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Research article

A new model for the inference of biological entities states: Ternary Entity State Inference System

Ziwei Zhao, Jingxuan Liang, Xianbao Zhang, Wenyan Li, Yun Wang *

Information Engineering Research Center for Traditional Chinese Medicines, Beijing University of Chinese Medicine, Beijing, 100029, China

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ABSTRACT

Understanding the state transitions in biological systems and identifying critical steady states are crucial for investigating disease development and discovering key therapeutic targets. To advance the study of state transitions in specific biological entities, we proposed the Ternary Entity State Inference System (T-ESIS). T-ESIS builds upon the Entity State Inference System by providing richer information on entity states, where states can take values of 0, 1, or 1/2, representing activation, inhibition, and normal states, respectively. This method infers state transition pathways based on interaction relationships and visualizes them through the Entity State Network. Furthermore, the cyclic structures within the Entity State Network capture positive and negative feedback loops, providing a topological foundation for the formation of steady states.

To demonstrate the applicability of T-ESIS, entity states were modeled, and attractor analysis was conducted in non-small cell lung cancer (NSCLC) networks. Our analysis provided valuable insights into targeted therapy for NSCLC, highlighting the potential of T-ESIS in uncovering therapeutic targets and understanding disease mechanisms. Moreover, the proposed T-ESIS framework facilitated the inference of entity state transitions and the analysis of steady states in biological systems, offering a novel approach for studying the dynamic principles of these systems. This ternary dynamic modeling approach not only deepened our understanding of biological networks but also provided a methodological reference for future research in the field.

1. Introduction

In the realm of systematic biology, there is substantial emphasis on the dynamic behaviors of the systems [1–3]. The systems consist of complex interactions, where each entity—whether protein or gene—can exhibit various dynamic states. These states and their transitions play a significant role in the progression of diseases, the determination of cell fate, and the regulation of biological responses. Furthermore, the ability to predict changes in system states, driving the system from a dysfunctional condition to a desired state, is essential for the design of disease treatment strategies [4,5]. Therefore, extensive research has been conducted on state analysis of biological networks [6]. Boolean network (BN) stands as a classical research paradigm in this domain [7]. It simplifies the encoding of system entity states into binary terms and employs Boolean functions to map out state transition diagrams. In practical applications, Boolean models have been proven effective for analyzing gene regulatory networks. Moreover, the attractors found in biological systems based on BN have also provided inspiration for the identification of therapeutic targets and the study of disease progression [8–10]. Building upon this foundation, some researchers have then proposed multi-valued networks to convey richer biological

* Corresponding author.
E-mail address: wangyun@bucm.edu.cn (Y. Wang).

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meanings and make them more applicable to specific biological contexts [11,12].

To further capture the detailed process of state changes within systems, we proposed a new model: the Entity State Inference System (ESIS). ESIS aimed to deepen our comprehension of complex systems by offering a refined depiction of biological entity states. Compared to current models that analyze system states, our approach emphasized individual entity states and their interrelationships within the system. Additionally, a three-value logic was employed to depict richer biological significance. In biological systems, some processes or states do not always present a binary of being fully activated or fully suppressed, but may instead exist in intermediate or transitional phases. The introduction of a third state, 1/2, alongside 0 and 1, enabled the modeling of these nuances, enhancing the representation of complex biological entity interactions. The flow chart for modeling was shown in Fig. 1. Firstly, an ESIS was introduced for the deduction of the state evolution of system entities based on their interacting relationships. Moreover, the inference results could be visualized as the Entity State Network (ESN). ESIS is equipped with reasoning capabilities and is adaptable to systems with known structures, whether they are macroscopic, microscopic, multi-hierarchical, discrete, or continuous. And ESN can depict the state transition pathways for each entity of the system, which provides a more comprehensive representation of system states. Secondly, in order to tailor the state network modeling of biological molecular networks, the entity state values were set to 0, 1, or 1/2, and logic rules were established for three-valued state transitions. Based on this, the Ternary Entity States Inference System (T-ESIS) was built to represent the Ternary Entity State Network (T-ESN). The T-ESIS utilized 1/2 to represent normal or intermediate entity states and 0 and 1 for abnormal states, which aligned more closely with the actual conditions of biological systems. Subsequently, given the biological significance of steady states in complex networks, we focused on the cyclic structures within the ESN, representing the system's periodic steady states. Our research revealed that these cycles in ESN, which served as the feedback loop of the system, contributed to the dysfunctional steady states or the maintenance of normal steady states. This observation led us to propose a method for analyzing the steady state of ESN based on the search for cyclic structure. Building upon the constructed system, non-small cell lung cancer (NSCLC) was chosen as an example to demonstrate the practicability of our method. Following the construction of a protein-protein interaction (PPI) network, the T-ESN of NSCLC was established, and attractor analysis was further conducted. This study proposed a new approach to infer entity state transitions and model a three-valued ESN, offering a methodological reference for dynamical studies on complex biological networks.

2. Methodology

In this study, the ESIS and the further developed biological T-ESIS are both conducted based on the entity grammar systems (EGS) [13]. Originating from Chomsky's formal language theory, EGS introduces a new set of operations, *F*. An entity grammar system *G* is defined as a quintuple, $G = (V_N, V_T, F, P, S)$, where V_N is a finite set of non-terminal symbols, V_T is a finite set of terminal symbols, and $V_N \cap V_T = \varnothing$. *F* is a finite set of operations: $F = \{ f_i \mid f_i: (E(V,F))^n \rightarrow E(V,F),\ 1 \leq i \leq m, m, n \in N \}$, where $V = V_N \cup V_T$. *P* is a finite set of productions $\alpha \rightarrow \beta$, *with* $\alpha \in E^+(V,F)$ *and* $\beta \in E(V,F)$ applied in parallel. *S* represents the starting entity. Compared to Chomsky's formal language theory, EGS retains the ability to depict and generate system structures while enhancing the expression of intricate entity systems, enabling greater computational and deductive capabilities [14]. Published in 2003, EGS has since been employed in establishing systems for the mechanistic analysis of traditional Chinese medicine and its formulations. It has been applied in modeling, learning, and simulating biological cells, as well as elucidating the mechanisms behind emergent phenomena in complex systems. These applications demonstrated the power of EGS in studying complex hierarchies of biological systems and controlling the

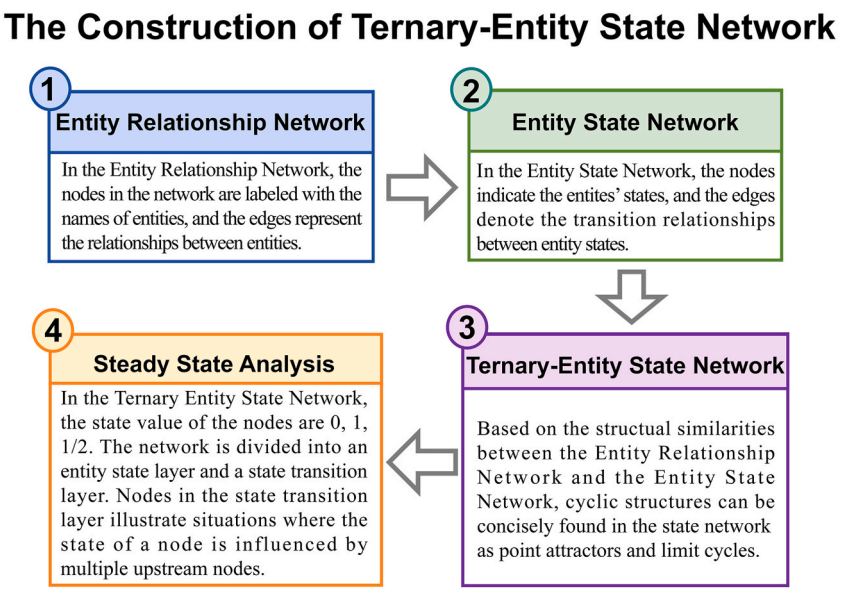

Fig. 1. The flow chart of constructing the Ternary Entity State Network and the steady state analysis.

generation of emergencies under certain conditions [15–19].

In our research, EGS was primarily applied to model and infer the states of biological systems. Moreover, the definitions of entities and their organizational structures from the EGS were adopted, with more details available in references [14]. Our emphasis was placed on the description of entity states, the formulation of transition rules, and the deduction of entity state evolution. Therefore, although the ESIS constructed in this study shared a similar form with EGS, further developments have been undertaken to make it more applicable to complex biological systems in the following aspects:

(1) The definition of the structure for a kind of entity: Each independent entity, according to its definition, can be expressed as $f(\xi 1, \ldots, \xi n)$, which is composed of a set of components $(\xi 1, \ldots, \xi n)$ and a new set of operations, F. In EGS, each element in the set F represents a way of character organization. The elements of the character set V, formed through these organizational methods, constitute a new unit $E(V, F)$, which is considered an entity. This study defined the entity states as non-terminal characters (V_N) and their values as terminal characters (V_T), with $V = V_N \cup V_T$. The relationships between entities were represented as operator F. The newly formed unit $E(V, F)$ possessed the dual functions of describing the organizational structure of the system and the states of its components.

(2) The definition of the production rules: In EGS, the set P comprises deduction rules that implement the system's generation function. These rules can be broadly categorized into recursive productions, context-sensitive productions, context-free productions, and regular productions. The formal expression of evolution rules in complex biological systems should ideally reflect the changing laws that govern the systems under study. Therefore, during the construction of the state inference system, mathematical and logical inference relationships were formulated based on actual interactions between biological entity states. Subsequently, we constructed the state inference system based on Boolean logic and ternary operators.

Grounded in grammar rules, this study primarily employed EGS to conduct the formal modeling and analysis of complex biological systems. Furthermore, the ESIS was established to provide a more comprehensive depiction of dynamic entity behaviors.

3. The definition of Entity State Inference System

To better understand the dynamic behaviors of complex systems, the ESIS was established to delineate the entity state transition diagram. ESIS possesses the capacity to deduce the transforming relationships among all the possible entity states based on the intrinsic entity relationships within the system. The deducing outcomes could be visualized as the ESN, where nodes served as the entity states and edges indicated state transition directions. The formal mathematical definition of ESIS was as follows:

Definition 1. The Entity State Inference System G was a quintuple,

$$G = (V_N, V_T, F, P, S)$$

(1) $V_N = V_{N1} \cup V_{N2} \cup V_{N3}$,

Here, V_N was a finite set of non-terminal symbols. V_{N1} was a finite set of entity names/IDs, V_{N2} was the finite set of all the entity variables, excluding entity names/IDs. V_{N3} was a finite set of units for entity variables.

(2) $V_T = V_{T1} \cup V_{T2} \cup V_{T3}$,

Here, V_T was a finite set of terminal symbols, and $V_N \cap V_T = V_{T1}$ was a finite set of values of entity names/IDs. V_{T2} was a finite set of values for all the entity variables, excluding entity names/IDs. V_{T3} was a finite set of units for entity variables.

(3) $F = \{ f_1(A, X, Y), f_2(A, B), f_3(A, X, Y, B, H, I, W), f_4(f_1(A, X, Y), f_1(B, H, I), f_1(B, G, I)), f_5(J, K), f_6(K, J), \ldots \mid f_i: (E(V, F))^n \rightarrow E(V, F),\ 1 \leq i \leq m,\ m, n \in N \}$,

with $A \in V_{T1}$, $C \in V_{T1}$, $D \in V_{T1}$, $X \in V_{T2}$, $Y \in V_{T3}$, $H \in V_{T2}$, $I \in V_{T3}$, $G \in V_{T2}$, $J \in E(V, F)$, $K \in E(V, F)$, $V = V_N \cup V_T$.

F was a finite set of operations or functions. In this definition, $f_1(A, X, Y)$ indicated that the entity A was in the state X with unit Y, and $f_2(A, B)$ represented a mutual interaction relationship between entities A and B, with the direction $A \rightarrow B$. The specific expressions could be configured according to the actual circumstances. For instance, when A inhibited B, it could be represented as inhibition (A, B), and when A activated B, it could be set as activation (A, B). $f_3(A, X, Y, B, H, I, W)$ denoted that the state of a system involving entities A and B was W, determined by their individual states. $f_4(f_1(A, X, Y), f_1(B, H, I), f_1(B, G, I))$ represented that entity A in state X regulated entity B from state H to G. In the ESN, f_4 was defined as a state transition node. $f_5(J, K)$ and $f_6(K, J)$ were the directed edges in ESN, indicating that $J \rightarrow K$ and $K \rightarrow J$, respectively.

(4) $P = P_1 \cup P_2 \cup P_3 \cup P_4 \cup P_5$,

$$P_1 = \{ f_1(A, X, Y), f_1(B, H, I), f_2(A, B) \Rightarrow f_1(B, G, I) \},$$

$$P_2 = \{ f_1(A, X, Y), f_1(B, H, I), {}_{f2}(A, B) \Rightarrow f_4(f1(A, X, Y), f_1(B, H, I), f_1(B, G, I)) \},$$

$P_3 = \{ f_4 (f_1 (A, X, Y), f_1 (B, H, I), f_1 (B, G, I)) \Rightarrow f_5 (f_1 (A, X, Y), f_4 ( ))\}$,

$P_4 = \{ f_4 (f_1 (A, X, Y), f_1 (B, H, I), f_1 (B, G, I)) \Rightarrow f_5 (f_1 (B, H, I), f_4 ( ))\}$,

$P_5 = \{ f_4 (f_1 (A, X, Y), f_1 (B, H, I), f_1 (B, G, I)) \Rightarrow f_6 (f_4 ( ), f_1 (B, G, I))\}$,

P was a finite set of generative inference $\alpha \rightarrow \beta$, with $\alpha \in E^+ (V, F)$ and $\beta \in E (V, F)$.

(5) S indicated the starting state of the system, serving as the starting point for reasoning.

To exhibit the ability of ESIS in transforming a static entity relationship network into a dynamic entity state transition network, a demonstrative network was built with three nodes and three edges, as shown in Fig. 2(a). The state values in this system were restricted to 0 or 1. The inference results of entity state relationships were visualized in Fig. 2(b) as an ESN, which encompassed all possible states of the three nodes, thereby mapping the complete state space. As illustrated in Fig. 2(c), the distribution patterns of entity states on the ESN varied with changes in the system state. Moreover, dynamic analysis of the system at the state level could be directly realized through ESN. For example, given the current system state of (a, 1, b, 1, c, 0), the ESN revealed that the antecedent state was (a, 1, b, 0, c, 0), and the subsequent state will evolve into (a, 1, b, 1, c, 1). In summary, the entity state transition diagram depicted by the ESN exhibits three salient characteristics. Firstly, it effectively portrays the transition pathways of all the entity states. Secondly, it illustrates the directional changes in the system's overall state. Meanwhile, it captures the holography of system states, representing the entire state space. This approach allows for the direct deduction of entity state evolution from entity relationships, serving as a potent tool for studying the dynamic behaviors of the system.

4. The construction of Ternary Entity State Inference System

BN, initially proposed by Kaufman in 1969 [20,21], is a classic model in the study of biological dynamics. A Kauffman random BN consists of multiple entity nodes, each endowed with a binary state variable, where the digit 0 denotes inhibition and 1 signifies activation. Given the state of the network at a particular time, the subsequent states are determined through Boolean functions, which can be done synchronously or asynchronously [22].

Currently, BN stands as a foundational paradigm for modeling the intricate dynamics in biological systems, such as gene regulatory networks, PPI networks, and signaling networks [23]. However, in some specific cases, a more detailed expression is needed. For

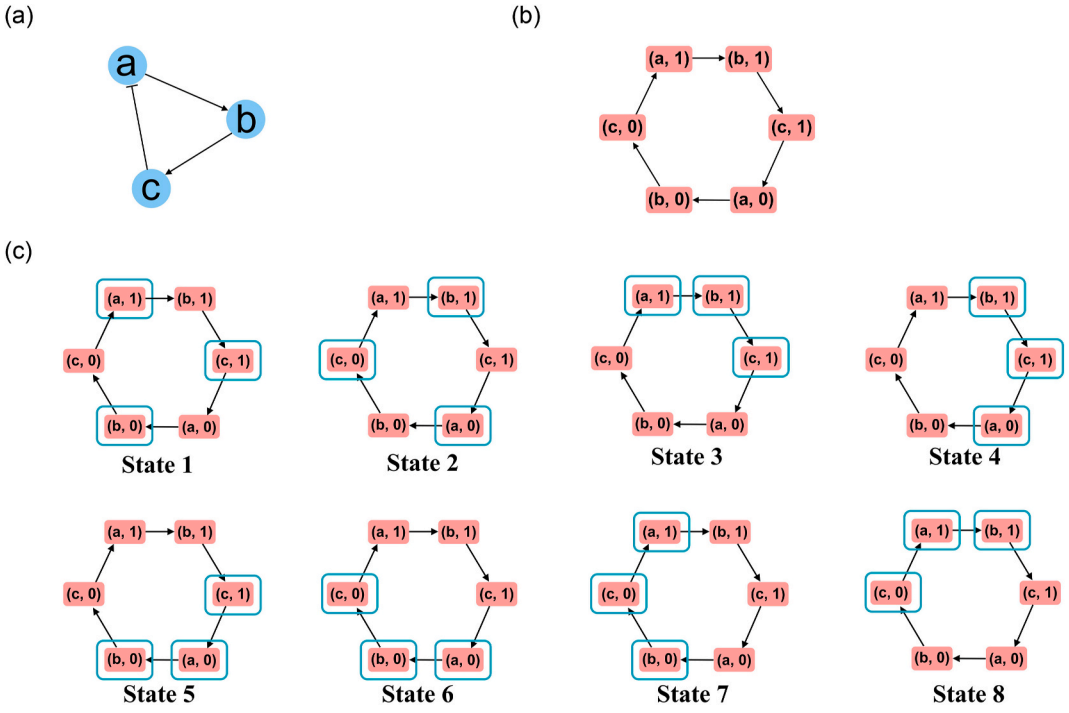

Fig. 2. An example entity relationship network and its Entity State Network.
(a) Net 1, a sample network with three nodes and three edges.
(b) The Entity State Network of Net 1.
(c) The entity state distribution patterns on the Entity State Network for eight different system states of Net 1.

example, experimental evidence suggests that gene expression levels in biological systems display distinct biological functions at an intermediate, low-level expression state, beyond the mere binary states of on and off [24]. Therefore, further research has explored the dynamic characteristics of multi-valued networks, revealing that ternary networks can yield more organized and stable dynamic behaviors. These networks provide more definitive markers for transitions between order and chaos, thereby offering potential control points for the effective directional influence of system behavior [25]. Based on the characteristics above, a ternary state characterization approach has been incorporated into the foundation of the ESIS, with 0, 1, and 1/2 employed to denote inactive, active, and intermediate or partial activation/expression states of biological entities, respectively. Consequently, a T-ESIS has been developed on the basis of ternary logic. Specifically, in biological networks, when node A activates node B, the state change of node B is consistent with that of node A, while when node A inhibits node B, the state change of node B is in the opposite direction. Namely, when the state of node A is p, the state of node B will update to ¬p in the next moment. In a three-valued network, the definition of ¬ and the state change rules between node A and node B were detailed in Tables 1 and 2.

T-ESIS was established by combining ternary logic operations with ESIS to realize the deduction of state transitions in three-valued logic. The mathematical definition of T-ESIS was as follows:

Definition 2. Ternary Entity State Inference System G was a quintuple,

$G = (V_N, V_T, F, P, S)$

(1) $V = V_N \cup V_{T1} \cup V_{T2} \cup V_{T3}$,

where V_N was a finite set of the variables of nodes, including node names, relations between nodes, and node states. V_{T1} was the set of values for node names. V_{T2} was the set of values of node relations, either activation or inhibition. Lastly, V_{T3} was the set of values of node states, including 1, 1/2, and 0.

(2) $F = \{$ *node (A), state (A, X), link (A, B, R), s_change (A, X, B, Z)*$\}$,

$A \in V_{T1}, B \in V_{T1}, R \in V_{T2}, X \in V_{T3}, Z \in V_{T3}$,

where *node (A)* indicated node A. *State (A, X)* denoted that node A was currently in state X. *Link (A, B, R)* represented that node A acted on node B through the relation R. *s_change (A, X, B, Z)* depicted the process of state change. Specifically, it indicated that when node A was in state X and acted on node B, the state of node B would turn to node Z.

(3) $P = P_1 \cup P_2 \cup P_3 \cup P_4$,

$P_1 = \{$ *state (A, X), state (B, Y), link (A, B, pos)* $\Rightarrow$ *s_change (A, X, B, X)*$\}$,

$P_2 = \{$ *state (A, 1), state (B, Y), link (A, B, neg)* $\Rightarrow$ *s_change (A, 1, B, 0)*$\}$,

$P_3 = \{$ *state (A, 1/2), state (B, Y), link (A, B, neg)* $\Rightarrow$ *s_change (A, 1/2, B, 1/2)*$\}$,

$P_4 = \{$ *state (A, 0), state (B, Y), link (A, B, neg)* $\Rightarrow$ *s_change (A, 0, B, 1)*$\}$,

$A \in V_{T1}, B \in V_{T1}, X \in V_{T3}, Y \in V_{T3}$,

P was defined based on the three-valued state transition rule.

(4) S was the initial entity relations and entity states.

T- ESN can provide more information about system states that the BN may ignore. For example, the T-ESN of Net 1, as shown in Fig. 1(a), was depicted for comparison with its binary state network. The inference results by T-ESIS in Net 1 were displayed in Table 3 and then visualized as T-ESN in Fig. 3. Compared to the ESN, the T-ESN had an additional circular structure, with all nodes having state values of 1/2. This extra circle represented that all entities in the system were at a normal level, forming a steady state. Unlike BN, which could only express states as high and low with 1 and 0, 1/2 in T-ESN represented the intermediate state or normal state of entities under unstimulated conditions, exhibiting more dynamical patterns of the system. Consequently, the T-ESN demonstrated better conformity with the dynamics of practical biological systems.

Table 1
The definition of ¬ in ternary logic.

p	¬p
1	0
1/2	1/2
0	1

Table 2
The state transition rules between node A and node B.

A activates B		A inhibits B	
A	B	A	B
1	1	1	0
1/2	1/2	1/2	1/2
0	0	0	1

Table 3
The inference results of entity state transitional relationships of Net 1.

The results of s_change ( )	Source	Target
s_change (a, 0, b, 0)	(a, 0)	(b, 0)
s_change (a,1/2, b, 1/2)	(a, 1/2)	(b, 1/2)
s_change (a, 1, b, 1)	(a, 1)	(b, 1)
s_change (b, 0, c, 0)	(b, 0)	(c, 0)
s_change (b, 1/2, c, 1/2)	(b, 1/2)	(c, 1/2)
s_change (b, 1, c, 1)	(b, 1)	(c, 1)
s_change (c, 0, a, 1)	(c, 0)	(a, 1)
s_change (c, 1/2, a, 1/2)	(c, 1/2)	(a, 1/2)
s_change (c, 1, a, 0)	(c, 1)	(a, 0)

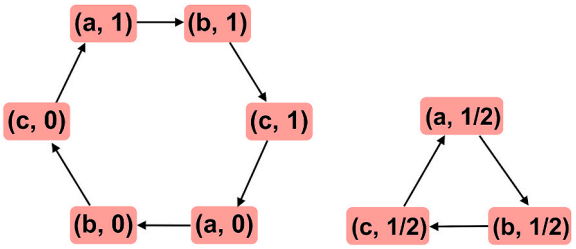

Fig. 3. The ternary entity state network of Net 1.

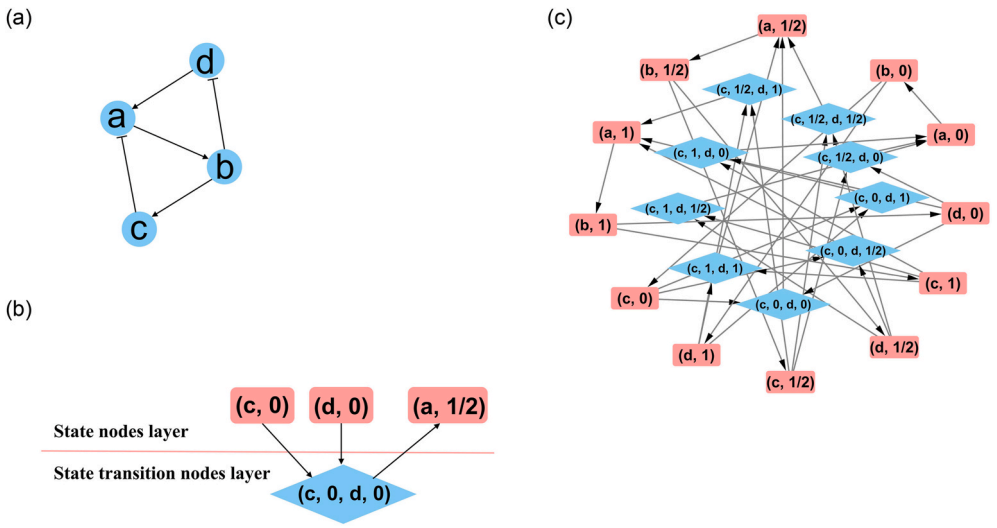

Fig. 4. An example complex entity relationship network and its Ternary Entity State Network.
(a) Net 2, a sample complex network with four nodes and five edges.
(b) The double layer of the complex Ternary Entity State Network, taking the process of node a regulated by node c and node d as an example.
(c) The Ternary Entity State Network of Net 2.

5. The establishment of Ternary Entity State Inference System for complex systems

In the previous section, the state transition rules were established for networks where each node was regulated by only one node. Biological networks, however, are complicated, with the states of a node usually influenced by multiple upstream nodes. Therefore, the state transition rules were further refined for complex T-ESIS.

When a node is regulated by multiple governing nodes, the approach involves two key steps. Firstly, inhibitory relationships should be transformed into equivalently promoting relationships through the logical rule ¬. Subsequently, the following rules need to be applied for state determination: after transformation, if the count of upstream nodes' states with a value of 1 surpasses the count of states with a value of 0, then the output state is 1. Conversely, if the count of upstream nodes' states with a value of 0 exceeds those with a value of 1, then the output state is 0. In instances where parity between states valued as 0 and 1 prevails or all states are 1/2, then the output state is 1/2. Based on these rules, a new function used to decide the output of the upstream nodes is added to the operator F in T-ESIS, and the inference rules of multi-upstream nodes need to be formulated in P.

To elucidate the modeling of T-ESIS for complex networks more clearly, Net 2 in Fig. 4(a) was taken as a demonstration. Net 2 was based on Net 1 with the addition of an upstream node for node a, so that node a was inhibited by node c and simultaneously activated by node d. To explain the state transitions of node a, the inhibitory relationship between ¬c and a was first transformed into the promotion relationship. Subsequently, the above rules were adopted to determine the final state of node a. For instance, time t was considered a temporal snapshot, at which the states of c and d were both 0, so that the inputs of node a were 1 (¬c (t)) and 0 (d (t)). As the upstream nodes of node a had an equal number of state values of 1 and 0 at time t, the state of node a would be 1/2 at the next time t + 1. Detailed transition rules for nodes with multiple upstream nodes were exhibited in Table 4, using node a as a typical example. The complete T-ESIS of Net 2 was as follows:

Example 1. The Ternary Entity State Networks G was a quintuple, $G = (V_N, V_T, F, P, S)$:

(1) $V = V_N \cup V_{T1} \cup V_{T2} \cup V_{T3}$,

$V_N = \{node\ name, relation, state\}$,

$V_{T1} = \{a, b, c, d\}$,

$V_{T2} = \{activation, inhibition\}$,

$V_{T3} = \{1, 1/2, 0\}$,

(2) $F = \{node\ (A), state\ (A, X), link\ (A, B, R), s\_change\ (A, X, B, Z), majority\ 2\ (A, X, B, Y, C, W, H), s\_change\ 2\ (A, X, B, Y, C, Z)\}$,

$A \in V_{T1}, B \in V_{T1}, C \in V_{T1}, R \in V_{T2}, X \in V_{T3}, Y \in V_{T3}, Z \in V_{T3}, W \in V_{T3}, H \in V_{T3}$,

where *s_change (A, X, B, Z)* indicated the state of *node B* turned to *Z* due to the influence of *node A* in *state X*. *Majority2 (A, X, B, Y, C, W, H)* functioned as a state judgment mechanism for a node with two upstream nodes, determining that when node A was in state X and node B in state Y, their states transformed into W and H, respectively, based on their relations with downstream node C. *S_change 2 (A, X, B, Y, C, Z)* was the output of *majority 2 (A, X, B, Y, C, W, H)*, signifying that the state of node C changed to node Z when node A was in state X and node B in state Y.

(3) $P = P_a \cup P_b \cup P_c \cup P_d$,

$P_a = P_{a1} \cup P_{a2} \cup P_{a3} \cup P_{a4}$,

$P_{a1} = \{state\ (c, 1), link\ (c, a, neg), state\ (d, Y), link\ (d, a, pos) \Rightarrow majority\ 2\ (c, 1, d, Y, a, 0, Y)\}$,

$P_{a2} = \{state\ (c, 1/2), link\ (c, a, neg), state\ (d, Y), link\ (d, a, pos) \Rightarrow majority\ 2\ (c, 1/2, d, Y, a, 1/2, Y)\}$,

$P_{a3} = \{state\ (c, 0), link\ (c, a, neg), state\ (d, Y), link\ (d, a, pos) \Rightarrow majority\ 2\ (c, 0, d, Y, a, 1, Y)\}$,

Table 4
The state transition rules of node A in Net 2.

a (t)	¬c (t)	D(t)	A (t + 1)
0	1	0	1/2
0	1	1/2	1
0	1	1	1
1/2	1/2	0	0
1/2	1/2	1/2	1/2
1/2	1/2	1	1
1	0	0	0
1	0	1/2	0
1	0	1	1/2

P_{a4} = {majority 2 (c, X, d, Y, a, W, Y) ⇒ s_change 2 (c, X, d, Y, a, Z)};

P_b = {state (a, X),link (a, b, pos) ⇒ s_change (a, X, b, X)};

P_c = {state (b, X), link (b, c, pos) ⇒ s_change (b, X, c, X)};

$P_d = P_{d1} \cup P_{d2} \cup P_{d3}$,

P_{d1} = {state (b, 1), link (b, d, neg) ⇒ s_change (b, 1, d, 3)},

P_{d2} = {state (b, 2), link (b, d, neg) ⇒ s_change (b, 2, d, 2)},

P_{d3} = {state (b, 3), link (b, d, neg) ⇒ s_change (b, 3, d, 1)};

$X \in V_{T3}$, $Y \in V_{T3}$, $Z \in V_{T3}$.

Since there were multi-upstream nodes, it was necessary to establish state transition rules for each node separately. P_i indicated the state transition rule of node i.

(4) S = {link (a, b, pos), link (b, c, pos), link (c, a, neg), link (d, a, pos), link (b, d, neg), state (a, 1), state (a, 1/2), state (a, 0), state (b, 1), state (b, 1/2), state (b, 0), state (c, 1), state (c, 1/2), state (c, 0), state (d, 1), state (d, 1/2), state (d, 0)}.

The inference results of the three-valued entity state relationships in Net 2 were shown in Table 5.

The visualization of ESN for complex networks, where nodes were regulated jointly by multiple nodes, revealed that the direct connectivity of state nodes, like in Fig. 3, was inadequate. Therefore, transitional nodes were introduced in the ESN to depict the multi-regulated node states. The upstream nodes converged into the transitional nodes, and they co-determined the downstream node states. Moreover, the ESN for complex networks adopted a bilayer configuration, comprising a state node layer and a state transitional layer, as illustrated in Fig. 4 (b). The state nodes layer showed direct transformational relationships between entity states, while the state transitional layer displayed the combined influence of multiple nodes on downstream entity states. These two layers provided a visual representation of the transformational relationships between entity states. Based on the above, the T-ESN was visualized for Net 2 in Fig. 4 (c).

The three-valued computation rules, deriving from Boolean functions, are adaptable to complex entity networks, allowing for synchronous updates of system states across all nodes. On the basis of this, the T-ESIS was established to provide a new method for representing and analyzing more systematic dynamical behaviors, especially those suited to intricate biological entity systems. In the following section, a steady state based on T-ESIS was further analyzed to better investigate the dynamics of complex systems.

6. Steady state analysis of Ternary Entity State Network

From the biological perspective, the steady states of a system are generally of important significance, commonly known as attractors [25–28]. An attractor represents a stable state of the system to which unstable neighboring states are drawn [29]. Deducing

Table 5
The inference results of entity state transitional relationships of Net 2.

The results of s_change ( )	Source	Target
s_change (a, 0, b, 0)	(a, 0)	(b, 0)
s_change (a,1/2, b, 1/2)	(a, 1/2)	(b, 1/2)
s_change (a, 1, b, 1)	(a, 1)	(b, 1)
s_change (b, 0, c, 1)	(b, 0)	(c, 0)
s_change (b, 0, d, 1)	(b, 0)	(d, 1)
s_change (b, 1/2, c, 1/2)	(b, 1/2)	(c, 1/2)
s_change (b, 1/2, d, 1/2)	(b, 1/2)	(d, 1/2)
s_change (b, 1, c, 1)	(b, 1)	(c, 1)
s_change (b, 1, d, 0)	(b, 1)	(d, 0)

The results of s_change 2 ( )	**Source1**	**Target 1/Target 2/Source 3**	**Source 2**	**Target3**
s_change 2 (c, 0, d, 0, a, 1/2)	(c, 0)	(c, 0, d, 0)	(d, 0)	(a, 1/2)
s_change 2 (c, 0, d, 1/2, a, 1)	(c, 0)	(c, 0, d, 1/2)	(d, 1/2)	(a, 1)
s_change 2 (c, 0, d, 1, a, 1)	(c, 0)	(c, 0, d, 1)	(d, 1)	(a, 1)
s_change 2 (c, 1/2, d, 0, a, 0)	(c, 1/2)	(c, 1/2, d, 0)	(d, 0)	(a, 0)
s_change 2 (c, 1/2, d, 1/2, a, 1/2)	(c, 1/2)	(c, 1/2, d, 1/2)	(d, 1/2)	(a, 1/2)
s_change 2 (c, 1/2, d, 1, a, 1)	(c, 1/2)	(c, 1/2, d, 1)	(d, 1)	(a, 1)
s_change 2 (c, 1, d, 0, a, 0)	(c, 1)	(c, 1, d, 0)	(d, 0)	(a, 0)
s_change 2 (c, 1, d, 1/2, a, 0)	(c, 1)	(c, 1, d, 1/2)	(d, 1/2)	(a, 0)
s_change 2 (c, 1, d, 1, a, 1/2)	(c, 1)	(c, 1, d, 1)	(d, 1)	(a, 1/2)

* In the results of s_change 2 ( ), target 1, target 2 and source 3 are merged into one column since the same value.

the state changes from a given initial state, the system eventually stabilizes, either into an endlessly repeating single state (defined as a point attractor), or into a limit cycle, where several states recur periodically [30].

Attractors can be obtained directly by inferring state changes in the system through ESI. For example, the system state transitions of Net 1 were inferred based on T-ESIS, resulting in one fixed point and five limit cycles, as shown in Fig. 5(a). For clarity, node names have been omitted in Fig. 5, retaining only the node state values. For instance, the system state (a, 1, b, 0, c, 1) is simplified as (1, 0, 1). The inference details were provided in Supplementary 1. S1, with all the nodes in the 1/2 state, indicated the normal steady states, while S2–S6 represented all the abnormal steady states of the system. Among them, S2 and S3 indicated completely abnormal steady states, as all entity state values were either 0 or 1, whereas other attractors represented partially abnormal states of the system. Furthermore, the system consistently transformed from a normal state through a partially abnormal state into a completely disorder state. For instance, if node a was constantly stimulated, the transition pathways of the system state would be S1→S4→S6→S2, as illustrated in Fig. 5 (b).

The completely normal and abnormal states of systems, such as S1, S2, and S3, could be clearly depicted in the ESN. Moreover, entity states propagate along the direction of entity relationships in the ESN. Specifically, an entity in abnormal states will cause abnormal states in its downstream entities, while an entity in normal states will also regulate the neighboring nodes into the same states. Therefore, the cycles can be initially detected in the entity network and reflected in the ESN based on a certain degree of opological similarities. These cycles in the ESN stand for the processes through which the system state evolves into either a perfectly normal or completely abnormal state, thereby forming the multi-stability of the system. As illustrated in Fig. 6, the signaling pathway in the entity network proceeded from node a to node b, then moved to nodes c and d simultaneously, and finally return to node a. By ranking the node states in this sequence within the ESN, the limit cycles became evident. The first cycle represented the formation of a completely abnormal state, indicating multi-stability, while the second one signified the regular normal state. It was also clearly shown that the system state could shift from abnormal to normal via transient states (c,1,d,1) or (c,0,d,0).

Research has demonstrated that the ESN was a bridge between the entity-relationship network and the system state network. The entity-relationship network highlights the static topological relationships within the system, whereas the system state network represents dynamic transition pathways through system states as nodes. Nevertheless, neither of them can capture the influence of mutual relationships among entity states on the emergence of macroscopic dynamic behaviors in the system. ESIS deduced the state relationships among entities and presented them as ESN, where circular structures demonstrated state propagation, contributing to the formation of both normal and abnormal steady states. Furthermore, circular structures also visually illustrated the transition of

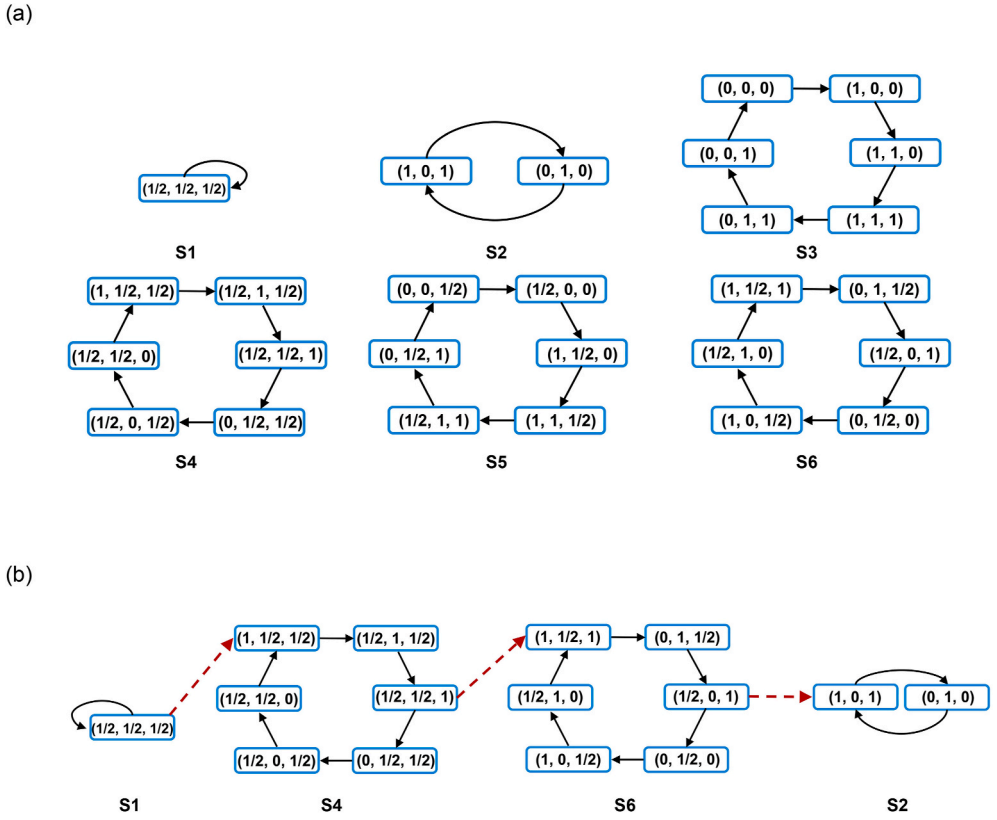

Fig. 5. The attractors of Net 1 and the transitional paths of attractors when node a is continuously activated.
(a) Six attractors (S1-S6) of Net 1.
(b) The transition pathways of attractors when *node a* is under a continuous activation.

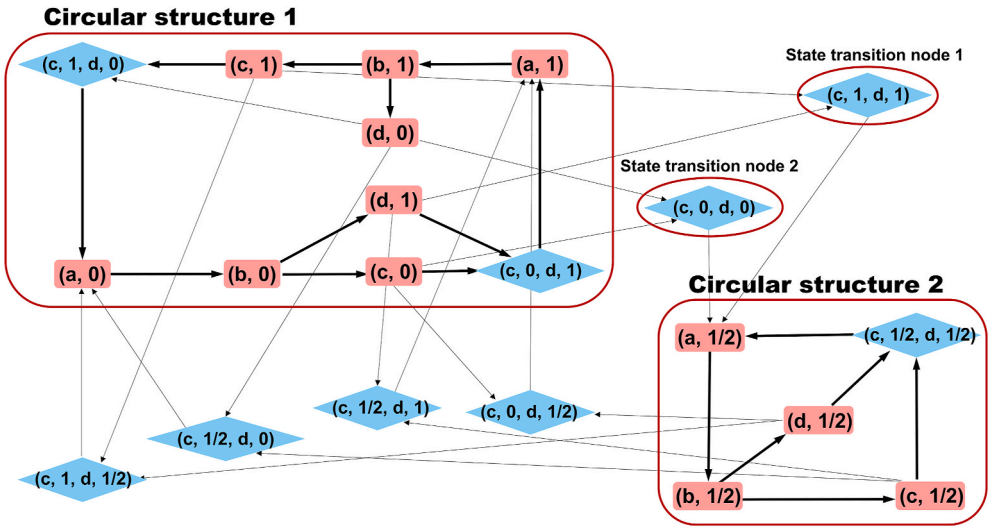

Fig. 6. Two steady states in the Ternary Entity State Network of Net 2, found through the structural similarity between entity relationships and entity state transitions.

transitional nodes from abnormal to normal, providing a novel quantitative perspective for the analysis of system dynamics.

7. Application of Ternary Entity State Inference System in non-small cell lung cancer

Lung cancer is the leading cause of cancer mortality globally, with 85 % of cases being NSCLC [31]. Identifying molecular drivers as therapeutic targets is a promising treatment for NSCLC [32]. Therefore, the positive and negative feedback loops were investigated within the PPI network of NSCLC, and the T-ESN was further established to elucidate the gene expression patterns that maintain a healthy system or contribute to cancerous states, aiding in the identification of the target genes from a dynamic system perspective.

The PPI network of NSCLC is constructed via EGS, based on 339 related genes and 37,786 proteins association. In our study, the data on related genes came from the Online Mendelian Inheritance in Man (OMIM) [33], Kyoto Encyclopedia of Genes and Genomes (KEGG) [34], and the Disease Gene Network [35]. Moreover, the data of protein associations were from Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) [36] (score $\geq$0.900). Further details on data and modeling were available in Supplementary 2. The final NSCLC entity network consisted of 89 nodes and 213 edges, including 130 activating and 83 inhibiting relations, as depicted in Fig. 7 (a).

The circular structures in the PPI network of NSCLC were then searched via Cypher on Neo4j. As illustrated in Fig. 7 (b), the cycles predominantly exhibited multiple positive feedbacks, multiple negative feedbacks, and combined positive and negative feedback loops. These nested feedback loops provided the systems with robust ways to adjust different steady state features. Fig. 7 (b3) was selected as the representative negative feedback loop for the subsequent analysis.

Utilizing the proposed method, we established the T-ESN of the negative feedback loop, as displayed in Fig. 7 (c). The detailed inference processes were provided in Supplementary 3. In the T-ESN, two circular structures represented two typical types of system states. The state values of nodes in cycle 1 were all 0 or 1, indicating a completely abnormal steady state. Conversely, the state values in cycle 2 were all 1/2, representing a normal steady state. The network also showed the transition pathways for the system to recover from abnormal to normal states. The trajectory was composed of state transition nodes: [Harvey rat sarcoma virus (HRAS), 1, Bruton's tyrosine kinase (BTK), 1], (HRAS, 0, BTK, 0), [HRAS, 1, vascular endothelial growth factor A (VEGFA), 0], and (HRAS, 0, VEGFA, 1). This suggested that regulating the system towards either (HRAS, 1, BTK, 1, VEGFA, 0) or (HRAS, 0, BTK, 0, VEGFA, 1) can gradually shift the system from an abnormal state to a normal state.

To depict the state space of the negative feedback loop, we simplified the negative feedback structure without changing the topology, as revealed in Fig. 8 (a). The resulting network comprised 5 entities, which were VEGFA, HRAS, Kirsten rat sarcoma virus (KRAS), vav guanine nucleotide exchange factor 1 (Vav1), and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA). VEGFA is an essential factor in the regulation of physiological and pathological angiogenesis and is also implicated in tumor expansion [37]. HRAS and KRAS, members of the rat sarcoma virus (RAS) family, encode a GTPase (guanosine triphosphatase) as a binary ON-OFF molecular switch, mediating the transduction of extracellular signals to the intracellular environment [38]. RAS is the most frequently mutated oncogene in human cancers [39]. Mutational activation of RAS has been shown to overstimulate downstream signaling pathways, leading to tumorigenesis. Vav1 is exclusively expressed in the hematopoietic system [40] and is involved in multiple biological processes, such as cell surface receptor activation, cytoskeleton reorganization, transcription regulation, cytokine production, and cell cycle progression [41]. In addition, Vav1 has also been proven to play a crucial role in the development and function of various types of immune cells [40]. Mutant PIK3CA induces an abnormal increase in the catalytic activity of PI3Ks, which subsequently activates AKT. Furthermore, this activation affects proliferation, metabolism, angiogenesis, and protein synthesis,

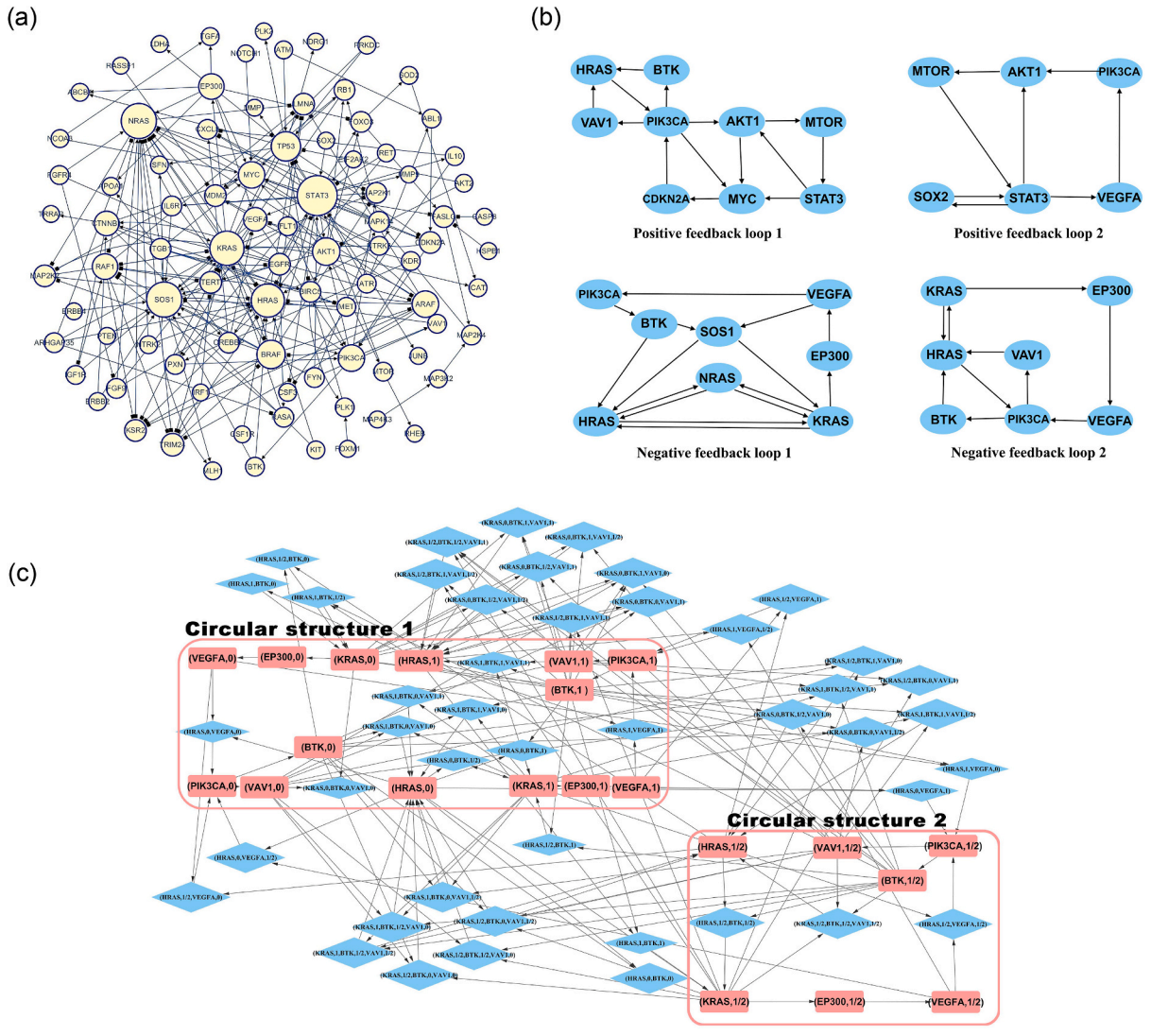

Fig. 7. The construction of Ternary Entity State Network for non-small cell lung cancer and steady-states analysis.
(a) The molecular entity network of non-small cell lung cancer.
(b) The circular structures which correspond to two negative feedback loops and two positive feedback loops in the entity network of non-small cell lung cancer.
(c) The Ternary Entity State Network of the negative feedback loop 2 in Fig. 7(b).

potentially leading to tumor formation [42–44]. These five entities formed a negative feedback structure, balancing the cell behaviors associated with tumors.

The negative feedback loop comprised 5 nodes, and each node had 3 states, resulting in the number of possible states was 3^5. The transformation relations among these states were then reasoned out, leading to the construction of the T-ESN, which was depicted in Fig. 8 (b). The computational process was detailed in Supplementary 4. To enhance clarity, node names have been omitted. For instance, the state (HRAS, 0, KRAS, 0, PIK3CA, 0, VAV1, 0, VEGFA, 0) was simplified to (0, 1, 0, 0, 1).

The attractors, searched as circular structures in the ESN, were highlighted in Fig. 8 (b). In addition, the details in Fig. 8 (c) revealed that the ESN contained 3 point attractors and 9 limit cycles, with S4 possessing the largest basin. Among the 12 attractors, the node states in S1 were all valued at 1/2, indicating that S1 represented a healthy system state. The other cancerous attractors indicated the gene expression patterns of different types or stages of NSCLC. Additionally, the ESN in Fig. 8 (b) suggested that most transient states were attracted to S4, which is an oscillatory pattern with normal PIK3CA and VAV1 expression levels. The system cycled between two states: one with overexpression of HRAS and KRAS as well as low expression of VEGFA, and another with the reverse, leading to a stable state. Overexpression of HRAS and KRAS implied highly metabolic activity and proliferative cell state, whereas overexpression of VEGFA signified continuous angiogenesis, forming a highly vascularized malignant tumor [45,46]. The persistent transitions between these two states implied a vigorous growth and expansion phase of the tumor. Consequently, S4 possessed strong attractiveness,

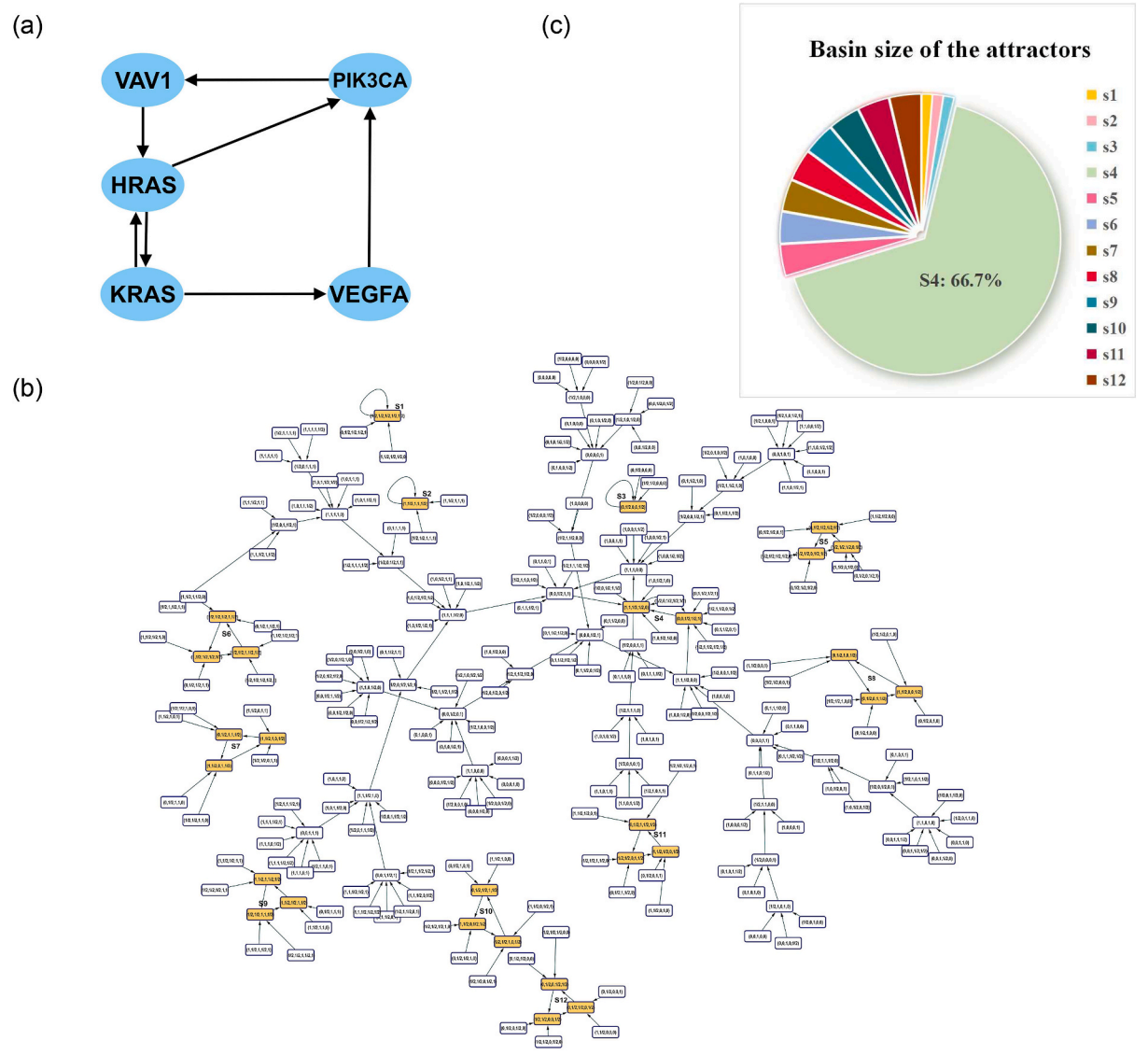

Fig. 8. The simplified negative feedback loop and its Ternary Entity State Network.
(a) The simplified negative feedback loop of non-small cell lung cancer.
(b) The Ternary Entity State Network of the simplified negative feedback loop and the attractors are highlighted.
(c) The basin size of the attractors in Ternary Entity State Network.

making it difficult for the system to revert to the cancer state. This conclusion was aligned with clinical data, which manifested that high expression of HRAS, KRAS, and VEGFA was closely correlated with the development and prognosis of NSCLC [47–50].

8. Conclusion

Our study emphasized the importance of understanding both the static and dynamic features of biological networks. Although topological features provide insights into the structure of networks, they only present a partial perspective on the characteristics of systems. Furthermore, dynamic modeling illustrated through T-ESN reveals higher-order collective behaviors driven by gene interactions, offering a more comprehensive understanding of biological complexity.

The T-ESN framework serves as a bridge between network topology and system dynamics, providing insights into the temporal interactions and influences among biological entities. This approach has significant implications for drug design and disease treatment, as it helps identify critical intervention points within the network.

In the context of NSCLC, our model successfully identified multiple stable states corresponding to different stages of disease progression. This understanding of state transitions can pinpoint critical targets for therapeutic intervention, potentially halting or

reversing disease progression. However, computational results require further experimental validation. In the future, we will focus on optimizing the T-ESN algorithm to manage larger biological networks, thereby extending its applicability to a broader range of biological research scenarios.

In conclusion, T-ESN offers a powerful tool for studying dynamic behavior in complex biological systems, providing a deeper understanding of disease mechanisms, and supporting the design of effective intervention strategies.

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Ethics approval and consent to participate section

The data mentioned in this study is publicly available for re-analysis, and no ethical approval was required by the local ethics committees. Therefore, this study does not require ethics approval.

Consent for publication

Not applicable.

Data availability statement

The datasets used and/or analyzed during the current study have not been deposited into a publicly available repository. However, the data will be made available to the corresponding author on reasonable request.

CRediT authorship contribution statement

Ziwei Zhao: Writing – review & editing, Writing – original draft, Visualization, Data curation, Conceptualization. **Jingxuan Liang:** Validation, Software, Formal analysis, Data curation. **Xianbao Zhang:** Visualization, Validation, Supervision, Formal analysis, Data curation. **Wenyan Li:** Project administration, Methodology, Investigation. **Yun Wang:** Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37578.

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