1   Degeneracy measures in biologically plausible random Boolean networks

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Abstract

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12   Biological systems show diversity in terms of the underlying network structure and the governing

13   rules of such networks. Yet, different types of biological networks may develop similar adaptation

14   strategies in face of environmental changes. Degeneracy refers to the ability to compensate for

15   compromised function without the need for a redundant component in the system. Highly degenerate

16   systems show resilience to perturbations and damage because the system can compensate for

17   compromised function due to reconfiguration of the underlying network dynamics.

18   Although formal definitions of degeneracy have been proposed, these definitions have only been

19   tested in relatively simple networks involving weighted connections between network nodes. In this

20   study, we test an information theoretic definition of degeneracy on random Boolean networks, frequently

21   used to model gene regulatory networks. Random Boolean networks are discrete dynamical systems with

22   binary connectivity and thus, these networks are well-suited for tracing information flow and the causal

23   effects. By generating networks with random binary wiring diagrams, we test the effects of systematic

24   lesioning of connections and perturbations of the network nodes on the degeneracy measure.

25   Our analysis shows that degeneracy, on average, is the highest in networks in which ~20% of the

26   connections are lesioned while 50% of the nodes are perturbed. Moreover, our results for the networks

27   with no lesions and the fully-lesioned networks are comparable to the degeneracy measures from

28   weighted networks, thus we show that the degeneracy measure is applicable to different networks. Such a

29   generalized applicability implies that degeneracy can be used to make predictions about the variety of

30   systems' ability to recover function.

Author Summary

32   Degeneracy – the ability of structurally different elements to perform similar functions – is a

33   property of many biological systems. Systems exhibiting a high degree of degeneracy continue to exhibit

34   the same macroscopic behavior following a lesion even though the underlying network dynamics are

35   significantly different. Degeneracy thus suggests how biological systems can thrive despite changes to

36   internal and external demands. Although degeneracy is a feature of network topologies and seems to be

37   implicated in a wide variety of biological processes, research on degeneracy in biological networks is

38   mostly limited to weighted networks (e.g., neural networks). To date, there has been no extensive

39   investigation of information theoretic measures of degeneracy in other types of biological networks. In

40   this paper, we apply existing approaches for quantifying degeneracy to random Boolean networks used

41   for modeling biological gene regulatory networks. Using random Boolean networks with randomly

42   generated rulesets to generate synthetic gene expression data sets, we systematically investigate the effect

43   of network lesions on measures of degeneracy. Our results are comparable to measures of degeneracy

44   using weighted networks, and this suggests that degeneracy measures may be a useful tool for

45   investigating gene regulatory networks.

Introduction

47   Biological systems can adjust their functioning dynamically in face of changing circumstances.

48   However, such functional adjustments are constrained by the structural properties of the components that

49   perform the functions [1–7] as well as the topology of the system. Biological systems as complex

50   networks have evolved multiple strategies to achieve a 'working' reconfiguration of the components that

51   ensures survival through shifts in environmental contingencies [8–15]. One strategy is redundancy which

52   means that a system has multiple structurally identical components serving the same function [16–19].

53   Systems also can utilize multifunctionality (or alternatively, pluripotentiality [20,21]) that is the capacity

54   for a single component to serve multiple functions [22–26]. Another strategy for biological systems to

55   respond flexibly to perturbations is called degeneracy [9,10,27–29].

56   Degeneracy (or alternatively, 'distributed redundancy' [30], 'distributed robustness' [31], 'functional

57   redundancy' [25], 'extrinsic buffering' [32]) describes the ability of components in a biological system

58   that are structurally different to carry out the same or similar functions [20,21,21,28,33–40]. As

59   difference in the structure implies different functions [41], under certain conditions degenerate

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60   components do not necessarily show functional variety [42,43] but instead, each degenerate component is

61   responsible for a (set of) function(s) which is initially determined by their biochemical(/physical)

62   structure [9,21,31]. Unlike multifunctionality and redundancy, degeneracy implies a change in the role

63   assignments among the components such that the system can continue to function even when its normal

64   processes have been compromised.

65   A biological network with high degeneracy means that the system can show the same macroscopic

66   behavior following a lesion even though the underlying network dynamics are significantly different. In

67   other words, if the system is highly degenerate, after a lesion, the function can be recovered by a

68   structurally different (i.e., performing a different function under normal conditions) component taking

69   over a new function. For example, in the brain many different neural clusters can affect the same motor

70   outputs, and if some of the brain areas are damaged, an alternative (non-redundant) pathway can be

71   recruited in order to generate functionally equivalent behaviors [44–48]. Degeneracy thus suggests how

72   biological systems can thrive despite changes to internal and external demands.

73   It has been shown that degeneracy also plays a role in complexity and evolvability of the biological

74   systems [8,10,27,28,32,36,49]. Higher levels of degeneracy correlate with an increase in the degree of

75   both the functional integration and local segregation of a system, and therefore, higher degeneracy is

76   accompanied by higher degree of complexity of the systems [33].While local segregation (namely,

77   functional specialization) enables system to be flexible against environmental stress (due to diversity of

78   functions), functional integration allows system to be robust [50–52]. If a component, or a group of

79   components are compromised in a highly degenerate system, functions can be reassigned among distinct

80   elements (that are locally segregated) while the macro-level behavior (which requires the system to be

81   functionally integrated) is conserved. This adaptability brings an obvious advantage over the course of

82   natural selection [44].

83   To measure degeneracy in systems, Tononi et al. [33] introduced a quantitative measure for

84   neural networks (see also alternatives [53]) using an information theoretic approach. Information theory

85   [54–56] provides a set of tools to describe how information is processed in systems. It allows us to

86   measure the statistical (in)dependencies in terms of the information content of the components. Mutual

87   information (MI), which is a measure provided by information theory, can also capture nonlinear

88   dependencies(/relationships) that are not detectable by correlation analysis [54,57]. However, direction of

89   the interaction between the components cannot be discerned from MI alone [33,57,58]. Incorporating MI

90   with systematic perturbations (to determine directionality), degeneracy is formalized [33] in terms of the

91   causal effects of the changes in the state of the subsets (components and/or subgroups of components) on

92   the system's output. If the output activity of the system is not affected by the change (e.g., perturbation)

93   in a subset's state, then the system is highly degenerate with regard to the function that is performed by

94   that subset. This information theoretic measure of degeneracy is, first, applied to highly abstract networks

95   in the work by Tononi et al. [33] which is followed by applications to the weighted networks with a high

96   degree of biological fidelity (e.g., Hodgkin-Huxley type neural networks [40] and genetic networks with

97   epistasis [59]).

98   Although it has been shown that degeneracy as a network property exists at different levels of

99   biological organization (from molecules to behavior [30,36,44]), a quantitative analysis of degeneracy at

100 such levels is sparse and methods are individualized to specific cases (see the different versions of

101 degeneracy measurements in other works [10,53,60]). In systems biology, information theoretic measures

102 are widely applied to many problems [61], yet, to date, there is not a comprehensive study applying this

103 measure for biologically realistic networks other than networks with weighted connections.

104   Neural networks offer one example of how biological systems can incorporate degeneracy to

105 ensure survival after being damaged. However, other biological networks are likewise capable of

106 recovering partial or full function following damage. For example, in between-species interaction

107 networks a species loss can be compensated by other species contributing to ecosystem functioning [62].

108 Likewise, on a smaller scale, it has been shown that loss of functioning in some (non-redundant) genes

109 has a weak or no effect on the fitness of the gene networks [8]. Although degeneracy might not be

110   detectable under normal conditions, after perturbating or lesioning the biological networks, changes in the

111   environment may also evoke degenerate responses ('degeneracy lifting' effect [9]). Environmental

112   (evolutionary) pressure in receptor-signal transduction networks [53] can push the signaling pathways to

113   reconfigure into a degenerate form.

114   Although degeneracy is a feature of biological gene networks, it is unclear whether models of

115   gene networks can be analyzed using the same information theoretic approach as used for neural network

116   models. A GRN is a network of gene-gene interactions through their regulators that control the gene

117   expression levels of the products (mRNAs and proteins) which, ultimately, determine the cell fate (final

118   cell type, i.e., function of the cell) [63]. Measurements of degeneracy at the level of gene transcription

119   control may provide insights on how functions of genes that determine the cell function, can be recovered

120   as a consequence of the network properties (GRN topology).

121   Random Boolean network (RBN) models, as discrete models of GRNs, are well-suited to study

122   degeneracy since with RBNs we can induce and trace the effects of targeted lesions while

123   environmental/external pressure is a parameter that can be controlled over in silico experiments. Unlike

124   neuronal networks where edges are (synaptic) weight vectors, RBNs have a static wiring diagrams [64]

125   governed by logic equations that represent the functions of gene regulatory factors (e.g., transcription

126   factors). Logic equations describe the underlying network architecture. For example, for a simple

127   network of 3 genes, if gene G1 is regulated both directly by G3 and through an indirect link from G2, this

128   architecture is represented by the logic function of "G1 AND (G2 OR G3)". Likewise, if there is an

129   inhibitory regulation of G1 through G2 while the same architecture is preserved from the network

130   described above, this structure can be represented by the logic equation of "G1 AND ((NOT G2) OR

131   G3)". Since each state of gene expression is the direct outcome of the activity of (regulatory interactions

132   in) the previous state, one can assess the effects of circuit architecture on gene expression levels [63].

133   Hence, in RBNs, it is feasible to trace the information flow at each (discrete) time step and so, causal

134   influences.

135   In this study we test to what extent the information theoretic measure of degeneracy applies to RBNs.

136   Furthermore, we test systematic lesions in randomly generated Boolean networks while varying the

137   number of perturbed nodes. This enables us to explore how degeneracy quantitively changes as a function

138   of interventions to the nodes and induced topological alterations in the networks. Results show that

139   degeneracy measures are comparable to different networks – not only to weighted networks.

140   **Results**

141   *Lesioning*

142   We first investigate the effects of systematic lesioning on measures of degeneracy in randomly

143   wired RBNs. Edges between network nodes were lesioned in two ways. In type-1 lesioning, only

144   outgoing edges were lesioned incrementally while it is possible (due to the pseudo-random algorithm to

145   generate the logic functions) that incoming edges stayed intact and for all the nodes, self-connections

146   were preserved in the network (for details see Methods). The networks with type-1 lesions decrease in

147   average degeneracy values as the cut percentage increases (Fig1). This validates that degeneracy emerges

148   as a network property.

149   In the second type of lesioning (hence the name type-2 lesion), we lesioned all incoming and outgoing

150   edges (but not the self-connections) for randomly chosen genes incrementally. Similar to the type-1

151   lesioning, average degeneracy decreases as a function of lesioning. We have anticipated that there could

152   be a difference between the effects of lesioning types as a direct consequence of the partial lesioning (the

153   incoming edges are preserved) in type-1 condition which can lead some nodes to become dead ends since

154   the activity ends in those nodes. Active nodes without outgoing edges means that such nodes do not serve

155   a function, and this eventually would result in lower degeneracy values. The comparison of two lesioning

156   types, in Fig 1, demonstrates that both conditions have similar effect on the average degeneracy, where

157   there is a no significant difference (two-way analysis of variance, ANOVA) found between both types of

158   lesioning. However, average degeneracy varies with cut conditions ($p = 0.004$).

159　　**Fig1. Average degeneracy values compared between type-1 (green line) and type-2 (blue**

160　　**line) lesioning.** On the x axis, the cut percentage represents the affected number of nodes (in total

161　　of 10 nodes) whose edges are lesioned given the networks. For both lesioning types, degeneracy

162　　was lowest in the 100% cut condition where edges of all nodes (10) were cut.

163　*Perturbation*

164　　Degeneracy is calculated as the area between the average $MI^P(X^k;O)$ (mutual information, MI,

165　between the portion of entropy shared by the system for *each* perturbed subset k and the output O) and

166　overall-MI (mutual information between the system and its output) for different perturbed subset sizes, k

167　(see Equation 3 in Methods). This area shows a characteristic shape of the degeneracy function: a non-

168　zero value that declines to zero as perturbed subset size k approaches $k = O$, following an increase that is

169　"higher than would be expected from a linear increase" [33] . This characteristic shape has furthermore

170　been replicated in networks composed of in Hodgkin-Huxley neurons [40]. In our study, the analogous

171　condition for such illustration of degeneracy is where no edges are lesioned in the networks (Fig2a).

172　In weighted networks with no connections, the average (overall-) MI shows a linear increase where

173　degeneracy is zero [33]. Here, we have replicated this condition (i.e., 100%-of-edges-cut) in a similar way

174　except, in our networks, nodes have preserved self-connections while all outgoing edges were lesioned.

175　These networks also have initial variance due to the model (see details in Methods and Supporting

176　Information) which is a system of stochastic differential equations (SDEs). Our results for no-edges-cut

177　condition and the characteristic profile of degeneracy are comparable to corresponding findings in

178　previous studies (Fig2 a, b).

179　　**Fig 2. Degeneracy, (grey area) is computed (see Equation 3 in Methods) as the average MI**

180　　**between subsets of *X* and *O* under perturbation over increasing perturbed subset size *k,* in**

181　　**networks with (a, c) no lesions and with (b, d) 100%-cut condition.**

182   Further inspection of partial degeneracy (see Methods for details) values from individual

183   simulations showed that partial degeneracy can have a negative value for some conditions (only two of

184   such conditions captured here for comparison, Figs 3a-b). Although we have observed that partial

185   degeneracy is negative for different cut-conditions and different sizes of perturbed subset, when MI is

186   averaged over the simulations for all the perturbed subset sizes k, $<MI^P(X^k;O)>$, degeneracy $D_N(X; O)$

187   was above zero in all conditions.

188   **Fig3. Partial degeneracy values from individual networks for each perturbed subset size k.**

189   Data from 10 (k) x 1000 simulations of networks with (a) no-cut condition and (b) 100%- cut

190   condition. The distribution of partial degeneracy values for (b) shows clear modes in the data

191   where there is an overlap of the perturbed subset and output sheet.

Interactions of lesions and perturbations

193   Increases in both lesion extent and the number of nodes perturbed contribute to decreases in the

194   degeneracy, raising the question of how these two factors may interact. We therefore conducted

195   additional simulations in which each lesion condition (0%-100%, see Methods) was crossed with each

196   perturbation condition (k = 1-10). Fig 4 (a-b) show how average partial degeneracy changes as a function

197   of perturbation subset size k, given cut percentages. For both lesioning types, partial degeneracy peaks

198   around when half of the nodes *(k ~ 5)* are perturbed in the system. The measure of degeneracy can detect

199   existing isofunctionality between the different structures only when one of the structures is perturbed.

200   When half of the nodes in the system are perturbed, we, thereby, maximize the probability of

201   selecting/measuring the right structure for given degeneracy.

202   **Fig4. Average degeneracy computed as a function of perturbation subset size k in type-1**

203   **lesioning (a) and type-2 lesioning (b).** Each line represents the cut condition for lesioned edges

204   given the percentage of number of nodes in networks.

Discussion

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206   Although a variety of types of biological networks are thought to exhibit degeneracy, previous

207   theoretical work has primarily focused on networks with weighted connections [33,40,59]. In this study,

208   we demonstrate that degeneracy measures are also suitable for RBNs, frequently used to model gene

209   regulation mechanisms. Although our simulations largely replicated previous studies investigating

210   degeneracy in neurally-inspired networks, RBNs use Boolean logic operators rather than weighted

211   connections to determine function. It therefore might have been the case that information-theoretic

212   approaches developed for one class of networks might not have generalized correctly to a new class. By

213   replicating previous findings using RBNs, we demonstrate that information-theoretic approaches are

214   applicable to a broad range of network types.

215   Network and graph theoretic approaches, frequently formulated in terms of information theory,

216   have been applied extensively to neuroscience [50–52,58,65–74] to predict individual differences,

217   consequences of lesions, and ability to recover function following injury. Extending this approach to the

218   study of GRNs opens the door to investigating the consequences of, and possible remedies for, genetic

219   dysfunction. Because most genetic functions are performed by subsets of many components within

220   functional modules [5,75], diseases may emerge due to disorganization of the components in these

221   modules. Degeneracy measures can be recruited for predicting and inducing topological modifications

222   (for example, 'rewiring of diseased modules' [75]) to achieve desired functional outcomes that have

223   clinical significance, such as enhanced pharmaceuticals with better drug targets.

224   In addition to replicating previous results, we explored the impact of systematically manipulating

225   network connectivity (lesioning) while decomposing degeneracy by size of the perturbed subset.   In

226   doing so, we identify a potential interaction between the number of perturbed nodes and the magnitude of

227   the impact of lesions on network degeneracy. In networks in which ~20% of the connections are lesioned

228   while 50% of the nodes are perturbed, it is observed that average partial degeneracy reaches its highest

229   value among all other cut conditions and for all perturbed subset sizes. This can be interpreted as, for

10

230   some conditions (here, k = 5 and ~20%-cut) might allow the expression of degenerate structures without

231   compromising their function, whereas more lesioning would diminish both primary and degenerate

232   structures, and more perturbation would confound the functions of the nodes. Likewise, if perturbation (of

233   the number of nodes) is smaller, not all possible degenerate structures might have expressed in the

234   network and, also, when the lesioning is less, degenerate structures might be unobservable since most of

235   the primary structures are intact.

236   In the literature it has been shown that additional damage (gene/node deletions) can restore the

237   function of previously compromised (metabolic) networks [76–78]. Here, we show that, up to a point,

238   progressively lesioning a network results in increased degeneracy. This finding suggests that it might be

239   possible to determine an optimum degree of lesioning and perturbation given a network to achieve higher

240   degeneracy in the systems. Thus, partial degeneracy measures might be helpful to develop strategies to

241   predict how to recover the function after damage.

242   As originally conceived, degeneracy was intended to capture the idea that identical functions

243   could be carried out by distinct network structures. Intuitively, therefore, degeneracy would seem to have

244   a lower bound at zero – in a network with no degeneracy, all structures would serve their own individual

245   functions, and perturbation of those structures would disrupt network output related to the function

246   served. Although on average degeneracy in our simulations tended to be above or equal to zero, we

247   observed individual simulations in which partial degeneracy values were below zero. In previous studies

248   ([40,59]), negative degeneracy has been observed especially for network models with increased biological

249   fidelity.

250   One possible reason for the observation of negative degeneracy may be that the information-

251   theoretic measure for degeneracy was originally developed for and tested on neural networks with no

252   initial variance. As the biological fidelity of the models (thus, inherent variance in the systems) increases,

253   for some conditions (lower coupling and lower connection probability [40] and networks with lower

254   complexity [59]) negative degeneracy has been shown. However, we have not observed such effects on

255   overall degeneracy measurements where 1000 MI values for each possible subset size k were averaged

256   across random networks. Mathematically, degeneracy gets a negative value when the portion (*k/n*) of the

257   MI between the whole system (*n*) and the output sheet (*n/2*) is higher than the average of the MI between

258   the (perturbed) subset of the system (*k*) and the output sheet (*n/2*). However, the biological

259   meaning/equivalence of negative degeneracy remains unclear. For studying more biologically realistic

260   complex networks, adjustments in the tools for quantifying degeneracy may be needed.

261   **Methods**

262   **Network Architecture**

263   RBNs were initially proposed as simplified models for gene regulatory networks by Kauffmann

264   [79] where network nodes represent the genes, and the edges represent the regulatory functions. A RBN

265   constitutes a discrete dynamical system that has N nodes with K incoming edges (hence, also referred to

266   as N-K models). Each node (gene) can be ON or OFF (1 or 0); a network of N binary nodes therefore has

267   2^N distinct states [79]. This system is state determined [79] according to Boolean functions that are

268   assigned to each node randomly (from K^N possible states) where each node has a minimum of zero to a

269   maximum of N inputs [80] . Such a state-space allows random network configurations which often leads

270   to nonlinear dynamics. In this study, the nodes can have no inputs (but the self-connection) without an

271   upper boundary, so that a gene can have a maximum of N inputs. The total number of nodes representing

272   the genes, here, is *N = 10*, thus there are 2^{10} possible states. In RBNs, the state of the nodes in the network

273   can be updated synchronously or asynchronously in discrete time steps. In this study, for simplicity

274   purposes, a synchronous update rule is chosen.

275   RBNs have a well-defined function mapping scheme through logic (Boolean) operators which

276   constitute the rules for connections that control the state of gene regulators. In our setting, operators *AND,*

277   *NOT, OR* are randomly placed to generate rulesets (functions). If a node has only one input (that is one

278   gene is connected to another gene) the probability of the function to have *NOT* operator is 0.5. If a node

279   has two genes assigned to it (outgoing edges), the probability of these two genes to be connected to the

280   node via *OR* operator or *AND NOT* operator is both 0.5. Outgoing edges are randomly distributed for each

281   node with the condition that each node has at least one (thereby, connectivity is preserved) and at most

282   two (more than one Boolean operator) edges mapped to the other nodes, while all the nodes have self-

283   connections Thus, incoming edges are assigned to the nodes in a completely random fashion (allowing for

284   the emergence of network hubs).

Network Lesioning

286   Degeneracy, as a strategy (or design principle [10,21,81]) for networks to recover their function,

287   refers to the rearrangement of (structurally different) components in a way that function/output remains

288   the same even after a damage. In a network with high degeneracy, there are many possible network

289   reconfigurations that can produce/recover the function. To test the potential factors that give rise to

290   (higher/lower) degeneracy in networks, here, we induce interventions to the systems at the network-level

291   by lesioning the edges.

292   Two different lesions were introduced to the synthetic networks. In type-1 lesioning, all outgoing

293   edges from randomly chosen nodes were cut while the self-connections of the nodes and incoming edges

294   were preserved. In the second type of lesioning, all incoming and outgoing edges were cut (except for

295   self-connections) from randomly selected nodes given the percentage of total lesioned edges. In both

296   types of lesioning, the edges are lesioned in increments of ten percent of the total number of the nodes

297   given a network. For example, in type-1 30% cut condition, we have lesioned all the outgoing edges

298   (except the self-connections) of the 3 randomly chosen nodes given a network of 10 nodes. Likewise, in

299   type-2 30% cut condition, all the edges (incoming and outgoing, except the self-connections) of 3

300   randomly chosen nodes (out of 10 nodes total in a network) were lesioned. By 100%-cut condition (in

301   both lesioning types), we refer to networks where no node is connected to the other, and the nodes have

302   only self-connections.

303   For both lesioning types, 0%-cut condition refers to networks that are not lesioned, yet the edges

304   are randomly disturbed (according to the method that is defined previously). This may lead some nodes to

305   not have any edges (but the self-connection) due to random assignments of the rules, therefore,

306   mimicking a (partially) lesioned condition.

Biologically realistic random Boolean networks: Discrete to Continuous

308   Boolean functions describe how the states of the regulators control the state of the target genes

309   [82]. In our study, Boolean functions are randomly generated for each simulation with incremental

310   lesioning. To execute numerical simulations, we used the BoolODE pipeline by Pratapa et al. [82].

311   BoolODE systematically converts a random Boolean network into a system of SDEs that is a continuous

312   model of gene regulation (for model specifications see SI Text 1 and SI Table). Time points in the

313   numerical solution result in vectors of gene expression values that correspond to individual cells. That

314   means for every analysis, each sampled time point is from a cell [82], and in this study, we sample from

315   990 time points (1000-10, first 10 timepoints treated as burn-in) for each gene in a single simulation and

316   total of 1000 simulations are run for each lesioning percentage increment of 10s (from no cut condition to

317   all 10 genes cut) which makes 10000 simulations for each type of lesioning and thus, 2 (lesioning type) x

318   10 (k subset of perturbed genes) x 11 (cut conditions, no-cut condition inclusive) x 10 synthetic cells with

319   random gene regulatory mechanisms.

Quantification of degeneracy in neural networks

321   To measure degeneracy, we used the mathematical framework described by Tononi et al. [33]. In

322   this framework, degeneracy is characterized in terms of the average mutual information between subsets

323   of elements within a system and an output sheet (which is also a subset of network *X*). The output sheet

324   is a set of randomly chosen nodes in a network and its activity is a result of the interactions the other

325   nodes in the system. Thus, activity in the output sheet represents the behavior or the response of the whole

326   system.

327   From information theory, entropy (Shannon entropy with log base 2 for binary representation) is

328   calculated from probability density functions for subsets of X (Equation 1). Then mutual information that

329   measures the portion of entropy shared by the system subset X_j^k and the output O, is calculated as follows

330   (*Equation 2*):

331
$$H(X) = -\sum_{i=1}^{n} P(x_i)\, log_2\, P(x_i)$$

332   (1)

333

334
$$MI\left(X_j^k; O\right) = H\left(X_j^k\right) + H(O) - H(X_j^k, O)$$

335   (2)

336    $H(X_j^k)$ and $H(O)$ are the entropies of X_j^k and O considered independently, whereas $H(X_j^k;O)$ is the

337   joint entropy of subset X_j^k and output O. To measure degeneracy in the network, we need to determine the

338   effects of the (subset of) element(s) on the entropy of the output - the behavior of the network. Since

339   mutual information does not capture direction, however, mere calculation of mutual information is not

340   enough to determine the contribution of the elements to the output of the system. To overcome this,

341   perturbations (variance) are injected to the system. If no initial variance is assumed in the system, the

342   value of mutual information between the network and the output is zero before any perturbation [33].

343   Variance (perturbation) is injected as uncorrelated random noise to each subset k.

344   Under such perturbations, mutual information of the system is computed as in *EQ3* and this

345   procedure is repeated for all subsets of sizes $1 \leq k \leq n$. Then, degeneracy $D_N(X;O)$ of X with respect to O

346   can be calculated as:

347
$$D_N(X; O) = \sum_{k=1}^{n} [< MI^P\left(X_j^k; O\right) > - \left(\frac{k}{n}\right) MI^P(X; O)]$$

348   (3)

349 $MI^P(X;O)$ is MI for all elements to the output sheet, and $<MI^P(X_j{}^k;O)>$ is the average of the contribution

350 of each perturbed subset size k to the output sheet.

Quantification of degeneracy in random Boolean networks

352   Biological RBN simulations (via BoolODE) results in continuous unit activity in terms of gene

353 expression vectors since the simulated networks are translated into nonlinear dynamical system (SI Text 1

354 and 2, SI Figure). To quantify degeneracy in RBNs we therefore discretize the gene expression vectors by

355 taking the median activity for a unit. Activity that is above the median is set to 1, and activity below the

356 median is set to 0.

357   We apply the degeneracy measures to the discretized gene expression vectors generated from the

358 simulations. In our simulations, perturbations are systematically injected to the subset size k of genes as

359 normally distributed (with *mean = 0,* and *standard deviation = 0.01*) random noise through the governing

360 SDE. The number of elements (namely, the genes) is $n = 10$ for all simulations with output sheet

361 consisted of the activity of $O = n/2 = 5$ elements, that is also randomized for each trial.

362 **Fig5. Illustration of RBNs under perturbation. A network of X, composed of nodes (light and**

363 **dark blue circles, n = 10) that are interconnected.** Arrows represent the edges for incoming,

364 outgoing, and self-connections. Light blue circles represent randomly chosen perturbation subsets of

365 nodes for k = 2 (a) and k = 7 (b). Perturbation (represented as syringes) of the nodes in boxes with k

366 notation, is injected as a variance (uncorrelated noise). The box with O notation represents output

367 sheet that is also consisted of randomly chosen set of (n/2 = 5) nodes (dark blue circles). For each

368 network, MI is calculated for perturbed set size k and the output sheet O, for all subset sizes of

369 perturbed set noted as j.

Partial degeneracy in random networks

370

371　　　Degeneracy can be measured by alternate ways that are mathematically equivalent (see other

372　definitions in [33]). The formal definition that we use in this study requires averaging over every MI

373　measured between each node (unit, j) which are incrementally perturbed (from $k = 1$ to $k = n$) and the

374　output sheet for a given network structure ($<MI^P(X_j^k;O)>$). However, in case where all the networks are

375　randomly generated and the output sheet units are randomly chosen, an alternate way of computing

376　$<MI^P(X_j^k;O)>$　is taking the average of MI measured for each random network that is perturbed once for a

377　particular perturbed subset size k in range of $1 \leq k \leq n$. This way, degeneracy is measured for a specific

378　subset given a network rather than for all possible subset sizes. Here, we call this measurement *partial*

379　*degeneracy*.

Supporting information

380

381　**S1 Text 1. Simulation platform.**

382　**S1 Text 2. Model specifications.**

383　**S1 Figure. A toy network with Boolean functions and translation into SDE.**

384　**References**

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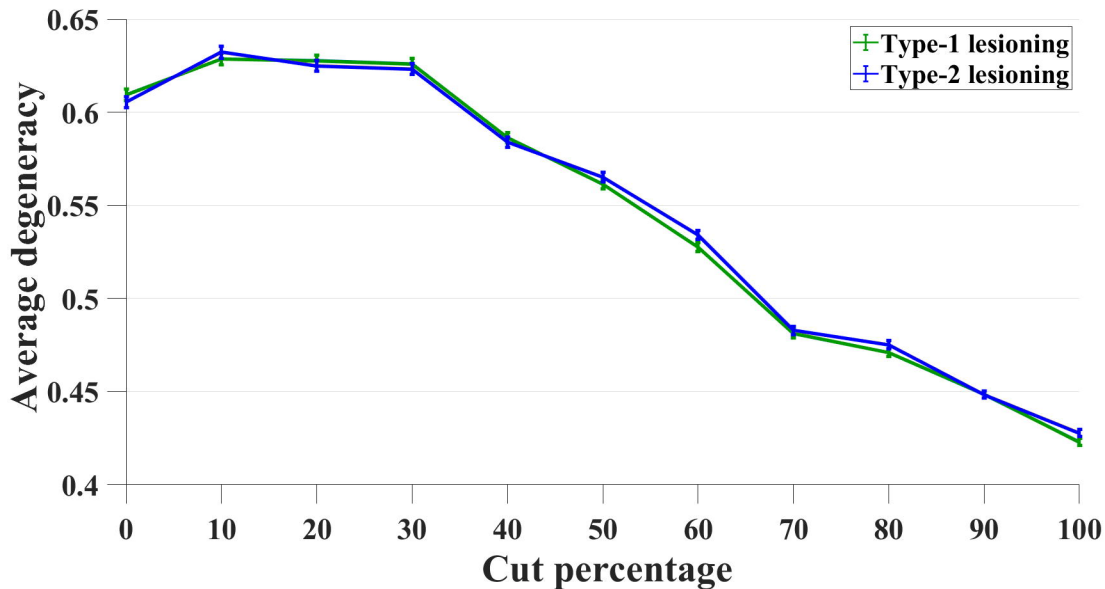
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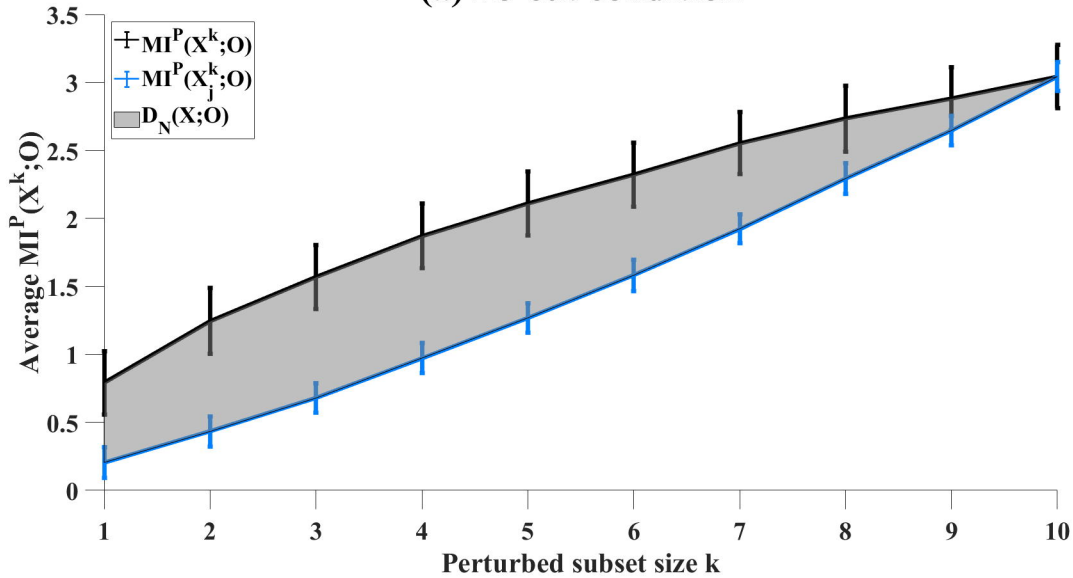
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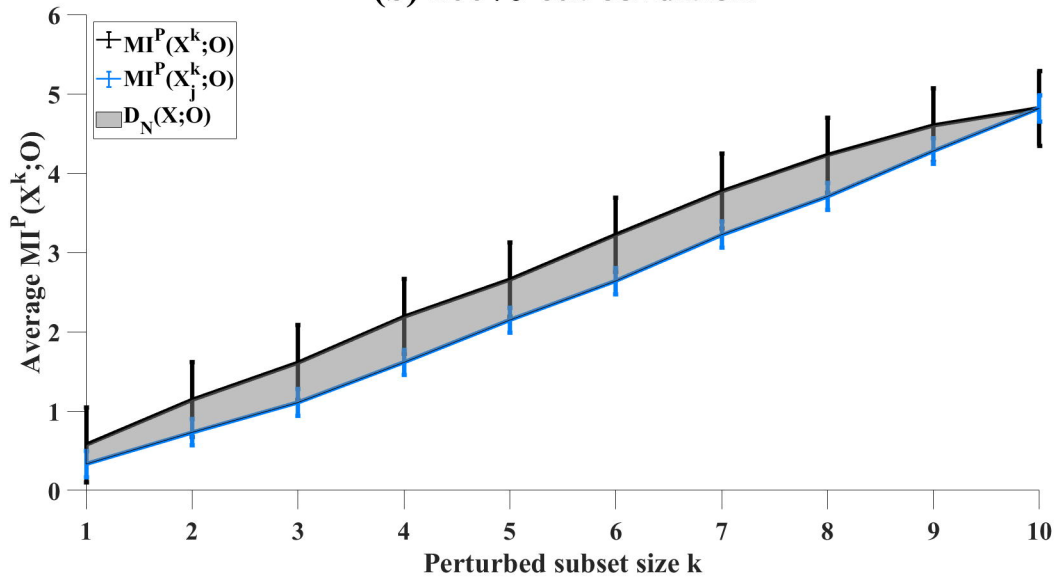
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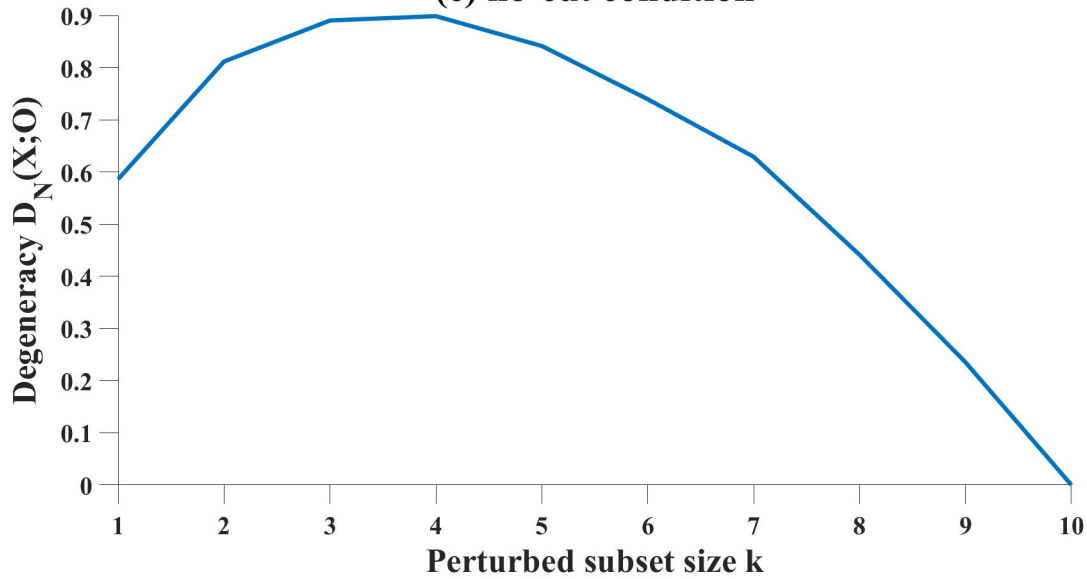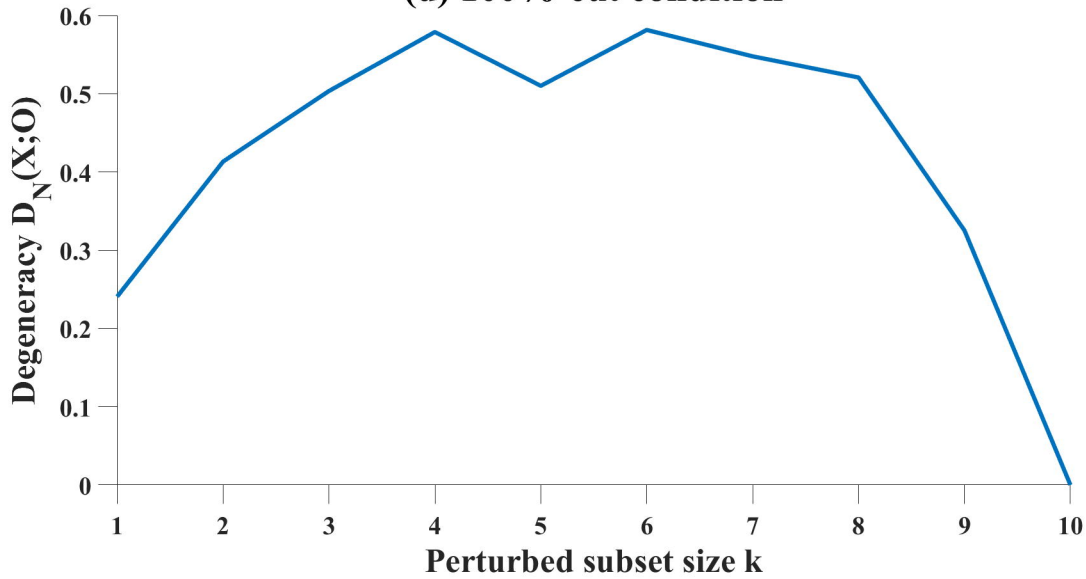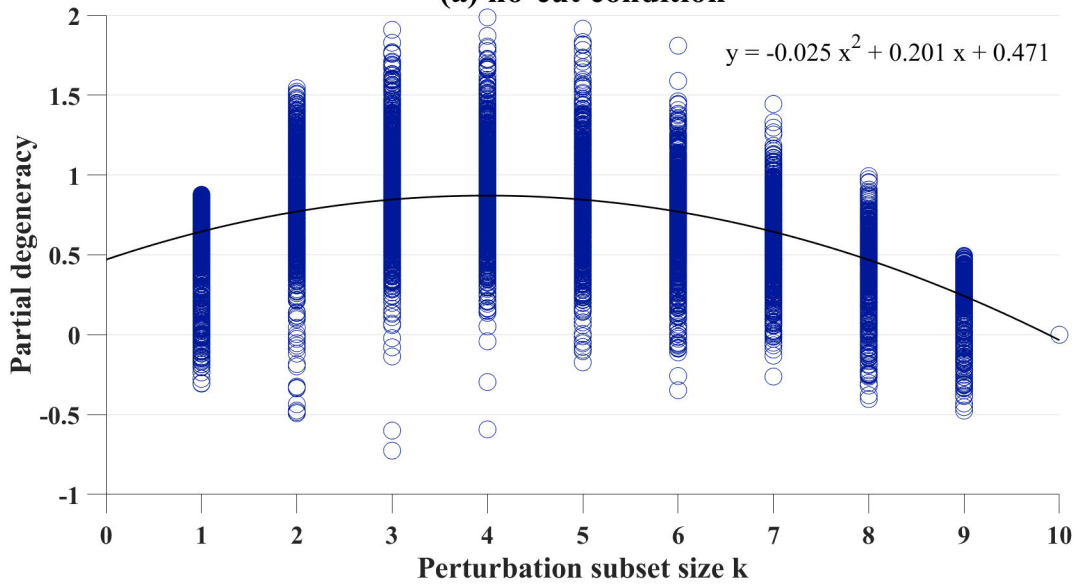
      Feb;17(2):147–54.

(a) no-cut condition

Legend:
- $MI^P(X^k; O)$
- $MI^P(X_j^k; O)$
- $D_N(X; O)$

Y-axis: Average $MI^P(X^k; O)$

X-axis: Perturbed subset size k

(b) 100%-cut condition

(c) no-cut condition

Degeneracy $D_N(X;O)$ vs. Perturbed subset size k

(d) 100%-cut condition

(a) no-cut condition

$y = -0.025\,x^2 + 0.201\,x + 0.471$

(b) 100%-cut condition

$y = -0.022 \, x^2 + 0.227 \, x + 0.025$

Partial degeneracy (y-axis)

Perturbation subset size k (x-axis)

(a) Type-1 Lesioning

(b) Type-2 Lesioning

Legend:
- 0% cut
- 10% cut
- 20% cut
- 30% cut
- 40% cut
- 50% cut
- 60% cut
- 70% cut
- 80% cut
- 90% cut
- 100% cut

X-axis: Perturbed subset size k

Y-axis: Average Degeneracy
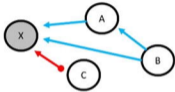
(a) **(b)**

$MI^P(X_j^k; O)$ $MI^P(X_j^k; O)$

k=2   k=7

O   O
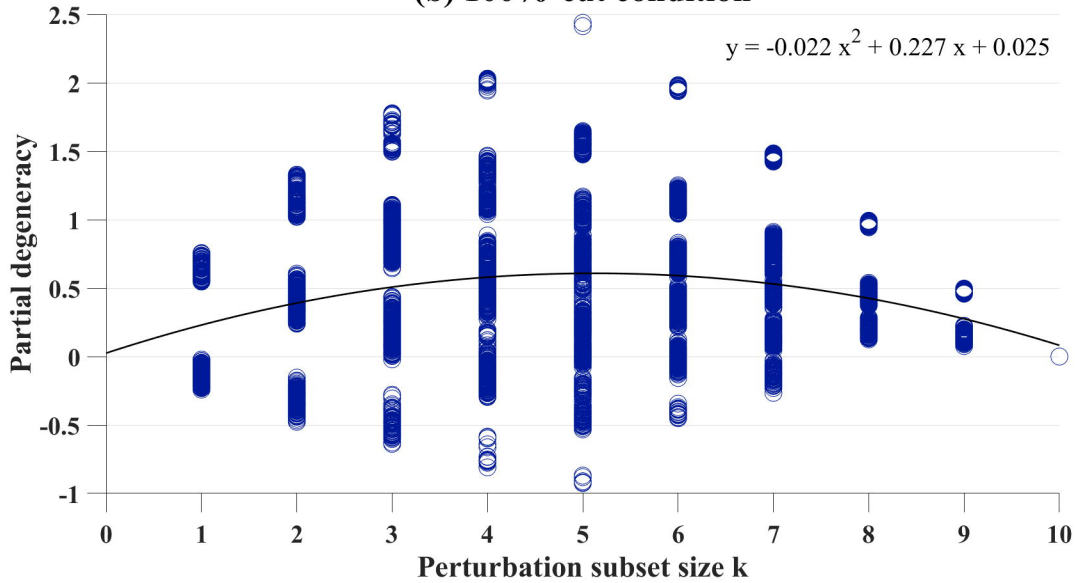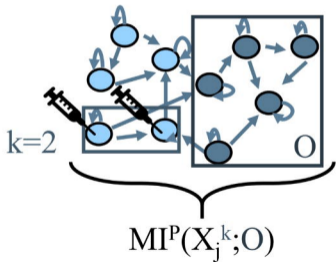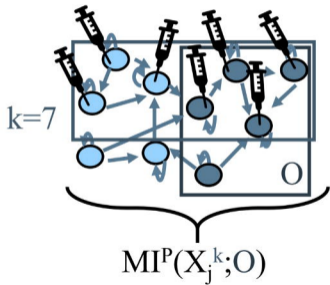
$$X = (A \lor B) \land \neg(C)$$

$$\frac{d[X]}{dt} = m \left(\frac{\alpha_0 + \alpha_A[A] + \alpha_B[B] + \alpha_C[C] + \alpha_{AB}[A][B] + \alpha_{AC}[A][C] + \alpha_{BC}[B][C] + \alpha_{ABC}[A][B][C]}{1 + [A] + [B] + [C] + [A][B] + [A][C] + [B][C] + [A][B][C]} \right) - l_x[X]$$

$$= m \left(\frac{[A] + [B] + [A][B]}{1 + [A] + [B] + [C] + [A][B] + [A][C] + [B][C] + [A][B][C]} \right) - l_x[X],$$