**Supplementary Material for**

**Analysis of Mammalian Succinate Dehydrogenase Kinetics and Reactive Oxygen Species Production**

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**Summary:** The supplementary material has four sections. The first section contains the heat map showing correlation between the adjustable parameters given in Table 1 of the main manuscript, the parameter table for fixed parameters, Table S1, and the environmental parameters, Table S2. It also contains a figure showing the model fits are unbiased and that the residuals follow a normal distribution. The second section contains the list of mathematical equations that govern the model behavior. The third section lists the equations for the ODE model used to simulate the results in Figure 9 of the main manuscript. The fourth section details the Matlab code used to simulate the model and generate the figures.

**Section 1**

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| **Figure S1. Methodology overview.** The general modeling approach involves a dynamic three-staged process of model construction, calibration and validation. In the initial construction stage (blue), a rudimentary model emerges from structural, thermodynamic and kinetic data. In the corroboration stage (orange), outputs of the rudimentary model are compared to experimental data. The model details, as well as the model parameters, are adjusted until the model outputs are consistent with experimental data. At this point, the model is revised, and the associated parameter set is described as “optimized.” During the final stage of model validation (yellow), the model outputs are compared to experimental data that were not used in the calibration stage. The revised model is considered refined if its outputs are consistent with experimental data. Otherwise, the model is sent back to the calibration stage. |

**Section 2**

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| **Figure S2. Net hydrogen peroxide emission rates were measured in-house using mitochondria isolated from guinea pig cardiomyocytes.** The net hydrogen peroxide emission rate was monitored using the Amplex UltraRed assay (excitation 560 nm, emission 590 nm). A) Succinate titration. Mitochondria were added to the reaction mixture containing myxothiazol and rotenone. After 2 minutes of stabilization, succinate was added to the final concentrations of 50, 100, 200, 300, 500, 1000, 3000 or 5000 µM. B) Decylubiquinol (DQH2) titration was obtained at 200 µM and 5 mM succinate concentrations. The initial stage of the experiment is similar to the succinate titration. After 10 minutes following succinate addition, DQH2 was added to the final concentrations of 12.5, 25, 50, 75, 100, 150 or 200 µM. In all experiments, the final concentrations are 10 µM Amplex UltraRed, 1 U/mL HrP, 10 U/mL SOD, 4 µM rotenone, 2 µM myxothiazol and 0.1 mg/mL mitochondria. At least 3 replicates were performed at each succinate or DQH2 concentration. |

**Section 3**

**Substrates:** succinate (SUC),quinone (Q), oxygen (O2)

**Products:** fumarate (FUM), quinol (QH2), superoxide, (O2•-), and hydrogen peroxide (H2O2)

**Inhibitors:** atpenin (A5), malonate (MALO), oxaloacetate (OAA), malate (MAL)

**Other notations:**

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|  |
| **Figure S3. Correlation heat map for adjustable parameters given in Table 2.** Matrix of correlation coefficient between the adjustable parameters. The normalized parameter sensitivity matrix is computed using Eq. S98. The sensitivity coefficients were computed from the parameter sensitivity matrix given in Eq. S199. Correlation coefficients range between −1 (negative correlation) to +1 (positive correlation). A coefficient value of 0 means the two parameters are uncorrelated. |

**Table S1. Fixed Model Parameters**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Definition | Values | Units | References |
|  | Ideal gas constant | 8.314 | J/mol/K | **-** |
|  | Faraday’s constant | 96.5 | J/mV/mol | **-** |
|  | pKa for flavin free radical | 8 | - | (1) |
|  | pKa for fully reduce flavin | 7.7 | - | (1) |
|  | FAD/FADH midpoint potential | 385 | mV | (1) |
|  | FADH/FADH2 midpoint potential | 284 | mV | (1) |
|  | FADH/FADH2 midpoint potential | 333.8 | mV | (1) |
|  | Midpoint potential of [*2Fe-2S*]ox,,red | 0 | mV | (2) |
|  | Midpoint potential of [*4Fe-4S*] ox,,red | -260 | mV | (2) |
|  | Midpoint potential of [*3Fe-4S*] ox,,red | 60 | mV | (2) |
|  | O2/O2•- midpoint potential | -160 | mV | (3) |
|  | O2/H2O2 midpoint potential | 940 | mV | (3) |
|  | FUM/SUC midpoint potential | 445 | mV | (4) |
|  | Q/QH2 midpoint potential | 464 | mV | (5) |
|  | Phenazine midpoint potential | 358 | mV | (6) |
|  | TMPD midpoint potential | 270 | mV | (7) |
|  | Total mitochondrial quinone concentration | 20 | mM | (8) |

Midpoint potentials are given at 273 K and pH 0 except for the ISCs, oxygen/superoxide, and oxygen/hydrogen peroxide couples. Those are given with respect to 273 K and pH 7. All potentials are given as reduction potentials.

**Table S2. Environmental Parameters**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Description | Value | Sensitivity | Rank |
| Q | succinate/QH2 constant | 1 M | 0.022 | 4 |
| Qbc1 | succinate/QH2 constant in the presence of complex III inhibitors | 211 nM | 0.052 | 3 |
| QA5 | atpenin inhibitory constant | 604 nM | 0.017 | 5 |
| QMAL | malate inhibitory constant | 31.6 mM | 2.73x10-6 | 6 |
| QOAA | oxaloacetate inhibitory constant | 70.6 µM | 6.87x10-6 | 7 |
| QFUM | fumarate inhibitory constant | 5.99 µM | 0.133 | 2 |
| QMALO | malonate inhibitory constant | 188 µM | 0.167 | 1 |

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| **Figure S4. Model Residual Analysis.** Left) Model residuals are randomly permuted and plotted as shown. Right) Model residuals are binned and plotted as a histogram indicating they follow a normal distribution. The normal distribution function is plotted with a mean of 0, standard deviation of 1.18, and a pre-exponential term equal to 74. These results show the model fits are unbiased and there are no systematic errors present. |

**Section 4**

**Quinol and quinone concentration equations**

|  |  |
| --- | --- |
|  | Eq. S1 |
| If complex III inhibitors are present, the following equation is used: | Eq. S2 |
|  | Eq. S3 |

**Oxidation-reduction reactions**

One-electron reactions

|  |  |
| --- | --- |
|  | Eq. S4 |
|  | Eq. S5 |
|  | Eq. S6 |
|  | Eq. S7 |
|  | Eq. S8 |

Two-electron reactions

|  |  |
| --- | --- |
|  | Eq. S9 |
|  | Eq. S10 |
|  | Eq. S11 |
|  | Eq. S12 |

**Binding polynomials for enzyme, substrates, products and regulators**

|  |  |
| --- | --- |
|  | Eq. S13 |
|  | Eq. S14 |
|  | Eq. S15 |

**Forward rate constants for succinate oxidation and quinol reduction**

|  |  |
| --- | --- |
|  | Eq. S16 |
|  | Eq. S17 |

**Flavin and [*3Fe-4S*] free radical production rates**

|  |  |
| --- | --- |
|  | Eq. S18 |
|  | Eq. S19 |
|  | Eq. S20 |

**Midpoint potential pH corrections**

|  |  |
| --- | --- |
|  | Eq. S21 |
|  | Eq. S22 |
|  | Eq. S23 |
|  | Eq. S24 |
|  | Eq. S25 |
|  | Eq. S26 |
|  | Eq. S27 |
|  | Eq. S28 |

**Midpoint potential bound state corrections**

|  |  |
| --- | --- |
|  | Eq. S29 |
|  | Eq. S30 |
|  | Eq. S31 |

**Equilibrium constants**

|  |  |
| --- | --- |
|  | Eq. S32 |
|  | Eq. S33 |
|  | Eq. S34 |
|  | Eq. S35 |
|  | Eq. S36 |
|  | Eq. S37 |

Here, GEA stands for “general electron acceptor” and is used when either phenazine or TMPD is the acceptor. The reaction is considered a concerted two-electron reduction of the acceptor. We use ISC3 and ISC1 as the electron source since these are the redox centers most highly reduced. Using ISC3 and ISC2 only causes the forward rate constant for phenazine and TMPD reduction to increase which does not change the simulation results.

**Boltzmann redox poise potentials**

|  |  |
| --- | --- |
|  | Eq. S38 |
|  | Eq. S39 |
|  | Eq. S40 |
|  | Eq. S41 |
|  | Eq. S42 |
|  | Eq. S43 |

**Formation energies for each redox center**

Before calculating the substate fractions for each electron state, the midpoint potentials are converted into the free energies using the following equation.

|  |  |
| --- | --- |
|  | Eq. S44 |

Here, n is the number of electrons transferred in the reaction.

**Substate fraction calculations.** To determining the transition rates, each combination of the redox centers (substates) reduced in each electronic state are calculated by Boltzmann distribution. Here, S*rk* is the fraction of redox centers *r* existing in the electronic state *k* that is reduced, and ΔG*rk* are the free energy change for each redox center *r* calculated from the linear superposition of the midpoint potentials.

|  |  |
| --- | --- |
|  | Eq. S45 |
| **Denominators for each electronic state** |  |
|  | Eq. S46 |
|  | Eq. S47 |
|  | Eq. S48 |
|  | Eq. S49 |
| ***E0* substates used in state transitions** |  |
|  | Eq. S50 |
| “*X*” represents any redox center as all are oxidized in the *E0* electronic state. |  |
| ***E1* substates used in state transitions** |  |
|  | Eq. S51 |
|  | Eq. S52 |
|  | Eq. S53 |
|  | Eq. S54 |
|  | Eq. S55 |
|  | Eq. S56 |
| ***E2* substates used in state transitions** |  |
|  | Eq. S57 |
|  | Eq. S58 |
|  | Eq. S59 |
|  | Eq. S60 |
|  | Eq. S61 |
|  | Eq. S62 |
|  | Eq. S63 |
|  | Eq. S64 |
|  | Eq. S65 |
| ***E3* substates used in state transitions** |  |
|  | Eq. S66 |
|  | Eq. S67 |
|  | Eq. S68 |
|  | Eq. S69 |
|  | Eq. S70 |
|  | Eq. S71 |
|  | Eq. S72 |
| ***E4* substates used in state transitions** |  |
|  | Eq. S73 |
|  | Eq. S74 |
|  | Eq. S75 |
|  | Eq. S76 |
|  | Eq. S77 |

**State transition details and rates**

|  |  |  |
| --- | --- | --- |
| 1) |  | Eq. S78 |
| 2) |  | Eq. S79 |
| 3) |  | Eq. S80 |
| 4) |  | Eq. S81 |
| 5) |  | Eq. S82 |
| 6) |  | Eq. S83 |
| 7) |  | Eq. S84 |
| 8) |  | Eq. S85 |
| 9) |  | Eq. S86 |
| 10) |  | Eq. S87 |
| 11) |  | Eq. S88 |
| 12) |  | Eq. S89 |
| 13) |  | Eq. S90 |
| 14) |  | Eq. S91 |

**Steady-state equations**

|  |  |
| --- | --- |
|  | Eq. S92 |

Five electron states were the minimal number required to fit all the data. Increasing the number to the maximum allowable of seven states does not significantly improve the model fits to the data.

**Succinate oxidation steady-state rate**

|  |  |
| --- | --- |
|  | Eq. S93 |

**Superoxide formation steady-state rate**

|  |  |
| --- | --- |
|  | Eq. S94 |

**Hydrogen peroxide formation steady-state rate**

|  |  |
| --- | --- |
|  | Eq. S95 |

**Quinol reduction steady-state rate**

|  |  |
| --- | --- |
|  | Eq. S96 |

**Phenazine and TMPD Reduction steady-state rate**

|  |  |
| --- | --- |
|  | Eq. S97 |

**Parameter sensitivity matrix and correlation coefficients.** The normalized parameter sensitivity matrix is computed using Eq. S98. Each model output, *fi*, is congruent with the experimental data. The parameter sensitivities were computed using the complex variable approach as described in Squire and Trap (9).

|  |  |
| --- | --- |
|  | Eq. S98 |

The sensitivity coefficients presented in Table 2 were computed by averaging all the non-zero sensitivity

coefficient for a given parameter. This was done by using Eq. S99.

|  |  |
| --- | --- |
|  | Eq. S99 |

Where *Ni* is the number of non-zero elements in *i*th row of *S*. The parameter correlation coefficients are pairwise linear correlation coefficients computed for each pair of columns in the normalized parameter sensitivity matrix.

**Section 5**

|  |  |
| --- | --- |
|  | Eq. S100 |
|  | Eq. S101 |
|  | Eq. S102 |
|  | Eq. S103 |
|  | Eq. S104 |
|  | Eq. S105 |
|  | Eq. S106 |

This system was integrated using ode15s with default tolerances and the backwards differentiation formulas. For Eq. S102, the oxygen consumption rate was taken from the data (10) (30 µM/sec) and converted to M/min. The enzyme concentration, [*Etot*] was 20 nM computed from 0.2 mg/ml SMP and 100 pmol SDH/mg taken from (11). The kinetic constants for QH2 and O2 were estimated from prior work (8,12). The quinone dynamics were computed with respect to a lipid volume fraction, *Vlp*, of approximately 50 µl lipid volume per liter assay buffer based on 250 nL/mg lipid volume (8) and 0.2 mg/ml SMP concentration.

**Section 6**

Model Code Description

SDH\_model\_code.zip - zip file containing the following files:  
data.mat - mat file containing data structure  
SDH\_parameters.mat - mat file containing model parameters  
main\_complexII.m - function containing SDH model code  
Generate\_Figures.m - function containing code to simulate the model and plot the figures

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