1 **Supporting Information**

2 # Substrate Redox Potential Controls Superoxide

3 # Production Kinetics in the Cytochrome *bc* Complex[†]

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9 **I. Chemical Syntheses.**

10 2,3-dimethoxy-5-chloro-6-undecyl-1,4-benzoquinone (**1**): 1 g of chloranil was suspended in

11 50 mL MeOH and refluxed for 3 hours to yield a bright red solution (*1*). A red residue was

12 obtained by evaporation of the solvent and was chromatographed on silica gel to yield 2-chloro-

13 3,5,6-trimethoxy-1,4-benzoquinone (~100mg) (along with other substituted chloro-

14 methoxybenzoquinones). Radical alkylation of 1,4-benzoquinone derivatives was carried out as

15 described by Gu et al. (*2*) and Folkers et al. (*3*). 100 mg of 2-chloro-3,5,6-trimethoxy-1,4-

16 benzoquinone and 0.200g lauroyl peroxide were dissolved in 25 mL acetic acid and refluxed for

17 4 hours. The red residue obtained upon evaporation of the acetic acid was chromatographed on

18 silica gel (CHCl$_3$), yielding ~50 mg of (**1**) as a red oil. This compound was not isolated in

19 sufficient yields for ^{13}C-NMR analysis. ^{1}H-NMR (300 MHz, CDCl$_3$); δ 4.03 (s, 3H, OMe), 3.99

Substrate Redox Potential in the *bc* Complex

1   (s, 3H, OMe), 2.05 (m, 2H, CH_2) 1.25 (m, 18H, CH_2), 0.87 (t, 3H, CH_3); MS(ESI): m/z= 356.4

2   [M$^-$] (calc. 356.5).

3

4   2-methoxy-5-methyl-6-undecyl-1,4-benzoquinone (**2**). 3.26 g (10.9 mmol) sodium dichromate

5   was added in small aliquots to 0.5 g (3.6 mmol) of 2-methoxy-5-methylaniline dissolved in a 1:1

6   mixture of H_2SO_4 and water over ice (*4*). The suspension was slowly warmed to room

7   temperature and stirred overnight. The reaction mixture was extracted with $CHCl_3$ (3X, 50 mL),

8   washed with concentrated sodium carbonate, dried over Na_2SO_4, and evaporated to dryness to

9   yield 200 mg of 2-methoxy-5-methyl-1,4-benzoquinone (~80% purity). The undecyl derivative

10   of the parent quinone was prepared as described above for compound (**1**). The reaction mixture

11   was dried by evaporation, re-dissolved in $CHCl_3$ and chromatographed on silica gel ($CHCl_3$).

12   Fractions containing the monosubstituted products were combined and dried over Na_2SO_4, and

13   evaporated to dryness to yield approximately 100 mg of a yellow oil (~23% yield). 2-methoxy-

14   3-undecyl-5-methyl-1,4-benzoquinone was also isolated as a minor product. ^{1}H-NMR (300

15   MHz, $CDCl_3$); δ 5.87 (s 1H, ring), 3.79 (s 3H, OCH_3), 2.48 (m 2H, CH_2), 2.04 (s 3H, CH_3), 1.57

16   (br, CH_2), 1.25 (br, CH_2), 0.85 (t 3H, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$); 188.3 (C=O), 182.3

17   (C=O), 158.5 (ring (**C**-OCH_3), 143.4 (ring **C**-CH_3), 141.4 (ring **C**-CH_2), 107.2 (ring CH), 56.3

18   (OCH_3), 32.1 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2),

19   28.7 (CH_2), 26.5 (CH_2), 22.8 (CH_2), 14.3 (ring CH_3), 12.3 (CH_3); MS(ESI) m/z = 307.6 (M+H$^+$)

20   (calc. 306.4).

21

22   2,6-dimethyl-5-undecyl-1,4-benzoquinone (**4**):   300 mg 2,6-dimethyl-1,4-benzoquinone (2.2

23   mmoles) and 1.31 g lauroyl peroxide (0.0033 mmoles) were dissolved in 20 mL glacial acetic

Substrate Redox Potential in the *bc* Complex

1 acid and refluxed for 48 hours. The reaction mixture was dried by evaporation, re-dissolved in

2 CHCl$_3$ and chromatographed on silica gel (1:1 hexanes:CHCl$_3$).   Three compounds were

3 collected corresponding to the starting quinone, mono-, and di-substituted products. Fractions

4 containing the monosubstituted product were combined and dried over Na$_2$SO$_4$, and evaporated

5 to dryness to yield approximately 300 mg of a yellow oil (~50%). ^{1}H-NMR (300 MHz, CDCl$_3$);

6 δ 6.42 (m 1H, ring)), 2.38 (m 2H, alkyl-CH$_2$), 1.98 (s 6H, alkyl-CH$_2$), 1.44 (m 2H, alkyl-CH$_2$),

7 1.23 (m 6H, ring-CH$_3$), 0.82 (t 3H, alkyl-CH$_3$). ^{13}C-NMR (75 MHz, CDCl$_3$); δ 188.6 (C=O),

8 187.6 (C=O), 145.4 (ring CH), 145.1 (ring CH), 140.7 (**C**-CH$_2$), 133.4 (**C**-CH$_3$), 32.1 (CH$_2$), 30.1

9 (CH$_2$), 29.8 (CH$_2$), 29.7 (CH$_2$), 29.6 (CH$_2$), 29.5 (CH$_2$), 29.4 (CH$_2$), 28.9 (CH$_2$), 26.6 (CH$_2$), 22.9

10 (CH$_2$), 16.1 (ring C-**C**H$_3$), 14.3 (ring C-**C**H$_3$), 12.3 (CH$_2$-**C**H$_3$).

11

12 2,6-dimethoxy-5-undecyl-1,4-benzoquinone (**5**): Prepared in a similar manner to (**1**) above.

13 160 mg 2,6-dimethoxy-1,4-benzoquinone (0.97 mmoles) and 0.53 g lauroyl peroxide (1.5

14 mmoles) were dissolved in 20 mL glacial acetic acid and refluxed for 8 hours. The reaction

15 mixture was dried by evaporation, re-dissolved in CHCl$_3$ and submitted to chromatography on

16 silica gel (CHCl$_3$). Fractions containing the monosubstituted product were combined and dried

17 over Na$_2$SO$_4$, and evaporated to dryness to yield approximately 200 mg of a yellow oil (~65%).

18 ^{1}H-NMR (300 MHz, CDCl$_3$); δ 5.83 (m 1H, ring), 3.96 (s 3H, OMe), 3.79 (s 3H, OMe), 2.42 (m

19 2H, CH$_2$), 1.56 (br m, CH$_2$), 1.27(br m, CH$_2$), 0.87 (t 3H, Me). ^{13}C-NMR (75 MHz, CDCl$_3$); δ

20 187.7 (C=O), 178.5 (C=O), 157.5 (ring **C**-OCH$_3$), 154.6 (ring **C**-OCH$_3$), 134.5 (ring **C**-CH$_2$),

21 107.2 (ring CH), 61.2 (OCH$_3$), 56.7 (OCH$_3$), 32.1 (CH$_2$), 30.2 (CH$_2$), 29.8 (CH$_2$), 29.7 (CH$_2$),

22 29.6 (CH$_2$), 29.5 (CH$_2$), 29.3 (CH$_2$), 23.6 (CH$_2$), 22.9 (CH$_2$), 14.3 (CH3); MS(ESI): m/z = 323.9

23 (M-H$^+$) (calc 324.4).

Substrate Redox Potential in the *bc* Complex

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2   2-(N-dimethylamino)-5-undecyl-6-methoxy-1,4-benzoquinone **(6)**:   50 mg of **(5)** was

3   dissolved in 20 mL acetonitrile on ice. 2 mL of 1M dimethylamine in MeOH was added to the

4   stirring solution, then warmed to room temperature and allowed to react for four hours.

5   Following evaporation of the solvent, the purple reside was submitted to chromatography on

6   silica gel ($CHCl_3$) to yield a purple band, which was collected and evaporated to dryness (~30

7   %). This compound was not isolated in sufficient yields for ^{13}C-NMR analysis. ^{1}H-NMR (300

8   MHz, $CDCl_3$); δ 5.48 (s, 1H), 3.85 (s, 3H), 3.07 (s, 6H), 2.43 (m, 2H), 1.25 (br m, CH_2), 0.87 (br

9   m, CH_3); MS(ESI): m/z = 366.4 (M+H^{+}) (calc. 365.5).

10

11   Hydroxy-3-amino-5-undecyl-methoxy-1,4-benzoquinones **(7):** 50 mg of **(5)** were dissolved in

12   20 mL EtOH. 40 mL concentrated NH_4OH was added to the solution and refluxed for 3 hours.

13   The red/purple solution was extracted twice with 30 mL $CHCl_3$, and dried over Na_2SO_4.

14   Chromatography on silica gel ($CHCl_3$) yielded multiple bands, of which a dark red band was

15   collected, dried, and re-purified by preparative TLC ($CHCl_2$) (~5 mg). Further TLC analysis

16   showed that this band was in fact two closely spaced bands, which we interpret as the isomers 2-

17   hydroxy-3-amino-5-undecyl-6-methoxy-1,4-benzoquinone and 6-hydroxy-3-amino-5-undecyl-2-

18   methoxy-1,4-benzoquinone based on broad singlet observed in ^{1}H-NMR spectra for the methoxy

19   protons. Further preparative TLC runs did not allow clean separation of these isomers. These

20   isomers, however, were found to have nearly the same redox properties (see below), thus

21   justifying the use of the mixture **(7)** in this study. This compound was not isolated in sufficient

22   yields for ^{13}C-NMR analysis. ^{1}H-NMR (300 MHz, $CDCl_3$); δ 3.92 (s, 3H, OMe), 1.62 (q, 2H,

1   CH$_2$), 1.25 (m, 18H, CH$_2$), 0.876 (t, 3H, Me); EI-MS (1% HOAc, MeOH): MS(EI): m/z = 324

2   (M$^+$) (calc 324).

3

4

5

6   **II. Calculation of substrate redox potentials.**

7

8   Equations (S1) – (S3) show the chemical equations and reaction free energies required for

9   correlation of quantum chemical calculations with experimental solution potentials, using

10   Hartree-Fock (HF) and Density Functional Theory (DFT) calculations as previously described

11   (5).   Table 1 below gives the calculated energies for the relevant redox and protonation states of

12   substrates **(1)**-**(7)**. In each of these equations the reaction energies are given as the difference in

13   the energy of the products and reactants. The term E_{elec} indicates the total electronic energy, and

14   the term $G_{correction}$ is the Gibbs free energy correction to the internal electronic energy that

15   derived from the molecular partition function. Derivation and application of this correction is

16   given in reference (6) and references therein.

17

18    $$Q(g) + H_2(g) \underset{\xleftarrow{\hspace{2cm}}}{\xrightarrow{E(Q/QH_2)_g}} QH_2(g) \; ;$$
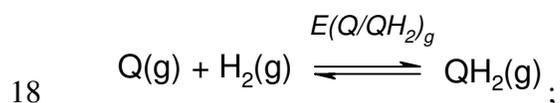
19    $$E(Q/QH_2)_{gas} = [E_{elec}(QH_2) + G_{correction}(QH_2)] - [E_{elec}(Q) + G_{correction}(Q)]$$
$$+ 2[E_{elec}(H_2) + G_{correction}(H_2) \qquad \qquad \textbf{(S1)}$$

20    $$Q(g) \underset{\xleftarrow{\hspace{2cm}}}{\xrightarrow{E(Q/Q^-)_g}} Q^-(g) \; ;$$
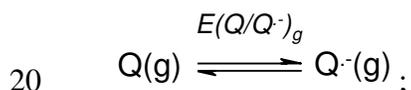
Substrate Redox Potential in the *bc* Complex

1     $E(Q/Q^{\bullet-})_{gas} = [E_{elec}(Q^{\bullet-}) + G_{correction}(Q^{\bullet-})] - [E_{elec}(Q) + G_{correction}(Q)]$ **(S2)**

                               $E(Q^{-}/QH\cdot)$

2     $Q^{-}(g) + H^{+}(g) \rightleftharpoons QH\cdot(g)$
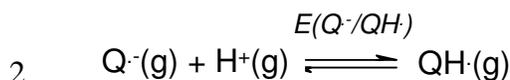
3     $E(Q^{\bullet-}/QH^{\bullet})_{gas} = [E_{elec}(QH^{\bullet}) + G_{correction}(QH^{\bullet})] - [E_{elec}(Q^{\bullet-}) + G_{correction}(Q^{\bullet-})]$ **(S3)**

4

5   Linear fits between experimental data and calculated redox potentials and pK_a values (within a

6   homologous series of water soluble benzo- and naphthoquinone species) for these reactions

7   yields the following correlations in equations (S4)-(S6), which can then be used to predict values

8   for homologous compounds for which no experimental data is available. (Note that these

9   correlations have been strictly applied only to other benzo- and naphthoquinone species, the

10   validity of applying these correlations outside these compounds has not been tested.) We have

11   found that calculating values of $E(Q/QH_2)$ and $E(Q/Q^{-})$ at the B3LYP/6-31G(d,p) and HF/6-

12   31G(d,p) levels of theory, respectively, yield the best results. All proton affinity values (eq. 8

13   above) were calculated with B3LYP/6-31G(d,p) , see (5) for details of this approach.

14     $E(Q/QH_2)_{estimate} = (E(Q/QH_2)_{calc} - 0.22146)/0.87508$ **(S4)**

15     $E(Q/Q^{-})_{estimate} = (E(Q/Q^{-})_{calc} - 2.79285)/1.04474$ **(S5)**

16     $E(Q^{-}/QH\cdot)_{estimate} = -22.55707 - (0.02007/E(Q^{-}/QH\cdot)_{calc})$ **(S6)**

17

18   Values of $E(Q/QH_2)$ given in equation (S4) give values at pH 7.0; the values in Table 1 of the

19   text are adjusted to pH 8.0. In equation (S6), $E(Q^{-}/QH\cdot)_{calc}$ is the gas phase proton affinity in

20   units of kJ/mole. All values for redox potentials are given in eV, with the estimates yielding

1   values relative to the standard hydrogen electrode. The results of applying the gas phase

2   energies in Tables 1 and 2 to equations (6)-(8) are shown in Table 3 below.

3   Good agreement is found between calculated values and those obtainable from experiment,

4   although in some cases the errors exceeded the range previously found (~30 mV) (*5*). However,

5   these errors also include experimental errors, which can be substantial considering the

6   difficulties in determining the experimental potentials by voltammetric techniques. As we noted

7   previously (*5*), the methoxy-substituted, high-potential quinone species' **(1)-(3)** Q/QH_2 midpoint

8   potentials represent a 'worst case' for this technique, and are overestimated by approximately 50-

9   100 mV, which underestimates the calculated stability constants. Even the largest expected

10   errors would not have affected the order of reactivity in this series, nor greatly affect the slope of

11   log k vs. ΔE, shown in Fig. 2 of the main text.

1   **Table 1.   Relevant gas phase energies calculated for substrates (1)-(7) calculated at the**

2   **B3LYP/6-31G(d.p) level.** All energies are given in hartrees, see (5) for details of this approach.

3

Substrate	E(Q)	E(QH$_2$)	E(Q$^-$)	E(QH$^-$)[1]
(1)	-1109.290491	-1110.502521	-1109.357423	-1109.886873 (4)
(2)	-574.495638	-575.70334	-574.550308	-575.090237 (1)
(3)	-688.984531	-690.194076	-689.040173	-689.579465 (1)
(4)	-499.299738	-500.498913	-499.35225	-499.883062(4)
(5)	-649.695882	-650.89606	-649.74782	-650.284932 (4)
(6)	-669.110202	-670.306076	-669.158187	-669.687653 (1)
(7)	-665.779149	-666.968632	-665.830668	-666.366088 (4)

4

5   [1] The protonated conformation yielding the lowest energy is given for structurally asymmetric
6   neutral semiquinone species. Since most of these quinone species are asymmetric with respect to
7   the plan bisecting the C=O two fold axis, the neutral semiquinones will have differing proton
8   affinities for either the 1- or 4- positions. We calculated both conformers, and show here the
9   energy of species with the position the highest proton affinity. The position of protonation is
10   given in parentheses, indicating either the 1- or 4- positions.

11

1 **Table 2. Relevant gas phase energies calculated for substrates (1)-(7) calculated at the**

2 **HF/6-31G(d.p) level for the Q/Q⁻ couple.** All energies are given in hartrees, see (5) for details

3 of this approach.

4

Substrate	E(Q)	E(Q⁻)
(1)	-1104.813253	-1104.9265
(2)	-571.074064	-571.176349
(3)	-684.901688	-685.004585
(4)	-496.239658	-496.337669
(5)	-606.90044	-607.003707
(6)	-665.077692	-665.176196
(7)	-661.938434	-662.042938

5

Substrate Redox Potential in the *bc* Complex

1 **Table 3. Gas phase reaction energetics obtained from DFT and HF calculations.**

2 Electrochemical values are given in eV and proton affinities are given in kJ/mole, see (5) for

3 details of this approach.

Substrate	$E(Q/QH_2)_{gas}$	$E(Q/Q^-)_{gas}$	$E(Q^-/QH^-)_{gas}$
(1)	0.42917	3.0173	-1358.52166
(2)	0.37142	2.72524	-1389.79882
(3)	0.38026	2.74154	-1390.89793
(4)	0.2577	2.61136	-1366.33902
(5)	0.27107	2.7514	-1382.55804
(6)	0.21367	2.6245	-1362.86664
(7)	0.12844	2.78436	-1378.19247

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Substrate Redox Potential in the *bc* Complex

1   References

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Substrate Redox Potential in the *bc* Complex

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Substrate Redox Potential in the *bc* Complex