Supplementary data

Table 1: The list of reaction kinetics for all nodes in the dynamic integrated model linking protein aggregation and proteasomal degradation of substrate proteins and its inhibition, mitochondrial dysfunction and GSH synthesis/metabolism.

1) Protein aggregation and proteasomal degradation				
(a) Protein aggregation and Lewy body formation				
NODE	Equation	Parameters (units)	Mechanism and assumption	Reference(s)
Seed formation (control α-syn)	syn_control --> seeds	Kf = 1.2E-6 (1/sec)	Irreversible mass action. Here during aggregation of a-syn a condition is given that aggregation will occur when concentration of A-Syn <= 8uM	[3,7]
Seed formation (WT α-syn)	syn_WT --> seeds	Kf =4.4E-2 (1/sec)	Irreversible mass action.	[1,2,4]
Seed formation (A53T α-syn)	syn_A53T --> Seeds	Kf = 8.79E-2 (1/sec)	Irreversible mass action. Assumed as the kinetics of Mutant A-syn aggregation is 2 fold higher than WT.	[1,2,4]
Seeds oligomerization	Seeds --> Oligomers	Kf = 3.9E-6 (1/sec)	Irreversible mass action.	[3,7]
Protofibril formation (oligomer polymerization)	Oligomers --> Protofibrils	Kf = 9.5E-7 (1/sec)	Irreversible mass action.	[3,7]
syn-agg Formation (Protofibril oligomerization)	Protofibrils --> synagg	Kf = 1E-4 (1/sec)	Irreversible mass action.	[3,7]

Lewy body formation (syn-agg polymerization)	syn-agg --> Lewy body	Kf = 8.35E-6 (1/sec)	Irreversible mass action.	[3,7]
(b) Proteasomal degradation and its inhibition by aggregates or exogenous inhibitor (PI)				
Protein Degradation	Proteins (a- syn/ synphilin / Pael-R) --> Sink	Kcatf_proteasome = 0.5 (μmol/sec) Km_protein = 5μM Ki_toxic aggregates = 0.001 μM Ki_PI = 1μM	Michaelis menten kinetics. 1. Sink is shown for the degradation of protein 2. Toxic aggregates (which comprise intermediates of aggregation namely, oligomers, protofibrils, synagg and their dopamine adducts) are shown to inhibit the proteasomal degradation. 3. Proteasoamal activity is modeled as michelis-menten kinetics.	[8,9]
2) Mitochondrial Bioenergetics and Dysfunction				
Electron transport chain-NADH oxidation	(a) CI + NADH --> CIbnnd + NAD (b) 3Hplus + UQ + CIbnd = 3 Hplus + Uqbnd + CI	(a)Km_CI= 0.3 μM, Km_NADH= 300 μM, Vf=7.5E-2 (μm/sec) (b) Vf = 8.5 E-2(μm/sec), Km_CIbnd = 0.15 μM, Km_UQ= 1.5 E-1 Ki_Toxic_Aggregate = 3.33E-6 μM Ki_PN = 7E-3 μM	Coupled reactions. Both reactions are modified Michelis-Menten equations.	[10-18]
ROS production coupled with CI oxidation	CIbnd + O_2Mt --> CI + ROSmt	Kf= 5E-5 ((Liter/μM)*(1/sec))	Irreversible mass action activated by ROS. This	[12,21-24]

		Kact_ROS_C1 = 0.1 μM	reaction remains coupled with CI reduction. Mitocondria produces SO and primary ROS at two complexes I and III .CI and CIII produces SO on the matrix side of membrane.	
CIII reduction coupled with UQ oxidation.	UQbnd + CIII --> UQ + CIIIbnd	Vf = 0.1 (μm/sec), Km_UQbnd = 0.15 (μM), Km_C3 = 0.15 (μM)	Modified Michaelis-Menten reaction.	[13-16,19,20]
CIII oxidation coupled with ROS production.	C3bnd + O2Mt --> CIII + ROSmt	Kf= 5E-5 ((Liter/μM)*(1/sec)) Kact_ROS_C3 = 0.1 μM	Irreversible mass action activated by ROS. This reaction remains coupled with CIII reduction. Mitochondria produces SO and primary ROS at two complexes I and III .CI and CIII produces SO on the matrix side of membrane.	[12,21-24]
UQ oxidation coupled with cytochrome C (CYCSi) reduction (During the process of electron flow, each complexes catalyzes the translocation of protons across inner membrane).	CIIIbnd + 3 Mtmatrix.Hplusi + CYCSi --> CYCSbnd + 3 IMS.Hpluso + CIII	Vf = 0.1 (μm/sec), Km_CIIIbnd = 0.15 (μM) Km_CYCSi = 0.15 (μM) Ki_PN = 0.007 (μM)	Modified Michaelis-Menten reaction. During the process of electron flow, each complex catalyzes the translocation of protons across IM.	[13-16,20]

CIV reduction coupled with cytochrome C Oxidation	CYCSbnd + CIV --> CYCSi + CIVbnd	Vf = 0.1 (μm/sec), Km_CYCSbnd = 0.15 (μM) Km_C4 = 0.15 (μM)	Modified Michaelis-Menten reaction. During the process of electron flow, each complex catalyzes the translocation of protons across IM.	[13-16,20}
CIV oxidation coupled with ROS production	O_2Mt + CIVbnd --> CIV + ROSmt	Kf= 5E-5 ((Liter/μM)*(1/sec)) Kact_ROS_C3 = 0.1 μM	Irreversible mass action.	[12,21-24]
H_2O Formation coupled with CIV oxidation.	CIVbnd + 5 Mtmatrix.Hplusi + Mtmatrix.O2Mt --> CIV + 3 IMS.Hpluso + Mtmatrix.H_2Omt	Vf = 1.25 E-1 (μmol/sec), Km_CIVbnd = 0.15 μM, Ki_NO = 20 μM Ki_PN = 0.007 μm.	Modified Michaelis-Menten.	[13-16,20}
(3) GSH Biosynthesis and metabolism (including GCL mRNA transcription)				
Glutathione synthesis Step 1- mediated by γ-GCL	Glut + Cys + ATPcyto --> GluCys + ADPcyto + Pi	Kcatf_GCL = 7.344 1/sec Km_Glut = 3600 μM, Km_Cys = 100 μM, Km_ATPcyto = 400 μM, Ki_GSH = 500 μM.	Modified Michaelis-Menten kinetics with product inhibition by GSH.	[28-32]
Glutathione synthesis Step 2- mediated by GS (glutathione synthetase)	GluCys + Gly + ATPcyto --> Pi + ADPcyto + GSH	Kcatf_GS = 4.9 E-1, Km_ATPcyto = 4.5 μM, Km_Gly = 2000 μM.	This is modeled as modified Michaelis-Menten reaction.	[29,30,33,34]

GPx – Glutathione Peroxidase	H_2O_2 + GSH --> H_2Oc + GSSG	Kcatf_GPx = 21 (1/sec), Km_GSH= 3000 μM, Km_H_2O_2 = 10 μM	This reaction is modeled as two substrate Michaelis-Menten mechanism	Values from Brenda and optimized [EC no. 1.11.1.9]
GR – Glutathione Reductase	GSSG + NADPH --> GSH + NADP	Kcatf_GR = 100 1/sec, Km_NADPH = 85 μM, Km_GSSG = 65 μm.	This reaction is modeled as two substrate Michaelis-Menten mechanism	Values from Brenda and optimized [EC no. 1.8.1.7]
S-Nitroso-glutathione formation	(a) GSH + ONOO --> GSNO (b) GSHc + N2O3 --> GSNOc + Hplus + NO2	(a) Kf = 3 E-4 (1/sec) (b) Kf = 1E-5 (1/sec)	Irreversible mass action.	[35,36]
GCL upregulation				
Keap1_Nrf2 complex dissociation	Keap1_Nrf2 --> Keap1 + Nrf2c	Vf = 0.01 1/sec, km_Keap1_Nrf2 = 50 μM Ka = 2 E-6 uM	Modified Michaelis Menten which is activated by ROS/electrophile stress	[38- 44]
Nrf2 translocation	Nrf2c --> Nrf2n	Kf = 1.0E-5 (1/sec)	Irreversible mass action.	[38-40]
EpRE complex formation	Nrf2n + JUNn + EpRE --> EpRE_Nrf2_JunD	Kf = .01 μM Kr = .008 μM	The binding of Nrf2 in nucleus to form EpRE complex is modeled with reversible mass action kinetics.	[38-40]
Transcription of γ- GCL gene induced by EpRE Transcription factor .	DNA --> GCL mRNA	Km_EpRE_Nrf2_JunD = 19 μM Vf = 0.0082 μmol/sec	This reaction is modeled as modified Michaelis-Menten mechanism.	[38-40]

Phosphorylation of Jun by JNK in the FosJun complex formation	FOSpn_JUNn --> FOSpnJUNpn	Kcatf_JNK = 25 (1/sec), Km_FosPn_JunN = 10 μM	This reaction is modeled as modified Michaelis-Menten mechanism.	[37-40]
Transcription of γ- GCL gene induced by AP1 Transcription factor.	DNA --> GCL mRNA	V max = 0.004 μmol/sec Km_ FOSpn_JUNn = 19 μM	This reaction is modeled as Michaelis-Menten mechanism.	[41-43]
Translation of GCL enzyme	GCLmRNAc --> GCL	Kf =3.5E-2 (1/sec)	The reaction is modeled with mass action kinetics.	[41-43]
Inhibition of proteasomal degradation of Nrf2 by toxic Aggregates.	Nrf2c --> Sink	Kcatf_proteasome = 0.01 (1/sec) Km_Nrf2c = μm Ki_Toxic aggregate = 1E-5 μM Ki_PI = 1 μM	This reaction is modeled as Modified Michaelis-Menten.	[8,40-45]

List of abbreviations:

CI – NADH Oxidoreductase (Complex I of Electron Transport Chain)
CIbnd – Electron bound CI
CIII – Cytochrome c Oxidase (Complex III of Electron Transport Chain)
CIIIbnd- Electron bound CIII
CIV- Cytochrome c Reductase (Complex IV of Electron Transport Chain)
CIVbnd- Electron bound CIV
UQ – Ubiquinone
Cytc – Cytochrome c
UQbnd - Electron bound Ubiquinone
cytcbnd - Electron bound Cytochrome c
ATP – Adenosine triphosphate
ADP – Adenosine diphosphate
Pi – Inorganic phosphate
NADP – Nicotinamide adenosine diphosphate
Arg – Arginine
Cit- Citrulline
NO -Nitric oxide
O_2 – Oxygen
ROS – Superoxide
H_2O_2 – Hydrogen peroxide
PN – Peroxynitrite
NO_2 – Nitrogen dioxide radical
DOPAL – Metabolite of dopamine
GSH – Glutathione
GSSG – Glutathione disulphide
GR – Glutathione reductase
GPx – Glutathione peroxidase
SOD – Super oxide dismutase
MAO– Monoamine oxidase
XO– Xanthine oxidase

NOS – Nitric oxide synthase
Gly – Glycine
GluCys -GlutamylCysteine
Cys – Cysteine
Glu – Glutamate
GSNO – S- Nitrosoglutathione
NO_2R – Nitrogen Dioxide radicals
N_2O_2- Anhydrous nitrous acid
ONOOH - Peroxinitirous acid
Hplusi – Proton concentration in mitochondrial matrix
Hpluso - Proton concentration in mitochondrial membrane space.
FOSpn_JUNn – With Phosphorylated FOS in AP 1 complex.
FOSpnJUNpn – Activated AP1
DNA – Deoxyribonucleic acid.
GCL mRNA – mRNA for GCL
γ-GCL – Gamma glutamylcysteine ligase
Nrf2c – Component of EpRE transcription factor in the cytosol.
Keap1 -A cytoskeleton binding protein
Keap1_Nrf2 - Inactive Nrf2 bound with Keap1
Nrf2n -Component of EpRE transcription factor in the nucleus.
JUNn - Nuclear JUN protein
EpRE - Electrophile Response Element or ARE
EpRE_Nrf2_JunD -Electrophile Response Element or ARE bound with JunD and Nrf2 to form an active Transcription factor
AP1- Activator Protein 1 - Activator protein 1 complex

Parameters:

Kf = Forward rate constant
Kcat = Catalytic rate constant
V max = The maximum rate of reaction
Km = Michaelis Menten constant
Ka = Activation constant
Ki = Inhibition constant

References:

1.El-Agnaf, O.M.A.; Jakes, R.; Curran, M.D.; Wallace, A. Effects of the mutations Ala30 to Pro and Ala53 to Thr on the physical and morphological properties of alpha-synuclein protein implicated in Parkinson's disease. *FEBS Lett.* **440:** 67–70; 1998.

2. El-Agnaf, O.M.A.; Jakes, R.; Curran, M.D.; Middleton, D.; Ingentto, R.; Bianchi, E.; Pessi, A.; Neill, D.; Wallace, A. Aggregates from mutant and wild-type alpha-synuclein proteins and NAC peptide induce apoptotic cell death in human neuroblastoma cells by formation of beta-sheet and amyloid-like Wlaments. *FEBS Lett.* **440:** 71–75; 1998.

3. Giasson, B.I.; Murray, I.V.; Trojanowski, J.Q.; Lee, V.M. A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly. *J. Biol. Chem.* **276:** 2380–2386; 2001.

4. Conway, K.A.; Lee, S.J.; Rochet, J.C.; Ding, T.T.; Williamson, R.E.; Lansbury, P.T. Jr. (2000). Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. *Proc. Natl Acad. Sci. U SA*. **97:** 571–576; 2000.

5. Lim, K.L.; Dawson, V.L.; Dawson, T.M. Parkin-mediated lysine 63-linked polyubiquitination: a link to protein inclusions formation in Parkinson's and other conformational diseases? *Neurobiol. Aging* **27:** 524-529; 2006.

6. Lim, K.L.; Chew, K.C.; Tan, J.M.; Wang, C.; Chung, K.K.; Zhang, Y.; Tanaka, Y.; Smith, W.; Engelender, S.; Ross, C.A.; Dawson, V.L.; Dawson, T.M. Parkin mediates nonclassical, proteasomal-independent ubiquitination of synphilin-1: implications for Lewy body formation. *J. Neurosci.* **25:** 2002-2009; 2005.

7. Raichur, A.; Vali, S.; Gorin, F. Dynamic modeling of alpha-synuclein aggregation for the sporadic and genetic forms of Parkinson's disease. *Neuroscience* **142:**859-870; 2006.

8. Lindersson, E.; Beedholm, R.; Højrup, P.; Moos, T.,; Gai, W.; Hendil, K.B.; Jensen, P.H. Proteasomal inhibition by alpha-synuclein filaments and oligomers. *J. Biol. Chem.* **279:** 12924-12934; 2004.

9. Snyder, H.; Mensah, K.; Theisler, C.; Lee, J.; Matouschek, A.; Wolozin, B. Aggregated and monomeric alpha-synuclein bind to the S6' proteasomal protein and inhibit proteasomal function. *J. Biol. Chem.* **278:** 11753-11759; 2003.

10. Watabe, M.; Nakaki, T. ATP depletion does not account for apoptosis induced by inhibition of mitochondrial electron transport chain in human dopaminergic cells. *Neuropharmacology* **52:**536-541; 2007.

[11] Tretter, L.; Adam-Vizi, V. Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **360:**2335-2345; 2005.

[12] Tretter, L.; Adam-Vizi, V. Moderate Dependence of ROS Formation on DeltaPsim in Isolated Brain Mitochondria Supported by NADH-linked Substrates. *Neurochem. Res.* **32:**569-575; 2007.

[13] Kadenbach, B. Intrinsic and extrinsic uncoupling of oxidative phosphorylation. *Biochim. Biophys. Acta.* **1604:**77-94; 2003.

[14] Jin, Q.; Bethke, C.M. Kinetics of electron transfer through the respiratory chain. *Biophys. J.* **83:**1797-1808; 2002.

[15] Schultz, B.E.; Chan, S.I. Structures and proton-pumping strategies of mitochondrial respiratory enzymes. *Annu. Rev. Biophys. Biomol. Struct.* **30:**23-65; 2001.

[16] Beard, D.A. A biophysical model of the mitochondrial respiratory system and oxidative phosphorylation. *PLoS Comput. Biol.* **1:**252-264; 2005.

[17] Kushnareva, Y.; Murphy, A.N.; Andreyev, A. Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)+ oxidation-reduction state. *Biochem. J.* **368:** 545-553; 2002.

[18] Hill, T.L. Steady-state kinetic formalism applied to multienzyme complexes, oxidative phosphorylation, and interacting enzymes. *Proc. Natl. Acad. Sci. USA.* **73:**4432-4436; 1976.

[19] Zu, Y.; Di Bernardo, S.; Yagi, T.; Hirst, J. Redox properties of the [2Fe-2S] center in the 24 kDa (NQO2) subunit of NADH:ubiquinone oxidoreductase (complex I). *Biochemistry* **41:**10056-10069; 2002.

[20] Nicholls, D.G. Mitochondrial membrane potential and aging. *Aging Cell* **3:**35-40; 2004.

[21] Sipos, I.; Tretter, L.; Adam-Vizi, V. Quantitative relationship between inhibition of respiratory complexes and formation of reactive oxygen species in isolated nerve terminals. *J. Neurochem.* **84:**112-118; 2003.

[22] Turrens, J.F. Mitochondrial formation of reactive oxygen species. *J. Physiol.* **552:**335-344; 2003.

[23] Saybasili, H.; Yuksel, M.; Haklar, G.; Yalcin, A.S. Effect of mitochondrial electron transport chain inhibitors on superoxide radical generation in rat hippocampal and striatal slices. *Antioxid. Redox. Signal.* **3:**1099-1104; 2001Cadenas, E.; Davies, K.J. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic. Biol. Med.* **29:**222-230; 2000.

[24] Han, D.; Antunes, F.; Canali, R.; Rettori, D.; Cadenas, E. Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. *J. Biol. Chem.* **278:**5557-5563; 2003.

[25] Kaim, G.; Dimroth, P. ATP synthesis by F-type ATP synthase is obligatorily dependent on the transmembrane voltage. *EMBO J.* **18:**4118-4127; 1999.

[26] Purdon, A.D.; Rapoport, S.I. Energy requirements for two aspects of phospholipid metabolism in mammalian brain. *Biochem. J.* **335:** 313-318; 1998.

[27] Bernstein, B.W.; Bamburg, J.R. Actin-ATP hydrolysis is a major energy drain for neurons. *J. Neurosci.* **23:**1-6; 2003.

[28] Huang, J.; Philbert, M.A. Distribution of glutathione and glutathione-related enzyme systems in mitochondria and cytosol of cultured cerebellar astrocytes and granule cells. *Brain Res.* **680:**16-22; 1995.

[29] Huang, J.; Philbert, M.A. Cellular responses of cultured cerebellar astrocytes to ethacrynic acid-induced perturbation of subcellular glutathione homeostasis. *Brain Res.* **711:**184-192; 1996.

[30] Jez, J.M.; Cahoon, R.E.; Chen, S. Arabidopsis thaliana glutamate-cysteine ligase: functional properties, kinetic mechanism, and regulation of activity. *J. Biol. Chem.* **279:**33463-33470; 2004.

[31] Nakayama, Y.; Kinoshita, A.; Tomita, M. Dynamic simulation of red blood cell metabolism and its application to the analysis of a pathological condition. *Theor. Biol. Med. Model.* **2:**18-29; 2005.

[32] Dringen, R. Metabolism and functions of glutathione in brain. *Prog. Neurobiol.* **62:**649-671; 2000.

[33] Njalsson, R.; Norgren, S.; Larsson, A.; Huang, C.S.; Anderson, M.E.; Luo, J.L. Cooperative binding of gamma-glutamyl substrate to human glutathione synthetase. *Biochem. Biophys. Res. Commun.* **289:**80-84, 2001.

[34] Jez, J.M.; Cahoon, R.E. Kinetic mechanism of glutathione synthetase from Arabidopsis thaliana. *J. Biol. Chem.* **279:**42726-42731; 2004.

[35] Clementi, E.; Brown, G.C.; Feelisch, M.; Moncada, S. Persistent inhibition of cell respiration by nitric oxide: crucial role of *S*-nitrosation of mitochondrial complex I and protective action of glutathione. *Proc. Natl. Acad. Sci. USA* **95:**7631–7636; 1998.

[36] Schild, L.; Reinheckel, T.; Reiser, M.; Horn, T.F.; Wolf, G.; Augustin, W. Nitric oxide produced in rat liver mitochondria causes oxidative stress and impairment of respiration after transient hypoxia. *FASEB J.* **17:**2194-2201; 2003.

[37] Mythri, R.B.; Jagatha, B.; Pradhan, N.; Andersen, J.; Bharath, M.M. Mitochondrial Complex I Inhibition in Parkinson's Disease: How Can Curcumin Protect Mitochondria? *Antioxid. Redox. Signal.* **9:**399-408; 2007.

[38] Dickinson, D.A.; Iles, K.E.; Zhang, H.; Blank, V.; Forman, H.J. Curcumin alters EpRE and AP-1 binding complexes and elevates glutamate-cysteine ligase gene expression. *FASEB J.* **17:**473-475; 2003.

[39] Zhu, Y.G.; Chen, X.C.; Chen, Z.Z.; Zeng, Y.Q.; Shi, G.B.; Su, Y.H.; Peng, X. Curcumin protects mitochondria from oxidative damage and attenuates apoptosis in cortical neurons. *Acta. Pharmacol. Sin.* **25:**1606-1612; 2004.

[40] Dickinson, D.A.; Levonen, A.L.; Moellering, D.R.; Arnold, E.K.; Zhang, H.; Darley-Usmar, V.M.; Forman, H.J. Human glutamate cysteine ligase gene regulation through the electrophile response element. *Free Radic. Biol. Med.* **37:** 1152-1159; 2004.

[41] Zhang, D.D. Mechanistic studies of the Nrf2-Keap1 signaling pathway. *Drug Metab. Rev.* **38:** 769-789; 2006.

[42] Kobayashi, A.; Kang, M.I.; Watai, Y.; Tong, K.I.; Shibata, T.; Uchida, K.; Yamamoto, M. Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. *Mol. Cell Biol.* **26:**221-229; 2006.

[43] Lee, J.M.; Shih, A.Y.; Murphy, T.H.; Johnson, J.A. NF-E2- related factor-2 mediates neuroprotection against mitochondrial complex I inhibitors and increased concentrations of intracellular calcium in primary cortical neurons. *J. Biol. Chem.* **278:** 37948-37956; 2003.

[44] D'Autréaux, B.; Toledano, M.B. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat. Rev. Mol. Cell Biol.* **8:** 813-824; 2007.

[45] Sekhar, K.R.; Yan, X.X.; Freeman, M.L. Nrf2 degradation by the ubiquitin proteasome pathway is inhibited by KIAA0132, the human homolog to INrf2. *Oncogene* **21:** 6829-6834; 2002.