**Supplemental file for "Enzyme Kinetics of the Mitochondrial Deoxyribonucleoside Salvage Pathway Are Not Sufficient to Support Rapid mtDNA Replication"**

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*Model constants*:

**Start of L strand replication**
Lstrandstart=10969;

**The fractions of A, C, G, T on the heavy and light strands of mtDNA**
fdTH=0.309;
fdTL=0.247;
fdCH=0.131;
fdCL=0.313;
fdAH=0.247;
fdAL=0.309;
fdGH=0.313;
fdGL=0.131;

**Hill coefficient of TK2-thymidine reaction**
Reference [8]
tk2hill=0.5;

**The length of both strands of mtDNA**
DNAlength=33136;
**The length of one strand of mtDNA**
strandDNA=DNAlength / 2;

**Volume of a mitochondrion**
Reference [25]
volmito=2 x $10^{-16}$;

**Conversion factor used to convert Kms and concentrations from micromolar to molecules per mitochondrion**
(conversion = 120.4;)
conversion=1 x $10^{-6}$ x 6.022 x $10^{23}$ x volmito;
secondsperminute=60;

**Factor used to decrease the Vmax of the polymerase on double stranded templates with lower primer density**
Reference [25]
dsfact=1 / 2;

**Polymerase kinetic constants**

Reference [14]
VmaxPoldT=25.0 x dsfact x secondsperminute;
VmaxPoldC=43.0 x dsfact x secondsperminute;
VmaxPoldA=45.0 x dsfact x secondsperminute;
VmaxPoldG=37.0 x dsfact x secondsperminute;
KmPoldT=0.63 x conversion;
KmPoldC=0.9 x conversion;
KmPoldA=0.8 x conversion;
KmPoldG=0.8 x conversion;

**Ki of dTTP on TK2**
Reference [21]
kidttptk2=2.3 x conversion;

**Ki of dUTP on TK2: geometric mean of dCTP and dTTP values**
kidutptk2=1.38 x conversion;

**Ki of dCTP on TK2**
Reference [21]
kidctptk2=0.83 x conversion;

**Ki of dU on TK2: geometric mean**
Reference [8]
kidutk2=227 x conversion;

**Ki of dC on TK2**
Reference [21]
kidctk2=40 x conversion;

**Ki of dT on TK2**
Reference [21]
kidttk2=4.9 x conversion;

**(Substrate Kis on DGUOK set equal to substrate Kms)**

**Ki of dI on DGUOK set equal to Km**
Reference [20]
kididgk=12 x conversion;

**Ki of dIMP on DGUOK**
Reference [36]
kidimpdgk=78 x conversion;

**Ki of dITP on DGUOK set equal to dATP Ki**
kiditpdgk=kidatpdgk;

**Ki of dGMP on DGUOK**
Reference [36]
kidgmpdgk=4 x conversion;

**Ki of dAMP on DGUOK**
Reference [36]
kidampdgk=28 x conversion;

**Ki of dATP on DGUOK**
Reference [36]
kidatpdgk=41 x conversion;

**Ki of dGTP on DGUOK**
Reference [36]
kidgtpdgk=0.4 x conversion;

**Estimated nucleoside transporter (ENT) molecular weight in kilodalton**
Reference [25]
transporterMW=50;

**TK2 and DGUOK molecular weights in kilodalton**
**(DGUOK is a dimer, TK2 exists both as dimer and tetramer (mean taken))**
Reference [37,38]
dgkMW=58;
tk2MW=87;

**Molecular weight of NT5M in kilodalton (dimer)**
Reference [19,24]
dnt2MW=46;

**Ectonucleotidase molecular weight in kilodalton (tetramer)**
Reference [23]
enMW=210;

**TMPK2 molecular weight in kilodalton**
Reference [39]
tmpk2MW=44;

**GMPK2 assumed molecular weight in kilodalton**
Reference [23]
gmpk2MW=22;

**CMPK2 molecular weight in kilodalton**
Reference [22]
cmpk2MW=44.5;

**AK2 molecular weight in kilodalton**
Reference [UniProt accession P54819]
akMW=26;

**NME4 molecular weight in kilodalton (homohexamer)**
Reference [17]
ndpkMW=120;

**Nucleoside kinase molecules in each mitochondrion**
Reference [25]
tk2moleculespermito=100;
dgkmoleculespermito=200;

**NT5M molecules in each mitochondrion**
Reference [25]
dnt2moleculespermito=50;

**Ectonucleotidase molecules in each mitochondrion**
enmoleculespermito=50;

**TMPK2 molecules in each mitochondrion**
Reference[25]
tmpk2moleculespermito=50;

**GMPK2 molecules in each mitochondrion**
Reference [25]
gmpk2moleculespermito=50;

**CMPK2 molecules in each mitochondrion**
Reference [25]
cmpk2moleculespermito=50;

**NME4 molecules in each mitochondrion**
Reference [25]
ndpkmoleculespermito=300;

**The factor that the reverse reaction is faster than the forward reaction for NMPK**
factorMD=0.1; (AMP/ADP)

**The factor that the reverse reaction is faster than the forward reaction for NDPK**
factorDT=0.1; (ADP/ATP)

**ENT molecules per mitochondrion**
Reference [25]
transportermoleculespermito=38;

**Adenylate kinase molecules per mitochondrion**
Reference [25]
akmoleculespermito=450;

**ENT Vmax converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [40,41]
transportervmax=0.000086/0.0000021 x transportermoleculespermito;

**Vmax of the first phosphorylation of dT in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [21]
Vmax1PfdT=1.288 x tk2MW x tk2moleculespermito;

**Vmax of the first phosphorylation of dC in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [21]
Vmax1PfdC=0.789 x tk2MW x tk2moleculespermito;

**Vmax of dC with DGUOK converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [20]
Vmax1PfdCdgk=0.059 x dgkMW x dgkmoleculespermito;

**Vmax of the first phosphorylation of dA in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [20]
Vmax1PfdA=0.429 x dgkMW x dgkmoleculespermito;

**Vmax of the first phosphorylation of dG in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [20]
Vmax1PfdG=0.043 x dgkMW x dgkmoleculespermito;

**Vmax of the first phosphorylation of dT in the reverse direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [16]
Vmax1PrdT=74 x dnt2MW x dnt2moleculespermito;

**Ectonucleotidase Vmax of the first phosphorylation of dT in the reverse direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Vmax1PrdTen=4.5 x enMW x enmoleculespermito;

**Vmax of the first phosphorylation of dC in the reverse direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**

Vmax1PrdC=4.5 x enMW x enmoleculespermito;

**Vmax of the first phosphorylation of dA in the reverse direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Vmax1PrdA=4.5 x enMW x enmoleculespermito;

**Vmax of the first phosphorylation of dG in the reverse direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Vmax1PrdG=4.5 x enMW x enmoleculespermito;

**Vmax of the second phosphorylation of dT in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [23]
Vmax2PfdT=0.821 x tmpk2MW x tmpk2moleculespermito;

**Vmax of the second phosphorylation of dC in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [22]
Vmax2PfdC=1.77 x cmpk2MW x cmpk2moleculespermito;

**Vmax of the second phosphorylation of dA in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [12]
Vmax2PfdA=272.8 x akMW x akmoleculespermito;

**Vmax of the second phosphorylation of dG in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [23]
Vmax2PfdG=1.54 x gmpk2MW x gmpk2moleculespermito;

**Vmax of the second phosphorylation of dT in the reverse direction**
Vmax2PrdT=Vmax2PfdT x factorMD;

**Vmax of the second phosphorylation of dC in the reverse direction**
Vmax2PrdC=Vmax2PfdC x factorMD;

**Vmax of the second phosphorylation of dA in the reverse direction**
Vmax2PrdA=Vmax2PfdA x factorMD;

**Vmax of the second phosphorylation of dG in the reverse direction**
Vmax2PrdG=Vmax2PfdG x factorMD;

**Vmax of the third phosphorylation of dT in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [42]
Vmax3PfdT=140 x ndpkMW x ndpkmoleculespermito;

**Vmax of the third phosphorylation of dC in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [42]
Vmax3PfdC=50 x ndpkMW x ndpkmoleculespermito;

**Vmax of the third phosphorylation of dA in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [17]
Vmax3PfdA=225 x ndpkMW x ndpkmoleculespermito; (set equal to dGDP Vmax)

**Vmax of the third phosphorylation of dG in the forward direction converting from micromoles substrate/mg enzyme/minute to molecules substrate/mitochondrion/minute**
Reference [42]
Vmax3PfdG=225 x ndpkMW x ndpkmoleculespermito;

**Vmax of the third phosphorylation of dT in the reverse direction**
Vmax3PrdT=Vmax3PfdT x factorDT;

**Vmax of the third phosphorylation of dC in the reverse direction**
Vmax3PrdC=Vmax3PfdC x factorDT;

**Vmax of the third phosphorylation of dA in the reverse direction**
Vmax3PrdA=Vmax3PfdA x factorDT;

**Vmax of the third phosphorylation of dG in the reverse direction**
Vmax3PrdG=Vmax3PfdG x factorDT;

**ENT Km**
Reference [40]
transporterkm=2 x conversion;

**Km of the first phosphorylation of dT in the forward direction**
Reference [21]
km1PfdT=13 x conversion;

**Km of the first phosphorylation of dC in the forward direction**
Reference [21]
km1PfdC=11 x conversion;

**Km of dC with DGUOK**
Reference [20]
km1PfdCdgk=336 x conversion;

**Km of the first phosphorylation of dA in the forward direction**
Reference [20]

km1PfdA=467 x conversion;

**Km of the first phosphorylation of dG in the forward direction**
Reference [20]
km1PfdG=4 x conversion;

**Km of the first phosphorylation of dT, dU in the reverse direction**
Reference [19]
km1PrdT=200 x conversion;
km1PrdU=100 x conversion;
km1PrrU=1.5 x km1PrdT;

**Ectonucleotidase data: geometric means for substrate Kms, higher Kms plugged for inhibitions to be conservative**
Reference [23,24]
**Ectonucleotidase Km of the first phosphorylation of dT, dU, rU in the reverse direction**
km1PrdTen=22.5 x conversion;
km1PrdUen=110 x conversion; (set equal to UMP Km)
km1PrrUen=110 x conversion; (set equal to Km)

**Ectonucleotidase Km of the first phosphorylation of dC, rC in the reverse direction**
km1PrdC=290 x conversion;
km1PrrC=360 x conversion;

**Ectonucleotidase Km of the first phosphorylation of da, rA in the reverse direction**
km1PrdA=62 x conversion;
km1PrrA=19 x conversion; (set equal to Km)

kiadpen=17 x conversion;
kiatpen=15 x conversion;

**Ectonucleotidase Km of the first phosphorylation of dG, rG in the reverse direction**
km1PrdG=48 x conversion;
km1PrrG=59 x conversion; (set equal to Km)

**Ectonucleotidase Km of the first phosphorylation of dI, rI in the reverse direction**
km1PrdI=100 x conversion; (set equal to Km of IMP)
km1PrrI=100 x conversion; (set equal to Km)

**Km of the second phosphorylation of dT in the forward direction**
Reference [12]
km2PfdT=20 x conversion;
km2PfdUtmpk2=2600 x conversion; (Km is 170, but Ki is 2600)

**Miscellaneous inhibitions**
Reference [23]

kidttptmpk2=700 x conversion;
kidttmpk2=180 x conversion;

**Km of the second phosphorylation of dC in the forward direction**
Reference [22]
km2PfdC=1310 x conversion;
km2PfrC=3090 x conversion;
km2PfrU=6300 x conversion;
km2PfdUcmpk2=100 x conversion;

**CMPK1 can phosphorylate AMP and dAMP**
Reference [43]
km2PfrAcmpk2=km2PrrAcmpk2=km2PfdAcmpk2=km2PrdAcmpk2=100 x 500 x conversion;
(Km of CMP is 500 micromolar)

**Km of the second phosphorylation of dA in the forward direction**
Reference [12]
km2PfdA=210 x conversion;
km2PfrA=80 x conversion;

**CMP and UMP have some reactivity with AK2 - included as inhibitions**
Reference [12]
km2PfrCak2=6000 x conversion;
km2PfrUak2=9000 x conversion;

**Km of the second phosphorylation of dG in the forward direction**
Reference [23]
km2PfdG=112 x conversion;
km2PfrG=18 x conversion;

**Km of the second phosphorylation of dT in the reverse direction**
km2PrdT=km2PfdT;
km2PrdUtmpk2=km2PfdUtmpk2;

**Km of the second phosphorylation of dC in the reverse direction**
km2PrdC=km2PfdC;
km2PrrC=km2PfrC;
km2PrrU=km2PfrU;
km2PrdUcmpk2=km2PfdUcmpk2;

**Km of the second phosphorylation of dA in the reverse direction**
km2PrdA=km2PfdA;
km2PrrA=km2PfrA;

km2PrrCak2=km2PfrCak2;
km2PrrUak2=km2PfrUak2;

**Km of the second phosphorylation of dG in the reverse direction**
km2PrdG=km2PfdG;
km2PrrG=km2PfrG;

**(Reaction is linear for dTDP and UDP until at least 1000 uM)**
**Km of the third phosphorylation of dT in the forward direction**
Reference [42]
km3PfdT=1000 x conversion;
km3PfdU=km3PfdT;
km3PfrU=km3PfdT;

**Km of the third phosphorylation of dC in the forward direction**
Reference [42]
km3PfdC=1000 x conversion; (dNDPs are weaker substrates than rNDPs: author statement but data n/a so same value used)
km3PfrC=1000 x conversion; (Reaction linear until at least 1000 uM)

**Km of the third phosphorylation of dA in the forward direction**
Reference [42]
km3PfdA=70 x conversion; (Km of ADP is about 70 micromolar, Km of dADP set equal to that of dGDP)
km3PfrA=300 x conversion; (substrate inhibition, Ki)

**Km of the third phosphorylation of dG in the forward directio**n
Reference [42]
km3PfdG=75 x conversion;
km3PfrG=100 x conversion; (substrate inhibition, Ki)

**Inosine inhibitions**
km3PfrI=km3PrrI=km3PfdI=km3PrdI=1000 x conversion;

**Km of the third phosphorylation of dT in the reverse direction**
km3PrdT=km3PfdT;
km3PrdU=km3PrdT;
km3PrrU=km3PrdT;

**Km of the third phosphorylation of dC in the reverse direction**
km3PrdC=km3PfdC;
km3PrrC=km3PrdC;

**Km of the third phosphorylation of dA in the reverse direction**
km3PrdA=km3PfdA;
km3PrrA=km3PrdA;

**Km of the third phosphorylation of dG in the reverse direction**
km3PrdG=km3PfdG;
km3PrrG=km3PrdG;

**(Initial concentrations selected randomly)**
**Initial dN concentrations**

dTcyto=RandomReal[{0.05 x conversion, 5 x conversion}];
dCcyto=RandomReal[{0.05 x conversion, 5 x conversion}];
dAcyto=RandomReal[{0.05 x conversion, 5 x conversion}];
dGcyto=RandomReal[{0.05 x conversion, 5 x conversion}];

dT0=dTcyto;
dC0=dCcyto;
dA0=dAcyto;
dG0=dGcyto;

**Initial dNXP and rNXP levels**
If[celltype==1,dTTPcyto=RandomReal[{0.1 x conversion, 10 x conversion}]];
If[celltype==1,dCTPcyto=RandomReal[{0.1 x conversion, 10 x conversion}]];
If[celltype==1,dATPcyto=RandomReal[{0.1 x conversion, 10 x conversion}]];
If[celltype==1,dGTPcyto=RandomReal[{0.1 x conversion, 10 x conversion}]];

dTMP0=RandomReal[{0.01 x conversion, 10 x conversion}];
dTDP0=RandomReal[{0.01 x conversion, 10 x conversion}];
dTTP0=dTTPcyto;

dCMP0=RandomReal[{0.01 x conversion, 10 x conversion}];
dCDP0=RandomReal[{0.01 x conversion, 10 x conversion}];
dCTP0=dCTPcyto;

dAMP0=RandomReal[{0.01 x conversion, 10 x conversion}];
dADP0=RandomReal[{0.01 x conversion, 10 x conversion}];
dATP0=dATPcyto;

dGMP0=RandomReal[{0.01 x conversion, 10 x conversion}];
dGDP0=RandomReal[{0.01 x conversion, 10 x conversion}];
dGTP0=dGTPcyto;

dU=dUcyto=dTcyto;
rU=rUcyto=dTcyto;
dI=dIcyto=0.1 x dAcyto;
rI=rIcyto=0.1 x dAcyto;
rC=rCcyto=dCcyto;
rA=rAcyto=dAcyto;
rG=rGcyto=dGcyto;

dUMP=1 x conversion; (0.1 x dTMP0;)
rUMP=10 x conversion; (10 x dTMP0;)
dIMP=1 x conversion; (0.1 x dAMP0;)
rIMP=1 x conversion; (0.1 x dAMP0;)
rCMP=10 x conversion; (10 x dCMP0;)
rAMP=10 x conversion; (10 x dAMP0;)
rGMP=10 x conversion; (10 x dGMP0;)

dUDP=1 x conversion; (0.1 x dTDP0;)
rUDP=10 x conversion; (10 x dTDP0;)
dIDP=1 x conversion; (0.1 x dADP0;)
rIDP=1 x conversion; (0.1 x dADP0;)
rCDP=10 x conversion; (10 x dCDP0;)
rADP=10 x conversion; (10 x dADP0;)
rGDP=10 x conversion; (10 x dGDP0;)

dUTP=1 x conversion; (0.1 x dTTP0;)
rUTP=10 x conversion; (10 x dTTP0;)
dITP=1 x conversion; (0.1 x dATP0;)
rITP=1 x conversion; (0.1 x dATP0;)
rCTP=10 x conversion; (10 x dCTP0;)
rATP=10 x conversion; (10 x dATP0;)
rGTP=10 x conversion; (10 x dGTP0;)

DNA0=0;
LDNA0=0;
HDNA0=0;