# 1. Results from Prior NSF Support

## 1.1 NSF CCLI Award # 0837075, $110K, 1/1/2009-12/31/2010, (Qin as PI 1/1/2009-12/31/2009), “Computing in Life Sciences through Hands-on Experience and Case Studies at Tuskegee University”. With this educational grant, Qin developed materials for teaching computing in biology, available at http://www.bioinformatics.org/ctls, and had a peer-reviewed teaching publication [[1](#_ENREF_1)]:

* Qin, H., Teaching computational thinking through bioinformatics to biology students. Proceedings of 40th ACM Technical Symposium on Computer Science Education, 2009: p. 188-191.

*Contributions to the development of Human Resources in STEM*: This grant has enabled Qin to develop and offer teaching materials on computing in biology at Tuskegee University (two course-offers and one three-day faculty workshop with 32 participants), Spelman College (three course offers), Lewis Clark College (one faculty workshop with 7 participants), Delaware State University (one guest lecture with computer lab of ~20 students), and Alabama State University (one workshop for ~15 participants). Four of these institutions are HBCUs. Qin has carried out this project beyond the support of the initial grant, and the developed teaching materials will contribute to the present proposal.

## 1.2 NSF RUI Award # 1022294, $293K, 9/1/2010-8/31/2012, with one year no-cost extension to finish manuscripts. PI Qin, “Testing the network hypothesis of cellular aging in *Saccharomyces cerevisiae*”, With this RUI research grant, Qin laid the groundwork for the present proposal (see also section 3.5). This RUI has led to 1 peer-reviewed publication [[2](#_ENREF_2)], 1 undergraduate honor thesis, and 3 manuscripts (Underlined authors are current and former Spelman students).

* GUO, Z., A. B. ADOMAS, E. D. JACKSON, H. QIN and J. P. TOWNSEND, 2011 SIR2 and other genes are abundantly expressed in long-lived natural segregants for replicative aging of the budding yeast *Saccharomyces cerevisiae*. FEMS Yeast Res 11: 345-355.
* Parnell, L, Undergraduate honor thesis, Study the links between oxidative stress, genomic instability, and cellular aging, May 2012.
* Parnell, L., E. D. Jackson, Parker, M., J. Rodrigues, N. Gupta, B. Mohanty, H. Qin, Hydrogen peroxide induced loss of heterozygosity offers insight on mitotic asymmetry and chronological aging in *Saccharomyces cerevisiae*. In preparation. (Poster accepted, 2012 yeast genetics annual meeting).
* Montgomery, C. K. Matheson, O. Morrison, A. Story, H. Qin, An analysis of genomic features associated with aging in *S. cerevisiae*. In preparation. (Poster, 2011 SMBE meeting by A. Story in Kyoto Japan)
* Qin, H. A network model of cellular aging. (Poster in 2011 computational cell biology meeting at CSHL. Oral presentation accepted, 2012 meeting of Society of Mathematical Biology).

*Contributions to the development of Human Resources in STEM*: This RUI has directly supported Qin to mentor over 25 African American female undergraduates to conduct computational and experimental research on cellular aging in less than two years. Two of them have won travel awards to attend the 2010 Yeast Genetics & Molecular Biology Meeting at Vancouver, Canada and the 2011 Society for Molecular Biology and Evolution at Kyoto, Japan, respectively. Part of this project has been integrated into two courses: BIO320 “Genomics, Proteomics, and Bioinformatics’ and BIO233 “Microbiology” with a combined total of ~30 enrolled students yearly. In BIO320, students learned to use R to conduct computational genomics projects. In BIO233, students learned to write mini research proposals on a Wiki- website (http://sunrays.spelman.edu/bgd/).

# 2. Specific Aims, Career Goals, and Institutional Environment

Built on PI’s prior work, the overarching goal of this 5-year CAREER proposal is to advance current knowledge of aging from network perspective through integrated research and teaching. Aging is a fundamental question in biology, but mechanistic understanding of aging is far from clear. PI approaches this question by focusing on cellular aging in *Saccharomyces cerevisiae*. One unique aspect of this proposal is PI’s network model of cellular aging, which will demonstrate theoretically that cellular aging is an emergent property of gene networks (section 3.3). The overall plan contains three major components – theoretical, empirical, and educational components (Table 1). Integrated natures of these components allow many sub-aims to proceed in parallel – Theoretical work provides directions for empirical studies. Empirical studies will test theoretical predictions and in turn refine and enhance theoretical models. These aims are based on the PI’s experiences, expertise, previous and preliminary findings, collection of yeast strains, and significance of expected results. PI has a demonstrated record of yeast aging research[[2-4](#_ENREF_2)], network analysis[[5](#_ENREF_5), [6](#_ENREF_6)], computational and experimental genomics[[2](#_ENREF_2), [7-9](#_ENREF_7)], mathematical modeling[[10](#_ENREF_10)] and integrating research into teaching (see Chair’s letter). The project is also designed to be both intellectually challenging and practically accessible to undergraduates.

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| **Table 1. Overview of planned project activities and time line** | |
| Years | Project Activities |
| 1-3  1-3  2-3  2-5 | **Theoretical Component –** *The foundation of the whole project.*  Aim 1. Develop network reliability model of cellular aging (NRMCA) to a sophisticated theoretical framework.  We will first focus on 3 important factors on network robustness, then expand to quantitative genetics. Aim 1.1. Study the impact of power-law and error tolerant network configurations on cellular aging.Aim 1.2. Introduce cooperativity and limiting interaction modules (LIMs) into NRMCA.Aim 1.3. Introduce renewals/repairs into NRMCA. Aim 1.4. Introduce ploidy and alleles into NRMCA and study aging as a quantitative trait. |
| 1-3  2-4  1-4  1-5  1-5 | **Empirical Componen**t – *Examine biological implications and provide feedback to theoretical work.*  Aim 2. Identify LIMs on robustness in *S. cerevisiae*, study their effect on cellular aging and related traits, and develop an alternative ODE network model on Calorie Restriction (CR) and ROS. Aim 2.1. Develop a comprehensive set of robustness proxies for yeast genes.Aim 2.2. Network clustering of robustness proxies and related traits to identify candidate LIMs. Aim 2.3. Refine candidate LIMs with natural variation in lifespan and related traits. Aim 2.4. Experimentally study how LIMs affect aging dynamics, tolerances to oxidative stress and genomic instability, and protein expressional robustness.Aim 2.5. Prototype an ODE network model on CR and ROS as an alternative approach to NRMCA. |
| 1-5  1-5  2-5  1-5 | **Educational Component** – *Operational plan of the research components through undergraduate research*  Aim 3. Integrated training on modeling, computing and genome biology to minority students. Aim 3.1. Engage minority undergraduates in research through independent studies.Aim 3.2. Integrate original research in computational and genome biology into four courses.Aim 3.3. Develop a new course on systems biology for undergraduates. Aim 3.4 Build a sustainable undergraduate program on computing and modeling through faculty workshops, an undergraduate minor, a student club, and outreach. |

PI Qin’s career goals are to become an effective teacher, a nurturing mentor, and an innovative scholar. Qin’s long-term research goal is to understand how gene networks influence complex traits, especially those with emergent aspects. Spelman College provides an excellent academic environment for Qin to pursue his career goals as a teacher-scholar and conduct the proposed research and educational activities. As a liberal arts college for women of African American descent, Spelman is a forward-looking college aiming to prepare students as future leaders. US News consistently ranks Spelman among the 100 best liberal arts colleges and the best Historically Black College and University (HBCU). More than one third of Spelman’s ~2000 students pursue degrees in Science and Engineering. Spelman has a long history of emphasizing research in student learning. The interdisciplinary nature of the proposed research and its close integration with education perfectly align with Spelman’s strategic plan for 2015 that aims to promote a campus culture of research and interdisciplinary training.

The Biology Department at Spelman is constantly revising its curriculum to provide students with the best available learning experience. Over 65% of the 2012 biology graduates have had at least one semester of mentored research experience. Since 2010, there are about 40~50 biology students presenting their research accomplishments at the annual Spelman Research Day. The department’s persistent drive of curriculum innovation has led to more than 20 years of continuous educational grant support from Howard Hughes Medical Institute (HHMI) to Spelman College through competitive renewals. In May 2012, Spelman College was selected for a HHMI Capstone Award for ”sustained excellence and important contributions to undergraduate science education” [[11](#_ENREF_11)]. Strong departmental support to PI’s proposed research and educational activities can be seen in the Departmental Chair’s support letter.

# 3. Background, Previous and Preliminary Findings, Significance and Intellectual Merit

## 3.1. Brief overview of yeast aging and current challenges

Aging is a fundamental question in biology [[12-14](#_ENREF_12)]. Aging likely occurred during evolution in unicellular organisms that predates eukaryotes because aging is observed in bacteria [[15-18](#_ENREF_15)]. Tremendous strides have been made toward the mechanistic understanding of aging over the past two decades; yet the very concept of aging is still under debate (For example, see [[19](#_ENREF_19)]).

As a unicellular organism, the budding yeast *Saccharomyces cerevisiae* has proven to be a good model system for studying mechanisms of cellular aging [[20-28](#_ENREF_20)]. Many key features of cellular aging were first discovered in yeast before they were established in metazoan cells [[17](#_ENREF_17), [21](#_ENREF_21), [29-31](#_ENREF_29)]. The lifespan of yeast can be measured in two ways: replicative and chronological lifespan. Replicative lifespan (RLS) is the number of cell cycles that individual mother cells produce before they senesce and cease to divide [[20](#_ENREF_20), [24](#_ENREF_24), [32](#_ENREF_32)], and is often determined by microdissection. Chronological lifespan (CLS) is how long cells can survive without dividing in stationary phase [[22](#_ENREF_22), [33](#_ENREF_33)], and is often assessed by quantifying colony-forming units (CFUs).

Paradoxically, biology of aging becomes evasive once we delve into molecular mechanisms. Although hundreds of genes in yeast have been found to influence aging, none of these genes suggests molecular mechanisms that are directly linked to aging. The effect of SIR2 on lifespan is attributed to the “toxic” effect due to accumulation of extrachromosomal rDNA circles [[34](#_ENREF_34)], a concept that is not only mechanistically obscure but has also been challenged [[35](#_ENREF_35)]. The effect of TOR pathway on replicative lifespan is attributed to the decreasing ribosome function and translation [[29](#_ENREF_29)] or to the hyper-activation of cellular functions [[19](#_ENREF_19), [36](#_ENREF_36)]. The mechanism of TOR on chronological lifespan remains unclear [[37](#_ENREF_37)]. In fact, it is speculated that bona fide aging genes do not exist because there are no conserved causes of aging [[23](#_ENREF_23), [38](#_ENREF_38), [39](#_ENREF_39)]. With no genes as direct causes of aging, it is surprising that calorie restriction (CR) is a universal way of intervention to extend lifespan. In yeast, CR can extend both RLS and CLS [[40](#_ENREF_40), [41](#_ENREF_41)], despite that there are substantial differences in active pathways between the two different aging processes [[42](#_ENREF_42)].

A large body of experimental data suggests that complex gene networks are involved in yeast aging. In a large-scale screen, 90 gene deletions were found to extend CLS in BY laboratory strains [[43](#_ENREF_43)], and 300 gene deletions can shorten CLS [[43](#_ENREF_43)]. In another screen of RLS, 20% of the gene deletions were found to shorten RLS, whereas 10 out of 564 genes significantly extended RLS [[44](#_ENREF_44)]. In a quantitative trait study, transgressive segregation of CLS was observed, indicating the involvement of many loci [[45](#_ENREF_45)]. In collaboration with Jeff Townsend at Yale University, we compared gene expressional profiles of short and long-lived segregants of a wild yeast isolate, and found 15 genes with consistent differential expression levels between the long- and the short-lived progenies[[2](#_ENREF_2)]. The complex nature of aging has motivated many authors to argue that network is the key to understanding aging [[46-57](#_ENREF_46)], and their work have provided inspirations to our model.

It is clear that cellular aging is a stochastic process to a great extent − Genotypically homogenous yeast cells from a single colony will live to different ages even when they are kept in the same laboratory environment. We found that genetic factors contributes ~22% of natural variation in individual RLS [[4](#_ENREF_4)]. Therefore, it is perplexing that there exists a universal characteristic of aging at the demographic level, known as the Strehler-Mildvan correlation [[4](#_ENREF_4)] (see section 3.2), despite its likely complex mechanisms and the great plasticity of individual lifespan. PI argues that this kind of universality suggests a common principle in the stochastic processes of aging.

## 3.2 Gompertz model – a quantitative definition of biological aging

The dynamics of biological aging can be defined by the two-parameter Gompertz model [[58](#_ENREF_58)]:

where, *m* is the mortality rate; *s* is the survival fraction of a population (i.e. viability); *t* is the time. Mortality rate, *m,* is basically the normalized declining rate of *s*. The initial mortality rate, *m0*, describes the innate susceptibility to dying. The Gompertz coefficient, *G*, determines acceleration rate of mortality rate over time and is therefore a parameter for rate of aging. The exponential increase of mortality rate is a universal characteristic of biological aging, and has been observed in bacteria, yeast, worms, fruit flies, mice, and humans [[4](#_ENREF_4), [12](#_ENREF_12), [15](#_ENREF_15), [16](#_ENREF_16), [59](#_ENREF_59), [60](#_ENREF_60)]. PI’s lab observed a negative linear correlation between *G* and ln(*m0*) in yeast [[4](#_ENREF_4)]. This negative linear correlation was first reported in humans [[61](#_ENREF_61)], known as the Strehler-Mildvan correlation. This kind of universality suggests a common principle and is a key motivation for our modeling effort.

An organism can be *non-aging*, given the Gompertz definition of biological aging. When *G*=0, mortality rate *m* becomes a constant. Hence, drop of viability becomes an exponential decay, which is basically a first-order chemical reaction, just like the exponential decay of radioactive isotopes. Individuals from these populations will then be as good as new at any time point, and are therefore *non-agin*g. Bacterial phages indeed display this kind of non-aging characteristics[[62](#_ENREF_62)]. It is worth clarifying that non-aging bacterial phages are not immortal, they just die with constant mortality rates.

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| C:\Users\hqin\Dropbox\Funds_and_proposals\career.proposals.dropbox\sandbox.career.dp\network-model-fig1.tif  Fig. 1. The basic network reliability model of cellular aging (NRMCA) and its equivalent pathway-based reliability model as in Gavrilov & Gavrilova 2001[[63](#_ENREF_63)]. Dark circles represent essential genes, and white circles represent non-essential genes. Biological functions of all gene interactions decay exponentially, i.e., *non-aging*. When an essential gene loses all of its interactions, it is equivalent to a gene-deletion and results in cell death. Consequently, only essential genes’ interactions influences aging, and are represented by solid links. Dashed links are interactions that will not affect aging. Interactions are stochastic. The failure of a two-module NRMCA is mathematically equivalent to failure of a two-block reliability model [[63](#_ENREF_63)]. To obtain analytic solutions, interactions between modules are assumed to have no effect on aging – Failures of modules are independent. This assumption will be replaced by cooperativity in Aim 1.2. |

## 3.3. A network reliability model of cellular aging (NRMCA) – Our unique mathematical approach

### 3.3.1 Rationale:

Emergent property refers to a feature formed at system levels but cannot be found at component levels. Classical examples include termite castles, schools of fishes, and flockings of birds. To prove that cellular aging is an emergent property of gene networks, components of our model network ought to be non-aging. Specifically, we need to demonstrate that Gompertzian aging at system level can arise from gene networks that are made of components with constant mortality rates*.* Although intracellular molecular networks can be partitioned into gene regulatory networks, protein networks, and metabolic networks etc, our network model is an abstractive one in order to demonstrate a basic principle.

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| **C:\Users\hqin\Dropbox\Funds_and_proposals\career.proposals.dropbox\sandbox.career.dp\figures\prediction.tif**  **Fig 2**. A counter-intuitive prediction of NRMCA: Stronger robustness increases *G*, as shown by the sharper transition of the dying off phase. For illustration, average lifespans are unchanged. |

### 3.3.2. The basic version of NRMCA

Network in the basic version of NRMCA contains *K* essential modules, and each module contains 1 essential and *n* non-essential genes (Fig. 1). The biological function of each interaction is assumed to be *non-aging* and decays exponentially with a constant rate of **µ**. Death of a cell occurs when an essential gene loses all of its interactions, equivalent to deletion of an essential gene. Interactions are assumed to be stochastic, and the initial probability of an interaction being active is assumed to be ***p***. When failures of modules are assumed to be independent, analytic approximation for the system mortality rate is

, Eq 2a,

Eq 2b

, (G is rate of aging) Eq 2c

where *C* is a normalization constant (Qin, manuscript in preparation).

Eq 2a is the Gompertz model of aging. Hence, the defining characteristics of biological aging, the exponential increase of mortality rate over time, can arise from a network model with *non-aging* components. By definition, we have shown that cellular aging is an emergent property of this model network. Our model has its roots in the reliability model of aging. Eq 2a was obtained using the ‘initial virtual age’ method developed by Gavrilov and Gavirolva [[63](#_ENREF_63), [64](#_ENREF_64)].

In Eq 2c, the rate of aging, *G*, is approximately proportional to the number of active interactions per essential gene (*n×p*) and will increase dramatically when chances of these interactions being active become higher (*p*). The number of active interactions per gene can be viewed as a measure of network robustness. Hence, stronger robustness would lead to a faster rate of aging (Fig.2). (Power-law configuration, cooperativity, renewals/repairs are 3 other important factors on cellular robustness, and will be studied in Aims 1.1, 1.2 and 1.3.)

### 3.3.3 Important Model prediction and property.

The most important prediction is the counter-intuitive positive correlation between the Gompertz coefficient *G* and network robustness – more robust cells have higher rates of aging (Fig. 2). It is counter-intuitive because aging dynamics is quantified here by two Gompterz parameters, *G* and m0, with only G as a measure of rate. In contrast, the colloquial meaning of aging rate actually contains information for both parameters.

The most important property of the model is the Strehler-Mildvan correlation – the trade-off between *G* and *m0*. Using a method developed by Gavrilov and Gavriolova [[63](#_ENREF_63), [64](#_ENREF_64)], it can be shown that ln(*m0*) ≈ -*BG + Intercept*, where *B* and *Intercept* are constants based on K, *µ,* and *p.* This property explains the universality of the Strehler-Mildvan correlation.

## 3.4 Intellectual merit, significance and implications

To our best knowledge, NRMCA is the first mathematical model to demonstrate the network emergent aspect of cellular aging. It provides a conceptual framework to explain the seemingly inconsistent experimental data, individual plasticity, and universal demographic characteristic of cellular aging in yeast. As an emergent property of networks, cellular aging is a system-level property, which explains the difficulty of pinpointing individual genes as direct causes of aging. The universal Strehler-Mildvan characteristic of aging can be attributed to the common interacting patterns of gene/protein networks shared among most species. The emergent aspect of aging also provides a link between RLS and CLS, even though specific pathways differ in the two ways of aging [[42](#_ENREF_42)].

NRMCA argues that heterogeneity of gene interactions is an important factor between biological aging and non-biological aging. If intrinsic stochastic noises are removed from our model, increase of mortality rate would follow the Weibull model, which is often the failure model of complex machinery[[63](#_ENREF_63), [64](#_ENREF_64)]. NRMCA can provide a mechanistic foundation for the antagonistic pleiotropy theory [[14](#_ENREF_14)] and the disposable soma theory [[65](#_ENREF_65)] on the evolution of aging. NRMCA is also consistent with the free radical theory that reactive oxygen species (ROS) is a major source of stochastic damages for aging [[13](#_ENREF_13)].

NRMCA suggests a mechanistic link among robustness, gene networks, and aging. NRMCA argues that *G* is a measure of robustness. Robustness can reconcile the mutational costs to individuals and the evolutionary benefits to the population [[66](#_ENREF_66)], because the phenotypic effects are hidden in most conditions. Robustness is related to canalization, and it has been argued that network buffering is a key mechanism of canalization[[66-70](#_ENREF_66)]. Our model also demonstrates that network biology is a useful way to tackle biological complexity, and can provide insight not easily achieved by reductionist approaches.

## 3.5. Other key previous and preliminary results in support of the proposed network model

PI has a demonstrated record of yeast aging research[[2-4](#_ENREF_2)], network analysis[[5](#_ENREF_5), [6](#_ENREF_6)], computational and experimental genomics[[2](#_ENREF_2), [7-9](#_ENREF_7)], and mathematical modeling[[10](#_ENREF_10)]. My group published the first quantitative study on yeast aging using the Gompertz model and reported the Strehler-Mildvan correlation in yeast[[4](#_ENREF_4)]. My lab has since reaffirmed the Strehler-Mildvan correlation by phenotyping more yeast strains. My lab reported that tolerance to genomic instability, measured by loss of heterozygosity, is associated with yeast replicative lifespan [[3](#_ENREF_3)]. My lab has collected more than 150 natural isolates of yeast, whose genomes have been sequenced, partially sequenced, or are being sequenced (see Botstein’s letter). Three more highlights are presented here.

### 3.5.1. Diploid cells have larger *G* than haploid cells.

Diploid cells are generally considered more robust than haploid cells. If stochastic variation in the number of key molecules causes gene expression noises, doubling their numbers ought to reduce the noises by , as argued by Schroedinger in 1944 [[71](#_ENREF_71)]. Yeast protein expression noises in G1 phase are much lower in diploids than that of haploid cells [[72](#_ENREF_72)], indicating that protein expressions are indeed more robust in diploid cells than in haploid cells. For a given set of measured lifespans of N cells (), the likelihood function is . Using maximum likelihood estimation (MLE) and likelihood ratio test, we compared the Gompertz parameters of diploid BY4743 with its haploid counterpart BY4741 and BY4742. We found that MLE of G=0.079 in the diploid strain and MLE of G=0.065 in haploid counterparts, and the difference is significant at p=3.610-10. Hence, more robust diploid cells age faster than less robust haploid cells.

### 3.5.2. Morphology robustness is correlated with RLS.

Yeast deletion mutation with known effects on morphology is available at the *Saccharomyces cerevisiae* Morphological Database (SCMD, http://scmd.gi.k.u-tokyo.ac.jp/). SCMD provides a list of 501 morphological parameters in four groups: cell shapes, bud sizes, nucleus locations, and actin localizations [[73](#_ENREF_73)]. Three Spelman students in PI’s lab, Ms. K. Matheson, O. Morrison, and R. Levy, calculated the coefficient of variation (CV) of the normalized 501 parameters for each yeast gene, which describes the unmasked morphological plasticity when the wildtype gene is removed and is therefore a proxy of morphological robustness for the wildtype gene function. This measure of morphological robustness was found to be negatively correlated with RLS (R2=0.034, p=1.3×10-5), indicating that large morphological variance corresponds to short RLS. This finding is consistent with the known role of morphology in the asymmetric partition of damaged proteins during mitosis [[74](#_ENREF_74)]. Although active transport is involved[[75](#_ENREF_75)], mitotic asymmetry can be sufficiently achieved by the slow diffusion of large aggregates, geometry of the mother and daughter cells, and the narrowness of the passage between them [[74](#_ENREF_74)]. The RLS data used by the students contain RLS for 564 genes measured by the Kaeberlein group [[76](#_ENREF_76)].

### 3.5.3. Inactivation of Hsp90 leads to smaller *G* in two yeast strains.

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| C:\Documents and Settings\hqin\My Documents\Dropbox\B3-Gompertz-fitting.tif  Fig. 3. Radicicol deceases *G* in strain K11. Concentrations of radicicol are in the inserted box. |

Hsp90 is a phenotypic capacitor that can buffer mutations in its substrate proteins [[77](#_ENREF_77), [78](#_ENREF_78)], and plays an important role in gene network robustness. Antibiotic radicicol can inactivate Hsp90 by occupying its nucleotide binding site [[79](#_ENREF_79)]. We preliminarily tested 4 strains to see how radicicol influence G of CLS, and found that radicicol decreased *G* in two yeast strains (K11 and DBVPG6765) in a dose-dependent way. (The other two strains are not responsive to radicicol, presumably due to antibiotic resistant mechanisms.) In strain K11, radicicol can then extend CLS at 1.25uM (p=0.027) but not at 5.0 uM (Fig. 3), indicating that the effect of radicicol may be hormetic.

Viability of yeast cells were measured by propidium iodide (PrI) staining followed by flow cytometery. PrI negative cells are considered viable cells, and PrI positive cells are considered dead[[80-83](#_ENREF_80)]. The fractions of live and dead cells were modeled by t-mixture models with Box-Cox transformation or log-transformation, and were estimated by expectation - maximization procedure using the R package flowClust [[84](#_ENREF_84), [85](#_ENREF_85)].

# 4. Research Plan, Expected Results, and Alternative Approaches.

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| Aim 1, *Theoretical Component*: Develop the network reliability model of cellular aging (NRMCA) into a sophisticated theoretical framework. Based on predicted connection between network robustness and cellular aging, we will focus on three factors with important roles in robustness: power-law configuration, cooperativity, and renewals/repairs. For limiting interaction module on robustness, we will focus on synthetic lethality, which will pave the way for network study on aging as a quantitative trait. The four intertwined and inter-dependent sub-aims will be carried out in parallel. |

### Aim 1.1 Study the impact of power-law and error tolerant network configurations on cellular aging.

Our NRMCA predicts that heterogeneity plays a key role during the emergence of biological aging. A key source of heterogeneity in gene networks is its power-law feature – the degree distribution of genes follows **,** where *k* is the number of connections per gene, *Z* represents the Ziemman function, and γ is a coefficient [[86](#_ENREF_86)]. When γ≤3, the variance of *P(k)* is infinite. For most biological networks, γ is often between 2 and 3 [[87](#_ENREF_87)], which indicates tremendous amount of heterogeneity in biological networks. In addition, networks with power-law features, such as Internet, are robust to random failures but are fragile to deliberate attacks [[88](#_ENREF_88), [89](#_ENREF_89)]. In yeast protein networks, highly connected genes, hub-genes, are less likely to directly interact with other hub genes in the protein interaction networks, which contributes to the error tolerance of protein networks [[90](#_ENREF_90)]. Perturbation of protein concentrations can be mostly buffered locally in yeast protein binding networks, but they can also cascade over more 4 interactions away in certain pathways [[91](#_ENREF_91)].

We plan to use simulation to study how power-law degree distribution and error tolerance features of gene networks influence aging dynamics, especially *G*. There are several ways to simulate power-law gene networks. The preferential attachment model is often used [[87](#_ENREF_87)]. Alternatively, we can generate the degree distribution based on , and then pair interacting nodes, in a similar way to a network simulation approach previously used by us[[5](#_ENREF_5)]. The parameter γ will be ranged from 1 to 3. Control studies will be conducted in networks with fixed numbers of interactions per gene, a Poisson distribution of degrees, and a log-normal distribution of degrees.

To simulate the error-tolerant features, we plan to choose 5%, 10%, and 15% of nodes with top-ranked connections as essential genes. For controls, essential genes will be chosen randomly, or deliberately assigned to nodes with few connections.

We plan to characterize the aging dynamics through *G*, *m0*, average and median lifespan, and tail distribution of lifespan. We plan to fit the simulated lifespans to Gompertz, Gompertz-Makeham, and Weibull distribution, and then evaluate them by Akai Information Index.

To simulate the lifespan of an individual cell using NRMCA as in Figure 1, we will first simulate the ages of all gene interactions based on exponential distributions (i.e., non-aging). The age of each essential module is the maximum age of its gene interactions. The age of this individual cell is the minimum age of all of its essential modules. Simulations will be run for a population of cells to generate a survival curve. Three Spelman undergraduates, Ms. E. Dommond, J. Williams and J. Christopher, have prototyped some R codes to simulate cellular aging based on the basic model of NRMCA.

As a proof of principle, in power-law configured networks, interaction failure rates will be set to a fixed constant, as in the basic version of NRMCA. We are aware that we may need to invoke complex gene interactions from Aim 1.2 for simulations that can better fit to the Gompertz model. However, our preliminary simulation suggests that the effect of network configuration on aging can be demonstrated using the basic version of NRMCA. In fact, PI’s students found that heterogeneity in degree distributions can dwarf the effect of heterogeneity in failure rates, at least in the parameter space that we tried.

We expect that power-law and error-tolerant feature would render networks more robust to random failures, and therefore lead to larger *G* values. Configuration heterogeneity may also influence the tail distribution of lifespan, as suggested by a study based on the reliability model[[92](#_ENREF_92)]. Plateau of mortality rate in late life is an empirical observation and has been attributed to population heterogeneity [[93](#_ENREF_93), [94](#_ENREF_94)]. It is worth discussing that power-law configuration is connected to Aim 1.2 and 1.3. Power-law configuration implies rate-limiting modules. Renewals and repairs in hub and non-hub nodes should have different effects on network robustness.

### Aim 1.2. Introduce cooperativity and limiting gene interaction modules (LIMs) into NRMCA.

In cells, loss of one gene’s function may activate another gene with overlapping functions [[70](#_ENREF_70), [95](#_ENREF_95)], which contributes to cellular homeostasis and robustness. This kind of gene interactions can be modeled by dependence of failure rates in NRMCA. Dependent failure rates of interactions also means cooperativity, which likely plays a role in network buffering mechanisms [[70](#_ENREF_70)]. We will limit dependence/cooperativity between modules, similar to the avalanche–like model of aging [[64](#_ENREF_64), [96](#_ENREF_96), [97](#_ENREF_97)].

First, linear cooperativity will be modeled. Failure rate of one module is assumed to be a linear function of the number of remaining active interactions in its ‘interacting’ modules, also similar to the avalanche-like model. Second, sigmoid cooperativity will be modeled using a Hill function, , where and are current and previous failure rates at time *t* and , is the previous number of active interactions in neighboring modules, *K* is a threshold, and *n* is the Hill coefficient. Hill coefficient can adjust the non-linearity and describe positive or negative cooperation. Hill function is also a generalized way to study cooperative changes, because linear model is a special case of the Hill function.

We expect that the extent of non-linearity would greatly influence cellular responses to random failures, and Hill coefficients can strongly influence *G* and *m0*. In Power-law networks, Hill cooperation between essential modules with dense interactions will also likely have stronger non-linearity. We will systematically compare aging dynamics in Poisson and power-law networks with either positive or negative cooperation of failure rates.

There are many theoretical possibilities on how a network module would be rate limiting in aging, including power-law configuration. We will focus on synthetic lethality – a biological relevant example that can be experimentally tested. Synthetic lethality occurs when two gene deletions are needed for cell death. Many single-gene deletions do not lead to cell death in yeast, but double deletion of two genes can result in lethality. When one of the synthetic lethal genes is deleted, it should decrease robustness and lead to smaller *G* and larger *m0*. This expected effect of synthetic lethal pairs will be empirically tested in Aim 2.4, using the yeast deletion collection available in PI’s laboratory.

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| C:\Users\hqin\Dropbox\Funds_and_proposals\career.proposals.dropbox\sandbox.career.dp\figures\synthetic-lethal-super-module.tif  Fig 4. A network with 3 essential genes (dark solid circles) and 1 pair of synthetic lethal genes (2 red solid circles). Open circles are non-essential genes. Failure of each purple essential module leads to cell death. Failure of the super-module occurs when the synthetic lethal pair loses all interactions, and leads to cell death. Only interactions that influence aging are shown for clarity. |

In NRMCA, synthetic lethality will occur when loss of two ‘semi-essential’ genes are required for cell death, which implies interaction between these two semi-essential genes (Fig. 4). We plan to merge the two synthetically lethal modules into a super-module for implementation (Fig. 4). Death of these super-modules needs additional book-keeping during simulation, but the rest of the modules will be simulated with minimal overhead. Synthetic lethality is also connected to Aim 1.4.

### Aim 1.3. Introduce renewals/repairs into NRMCA.

Renewals and repairs play an important role in biological robustness. Self-repairing is also an important factor in several theories of aging [[98-100](#_ENREF_98)]. From a mathematical perspective, both renewals and repairs can be modeled exactly in the same way, as in repairable engineering systems [[92](#_ENREF_92), [101](#_ENREF_101)]. Renewals/Repairs can be imperfect or perfect. To explore the theoretical impact, we will focus on perfect repairs as in previous studies[[101](#_ENREF_101)].

Perfect repairs are equivalent to replacement of machinery components in reliability engineering [[102](#_ENREF_102), [103](#_ENREF_103)]. In reliability engineering, repairs are modeled by a specified number of stand-by components, and the system fails as the last reserved component fails [[103](#_ENREF_103)]. This approach was used in a reliability study that assumed one replaceable component, and counter-intuitively found that repairs can decrease the relative tail of longevity [[92](#_ENREF_92), [101](#_ENREF_101)]. As a proof of principle, we will also assume one replaceable interaction in NRMCA. We expect that repairs should increase network robustness, lead to larger *G* and smaller *m0*. Intuitively, repairable organisms are more robust, and hence more homogenous. Consequently, the average lifespan of a repairable population should be higher, but relatively few individuals should have extremely long lifespans. These predictions will be tested in yeast null mutants of protein chaperons (Aim 2.4). Protein chaperons prevent mis-folding of their substrate proteins and therefore play an important role in cellular repairs. Our preliminary results on Hsp90 are consistent with these predictions (section 3.5.3).

We are also interested in how differential repair mechanisms influence aging. We will compare repairs that preferentially target highly connected genes versus less-connected genes in power-law networks, and repairs that preferentially target limiting interaction/modules versus random repairs. As control, repairs in random networks will also be studied.

### Aim 1.4. Introduce ploidy and alleles into NRMCA and study aging as a quantitative trait.

Ploidy and alleles are required to study aging using quantitative genetics. An important question in quantitative genetics is the so-called ‘missing heritability’ of complex traits [[104](#_ENREF_104)]. Genome-wide association (GWA) studies have revealed many loci for complex human traits, however, these loci can only explain a small proportion of the “total heritability” of the traits[[104](#_ENREF_104)]. To address this problem, Zuk and colleagues from the Lander group proposed a limiting pathway (LP) model for complex trait [[104](#_ENREF_104)], which is analogous to the classic reliability models. Zuk et al 2012 found that gene interactions (epistasis) can inflate the estimation of “total heritability”.

Based on the Gompertz model, the average lifespan can be determined by *G* and *m0*:

This non-linear form suggests that it is unlikely genes’ effects on lifespan would be an additive linear model which is the standard practice in quantitative genetics. PI and two students conducted a preliminary GWA study on CLS using 33 sequenced yeast strains. We indeed found that linear model has very poor statistical power to detect association between average CLS and segregating sites. We also used Fisher’s exact test on genotype-counts for categorical lifespan (long-, short-, and averaged-lived), and found it to be more useful presumably because it does rely on linear assumption. Here, we plan to study network influence on aging as a quantitative trait, evaluate different GWA approaches for empirical study in Aim 2.3, and investigate the problem of ‘missing heritability’.

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| C:\Users\hqin\Dropbox\Funds_and_proposals\career.proposals.dropbox\sandbox.career.dp\figures\synthetic-lethal-and-alleles2.tif  Figure 5. Synthetic lethality is mathematically analogous to alleles in essential genes in NRMCA. Blue dots are 2 alleles for an essential gene, and two red dots are a synthetic lethal pair. Cell deaths in both networks occur when two blue/red dots lose all interactions in solid links. Dashed links does not affect aging. |

Alleles will only be introduced to essential genes (nodes) because only their interactions will influence aging in NRMCA.

First, we will assume failure of modules are independent, i.e. no epitasis between essential nodes. When two alleles of an essential gene are expressed in the same cell, we need to decide how to model cell death. A straightforward option is to treat the two alleles as redundant components (i.e., no genetic dominance), which is equivalent to parallel components in reliability engineering models. Serendipitously, this scenario of two alleles in one essential gene is mathematically analogous to synthetic lethality – The two alleles can be treated as a synthetically lethal pair. This insight actually has an evolutionary root: Some synthetically lethal gene pairs are evolutionary duplicates that were alleles in the ancestral species [[105](#_ENREF_105)].

We plan to simulate 2 alleles in K number of essential genes, which means K essential modules. These 2K allelic modules will randomly have fast, slow, and average failure rates. The modules with fast failure rates are rate limiting for cell death in NRMCA, however, whether they can be detected by quantitative genetics is exactly the question to be studied here. We may randomly pick 150 genotypes (“strains”) out of 3K possible genotypes (We have 150 strains for GWA in Aim 2.3). For each “strain”, we will simulate aging for *N*=103 cells to estimate average and median lifespan, *G* and *m0*. Preliminary results showed that 1000 cells can offer sufficient details on aging dynamics. Association between average lifespan and essential loci will be tested by various parametric methods and non-parametric methods using genotype-counts, as in the proposed GWA study in Aim 2.3. Because of the simulated ‘truth’, we can compare the type I and type II errors of different methods. This comparison will be conducted in network with regular, Poisson, or Power-law configurations. We expect that power-law configuration will introduce more non-linearity and render the contrast between linear and non-linear methods more apparent. For comparison, we will also introduce population structures by introducing low levels of mutations into a particular ‘strain’.

Another approach to introduce diverse genotypes into NRMCA is to use observed genotypic variations of known lifespan influencing genes. Yeast genes with known aging effect are available at Sageweb and their genotypic variations are available in the Sanger sequenced strains.

To address the problem of ‘missing heritability’, the “total heritability” will be calculated by linear regression between individual lifespan and “strains”, as in previous studies [[4](#_ENREF_4), [104](#_ENREF_104)]. We will then calculate the “known heritability” using additive linear model for the *K* number of loci, as in Zuk et al 2012 [[104](#_ENREF_104)]. The ratio of “known heritability” versus “total heritability” is the “explained heritability”, which should be 100% for perfectly additive traits.

Second, we will introduce ‘epistasis’ between two essential modules, based on the first part of this sub-aim. In general, epistasis means the effect of one locus is altered by another [[106](#_ENREF_106)]. Hence, ‘epistatsis’ between two essential genes (modules) means that failure rate in one module will be altered by another module, which is cooperativity between modules discussed in Aim 1.2. The subtle difference here is that cooperativity between different allelic combinations has to be modeled differently. Let’s assume Gg and Bb are two alleles at two essential loci, and their nine combinations are GGBB, GGBb, GGbb, GgBB, GgBb, Ggbb, ggBB, ggBb, and ggbb. For simplicity, let us assume G and B share the same slow failure rates, and g and b share the same fast failure rates. As a proof of principle, we will assume that ‘g’-module and ‘b’-module will influence each other’s failure rates only in ggbb genotype, as in the classical example of epistasis [[106](#_ENREF_106)]. Similar to Aim 1.2, both positive and negative Hill cooperativity will be simulated. Similarly to the non-epistatic simulation plan, we will compare type I and type II errors for linear and non-linear GWA methods, and also study the ‘missing heritability’ problem.

**Future theoretical directions and career plans:**

With development through this five-year career plan, PI will expand the research on networks and robustness to other important topics, such as dominance, canalization, antibiotic persistence, evolution in age-structured populations [[107](#_ENREF_107)], and speciation in the future. The proposed research will indeed build a foundation for a life-long endeavor on network biology.

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| **Aim 2, *Empirical Component*:** **Identify limiting interaction modules (LIMs) on robustness, study their effect on aging and related traits, and develop an ODE network model on CR and ROS.** Based on the theoretical predicted connection between robustness and cellular aging, we will study how limiting modules on robustness influence yeast aging. We will first generate a large set of proxies for gene’s robustness, infer LIMs, refine them by natural variation, and then experimentally examine them. |

### Aim 2.1. Develop a comprehensive set of robustness proxies for yeast genes.

Biological robustness means persistence of phenotype in the presence of genetic, environmental, or stochastic perturbations [[66-68](#_ENREF_66), [108-113](#_ENREF_108)]. One way to gauge a gene’s role in robustness is the phenotypic variation in its null mutant. Larger phenotypic variation of the null mutant indicates a stronger role in robustness for the wildtype gene. Variation in morphology and expression levels have been used as proxies of robustness [[56](#_ENREF_56), [67](#_ENREF_67), [108](#_ENREF_108), [114](#_ENREF_114)]. Coefficient of variation (CV), which is the standard deviation divided by the mean, is a normalized robustness proxy to compare phenotypic measures at different levels. Gene functions are context-dependent, and essentiality and non-essentiality can vary by experimental conditions. Hence, practical measures of robustness depend on the source of perturbations, i.e, experimental conditions. For example, genes in galactose catabolism are non-essential in glucose-containing media, but can become essential when galactose is the limiting nutrient. To obtain a big picture for gene robustness, here, we propose to collect and compute a large set of proxy measures of gene robustness under various experimental conditions. This resource can not only help us to infer LIMs on robustness, it is likely a useful resource for a broader community [[115](#_ENREF_115), [116](#_ENREF_116)].

First, we will focus on phenotypic proxies of robustness and curate a large set of relevant genome-scale phenotypic datasets. We will start with Saccharomyces Genome Database (SGD) that provides many published functional genomic datasets, Gene Expression Ominibus (GEO), and Yeast Fitness Database [[117](#_ENREF_117), [118](#_ENREF_118)]. Preliminary search of GEO showed that many datasets of gene expression are potentially useful, including studies on cell cycles (GDS2347), heat shock stress (GDS2343, 1711, 281, 36, and 35), oxidative stress (GDS108), rapamycin effect (GDS2338), various growth inhibitions (GDS2196 and 1636), polymorphic variation (GDS1115 and 1116), and nutrient limitations (GDS777, 115, 112, and 111). Other interesting datasets include protein abundance [[119](#_ENREF_119)], protein expression noises [[120](#_ENREF_120)], half-lives [[121](#_ENREF_121)], dosage effect [[122](#_ENREF_122)], mRNA half-lives [[123](#_ENREF_123)], antioxidant responses[[124](#_ENREF_124), [125](#_ENREF_125)], and survival in nutrient limiting conditions [[126](#_ENREF_126)]. We will group datasets by strains and experimental conditions, convert and/or normalize different data when necessary, and compute CVs as proxies for robustness. Principal components will be used to evaluate interdependency. PI’s group has already analyzed growth fitness measures in various conditions [[127](#_ENREF_127), [128](#_ENREF_128)] and morphological robustness (section 3.5.2).

These research activities clearly require substantial effort of careful reading and critical thinking, and it is therefore an ideal project for crowd-sourcing. PI will lead students to exhaustively survey literature, critically read and evaluate experimental findings in four courses (Fig 8 in section 5, page 14). Students will use PI’s wiki web-server to work collaboratively (http://sunrays.spelman.edu/bgd/). PI will also lead students to perform bioinformatics and computational tasks in two courses (Fig 8 in section 5). Parsing and merging of heterogeneous datasets are suitable bioinformatics projects for undergraduates.

Second, we will focus on mutational robustness. Mutation rates in each gene indicate the extent of mutational perturbation that cells can tolerate, and hence is a proxy of mutational robustness. One accepted metric for mutation rate is the synonymous nucleotide diversity, as in a recent study in *E. coli* genes [[129](#_ENREF_129)]. We will use the same approach to estimate the synonymous and nonsynonymous nucleotide diversity in sequenced strains of *S. cerevisiae*. Nucleotide diversity in the 5’ UTR regions will be studied for comparison. {Kobayashi, 2006 #2029;Kobayashi, 2004 #2025;Kobayashi, 2011 #691;Ganley, 2009 #644}Currently, there are ~70 yeast strains with sequenced genomes: 36 genomes by the *Saccharomyces* Genome Resequencing Project (SGRP) at the Wellcome Trust Sanger Institute, at least 25 strains with contigs available at the *Saccharomyces cerevisiae* Strain Project at Genome Institute at Washington University. There are also some additional genomes available at Saccharomyces Genome Database (SGD) and NCBI. In collaboration with Dr. David Botstein (see his letter), we are also sequencing 11 natural isolates that we have measured lifespans, genomic instability, and tolerance to oxidative stress [[3](#_ENREF_3), [4](#_ENREF_4)]. PI will also collaborate with Dr. Titus Brown on NGS genome analysis (see his letter).

We are aware that phenotypic changes of null mutants are sometimes caused by their neighboring genes on the same chromosomes [[130](#_ENREF_130)], an artifact that will be addressed in Aim 2.2. We are aware of the low-coverage problem in the Sanger sequenced strains [[131](#_ENREF_131)], and the potential bias by its imputed SNPs based on ancestral recombination graphs using closely related genomes. This approach likely will remove rare SNPs and indels from the samples. Because we are interested in the overall mutational pattern of each gene, rare SNPs should not substantially affect our estimations.

PI is experienced with analyzing genome scale data, comparative genomics, gene expression analysis, and network inference [[5-10](#_ENREF_5)]. PI has established a batch query procedure to GEO in the R environment through a client-server exchange protocol implemented in the GEOquery package [[6](#_ENREF_6), [132](#_ENREF_132)]. PI has also led undergraduates to conduct computing projects in BIO386, and annotate newly sequenced genomes through crowd-sourcing in a HHMI phage genomics course. PI’s students found that morphological robustness is significantly correlated with RLS though only explains 3.4% of RLS variance (section 3.5.2).

### Aim 2.2. Network clustering of robustness proxies and related traits to identify candidate LIMs.

Here, we will perform network clustering analysis to identify gene clusters based on robustness proxies. For each gene, its observed phenotypic and mutational variations could be due to either its intrinsic robustness or extrinsic robustness of its interacting genes (i.e. genetic capacitors such as Hsp90). Some phenotypes in the yeast deletion collection are also caused by artifacts [[130](#_ENREF_130)]. These two problems can be addressed by network clustering analysis.

There are many options to perform clustering analysis of genomic data based on networks. One straightforward approach is to convert genomic data into distances, map them to interactions (also called edges or links) in networks, and apply various clustering algorithms on weighted networks. PI has previously converted a gene expression dataset into edge-weight and applied shorted-path clustering method on weight protein interaction network[[6](#_ENREF_6)]. Euclidean distance is a standard method to convert normalized genomic data into edge-weights, though many other transformations are available, such as logit-like transformation [[5](#_ENREF_5)]. We will apply standard hierarchical clustering based on network traversal distances using shortest-path. In addition, we will try other clustering methods, such as K-means and Markov clustering using MCL [[133](#_ENREF_133)].

Several network datasets will be used, including protein-protein interactions from DIP, BioGRID, and BIND [[134-136](#_ENREF_134)], protein complexes [[137](#_ENREF_137), [138](#_ENREF_138)], genetic interactions [[139](#_ENREF_139)], and a probabilistic interaction network inferred from heterogeneous dataset from bioPIXIE [[140](#_ENREF_140), [141](#_ENREF_141)].

We will evaluate robustness-clusters with RLS and CLS measures from the Kaeberlein group [[43](#_ENREF_43), [44](#_ENREF_44), [53](#_ENREF_53), [76](#_ENREF_76), [142](#_ENREF_142), [143](#_ENREF_143)]. A statistic measure of lifespan of each module, mean, median, or standard deviation, will be evaluated based on null distributions generated by permutation of lifespans. Missing lifespan values will be treated as ‘NA’s during analysis and permutations. Recent and unpublished RLS and CLS data from the Kaeberlein group will also be incorporated into the analysis. PI is currently collaborating with the Kaeberlein group to predict longevity genes using machine-learning methods. PI has planed visits to the Kaeberlein laboratory for more effective collaborations (see Kaeberlein support letter). Tentatively, we plan to pick network clusters/modules with top 5% of averages and bottom 5% of variances for robustness proxies, RLS, and CLS.

PI has led students in his laboratory and courses to conduct large scale analysis of fitness data, morphology data, RLS and CLS. In addition to morphological robustness, students found that RLS is significantly correlated with fitness measured in media with ethanol as the main carbon source (p=0.00159, R2=0.04). Based on these prior results, it is very promising that larger scale analysis can lead to gene interaction clusters associated with robustness and aging.

### Aim 2.3 Refine candidate LIMs with natural variation in lifespan and related traits. {Gat-Viks, 2010 #1972}

Our goal here is to examine whether our candidate LIMs tend to be associated with natural variation in lifespan and related traits. Genetic variants associated with natural lifespan variation are likely involved in limiting steps during aging. With the relatively large number of sequenced yeast genomes, it is conceptually straightforward to conduct genome-wide association (GWA) studies [[144](#_ENREF_144)]. PI and two Spelman students have preliminarily measured 33 Sanger yeast strains using propidium iodide (PrI) at Princeton during the summer of 2011. A preliminary GWA study showed that linear model has extremely poor statistical power (see also aim 1.4), but Fisher’s exact test on genotype-counts for long-, short- and average CLS categories gave 37K ‘positive’ SNPs out of 200K SNPs in the dataset, using a false discovery rate of 10%. Many of the ‘positive’ SNPs are located in biological meaningful genes, but a large number of them are likely caused by population structure, similar to another study [[144](#_ENREF_144)]. These preliminary results suggest that our main concern is the amount of false-positives in GWA. To address this problem, we plan to scale up GWA to ~150 strains with 6 phenotypic measures and use sophisticated analysis.

We will focus on CLS because it can be straightforwardly carried out by undergraduates. In addition to CFU measure of CLS, PI’s laboratory has developed a highthroughput approach to measure CLS using PrI permeability (section 3.5.3). We will also measure intracellular H2O2 and superoxide using flow cytometry (see also Aim 2.5), and measure tolerance to H2O2, paraquat, and menadione using Bioscreen growth curves assay. Overall, we will quantify 6 phenotypes for over 150 strains with sequenced or partially sequenced genomes [[131](#_ENREF_131), [145](#_ENREF_145)] (see Aim 2.1 for details on strains).

To combine 6 phenotypic measures for each SNP, one way is the Fisher’s method. When null hypotheses are true for all association tests, they are independent by definition, and their p-value can be combined as for *K* number of traits. To correct for multiple tests, phenotypic data will be permuted 1000 times, and lowest p-value from each test will be used to generate a distribution to determine proper genome-wide significance [[144](#_ENREF_144)].

To combine multiple SNPs in one gene, one option is to sum *M* number of largest SNP as as recommend in Hoh and Ott 2003 [[146](#_ENREF_146)]. Significance of *sM* can be evaluated by permutation of phenotypic data [[146](#_ENREF_146)].

Population structure can be addressed by the first principal component of genotypes [[147](#_ENREF_147)] or linear mixed model based EMMA [[148](#_ENREF_148)]. Alternatively, we plan to choose ‘unstructured’ strains based on neighbor-joining tree calculated from whole genome genetic variations as in previous work [[131](#_ENREF_131), [144](#_ENREF_144), [145](#_ENREF_145)]. Association tests on genotype-counts of categorical phenotypic data can be applied using ‘unstructured’ strains. Choosing ‘unstructured’ strains should mitigate false positives, but it also decreases sample size and likely increases false negatives. On the other hand, linear assumptions in model-based approach may not work for aging as a quantitative trait. Comparison of these two approaches will also be addressed by simulation studies in Aim 1.4.

Joint analysis of all genes in LIMs is basically pathway association analysis in GWA [[149-153](#_ENREF_149)]. One option is to permute single-gene statistic such as *sM*  in the empirical interaction network (as in Aim 2.2), generate null distributions of the mean, median, and variance for all genes in each LIM, and use these null distributions to evaluate the significance for each LIM. This permutation approach is analogous to PI’s previous work on network evolution [[5](#_ENREF_5)]. For comparison, we will apply several ‘standard’ pathway-association tests, such as gene set enrichment, SNP ratio test, GSA-SNP, ALIGATOR, as reviewed recently [[153](#_ENREF_153)].

In the event that GWA would not be helpful to refine candidate LIMs, we would like to emphasize that it would **not** prevent us to study Aim 2.4, and it will **not** determine the success of the entire proposal (see Aim 2.4). Study here and in Aim 1.4 will clearly improve the understanding of aging as a quantitative trait. PI plans a two-month visit to Princeton’s Lewis-Sigler Genome Center in Year 2 in order to effectively collaborate with the Kruglyak laboratory (see letters from Kruglyak and Botstein).

### Aim 2.4. Experimentally study how LIMs affects aging dynamics, tolerances to oxidative stress and genomic instability, and protein expressional robustness.

The goal here is to experimentally test the link between network robustness and ***G***, the rate of aging, in contrast to many other study’s focus on average lifespan. In Year 1-3, we will study two examples of limiting interactions on robustness – synthetic lethal pairs and protein chaperons, in conjunction with Aim 1.2 and 1.3. PI will lead students in two courses to investigate candidate genes with limiting effect on robustness, formulate hypotheses, design experiments, and carry them out in a manner of crowd-sourcing (Fig 8 on page 14). This empirical approach will focus on genes with known effects on lifespan, using information at Sageweb (lifespandb.sageweb.org/). For controls, we can randomly pick genes from the yeast genome, and pick genes whose null mutations have no effect on lifespan.

In Year 3-5, experimental study will be expanded to LIMs predicted in Aims 2.2 and 2.3. Though priority will be given to LIMs associated with both natural variations and lifespan changes in null mutants, top-ranked LIMs predicted in Aim 2.2 can be used directly. Evaluation of promising LIMs for experiments will also be done empirically by student groups, as in Year 1-3. For control, we plan to use least-likely candidates LIMs. These results will help us to evaluate the computational approaches in Aim 2.2 and 2.3.

We will focus on CLS because it can be effectively carried out by undergraduates. CLS for both deletion mutants and GFP-fusion derivatives will be measured by colony forming units (CFUs) and by flow cytometry measure of PrI permeability. Intracellular H2O2 and superoxide during chronological aging will be monitored by DHR and DHE in the deletion mutant [[154](#_ENREF_154), [155](#_ENREF_155)]. Coefficients of Variation (CVs) of GFP, DHR, and DHE are inverse proxies of robustness [[120](#_ENREF_120), [156](#_ENREF_156), [157](#_ENREF_157)], and their changes during aging are informative on how aging affect network robustness. Alternatively, RLS in null mutants for some promising candidates will also be measured, in collaboration with the ongoing RLS screen project in the Kaeberlein group (see his letter). PI’s own laboratory also has the capability to measure RLS.

We will also measure tolerance to genomic instability by loss of heterozygosity (LOH) at the MET17 loci during CLS using null mutants from the yeast deletion collection [[3](#_ENREF_3), [158](#_ENREF_158)]. The effect of oxidants on LOH will also be investigated. PI is experienced in studying genomic instability using LOH at the MET17 locus [[3](#_ENREF_3)]. Our previous results show that LOH is informative on both RLS and CLS. Recently, a student in PI’s laboratory studied H2O2-dose dependent LOH for her honor thesis.

Students will learn to analyze experimental data in R, study change of robustness during aging, and use regression to evaluate all the measured robustness proxies: *G*, CV of H2O2, CV of superoxide, CV of GFP, changes of LOH, and tolerance to oxidants as in previous studies [[3](#_ENREF_3), [4](#_ENREF_4)].

### Aim 2.5. Prototype an ODE network model on CR and ROS as an alternative approach.

PI will prototype an ODE model to study how glucose influences intracellular ROS – an important aspect of CR. It was recently reported that CR (0.5% glucose) extends CLS by initially inducing H2O2 and superoxide dismutase *Sod2p* activity [[154](#_ENREF_154), [155](#_ENREF_155)]. Though it was not discussed in the original publication, the reported signals of H2O2 and superoxide are conspicuously multi-modal in CR treated cells but less obvious in 2% glucose (Figure 2A in Mesquita et al 2010). PI hypothesizes that low glucose levels induce heterogeneity in cell populations that may partially explain the reported changes of average signals. Two Spelman students recently measured glucose-dependent H2O2 and superoxide signals in one yeast strain (Figure 6). These preliminary results strongly suggest that glucose can lead to two cell populations with different levels of H2O2 and superoxide. Hence, we propose to characterize these changes in great detail and prototype a mathematical model to study its mechanisms.

We will conduct CLS assays in synthetic media with various glucose levels, and monitor intracellular H2O2 and superoxide. Intracellular H2O2 can be detected by dihydrorhodamine 123 (DHR) [[3](#_ENREF_3), [154](#_ENREF_154), [155](#_ENREF_155)], and intracellular superoxide can be detected by dihydroethidium (DHE) [[154](#_ENREF_154), [155](#_ENREF_155)]. PI has established a DHR-DHE double staining procedure similar to Cossarizza et al 2009 [[159](#_ENREF_159)] (see Figure 6). Noticeably, the glucose levels in bulk growth assays are constantly changing. To address this problem, we will grow yeast cells in chemostat with constant glucose levels that can precisely reflect glucose-dependent changes H2O2 and superoxide levels (see Botstein letter). For comparison, yeast cells will be treated with rapamycin, H2O2 and menadione. Null mutants sod2Δ, cta1Δ, and tor1Δ will be studied. Sytox Green will be used to determine cell cycle states [[160](#_ENREF_160)]. Viability will be monitored by PrI permeability.

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| :2012JUNE28DHRDHE.M5.Day3.FL1-3marginal.tiff  Figure 6. Intracellular H2O2 and superoxide distributions on the 3rd day in chronological aging of strain M5. Bimodal distributions are apparent in mid-ranged glucose concentrations, indicating bistablilty. Signals are in log10 scale. |  | C:\Documents and Settings\hqin\My Documents\Dropbox\Funds_and_proposals\career.proposals.dropbox\sandbox.career.dp\figures\ODE-model2.tif  Figure 7. A four-node network model with glucose as an input signal based on literature [[154](#_ENREF_154), [161-168](#_ENREF_161)]. Arrows are positive cooperativity, dots are negative cooperativity, and dashed lines are uncertain ones. Question marks indicate plausible interactions. *xj* is the concentration (activity) of species *j*, *γj* is a time scale, ωj express weight and direction of interactions, ωj0 describes a threshold, σ describes nonlinearity of regulation, *Sj* is external signal strength [[169](#_ENREF_169)] |

We will prototype an ODE-based four-node network model (Fig. 7), inspired by a recent work from Xing’s group [[169](#_ENREF_169)], and use simulations to explore plausible mechanisms on CR and ROS. This prototype dimensionless network model will focus on two high-level functional modules – respiration and stress defenses. Regulation of the *j-th* species (node j) by *i-th* species is described by ji, with its absolute value for regulation strength and its sign for positive or negative regulations. *Wj* sums up the net regulation from interacting nodes. Parameter space will be searched by a two-stage random walk strategy using a Metropolis algorithm developed by Xing’s group [[169](#_ENREF_169)]. In the 1st search stage, random walks have higher chances of leaving local optima. ‘Good’ parameter sets from the 1st stage will be analyzed by K-means clustering. One parameter set from each cluster will be used in the 2nd search stage, in which random walk is only around the local optima. To reduce parameter search space, ji can be set to zero, positive or negative ranges based on empirical observations in Fig. 7. Finally, ji will be descretized to generate topological matrices [[169](#_ENREF_169)], and common network motifs can be identified [[169](#_ENREF_169)].

PI is experienced in mathematical modeling and attended a CSHL course on dynamic modeling. PI will also visit Xing’s group for close collaboration (see Xing’s letter). True to the career development nature of this proposal, this sub-aim will lay a ground work for PI’s future research on CR.

# 5. Educational Plan (Broader impact)

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| Aim 3. Integrated training on modeling, computing and genome biology to minority students. |

Our educational plan is essentially the operational plan to carry out the research plans through undergraduate based research and teaching activities. It is important to emphasize that most project activities are practically accessible to undergraduates and intellectually challenging. PI will lay out his plan to engage students in research through independent studies and courses, and build a sustainable program for computing and systems biology (see Chair’s letter for departmental support).

### Aim 3.1. Engage minority undergraduates in research through independent studies.

This project will mainly be carried out by students at Spelman College, a historically black college for women. Students will be trained in mathematical modeling, computational simulation, genomics, next-generation sequence analysis, experimental genetics, and flow cytometry. The interdisciplinary nature and genome-scale studies of this project will add a sense of excitement to students. PI strives to instill the fundamental values and ethics of research into the students. By taking ownerships of a unique body of data and knowledge through original research, students may discover their love of research and may choose science-related careers. PI is experienced in training undergraduate researchers for both experimental and computing skills. Data from aging experiments and flow cytometry are routinely analyzed by students using R scripts. PI has been using YouTube to facilitate student learning (www.youtube.com/qinstat). PI has trained over 35 Spelman students in three years, among which four are math majors, two are computer science majors, two have won competitive travel awards to attend international meetings, several are pursuing graduate trainings in STEM, and at least two are pursuing Ph.D. training in computing and/or genomics related fields.

### Aim 3.2. Integrate original research in computational and genome biology into courses.

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| C:\Users\hqin\Dropbox\Funds_and_proposals\career.proposals.dropbox\sandbox.career.dp\figures\teaching-research.tif  Figure 8. Integration of research into courses. Arrow thickness indicates the extent of integration. Community building helps for recruiting and sustaining the teaching effort. Spelman Biology uses four 100 level courses for core courses. Consequently, many 200 and 300 level courses targets juniors and seniors. |

Integration of research into teaching through crowd-sourcing is outlined in Figure 6. As a tenure track faculty in the Spelman Biology department, PI teaches 9 credit/contact hours per semester, which provides excellent opportunity for interactions with students.

In CIS115 Introduction to Computing and Informatics (4 credits, co-instructor), PI will lead student groups to work on parsing genomics data from various sources, pipe-line design for large scale analysis, and network/graph analysis, as in Aim 2.1.

In BIO125 Molecular Biology and Genomics (4 credits, instructor for 1 section), PI will lead students to measure CLS, study LOH, ROS and protein expression changes during chorological aging, and the effect of oxidants, using the diploid deletion mutants and GFP fusion strains, as in Aim 2.4. Students are encouraged to propose their own experiments. Students will learn to take research notes properly and write research reports. Research integrity and ethics will also be emphasized. With departmental support (See Chair’s letter), PI plans to first pilot this course in Year 1 and 2, and then expand to all four sections with help of other instructors. PI will parlay his past experience of teaching an HHMI phage genomics course and bioinformatics courses into BIO125. BIO125 is a core course for biology majors and aims to prepare students for independent research.

In BIO233 Microbiology (4 credits, instructor), PI plans to lead students to work on mini research proposals to evaluate candidate limiting interaction modules for Aim 2.4. Students will be guided to formulate a hypothesis and propose experiments to test them. Students will learn to use bioinformatics tools to identify homolog/orthologs, perform domain analysis, gene/protein interaction analysis, and generate their hypothesis. Students are required to write their mini proposals on a wiki based website developed by PI (http://sunrays.spelman.edu/bgd/ ). This approach will also be used in BIO120.

In BIO386 Genomics, Proteomics, and Bioinformatics (4 credits, instructor), PI teaches R programming, data analysis, and computational methods. BIO386 targets upper-class students and honor students. BIO386 is revised from BIO320, a project-based computing course that PI has taught for three years at Spelman. Past students reflected that BIO320 is like a rotation experience in graduate schools and made them prepared for graduate training. BIO386 students are required to write manuscript-styled project reports. PI plans to lead BIO386 students in groups to carry out the proposed computational modeling, simulation, and analysis in Aims 1, 2.1, 2.2, and 2.3.

PI is experienced in integrating original research into courses (see Chair’s letter). He has developed many hands-on laboratory modules for bioinformatics (see www.bioinformatics.org/ctls) and has been using tutorial videos to facilitate student training and learning.

### Aim 3.3. Develop a new course of systems biology for undergraduates.

The core mission of this proposal is to understand cellular aging from the systems perspective. PI believes that an introductory course of systems biology will not only better prepare Spelman students to participate in the proposed research, but also provide a venue to synthesize and disseminate the research findings of this project. In addition, this course can expose many biology students to the power of mathematical modeling and computational analyses. The proposed course will cover basic concepts of dynamic systems, ordinary differential equations, phase diagrams, and bifurcation analysis. Classical examples of toggle switch, and cell cycle model will be discussed. Students will learn to use Xpp and R to study system behaviors. PI also plans to guide students to develop ODE based models in Aim 2.5. PI plans to develop this course by simplifying the CSHL course materials of Computational Cell Biology (PI attended in 2011). PI will also learn from similar courses offered at the College of William and Mary (CWM) and Virginia Tech (VT). Dr. Gregory Smith at CWM has generously shared his entire course materials with PI. PI has budgeted visits to Dr. Xing’s group at VT (see his letter). PI plans to pilot this new course in Year 2 and 3, and then propose to the college curriculum committee for formal offering in Year 4.

### Aim 3.4. Build a sustainable undergraduate program on computing through faculty workshops, an undergraduate minor, a student club, and outreach.

The primary goal here is to build a local community of computing, in order to better recruit and retain undergraduates, and go beyond the effort of PI as a single faculty. First, PI plans to organize tutorial workshops for faculty in nearby colleges to adopt R and other computing and modeling tools in research and education in Year 1-4. Virtual Cell (Vcell) is a problem solving environment used by many biologists and also has well developed educational resources. Dr. Raquell Holmes from the Center for Cell Analysis and Modeling at the University of Connecticut Health Center which develops VCell will visit Spelman to deliver a workshop (see her letter of support). PI will also further develop Youtube videos on computing, modeling, and systems biology.

Second, PI plans to propose an undergraduate minor of bioinformatics and systems biology. CIS115, BIO386, and the new course on systems biology will likely be part of the minor (see Chair’s letter for Departmental support). We plan to propose this new minor to curriculum committee in Year 4. To cultivate student interests, PI plans to guide a group of students to form a student club of computing. Promotional items (such as pens) with “Computing @ Spelman” will be designed and distributed for recruitment events held on campus. Students will meet regularly in journal clubs. A Facebook group has been formed for students and alumni from the Spelman computing program to share their career experiences. PI teaches First Yeast Experiences (FYE), a 0.5 credit seminar course, which will help recruit freshmen.

For outreach, PI will host a high-school teacher during the summer for professional development. PI has cultivated a partnership with Science teachers at the Cedar Grove High School (CGHS) with a predominately African American student enrollment (see Hairston’s letter). 1. Qin, H. (2009). Teaching computational thinking through bioinformatics to biology students. *Proceedings of the 40th ACM technical symposium on computer science education*, 188-191.

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