

1 **Article type: Advanced Review**

2 **Article title: Aging and Computational Systems Biology**

3

4 **Authors:**

5 **Full name and affiliation; email address if corresponding author; any conflicts of interest**

**First author**

Kathleen M Mooney  
Faculty of Health and Social care  
Edge Hill University  
L39 4QP  
UK

**Second author**

Amy Morgan  
Faculty of Science and Engineering  
University of Chester  
CH2 4 NU  
UK

**Third author**

Mark T Mc Auley  
Faculty of Science and Engineering  
University of Chester  
CH2 4 NU  
UK  
Correspondence to [m.mcauley@chester.ac.uk](mailto:m.mcauley@chester.ac.uk)

6

7

8

9

10

11

12

13

14 **Abstract**

15 Aging research is undergoing a paradigm shift, which has led to new and innovative methods of  
16 exploring this complex phenomenon. The systems biology approach, endeavours to understand  
17 biological systems in a holistic manner, by taking account of intrinsic interactions, whilst also  
18 attempting to account for the impact of external inputs, such as diet. A key technique employed in

19 systems biology, is computational modeling, which involves mathematically describing and  
20 simulating the dynamics of biological systems. Although a large number of computational models  
21 have been developed in recent years, these models have focused on various discrete components of  
22 the aging process, and to date no model has succeeded in completely representing the full scope of  
23 aging. Combining existing models or developing new models may help to address this need and in so  
24 doing could help achieve an improved understanding of the intrinsic mechanisms which underpin  
25 aging.

26

## 27 INTRODUCTION- AGING AND THE NEED FOR COMPUTATIONAL SYSTEMS BIOLOGY

28 The world's population is aging. Globally, the number of older people (aged 60 years or over) is  
29 expected to more than double, from 841 million people in 2013 to more than 2 billion in 2050<sup>1</sup>.  
30 Those aged 80 years and over, the fastest growing group of older people, make up approximately  
31 14% of the global population, and it is projected by 2050 there will be more than three times the  
32 present number of this age group. To help put this demographic shift into perspective, it is worth  
33 noting, that the number of older people in the world's population will exceed the number of  
34 younger people by 2047<sup>1</sup>. An aging population poses many challenges for all sectors of society.  
35 Particularly as advancing age is associated with an increased risk of developing many disease states,  
36 such as cancer<sup>2</sup>, cardiovascular disease (CVD)<sup>3</sup>, Alzheimer's disease (AD)<sup>4</sup> and Parkinson's disease<sup>5</sup>.  
37 Thus, there is a growing imperative to better understand the aging process and health-span.  
38 However, to date, there is no overall consensus as to what constitutes healthy-span<sup>6</sup> or what the key  
39 mechanisms are that underpin human aging. This is partly due to the inherent complexity of aging,  
40 which effects every component of a living system, from the disruption of DNA integrity to the  
41 dysregulation of whole-body homeostatic mechanisms (Figure 1)<sup>7</sup>. Thus, aging is especially  
42 challenging to investigate. Consequently there are many approaches to study the complexities of  
43 this phenomenon, from studying single genes in isolation, to using simple organisms such as yeast,  
44 or employing epidemiological studies. Over the last decade and half, aging research has become  
45 increasingly affected by the systems biology paradigm, which eschews reductionism and treats the  
46 organism as a whole<sup>8, 9</sup>. By placing aging research firmly within a systems biology framework a  
47 means of dealing with its intrinsic complexity is provided. A key element of this approach is the  
48 juxtapositioning of computational modelling with experimental investigations<sup>10-12</sup>. These models  
49 both compliment and inform the experimental work by facilitating hypothesis testing, generating  
50 new insights, deepening biological understanding, making predictions, tracing chains of causation,  
51 integrating knowledge, and inspiring new experimental approaches<sup>13-15</sup>. Computational models  
52 developed to date to understand the aging process, have in the main represented several discrete  
53 mechanisms that are associated with aging. Examples include models of mitochondrial  
54 dysregulation<sup>16</sup>, telomere attrition<sup>17</sup> and the disruption of protein turnover<sup>18</sup>. Despite this, there are  
55 relatively few examples whereby aging has been represented using a computational model in a  
56 holistic fashion. In this paper we will 1) use oxidative stress as a framework to discuss the  
57 interconnectivity of aging 2) briefly outline the two main theoretical approaches used to assemble  
58 computational models in systems biology 3) discuss recent models that have been used to represent  
59 various aspects of aging 4) suggest how these models could be further developed in the future to  
60 lead to a more holistic representation of aging.

61

## 62 THE QUEST FOR A COMMON THREAD

63 Many theories have been proposed to explain the aging process. From an evolutionary standpoint  
64 aging is generally regarded as a non-adaptive process which is a by-product of evolution (for a  
65 review of the main evolutionary theories see Gavrilov and Gavrilova (2002)<sup>19</sup>). If we assume that  
66 aging is a by-product of evolution, the question remains, how does this process unfold? Moreover, is  
67 there a common thread that regulates aging in all organisms? It is generally accepted that **aging is  
68 not underpinned by one biological mechanism, rather it is the result of the interaction between an  
69 array of processes that act over a diverse range of spatial and temporal scales. As a result of this  
70 consensus, it has been recognized that in order to gain a more complete understanding of the  
71 mechanics of aging, integration of multiple biological pathways need to be considered. However,  
72 despite this complexity, the free radical theory of aging is arguably the closest gerontology has come  
73 to a framework, which connects together the disparate aspects of the aging process.** The free radical  
74 theory of aging proposes that damage to biological macromolecules by reactive oxygen species  
75 (ROS) accounts for aging<sup>20</sup>. Due to the role of the mitochondrial electron transport chain (ETC) in  
76 cellular respiration, mitochondria are central to this theory and are regarded as the main producers  
77 of ROS<sup>21</sup>. Together with other cellular organelles and macromolecules, mitochondria are vulnerable  
78 to the destructive capabilities of ROS. During aging mitochondrial DNA (mtDNA) accumulate  
79 deletions across a variety of somatic cell types<sup>22, 23</sup>. These deletions contribute to the overall decline  
80 in mitochondrial dysfunction<sup>24</sup>. Specifically, age-related mitochondrial changes include fusion and  
81 fission dysregulation<sup>25</sup>, impaired proteostasis<sup>26</sup>, diminished mitophagy<sup>27</sup> and diminished ATP  
82 production<sup>28</sup>. This damage to mitochondria affects their integrity, exacerbating ROS emissions and  
83 driving the aging process. This assertion is backed up by experimental evidence, which has shown  
84 that mitochondrial emission rates of O<sub>2</sub><sup>-•</sup> and H<sub>2</sub>O<sub>2</sub> increase continuously with age at species-specific  
85 rates<sup>29</sup>. **In this paper we will use ROS as a conduit to emphasise the interconnected nature of the  
86 aging process and we will stress that no single factor is responsible for the aging process but rather a  
87 multitude of overlapping mechanisms. Moreover, it is imperative at this point, to emphasise that  
88 low levels of ROS have also been suggested to improve host resistance to oxidative damage in a  
89 process termed mitohormesis<sup>30</sup>. Thus, although it is generally regarded that ROS cause cellular  
90 damage, their role within the aging process maybe much broader.**

91

92

93

94

95

## 96 Telomeres Attrition, Cellular Senescence and Oxidative Stress

97 The free radical theory of aging converges with a multitude of other cellular processes, which have  
98 been implicated with aging, including the maintenance of telomere integrity<sup>31</sup>. Telomeres are

99 repetitive TTAGGG sequences at the ends of chromosomes. Telomeres operate like a protective cap  
100 while telomerase, the enzyme responsible for maintaining telomere length, is largely absent from  
101 human somatic cells<sup>32</sup>. Consequently, each time a somatic cell divides, some of the telomere is lost.  
102 Hence, in humans, telomeres are shorter in older individuals. This was initially confirmed  
103 experimentally by the seminal work of Harley et al. (1990), who showed that both the quantity and  
104 length of telomeric DNA in human fibroblasts decrease during aging *in vitro*<sup>33</sup>. Moreover, the  
105 relationship between telomeres and cellular senescence was further cemented when telomerase-  
106 negative normal human cells were transfected with the telomerase catalytic subunit<sup>34</sup>. As a result,  
107 these cells had elongated telomeres, divided vigorously and displayed reduced senescence, when  
108 compared to telomerase-negative control clones, which exhibited telomere shortening and  
109 senescence<sup>34</sup>. More recently, investigations using telomerase knock-out rodents and human studies  
110 with telomere maintenance disorders have shown that a reduction in telomere length is associated  
111 with functional decline in a wide variety of tissues<sup>35</sup>. This brings us to oxidative stress and telomere  
112 shortening; experimental studies have determined that telomerase is not the sole factor governing the  
113 rate of loss of telomeric DNA. It has been shown that mild oxidative stress, as demonstrated by the  
114 culturing of human fibroblasts under 40% oxygen partial pressure, resulted in an increase telomere  
115 shortening from 90 base pairs(bp) per population doubling under normoxia, to more than 500 bp  
116 per population doubling under hyperoxia<sup>36</sup>. Thus, further embedding the free radical theory and  
117 oxidative stress as the epicentre of the aging process.

118

## 119 **Caloric Restriction and Oxidative Stress**

120 Oxidative stress is one possible mechanism which might explain the effect of caloric restriction (CR)  
121 on longevity. However, it is important to again stress at this point that oxidative damage is likely to  
122 be one key mechanism among many deleterious processes that underlie aging<sup>37</sup>. For instance, it is  
123 suggested that the beneficial effects of CR are mediated via a reduction in the production of ROS<sup>36</sup>.  
124 CR is a dietary regime that involves reducing nutrient intake without inducing malnutrition (usually a  
125 20–40% reduction in calorie intake)<sup>38</sup>. CR has been demonstrated to extend lifespan in a diverse  
126 range of organisms<sup>39-41</sup>; although its effect on humans is yet to be fully established. What has been  
127 established is that CR positively effects mitochondrial function in a number of ways. Most notably,  
128 CR has been shown to reduce the emission of ROS. For example, CR dampens the release of ROS  
129 from complex I of mitochondria in cardiac tissue of rats<sup>42</sup>. Furthermore, it has also been found that  
130 CR lessens the accumulation of oxidative damage. This damage characterises aging, in many tissue  
131 types across a diverse array of species<sup>43</sup>.

132

133

134

135

## 136 **Sirtuins and Caloric Restriction**

137 Metabolically, the effects of CR on the mitochondria could be modulated by several important  
138 biochemical pathways which have been implicated with increased longevity. For instance, in yeast  
139 mother cells the NAD<sup>+</sup> dependent class III of histone deacetylase enzymes (sirtuins) have been  
140 suggested to mediate the life-extending effects of CR<sup>44</sup>. In particular sirtuin 2 (Sir2) is implicated in  
141 the response to CR in yeast models<sup>45</sup>. Homologues of Sir2 have been shown to mediate some of the  
142 effects of CR in other organisms. For instance, it has been reported that an increase in *Drosophila*  
143 Sir2 extends life span, whereas a decrease in Sir2 blocks the life-span-extending effect of CR<sup>46</sup>, while  
144 similar findings have been reported in *Caenorhabditis elegans*<sup>47</sup>. Mammals possess 7 homologues of  
145 the Sir2 protein, which have been implicated in the regulation of a number of processes, from cell  
146 growth and apoptosis, to mitochondrial metabolism<sup>48</sup>. SIRT1, is the homologue of Sir2, a gene whose  
147 activity has also been shown to be modulated by CR<sup>49</sup>. For instance, it has been shown that  
148 expression of mammalian Sir2 (SIRT1) is induced in CR rats as well as in human cells that are treated  
149 with serum from these animals<sup>50</sup>. In certain cells this response could be induced by nitric oxide  
150 synthase (eNOS), which can activate the SIRT1 promoter<sup>51</sup>. This view is tentatively supported by  
151 recent findings from Shinmura *et al.* (2015), who showed that eNOS knock-out mice exhibited  
152 elevated blood pressure and left ventricular hypertrophy compared with wild-type mice, although  
153 they underwent CR<sup>52</sup>. Other sirtuins have also been implicated as mediators of the effects of CR<sup>52</sup>.  
154 For instance, mice lacking the mitochondrial deacetylase SIRT3 have been shown to suffer from  
155 increased levels of oxidative damage<sup>53</sup>. Specifically, this study showed that SIRT3 reduced cellular  
156 ROS levels by deacetylating superoxide dismutase 2 (SOD2), a major mitochondrial antioxidant  
157 enzyme. This alteration promoted its antioxidative activity, thus emphasising the close coupling of  
158 many of the factors that have been implicated in aging and longevity.

159

### 160 **mTOR the Missing Metabolic Link?**

161 Another key pathway implicated in longevity is the pathway defined by the mammalian target of  
162 rapamycin (mTOR)<sup>54</sup>. mTOR is a serine/threonine protein kinase of the phosphatidylinositol-3-OH  
163 kinase (PI(3)K)-related family. mTOR comprises of two separate complexes, mTORC1 and mTORC2,  
164 which coordinate a variety of nutrient and hormonal cellular signals, which control a variety of  
165 cellular processes including cell growth, cell size, and metabolism<sup>55</sup>. The connection between mTOR  
166 and longevity was first identified over two decades ago, when it was found that knocking out Sch9,  
167 the homolog of the mTORC1 substrate S6K, augmented chronological lifespan<sup>56</sup>. Subsequently, a  
168 number of key studies using a variety of organisms have revealed that the mTOR is highly  
169 conserved<sup>57</sup>. For example, mutations in *daf-15* a homolog of Raptor, a constituent of mTORC1, can  
170 extend the lifespan of *C. elegans*. The mutants adapted their metabolism to accumulate lipids, while  
171 there was also an increase in adult life span<sup>58</sup>. Moreover, it has been suggested that the effects of CR  
172 are coordinated by mTOR. For instance, CR has been shown to activate eukaryotic translation  
173 initiation factor 4E-binding protein 1 in *Drosophila*<sup>59</sup>. Activation of this translation protein provoked  
174 an increase in the translation of several molecules involved in the mitochondrial electron transport  
175 chain and an increase in lifespan. This lifespan increase could be due to a concomitant drop in  
176 oxidative stress. This assertion is supported by experimental evidence, which has shown that the  
177 inhibition of mTORC1 lowers mitochondrial membrane potential, O<sub>2</sub> consumption and ATP levels<sup>60</sup>.  
178 In addition, mTOR has been shown to interact with other aspects mitochondrial function including  
179 biogenesis, apoptosis and mitochondrial hormesis<sup>61</sup>.

180

## 181 Mitochondrial Function and Epigenetic Processes

182 Given the key role mitochondrial metabolism plays in ROS generation, and its putative connection  
183 with CR, it is worth considering how both mitochondrial function and the emission of ROS interact  
184 with other important biochemical and genetic processes. The Krebs cycle occurs in the mitochondrial  
185 matrix and intermediates of this fundamental metabolic pathway are required for epigenetic  
186 processes. Epigenetic processes are those factors that influence gene expression without changing  
187 the actual nucleotide sequence of the DNA molecule<sup>62</sup>. One of the best characterised epigenetic  
188 processes is DNA methylation, a process key to the regulation of gene expression<sup>63</sup>. Methylated DNA  
189 have a covalently bonded methyl group at the carbon-5 position of a deoxycytidine. This is followed  
190 by a deoxyguanine, to form tissue specific methyl patterns<sup>64</sup>. Advancing age has been associated  
191 with the disruption of these DNA methylation patterns which are key to the fidelity of gene  
192 expression<sup>65</sup>. Specifically, during aging, human DNA undergoes genome wide hypomethylation  
193 across a variety of different tissues<sup>66</sup>. Moreover, advancing age also results in regional increases in  
194 DNA methylation at the promoter regions of a multitude genes<sup>67</sup>. This alteration, which is referred to  
195 as site-specific hypermethylation has significant implications for health<sup>68</sup>. For example, cancers  
196 regularly display global hypomethylation and concomitant gene specific hypermethylation<sup>69</sup>, while it  
197 has also been observed that autoimmune diseases<sup>70</sup> and CVD<sup>71</sup> also manifest this phenomenon.

198

199 We derive methyl groups from the B vitamin folate in our diet<sup>72</sup>, however deficiencies in the intake of  
200 this vitamin or other B vitamins can disrupt the methylation process. However, it has been recently  
201 acknowledged that intrinsic aging is also a contributing factor to age-related aberrant DNA  
202 methylation<sup>73</sup>. It has been found that with age changes occur to the activity of the enzymes that  
203 dynamically regulate DNA methylation patterns<sup>74</sup>. Of these enzymes, DNA methyltransferase 1  
204 (Dnmt1) is primarily responsible for maintaining genomic DNA methylation<sup>75</sup>. DNA methylation  
205 events are counterbalanced by active and passive demethylation<sup>76</sup>. Passive demethylation occurs  
206 during replication, while active methylation involves ten eleven translocation (TET) dioxygenases,  
207 which oxidize the methyl groups of cytosine and appear central to demethylation<sup>77</sup>. Intriguingly, the  
208 activity of the TET demethylation enzymes is dependent on fluctuations in  $\alpha$ -ketoglutarate an  
209 important intermediate in the Krebs cycle<sup>78</sup>. Moreover, several enzymes involved in the Krebs cycle  
210 including, isocitrate dehydrogenase, fumarate hydratase and succinate dehydrogenase (SDH) are  
211 also known to modulate TET enzymes<sup>78</sup>. Adding further intrigue to the connection between  
212 methylation and metabolism, recent experimental evidence has shown that Dnmt1 activity is  
213 elevated in response to caloric (CR) in human fibroblast cell lines<sup>79</sup>. Importantly, it has also been  
214 suggested that the response of Dnmt1 to CR is mediated by SIRT1, which has been shown to  
215 modulate the activity of this key methylation enzyme<sup>80</sup>. Finally, there is also experimental evidence  
216 that age related changes to the DNA methylation landscape are at least in part impacted by  
217 increases in oxidative stress<sup>81</sup>. **For example, it has been shown that DNA lesions, caused by oxidative  
218 stress, can disrupt the ability of DNA to function as a substrate for the DNMT1<sup>82</sup>.** Taken together,  
219 these findings suggest that both ROS emissions by the mitochondria and mitochondrial metabolism  
220 could be key players that mediate how DNA methylation changes unfold with age.

221

## 222 **Reasons for Adopting Mechanistic Computational Modelling for Aging Research**

223 From our discussion of the aging process, it is apparent that it is an inherently complex process.  
224 Traditionally, aging has been investigated like many other aspects of biology in a reductionist  
225 manner. However, investigating aging cannot be viewed as just one single aspect of biology. Thus, it  
226 is important to acknowledge and appreciate the biological uniqueness of aging and that aging needs  
227 to be studied in a holistic manner. Fortunately, there is an increasing appreciation in recent years  
228 that biological systems need to be studied within integrated frameworks, and that viewing complex  
229 biological systems through a reductionist lens is no longer an adequate experimental paradigm<sup>83</sup>.  
230 The aim of systems biology is to provide an integrated understanding of biological processes from  
231 the molecular through to the physiological<sup>84</sup>. Computational modelling is an ideal means of  
232 facilitating this paradigm shift and they are now increasingly used alongside more conventional  
233 biological approaches. The contributions such models can make to the understanding of aging are  
234 clear. 1) Computational models can represent the intrinsic complexity associated with aging. 2)  
235 Modelling can improve our understanding of the biology underpinning aging and help to generate  
236 new insights. 3) It can highlight gaps in current knowledge. 4) A model can help to develop clear,  
237 testable predictions about aging that are not always possible to do using conventional means. 5) A  
238 model may lead to counterintuitive explanations and unusual predictions about aging that would  
239 otherwise be unapparent if the system was not studied in an integrated manner. 6) Models can  
240 provide a quick way to analyse a biological system under a wide range of conditions, for example by  
241 examining the effects of an array of dietary components. 7) There are many conflicting ideas about  
242 aging and models can be used to test a particular hypothesis which may lead to counterintuitive  
243 explanations.

244

## 245 **APPROACHES TO MODELING AGING**

246 In order to appreciate what computational modeling is, and how it is used in systems biology, it is  
247 firstly necessary to give an overview of what it is. Computational modeling is an abstract process  
248 which uses mathematics to dynamically represent the components of a biological system and their  
249 interactions within a mathematical framework. *A key aspect of this techniques is that it allows the*  
250 *simulation of a system's dynamic behaviour. At the heart of computational modeling is mathematics,*  
251 *and there are a number of theoretical frameworks that can be used to construct a computational*  
252 *systems model<sup>85</sup>. The approach that is adopted is largely dependent on the nature of the system*  
253 *that is to be modelled<sup>86</sup>. Recently, Petri nets have been used to model a variety of process in*  
254 *biology<sup>87</sup>. These are a directed bipartite graph, with two types of nodes, called places and*  
255 *transitions, which are represented diagrammatically by circles and rectangles, respectively. Places*  
256 *and transitions are connected via arrows/arcs. Each circle or place contains a number of tokens,*  
257 *which is a kin to a discrete number of biochemical molecules, while the stoichiometry is indicated by*  
258 *the weight above the arrow/arc. Tokens can be both consumed and produced within the Petri net. A*  
259 *Petri net functions by input-output firing at the transitions within the network. The 'firing' of*  
260 *transitions is a kin to a biochemical reaction taking place. Biological systems can also be represented*  
261 *with a Bayesian network (BN)<sup>88</sup>. BNs are a type of probabilistic network graph, where each node*  
262 *within the graph represents a variable. Nodes can be discrete or continuous and are connected to a*  
263 *probability density function, which is dependent on the values of the inputs to the nodes. Agent-*



264 based models have been increasingly used in aging research also<sup>89</sup>. This is a rule-based approach  
265 which is used to investigate biological systems using clusters of independent agents whose  
266 behaviour is underpinned by simple rules. These agents are capable of interacting with one another  
267 through space and time. However, by far the most commonly adopted theoretical approach to  
268 modelling in systems biology is a deterministic framework. However, more recent developments  
269 have witnessed the adoption of stochastic modelling. In the next sections we will introduce these  
270 two important approaches and will highlight some examples that have been used in recent aging  
271 research.

272

### 273 **Deterministic models versus Stochastic Models**

274 **Deterministic models can be represented mathematically** by ordinary differential equations (ODEs).  
275 ODEs are known as ordinary because they depend on one independent variable (time), and use the  
276 assumption that biological species exist in a well-mixed compartment, where concentrations can be  
277 viewed as continuous. These systems can be defined as follows

$$278 \frac{dx}{dt} = f_x(x, y \dots \dots t)$$

279

$$280 \frac{dy}{dt} = f_y(x, y \dots \dots t)$$

281

282 x, and y are referred to as state variables, for example these could be the concentration of ROS in a  
283 cell, the length of a telomere or the concentration of mTORC1. Species concentration is generally  
284 denoted by the state variable enclosed within a square bracket. In the equations  $f_x$ ,  $f_y$ , are the  
285 functions describing the molecular interactions. Systems of ODEs that are used to represent  
286 biological processes are generally too complex to solve analytically. Therefore, numerical integration  
287 is used to simulate their behaviour using a computer. Computational systems biology software tools  
288 come equipped with algorithms for doing this, which helps to facilitate the modeling process for  
289 those less familiar with mathematics<sup>90</sup>.

290 Continuous deterministic ODEs are based on the assumption that large numbers of molecules are  
291 involved in biological reactions and that the random interactions between these molecules has a  
292 negligible impact on the behaviour of the system. This makes continuous deterministic models  
293 unsuitable for representing process which are governed by stochasticity or randomness within cells.  
294 The main sources of stochastic variability at the cellular level are fluctuations in biochemical  
295 reactions, which drive a number of processes including gene expression, transduction signalling, and  
296 biochemical pathway signalling<sup>91</sup>. These reactions occur through random collisions and transient  
297 binding of various molecular species within the cell. This makes these reactions prone to significant  
298 noise. In order to deal with this noise stochastic reaction models attempt to represent the discrete  
299 random collisions between individual molecules. These type of models treat molecule interactions as  
300 random events. A stochastic model is usually underpinned by a propensity function, known as the



301 Gillespie equation<sup>92</sup>. This equation explicitly gives the probability  $a_{\mu}$  of a reaction  $\mu$  occurring in time  
302 interval  $(t, t + dt)$ .

303

$$a_{\mu} dt = h_{\mu} c_{\mu} dt$$

304

305 The  $M$  reactions in the system are given an index value of  $\mu$  ( $1 \leq \mu \leq M$ ) and  $h_{\mu}$  implies the number of  
306 possible combinations of reactant molecules involved in reaction  $\mu$ . In essence each reaction within  
307 the system has a different probability of occurring. In practice the Gillespie algorithm or one of its  
308 variants<sup>92-94</sup> is embedded within a computational modelling tool. Therefore, it is only necessary for  
309 the user to have a reasonable understanding of underlying theory of the Gillespie algorithm in order  
310 to build a stochastic model of a biological system. At this point it is important to acknowledge that in  
311 addition to these approaches, there are a number of other theoretical frameworks that can be  
312 employed to model biological systems. These include Petri nets, which are a graphical tool for the  
313 description and analysis of concurrent processes<sup>95</sup>, Bayesian networks, which are probabilistic  
314 graphical models<sup>96</sup>, Boolean networks in which entities are either in an on or off state<sup>97</sup>, systems of  
315 partial differential equations (PDEs), which are multivariable functions that deal with partial  
316 derivatives<sup>98</sup> and agent based modelling, which is a rule-based, discrete-event and discrete-time  
317 approach that uses objects and rules to simulate interactions among the individual components of  
318 the model<sup>99</sup>.

319

## 320 **Modelling Tools and Model Exchange**

321 A variety of software tools are available for building models and the choice of software tool is  
322 dependent on the level of experience of the individual assembling the model. Certain tools are more  
323 suitable than others for novice model builders. ODEs can be coded manually by using a commercial  
324 software tool such as Matlab or Mathematica. Non-commercial software tools such as Copasi<sup>100</sup> or  
325 CellDesigner<sup>101</sup>, which have graphical user interfaces, allow the user to build the model by creating a  
326 succession of kinetic reactions/a process diagram, which in the in the case of a deterministic model  
327 is then converted to a series of coupled ODEs. As discussed in the previous section the software tool  
328 then uses an algorithm to solve the ODEs and produce a deterministic output. Once a computational  
329 model has been assembled, it is important that it can be both easily accessed and updated by the  
330 community as a whole. To facilitate model portability a number of exchange frameworks have been  
331 developed<sup>102</sup>. These frameworks allow models to be shared and reused by researchers even if they  
332 do not use the same modeling software tool. At present, the leading exchange format is the systems  
333 biology markup language (SBML)<sup>103</sup>. This framework is supported by a broad range of modelling  
334 software tools ([http://sbml.org/SBML Software Guide/SBML Software Summary](http://sbml.org/SBML_Software_Guide/SBML_Software_Summary)). Models that  
335 have been encoded in this format can be archived in the BioModels database, a repository designed  
336 specifically for archiving models of biological systems<sup>104</sup>.

337

338

339

## 340 **Computational Models of Mitochondrial Dynamics**

341 As outlined, oxidative stress and the emission of ROS by mitochondria is one of the fundamental  
342 cellular processes that impacts aging. Therefore, it is unsurprising that various aspects of  
343 mitochondrial dynamics have been modelled over the years (for a comprehensive review see Kowald  
344 and Klipp (2014)<sup>16</sup>). An early network model of mitochondrial dynamics that examined this was  
345 developed by Kowald and Kirkwood (1994). This model showed that during increased free radical  
346 production and/or inadequate protection from these free radicals, damage can occur to an  
347 otherwise stable translation system<sup>105</sup>. Another area of keen focus is mitochondrial fission and  
348 fusion. Briefly, fission and fusion events can be viewed as mitochondrial caretakers whose  
349 responsibility it is to control cellular ATP concentration, and to mitigate against the accumulation of  
350 damage to mitochondrial DNA (mtDNA). One of the earliest models that focused on these processes  
351 was the model developed by Kowald et al. (2005). In this model stochastic simulation of  
352 mitochondrial replication, mutation and degradation showed a low mosaic pattern of oxidative  
353 phosphorylation (OXPHOS) impaired cells in old organisms<sup>106</sup>. More recently, Tam and colleagues  
354 (2013) used computational modelling to investigate the effects of mitochondrial fusion and fission  
355 dynamics on mutant mtDNA accumulation<sup>107</sup>. In this stochastic model, simulations indicated that the  
356 slowing down of mitochondrial fusion-fission results in higher variability in the mtDNA mutation  
357 burden among cells over time, and mtDNA mutations have a higher propensity to clonally expand  
358 due to an increase in stochasticity. The model was able to suggest that the protective ability of  
359 retrograde signalling (biochemical communication between mitochondria and nucleus) depends on  
360 the efficiency of fusion-fission process<sup>107</sup>. Another model which focuses on fusion-fission cycles is the  
361 model by Figge and colleagues (2012). This probabilistic model demonstrated that cycles of fusion and  
362 fission and mitophagy are needed to maintain a high average quality of mitochondria, even under  
363 conditions in which random molecular damage is present<sup>108</sup>. Recent mitochondrial models have also  
364 focused on specific regions within the mitochondrial ETC. For instance, a model of superoxide  
365 production at complexes I and III of the ETC, was able to generate an improved mechanistic  
366 understanding of how ROS are generated by complex III. This model also described ROS production  
367 by antimycin A inhibited complex III. In order to validate the model, output from its simulations was  
368 matched to experimental data from rodents<sup>109</sup>. On a similar theme Markevich and Hoek (2015) used  
369 a computational model of mitochondrial bioenergetics to monitor superoxide production under  
370 different substrate conditions. Their model suggested that the semiquinone of Complex I should be  
371 included as an additional source of ROS<sup>110</sup>.

372

373

374

375

376

377

378

## 379 **Telomere Models**

380 A number of models have explored telomere dynamics. Most recently, Bartholomäus and colleagues  
381 (2014) used a computational model to investigate telomere length under a variety of  
382 perturbations<sup>111</sup>. The model was used to explore telomeres during different conformational states,  
383 specifically t-loops, G-quadruplex structures and those being elongated by telomerase. This  
384 deterministic model was used to examine how different levels of telomerase impacted telomere  
385 length. Moreover, the authors used the model to explore the impact of adding different levels of a  
386 G4-stabilising drug on the distribution of telomere lengths. Several older models can be found in the  
387 literature. Others of note include the model by Rodriguez-Brenes and Peskin (2010) who modelled  
388 telomere state on the basis of the biophysics of t-loop formation<sup>112</sup>. The model was able to predict  
389 the steady-state length distribution for telomerase positive cells, the time evolution of telomere  
390 length, and the life span of a cell line on the basis of the levels of telomeric repeat-binding factor 2; a  
391 protein that protects telomeres from end-to-end fusion of chromosomes. The model was also able  
392 to predict the life span of a cell line based on telomerase levels. Stochastic models of telomere  
393 dynamics include the model by Portugal et al. (2008) which made the assumption that cell division is  
394 a stochastic phenomenon whose probability decreases linearly with telomere shortening<sup>113</sup>. Proctor  
395 and Kirkwood (2003) also used a model informed by probability to model cellular senescence as a  
396 result of telomere state<sup>17</sup>. From an oxidative stress perspective Trusina (2014) recently used a  
397 computational model to investigate the effect of genotoxic stress on telomere attrition<sup>114</sup>. Virtual  
398 populations of cells were compared and it was found that when ROS was distributed unequally  
399 among cells, telomere shortening increased longevity, while also reducing the DNA mutation rate.

400

## 401 **Computational Modelling of Metabolic Signalling**

402 In the first section of this paper we described the increasing attention there has been on certain  
403 metabolic pathways and how they may have a significant role to play in longevity. Most notably we  
404 identified those metabolic pathways that are defined by mTOR and by sirtuins. Several attempts  
405 have been made to computationally model various aspects of these pathways<sup>115</sup>. For example, Kriete  
406 et al. (2010) developed a computational model that included the mTOR pathway together with other  
407 pathways associated with intrinsic aging<sup>116</sup>. This rule based model is of note as it encapsulated many  
408 important aspects of aging, including mitochondrial biosynthesis, metabolic fluxes, mTOR as an  
409 energy sensor and NF-κB, to detect oxidative stress. Another model which successfully included  
410 oxidative stress is that developed by Smith and Shanley (2013)<sup>117</sup>. By building a model of insulin  
411 signalling in rodent adipocytes that included transcriptional feedback through the Forkhead box type  
412 O (FOXO) transcription factor, it was demonstrated that oxidative stress can have a significant effect  
413 on insulin signalling and aging. The model produced a range of findings including the combination of  
414 insulin and oxidative stress produced a lower degree of activation of insulin signalling than insulin  
415 alone. Antioxidant defences were upregulated in the presence of fasting and weak oxidative stress,  
416 whereas, stronger oxidative stress caused short term activation of insulin signalling. The model also  
417 demonstrated that if prolonged high insulin may negate the protective effects of moderate oxidative  
418 stress. The complex nature of this model is evident, but, combining it with other factors that can  
419 influence insulin signalling such as the mTOR pathway could add to our understanding of insulin  
420 signalling.

## 421 **Computational Models of DNA Methylation Dynamics and Aging**

422 In spite of increasing age related experimental data there is a paucity of computational models that  
423 have focused specifically on intrinsic aging and DNA methylation dynamics. However, methylation  
424 dynamics have been represented computationally within a number of disease states. For instance,  
425 Mc Govern et al. (2012) developed a dynamic multi-compartmental model of DNA methylation,  
426 which was used as a predictive tool for hematological malignancies<sup>118</sup>. The model centred on the  
427 activity of DNMTs. PDEs were used to represent methylation reactions and the model was able to  
428 predict the relative abundances of unmethylated, hemimethylated, fully methylated, and  
429 hydroxymethylated CpG dyads in the DNA of cells with fully functional Dnmt and Tet proteins. It  
430 would be worthwhile adapting this model to include oxidative stress, folate biochemistry and the  
431 effects of aging on the activity of the methylation enzymes. This model is also deterministic in  
432 nature. However, it has been recognised that DNA methylation dynamics are susceptible to inherent  
433 stochasticity<sup>119</sup>. Consequently a number of theoretical frameworks have been proposed for modeling  
434 the noise associated with DNA methylation dynamics. For example, reduced mathematical  
435 representations of methylation dynamics have been proposed by Riggs and Xiong (2004)<sup>120</sup> and  
436 more recently by Jeltsch and Jurkowska (2014), in which DNA methylation at each genomic site is  
437 determined by the activity of Dnmts, demethylation enzymes, and the DNA replication rate<sup>121</sup>. An  
438 awareness of the stochastic nature of these mechanisms has important implications for the aging  
439 process, as experimental evidence indicates that the persistent nature of the human methylome  
440 results give rise to this noise<sup>122</sup>. Accordingly, it is imperative that computational models which seek  
441 to represent the dynamics of DNA methylation need to account for this inherent variability. **One**  
442 **such recent model that has dealt with the intrinsic stochasticity associated with DNA methylation is**  
443 **the model developed by Przybilla et al. (2014), which simulated age-related changes of DNA**  
444 **methylation in stem cells. The findings of this model, which compared age-related changes of**  
445 **regulatory states in quiescent stem cells, with those observed in proliferating cells, suggest that**  
446 **epigenetic aging strongly affects stem cell heterogeneity and that homing at stem cell niches**  
447 **retarded epigenetic aging<sup>123</sup>.**

448

## 449 **Cholesterol Metabolism and Aging**

450 **The aging process results in the gradual decline of a biological system. This decline is associated with**  
451 **a broad range of pathological states. An example of this decline is the dysregulation of cholesterol**  
452 **metabolism which is inextricably linked to CVD. Therefore, a keen area of focus is how intrinsic aging**  
453 **impacts whole-body cholesterol metabolism<sup>124-127</sup>.** Recently we developed a whole-body model that  
454 attempted to capture whole-body cholesterol metabolism. The model was used to examine how age  
455 related mechanistic changes to the intestinal absorption of cholesterol resulted in a rise in low-  
456 density lipoprotein cholesterol (LDL-C), as increased levels are a risk factor for CVD. The model also  
457 revealed that an age related decrease in the hepatic clearance of LDL-C resulted in significant rise in  
458 LDL-C by 65 years of age. This model is coded in SBML and is archived in the BioModels database  
459 (<http://www.ebi.ac.uk/biomodels-main/BIOMD0000000434>). In theory this model should be  
460 straightforward to update and expand to include other important aspects of aging. As we have  
461 eluded to, the free radical theory of aging is a useful means of gluing together disparate aspects of  
462 the aging process. It is therefore possible to extend this model by framing it around the insidious rise

463 in ROS that occurs with age in endothelial, vascular smooth muscle, and adventitial cells. This rise in  
464 ROS is suggested to be the key driver in a signalling cascade that results in atherosclerosis.  
465 Atherosclerosis occurs when LDL molecules migrate into the artery wall at a site which is  
466 undermined by endothelial damage. The LDLs are then oxidised upon coming into contact with ROS.  
467 The oxidatively modified lipoproteins (oxLDL) are more atherogenic than the native LDL and lead to  
468 the recruitment of the macrophages to the site of the lesion. Monocytes pass into the intima before  
469 differentiating into macrophages. These molecules engulf oxidized LDL to form cholesterol-laden  
470 foam cells. This ultimately results in the formation of an atherosclerotic plaque which eventually  
471 ruptures and causes an artery to block<sup>128</sup> (Figure 2). This can lead to a stroke or myocardial  
472 infarction<sup>129</sup>. Computational modeling offers a way of dealing with the different molecular, cellular  
473 and hemodynamic events associated with this process.

474

### 475 **Brain Aging and Pathology**

476 Recently, we also created a computational model which incorporated key brain regions that  
477 characterise AD and combined these with the homeostatic regulation of the stress hormone  
478 cortisol<sup>130</sup>. The aim of this model was to examine how increased levels of cortisol impinge on the  
479 integrity of the hippocampal region of the brain, which is the core pathological substrate for AD. The  
480 model was able to replicate the *in vivo* aging of the hippocampus. Moreover, both acute and chronic  
481 elevations in cortisol increased aging-associated hippocampal atrophy and concomitant loss in the  
482 activity of the hippocampus. This computational systems model could be updated to include a  
483 number of other processes. For instance, cortisol is synthesised from cholesterol and also acts is also  
484 involved in provoking the breakdown of lipids, and a wide variety of other metabolites<sup>131</sup>. Therefore,  
485 the model could be integrated with the cholesterol model discussed previously. Moreover, this  
486 model could be used as a framework for investigating vascular dementia (VAD). VAD is underpinned  
487 by a dysregulation in the supply of O<sub>2</sub> following a stroke or small vessel deterioration, and oxidative  
488 stress is central to the processes that underpin the progression of VAD<sup>132</sup>. Oxygen deprivation results  
489 in mitochondrial dysregulation and the release of ROS<sup>133</sup>. This increase in oxidative stress damages  
490 blood vessels and neurons, resulting in a process which has been termed neurovascular  
491 uncoupling<sup>134, 135</sup>. Moreover, this burst of ROS can disrupt mitochondrial function and further induce  
492 hypoxia and oxidative stress<sup>136</sup>.

493 A recent ODE model explored a number of the cellular processes associated with Parkinsons Disease  
494 (PD). Among the many cellular features of this model, the feedback interactions between damaged  
495  $\alpha$ -synuclein and ROS<sup>137</sup> were explored. Simulation results showed, hat the Parkinsonian condition,  
496 with elevated oxidative stress and misfolded  $\alpha$ -synuclein accumulation, can be induced in the model  
497 by intrinsic aging, together with exposure to toxins and genetic defects. Computational approaches  
498 could also be used to investigate other key aspects of brain aging. For instance, many individuals  
499 with Parkinson's disease report problems with their respiratory, cardiovascular, and gastrointestinal  
500 systems<sup>138</sup>. There is also ample evidence of increased neuroinflammation in Parkinsons individuals,  
501 due to oxidative stress, with reports of increased levels of cytokines, macrophages and microglia  
502 activation in brain tissues<sup>139,140</sup>. A computational model could thus consider abnormalities in central  
503 autonomic nuclei, as to our knowledge, there have been no studies to determine whether  
504 abnormalities in central autonomic nuclei contributes to autonomic dysfunction or whether

505 peripheral autonomic nuclei also show perturbed development and increased inflammation in PD.  
506 Autonomic dysfunction could be reflective of systemic autonomic pathology in PD, and that in fact  
507 PD is, in part, an autonomic disorder. It is therefore logical that integrated approaches are required  
508 to disentangle the pathological onset of this disease. A worthwhile approach that could address  
509 these questions would be to construct a computational systems model of these key processes. In  
510 Figure 3, we have used Systems Biology Graphical Notation (SBGN) to represent these processes,  
511 which could be modelled computationally.

512

### 513 **Other recent Models that have focused on Integrating Aspects of Aging**

514 To date, no model has been able to represent aging in its entirety. However, there have been a  
515 number of recent examples, whereby various components associated with aging have been  
516 integrated together within a mathematical framework, in an attempt to complete a more global  
517 view of how aging impacts a particular biological system. For example, Xue and colleagues (2007)  
518 demonstrated that aging is associated with the alteration of a few key brain network modules  
519 instead of many, and that the aging process preferentially affects regulatory nodes involved in  
520 network stability<sup>141</sup>. Multi-level aging based models have also been used to gain an insight into  
521 intracellular protein aggregate damage, during aging in *Escherichia coli*<sup>142</sup>. Moreover, multi-scale  
522 models have also had a mammalian focus, for example to examine collagen turnover and the  
523 adaptive nature of arterial tissue, in response to mechanical and chemical stimuli<sup>143</sup>. Furthermore,  
524 this type of modelling has also been utilised to examine disease pathophysiology, such as the muscle  
525 fibre arrangement and damage susceptibility in Duchenne muscular dystrophy<sup>144</sup>.

526

527

## 528 **FUTURE OPPORTUNITIES AND CHALLENGES**

529 As outlined, the intrinsic biological mechanisms which characterise the aging process are complex  
530 and their activities transcend scale and time. In addition, they involve the interplay of a broad range  
531 of molecular, biochemical and physiological processes. In the main, computational models have  
532 focused on these process at a cellular level. However, these models are not an adequate  
533 representation of whole body human aging. In the final section, we will explore the challenges and  
534 opportunities for the future integration of mechanistic models associated with the aging process.

535

### 536 **Embedding Existing Models into a Multi-Scale Holistic Framework of Aging**

537 A long term goal of aging research is to have whole-body mechanistic models of the aging process. It  
538 is important to note that there are currently no models of this nature in existence. However, in order  
539 to fully computationally represent aging from cell to tissue level, there are a number of outstanding  
540 challenges that remain. Rather than reinventing the wheel it is worth considering extending existing  
541 models. In this final section we will outline some of the challenges that exist in combining models  
542 and will propose a number of potential solutions. It is important to recognise that a number of these

543 biological systems need to be further characterised before they can be successfully represented by a  
544 computational model. A solution to this problem could be to firstly work on aspects of the aging  
545 process **that are reasonably well characterised**, so that future models are founded upon well  
546 characterised biological mechanisms. Moreover, it is important that model building is coupled  
547 closely with wet-laboratory experimentation. Systems biology experiments that are designed with  
548 existing *in silico* models firmly embedded within their methodology would significantly improve both  
549 the model and extend our understanding of the underlying biology. Another significant issue relates  
550 to representing biological systems at different levels of scale. It is common place to represent  
551 biological systems using models which consist of a system of ODEs that can be analysed, whose  
552 dynamics can be solved using a computer. This deterministic approach neglects those reactions that  
553 occur at a much smaller scale and involve fluctuations in low molecular populations. Implementing  
554 models which combine both the deterministic and stochastic features of biological systems is  
555 challenging. However, recently there have been some examples of computational models that have  
556 succeeded in accounting for both these effects. For example, Singhanian (2011)<sup>145</sup> used a hybrid  
557 approach that combined differential equations and discrete Boolean networks to represent  
558 mammalian cell cycle regulation. This is particularly important from the perspective of the aging  
559 process as in order to truly represent it requires the integration of a variety of processes which  
560 traverse different biological and temporal scales. Assembling holistic models which represent the  
561 aging process is also hindered by the need to determine realistic values for the many parameters  
562 that are the essence of large complex models of biological systems. Due to the nature of the  
563 experiments it can be difficult to estimate these parameters from existing experimental data. It is  
564 important to recognise however that this is a persistent problem within systems biology generally.  
565 Thus, as previously eluded to it is necessary to align computational modelling within any future  
566 experimental methodology. In addition a broad range of statistical techniques have been applied to  
567 this area recently. For instance, Aitken et al. (2015) embedded an algorithm based on Bayesian  
568 inference within the computational systems biology software tool Dizzy<sup>146, 147</sup>. There are several  
569 other approaches in which statistical techniques can be used to estimate unknown parameters in  
570 systems biology<sup>148</sup>. Continuing developments in this area will no doubt increase in the utility of  
571 computational systems models, and this will be of benefit to those models which represent aging.

572

573

## 574 **Conclusion**

575 In this paper we have presented a broad overview of some of the processes associated with the  
576 biology of aging. We have also introduced a number of approaches that are currently used to  
577 computationally model biological systems and have described in detail a number of models that  
578 have been developed to represent a wide variety of discrete components of the aging process.  
579 Some of these models include the key role of ROS in the aging process, while others do not. From  
580 our perspective, it is hoped that by converging around ROS in coming years we will witness a more  
581 comprehensive view of aging that encapsulates the various different mechanisms and their  
582 interactions, whose dysregulation result in age associated disease.

583



584

585

586 **References**

587

- 588 1. WHO. World Population Ageing 2013. *Department of Economic and Social Affairs Population*  
589 *Division* 2013.
- 590 2. Yancik R, Ries LA. Aging and cancer in America. Demographic and epidemiologic  
591 perspectives. *Hematol Oncol Clin North Am* 2000, 14:17-23.
- 592 3. Jousilahti P, Vartiainen E, Tuomilehto J, Puska P. Sex, age, cardiovascular risk factors, and  
593 coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and  
594 women in Finland. *Circulation* 1999, 99:1165-1172.
- 595 4. Lindsay J, Laurin D, Verreault R, Hebert R, Helliwell B, Hill GB, McDowell I. Risk factors for  
596 Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging. *Am*  
597 *J Epidemiol* 2002, 156:445-453.
- 598 5. Reeve A, Simcox E, Turnbull D. Ageing and Parkinson's disease: why is advancing age the  
599 biggest risk factor? *Ageing Res Rev* 2014, 14:19-30.
- 600 6. Kelly GA, Lazarus J. Perceptions of Successful Aging: Intergenerational Voices Value Well-  
601 Being. *Int J Aging Hum Dev* 2015.
- 602 7. Chauhan A, Liebal UW, Vera J, Baltrusch S, Junghanss C, Tiedge M, Fuellen G, Wolkenhauer  
603 O, Kohling R. Systems biology approaches in aging research. *Interdiscip Top Gerontol* 2015,  
604 40:155-176.
- 605 8. Auffray C, Imbeaud S, Roux-Rouquie M, Hood L. From functional genomics to systems  
606 biology: concepts and practices. *C R Biol* 2003, 326:879-892.
- 607 9. Hou L, Huang J, Green CD, Boyd-Kirkup J, Zhang W, Yu X, Gong W, Zhou B, Han JD. Systems  
608 biology in aging: linking the old and the young. *Curr Genomics* 2012, 13:558-565.
- 609 10. Choi H, Mc Auley MT, Lawrence DA. Prenatal exposures and exposomics of asthma. . *AIMS*  
610 *Environmental Science* 2015, 2:87-109.
- 611 11. Kriete A, Lechner M, Clearfield D, Bohmann D. Computational systems biology of aging.  
612 *Wiley Interdiscip Rev Syst Biol Med* 2011, 3:414-428.
- 613 12. Kilner J, Corfe BM, McAuley MT, Wilkinson SJ. A deterministic oscillatory model of  
614 microtubule growth and shrinkage for differential actions of short chain fatty acids. *Mol*  
615 *Biosyst* 2015.
- 616 13. Brodland GW. How computational models can help unlock biological systems. *Semin Cell Dev*  
617 *Biol* 2015.
- 618 14. Mc Auley MT, Choi H, Mooney K, Paul E, Miller VM. Systems Biology and Synthetic Biology: A  
619 New Epoch for Toxicology Research. *Advances in Toxicology* 2015, 2015:14.
- 620 15. Mc Auley MT, Mooney KM. Computational systems biology for aging research. *Interdiscip*  
621 *Top Gerontol* 2015, 40:35-48.
- 622 16. Kowald A, Klipp E. Mathematical models of mitochondrial aging and dynamics. *Prog Mol Biol*  
623 *Transl Sci* 2014, 127:63-92.
- 624 17. Proctor CJ, Kirkwood TB. Modelling cellular senescence as a result of telomere state. *Ageing*  
625 *Cell* 2003, 2:151-157.
- 626 18. Proctor CJ, Soti C, Boys RJ, Gillespie CS, Shanley DP, Wilkinson DJ, Kirkwood TB. Modelling  
627 the actions of chaperones and their role in ageing. *Mech Ageing Dev* 2005, 126:119-131.
- 628 19. Gavrilov LA, Gavrilova NS. Evolutionary theories of aging and longevity.  
629 *ScientificWorldJournal* 2002, 2:339-356.
- 630 20. Sanz A, Stefanatos RK. The mitochondrial free radical theory of aging: a critical view. *Curr*  
631 *Aging Sci* 2008, 1:10-21.

- 632 21. Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc* 1972, 20:145-147.
- 633 22. Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, Jaros E, Hersheson JS,  
634 Betts J, Klopstock T, et al. High levels of mitochondrial DNA deletions in substantia nigra  
635 neurons in aging and Parkinson disease. *Nat Genet* 2006, 38:515-517.
- 636 23. Yu-Wai-Man P, Lai-Cheong J, Borthwick GM, He L, Taylor GA, Greaves LC, Taylor RW, Griffiths  
637 PG, Turnbull DM. Somatic mitochondrial DNA deletions accumulate to high levels in aging  
638 human extraocular muscles. *Invest Ophthalmol Vis Sci* 2010, 51:3347-3353.
- 639 24. Trifunovic A, Larsson NG. Mitochondrial dysfunction as a cause of ageing. *J Intern Med* 2008,  
640 263:167-178.
- 641 25. Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. *Science* 2012, 337:1062-  
642 1065.
- 643 26. Jensen MB, Jasper H. Mitochondrial proteostasis in the control of aging and longevity. *Cell*  
644 *Metab* 2014, 20:214-225.
- 645 27. Kim I, Rodriguez-Enriquez S, Lemasters JJ. Selective degradation of mitochondria by  
646 mitophagy. *Arch Biochem Biophys* 2007, 462:245-253.
- 647 28. Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS. Decline  
648 in skeletal muscle mitochondrial function with aging in humans. *Proc Natl Acad Sci U S A*  
649 2005, 102:5618-5623.
- 650 29. Barja G. Aging in vertebrates, and the effect of caloric restriction: a mitochondrial free  
651 radical production-DNA damage mechanism? *Biol Rev Camb Philos Soc* 2004, 79:235-251.
- 652 30. Ristow M, Schmeisser K. Mitohormesis: Promoting Health and Lifespan by Increased Levels  
653 of Reactive Oxygen Species (ROS). *Dose-Response* 2014, 12:288-341.
- 654 31. Aubert G, Lansdorp PM. Telomeres and aging. *Physiol Rev* 2008, 88:557-579.
- 655 32. Collins K, Mitchell JR. Telomerase in the human organism. *Oncogene* 2002, 21:564-579.
- 656 33. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts.  
657 *Nature* 1990, 345:458-460.
- 658 34. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW,  
659 Lichtsteiner S, Wright WE. Extension of life-span by introduction of telomerase into normal  
660 human cells. *Science* 1998, 279:349-352.
- 661 35. Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells  
662 during ageing. *Nature* 2010, 464:520-528.
- 663 36. Weindruch R, Walford R. The Retardation of Aging and Disease by Dietary Restriction.  
664 *Charles C Thomas, Springfield, Illinois* 1988.
- 665 37. Masoro EJ. Overview of caloric restriction and ageing. *Mech Ageing Dev* 2005, 126:913-922.
- 666 38. Sinclair DA. Toward a unified theory of caloric restriction and longevity regulation. *Mech*  
667 *Ageing Dev* 2005, 126:987-1002.
- 668 39. Anderson RM, Shanmuganayagam D, Weindruch R. Caloric restriction and aging: studies in  
669 mice and monkeys. *Toxicol Pathol* 2009, 37:47-51.
- 670 40. Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, Anderson RM. Caloric  
671 restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat Commun*  
672 2014, 5:3557.
- 673 41. Lin SJ, Ford E, Haigis M, Liszt G, Guarente L. Calorie restriction extends yeast life span by  
674 lowering the level of NADH. *Genes Dev* 2004, 18:12-16.
- 675 42. Gredilla R, Sanz A, Lopez-Torres M, Barja G. Caloric restriction decreases mitochondrial free  
676 radical generation at complex I and lowers oxidative damage to mitochondrial DNA in the rat  
677 heart. *Faseb j* 2001, 15:1589-1591.
- 678 43. Agarwal S, Sohal RS. Relationship between susceptibility to protein oxidation, aging, and  
679 maximum life span potential of different species. *Exp Gerontol* 1996, 31:365-372.
- 680 44. Guarente L. Calorie restriction and sirtuins revisited. *Genes & Development* 2013, 27:2072-  
681 2085.

- 682 45. Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by  
683 calorie restriction in *Saccharomyces cerevisiae*. *Science* 2000, 289:2126-2128.
- 684 46. Rogina B, Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie  
685 restriction. *Proc Natl Acad Sci U S A* 2004, 101:15998-16003.
- 686 47. Tissenbaum HA, Guarente L. Increased dosage of a sir-2 gene extends lifespan in  
687 *Caenorhabditis elegans*. *Nature* 2001, 410:227-230.
- 688 48. Haigis MC, Guarente LP. Mammalian sirtuins--emerging roles in physiology, aging, and  
689 calorie restriction. *Genes Dev* 2006, 20:2913-2921.
- 690 49. Canto C, Auwerx J. Caloric restriction, SIRT1 and longevity. *Trends Endocrinol Metab* 2009,  
691 20:325-331.
- 692 50. Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de  
693 Cabo R, Sinclair DA. Calorie restriction promotes mammalian cell survival by inducing the  
694 SIRT1 deacetylase. *Science* 2004, 305:390-392.
- 695 51. Xia N, Strand S, Schlufte F, Siuda D, Reifenberg G, Kleinert H, Forstermann U, Li H. Role of  
696 SIRT1 and FOXO factors in eNOS transcriptional activation by resveratrol. *Nitric Oxide* 2013,  
697 32:29-35.
- 698 52. Shinmura K, Tamaki K, Ito K, Yan X, Yamamoto T, Katsumata Y, Matsushashi T, Sano M,  
699 Fukuda K, Suematsu M, et al. Indispensable role of endothelial nitric oxide synthase in  
700 caloric restriction-induced cardioprotection against ischemia-reperfusion injury. *Am J Physiol*  
701 *Heart Circ Physiol* 2015, 308:H894-903.
- 702 53. Qiu X, Brown K, Hirschey MD, Verdin E, Chen D. Calorie restriction reduces oxidative stress  
703 by SIRT3-mediated SOD2 activation. *Cell Metab* 2010, 12:662-667.
- 704 54. Mazucanti CH, Cabral-Costa JV, Vasconcelos AR, Andreotti DZ, Scavone C, Kawamoto EM.  
705 Longevity Pathways (mTOR, SIRT, Insulin/IGF-1) as Key Modulatory Targets on Aging and  
706 Neurodegeneration. *Curr Top Med Chem* 2015, 15:2116-2138.
- 707 55. Laplante M, Sabatini DM. mTOR signaling at a glance. *Journal of Cell Science* 2009, 122:3589-  
708 3594.
- 709 56. Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant  
710 rapamycin in yeast. *Science* 1991, 253:905-909.
- 711 57. Takahashi T, Hara K, Inoue H, Kawa Y, Tokunaga C, Hidayat S, Yoshino K, Kuroda Y, Yonezawa  
712 K. Carboxyl-terminal region conserved among phosphoinositide-kinase-related kinases is  
713 indispensable for mTOR function in vivo and in vitro. *Genes Cells* 2000, 5:765-775.
- 714 58. Jia K, Chen D, Riddle DL. The TOR pathway interacts with the insulin signaling pathway to  
715 regulate *C. elegans* larval development, metabolism and life span. *Development* 2004,  
716 131:3897-3906.
- 717 59. Zid BM, Rogers AN, Katewa SD, Vargas MA, Kolipinski MC, Lu TA, Benzer S, Kapahi P. 4E-BP  
718 extends lifespan upon dietary restriction by enhancing mitochondrial activity in *Drosophila*.  
719 *Cell* 2009, 139:149-160.
- 720 60. Schieke SM, Phillips D, McCoy JP, Jr., Aponte AM, Shen RF, Balaban RS, Finkel T. The  
721 mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen  
722 consumption and oxidative capacity. *J Biol Chem* 2006, 281:27643-27652.
- 723 61. Wei Y, Zhang Y-J, Cai Y, Xu M-H. The role of mitochondria in mTOR-regulated longevity.  
724 *Biological Reviews* 2015, 90:167-181.
- 725 62. Brunet A, Berger SL. Epigenetics of aging and aging-related disease. *J Gerontol A Biol Sci Med*  
726 *Sci* 2014, 69 Suppl 1:S17-20.
- 727 63. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates  
728 intrinsic and environmental signals. *Nat Genet* 2003, 33 Suppl:245-254.
- 729 64. Guo H, Zhu P, Yan L, Li R, Hu B, Lian Y, Yan J, Ren X, Lin S, Li J, et al. The DNA methylation  
730 landscape of human early embryos. *Nature* 2014, 511:606-610.
- 731 65. Jung M, Pfeifer GP. Aging and DNA methylation. *BMC Biol* 2015, 13:7.

- 732 66. Liu L, Wylie RC, Andrews LG, Tollefsbol TO. Aging, cancer and nutrition: the DNA methylation  
733 connection. *Mech Ageing Dev* 2003, 124:989-998.
- 734 67. Esteller M, Fraga MF, Guo M, Garcia-Foncillas J, Hedenfalk I, Godwin AK, Trojan J, Vaurs-  
735 Barriere C, Bignon YJ, Ramus S, et al. DNA methylation patterns in hereditary human cancers  
736 mimic sporadic tumorigenesis. *Hum Mol Genet* 2001, 10:3001-3007.
- 737 68. Esteller M, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human  
738 cancer. *Cancer Res* 2001, 61:3225-3229.
- 739 69. Kulis M, Esteller M. DNA methylation and cancer. *Adv Genet* 2010, 70:27-56.
- 740 70. Quintero-Ronderos P, Montoya-Ortiz G. Epigenetics and Autoimmune Diseases.  
741 *Autoimmune Diseases* 2012, 2012:16.
- 742 71. Glier MB, Green TJ, Devlin AM. Methyl nutrients, DNA methylation, and cardiovascular  
743 disease. *Mol Nutr Food Res* 2014, 58:172-182.
- 744 72. Cuskelly GJ, Mooney KM, Young IS. Folate and vitamin B12: friendly or enemy nutrients for  
745 the elderly. *Proc Nutr Soc* 2007, 66:548-558.
- 746 73. Zampieri M, Ciccarone F, Calabrese R, Franceschi C, Burkle A, Caiafa P. Reconfiguration of  
747 DNA methylation in aging. *Mech Ageing Dev* 2015.
- 748 74. Zhang Z, Deng C, Lu Q, Richardson B. Age-dependent DNA methylation changes in the ITGAL  
749 (CD11a) promoter. *Mech Ageing Dev* 2002, 123:1257-1268.
- 750 75. Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA, Jones PA. The human  
751 DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal  
752 tissues and overexpression in tumors. *Nucleic Acids Res* 1999, 27:2291-2298.
- 753 76. Jurkowska RZ, Jurkowski TP, Jeltsch A. Structure and function of mammalian DNA  
754 methyltransferases. *Chembiochem* 2011, 12:206-222.
- 755 77. Scourzic L, Mouly E, Bernard OA. TET proteins and the control of cytosine demethylation in  
756 cancer. *Genome Med* 2015, 7:9.
- 757 78. Salminen A, Kauppinen A, Hiltunen M, Kaarniranta K. Krebs cycle intermediates regulate  
758 DNA and histone methylation: epigenetic impact on the aging process. *Ageing Res Rev* 2014,  
759 16:45-65.
- 760 79. Li Y, Liu Y, Strickland FM, Richardson B. Age-dependent decreases in DNA methyltransferase  
761 levels and low transmethylation micronutrient levels synergize to promote overexpression  
762 of genes implicated in autoimmunity and acute coronary syndromes. *Exp Gerontol* 2010,  
763 45:312-322.
- 764 80. Peng L, Yuan Z, Ling H, Fukasawa K, Robertson K, Olashaw N, Koomen J, Chen J, Lane WS,  
765 Seto E. SIRT1 deacetylates the DNA methyltransferase 1 (DNMT1) protein and alters its  
766 activities. *Mol Cell Biol* 2011, 31:4720-4734.
- 767 81. Cencioni C, Spallotta F, Martelli F, Valente S, Mai A, Zeiher A, Gaetano C. Oxidative Stress  
768 and Epigenetic Regulation in Ageing and Age-Related Diseases. *International Journal of*  
769 *Molecular Sciences* 2013, 14:17643.
- 770 82. Campos AC, Molognoni F, Melo FH, Galdieri LC, Carneiro CR, D'Almeida V, Correa M,  
771 Jasiulionis MG. Oxidative stress modulates DNA methylation during melanocyte anchorage  
772 blockade associated with malignant transformation. *Neoplasia* 2007, 9:1111-1121.
- 773 83. Loscalzo J, Barabasi A-L. Systems biology and the future of medicine. *Wiley Interdisciplinary*  
774 *Reviews: Systems Biology and Medicine* 2011, 3:619-627.
- 775 84. Weston AD, Hood L. Systems biology, proteomics, and the future of health care: toward  
776 predictive, preventative, and personalized medicine. *J Proteome Res* 2004, 3:179-196.
- 777 85. Vallabhajosyula RR, Raval A. Computational modeling in systems biology. *Methods Mol Biol*  
778 2010, 662:97-120.
- 779 86. Mc Auley MT, Proctor CJ, Corfe BM, Cuskelly CJ, Mooney KM. Nutrition Research and the  
780 Impact of Computational Systems Biology. *Journal of Computer Science and Systems Biology*  
781 2013, 6:271-285.

- 782 87. Machado D, Costa RS, Rocha M, Ferreira EC, Tidor B, Rocha I. Modeling formalisms in  
783 Systems Biology. *AMB Express* 2011, 1:45.
- 784 88. Seixas FL, Zadrozny B, Laks J, Conci A, Muchaluat Saade DC. A Bayesian network decision  
785 model for supporting the diagnosis of dementia, Alzheimers disease and mild cognitive  
786 impairment. *Comput Biol Med* 2014, 51:140-158.
- 787 89. Figueredo GP, Siebers PO, Aickelin U, Whitbrook A, Garibaldi JM. Juxtaposition of system  
788 dynamics and agent-based simulation for a case study in immunosenescence. *PLoS One*  
789 2015, 10:e0118359.
- 790 90. Ghosh S, Matsuoka Y, Asai Y, Hsin K-Y, Kitano H. Software for systems biology: from tools to  
791 integrated platforms. *Nat Rev Genet* 2011, 12:821-832.
- 792 91. Springer M, Paulsson J. Biological physics: harmonies from noise. *Nature* 2006, 439:27-28.
- 793 92. Gillespie D. A general method for numerically simulating stochastic time evolution of  
794 coupled chemical-reactions. *Journal of Computational Physics* 1976, 22::403-434.
- 795 93. Gibson MA, Bruck J. Efficient exact stochastic simulation of chemical systems with many  
796 species and many channels. . *J. Phys. Chem* 2000, A 104:1876-1889.
- 797 94. Gillespie DT, Hellander A, Petzold LR. Perspective: Stochastic algorithms for chemical  
798 kinetics. *J Chem Phys* 2013, 138:170901.
- 799 95. Goss PJ, Peccoud J. Quantitative modeling of stochastic systems in molecular biology by  
800 using stochastic Petri nets. *Proc Natl Acad Sci U S A* 1998, 95:6750-6755.
- 801 96. Needham CJ, Bradford JR, Bulpitt AJ, Westhead DR. A primer on learning in Bayesian  
802 networks for computational biology. *PLoS Comput Biol* 2007, 3:e129.
- 803 97. Wang RS, Saadatpour A, Albert R. Boolean modeling in systems biology: an overview of  
804 methodology and applications. *Phys Biol* 2012, 9:055001.
- 805 98. Farlow SJ. *Partial Differential Equations for Scientists and Engineers: Dover Books on*  
806 *Mathematics*; 2003.
- 807 99. An G, Mi Q, Dutta-Moscato J, Vodovotz Y. Agent-based models in translational systems  
808 biology. *Wiley Interdiscip Rev Syst Biol Med* 2009, 1:159-171.
- 809 100. Hoops S, Sahle S, Gauges R, Lee C, Pahle J, Simus N, Singhal M, Xu L, Mendes P, Kummer U.  
810 COPASI—a COMplex PATHway Simulator. *Bioinformatics* 2006, 22:3067-3074.
- 811 101. Matsuoka Y, Funahashi A, Ghosh S, Kitano H. Modeling and simulation using CellDesigner.  
812 *Methods Mol Biol* 2014, 1164:121-145.
- 813 102. Sauro HM, Bergmann FT. Standards and ontologies in computational systems biology. *Essays*  
814 *Biochem* 2008, 45:211-222.
- 815 103. Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, Arkin AP, Bornstein BJ, Bray D,  
816 Cornish-Bowden A, et al. The systems biology markup language (SBML): a medium for  
817 representation and exchange of biochemical network models. *Bioinformatics* 2003, 19:524-  
818 531.
- 819 104. Le Novère N, Bornstein B, Broicher A, Courtot M, Donizelli M, Dharuri H, Li L, Sauro H,  
820 Schilstra M, Shapiro B, et al. BioModels Database: a free, centralized database of curated,  
821 published, quantitative kinetic models of biochemical and cellular systems. *Nucleic Acids*  
822 *Research* 2006, 34:D689-D691.
- 823 105. Kowald A, Kirkwood TB. Towards a network theory of ageing: a model combining the free  
824 radical theory and the protein error theory. *J Theor Biol* 1994, 168:75-94.
- 825 106. Kowald A, Jendrach M, Pohl S, Bereiter-Hahn J, Hammerstein P. On the relevance of  
826 mitochondrial fusions for the accumulation of mitochondrial deletion mutants: a modelling  
827 study. *Aging Cell* 2005, 4:273-283.
- 828 107. Tam ZY, Gruber J, Halliwell B, Gunawan R. Mathematical modeling of the role of  
829 mitochondrial fusion and fission in mitochondrial DNA maintenance. *PLoS One* 2013,  
830 8:e76230.

- 831 108. Figge MT, Reichert AS, Meyer-Hermann M, Osiewacz HD. Deceleration of fusion-fission  
832 cycles improves mitochondrial quality control during aging. *PLoS Comput Biol* 2012,  
833 8:e1002576.
- 834 109. Guillaud F, Drose S, Kowald A, Brandt U, Klipp E. Superoxide production by cytochrome bc1  
835 complex: a mathematical model. *Biochim Biophys Acta* 2014, 1837:1643-1652.
- 836 110. Markevich NI, Hoek JB. Computational modeling analysis of mitochondrial superoxide  
837 production under varying substrate conditions and upon inhibition of different segments of  
838 the electron transport chain. *Biochim Biophys Acta* 2015, 1847:656-679.
- 839 111. Hirt BV, Wattis JA, Preston SP. Modelling the regulation of telomere length: the effects of  
840 telomerase and G-quadruplex stabilising drugs. *J Math Biol* 2014, 68:1521-1552.
- 841 112. Rodriguez-Brenes IA, Peskin CS. Quantitative theory of telomere length regulation and  
842 cellular senescence. *Proc Natl Acad Sci U S A* 2010, 107:5387-5392.
- 843 113. Portugal RD, Land MG, Svaiter BF. A computational model for telomere-dependent cell-  
844 replicative aging. *Biosystems* 2008, 91:262-267.
- 845 114. Trusina A. Stress induced telomere shortening: longer life with less mutations? *BMC Syst Biol*  
846 2014, 8:27.
- 847 115. Auley MT, Mooney KM, Angell PJ, Wilkinson SJ. Mathematical modelling of metabolic  
848 regulation in aging. *Metabolites* 2015, 5:232-251.
- 849 116. Kriete A, Bosl WJ, Booker G. Rule-based cell systems model of aging using feedback loop  
850 motifs mediated by stress responses. *PLoS Comput Biol* 2010, 6:e1000820.
- 851 117. Smith GR, Shanley DP. Computational modelling of the regulation of Insulin signalling by  
852 oxidative stress. *BMC Syst Biol* 2013, 7:41.
- 853 118. McGovern AP, Powell BE, Chevassut TJ. A dynamic multi-compartmental model of DNA  
854 methylation with demonstrable predictive value in hematological malignancies. *J Theor Biol*  
855 2012, 310:14-20.
- 856 119. Landan G, Cohen NM, Mukamel Z, Bar A, Molchadsky A, Brosh R, Horn-Saban S, Zalcenstein  
857 DA, Goldfinger N, Zundelovich A, et al. Epigenetic polymorphism and the stochastic  
858 formation of differentially methylated regions in normal and cancerous tissues. *Nat Genet*  
859 2012, 44:1207-1214.
- 860 120. Riggs AD, Xiong Z. Methylation and epigenetic fidelity. *Proc Natl Acad Sci U S A* 2004, 101:4-  
861 5.
- 862 121. Jeltsch A, Jurkowska RZ. New concepts in DNA methylation. *Trends Biochem Sci* 2014,  
863 39:310-318.
- 864 122. Shipony Z, Mukamel Z, Cohen NM, Landan G, Chomsky E, Zelig SR, Fried YC, Ainbinder E,  
865 Friedman N, Tanay A. Dynamic and static maintenance of epigenetic memory in pluripotent  
866 and somatic cells. *Nature* 2014, 513:115-119.
- 867 123. Przybilla J, Rohlf T, Loeffler M, Galle J. Understanding epigenetic changes in aging stem cells-  
868 -a computational model approach. *Aging Cell* 2014, 13:320-328.
- 869 124. Mc Auley MM, Wilkinson DJ, Jones JJ, Kirkwood TT. A whole-body mathematical model of  
870 cholesterol metabolism and its age-associated dysregulation. *BMC Syst Biol* 2012, 6:130.
- 871 125. Mc Auley M, Jones J, Wilkinson D, Kirkwood T. Modelling Lipid Metabolism to Improve  
872 Healthy Ageing. *BMC Bioinformatics* 2005, 6:P21.
- 873 126. Mc Auley MT, Mooney KM. Computationally Modeling Lipid Metabolism and Aging: A Mini-  
874 review. *Computational and Structural Biotechnology Journal* 2015, 13:38-46.
- 875 127. Mooney KM, Mc Auley MT. Cardiovascular disease and healthy ageing. *Journal of Integrative*  
876 *Cardiology* 2015, 1:76-78.
- 877 128. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of Plaque Formation and Rupture.  
878 *Circulation Research* 2014, 114:1852-1866.
- 879 129. Badimon L, Vilahur G. Thrombosis formation on atherosclerotic lesions and plaque rupture.  
880 *Journal of Internal Medicine* 2014, 276:618-632.

- 881 130. McAuley MT, Kenny RA, Kirkwood TB, Wilkinson DJ, Jones JJ, Miller VM. A mathematical  
882 model of aging-related and cortisol induced hippocampal dysfunction. *BMC Neurosci* 2009,  
883 10:26.
- 884 131. Mc Auley MT, Mooney KM. Lipid metabolism and hormonal interactions: impact on  
885 cardiovascular disease and healthy aging. *Expert Review of Endocrinology & Metabolism*  
886 2014, 9:357-367.
- 887 132. Iadecola C. The pathobiology of vascular dementia. *Neuron* 2013, 80:844-866.
- 888 133. Popa-Wagner A, Mitran S, Sivanesan S, Chang E, Buga AM. ROS and brain diseases: the good,  
889 the bad, and the ugly. *Oxid Med Cell Longev* 2013, 2013:963520.
- 890 134. Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other  
891 disorders. *Nat Rev Neurosci* 2011, 12:723-738.
- 892 135. Rancillac A, Geoffroy H, Rossier J. Impaired neurovascular coupling in the APPxPS1 mouse  
893 model of Alzheimer's disease. *Curr Alzheimer Res* 2012, 9:1221-1230.
- 894 136. Stanimirovic DB, Friedman A. Pathophysiology of the neurovascular unit: disease cause or  
895 consequence? *J Cereb Blood Flow Metab* 2012, 32:1207-1221.
- 896 137. Cloutier M, Wellstead P. Dynamic modelling of protein and oxidative metabolisms simulates  
897 the pathogenesis of Parkinson's disease. *IET Syst Biol* 2012, 6:65-72.
- 898 138. Pfeiffer RF. Autonomic dysfunction in Parkinson's disease. *Expert Rev Neurother* 2012,  
899 12:697-706.
- 900 139. Perry VH. Innate Inflammation in Parkinson's Disease. *Cold Spring Harbor Perspectives in*  
901 *Medicine* 2012, 2.
- 902 140. Hwang O. Role of oxidative stress in Parkinson's disease. *Exp Neurobiol* 2013, 22:11-17.
- 903 141. Xue H, Xian B, Dong D, Xia K, Zhu S, Zhang Z, Hou L, Zhang Q, Zhang Y, Han J-DJ. A modular  
904 network model of aging. *Molecular Systems Biology* 2007, 3:n/a-n/a.
- 905 142. Koleva KZ, Hellweger FL. From protein damage to cell aging to population fitness in E. coli:  
906 Insights from a multi-level agent-based model. *Ecological Modelling* 2015, 301:62-71.
- 907 143. Sáez P, Peña E, Tarbell JM, Martínez MA. Computational model of collagen turnover in  
908 carotid arteries during hypertension. *International Journal for Numerical Methods in*  
909 *Biomedical Engineering* 2015, 31:n/a-n/a.
- 910 144. Virgilio KM, Martin KS, Peirce SM, Blemker SS. Multiscale models of skeletal muscle reveal  
911 the complex effects of muscular dystrophy on tissue mechanics and damage susceptibility.  
912 *Interface Focus* 2015, 5.
- 913 145. Singhania R, Sramkoski RM, Jacobberger JW, Tyson JJ. A hybrid model of mammalian cell  
914 cycle regulation. *PLoS Comput Biol* 2011, 7:e1001077.
- 915 146. Aitken S, Kilpatrick AM, Akman OE. Dizzy-Beats: a Bayesian evidence analysis tool for  
916 systems biology. *Bioinformatics* 2015, 31:1863-1865.
- 917 147. Ramsey S, Orrell D, Bolouri H. Dizzy: stochastic simulation of large-scale genetic regulatory  
918 networks. *J Bioinform Comput Biol* 2005, 3:415-436.
- 919 148. Ashyraliyev M, Fomekong-Nanfack Y, Kaandorp JA, Blom JG. Systems biology: parameter  
920 estimation for biochemical models. *Febs j* 2009, 276:886-902.

921

922

## 923 **Figure Legends**

924 **FIGURE 1.** An integrated overview of aging and some of its key players. This figure emphasises the  
925 extent of interplay between the different components that underpin intrinsic aging, and how age-  
926 related changes to these components affect health-span and longevity. The integrated nature of this  
927 diagram highlights the complexities of ageing and why computational models are needed to help



928 study its dynamics. IGF-1, insulin-like growth factor-1; ROS, reactive oxygen species; PARP, poly ADP  
929 ribose polymerase; mTOR, mammalian target of rapamycin.

930

931 **FIGURE 2.** Integrating a computational model of cholesterol metabolism with a variety of other  
932 factors involved in the onset of CVD. Our extended model is framed around the insidious rise in ROS  
933 that occurs with age. This rise in ROS is a key driver which underpins a pathological cascade that  
934 ultimately results in CVD.

935

936 **FIGURE 3.** An SBGN representation of the autonomic nervous system. The aim of this proposed  
937 model would be to simulate physiological responses associated with the autonomic nervous system  
938 such as heart rate, rate of movements in the gastrointestinal tract, or synthesis of B cells by the  
939 spleen. These processes are regulated in part by neurotransmitters and cytokines. Dysregulation of  
940 these processes together with oxidative stress have been strongly implicated in the pathology which  
941 underpins Parkinson's disease. NE, Norepinephrine; 5HT, serotonin; Ach, acetylcholine.

942

#### 943 **Further Reading**

944 Systems Biology

945 Edda Klipp, Wolfram Liebermeister, Christoph Wierling, Axel Kowald, Hans Lehrach, Ralf Herwig

946 ISBN: 978-3-527-31874-2

947 2009, Wiley-Blackwell

948

949 Aging and Health - A Systems Biology

950 Perspective. Interdiscipl Top Gerontol. Basel, Karger, 2015

951

952 [Systems Biology of Parkinson's Disease](#)

953 [Peter Wellstead & Mathieu Cloutier](#)

954 [ISBN: 1493901265](#)

955 [2012, Springer](#)

956

957 [Systems Biology in Practice: Concepts, Implementation and Application](#)

958 [Edda Klipp, Ralf Herwig, Axel Kowald, Christoph Wierling & Hans Lehrach](#)

959 [ISBN: 3527310789](#)

960 [2005, Wiley VCH](#)

961

962 [A First Course in Systems Biology](#)

963 [Eberhard Voit](#)

964 [ISBN: 0815344678](#)

965 [2012, Garland Science](#)

966

967

968

969

970

971 **Related Articles**

<b>Article ID [or Subtopic]</b>	<b>Article title</b>
WSBM.1209	Modeling cellular compartmentation in one-carbon metabolism
wsbm.126	Computational systems biology of aging
wsbm.52	Computational modeling of mammalian signaling networks

972