# CHEMISTRY 320

Randolph Coleman

# Biochemistry

Develop a research review paper in ANY area of biochemistry or neurochemistry.

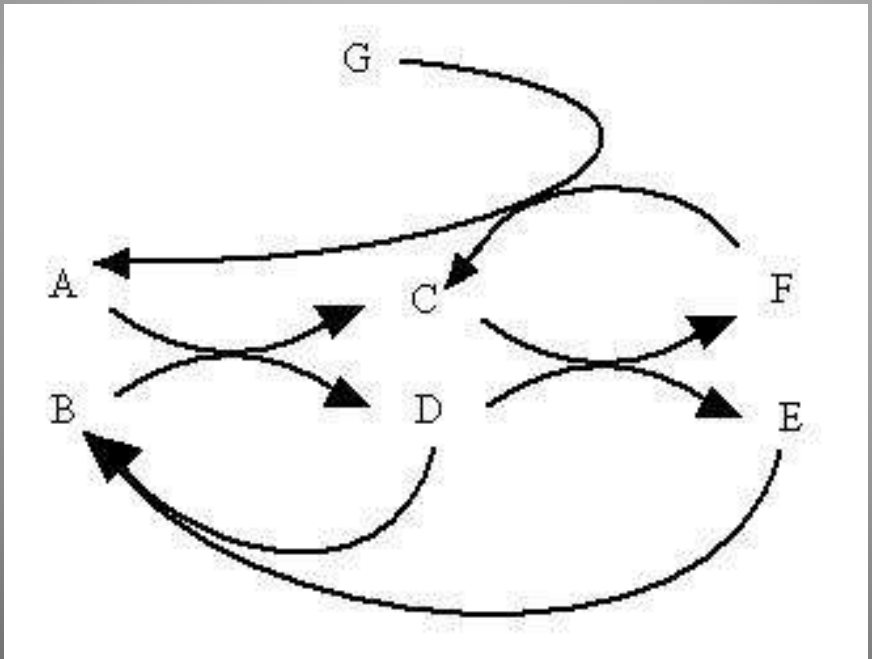
Past topics have included:

- Diseases associated with membrane transport
- Toxic materials and metabolic processing
- Defects in metabolism
- Current understanding of the metabolism of neurodegenerative diseases such as Alzheimer's, Parkinson's, and others.
- Current understanding of the neuropharmacology of a class of drugs

# COMPUTATIONAL STUDIES OF NEURODEGENERATIVE DISEASES

# Introduction to Computational Studies

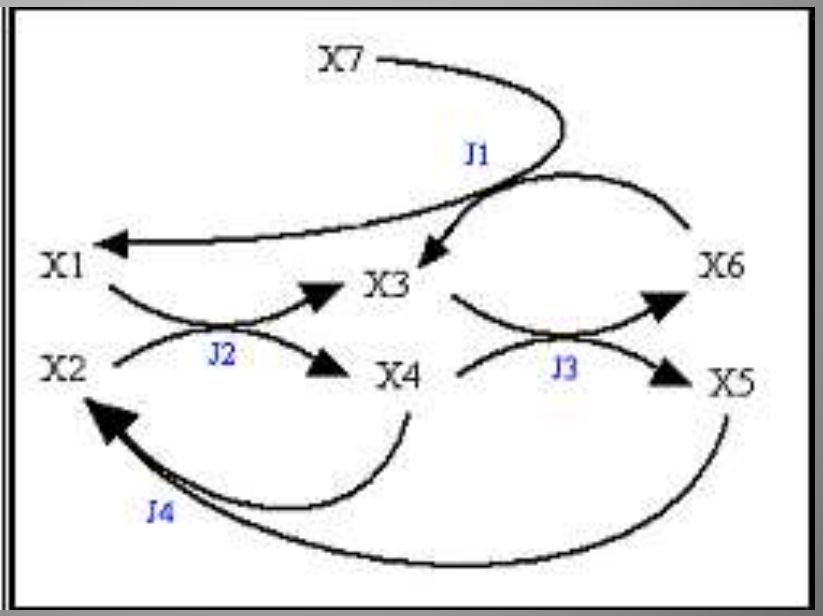
(1) Gather together metabolic data



# Introduction to Computational Studies

(2) Make mathematical assignments

A = X1
B = X2
C = X3
D = X4
E = X5
F = X6
G = X7
Flux 1 = J1
Flux 2 = J2
Flux 3 = J3
Flux 4 = J4



# Introduction to Computational Studies

(3) Define mathematical relationships

| Flux Rate Equations | Dependent Variable Differential Equations |
|---|---|
| | $\dot{X}1 = 2*J1 - J2$ |
| $J1 = k_1 X6^{g_{16}} X7^{g_{17}}$ | $\dot{X}2 = 2*J4 - J2$ |
| $J2 = k_2 X1^{g_{21}} X2^{g_{22}}$ | $\dot{X}3 = J1 + J2 - 3*J3$ |
| $J3 = k_3 X3^{g_{33}} X4^{g_{34}}$ | $\dot{X}4 = J2 - J3 - J4$ |
| $J4 = k_4 X4^{g_{44}} X5^{g_{45}}$ | $\dot{X}5 = J3 - J4$ |
| | $\dot{X}6 = J3 - J1$ |

# Introduction to Computational Studies

(4) Organize data in spreadsheet format

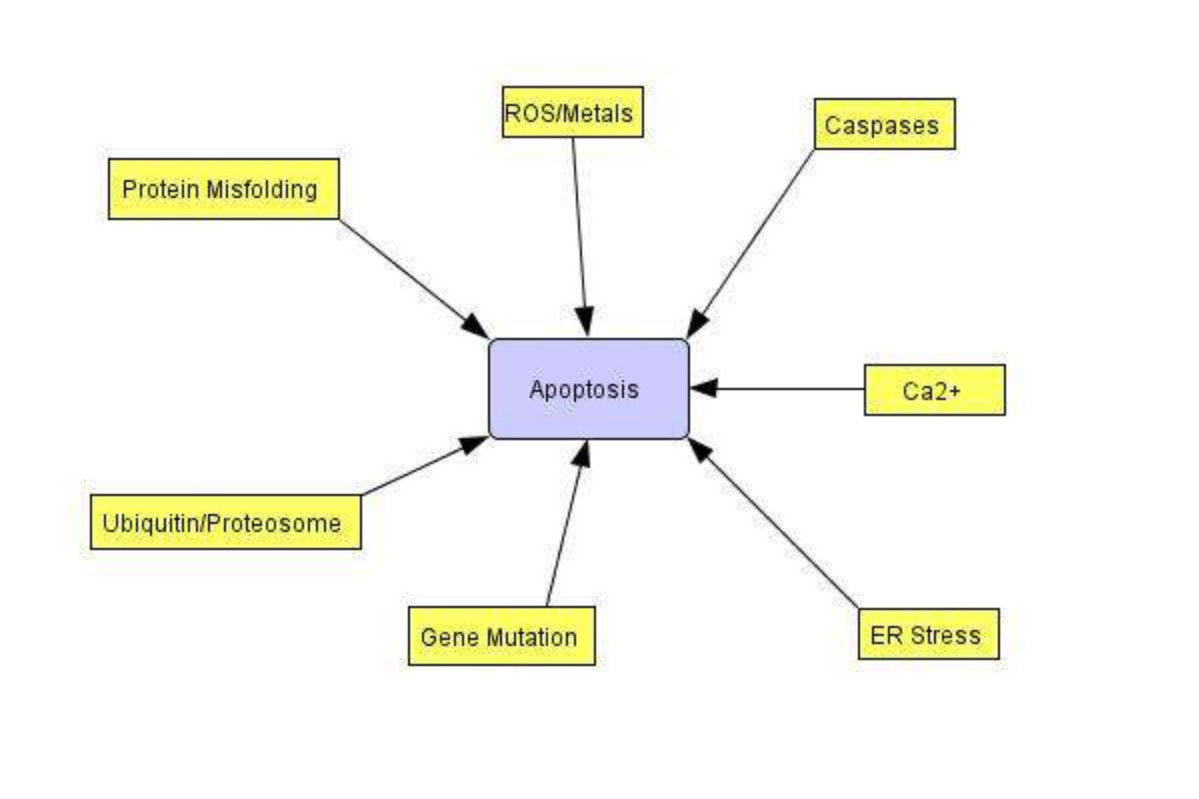
| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | **Dependent variables:** | | | | | |
| 2 | Component | Index | ss Value (mM) | ref | rationale | |
| 3 | A | X1 | 7.24E-08 | [3] | notes... | . |
| 4 | B | X2 | 1.50E+02 | [4] | notes... | . |
| 5 | C | X3 | 5.00 | guess | notes... | . |
| 6 | D | X4 | 0.861 | [1],[2] | notes... | . |
| 7 | E | X5 | 0.392 | [5] | notes... | . |
| 8 | F | X6 | 0.459 | [1] | notes... | . |
| 9 | | | | | | . |
| 10 | **Constrained Variables:** | | | | | . |
| 11 | Component | Index | ss Value (mM) | ref | rationale | . |
| 12 | none | --- | --- | --- | --- | . |
| 13 | | | | | | . |
| 14 | **Independent Variables:** | | | | | . |
| 15 | Component | Index | ss Value (mM) | ref | rationale | . |
| 16 | G | X7 | 2.00 | [1] | notes... | . |

# Neurodegenerative Diseases

AD PD HD

MS ALS Prion

# Neurodegenerative Triggers



# Parkinson's Disease

Alpha-synuclein aggregation mediated by:

- Dopamine metabolism
- Ubiquitin-proteosome system
- Lysosomal degradation
- Reactive oxygen species

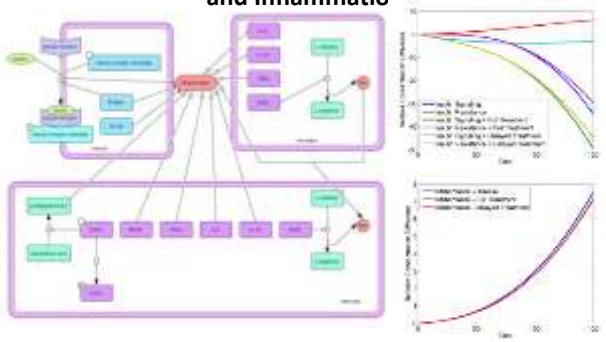
# A Mathematical Model of Insulin Resistance in Parkinson's Disease

E.M. Braatz, R.A. Coleman
Department of Chemistry

## Abstract

**This project introduces a mathematical model representing the biochemical interactions between insulin signaling and Parkinson's disease. The model can be used to examine the changes that occur over the course of the disease as well as identify which processes would be the most effective targets for treatment. The model is mathematized using Biochemical Systems Theory. It incorporates a treatment strategy that includes several experimental drugs along with current treatments. In the past, Biochemical Systems Theory models of neurodegeneration have used the Power Law Analysis and Simulation tool to model the system. This project suggests the use of MATLAB instead. MATLAB allows for more flexibility in both the model itself and in data analysis. Previous Biochemical Systems Theory analyses of neurodegeneration began treatment at disease onset. As shown in this model, the outcomes of delayed, realistic treatment and full treatment at disease onset are significantly different. The delayed treatment strategy is an important development in Biochemical Systems Theory modeling of neurodegeneration. It emphasizes the importance of early diagnosis, and allows for a more accurate representation of disease and treatment interactions.**

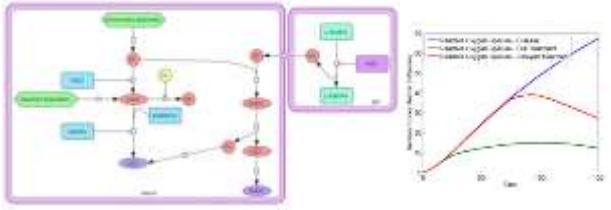
## Effects of Treatment on Insulin Signaling and Inflammation



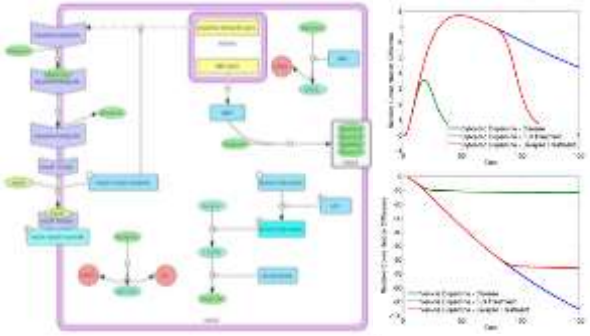
## Introduction

- Parkinson's disease is a neurodegenerative disorder resulting in the death of dopaminergic neurons in the substantia nigra pars compacta region of the brain
- Insulin resistance is a precursor to type-II diabetes mellitus caused by a diet high in fats and sugars
- Parkinson's disease and insulin resistance are mutually intensifying

## Effects of Treatment on Reactive Oxygen and Nitrogen Species



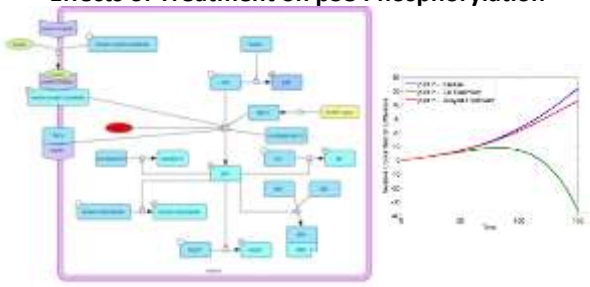
## Effects of Treatment on Dopamine



## Methods: Biochemical Systems Theory

- Initial values are assigned on a relative basis
- Flux equations describe relative reaction rates and consist of the product of the relative concentrations of the reactants multiplied by a rate constant or rate equation.
  - Ex. J(88) = X(258)*Xind(75)
- Rate equations describe the effect of modifiers on the reactions. Promoters have positive effects while inhibitors have negative effects.
  - Ex. X(258) = 0.00001*X(92)
- Systems equations describe the change to the relative species concentrations as the disease progresses. The equations consist of the flux equations detracting from the species concentration subtracted from the flux equations contributing to its concentration.
  - Ex. X(93) = X(258)*Xind(75) – X(259)*X(93)
- Data is analyzed comparatively by subtracting the disease or treatment state from a baseline state depicting a healthy cell system.

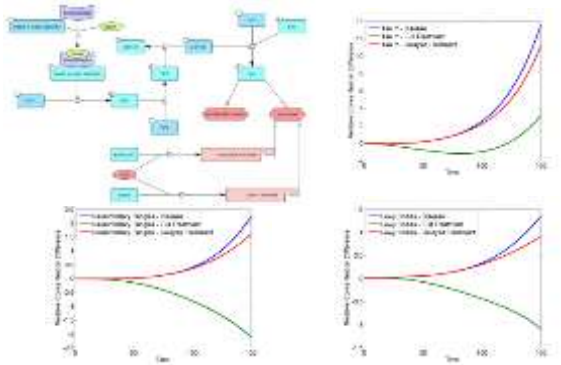
## Effects of Treatment on p38 Phosphorylation



## Results

- The disease state demonstrates an increase in neurotoxic species compared to the baseline state.
- The full treatment state significantly decreased the effects of the disease state.
- The delayed treatment was somewhat effective against degeneration.

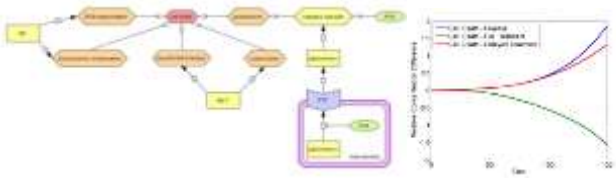
## Effects of Treatment on Phosphorylated Tau, Neurofibrillary Tangles, and Lewy Bodies



## Treatment Summary

| Treatment | Main Processes Affected |
|---|---|
| Vitamin D | Inflammation, Reactive Oxygen Species Scavenging |
| Edaravone | Tau Phosphorylation, Dopamine Vesicle Formation |
| Lithium | Tau Phosphorylation |
| Sodium Butyrate | p38 Phosphorylation |
| N-Acetylcysteine | Reactive Oxygen Species Scavenging, Insulin Sensitization |
| Pioglitazone | Insulin Sensitization |
| Aspirin | Inflammation |
| Cyclosporin A | Apoptosis |
| Nortriptyline | Apoptosis |

## Effects of Treatment on Cell Death



## Conclusion

This model predicts that the treatment combination summarized in the table above is effective in slowing the advancement of Parkinson's disease on pathways that are influenced by insulin signaling. The earlier the treatment is introduced, the more likely it is to prevent disease progression.

## Acknowledgements

- Howard Hughes Medical Institute
- Roy R. Charles Center for Academic Excellence
- Douglas Morton, Marilyn Brown
- College of William and Mary

# Huntington's Disease

Mutant (expanded) Huntington Protein

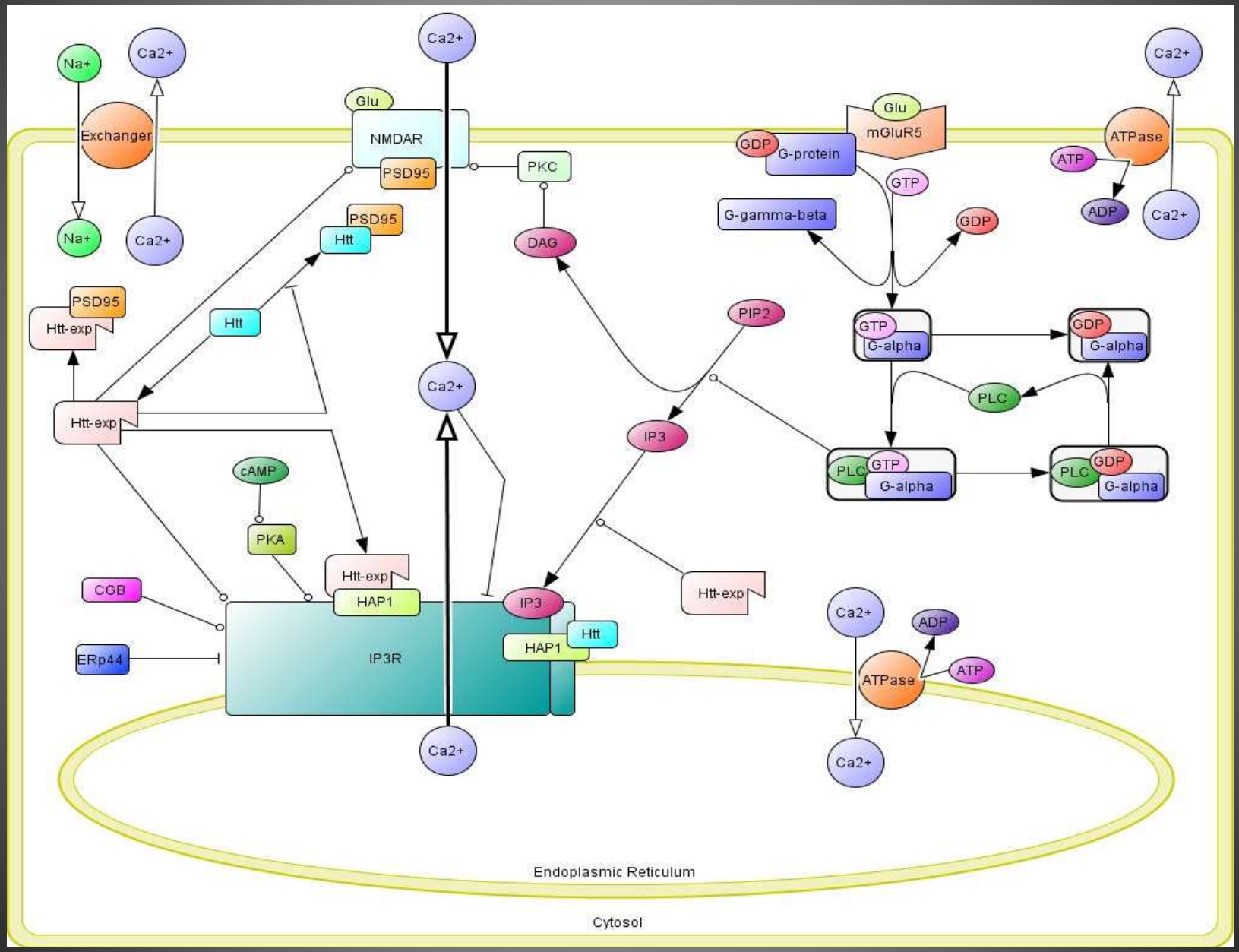
Leads to:

Dyshomeostasis of $Ca^{2+}$

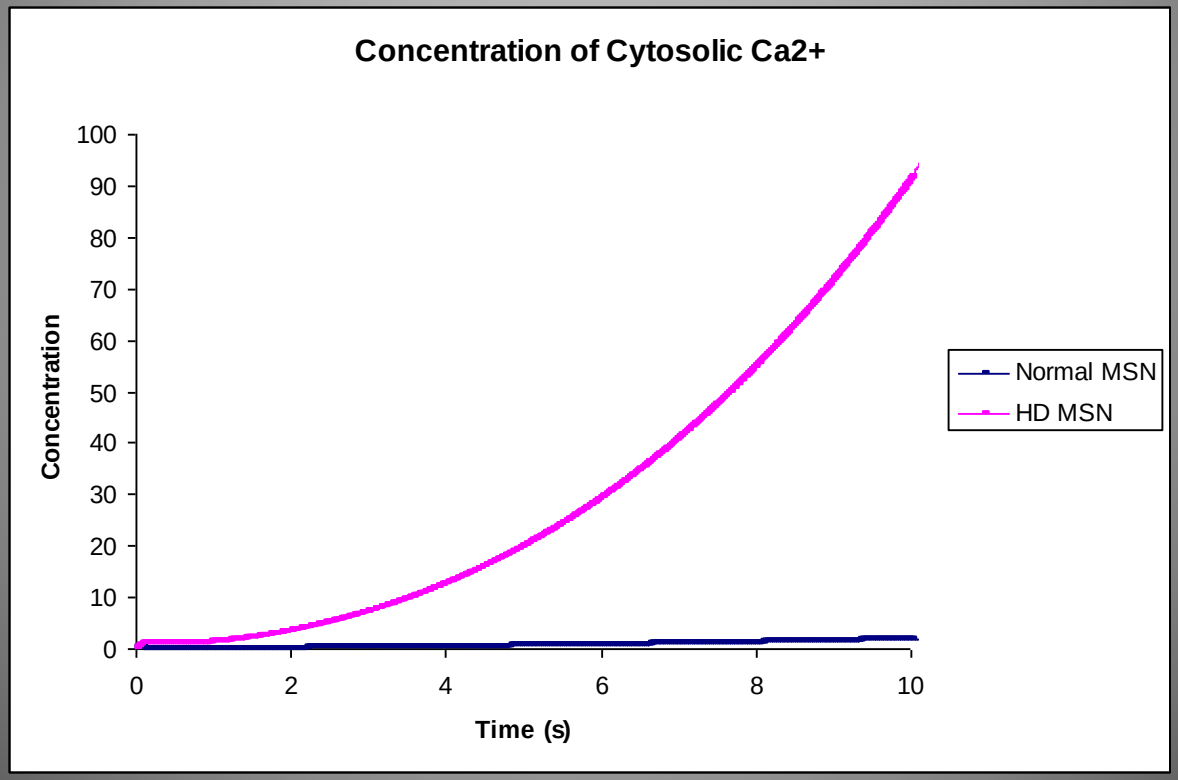
Mitochondrial Dysfunction

Caspase activation

# Calcium Metabolism in HD



# Cytosolic $Ca^{2+}$ HD MSN vs Normal MSN



# Multiple Sclerosis

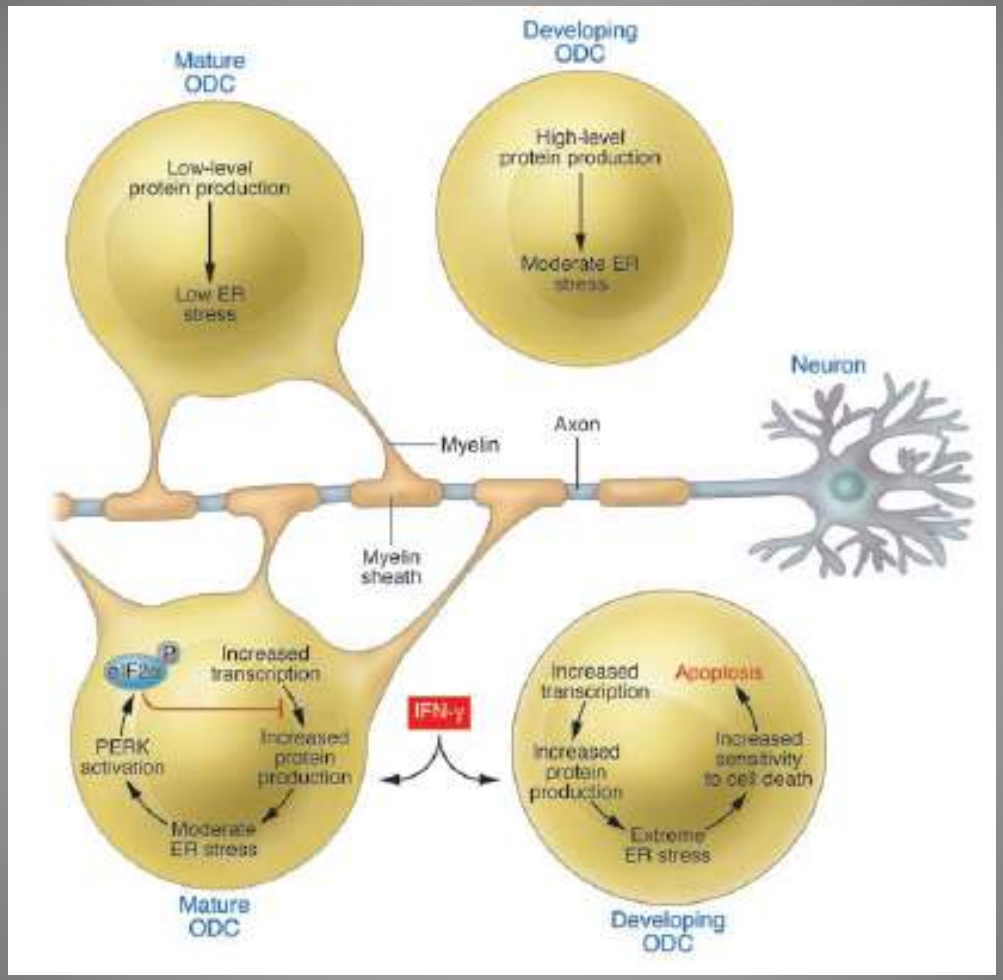
Immune System Involvement:

Oligodendrocytes are focus

ER stress involved

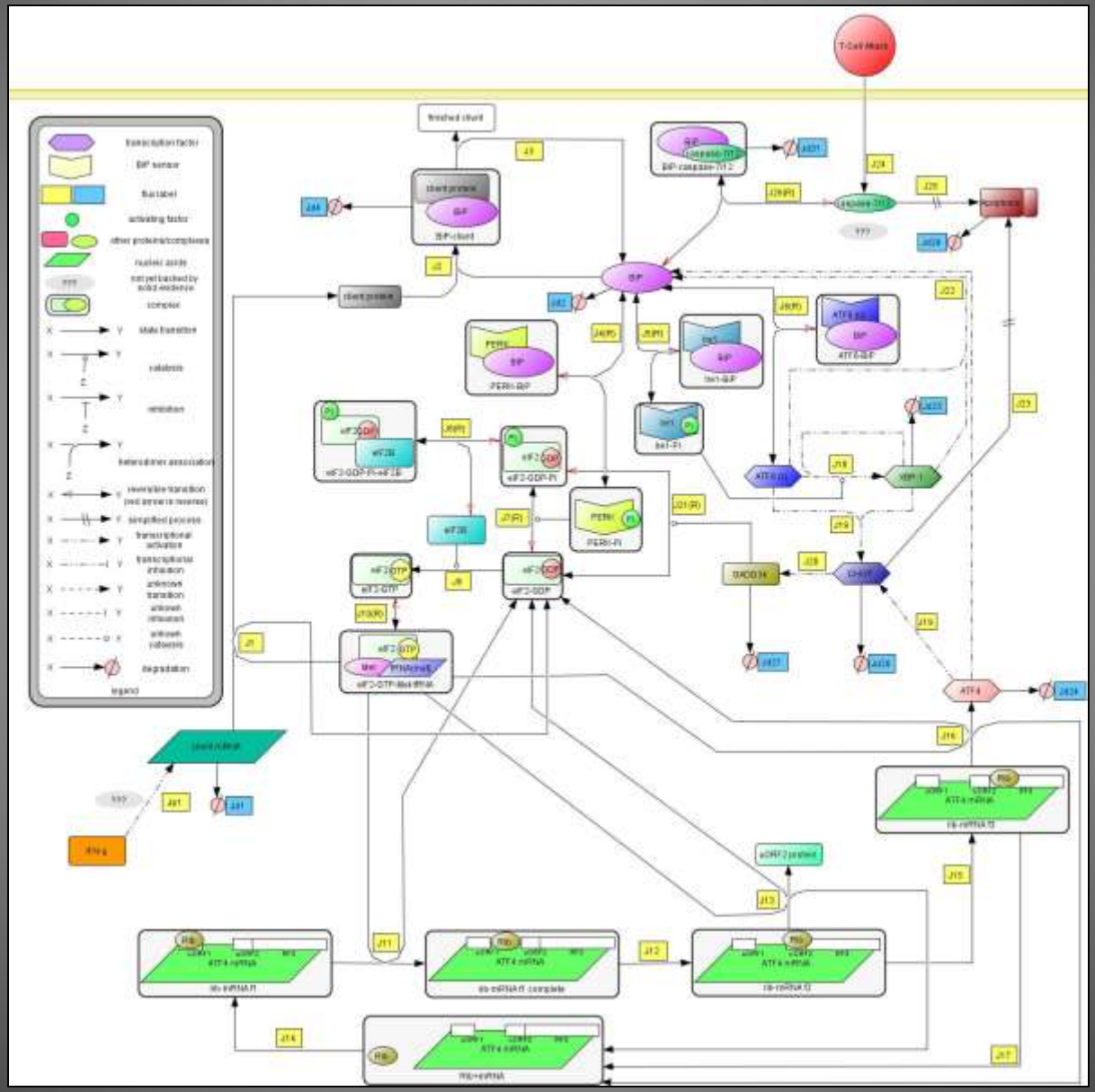
Protein synthesis affected

# ER Stress vs ODC Survival

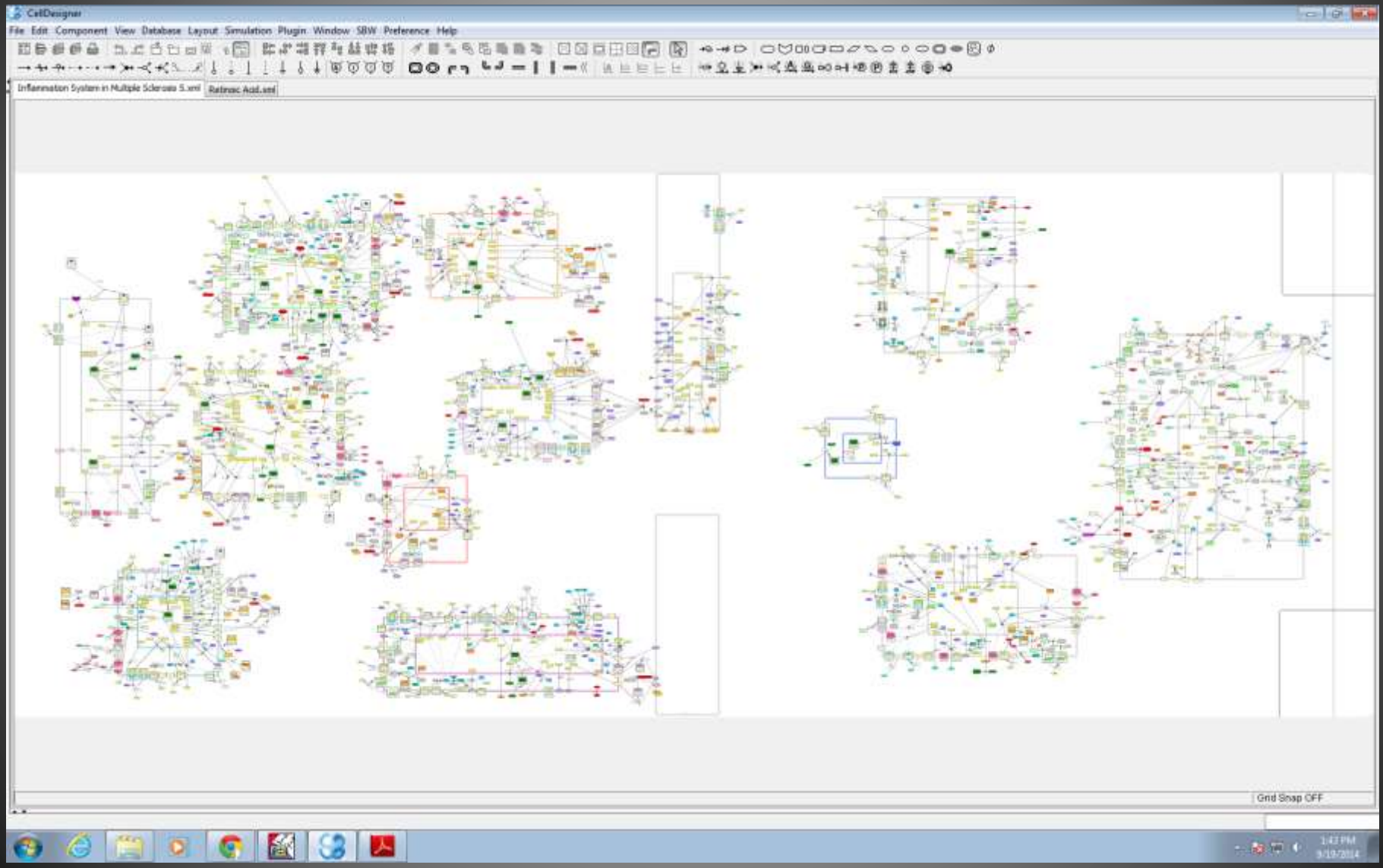



Lees JR, Cross AH (2007) *J of Clinical Investigation*
Used with permission

# MS Model with ER Stress



# Carrie Sheeler MS Model



# Prion Disease

Prion Protein (PrP) Processing:

Lipid raft trafficking

Metal ion involvement

Reactive oxygen species implicated

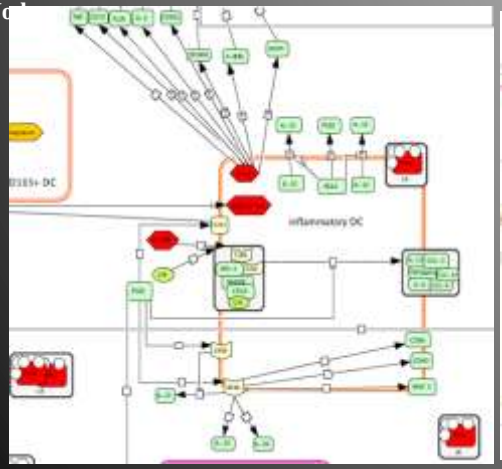
# Neuroinvasion and Proliferation of $PrP^{Sc}$ in Humans Following Prion Infection

C.A. Stephens, R. A. Coleman
Department of Chemistry

## Abstract

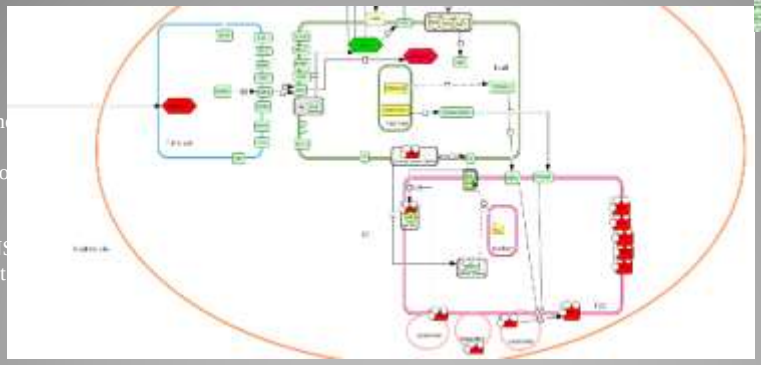
Prions are misfolded isoforms of the normal membrane protein $PrP^{C}$, called $PrP^{Sc}$. This scrapie protein can through an unclear mechanism convert the normal isoform to the 'bad' one and cause a loss of function within infected cells. Many cells express $PrP^{C}$, but neurons and glia are particularly susceptible to infection, which ultimately results in apoptosis and symptoms related to neurodegeneration. Using the program CellDesigner and Matlab this model simulates oral ingestion of $PrP^{Sc}$, its uptake through the intestinal epithelia (via M cells), into Peyer's Patches and lymph nodes where neutrally circulating immune cells, such dendritic cells, macrophages, B cells and T cells phagocytized $PrP^{Sc}$. and consequently are infected with. $PrP^{Sc}$. Circulation through the blood stream and transendothelial migration into the brain infects the CNS with prions. The subsequent damage done to local cells initiates an inflammatory response, further circulating the prions, not recognized as an antigen, through the lymphatic and nervous system. Cell death within the CNS causes neurodegeneration and subsequent death. There is yet no cure for prion disease. Highly specific monoclonal antibodies for $PrP^{Sc}$ are being investigated.

**Initial Infection Through Intestinal Lining and Uptake Into Peyer's Patches and Lymph Nodes**



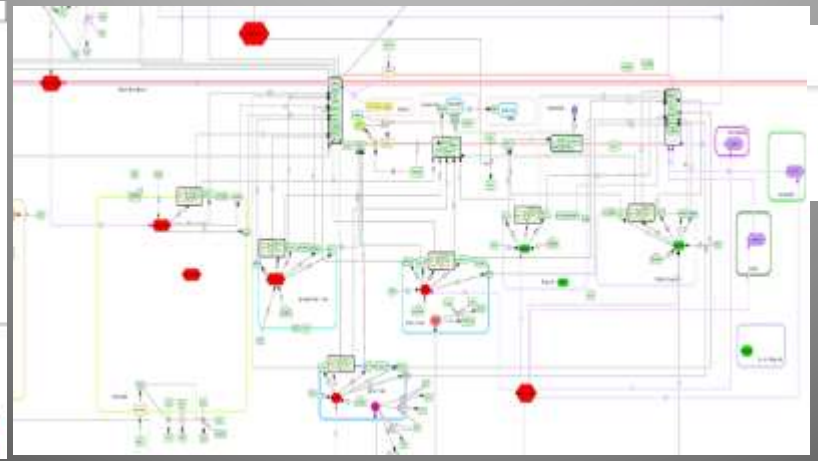
### Replication in Lymph Node FDCs

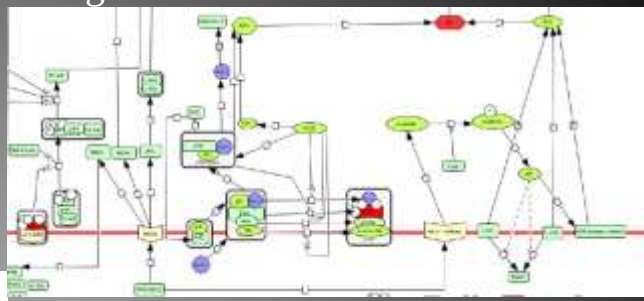
- FDC naturally express high levels of $PrP^{C}$ therefor serving as a site for conversion and replication of $PrP^{Sc}$.
- Interactions with B cells cause cross infection and subsequent infection of T cells coming into contact with B cells.



### Trojan Horse Neuroinvasion of CNS

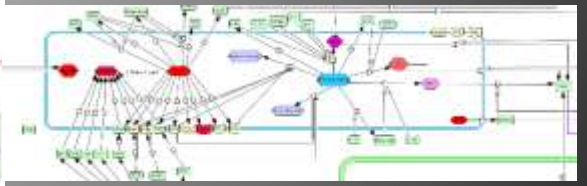
- After reaching a certain threshold titer load of replicated $PrP^{Sc}$, cells carrying the scrapie form (DC, macrophages and T cells) recirculate into the blood and cross infect monocytes that regularly cross the blood brain barrier (BBB) into the perivascular space to differentiate and replace local DC.
- Normal interaction between DC and CNS neurons/glia cause localized infection with $PrP^{Sc}$.





### Damage and Inflammation

- A build-up of reactive oxygen species (ROS) follows infection and contributes to oxidative stress, ER stress, release of $Ca^{2+}$, metal ion irregularity, upregulation and releases of pro-inflammatory agents (TNF-alpha, TGF-beta, CXCL10, CX3C1, CCL21, IL-1beta, and IL-6) and direct/indirect induction of multiple apoptotic pathways.
- Similar effects are seen following infection of the microglia, astrocytes, and oligodedrocytes that regulate normal function of neurons.
- Activation of ant-inflammatory cells and their migration across the blood brain barrier. These cells include monocytes, CD4+ and CD8+ T cells, $T_{reg,}$ FoxP3+ $T_{reg}$ cells, neurtrophils, and B cells.
- Both para- and transcellular migration across endothelia causes disruption of the BBB.
- Inside the brain these cells work to phagocytize and degrade $PrP^{Sc}$ and damaged cells, but bring more PrPSc with them, causing more damage, excitement of cytokines and pro-inflammatory agents like IL-27, IL-17, TGF, Il-6, IL-2, TNF-alpha, and MMP9, induction of macrophage-acting DC, and unchecked inflammation.



### Conclusions

Potential target areas for treatment of prion infection could include limited immobilization of immune response as overacting inflammation stimulates the clinical phase of the disease.

### Acknowledgements

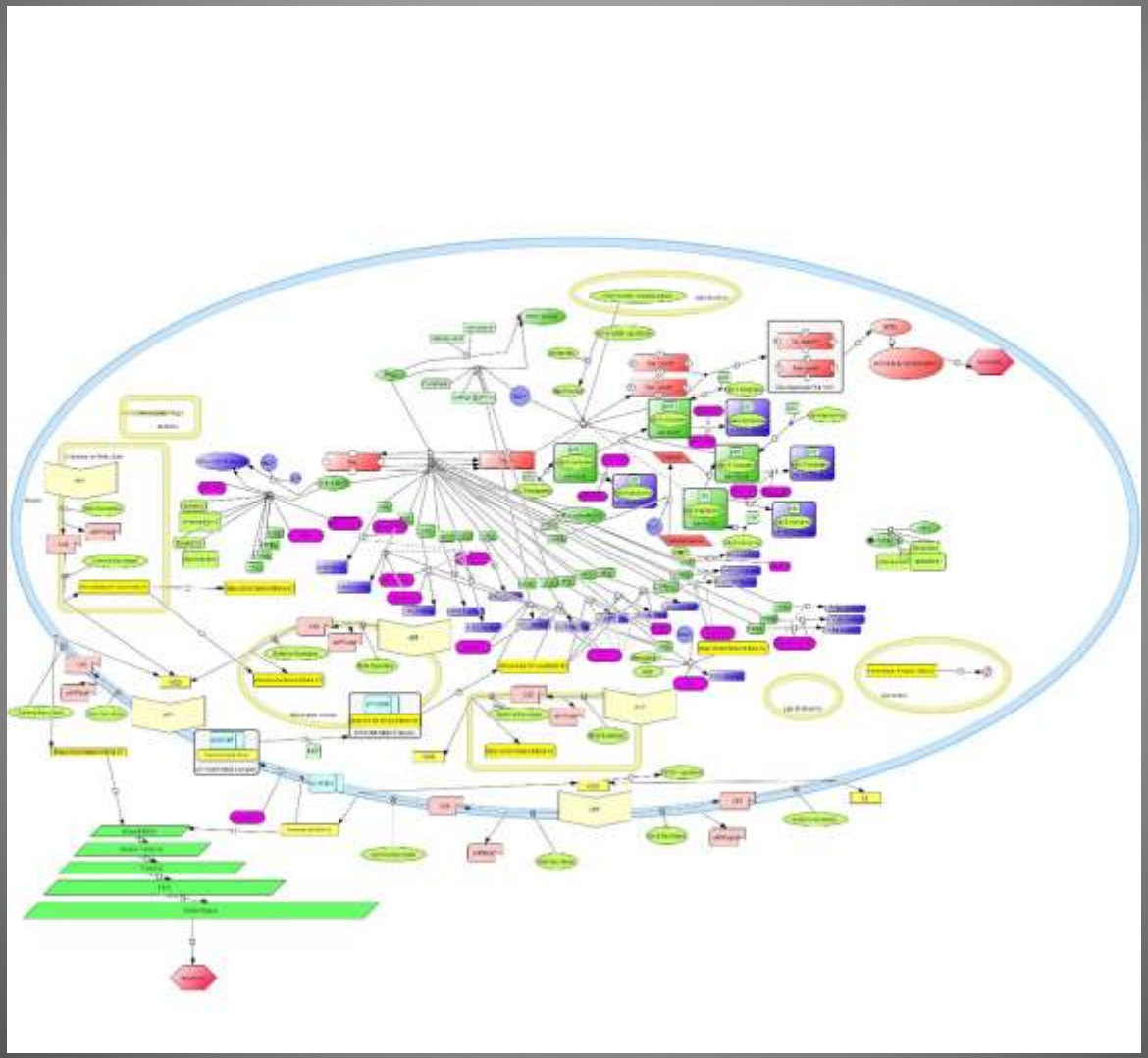
- Cummings Memorial Fund
- Roy R. Charles Center for Academic Excellence
- The College of William and Mary

# Alzheimer's Disease

Improper APP processing :

- Accumulation of A-beta fibrils & plaques
- Hyperphosphorylated tau-protein tangles
- Reactive oxygen species implicated
- Metal ion involvement

# Alzheimer's Model



# A mathematical model for Alzheimer's Disease predicts that mitochondrial dysfunction is linked to changes in expression of 5 genes impacting mitochondrial ROS and apoptosis.

Randolph A. Coleman, PhD, Morgan Shelton, BS, Ceyda Durmaz, BS, Elena Gavrila, College of William and Mary, Williamsburg, VA, USA
Frank J Castora, PhD, Eastern Virginia Medical School, Norfolk, VA, USA

## Abstract

Abnormal mitochondrial function has become recognized as a critical component in the pathogenesis of a variety of neurodegenerative diseases, including AD. We have recently found abnormal expression of several genes critical to mitochondrial biogenesis in AD brains. Using this subset of mitochondrial genes, we have begun to build a mathematical model of AD using Biochemical System Theory (BST). Through the development and application of appropriate differential equations, the flux of various metabolites and small molecules will be simulated and used to generate a testable model of mitochondrial involvement in AD pathogenesis. Methods: Human Mitochondrial Biogenesis and Human Alzheimer Disease RT² Profiler PCR Arrays were used to assess expression of 168 mitochondrial function and AD genes in two control and five age- and gender-matched AD brains. These gene expression changes served as the starting point for MatLab-based mathematical analysis and BST dynamic computer simulations. Results: Our preliminary PCR array analysis identifies significant expression changes in genes involved with maintaining mitochondrial morphology or regulating mitochondrial membrane potential. Combining these data with the mathematical modeling of these interactions using BST, we have generated a tentative working model shown here. Conclusions: Our preliminary model depicts and predicts the relationships of HSPD1, DNM1L, CDKN2A,

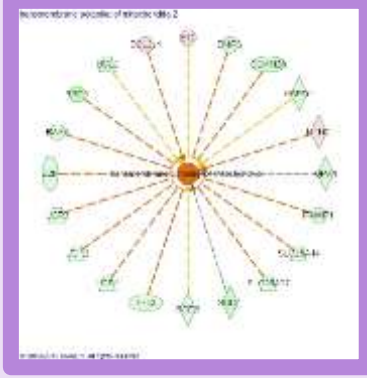


**Fig. 1** Diagram showing the interaction of 3 up-(red) and 17 down-(green) regulated mitochondrial biogenesis genes acting to activate (orange lines) effects on the transmembrane potential of the mitochondria. There are four (yellow) interactions inconsistent with this effect and 2 (gray) that are unpredicted.

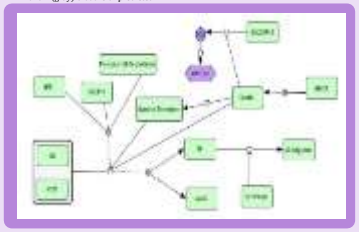


**Fig. 2** Initial model based on PCR array data provided by Frank Castora (EVMS) This became the platform for creating the large, complex model as seen in **Fig. 8**

**Fig. 3** Diagram showing the interactions of BBC3, GAPDH, NEFL, and SLC25A13 with genes known to contribute to the pathogenesis of AD. Also shown are transcription factors acting as upstream regulators of these four

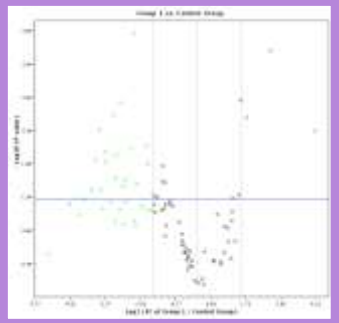
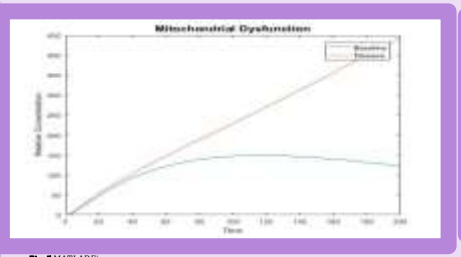
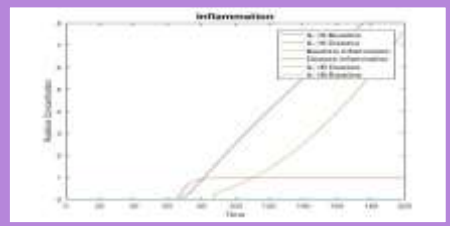


**Fig. 4** Volcano plot showing up (red) and down (green) regulated mitochondrial biogenesis genes in AD (Group 1) vs matched control brains. The further left or right from the triplet of vertical lines, the greater the fold-change in expression level of the specific gene. The vertical lines enclose all genes with less than or equal to 3-fold change relative to controls. The genes in the upper left and right quadrants show statistically significant ($p \leq 0.05$) changes.

**Biochemical Systems Theory**

- Utilizes Mass Action Kinetic Modeling
- Two forms of equations
  - **Rate Equations** determine the reaction rates based on the concentrations of relevant catalysts and inhibitors
    dX(171)=0.1*(X(25)-X(65))
  - **System Equations** illustrate changes in protein concentration
    dX(20)=X(21)*0.01-X(20)*0.001
- Transcription rates were adjusted based on PCR array data to simulate the diseased state (**Fig. 1**)
- Concentration of dependent variables are altered by differential equations containing the initial concentrations of reactants and their reaction rates (**Fig. 9**).
- The difference between Diseased and Baseline states was used for qualitative analysis
- The difference data was used to identify the pathways and protein species affected by the upregulation of protein transcription (**Fig. 7**)




**Upregulated in Disease State**

| Variable | Relative_Difference |
|---|---|
| max_X177 | 4.4899e+10 |
| max_Deg | 5.2543e+07 |
| max_X186 | 1.3435e+07 |
| max_X187 | 1.3435e+07 |
| max_p53monoUb | 5.1947e+06 |
| max_NO | 4.4578e+06 |
| max_X166 | 3.0543e+06 |
| max_ONOO | 2.0681e+06 |
| max_X173 | 3.0543e+05 |
| max_BID | 2.884e+05 |
| max_p53 | 2.7765e+05 |
| max_X147 | 1.9637e+05 |
| max_BCL2 | 1.4432e+05 |
| max_NMDAR | 98177 |
| max_X144 | 79973 |

**Downregulated in Disease State**

| Variable | Relative_Difference |
|---|---|
| min_BCL2rna | -2.8137e+05 |
| min_C99mem | -93.482 |
| min_C99mito | -92.523 |
| min_NEFLrna | -79.647 |
| min_SOD2 | -41.777 |
| min_SOD2rna | -39.987 |
| min_X192 | -26.852 |
| min_X193 | -26.852 |
| min_X190 | -26.507 |
| min_SERPINA3rna | -19.285 |
| min_TOMM40rna | -19.285 |
| min_AIFM2rna | -19.285 |
| min_Deg | -10.978 |
| min_BCLSBakComplx | -3.1639 |
| min_NADH | -2.7555 |

**Fig 7** Data was found by subtracting Baseline Values from Disease State Values
Results are relative concentration in nM

## Explanation of MATLAB Coding

- MATLAB code (**Fig. 9**) is based off interactions visualized in CellDesigner (**Fig. 8**).
- A system of nonlinear ordinary differential equations was created with each species being represented by a unique equation (**Fig. 9**)

Ex. dX(6)=X(12)*k(6)-X(6)*k(74)

positive values (X(12)*k(6)) depict the creation of the target species based on a preset rate (k(6)) the concentration of precursor(s) (X(12))

negative values (-X(6)*k(74)) depict the loss of the target species as it reacts to form a new species

- Phenotypic species, such as inflammation, mitochondrial dysfunction and apoptosis, set a baseline for system perturbations.
- Baseline perturbation values were then used to set thresholds for activation of apoptotic and inflammatory pathways in the disease state. (Seen as "if else" statements in **Fig. 9**)

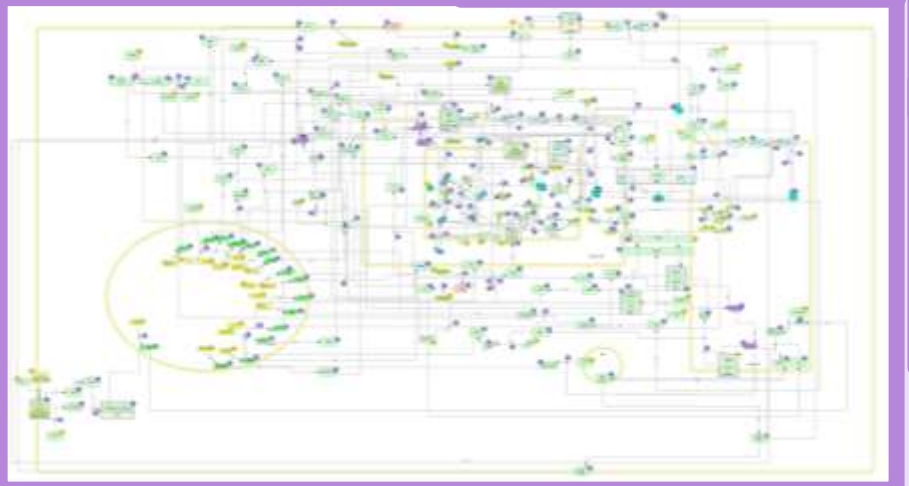


**Fig. 8** Cell Model

**Fig. 9** MATLAB code

## Results

- This model presents a relationship between DNM1L (DRP1), MFN2, AIFM2, and BCL2L1 upregulation and pathogenic Amyloid-Beta leading to Mitochondrial dysfunction and Inflammation (**Fig. 5 and 6**)
- Allowing for a certain level of dysfunction within the mitochondria, **Fig. 6** shows the increase in Interleukin release as well as an increase in overall inflammation
- **Fig 7** presents the top fifteen up- and down-regulated species. including reaction rates (presented as X##)
- Relative Values were determined by finding the difference between the Disease and Baseline species concentrations

depict the relationship between mitochondrial dysfunction and inflammatory pathways.

- The increase in pathogenic Amyloid Beta production with the upregulation of key proteins induces increased levels of mitochondrial dysfunction
- Future work will build upon this principle relationship and incorporate additional data from Alzheimer's PCR arrays to simulate the abnormal mitochondrial activity seen in actual cellular functioning. Further work will be done to incorporate the effects of pathogenic Amyloid Beta.

# Support provided by:

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# Questions?

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