Mitochondria, oxygen and cardiac PCr/ATP

## Supplementary materials

There now follows a complete mathematical description of the basic model used in the present study. Subscripts: e, external (cytosolic); i, internal (mitochondrial); t, total; f, free; m, magnesium complex; j, monovalent. All reaction rates are expressed in μM min$^{-1}$. The Mathematica notebook file of the basic model can be downloaded here:

http://dl.dropbox.com/u/1998606/heart_model_molbiosyst2011.nb

Kinetic equations

Substrate dehydrogenation:

$$v_{DH} = k_{DH} \frac{1}{\left(1 + \frac{K_{mN}}{NAD^+/NADH}\right)^{p_D}}$$

$k_{DH}$= 96293 μM min$^{-1}$, $K_{mN}$=100, $p_D$=0.8

Complex I:

$$v_{C1} = k_{C1} \cdot \Delta G_{C1}$$

$k_{C1}$= 819.61 μM mV$^{-1}$ min$^{-1}$

Complex III:

$$v_{C3} = k_{C3} \cdot \Delta G_{C3}$$

$k_{C3}$= 467.90 μM mV$^{-1}$ min$^{-1}$

Complex IV:

$$v_{C4} = k_{C4} \cdot a^{2+} \cdot c^{2+} \frac{1}{1 + \frac{K_{mO}}{O_2}}$$

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$k_{C4}$= 12.348 μM$^{-1}$ min$^{-1}$, $K_{mO}$=120 μM (apparent $K_{mO}$=0.8 μM)

ATP synthase:

$$v_{SN} = k_{SN} \frac{\gamma - 1}{\gamma + 1}$$

$k_{SN}$= 117706 μM min$^{-1}$, $\gamma = 10^{\Delta G_{SN}/Z}$

ATP/ADP carrier:

$$v_{EX} = k_{EX} \cdot \left( \frac{ADP_{fe}}{ADP_{fe} + ATP_{fe} \cdot 10^{-\Psi_e/Z}} - \frac{ADP_{fi}}{ADP_{fi} + ATP_{fi} \cdot 10^{-\Psi_i/Z}} \right) \cdot \left( \frac{1}{1 + K_{mADP} / ADP_{fe}} \right)$$

$k_{EX}$= 187185 μM min$^{-1}$, $K_{mADP}$=3.5 μM

Phosphate carrier:

$$v_{PI} = k_{PI} \cdot \left( Pi_{je} \cdot H_e - Pi_{ji} \cdot H_i \right)$$

$k_{PI}$= 238.11 μM$^{-1}$ min$^{-1}$

ATP usage:

$$v_{UT} = k_{UT} \frac{1}{1 + \frac{K_{mA}}{ATP_{te}}}$$

$k_{UT}$= 12244 μM min$^{-1}$ (low work) – 61220 μM min$^{-1}$ (high work), $K_{mA}$=150 μM

Proton leak:

$$v_{LK} = k_{LK1} \cdot \left( e^{k_{LK2} \cdot \Delta p} - 1 \right)$$

$k_{LK1}$= 8.5758 μM min$^{-1}$, $k_{LK2}$=0.038 mV$^{-1}$

Adenylate kinase:

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$$v_{AK} = k_{fAK} \cdot ADP_{fe} \cdot ADP_{me} - k_{bAK} \cdot ATP_{me} \cdot AMP_{e}$$

$k_{fAK}$= 862.10 μM$^{-1}$ min$^{-1}$, $k_{bAK}$= 22.747 μM$^{-1}$ min$^{-1}$

Creatine kinase:

$$v_{CK} = k_{fCK} \cdot ADP_{te} \cdot PCr \cdot H_e^+ - k_{bCK} \cdot ATP_{te} \cdot Cr$$

$k_{fCK}$ = 1.9258 μM$^{-2}$ min$^{-1}$, $k_{bCK}$ = 0.00087538 μM$^{-1}$ min$^{-1}$

Set of differential equations

$$\dot{NADH} = (v_{DH} - v_{C1}) \cdot R_{cm} / B_N$$

$$\dot{UQH_2} = (v_{C1} - v_{C3}) \cdot R_{cm}$$

$$\dot{c}^{2+} = (v_{C3} - 2 \cdot v_{C4}) \cdot 2 \cdot R_{cm}$$

$\dot{O}_2 = 0$ (constant saturated oxygen concentration = 240 μM, or $\dot{O}_2 = -v_{C4}$)

$$\dot{H}_i^+ = -\left(2 \cdot (2 + 2 \cdot u) \cdot v_{C4} + (4 - 2 \cdot u) \cdot v_{C3} + 4 \cdot v_{C1} - n_A \cdot v_{SN} - u \cdot v_{EX} - (1-u) \cdot v_{PI} - v_{LK}\right) \cdot R_{cm} / r_{buffi}$$

$$\dot{ATP}_{ti} = (v_{SN} - v_{EX}) \cdot R_{cm}$$

$$\dot{Pi}_{ti} = (v_{PI} - v_{SN}) \cdot R_{cm}$$

$$\dot{ATP}_{te} = v_{EX} - v_{UT} + v_{AK} + v_{CK}$$

$$\dot{ADP}_{te} = v_{UT} - v_{EX} - 2 \cdot v_{AK} - v_{CK}$$

$$\dot{Pi}_{te} = v_{UT} - v_{PI}$$

$$\dot{PCr} = -v_{CK}$$

$R_{cm}$ = 3.35 (cell volume/mitochondria volume ratio)

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$B_N = 5$ (buffering capacity coefficient for NAD)

Calculations

$c^{3+} = c_t - c^{2+}$

$c_t = 270\ \mu M$ (= $c^{2+} + c^{3+}$, total concentration of cytochrome c)

$UQ = U_t - UQH_2$

$U_t = 1350\ \mu M$ (= $UQH_2$ + UQ, total concentration of ubiquinone)

$NAD^+ = N_t - NADH$

$N_t = 2970\ \mu M$ (= NADH + $NAD^+$, total concentration of NAD)

$AMP_e = A_{eSUM} - ATP_{te} - ADP_{te}$

$A_{eSUM} = 6700.2\ \mu M$ (= $ATP_{te}$ + $ADP_{te}$ + $AMP_e$, total external adenine nucleotide concentration)

$ADP_{ti} = A_{iSUM} - ATP_{ti}$

$A_{iSUM} = 16260\ \mu M$ (= $ATP_{ti}$ + $ADP_{ti}$, total internal adenine nucleotide concentration)

$Cr = C_{SUM} – PCr$

$C_{SUM} = 25000\ \mu M$ (= Cr + PCr, total creatine concentration)

$P_{SUM} = 45582\ \mu M$ (=$PCr+3ATP_{te}+2ADP_{te}+AMP_e+Pi_{te}+(3ATP_{ti}+2ADP_{ti}+Pi_{ti})/R_{cm}$, total phosphate pool)

$Mg_{fe} = 4000\ \mu M$ (free external magnesium concentration)

$ATP_{fe} = ATP_{te}/(1+Mg_{fe}/k_{DTe})$

$k_{DTe} = 24\ \mu M$ (magnesium dissociation constant for external ADP)

$ATP_{me} = ATP_{te} - ATP_{fe}$

$ADP_{fe} = ADP_{te}/(1+Mg_{fe}/k_{DDe})$

$k_{DDe} = 347\ \mu M$ (magnesium dissociation constant for external ATP)

$ADP_{me} = ADP_{te} - ADP_{fe}$

$Mg_{fi} = 380\ \mu M$ (free internal magnesium concentration)

$ATP_{fi} = ATP_{ti}/(1+Mg_{fi}/k_{DTi})$

$k_{DTi} = 17\ \mu M$ (magnesium dissociation constant for internal ATP)

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$ATP_{mi} = ATP_{ti} - ATP_{fi}$

$ADP_{fi} = ADP_{ti}/(1+Mg_{fi}/k_{DDi})$

$k_{DDi} = 282\ \mu M$ (magnesium dissociation constant for internal ADP)

$ADP_{mi} = ADP_{ti} - ADP_{fi}$

T = 298

$R = 0.0083\ kJ{*}mol^{-1}{*}K^{-1}$

$F = 0.0965\ kJ{*}mol^{-1}{*}mV^{-1}$

S = 2.303*R*T

Z = 2.303*R*T/F

u = 0.861 $(=\Delta\Psi/\Delta p)$

$pH_e = 7.0$ (= constant)

$pH_i = -\log(H_i/1000000)$ ($H_i$ expressed in µM)

$\Delta pH = Z\ (pH_i - pH_e)$

$\Delta p = 1/(1-u)\ \Delta pH$

$\Delta\Psi = -\ (\Delta p - \Delta pH)$

$\Psi i = 0.65{*}\Delta\Psi$

$\Psi e = -\ 0.35{*}\Delta\Psi$

$c_{0i} = (10^{-pHi} - 10^{-pHi-dpH})/dpH$ (‘natural’ buffering capacity for $H^+$ in matrix)

dpH = 0.001

$r_{buffi} = c_{buffi}/c_{0i}$ (buffering capacity coefficient for $H^+$ in matrix)

$c_{buffi} = 0.022\ M\ H^+/pH$ unit (buffering capacity for $H^+$ in matrix)

$c_{0e} = (10^{-pHe} - 10^{-pHe-dpH})/dpH$ (‘natural’ buffering capacity for $H^+$ in cytosol)

dpH = 0.001

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$Pi_{je} = Pi_{te}/(1+10^{pHe-pKa})$

$Pi_{ji} = Pi_{ti}/(1+10^{pHi-pKa})$

$pK_a = 6.8$

$\Delta G_{SN} = n_A * Dp - DG_P$ (thermodynamic span of ATP synthase)

$\Delta G_P = DG_{P0}/F + Z * \log(1000000 * ATP_{ti}/(ADP_{ti} * Pi_{ti}))$ (concentrations expressed in μM)

$n_A = 2.5$ (phenomenological $H^+$/ATP stoichiometry of ATP synthase)

$\Delta G_{P0} = 31.9$ kJ $*mol^{-1}$

$E_{mN} = E_{mN0}+Z/2 * \log(NAD^+/NADH)$ (NAD redox potential)

$E_{mN0} = -320$ mV

$E_{mU} = E_{mU0}+Z/2 * \log(UQ/UQH_2)$ (ubiquinone redox potential)

$E_{mU0} = 85$ mV

$E_{mc} = E_{mc0}+Z * \log(c^{3+}/c^{2+})$ (cytochrome c redox potential)

$E_{mc0} = 250$ mV

$E_{ma} = E_{mc}+\Delta p*(2+2u)/2$ (cytochrome $a_3$ redox potential)

$A_{3/2} = 10^{(Ema-Ema0)/Z}$ ($a^{3+}/a^{2+}$ ratio)

$a^{2+} = a_t/(1+A_{3/2})$ (concentration of reduced cytochrome $a_3$)

$a^{3+} = a_t - a^{2+}$

$a_t = 135$ μM

$E_{ma0} = 540$ mV

$\Delta G_{C1} = E_{mU}-E_{mN}-\Delta p*4/2$ (thermodynamic span of complex I)

$\Delta G_{C3} = E_{mc}-E_{mU}-\Delta p*(4-2u)/2$ (thermodynamic span of complex III)

**Supplementary figure legends**

**Figure S1:** The effect of changes in the activity of proteins that either contribute to (A-C) or consume (D-F) the proton gradient on mitochondrial ATP synthesis flux ($V_{ATP}$ in μM/min) and the mitochondrial membrane potential (ΔΨ). (A, D) saturating [$O_2$]; (B, D) normoxia; (C, F) hypoxia.

**Figure S2:** The effect of changes in oxygen concentration on ΔΨ (delta psi in mV) and mitochondrial ATP synthesis flux ($V_{ATP}$ in μM/min) at various rates of proton leak

Electronic Supplementary Material (ESI) for Molecular BioSystems


**Figure S1**

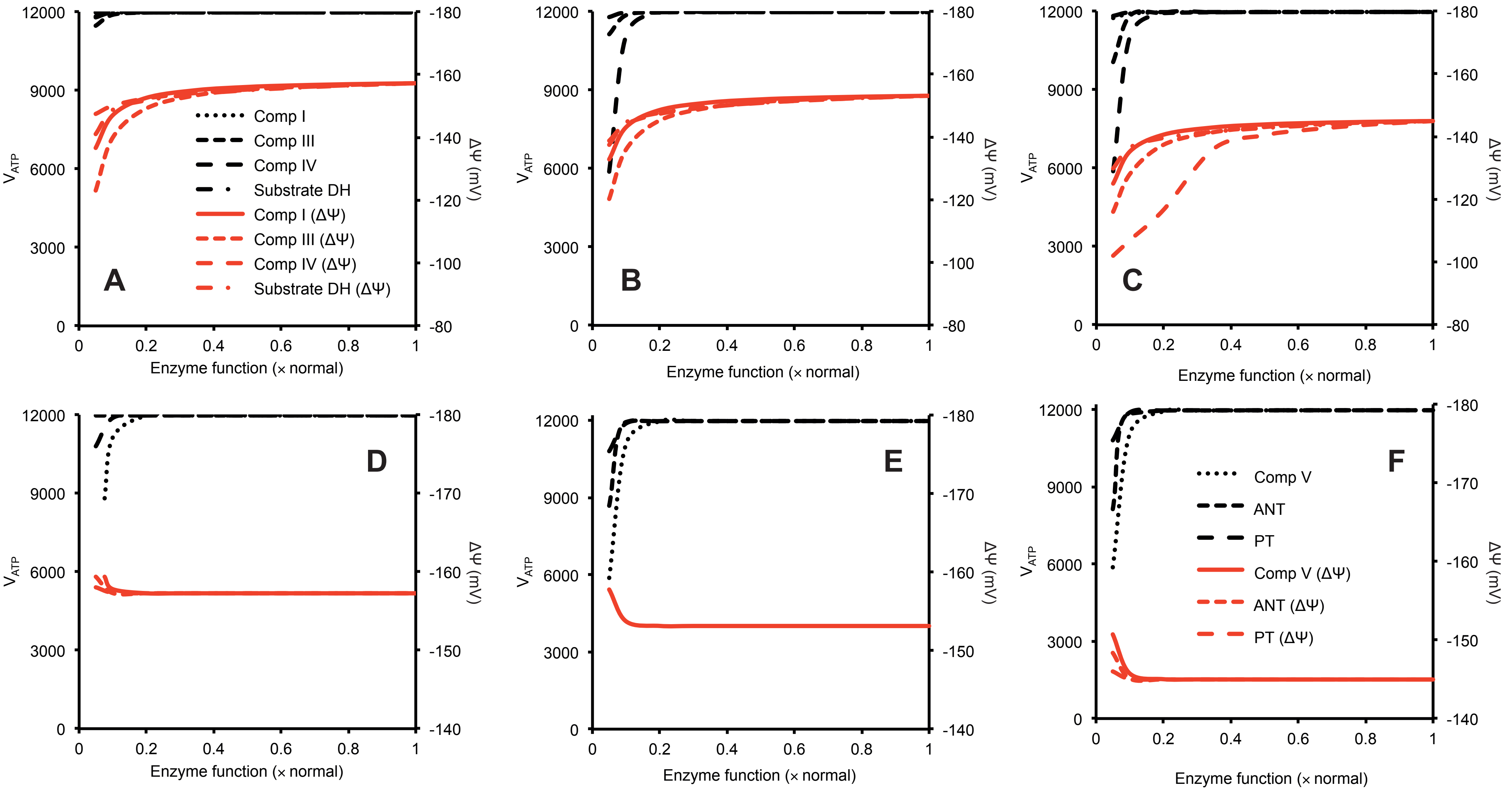


**Figure S1:** The effect of changes in the activity of proteins that either contribute to (A-C) or consume (D-F) the proton gradient on mitochondrial ATP synthesis flux ($V_{ATP}$ in uM/min) and the mitochondrial membrane potential (ΔΨ)

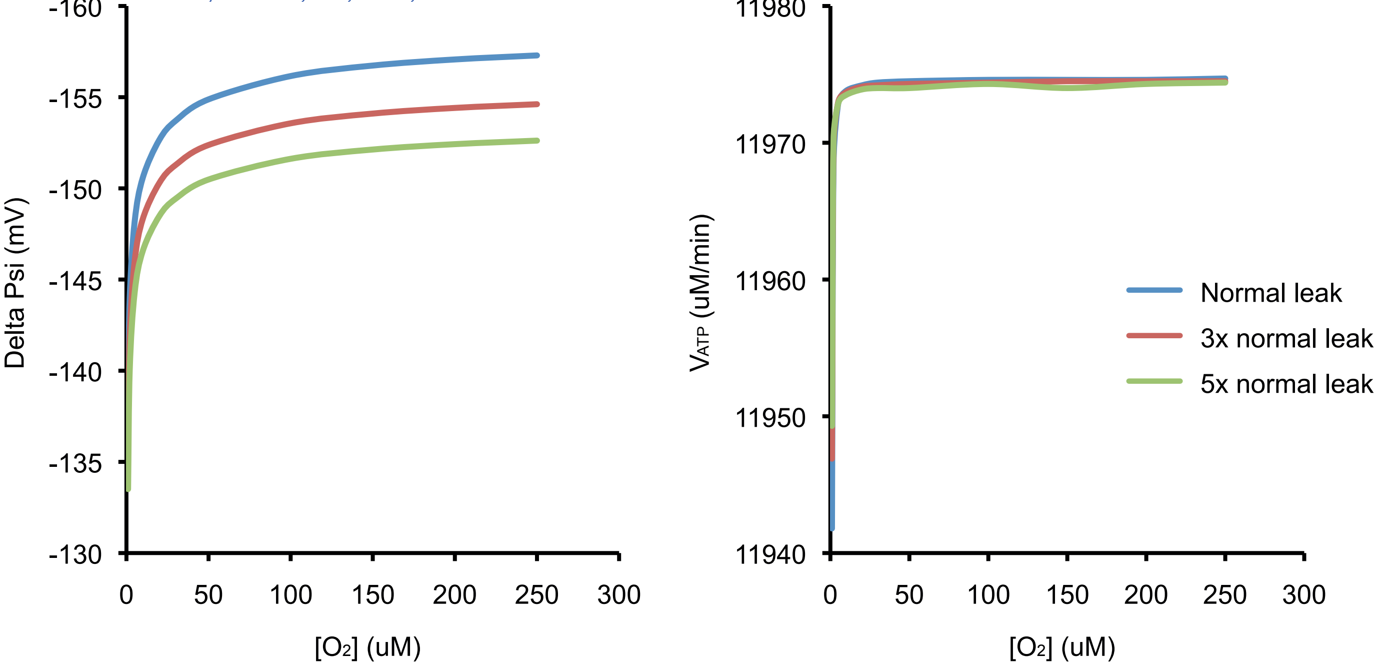
-160
-155
-150
-145
-140
-135
-130
Delta Psi (mV)
0
50
100
150
200
250
300
[O2] (uM)
11980
11970
11960
11950
11940
VATP (uM/min)
0
50
100
150
200
250
300
[O2] (uM)
Normal leak
3x normal leak
5x normal leak

**Figure S3**

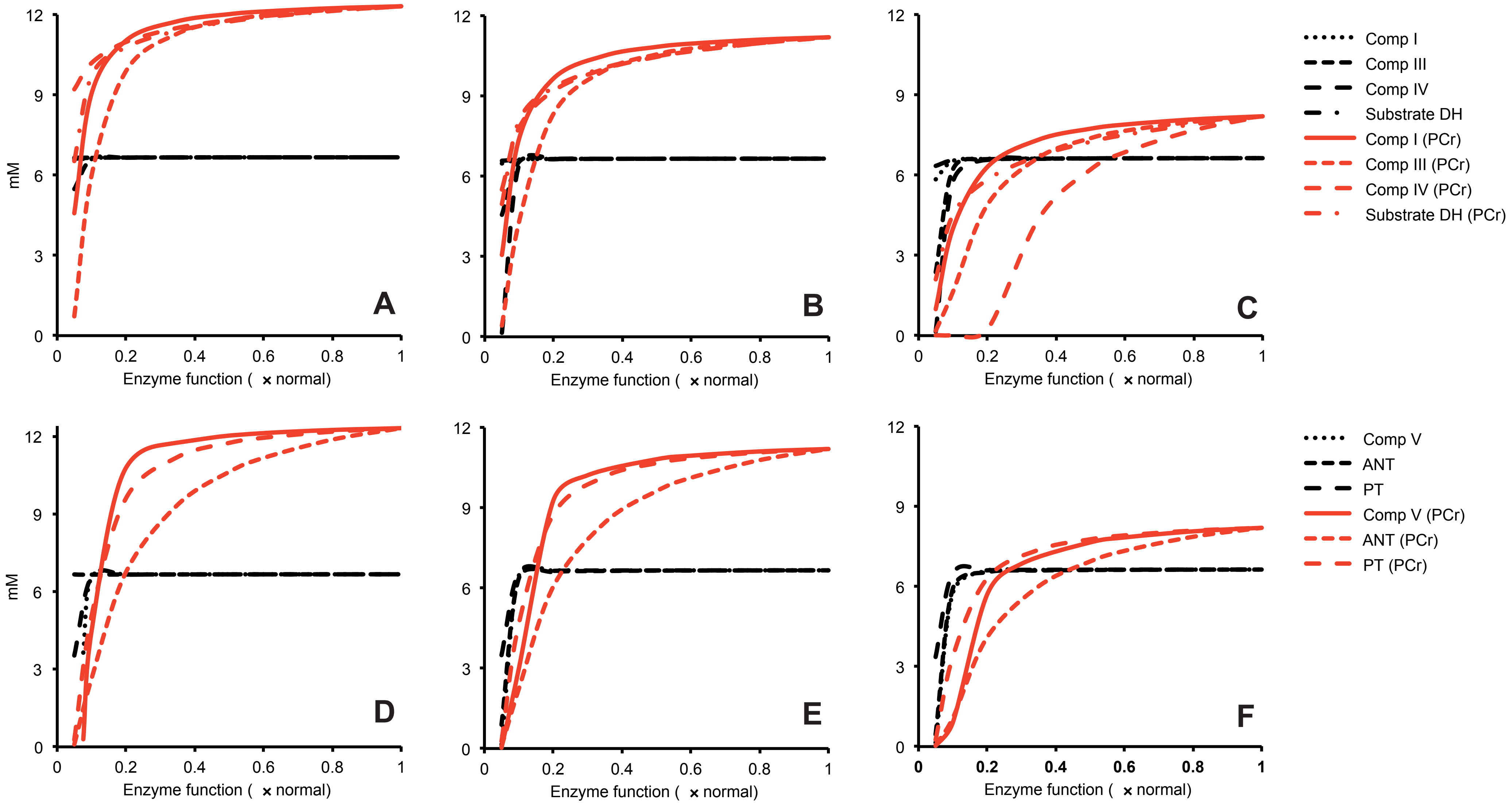


**Figure S3:** The effect of changes in the activity of proteins that either contribute to (A-C) or consume (D-F) the proton gradient on [ATP] (in black) and [PCr] (in red).