Table S2:** Primers for the insertion of the *nptI-sacRB* cartridge and for amplification of the PCR fragment carrying the point mutation. Regions homologous to *nuoF* are underlined. Regions homologous to the *nptI-sacRB* cartridge are marked bold.

| **Primer** | **Sequence** |
|---|---|
| *nuoF::nptI-sacRB*_fwd | 5´-CCGCTGACCTGGCGTCTGCGCGATGACAAACAGCCAGTG TGGCTGGACG**GTACCGGATCCGTCGACCTG**-3´ |
| *nuoF::nptI-sacRB*_rev | 5´- GCCAGGCTTTAAATTTCAGACCATCACGCATACCACCGGC GTAATCTTC**GGAATTCCCCGGGGGATCCG**-3´ |
| *nuoF*2_fwd | 5´-AACATTATCCGTACTCCCGAAACG-3´ |
| *nuoF*_rev | 5´-CAGATCAAGGTGCGCTTC-3´ |

**Table S3:** Isolation of the complex I variant Glu183Asp$^{F}$ from 25 g cells (wet weight).

| preparation | NADH/ferricyanide-oxidoreductase activity | | | | |
|---|---|---|---|---|---|
| | volume | protein | total | specific | yield |
| | [mL] | [mg] | [µmol·min$^{-1}$] | [µmol·min$^{-1}$·mg$^{-1}$] | [%] |
| membranes | 17.8 | 1337 | 4146 | 3.1 | 100 |
| extract | 59.5 | 1021 | 3469 | 3.4 | 82 |
| Fractogel EMD | 70 | 154 | 2961 | 19.2 | 71 |
| ProBond $Ni^{2+}$-IDA | 1 | 2.4 | 185 | 77 | 4 |

**Table S4:** Isolation of the complex I variant Glu183Gln$^{F}$ from 22 g cells (wet weight).

| preparation | NADH/ferricyanide-oxidoreductase activity | | | | |
|---|---|---|---|---|---|
| | volume | protein | total | specific | yield |
| | [mL] | [mg] | [µmol·min$^{-1}$] | [µmol·min$^{-1}$·mg$^{-1}$] | [%] |
| membranes | 10,1 | 714 | 3414 | 4.8 | 100 |
| extract | 60 | 409 | 3150 | 7.7 | 92 |
| Fractogel EMD | 35 | 322 | 3115 | 9.7 | 91 |
| ProBond $Ni^{2+}$-IDA | 0.3 | 2.9 | 237 | 81 | 7 |

**Table S5:** Isolation of the complex I variant Glu183Asn$^{F}$ from 17 g cells (wet weight).

| preparation | NADH/ferricyanide-oxidoreductase activity | | | | |
|---|---|---|---|---|---|
| | volume | protein | total | specific | yield |
| | [mL] | [mg] | [µmol·min$^{-1}$] | [µmol·min$^{-1}$·mg$^{-1}$] | [%] |
| membranes | 6 | 398 | 2430 | 5.4 | 100 |
| extract | 31 | 548 | 1717 | 3.1 | 71 |
| Fractogel EMD | 2.5 | 121 | 1433 | 11.8 | 59 |
| ProBond $Ni^{2+}$-IDA | 0.3 | 2.0 | 120 | 60 | 5 |

**Table S6:** Isolation of the complex I variant Glu183His$^{F}$ from 20 g cells (wet weight).

| preparation | NADH/ferricyanide-oxidoreductase activity | | | | |
|---|---|---|---|---|---|
| | volume | protein | total | specific | yield |
| | [mL] | [mg] | [µmol·min$^{-1}$] | [µmol·min$^{-1}$·mg$^{-1}$] | [%] |
| membranes | 8,2 | 613 | 1542 | 2.5 | 100 |
| extract | 30 | 454 | 1269 | 2.8 | 82 |
| Fractogel EMD | 21 | 153 | 1163 | 7.6 | 75 |
| ProBond Ni$^{2+}$-IDA | 0.5 | 4.4 | 342 | 78 | 22 |

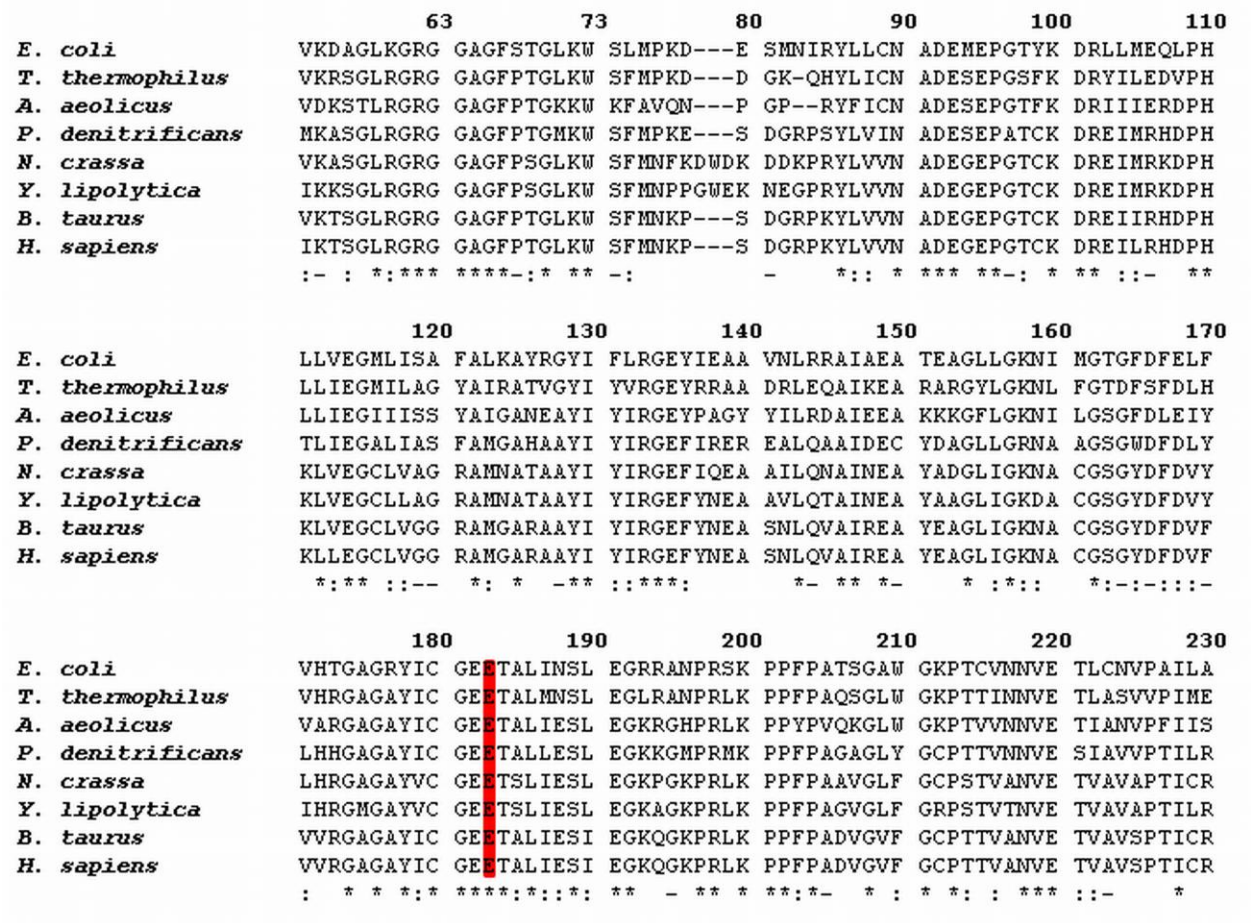


Fig. S1; Morina *et al.*

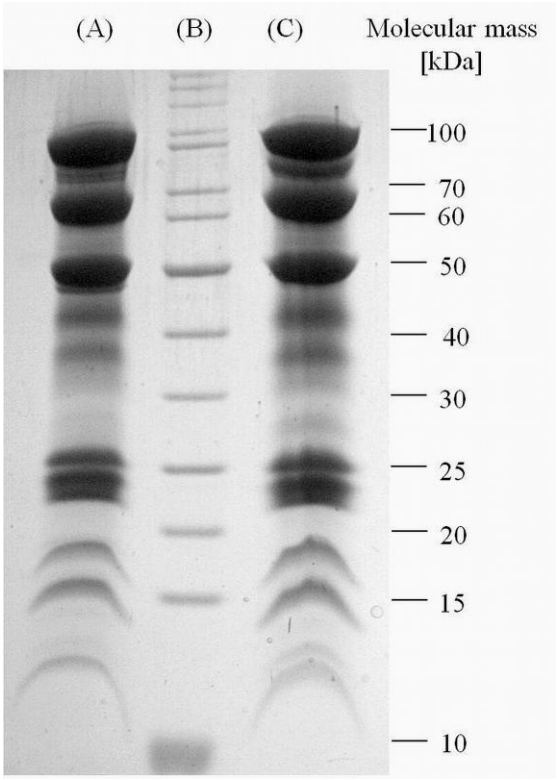


Fig. S2; Morina *et al.*

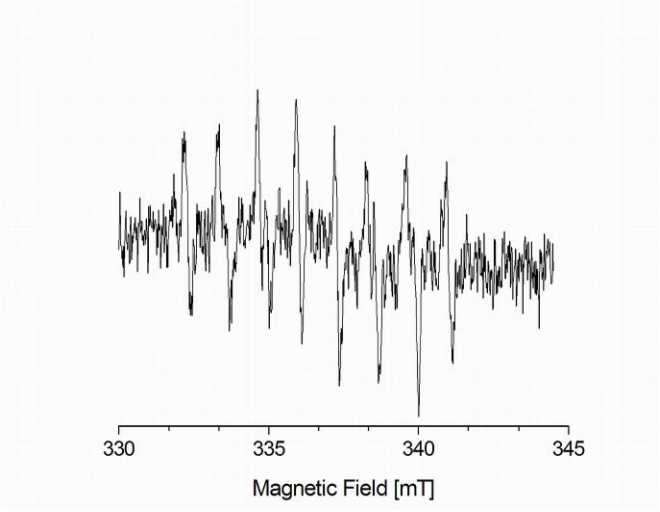


Fig. S3; Morina *et al.*