OPINION ARTICLE

REVISED A general hypothesis of multistable systems in pathophysiology [version 3; peer review: 2 approved]

Previously titled: A general theory of multistable systems in pathophysiology

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Abstract

Despite intensive investigations numerous diseases remain etiologically puzzling and recalcitrant to treatments. A hypothesis is proposed here assuming that these difficulties are due to an unsuitable approach to the mechanisms of life, which is subjugated by an apparent complexity and fails to grasp the uniformity that lays behind. The stability of metabolism, despite the enormous complex of chemical reactions, suggests that reciprocal control is a prerequisite of life. Negative feedback loops have been known for a long time to maintain homeostasis, while more recently, different life processes involved in transitions or changes have been modeled by positive loops giving rise to bistable switches, also including various diseases. The present hypothesis makes a generalization, by assuming that any functional element of a biological system is involved in a positive or a negative feedback loop. Consequently, the hypothesis holds that the starting mechanism of any disease that affects a healthy human can be conceptually reduced to a bistable or multistationary loop system, thus providing a unifying model leading to the discovery of critical therapeutic targets.

Keywords

bistable switch, feedback loops, pathogenesis, pathophysiology, systems and control theory, systems biology

Open Peer Review

Approval Status ✓ ✓

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version 3 (revision) 30 Sep 2022		✓ view
version 2 (revision) 24 Aug 2022	✓ view	? view
version 1 05 Aug 2022	? view	

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2. **Sudin Bhattacharya**, Michigan State University, East Lansing, USA

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Burlando B: Conceptualization, Funding Acquisition, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

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REVISED **Amendments from Version 2**

Major differences with respect to the previous version.

Two new paragraphs have been introduced: "Limitations to the hypothesis" and "Hypothesis validation". A total of five new references have been introduced.

In the paragraph "A loopomic hypothesis in pathophysiology", it has been clarified that the development of diseases observed in clinical setting, appearing as gradual progressions rather than abrupt changes, is the result of cascade events realizing complex patterns that hinder disease management. Conversely, the article hypothesis assumes the occurrence of, and is focused on, multistable transitions as point-like events at the root of these processes.

In the paragraph "Limitations to the hypothesis", it has been considered that the multistability of molecular feedback loops concerns behaviors at the level of individual cells that not necessarily spread uniformly to whole tissues or organs. It has been suggested that multi-omic, single-cell studies may be deployed to disclose the underlying switches or bifurcations behind the development of a disease.

In the paragraph "Hypothesis validation", the theory of critical state transitions in dynamic systems and its application to disease development have been discussed, highlighting the importance of multistable transition analysis also in preventive healthcare survey.

Any further responses from the reviewers can be found at the end of the article

Introduction

Complexity is one of the most addressed features of life and a possible major hindrance to the advancement of knowledge in life science. This inevitably makes life science a non-exact scientific field largely operating through qualitative, verbose descriptions, as opposed to the quantitative, mathematical models of physics. Consequently, the predictivity of theoretical models in life science is generally low, posing serious limits to their application to the real world. This is mirrored by serious difficulties in biomedical investigations, with several diseases remaining etiologically puzzling and recalcitrant to treatments, including tumor malignancies, neurodegenerative diseases, immune disorders, metabolic syndromes, and different infectious diseases. However, rather than being due to the excessive complexity of life, these drawbacks could derive from an unsuitable approach to the study of life processes, including transitions from physiological to pathological conditions.

The human body is believed to consist of about 10^{13} cells (Bianconi *et al.*, 2013), while about 10^{10} s^{-1} chemical reactions can be roughly calculated to occur inside each cell, considering basal metabolism and the energy released per mole by ATP hydrolysis (Wackerhage *et al.*, 1998), thus making up a total of about 10^{23} s^{-1} chemical reactions in the whole body. The ability of maintaining a metabolic steady state, despite such a huge complex of events, seems statistically paradoxical. However, this would be true if metabolic processes were uncorrelated from each other, whereas they are supposed to be strictly regulated and cross-adjusted (Burlando, 2017; El-Samad, 2021). Hence, if reciprocal control is the prerequisite of life processes, their study cannot be exempted from an appropriate analysis of control mechanisms.

Seminal theoretical work by Ludwig Von Bertalanffy has led to the development of systems theory (von Bertalanffy, 1968), which in the beginning was inspired by living systems, and thereafter was widely applied to engineering. Thereafter, systems and control theory has developed the study of dynamic systems consisting of negative or positive feedback loops, i.e. sequences of interactions among functional agents arranged as closed chains (Blanchini *et al.*, 2014). Negative loops have an uneven number of inhibitory steps, in addition to possible activation steps, and one stable equilibrium point or a limit cycle with oscillatory behavior. By contrast, positive loops have an even number of inhibitory steps, or none, and admit multiple stable equilibrium points.

Physiological mechanisms based on negative feedback loops are known to maintain homeostasis, or induce oscillations within fixed boundaries, thus ensuring the body's steady state (Blanchini *et al.*, 2014; Burlando *et al.*, 2019). Conversely, positive feedback loops have been classically underestimated and assumed to occur sparingly, due to their supposed destabilizing effect in need of compensation by negative loops. However, the advent of systems biology has favored a new analysis of life complexity, by focusing not so much on the biodiversity of life constituents, but rather on the interactions that are established between them (Wolkenhauer and Mesarovic, 2005; Wolkenhauer *et al.*, 2005). Such a new trend has proved that several life processes, especially those that produce irreversible changes, can be modeled by positive loops. A noncomprehensive list includes bistable gene expression (Olivenza *et al.*, 2019), cell cycle (Mochida *et al.*, 2016; Vigneron *et al.*, 2018; Stallaert *et al.*, 2019), mitosis (Hutter *et al.*, 2017), cell migration (Nguyen *et al.*, 2018), cell differentiation (Wang *et al.*, 2009), and axon growth (Padmanabhan and Goodhill, 2018).

A loopomic hypothesis in pathophysiology

The accumulating evidence that loops play an essential role in several functions accomplished by living beings (El-Samad, 2021) has inspired the loopomics paradigm, i.e. the assumption that any functional element of a biological system is somehow involved in a loop mechanism, while the whole system can be conceptualized as an intertwined array of loops (Burlando, 2017). Hence, the dynamics of the whole system obey to a restricted number of rules, but nevertheless, they give rise to a highly complex biodiversity, expressed in terms of epiphenomena occurring at different dimensional scales, e.g. subcellular organelles, cells, tissues, human beings, etc. Hence, given the above dynamical behavior of functional loops, any change or transition that can be observed within the human body would be the result of a multistable positive loop system switching among different equilibrium points (Laurent and Kellershohn, 1999).

This is a completely new redefinition of life, shifting from complexity to uniformity through a comprehensive and unifying modeling in terms of loops. Such a new paradigm has deep repercussions in the field of medicine because any disease that affects a healthy organism must have at its origin an early event in the form of a change that drives the system from a physiological to an altered functional regimen.

A rapidly increasing number of pathogenic processes are being modeled as bistable switches, a few representative examples of which include cancer (Kim *et al*., 2007; Huang *et al*., 2014), prion infections (Kellershohn and Laurent, 2001), immunological disorders (Beutler, 2009), dermatitis (Dominguez-Huttinger *et al*., 2017), neurological problems (Mucci *et al*., 2020), and neurodegenerative diseases (De Caluwe and Dupont, 2013; Burlando *et al*., 2020). In other cases, a bistable model has not been explicitly used to describe pathogenesis, but a positive loop has been nevertheless invoked (Beutler, 2009; Norwitz *et al*., 2019). However, this kind of approach is generally conducted on a case-by-case basis, without making any attempt at proving generalizability, despite long established evidence that could be used to move towards this direction, as explained below. First, it is obvious that diseases affecting a healthy human must involve at their origin pathophysiological changes leading to transitions from a physiological to pathological condition. Second, diseases are classifiable (e.g. https://www.who.int/standards/classifications/classification-of-diseases), i.e. they make up an ordered set, showing that the above changes are predictable. Third, diseases are recurrent through human generations, showing that pathophysiological changes depend on the activity of clonable physiological pathways that have been selected for by natural selection. Therefore, these changes must admit stability as a starting point, otherwise their selection would not have been possible. However, stability must also characterize their ending point, otherwise disease classification would not be possible. In summary, diseases depend on predictable changes carried out by multistable systems, and therefore a generalization of these processes can be achieved by using dynamic models able to develop transitions between different stable equilibrium points.

Such a view is to some extent counterintuitive because diseases are generally thought as complex processes involving many factors, progressing as gradual processes rather than by abrupt switch-like transitions, like for example diabetes, cardiovascular disorders, and obesity. However, the clinical presentation of a disease, independently from its severity, generally consists of several altered metabolic and physiological conditions deriving from widespread cascade effects. This can be inferred e.g. from the SOD1-G93A mouse model of amyotrophic lateral sclerosis, in which metabolic alterations are detectable well before symptom onset (Ravera *et al.* 2019). In contrast, the present analysis is aimed at understanding the very early events located at the boundary between the physiological and the pathogenic condition, i.e. the seminal etiological processes.

Given the above considerations, a new hypothesis can be proposed, stating that any disease that affects a healthy human involves at its primary causal event a change that can be conceptually reduced to a multistable system (typically a bistable one) depending on a positive functional loop consisting of cellular, molecular, or biochemical elements interplaying with each other. Also, the mathematical study of loop dynamics can allow the identification of bifurcation parameters that drive the transition from monostability to multistability (Freyer *et al.*, 2012), thus realizing the condition for the switching of the system from a "physiological" to a "pathological" stable equilibrium point, or steady state. Hence, the physical correspondents of these parameters, which could be single factors or pathways, are to be recognized as best therapeutic targets, potentially allowing the achievement of disease manageability via pharmacological or non-pharmacological treatments. Moreover, this kind of system is characterized by hysteresis, i.e. the transitions from physiological to pathological steady states occur at different threshold values of the bifurcation parameter, according to the direction of movement along the steady state trajectory, and therefore, irreversibility is also possible for a strengthening of the interactions among the functional agents of the loop (Figure 1).

Limitations to the hypothesis

Given the elevate number of elements and interactions that constitute metabolic and physiological pathways, the problem of identifying analytically manageable feedback loops that realize switch mechanisms, and isolating the essential elements that participate in these systems, is a main challenge. A first possibility of simplification derives from the

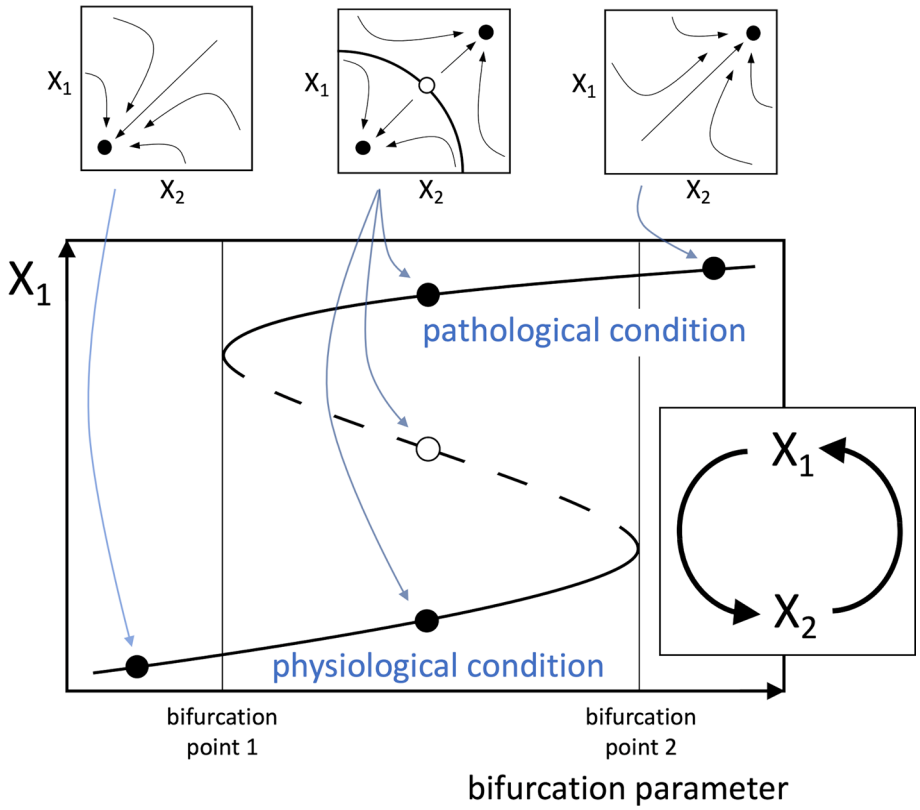

Figure 1. Schematic positive loop, representing a simplified model of a hypothetical pathogenic process. The illustrated system captures the essential elements of the proposed theory, though more complex loops are likely to occur in real pathophysiological processes. The loop (inset below) consists of two functional agents, indicated by X_1 and X_2, which may represent the amounts or activities of cells (e.g. lymphocytes), enzymes (e.g. kinases), signal molecules (e.g. interleukins), etc. In the mathematical analysis of the loop, the dynamical interactions between the functional agents are described by a system of differential equations yielding the rate of change of the functional agents. In commonly used models of biological systems dynamics, each functional agent X_i is assumed to undergo spontaneous inactivation and evolve along time with time constant τ_{X_i}. The system of differential equations, in a schematic form, is the following:

$$\tau_{X_1}\dot{X}_1 + X_1 = f(X_2)$$

$$\tau_{X_2}\dot{X}_2 + X_2 = f(X_1)$$

A possible example for the functions appearing in the differential equations is the Hill function $f(x) = \frac{\alpha x^p}{1+\beta x^p}$, which is a suitable model for different stimulus-response relationships in biological systems (Ferrante *et al.*, 2009; Huang *et al.*, 2014). However, $f(X_1)$ and $f(X_2)$ need not have the same form. By solving the system of differential equations, the trajectories followed by the system in the X_1/X_2 phase portrait can be traced (small plots at the top), showing the attraction basins of the stable equilibrium points (full dots), i.e. the system steady-states, and an unstable equilibrium point (empty dot). The bifurcation diagram (large plot below) shows the plot of the equilibrium values of X_1 as a function of a bifurcation parameter, represented by one of the parameters of the differential equations. A similar plot can be derived for X_2. The continuous lines represent stable equilibrium points and the dashed line unstable equilibrium points. As the bifurcation parameter increases (or decreases, depending on cases), the system can change from monostable (left), i.e. having a single stable equilibrium point, to bistable (middle), i.e. with two stable equilibrium points and an intermediate unstable equilibrium point, and eventually to monostable again (right). The two alternative stable equilibrium points represent the physiological and pathogenic conditions. Therefore, depending on the direction of variation, the bifurcation factor creates the conditions for the switch from physiological to pathogenic condition, or promotes the backward transition, respectively. The system also shows hysteresis, i.e. as shown in Figure 1, as the bifurcation parameter increases, and in the absence of other stimuli, the system is forced to abruptly jump from the physiological to the pathological condition at bifurcation point 2. However, once the system resides on the pathological condition, the reverse transition to physiological condition requires that the bifurcation parameter decreases until it reaches bifurcation point 1.

notion that metabolic pathways can be often considered as aggregates of input/output monotone subsystems, allowing to combine different chain elements into a single element (Blanchini *et al.*, 2018). Moreover, it has been shown that, if monotonicity is satisfied, conditions for bistability can be mathematically assessed in feedback systems of arbitrary order (Angeli *et al.*, 2004). This kind of theoretical achievements are fundamental for the herein proposed approach to the study of diseases, since they provide hints for the identification of biological elements acting as critical bifurcation parameters.

Another issue to be considered is that the multistability of molecular feedback loops concerns behaviors at the level of individual cells that not necessarily spread uniformly to whole tissues or organs. Even among cells of the same type, noisy gene expression can obscure discrete switching and make the transition from one state to another more gradual at the aggregate level. This has been clearly highlighted in cancer, with variable tumorigenic features being possibly observed within the individual malignant cells of a tumor (Kreso and Dick, 2014). To address this kind of problems, multi-omic, single-cell studies may be deployed to disclose the underlying switches or bifurcations behind the development of a disease (Zhang *et al.*, 2022).

Hypothesis validation

In operational terms, if a completely new disease is discovered, what is the main question to be addressed in order to manage it? Of course, this question concerns etiology, i.e. identifying the pathophysiological process leading to the primary causes of the disorder. Until we have an answer to this question, few possibilities exist to find a complete solution to the disease, and this is the main drawback that frequently affects biomedical research. According to the herein proposed hypothesis, the above unexplored disease should be investigated by using currently available and newly acquired knowledge through the following steps from bench to bed: (i) localize the site of insurgence, (ii) identify major biological (molecular) factors involved, (iii) identify a positive loop (or a loop system collectively behaving as a positive loop) that could allegedly drive the early pathogenic transition, (iv) develop formal mathematical analysis of the loop leading to the identification of critical bifurcation parameter(s), (v) design *in vitro* and/or *in vivo* experiments to verify that the loop and its bifurcation parameter(s) are responsible for the development of the disease. If successfully accomplished, these investigations would open the way to finely oriented pharmacological and pharmaceutical research directed to bifurcation parameter(s), followed by clinical trials.

Besides finding solutions to ongoing pathological conditions, the analysis of multistable systems could also be determinant in preventive healthcare. A theory of critical state transitions in dynamic systems predicts that in proximity to a bifurcation point the return to the current equilibrium state upon random perturbation shows critical slowing down, together with an increased fluctuation of system variables (Scheffer *et al.*, 2009). This idea has been applied to the study of disease progression by developing dynamic network biomarkers, e.g. by high-throughput gene expression analysis, able to reveal the approach to a critical pre-disease state (Liu *et al.*, 2013; Deb *et al.*, 2022).

This kind of approaches is assumed to be the most suitable to fit the nature of life processes and therefore, given that health problems also derive from life processes, it is expected to maximize the chance of finding solutions to diseases. Although not intentionally hinged in the present hypothesis, a study in this direction has already been done through a large-scale characterization of bistable switch-like, gene-gene interactions in cancer progression (Shiraishi *et al.*, 2010).

Conclusions

A huge set of data from systems biology suggests that changes occurring within living systems are the result of the activity of multistable switches depending on positive functional loops. The theory of systems and control provides a description of loop system dynamics in terms of a restricted set of mathematical rules, providing a basis for bypassing the drawbacks caused by the biodiversity of life constituents, spanning from molecules to individuals. Such a new conceptualization, focused on interactions rather than on objects, results in a recodification of life in terms of biouniformity, instead of biodiversity, allowing the application of unifying formal analyses on a ground where this approach seemed almost impossible. Accordingly, provided that pathogenic processes affecting healthy individuals must themselves be the result of functional changes, a general hypothesis of pathogenesis can be formulated. Based on this theory, the enormous diversity of pathologies that have been described in the human body can be essentially reduced to a single, unifying pathogenic model, of which any disease would represent a variant. Consequently, each disease can be approached by a standard procedure of analysis aimed at identifying positive loops and their bifurcation parameters, thus addressing critical pharmacological targets. Such a theoretical framework could have a huge impact on pharmacology, medicinal chemistry, and clinical practice. Therefore, given the impasse of many approaches to the study of pathogenesis, this hypothesis would deserve to be validated on specific cases by preclinical and clinical studies, in order to explore the extent to which it can be generalized and represent a turning point in biomedical and pathophysiological research.

Data availability

No data are associated with this article.

Acknowledgments

I wish to thank Giulia Giordano and Franco Blanchini for their advice and suggestions.

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Current Peer Review Status: ✔ ✔

Version 3

Reviewer Report 03 October 2022

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✔ **Sudin Bhattacharya**
Department of Biomedical Engineering, Center for Research on Ingredient Safety, Michigan State University, East Lansing, MI, USA

The author has responded to my comments in a satisfactory manner. Thanks for considering my suggestions.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Computational biology, Bioinformatics, Computational toxicology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 22 September 2022

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? **Sudin Bhattacharya**
Department of Biomedical Engineering, Center for Research on Ingredient Safety, Michigan State University, East Lansing, MI, USA

The author offers an interesting hypothesis: that multistability, specifically bistability, underlies

transitions from health to disease. The basis and implications of this hypothesis are discussed, and a quantitative analysis of diseases is proposed by identifying underlying molecular feedback loops and bifurcation parameters, which can then be used as potential therapeutic targets.

However, while switching among multiple stable steady states does indeed underlie many physiological processes like the cell cycle, it is not obvious that all disease progression is characterized by abrupt switch-like transitions from healthy to diseased states. For example, development of diabetes, heart disease, or obesity is presumably a gradual process. This is a distinction that the author may want to address. Further, even for diseases that are indeed marked by multistability, identification of all underlying molecular feedback loops and the bifurcation parameters is likely to be a daunting task.

Another issue worth noting is that the phenomenon of bistability arising from molecular feedback loops concerns behavior at the level of individual cells, not necessarily for whole tissues or organisms. Even among cells of the same type, noisy gene expression can obscure discrete switching and make the transition from one state to another more gradual at the aggregate level. Multi-omic single-cell studies may help clarify whether there are indeed underlying switches or bifurcations behind development of different diseases. It would be helpful to address this point in the manuscript.

As a final point, I would suggest that the author include some discussion of the theory of critical state transitions as proposed by Scheffer et al[1], which suggests that switch-like transitions in natural phenomena may be predicted by early warning signals like increased fluctuation of system variables. This idea has been explored in the context of disease progression[2].

Overall, this manuscript provides an interesting and potentially useful hypothesis about disease development.

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Is the topic of the opinion article discussed accurately in the context of the current literature?

Partly

Are all factual statements correct and adequately supported by citations?

Yes

Are arguments sufficiently supported by evidence from the published literature?

Partly

Are the conclusions drawn balanced and justified on the basis of the presented arguments?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Computational biology, Bioinformatics, Computational toxicology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 Sep 2022

Bruno Burlando, University of Genoa, Genoa, Italy

I wish to thank the reviewer for appreciating the article and providing various useful suggestions. I have now realized and submitted a new version. Responses to the reviewer's comments and main changes in the new version follow (author in bold):

Rev. However, while switching among multiple stable steady states does indeed underlie many physiological processes like the cell cycle, it is not obvious that all disease progression is characterized by abrupt switch-like transitions from healthy to diseased states. For example, development of diabetes, heart disease, or obesity is presumably a gradual process. This is a distinction that the author may want to address.
Au. This point deserved to be discussed and has been addressed in the paragraph entitled "A loopomic hypothesis in pathophysiology". Specifically, it has been clarified that the development of diseases observed in clinical setting, appearing as gradual progressions rather than abrupt changes, is the result of cascade events realizing complex patterns that hinder disease management. Conversely, my hypothesis assumes the occurrence of, and is focused on, multistable transitions as point-like events at the root of these processes.

Rev. Further, even for diseases that are indeed marked by multistability, identification of all underlying molecular feedback loops and the bifurcation parameters is likely to be a daunting task
Au. This point had already been addressed after previous revision. But now, also due to other changes in this revision, I have subdivided the former paragraph "A loopomic hypothesis in pathophysiology" into three ones, by introducing two new paragraphs "Limitations to the hypothesis" and "Hypothesis validation".

Rev. Another issue worth noting is that the phenomenon of bistability arising from molecular feedback loops concerns behavior at the level of individual cells, not necessarily for whole tissues or organisms. Even among cells of the same type, noisy gene expression can obscure discrete switching and make the transition from one state to another more gradual at the aggregate level. Multi-omic single-cell studies may help clarify whether there are indeed underlying switches or bifurcations behind development of different diseases. It would be helpful to address this point in the manuscript.
Au. This point has been addressed in the new paragraph "Limitations to the hypothesis" by closely following the reviewer indications.

Rev. As a final point, I would suggest that the author include some discussion of the theory

of critical state transitions as proposed by Scheffer et al1, which suggests that switch-like transitions in natural phenomena may be predicted by early warning signals like increased fluctuation of system variables. This idea has been explored in the context of disease progression2.
Au. This point has been addressed in the new paragraph "Hypothesis validation" by highlighting the importance of multistable transition analysis also in preventive healthcare survey.

A total of five additional references have been quoted in the new version of the manuscript and added to the list at the end of text. The complex of changes has considerably improved the contents of the article.

Competing Interests: No competing interests.

Reviewer Report 01 September 2022

https://doi.org/10.5256/f1000research.137760.r148428

Rodrigo A. Mora-Rodríguez
Research Center on Surgery and Cancer (CICICA), Campus Rodrigo Facio, University of Costa Rica, San José, Costa Rica

The author has addresses all my concerns. Congratulations on this beautiful review.

Is the topic of the opinion article discussed accurately in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Partly

Are arguments sufficiently supported by evidence from the published literature?
Partly

Are the conclusions drawn balanced and justified on the basis of the presented arguments?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Systems Biology, miRNA research, cancer, virology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 18 August 2022

https://doi.org/10.5256/f1000research.135266.r146862

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Rodrigo A. Mora-Rodríguez
Research Center on Surgery and Cancer (CICICA), Campus Rodrigo Facio, University of Costa Rica, San José, Costa Rica

This is a beautiful opinion article accurately summarizing what we, many systems biologists think and see biological complexity.

Complexity implies that almost everything is connected within an interaction network and the influence of a perturbation could impact other parts of that network. This network could offer robustness but also, beyond certain level of perturbation lead to a different steady state.

Although very interesting, I think that the problem is the word theory. To speak about a general theory, more systematic (and directed) validation is required. I would suggest to change that word to "hypothesis" in the title and along the text.

Apart from that, this hypothesis is fantastic and I would just like to see something more about how the author suggest that bistability can be identified, specially in complex loops since this is one the main limitations of the study of bistability. Perhaps the work of Angeli et al could be of some interest for the author.[1]

References

1. Angeli D, Ferrell JE, Sontag ED: Detection of multistability, bifurcations, and hysteresis in a large class of biological positive-feedback systems.*Proc Natl Acad Sci U S A*. 2004; **101** (7): 1822-7 PubMed Abstract | Publisher Full Text

Is the topic of the opinion article discussed accurately in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Yes

Are arguments sufficiently supported by evidence from the published literature?
Yes

Are the conclusions drawn balanced and justified on the basis of the presented arguments?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Systems Biology, miRNA research, cancer, virology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 21 Aug 2022

Bruno Burlando, University of Genoa, Genoa, Italy

I wish to thank the reviewer for the appreciation of my article. Based on the peer review comments, I have prepared a new version of the article. Responses to the reviewer's comments and main changes in the new version follow (author in bold):

Rev. Complexity implies that almost everything is connected within an interaction network and the influence of a perturbation could impact other parts of that network. This network could offer robustness but also, beyond certain level of perturbation lead to a different steady state.
Author. In the new version I have more accurately addressed the problem of complexity in the interactions among biological functional agents. Specifically, input/output monotonicity has been addressed, being a distinctive feature of these interactions and a key element for the possibility of realizing mathematical models able to provide information for operational interventions on these systems, e.g. resolving a pathological condition.

Rev. Although very interesting, I think that the problem is the word theory. To speak about a general theory, more systematic (and directed) validation is required. I would suggest to change that word to "hypothesis" in the title and along the text.
Author. The word "theory", when referred to the present proposal, has been substituted with "hypothesis". By this way, also the title has been modified.

Rev. Apart from that, this hypothesis is fantastic and I would just like to see something more about how the author suggest that bistability can be identified, specially in complex loops since this is one the main limitations of the study of bistability. Perhaps the work of Angeli et al could be of some interest for the author.
Author. Together with complexity, the issue of bistability has also been addressed and it has been explained more clearly how it is linked to the identification of bifurcation parameters. These latter are a crucial element of the proposed hypothesis, as being considered main sites of "engineering" interventions on biological systems, obviously including pathological conditions. The suggested reference has been quoted, together

with a few others.

Competing Interests: No competing interests were disclosed.