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REVIEW

Perspectives on the landscape and flux theory for describing emergent behaviors of the biological systems

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Abstract
We give a review on the landscape theory of the equilibrium biological systems and landscape-flux theory of the nonequilibrium biological systems as the global driving force. The emergences of the behaviors, the associated thermodynamics in terms of the entropy and free energy and dynamics in terms of the rate and paths have been quantitatively demonstrated. The hierarchical organization structures have been discussed. The biological applications ranging from protein folding, biomolecular recognition, specificity, biomolecular evolution and design for equilibrium systems as well as cell cycle, differentiation and development, cancer, neural networks and brain function, and evolution for nonequilibrium systems, cross-scale studies of genome structural dynamics and experimental quantifications/verifications of the landscape and flux are illustrated. Together, this gives an overall global physical and quantitative picture in terms of the landscape and flux for the behaviors, dynamics and functions of biological systems.

Keywords Landscape · Flux · Nonequilibrium · Systems biology · Biophysics · Molecular biology

1 Introduction

The world around us is made of large number of the atoms and molecules. Although we now understand the fundamental laws governing how these particles move, it does not automatically guarantee the understanding how the world is working on the meso- or macro-scales with large number of basic elements. For example, we do not understand exactly how life works or how our atmosphere works. P.W. Anderson pointed this out as "more is different." The behavior of the whole can be very different from the individual. In fact, many new features can be emerged from the interactions among the underlying elements distinct from the individuals. Therefore, one expects laws of nature to be dependent on the scales. Different scales can have different laws. It is in this sense that theory of everything has to include all the laws at different scales, not only the law at the most micro-level. From this perspective, significant efforts

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have been made toward the understanding of the underlying laws and mechanisms at different scales including physics, astronomy, chemistry, biology, geology, economics and society. The grand challenges for understanding the associated complex systems and phenomena are both conceptual and quantitative. On the one hand, one needs to develop concepts to guide the understanding. On the other hand, one needs to develop quantitative methods and tools to probe and understand these complex systems [1–6].

Prof. Hans Frauenfelder has been a pioneer in investigating the fundamental laws across scales [7–10]. He and his collaborators have focused on the basic functional unit of life, the protein and tried to explore the relationship between the structure and dynamics to understand how the protein performs its function. A protein is a complex system made of thousands of atoms. Even one has a good understanding of the individual atoms, the understanding of the protein as a whole has been still very challenging. Notice that it is often difficult to track the dynamics of each individual atom to understand the behavior of the whole protein [7–10]. Therefore, one should look for statistical way of understanding this complex system in analogy to the understanding of the liquid and gas phenomena from statistical mechanics rather than the Newtonian dynamics.

Prof. Frauenfelder developed the concept of energy landscapes and hierarchy structure to explain the different dynamical behaviors of the proteins using the examples of ligand-heme protein binding (Myoglobin and Hemeglobin) [7–10]. The energy landscape is formed from the interactions among the individual atoms which are often known from the microscopic physics. Therefore, this transforms the concept of studying the complex system from following the individual trajectories to the evolutions of the system states. The system as a whole can be characterized by the states with the weights specified based on the depths of the underlying landscape. The different energy valleys or basins represent possible functional states of the protein and the importance of these functional states is determined by their associated weights [7–10].

The energy landscape of the whole protein state space is still complex with many valleys or basins separated by different heights of the barriers [11, 12]. Hierarchy provides a principle for organization of these states. On the mesoscopic scale, the behaviors of the states and associated landscape valleys are the result of the interactions among the individual elements just a level (microscopic level) beneath it [1–10]. Furthermore, on the macroscopic scale, the behaviors of the states and associated landscape valleys are the result of the interactions among those states corresponding to the energy valleys at (the level just beneath the current level of interest) the mesoscopic level. Therefore, one can see that in the hierarchical picture, at each level or layer of the landscape, the functional states are the result of the interactions among the individual valley states at the level just beneath it. Frauenfelder's team over the years has shown experimentally clear evidences of the presence of the energy landscape and the associated hierarchical structure for the protein dynamics [13]. This guides generations of researchers to probe the complex systems using these ideas and concepts.

We are going to give a few examples of the recent progress in biological physics on enriching and further developing the energy landscape concepts and theories beyond protein dynamics. This includes the study of at the molecular scale of protein folding, biomolecular recognition, molecular evolution and design, genome folding and dynamics as well as study at the gene and cell scale of cell cycle, differentiation and development, cancer, evolution and neural networks.

2 Equilibrium landscapes

2.1 Protein folding

Proteins are the fundamental building blocks of biology. In fact, many of the biological functions are realized through the protein molecules. In order to understand the protein functions, one needs to first know the associate structures. With the rapid development of the technologies, the primary sequences are relatively easily obtained. Great progresses have been made in obtaining the three-dimensional structures of proteins through the X-ray crystallography, NMR and more recently EM methods. However, there is a deficit in structure determination compared with the sequencing information. This is the origin of the protein folding problem in biology. Given a sequence, how can one determine the structure of a protein? In 1969, Levinthal proposed a paradox for the protein folding problem: How does a protein select a specific native structure state among a huge number of the available conformational states of the proteins [14, 15]. If one assumes that there are 10 possible conformations of an amino acid residue and a protein is made of 100 such residues, then there are 10^{100} possible conformations. If the search time for the conformation is fast on the order of 10^{-15} s, then the estimated time for folding through the random search takes about 10^{85} s which is even longer than the age of our universe. In fact, the protein folding completes in the laboratory experiments ranging from microseconds to minutes. This creates the Levinthal paradox [14].

The new view of protein folding has recently emerged by the energy landscape theory [16–18]. The folding landscape has roughness originated from the conflicts in the interactions between different amino acid residues while there is an overall bias toward the native folded state which is due to the natural evolution selection. This forms a funneled landscape against the roughness toward the native structural state for the protein folding [19]. At the early stages of folding, multiple paths direct toward the folded state. At a later stage of folding, local traps can be effective leading to discrete paths toward folding. Due to the biasing, the searching for the native state can be greatly accelerated and therefore the protein folding paradox is resolved by the funnel. Performing the molecular dynamics simulations, the funneled landscape can be quantified through transforming the results of the canonical ensemble to that of the micro-canonical ensemble. This is illustrated for a few proteins in Fig. 1. The funneled landscape theory is supported by many experimental results.

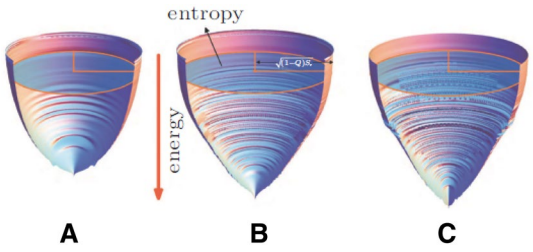

Fig. 1 Quantified funneled landscapes for villin headpiece (Left), CI2 (Center) and P13 (Right) obtained from simulation. The depth of the funnel is the energy and the cross section perpendicular to the energy axis is an ellipsoid with an area equal to the configurational entropy SeET. Both energy and configurational entropy are normalized by the protein size [19]

2.2 Specificity and drug discovery

Once the protein structure is known, the next challenge is to understand how recognition between two biomolecules is achieved which is crucial for realizing the biological functions. Two key elements determine the recognition. One is the affinity quantifying the stability of the binding between the two molecules. The other is the specificity in distinguishing the one target from another. In the practical drug discovery design, while affinity can often be estimated, the specificity is very hard to characterize. However, both accurate quantifications of the affinity and the specificity are required for tight binding and avoidance of side effects [11, 15, 20–22]. Recent developments in combinatorial chemistry provide a way of screening large number of compounds for the drug design industry [11, 15, 20–22]. This allows the use of the statistical energy landscape approach to improve the design [11, 15, 20–22].

The main difference between protein folding and binding is the polymer chain connectivity. Protein folding can be seen as self-binding while binding can be seen as folding between two disconnected domains. It is thus natural to think about the folding and binding as similar processes since the underlying driving forces for folding and binding are the hydrophobic and electrostatic interactions [15, 21, 22]. Similar to protein folding, the underlying binding energy landscape naturally should have a funneled shape toward the native binding state to guarantee the stability and the distinction of the native binding against the roughness [15, 21, 22].

The conventional definition of specificity is the ability of distinguishing a specific ligand against different receptors Fig. 1a. To do so, one needs to search for all the receptors to get the ones with a clear separation in binding free energy from the rest. This is often impossible to realize. A new way of quantifying the specificity is the preference for a (set of) binding mode(s) of a ligand to its receptor Fig. 1b separated against others [15, 21, 22]. When the receptor protein is large enough, these two ways of quantifying the specificity should be the same. While searching for the receptor universe is impractical, the searching for the binding modes for a specific ligand-receptor binding can often be relatively easy to be performed. A funneled binding landscape gives a criterion quantifying the binding specificity: the energy gap or biasing toward the native state δE should be large compared to the underlying energy landscape roughness ΔE and the size of the system measured by the entropy ΔS. In other words, the intrinsic specificity ratio defined as $\frac{\delta E}{\Delta E\sqrt{2\Delta S}}$ should be larger than 1. The larger this ratio is, the better discrimination of the native binding mode against traps and conformational states is and therefore the higher specificity it is [15, 21, 22].

The new way of defining the specificity for binding has been used to generate the optimized scoring function for reaching both high affinity and specificity for ligand binding, protein–protein binding, and protein-RNA(DNA) (include references binding in Fig. 2. This leads to very high performances against other academic and industrial scoring functions in terms of affinity and specificity [24–26]. The new way of quantifying the specificity has been successfully applied to the drug discovery for identifying the lead compounds with specificity against the targets. This includes the selectivity for the Cox-2 inhibitors (pain relievers), selective Ras intermediate state (cancer target) inhibitors, the small molecules against BLVRB for the treatment of patients with high incidence of thrombocytopenia and the lead compound originally for cancer inhibition but now with a:significant effect on Alzheimer's disease [27–44].

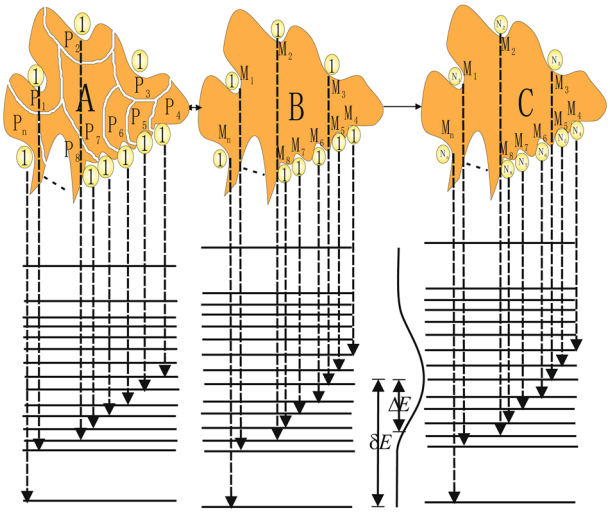

Fig. 2 Illustration of Specificity Concept. Left panel illustrates the conventional specificity of a ligand against many receptors (1 against Pn) with high affinity and high specificity (discrimination against rest). Middle panel illustrates our intrinsic specificity definition of a ligand with a large receptor (composed of many other receptors in a thought experiments) as the discrimination of native binding mode (1 versus Mn) against the rest. The right panel illustrates another way of defining the specificity of many ligands against one receptor (Nn versus M) with high affinity and high specificity (discrimination against others) [23]. Reprinted from Curr. Pharm. Des.19, Zheng, X. L., Liu, Z. J., Li, D., Wang, E. K., Wang, J., Rational Drug Design: The Search for Ras Protein Hydrolysis Intermediate Conformation Inhibitors with Both Affinity and Specificity, 2246-2258, Copyright (2013), with permission from Bentham Science

2.3 Protein evolution and design

As discussed above, the funneled landscape is the prediction for protein folding and binding for stability and function. The reason behind should be the natural evolution. Nature selects the sequences with funneled shape for realizing the stability and kinetic accessibility of the three dimensional structure. The nature selects binding sequences and structures for the stability and kinetic accessibility for successful binding and function. In fact, the biological function is the result of evolution. To search for the origin of the protein folding and protein binding, one should not only focus on the folding and binding at a given sequence, but also explore the evolution of arbitrary sequences and see whether and how they reach to the native folding and recognition [24–26, 45–47].

For a given sequence, the random conformational structural state search space is huge (10^{100} for a 100 amino acid residue protein with 10 conformations of each residue at a given sequence). On the other hand, the random search in sequence space is also huge. For 100 residue protein, since there are 20 possible amino acid residues in each position, the sequence space can be as large as 20^{100}. Thus, one runs into the Levinthal paradox in both conformational structure and sequence space since random search takes too long to reach this far. The evolution likely only has explored a small region of the state space accessible to folded and functional proteins. Therefore, the search cannot be entirely random. Environmental constraints on the evolution and selection of proteins and their interactions must be in action in additional to the random mutations [24–26, 45–47], according to Darwin's fittest survival theory of natural evolution. The stable and functional folding

and recognition should be the results of the selection and random mutations. To resolve the Levinthal paradox and realize the function, a funneled landscape in both sequence space and conformational structural state space has been proposed for either folding or binding.

In Fig. 3, one can see the landscape has a super-funneled bowl shape toward the native states in both sequence and conformational structure state space. The bottom of the funnel has a finite size representing there are certain numbers of sequences with the similar folding structures (3A and 3B) [46]. A stable folded structure can be generated as a result of this evolution funnel with distinct hydrophobic core consistent with the existing data (3C)

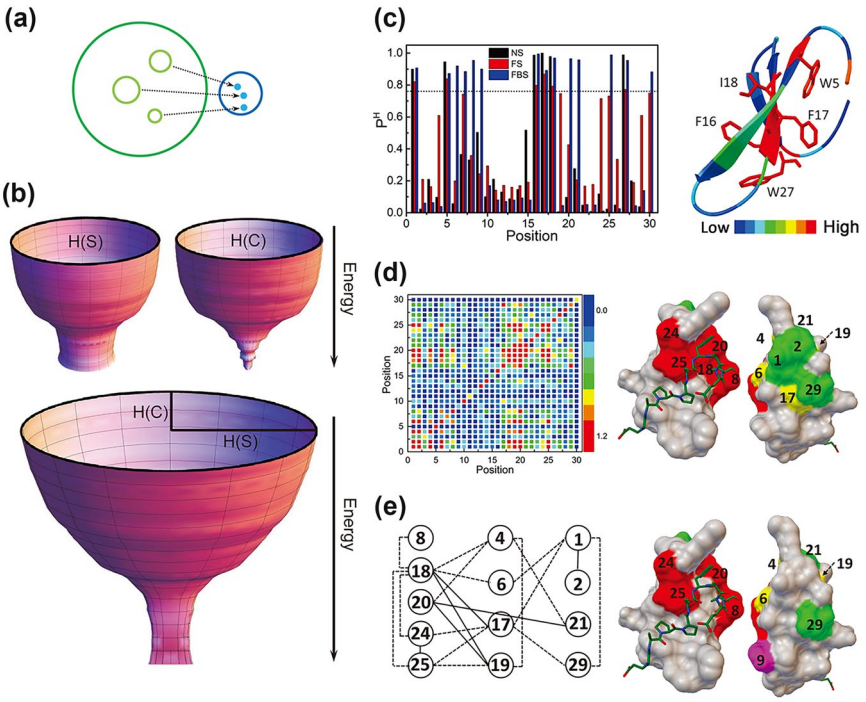

Fig. 3 **a** Schematic diagram for the naturally occurring proteins in the sequence and structure space. Each subspace (areas inside the light green circle) corresponds to a set of natural occurring sequences that possess the same structure as their ground-state native structure (light blue point) [46]. Reprinted from Phys. Rev. Lett. 122, Yan, Z., Wang, J., Superfunneled Energy Landscape of Protein Evolution Unifies the Principles of Protein Evolution, Folding, and Design, 018103, Copyright (2019), with permission from American Physical Society. **b** Quantified energy landscape of protein evolution in sequence and structure space [46]. Reprinted from Phys. Rev. Lett. 122, Yan, Z., Wang, J., Superfunneled Energy Landscape of Protein Evolution Unifies the Principles of Protein Evolution, Folding, and Design, 018103, Copyright (2019), with permission from American Physical Society. **c** Hydrophobic core for folding. Hydrophobic preferences (PH) of residual positions for NSs, FSs, and FBSs. The structure of the WW domain (PDB ID code 4N7H) with the residual positions shown and labeled for the hydrophobic core; hydrophobic preferences of NSs are represented with color spacing (From Ref [45]). **d** Coupling conservations for binding. The map of coupling conservations from NSs; the points with values larger than 1.2 are colored in red. The native complex structure of WW domain binding with the peptide (PDB ID code 4N7H) in front and back views; the positions with large coupling values are labeled, the positions in the binding site are colored in red, the bridging positions are colored in yellow, and the opposite positions are colored in green [45]. **e** The positions with large coupling values physically constitutes a network by contacts (dashed line) and bonds (solid line). The positions having large differences of hydrophobic preference between NSs/FSs and FBSs are labeled and colored as in B; the additional binding position 9 is colored in purple [45]

[45]. On the other hand, the evolutionary funnel can also generate binding complex which is similar to the native ones in terms of the coupling interactions (3 D and 3E) [45]. This shows the evidences for supporting the funneled landscape in both sequence and structural space as the guiding principle for the protein evolution. This also suggests a new basis and strategy for the protein design of stability and function [45].

2.4 Biomolecular binding

Biomolecular binding is essential for realizing the function. Several mechanisms have been suggested for the recognition [8, 11, 15, 20, 48–51]. Lock-and-key mechanism is used for explaining the rigid binding. However, conformational changes are often accompanied with the binding process. Induced fit and conformational selection have been suggested accounting the flexibility of protein binding [48–51]. Recently, more evidences have accumulated that significant numbers of the proteins known as "intrinsically disordered proteins" (IDPs) when isolated do not have fixed structures in physiological conditions. These proteins gain their structures only upon binding with the partners [8, 11, 15, 20, 48–51]. The IDP binding is associated with the conformational changes. Due to similarity between folding and binding in terms of the driving force of hydrophobic interactions and electrostatic interactions, binding can be viewed as folding of two disconnected domains. Therefore, one expects that the global flexible recognition landscape should be funneled toward the native binding state. The global binding-folding energy landscape should involve an interfacial binding energy landscape and two folding energy landscapes [11, 20, 52]. The flexible recognition landscape is then a result of the delicate balance between the folding and binding for realizing its biological function. Notice that the individual folding landscape such as for the IDP does not have to be funneled as long as the whole global binding landscape is funneled toward the native binding state.

The binding-folding landscape is shown in Fig. 4 [20]. There are two scenarios of flexible recognition. When the binding is strong or comparable with the folding (such as IDP), the binding can occur prior to or simultaneously as folding. This favors first

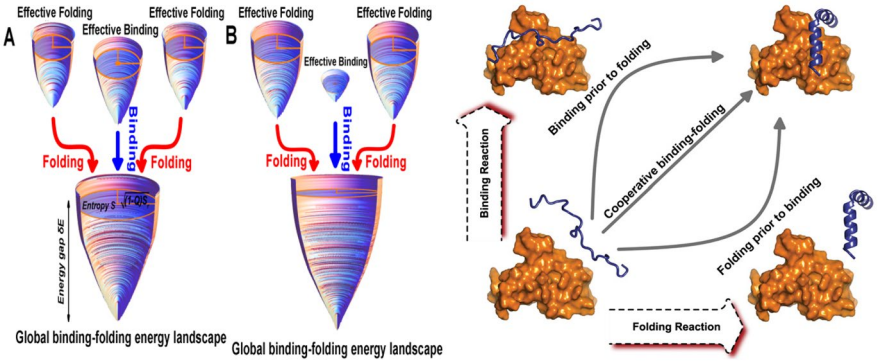

Fig. 4 Left: The individual effective binding and folding as well as the whole global binding–folding energy landscapes. The energy and entropy are normalized by the sizes of the homodimers for a better visualization. Right:The schematic diagram of three typical association mechanisms for IDPs. The diagonal line represents the cooperative process with binding and folding strongly coupled. The noncooperative processes are represented by the two lines along the rectangular edge, corresponding to binding prior to folding (up) and folding prior to binding (down), respectively [20]

binding then folding with distinct intermediate state or simultaneous binding-folding with no apparent intermediate state. On the other hand, when the folding is strong compared with the binding (stable folded structures), this favors first folding and then binding scenario where intermediate state is expected. One finds that the binding interface contacts and individual folding contacts of the native state determine the mechanisms of flexible recognition. When the interface binding contacts are comparable to the folding contacts, 2 state binding is preferred. When the folding contacts are more than the interface binding contacts, then the 3 state binding is preferred. One can further quantify the global landscape topography measure of the ratio of the energy gap against the roughness modulated by the entropy. It was demonstrated that the ratio is typically larger than 1 for many protein binding complexes, suggesting an overall funneled landscape for the flexible binding or binding-folding processes of IDP. On the other hand, strong correlations suggests that the landscape topography determines both the thermodynamics and the kinetics of the flexible recognition [20]. Many examples have been studied on the underlying mechanisms of the IDP flexible recognition through quantifying the global binding landscapes [53–68].

One interesting prediction of the landscape theory of flexible recognition is that the flexibility in biomolecular recognition can lead to moderate affinity but high specificity [11]. This is in contrast with the conventional view that high affinity goes hand in hand with the high specificity. The thermodynamic specificity can be defined by the topography of the intrinsic binding energy landscape with the distinction of native complex from the rest and the kinetic specificity by the contrasts in the association rate. The flexibility is found to decrease the binding affinity but increase the binding specificity. The degree of the change in affinity and specificity are closely related to the degree of the flexibility [11]. This demonstrates that the flexibility can lead to decoupling between the affinity and the specificity. It provides a physical basis for quantifying the relationships among flexibility, affinity and specificity.

3 Landscape and flux theory of nonequilibrium systems

For complex systems, the dynamics are often determined by the driving force obeying a set of nonlinear dynamics equations [69–72]: $\frac{\mathrm{d}\vec{C}}{\mathrm{d}t} = \vec{F}(\vec{C})$, where $\vec{C} = \{c_1, c_2, c_3, \ldots c_n\}$ denotes the density, concentration or populations of the species of interests and $\vec{F}(\vec{C}) = \{F_1(\vec{C})F_2(\vec{C})F_3(\vec{C}) \ldots F_n(\vec{C})\}$ denotes the driving force based on the interactions among the populations. One can then define the state of the system through the combination of the population components, $\vec{C} = \{c_1, c_2, c_3, \ldots c_n\}$ [69]. If there are N components of the populations while each component can have M values, then the total number of the states is M^N. The important and functional states of the interests should be among these states. The dynamics of the system can then be described as the evolution from one state to another [69].

Traditionally, the dynamics and associated stability can be studied first by identifying the fixed points and performing the local stability analysis around those fixed point. However, the local stability analysis does not give the information on the connections between the local stable states which are critical in understanding the dynamics as the transitions among the states. In addition, fluctuations are inevitable. Instead of following the evolution trajectories which are not predictable due to the nonlinearity and fluctuations (such as nonlinear Langevin equation), the focus should be on the

evolution of the associated probability which follows the linear law (such as Fokker–Planck equation or master equation) [69]. For general dynamical systems, there are often energy, material and information exchanges with the environment. Therefore, one needs to study the nonequilibrium dynamics in understanding the underlying principles and mechanisms.

3.1 Nonequilibrium landscape and flux as the driving force for the general dynamics

Under the fluctuations, the deterministic nonlinear dynamics become stochastic dynamics which can be described by the Langevin equation [69–74]:

$$\frac{\mathrm{d}\vec{C}}{\mathrm{d}t} = \vec{F}(\vec{C}) + \vec{\eta}$$

where $\vec{\eta}$ represents the fluctuation force [69]:

$$< \vec{\eta}(t)\vec{\eta}(0) >= 2D\mathbf{D}\delta(t)$$

where D is the strength of the fluctuations while **D** is the scaled diffusion matrix of the fluctuation force. The associated probability evolution is dictated by the conservation law [69]: $\partial \mathrm{P}(\mathbf{C}, \mathrm{t})/\partial \mathrm{t} = -\nabla \cdot \mathbf{J}$. The local probability change is due to the net flux in or out. The probability flux $\mathrm{J} = (\mathrm{F}(\mathbf{C})\,\mathrm{P}(\mathbf{C}, \mathrm{t})) - (\nabla \cdot (\mathrm{D}\mathbf{D}\mathrm{P}(\mathbf{C}, \mathrm{t})))$ is determined by the driving force F(C) and the stochastic fluctuation contribution along with the probability. At steady-state $\partial \mathrm{P}(\mathbf{C}, \mathrm{t})/\partial \mathrm{t} = 0$, the divergence of the flux is zero, $\nabla \cdot \mathbf{J} = 0$. If the steady-state probability flux is zero, $\mathbf{J}\mathrm{ss} = 0$, then there is no net energy or particle flow in or out of the system. This implies that the system satisfies a detailed balance condition and is therefore in equilibrium [5, 6, 75].

One can obtain readily that the force is just a gradient of the potential $\mathbf{F} = -\mathrm{D}\mathbf{D}\nabla \cdot \mathrm{U}$, where the potential is determined by the equilibrium probability distribution through the Boltzmann law $\mathrm{U} = -\ln \mathrm{Peq}$. Therefore, in equilibrium systems under detailed balance, the global state can be characterized by the equilibrium probability while the dynamics is dictated by the gradient of the probability or the associated potential landscape [5, 6, 75].

On the other hand, a nonzero Jss gives rise to the net energy or particle flow in or out of the system. Furthermore, due to the steady-state condition, $\nabla \cdot \mathbf{J} = 0$. This steady-state probability flux is divergent free and therefore rotational [5, 6, 75]. The non-vanishing steady-state flux Jss breaks the detailed balance and provides a distinct signature of the nonequilibrium system. Deviated from the equilibrium system where the dynamics is dictated by the gradient of the equilibrium potential, the dynamics of the nonequilibrium systems are dictated by the two forces $\mathrm{F} = -\mathrm{D}\mathbf{D}\nabla \cdot \mathrm{U} + \mathbf{J}\mathrm{ss}/\mathrm{Pss}$: One driving force is the gradient of the potential U where U is directly linked to the steady-state probability landscape of the system where $\mathrm{U}(\mathbf{C}) = -\ln(P_{SS}(\mathbf{C}))$ while the other driving force is linked to the rotational steady-state probability flux [75]. The steady-state probability landscape Pss quantifies the weight of each state while the rotational flux Jss quantifies the flow around the states. In fact, the rotational flux provides a quantitative measure of the degrees of the detailed balance breaking as a nonequilibrium signature of the matter, energy or information exchange in or out of the system from or to the environments [5, 6, 75].

While the potential landscape can be used to quantify the weights of the system, the actual dynamics are dictated by both the landscape and the flux. The landscape tends to

stabilize the system toward the state or the basin of attraction with higher probability or lower potential, while the flux provides an additional force from the net flow in or out of the state. One can view the dynamics in analogy to a charged particle moving in an electric field under the electric potential gradient and a magnetic field giving a spiral motion [5, 6, 75].

Under zero fluctuation limit, one can perform the leading order expansion of the potential U(x) upon the strength of the fluctuations from the Fokker–Planck equation and obtain the Hamilton–Jacobi equation for the intrinsic potential ϕ_0 under the zero fluctuation limit [69, 72, 73, 76–84].

$$\sum_{i=1}^{n} F_i(\mathbf{C})\frac{\partial \phi_0(\mathbf{C})}{\partial C_i} + \sum_{i=1}^{n}\sum_{j=1}^{n} D_{ij}(\mathbf{C})\frac{\partial \phi_0(\mathbf{C})}{\partial C_i}\frac{\partial \phi_0(\mathbf{C})}{\partial C_j} = 0.$$

Based on $\frac{d\vec{C}}{dt} = \vec{F}(\vec{C})$, one can check that $\frac{d\phi_0(\mathbf{C})}{dt}$ is always less or equal to zero along the trajectory **C**. Therefore, ϕ_0 is a Lyapunov function which can be used to quantify the global stability.

3.2 Dominant paths and kinetics among states

To understand the dynamics, one needs to find out how fast the process is and how it actually occurs. To address both issues, one can quantify the dominant paths between the states and characterize the associate rates. A path integral formulation can be developed under the driving forces from both the landscape gradient **F**grad and rotational flux **F**curl [81, 82, 85–90]. The path probability initially at the state **C**i and time t=0 and finally at the state of **C**f at time t, can be quantified by a path integral formulation [86]: $P(\mathbf{C}f, t|\mathbf{C}i, 0) = \int D\mathbf{C}\exp[-S[\mathbf{C}]]$ where the action $S[\mathbf{C}] = \int (1/4\, d\mathbf{C}/dt \cdot (D\mathbf{D})^{-1} \cdot d\mathbf{C}/dt - 1/2(D\mathbf{D})^{-1} \cdot \mathbf{F} \cdot d\mathbf{C}/dt + Veff)dt$ and $Veff = 1/4\mathbf{F} \cdot (D\mathbf{D})^{-1} \cdot \mathbf{F} + 1/2\nabla \cdot (D\mathbf{D}^{-1} \cdot \mathbf{F})$. Each path **C**(t) linking the initial and the final state is associated with a weight exp[−S[**C**]]. S gives the action or the weight of the path. The Veff is the effective potential of the system. The probability of the path is then equal to the sum of the weights of all the possible paths DC. The paths can have different weights. Since the weight depends on the action S exponentially, the sub-leading paths will have exponentially

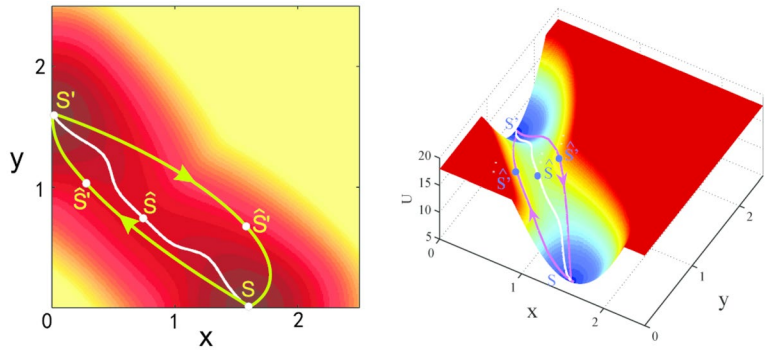

Fig. 5 2D and 3D illustrations of nonequilibrium landscape with the irreversible dominant transition paths between basins [88]

smaller weights than the dominant paths. One can then focus on the dominant paths. The dominant paths can be identified through maximizing the weights, exp[−S[x]] or minimizing the action. This can be realized even in the high dimensional state space through the optimization of a line integral by the Monte Carlo sampling [86].

One can now quantify how fast of the dynamics from one state to another are. Due to the rotational nature of the flux force, the nonequilibrium transition states can be identified and will be shifted away from the saddle point of the landscape as shown in Fig. 5 [88]. The kinetic rate from one state to another is then determined by the effective barrier (action) between the starting state and the nonequilibrium transition state.

3.3 Nonequilibrium thermodynamics, intrinsic energy, entropy and free energy of general neural networks

In addition to the nonequilibrium dynamics, the nonequilibrium thermodynamics for general dynamical systems can be also developed for characterizing the global emergent natures [5, 6, 75, 82, 91–94]. For deterministic dynamics, the nonequilibrium intrinsic potential ϕ_0 is related to the steady-state probability distribution as $P_{ss}(x) = \exp\left(-\phi_0/D\right)/Z$, where $Z = \int \exp\left(-\phi_0/D\right)dx$ can be defined as the nonequilibrium partition function, the intrinsic energy and entropy of the nonequilibrium dynamical sytems can then be naturally defined as $E = \int \phi_0 P(\mathbf{C}, t)dx = -D\int \ln\left[ZP_{ss}\right]P(\mathbf{C}, t)dx$, $S = -\int P(\mathbf{C}, t)\ln P(\mathbf{C}, t)dx$. Therefore, the intrinsic free energy can be defined as $\mathcal{F} = E - DS = D\left(\int P\ln\left(P/P_{ss}\right)dx - \ln Z\right)$ [5, 6, 75, 82, 91–94]. The free energy will always be minimized following the second law of thermodynamics.

On the other hand, under the finite fluctuations, evolution of the system entropy can be divided into two terms: $dS/dt = dS_t/dt - dS_e/dt$. Here the entropy production rate is given as $dS_t/dt = \int dx\left(\mathbf{J}\cdot(D\mathbf{D})^{-1}\cdot\mathbf{J}\right)/P$ which is larger or equal to zero[5, 6, 75, 79, 80, 82, 93, 94]. The heat dissipation rate or entropy flow rate from the environment is given as $dS_e/dt = \int dx\left(\mathbf{J}\cdot(D\mathbf{D})^{-1}\cdot\mathbf{F}'\right)/P$ can be positive or negative, while the effective force is defined as $\mathbf{F}' = \mathbf{F} - \nabla\cdot(D\mathbf{D})$. Since the total entropy change of the system and environment dS_t/dt is always larger or equal to zero, this is consistent with the second law of thermodynamics. However, the entropy change of the system dS/dt is not guaranteed to be positive. This illustrates that the entropy of the system is not always necessarily maximized for the general dynamical systems. Notice that even though the entropy change of the system is not always positive, the free energy of the system is minimized according to the second law of thermodynamics [5, 6, 75, 79, 80, 82, 93, 94]. In fact, the entropy production rate can be used to quantify the thermodynamic cost for maintaining the function of the dynamical system. From the definition of the entropy production, one can see that it is directly related to the rotational flux which is the nonequilibrium driving force. The origin of the flux force has been shown in certain biological examples from the energy pumps in the form of ATP, GTP and phosphorylation related processes [95]. Both the landscape and flux are important in determining the global natures of the dynamical system. One can perform the global sensitivity analysis of the underlying landscape topography and flux upon the changes of the underlying interactions. The key types of interactions can then be identified crucial for the stabilities and state transformation dynamics. This provides possible new ways for function and design from the global systems stand [5, 6].

4 Examples of nonequilibrium landscape and flux

4.1 Cell Cycle

The cell is the basic unit of life. A cell can grow, proliferate, divide and differentiate. Division or cycle is essential for the life of the cell for passing the genetic information to the next generations [96–102]. The cell cycle process is tightly regulated by the underlying gene regulatory networks. A wiring diagram for the model of a mammalian cell cycle is shown in Fig. 6 [103]. Arrows denote the activation regulation and dotted lines with short bar denotes the repression regulation. The model involves four major cyclin/Cdk complexes centered on cyclin D/Cdk4-6, cyclin E/Cdk2, cyclinA/Cdk2 and cyclin B/Cdk1. The opposite effects of pRB and E2F direct the cell cycle progression. The combined effects of the four modules determine the cell cycle oscillation dynamics [103]. From the Michaelias–Menton kinetics, the underlying dynamics is dictated by a set of nonlinear ordinary differential equations, $d\mathbf{C}/dt = F(\mathbf{C})$, where C represent the protein concentration or the gene expression vector and F represents the underlying chemical driving force [5, 6, 75].

The landscape can be obtained by the steady-state probability distribution through the stochastic simulations of the Langevin equations for the corresponding deterministic dynamics specified above. The steady-state probability flux can be obtained by subtracting the gradient force from the total driving force. There are several phases in the typical biology textbook description such as G1, S/G2, and M signifying the different stages of the cell cycle with check points in between for examining the progressing [103]. The landscape for the cell cycle projected into two gene expressions CycA and CycE shows an irregular Mexican hat shape with a close ring valley at the bottom [103]. From the landscape gradient, the system will tend to be attracted to the ring valley with lower potentials. There are

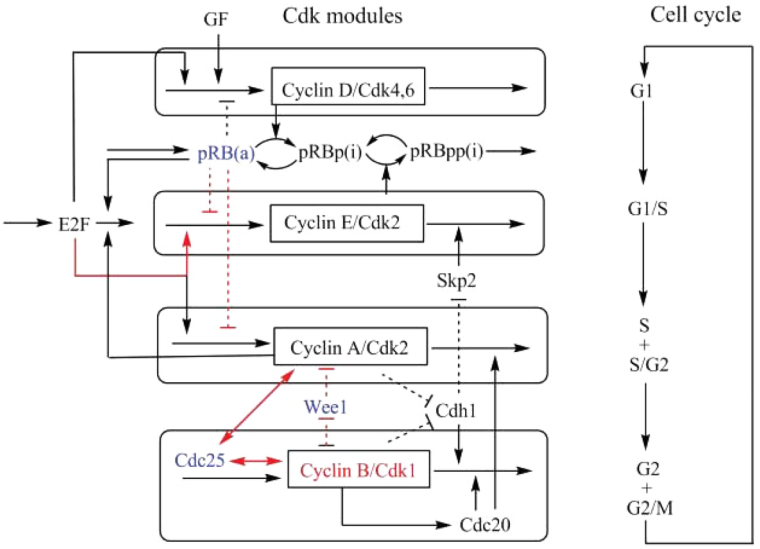

Fig. 6 The diagram for the mammalian cell cycle model. Red colors denote the key genes and regulations from the global sensitivity analysis. Blue colors denote the key genes and regulations from the global sensitivity analysis consistent with the experiments [103]

three local minimum along the close ring valley on the landscape which can be identified as the G1, S/G2 and M phases with local barriers between the different phases. At these phases, the chance of staying is higher due to the lower potential. Furthermore, the barrier tops as the local transition state between these phases can be identified as the check points [103]. On the other hand, if there is no other driving force other than the landscape gradient, then eventually the system will settle to the lowest potential one which is the G1 state. This is obviously not the case for cell cycle. In order to have the cell cycle oscillation along the low potential ring valley, there must be another force driving the rotational flow. This is where the curl flux comes into the play. In fact, while the landscape gradient attracts the system down to the ring valley, it is the curl flux which drives the cell cycle oscillations along the ring valley [5, 6, 75, 103–105]. This shows the necessary for both the landscape and flux as the driving force for the cell cycle oscillations. The landscape is from the regulations among the genes in the network [5, 6, 75, 103–105].

The origin of the flux force is from the nutrition supply and energy pump of ATP/ADP in the phosphorylation and de-phosphorylation processes [95]. More nutrition supply gives rise to larger flux and higher speed and more coherent oscillations of the cell cycle. There appears a threshold of the flux beyond which the cell cycle oscillation can proceed. This indicates that there is a minimum energy required for the birth of a single-cell life. The energy pump or driving is essential for the origin of life [95, 106]. On the other hand, a cancer cell has considerably faster cell cycle oscillation than that of a normal cell. This is because faster cell cycle gives rise to faster division of the cells and more chances of survival for the population as a whole. One can perform the global sensitivity analysis for the landscape topography (barrier) and flux upon the changes of the underlying regulation strengths and gene expressions. This can lead to the identifications of the critical gene regulations and genes for the cell cycle speed and function which are marked in red and blue in Fig. 7. These hot spot regulations and genes provide the potential targets for the drug discovery and the development of pharmaceutical strategy against various diseases.

4.2 Cell differentiation and development

One of the crucial functions of the cell is the differentiation. A primary stem cell can develop and turn into differentiated cell with specific function such as skin, heart, lung etc. Uncovering the underlying mechanism is not only for understanding the function but also important for the practice of tissue engineering [107–109]. In 1950s, Waddington proposed a picture for understanding the differentiation and development. The stem cell is assumed at the top of the hill while the differentiated cells are at the bottom of the valley. The differentiation process is thus mimicked by a ball rolling down the hill to the valley. Waddington's picture has become a metaphor for guiding the thinking for the differentiation and development [110]. However, Waddington's proposal is just a picture with no firm physical basis. Therefore, it is necessary to provide a solid physical and quantitative basis for differentiation/development and compare with the original Waddington picture. Researchers have found typical core gene motif responsible for the differentiation involve two self-activating genes mutually repressing each other [12, 78, 81, 89, 111–118]. Examples of such gene motifs include the PU.1 and GATA1 gene pair, Oct4 and Cdx2 gene pair, Nanog and Gata6 gene pair, Oct-4 and NANOG gene pair for differentiation. The gene regulation dynamics of these motifs can be described by the corresponding ordinary differentiation equations $dC/dt = F(C)$, with both self-activation and mutual repressions implemented. The landscape can be quantified and can be illustrated in the gene expressions. Furthermore,

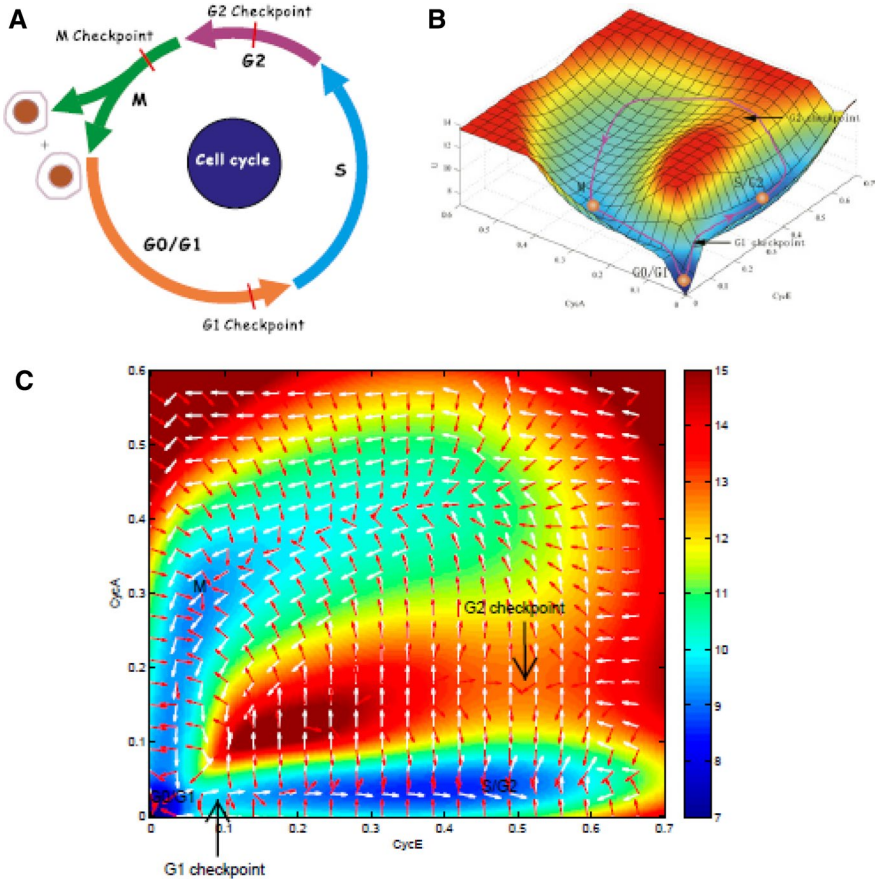

Fig. 7 **A** The phases of cell cycle with the checkpoints: G1,S, G2, and M phases. **B** Shows the three phases (G1, S/G2, and M) and the two checkpoints (G1 checkpoint and S/G2 checkpoint) on the landscape with respect to CycA and CycE. **C** Shows the 2D landscape. The red arrows denote the negative gradient of potential landscape while the white arrows denote the probabilistic flux (from Ref. [103])

since the experiments show the evidences that the regulation strengths change during the differentiation process. This provides a direction for the development. By quantifying the steady-state probability distribution of the corresponding stochastic dynamics for the gene motif dynamics along the development characterized by the regulation strength changes, one can quantify the landscape for differentiation as illustrated in Fig. 8 [78, 115].

One can clearly see that the quantified landscape for differentiation and development is a result of the gene regulations. This gives a physical basis for the original Waddington picture [78, 115]. The quantified landscape for development here echoes the basic Waddington landscape in terms of the ball from the stem cell state rolling down to the valleys of the differentiated states. However, there are a few distinct differences. First, the stem cell state is locally stable sitting in a valley instead of sitting on top of the hill as suggested by the original Waddington picture [78, 81, 89, 115, 119]. Second, the differentiation from stem cell state to the differentiated state and reprogramming from differentiated state back to the stem cell state paths are different. Third, the differentiation paths do not follow the gradient

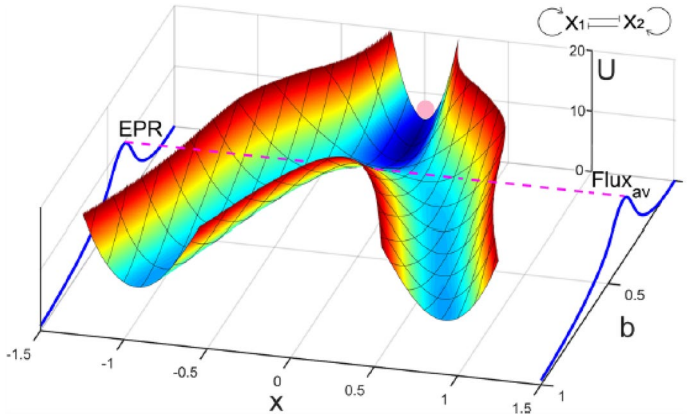

Fig. 8 The quantified Waddington landscape on the gene expression (x) along the developmental direction characterized by the regulation strength (b) changes for the self-activation and mutual repression gene regulatory motif crucial for the differentiation and development [78]. Reprinted from J. Phys. Chem. B. 124, Xu, L., Wang, J., Curl Flux as a Dynamical Origin of the Bifurcations/Phase Transitions of Nonequilibrium Systems: Cell Fate Decision Making, 2549-2559, Copyright (2020), with permission from American Physical Society

of the underlying landscape. To accomplish the differentiation process, the energetics such as phosphorylation/de-phosphorylation and ATP pumping are needed. This provides the input energy source and gives rise to the net flux which breaks the detailed balance. Since the driving force for the dynamics is determined by both the landscape and flux, the differentiation paths are thus deviated from the naively expected gradient ones [78, 115].

One can even find the close relationship among the peak of the average flux, the peak of the entropy production rate and the emergence of the bifurcation from the stem cell state basin to the differentiated state basins. This leads to a physical origin of the bifurcation or nonequilibrium phase transition between the stem cell and differentiation states. It also provides the quantitative indicators for the cell fate decision making. While the gradient force tends to attract the system and stabilize the point attractors, the flux force being rotational in nature tends to destabilize the point attractors [78, 115]. The higher average flux is associated with the higher nonequilibriumness and therefore the higher thermodynamic cost characterized by the entropy production rate. The higher nonequilibrium force and nonequilibrium thermodynamic cost can drive the point attractor (in this case the stem cell) to be unstable and eventual leads to the bifurcation or nonequilibrium phase transition to the bi-stability of the differentiated states, and vice versa. One can therefore use the nonequilibrium driving force and nonequilibrium thermodynamic cost to predict the onset and offset of the bifurcations for differentiation and reprogramming [78, 115].

4.3 Cancer

Cancer is a major cause of deaths in humans. Great efforts have been made in searching for the underlying cause of cancer, but challenges still remain [119, 120]. Ten hallmarks of cancer were identified [119, 120] for the target of treatment. Cancer is conventionally thought of being generated by the mutations. However, more evidences show that the epigenetics such as histone re-modification, DNA methylation, post-translational modifications etc. can

play a very important role [103, 113, 119, 121–130]. Therefore, from the gene regulatory network perspectives, it is better to think of both the cancer and normal as the states of the network as a whole, not being dependent only on the network gene nodes (genetics) or network links or gene regulations (epigenetics) alone. Micro-environmental changes such as epigenetics can perturb the gene regulations in favor of either normal or cancer state. It indicates the possible strategy against cancer should be to identify both the key genes and the key regulations. Landscape and flux theory can help to identify cancer state as well as the key associated genes and regulations. It can also help to find out the cancer formation and possible reversion paths [131–135].

One can illustrate the idea using an example of gastric cancer. It is recently realized that the *Helicobacter pylori* (*H. pylori*) infection can accelerate the development of gastritis and gastric cancer. Based on the many previous studies, a gene network can be developed to illustrate the regulatory interactions related to the cancer where red indicates the activated regulations while the blue indicates the repressive regulations. This is illustrated in Fig. 9 [130].

Based on the gene regulatory network for the gastric cancer, one can write down the corresponding dynamical equations for the gene expression dynamics by explicitly quantifying the gene regulations using Hill functions. The landscape can be quantified by solving the corresponding stochastic Langvin equations. Three stable state attractors emerge on the landscape: normal, gastritis and gastric cancer, each with distinct gene expression profiles [130].

The stabilities of these states can be addressed by the switching time among those states in Fig. 10. The dominant paths among normal, gastritis and gastric cancer states can be identified. This demonstrates that the cancer is most likely to be formed by first reaching the intermediate state of gastritis where inflammation is the signature. When

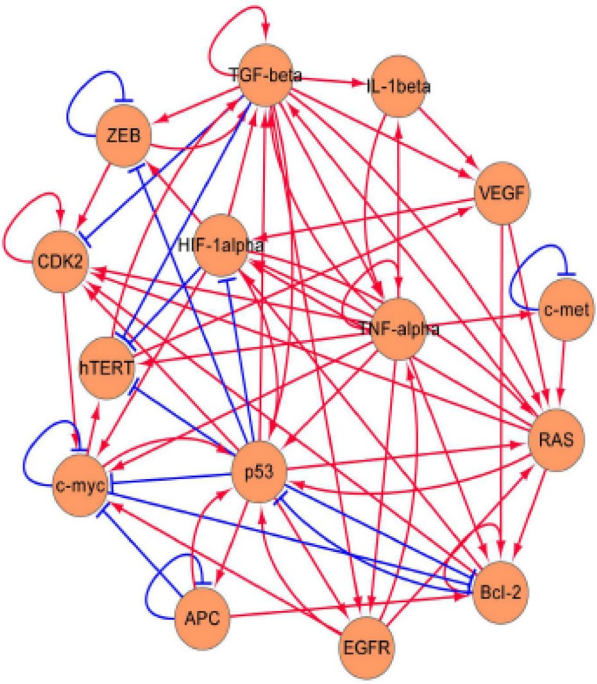

Fig. 9 The regulatory network of the gastric cancer with 15 nodes and 72 regulations. (57 activations and 15 repressions. The arrows represent the activating regulations and the short bars represent the repressing regulations) [130]. Reprinted from J. Theor. Biol. 124, Yu, C., Xu, H., Wang, J., A global and physical mechanism of gastric cancer formation and progression, 110643, Copyright (2021), with permission from Academic press

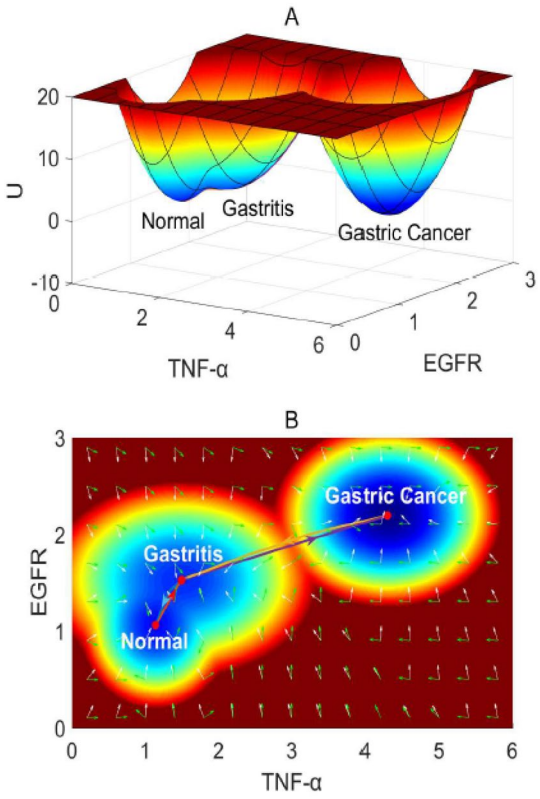

Fig. 10 The tristable state landscape of the gastric cancer. **A** The three dimensional landscape and dominant kinetic paths. **B** The corresponding two dimensional landscape of the gastric cancer. The lines in red, blue, voilet and yellow represent, respectively, the dominant kinetic path from the normal to the gastritis state, from the gastritis to the normal state, from the gastritis to the gastric cancer state, and from the gastric cancer to the gastritis state. White arrows and green arrows represent the negative gradient of the potential landscape and the steady-state probability curl flux force, respectively [130]. Reprinted from J. Theor. Biol. 124, Yu, C., Xu, H., Wang, J., A global and physical mechanism of gastric cancer formation and progression, 110643, Copyright (2021), with permission from Academic press

the situation deteriorates, this gastritis intermediate state has the chance of switching over the barrier to the cancer state. It is quantitatively demonstrated that increasing the degree of the *H. pylori* infection can accelerate the switching process from gastritis to gastric cancer. Based on the global sensitivity analysis of the landscape topography characterized by the barrier heights and switching time, key genes and regulations were identified. This is useful for the design against gastric cancer. The landscape and flux approach gives a new way of analyzing gastric cancer, providing a possible treatment strategy through modifying the key genes and key regulations [5, 6, 128–130, 132–135].

4.4 Neural networks and function of the brain

Understanding the function of the brain is one of the ultimate goals for biology. The brain is made of neurons interconnected to each other by synapses which give rise to neural networks [136–138]. The neural networks exhibit many important functions, such as learning, memory, and decision making [82, 91–93], etc. For single neurons, Hodgkin-Huxley model provides a quantitative description [139]. However, it is still challenging to understand the function of the neural networks made of many neurons. Hopfield proposed an associate memory model [136, 139] to study the global behavior of the neural networks. For symmetric connected neural networks, an energy landscape can be quantified. As shown in Fig. 11, from any state, the dynamics move along a gradient path down to the

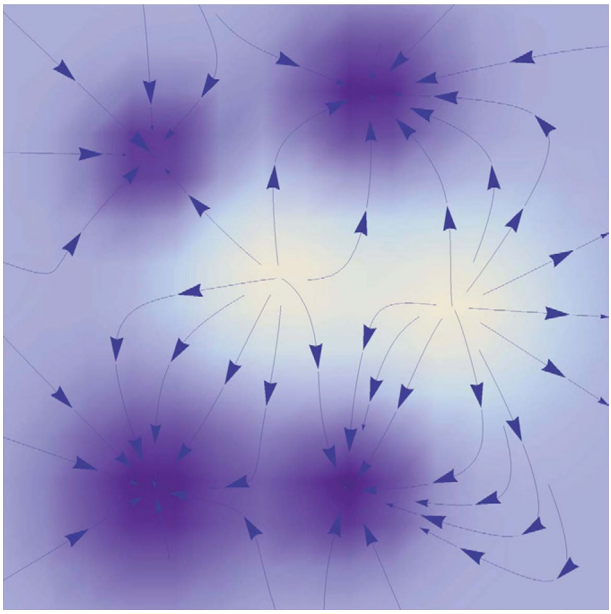

Fig. 11 The schematic diagram of the original computational energy function landscape of Hopfield neural network [93]

closest attractor of the energy landscape. Each attractor represents the information memory storage from learning. The whole dynamics describe the memory retrieving process from the initial cue. However, in real neural circuits, the neural connections are often asymmetric. The original Hopfield model cannot provide the underlying energy to describe the global function [91–93].

Realizing that the neural networks are nonequilibrium dynamical systems, one can apply the potential and flux landscape theory to study the global behavior of the neural circuit dynamics. Instead of the energy landscape alone for describing the global behavior of the neural network function such as given by Hopfield associate memory model [4, 136], the neural network dynamics is determined by both the landscape gradient and the nonequilibrium rotational flux. The rotational flux can give rise to spiral motion, leading to coherent oscillations which do not appear in the original Hopfield model under the pure landscape gradient force. The limit cycle provides the opportunities of continuous attractors with the directions stored in the oscillation attractors. Both landscape and flux are important for determining continuous memory retrieving rather than discrete memory retrieving dynamics. While the landscape attracts the system down to the oscillation attractor, the flux provide the driving force for the coherent oscillation flow on the oscillation attractor and therefore the possible associations or connections among different memories (each point on the oscillation path). This can also enhance the information storage capacity [91–93].

One can also show that a Lyapunov function monotonically decreasing along the trajectories emerges in the deterministic case under even the asymmetric connections for the neural networks. Such Lyapunov function can be readily used to quantify the global stability of the neural networks [93].

For a rapid eye movement (REM) sleep cycle model, using the landscape and flux theory and performing the global sensitivity analysis, one can find the key elements for the function as the release of acetylcholine (Ach) and norepinephrine in Fig. 12.

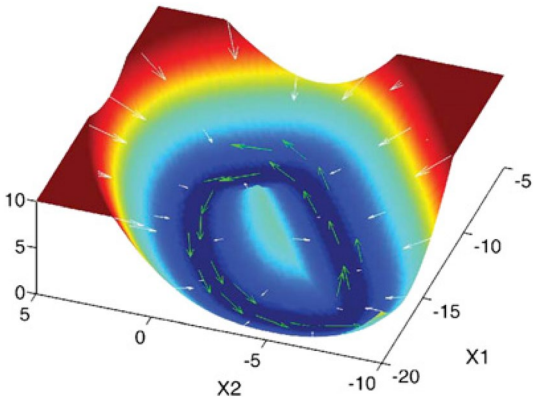

Fig. 12 The potential landscape ϕ0 as well as corresponding force: the green arrows represent the flux, and the white arrows represent the force from negative gradient of the potential landscape [93]

The flux is found to be important for both the stability and the speed of the REM sleep rhythms. This is consistent with the experimental finding and provides predictions for future experiments to be tested [93].

The nonequilibrium landscape and flux theory has also been applied to decision making, fear response, working memory, Parkinson's disease for the brain functions [91–93, 140, 141]. One expects to have more applications in the near future [93].

4.5 Evolution

Biology is dictated by the evolution. Many efforts have been devoted to uncover the laws of evolution [142–144]. Darwin first proposed the natural selection principle of fittest survival. Two eminent scientists provided the quantification of Darwin's evolution idea [143]. Fisher's fundamental theorem of natural selection suggests that the increase rate of mean fitness is equal to its genetic variance [142]. Wright developed a fitness landscape idea to quantifying the evolution adaptation process as a mountain-climbing process until reaching a local fitness peak shown in Fig. 13 [144, 145]. Wright's fitness landscape and Fisher's fundamental theorem of natural selection have been widely used to explain the evolution as the fitness maximization. These theories apply to the cases where the interactions within or among species can be ignored (allele frequency independent selection) [146]. However, the interactions within or among species can give rise to the frequency-dependent selection. The evolution dynamics under this general scenario does not follow the gradient of the mean fitness any longer and can enter into an endless cycle instead of reaching the fitness peaks. This cycle behavior in evolution is termed as Red

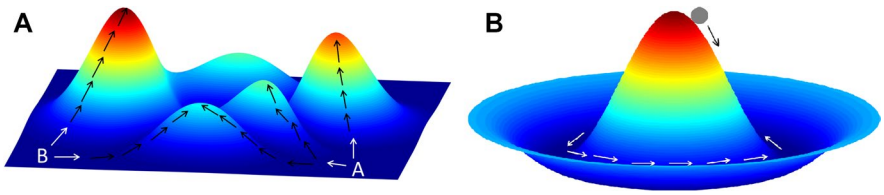

Fig. 13 A sketch fitness landscape to quantifying the evolution adaptation process as a mountain-climbing process until reaching a local fitness peak

Queen hypothesis proposed by Van Valen and cannot be explained by the Wright and Fisher theory of evolution [146]. Red Queen hypothesis originates from Alice in Wonderland: One needs to keep running in order to survive. The Red Queen hypothesis in the evolution context states that the biotic interactions between different species can lead to endless evolution for certain species even when the evolution optimum has already been reached. The coevolving systems can enter into cycles. This behavior is called Red Queen dynamics. The potential and flux landscape theory can give a physical foundation and quantitative explanation for effect. The maintenance of the genetic variance even when the evolution optimum is reached for the Red Queen hypothesis is supported and sustained by the curl flux originated from the bio-interactions.

By applying the landscape and flux landscape theory to evolution, one can demonstrate that the conventional Wright's gradient fitness landscape is inadequate to determine the general evolutionary dynamics [5, 6, 76, 82]. However, a Lyapunov function as an intrinsic adaptive landscape can be found to quantify the global stability for general evolution dynamics. The evolution dynamics is determined by both the landscape and the rotational flux. The rotational flux can be originated from the interactions within and among species, mutations, recombination and epistasis. The intrinsic energy, entropy and free energy of the evolution can be defined and the associated nonequilibrium thermodynamics can be developed [5, 6, 76, 82]. Both intrinsic landscape and free energy can be used to quantify the global stability and robustness of the evolution.

There are several distinct natures for the general evolution dynamics revealed by the landscape and flux theory of evolution different from the Wright-Fisher theory of evolution [142, 144]. First, the general evolution dynamics can generate point attractors and limit cycle oscillations while the Wright and Fisher's gradient evolution dynamics can only generate point attractors. Second, one can generalize the original Fisher's fundamental theorem of natural selection to the general evolution dynamics by connecting the mean fitness adaptation rate with not only the genetic variance associated to the landscape but also to the flux which gives [5, 6, 76, 82]: $\frac{d\phi_0}{dt} = -\frac{1}{2}\frac{V_A}{\overline{w}^2} + \mathbf{V}\cdot\mathbf{G}^{-1}\cdot\mathbf{V}$. Here ϕ_0 represents the intrinsic potential landscape for evolution while V_A represents the genetic variance, $\overline{w}$ represents the mean fitness and V represents the flux velocity. For Fisher's case, the V = 0 which leads to [5, 6, 76, 82]: $\frac{d\phi_0}{dt} = -\frac{1}{2}\frac{V_A}{\overline{w}^2}$. One can demonstrate that the rotational flux resulting from interactions within or among the species can drive an endless oscillation cycle evolution even when an optimum adaptation potential is reached. In fact, this gives rise to the nonzero genetic variance even when the adaptation has reached the optimum, in contrast to the prediction of Fisher's fundamental theorem of natural selection. This provides a physical and quantitative basis to explain the Red Queen hypothesis. Third, since there is a flux component in the driving force for the general evolution dynamics, the mean fitness landscape and its peaks may not coincide with the probability landscape and its peaks. This means that the mean fitness may not be the ultimate destiny for the general evolution dynamics. The discrepancy of the mean fitness landscape and the probability landscape can be from the interactions within and among the species (allele frequent dependent selection), mutations, recombination and epistasis, etc. For the general evolution dynamics, the aims for the targets are at the probability peaks rather than the fitness peaks. The landscape and flux theory has been applied to the more general multi-allele multi-locus evolution dynamics including the allele frequency-dependent selection, mutation, drift, recombination and epistasis effects. The landscape and flux theory can provide a theoretical foundation for the general evolutionary dynamics [5, 6, 76, 82].

5 Example of crossing scales

5.1 Genome dynamics

We have reviewed some efforts in understanding the dynamics and function at the molecular level and cellular level. However, there is a missing link between the two approaches. On the one hand, the molecular dynamics can be modeled using simulations at the atomic level. On the other hand, the cellular level dynamics and function are modeled by the chemical reaction kinetics at the concentration or expression level. In fact, these expression level dynamics of the cellular networks are the reflections of the underlying molecular dynamics of genes and proteins. On the other hand, due to the rapidly advances in technology especially the Hi-C methods, the ensemble averaged structures of the chromosomes and genome can be obtained through the contact map between individual elements [147–152]. This is in the similar spirit on using the NMR measured residue contacts for determining the protein structures [147–152]. One can use the Hi-C contact information to infer the genome ensemble averaged structures such as the multi-megabase compartments and the sub-megabase topologically associating domains (TADs) [147–152]. However, Hi-C data only provide genome organization at one cell state, the information on the genome or chromosome structural changes in the switching between two cell states is not available, yet this is critically important for the cell function such as differentiation and development, cancer formation, cell cycle etc. Clearly a microscopic description for the structural transformation for the cell fate changes and decision making is in great demand [12, 153–155].

On the other hand, genome is huge involving 10^9 base pairs. The barrier between the two cell states is expected to be huge. This leads to great challenge of how to model the cell state transition process [12, 153, 154]. In fact, exploring how nature resolves this issue may provide us a clue. It appears that all these cell fate decision making processes are involved with the phosphorylation/de-phosphorylation processes with many ATP energy pumps. This shows that energy activation is inevitable and can be used to facilitate the cell state switching. This motivates the landscape excitation-relaxation model for quantifying the cell fate switching process. See the following illustration. Two cell fates are described by the two basins of individual landscapes. The ATP energy pumps provide the opportunity for jumping up from one initial landscape to another and then relax to the basin of attraction instead of direct crossing the barrier which is too high to overcome. This simulates the naturally occurring process of the cell fate decision making with state switching [12, 153, 154].

When applying this model to cancer and development, one explores the chromosome structural dynamics during the cell state switching among the pluripotent embryonic stem cell (ESC), the differentiated normal cell and the cancer cell shown in Fig. 14. Six transformations for cell fates involving differentiation, reprogramming, and cancer formation are considered. Pathways can merge in the process of state switching toward the stem cell or the normal cell. Before the merging, the two pathways are distinct in cell types in Fig. 15. High structural similarities are found at the merging point compared to the final cell destiny states in terms of the contact maps, TADs and compartments. After the merging point, it appears that the process proceeds with the adaption of the chromosome shape through its compaction with no clear influence on the contact formation. There is no merging of the cancer formation pathways from either the stem the normal cell state or the stem cell state. This illustrates that the cancer formation might involve multiple pathways. The genome model can provide a microscopic molecular level description of the cell differentiation and

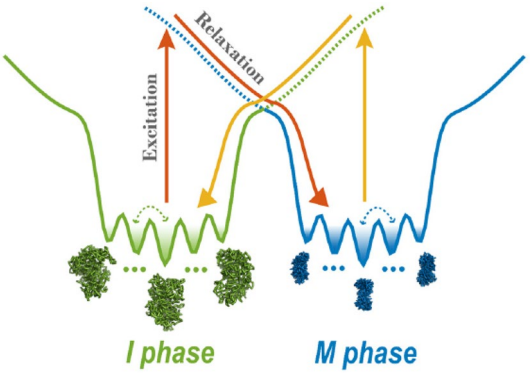

Fig. 14 Excitation-relaxation model for cell fate decision making process [153]. Reprinted from Appl. Phys. Rev. 7, Chu, X., Wang, J., Conformational state switching and pathways of chromosome dynamics in cell cycle, 031403, Copyright (2020), with permission from AIP Publishing

cancer formation from the dynamical chromosome structural perspectives. It can facilitate our understanding of the underlying molecular mechanisms of the cell fate decision making processes [12, 153–155].

5.2 Hierarchical organizations across scales

As we have seen, the larger scale emergent behaviors are usually the results of the smaller scale elements interacting with each other. The larger scale emergent behaviors can be very different from the behaviors of the smaller scale elements. Hans and other thinkers in the field suggested the hierarchical organization of the complex systems [1, 2, 4, 9, 74, 156] including the concepts of symmetry breaking, bifurcation, and emergence. These concepts can be combined together and provide a landscape picture of the emergence from the smaller scale to the larger scale. The intermediate scale dynamics can be emergent from the microscopic dynamics at the smaller scale. The microscopic interactions give constraints to the system so that not all the degrees of freedom are equally probable. In fact, some states are more preferred than the other. This naturally forms a probability or potential landscape. The higher probability states are emergent from the underlying microscopic interactions and give rise to the basins of attractions on the landscape. At the intermediate scale, the

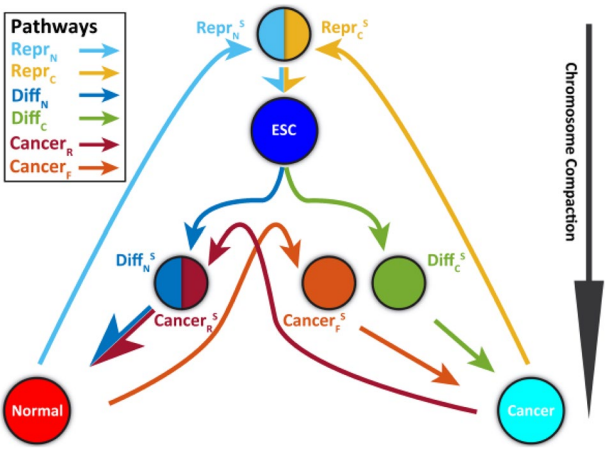

Fig. 15 Scheme illustrating the 6 cell state transition processes among the ESC, the normal and cancer cell involved with the transient states from the chromosome structural dynamics perspective. The vertical arrow from the top to the bottom indicates the degree of chromosome compaction [154]

dynamics can be described by the motions within each state basin and the state switching among the basins of attractions. It is also influenced by the underlying fast microscopic dynamics which lead to the effective friction and stochastic component of the driving force in addition to the emergent landscape [3], as shown in Fig. 16.

At the intermediate scale, the stochastic switching dynamics between the basins of attraction provide the interaction elements for the even larger scale. This leads to the emergent behavior at the larger scale from the underlying interactions among intermediate scale elements. Therefore, one can reach the hierarchical organization for the complex systems across the scales. In fact, for nonequilibrium systems, both the landscape and flux can be emergent from the underlying interactions among the elements at a smaller scale. The hierarchical organizations of the protein dynamics characterized by the energy landscapes have been demonstrated experimentally across different scales [7–9]. The nonequilibrium landscape and flux framework across the scales can help to reveal the underlying the hierarchical organization and emergence of the complex systems [3].

6 Experimental quantifications of the landscape and flux

The landscape and flux theory can be tested and quantified in the experiments. Pioneering work led by Hans on ligand binding to myoglobin and hemoglobin in the early days revealed the distributed kinetics and the associated underlying complex energy landscape structure [7–10].

For the larger scales, rapid developments of the technology make it possible to observe the real-time gene expression dynamics. This can be realized for example by integrating the GFP fluorescence molecule and attach to the gene expression product protein so that the associated fluorescence light intensity can be used to quantify the gene expression level or the concentration of the proteins. The light intensity fluctuates stochastically in time. The individual intensity in time is uncertain and not predictable. However, the statistical patterns of the intensity can be regular and be used to characterize the dynamics shown on the left panel in Fig. 17. Therefore, one can collect the statistics of light intensity in time

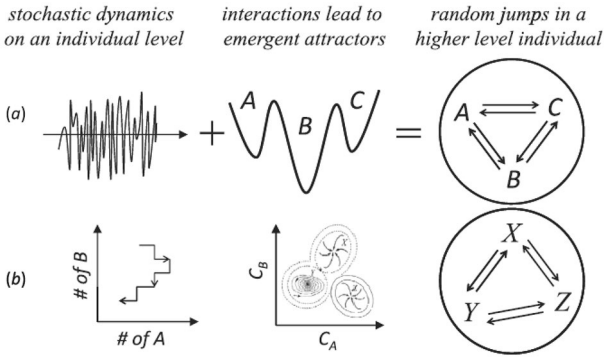

Fig. 16 **a** A schematics showing rapid solvent-macromolecule collisions, as a source of stochasticity and together with a multi-energy-well landscape, gives rise to a kinetic jump process for an individual macromolecule with multiple states (shown within the circle). **b** A level higher, many interacting chemical individuals each with multiple discrete states form mesoscopic nonlinear reaction systems [3]. Reprinted from Chem. Phys. Lett. 665, Qian, H., Ao, P., Tu, Y. H., Wang, J., A framework towards understanding mesoscopic phenomena: Emergent unpredictability, symmetry breaking and dynamics across scales, 153-161, Copyright (2016), with permission from Elsevier

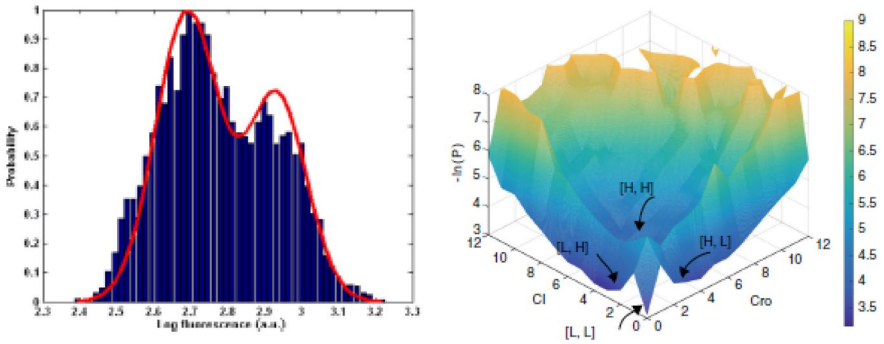

Fig. 17 Left:The histogram gives the intensity distribution of the 163 single-cell fluorescence trajectories induced at 1500 ng/mL aTc collected from the time-lapse experiments. The red solid curve is the fitted intensity distribution from HMM [158]. Right: The potential landscape was calculated using the experimentally measured 2D histogram of CI and Cro expression numbers in every 5-min frame and interpolated [157]

and obtain the associated distribution. This gives the probability or chance of observing the specific values of the intensity. The negative logarithm of this distribution can be used to quantify the potential landscape [157, 158].

For a self-repressive gene as TetR in the bacteria E-Coli, one expects to observe a single peak distribution centered around the repressive gene expression. By introducing the inducer molecules to disrupt the binding process of the self-repressor to the gene, one can effectively slow down the gene regulation process. This can give a chance of the genes being on when the self-repressors are not bound to the gene. Experimental real-time observations of the fluorescence intensity demonstrate the emergence of the bimodal distribution at certain inducer concentrations. This quantifies the underlying landscape with double basins of attraction [158].

Another example comes in for a lambda phage system in bacteria E. Coli which has been viewed as the hydrogen atom of the molecule biology [159]. Lambda phase is expected to have two fates controlled by the two genes mutually repressing each other. By the new developed technique of the co-localization, one can use the same color of the fluorescence molecules to label both gene expressions in different locations avoiding the different maturation time problem for synchronization. This allows one to follow the dynamics of a synthetic circuit of the lambda phase of CI and Cro under temperature sensitive mutation. Interestingly, four peaks have been observed from the statistics of the real-time traces shown on the right panel in Fig. 17. A four basin landscape emerges. Furthermore, the switching rates among these states can be quantified based on the hidden Markov chain analysis of the real-time data. Furthermore, one finds that the major pathway of switching from CI to Cro or vice versa is not the direct one but instead realized through the high expression state of both CI and Cro [157].

As mentioned, the landscape can be quantified experimentally through the statistics of the real-time traces shown in Fig. 18. One can also quantify the flux experimentally. Through the study of a single-molecule enzyme dynamics of horseradish peroxidase with the substrates of dihydrorhodamine 123 and hydrogen peroxide (H2O2), deviations from the traditional Michaelis–Menten kinetics were experimentally seen. Through the detailed analysis, the nonequilibrium flux is identified as the source of this non-Michaelis–Menten behavior. The flux can be quantified through fluorescence correlation spectroscopy from

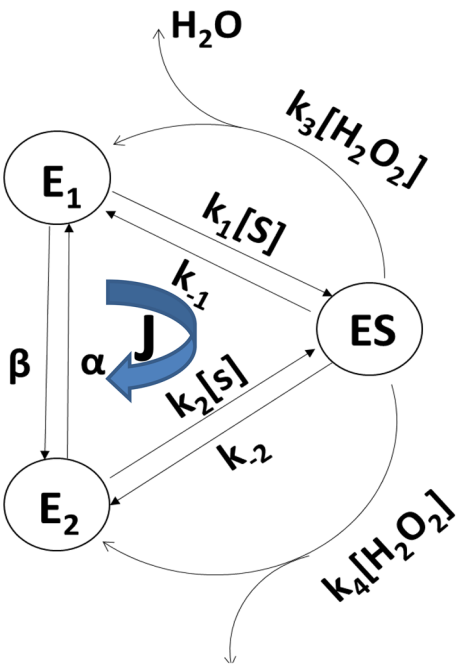

Fig. 18 The simplest kinetic scheme for HRP with 2 unbound enzyme states with nonzero internal loop flux J. E1 and E2 are the different conformations of free HRP, and ES is the substrate-bound state of HRP [160]

the real-time trace of the single enzyme dynamics. From this, one can further quantify the chemical potential and entropy production as the nonequilibrium thermodynamic driving force for the non-Michaelis–Menten behavior. Through the isothermal titration calorimetry, the heat absorbed into the enzyme reaction can be identified as the source for the nonequilibrium flux and nonequilibrium thermodynamics. This can provide a general way to quantify the dynamical and thermodynamic driving forces of the nonequilibrium systems [5, 160].

7 Outlook

In this review, I concentrate on the concepts of the landscape pioneered by Hans Frauenfelder and the flux for understanding the emergent behaviors of the complex systems. As seen, in general, the landscape and flux are the driving forces for the equilibrium and nonequilibrium dynamical systems. It also gives rise to the equilibrium and nonequilibrium thermodynamics. From this, many interesting behaviors such as the emergence of the basin of attraction state, the switching among these basins, the dominant paths connecting these states, the bifurcations and phase transitions can be quantified. Furthermore, the landscape and flux theory has found its wide applications. In protein folding, the funneled shaped landscape is crucial for folding stability and kinetic accessibility, and also for the evolution and design [16–19, 24–26, 45–47, 81, 89, 114]. For biomolecular recognition, the binding landscape can be used to quantify the specificity critical for the lead compound screening and drug discovery [11, 15, 20–32, 47]. For biomolecular conformational dynamics and enzyme dynamics, the landscape topography is important for addressing the underlying mechanisms in population shift and induced fit [11, 12, 19, 20, 42–44, 55–68, 153–155,

161]. For signal transduction, the landscape and flux theory can guide the information flow [162, 163]. For the metabolism, the landscape and flux theory can be used to find out the metabolic flow and stability [164]. For cell cycle, the landscape and flux theory can be used to identify the driving forces and the critical elements for maintaining the period relevant for the disease control [5, 6, 75, 103–106, 116, 165]. For cell differentiation and development, the landscape and flux theory helps to quantify the Waddington landscape and the pathways for differentiation and reprograming relevant to tissue engineering [78, 81, 89, 114, 115, 117]. For cancer, the landscape and flux theory can help to identify the disease state and key genes and regulations response for the cancer formation relevant to network medicine [5, 6, 128–130, 132–135]. For immunity, the landscape and flux theory can help to reveal different immune mechanisms through the cell–cell interactions [166]. For aging, the landscape and flux theory can help to figure out key elements for aging and rejuvenation [167, 168]. For brain science, the landscape and flux theory can help to reveal the underlying cognition mechanisms of the decision making, fear response, working memory, Parkinson's disease [91–93, 140, 141]. For evolution, the landscape and flux theory provides a physical foundation for general scenarios [5, 6, 76, 82]. For ecology, the landscape and flux theory can quantify the underlying global stability and associated bifurcations of the ecological states [80, 169, 170]. For game theory, the landscape and flux theory reveals the global quantification and physical mechanisms of the strategy state switching dynamics [77]. For economic theory, the landscape and flux theory can provide a physical basis for the complex nonequilibrium economy [171, 172]. For chaos, the landscape and flux theory can provide the quantitative origin of onset and off set of chaos formation [173, 174]. For turbulence, the landscape and flux theory leads to the nonequilibrium perspective and angle of the turbulence dynamics and thermodynamics [175, 176]. For open quantum systems, the landscape and flux theory can address the nonequilibrium dynamics and thermodynamics of the open quantum systems [177–182]. For quantum information, the landscape and flux theory can help to reveal the nonequilibrium effects on the quantum information measures such as entanglement, coherence, mutual information, fidelity etc. [183–187]. For black holes, the landscape and flux theory provides equilibrium and nonequilibrium views on the black hole dynamics and thermodynamics [188–192]. For cosmology, the landscape and flux theory provides the quantifications of the multiverses through cycles [193].

The landscape and flux theory for nonequilibrium systems provides a different view than the conventional ones for the equilibrium systems. The traditional logic is that the emergence behaviors of the complex systems come from the underlying interaction formed energy landscape which is a priori known. This is certainly true for the equilibrium systems. However, for the nonequilibrium systems, the traditional description fails since there is often no a priori known energy landscape for the dynamics to follow. In fact, the dynamics is not only determined by the landscape but also the nonequilibrium flux while the landscape and the flux themselves are the results of the dynamical process (steady-state outcomes). This indicates that the global driving force for the dynamics may not always be determined by the interaction energies as done conventionally since they are not necessarily known a priori. However, it appears that the probability landscape and the probability flux can still be used globally to determine the dynamics.

Since the landscape and flux can distinguish the weights of the states and the flows in between, one can focus on the states of high chance of appearance and the correlations among them. This can effectively reduce significantly the dimensionality of the system by concentrating on the dynamics of these relatively "stable" (high weight) or "slow" state variables while averaging out or coarse-graining the "transient" or "fast" state variables [5, 6, 74, 156]. The remaining "slow" degrees of the freedom with the reduced

dimensionality live on a new mesoscopic scale emerged from the underlying degrees of the freedom at the microscopic scale [3, 5, 6]. The laws for the dynamics at this mesoscopic scale are thus dictated by the correlations or transitions among those states emerged from the underlying dynamics of the microscopic degrees of freedom in the reduced dimension of the state space. In fact, the stochastic dynamics at the mesoscopic level is again determined by the landscape and flux emerged from the correlations among these "slow" states in the reduced dimensions of the state space. The landscape and flux determining the dynamics can also be used to quantify the nonequilibrium thermodynamics and statistical mechanics at both the microscopic and mesoscopic scales (see details in Sect. 3). One expects that from the results of the dynamics at the mesoscopic scale in terms of the landscape and flux can lead to further discriminations of the higher weighted states and the flows in between in the mesoscopic scale. Therefore, this provides the basis for building up the dynamics and their associated laws as well as the corresponding nonequilibrium thermodynamics for another higher scale (which we expect to be governed again by the landscape and flux), say macroscopic scale. The procedure can keep on going to generate the different scales or levels of hierarchies of organizations for the states of the matter and their associated dynamical laws as well as the corresponding nonequilibrium thermodynamics from the bottom up approach.

Importantly, the information on the landscape and flux can be obtained from the statistics and the correlations of the experimental observations for the real-time traces. Since the probability landscape and probability flux can drive the global dynamics, this implies that the fundamental force law determining the dynamics can be emerged from the statistics and correlations of the observations (data). Instead of building the fundamental force law from the bottom level as Newtonian mechanics, we now can see that the fundamental force laws for the dynamics can emerge from the top down in a statistical way by the number counting and the statistical correlations among the numbers in the observational data. While the equilibrium thermodynamics and statistical mechanics are usually determined by the underlying interaction energies from the bottom up, the general equilibrium and nonequilibrium thermodynamics as well as the associated statistical mechanics can be developed through the probability landscape and the probability flux emerged from the dynamics with the top down approach. This again indicates the possibility that the fundamental force laws and the corresponding emergent thermodynamics as well as the associated statistical mechanics can all come from the probability landscape and probability flux [6]. This is in some sense intuitive since the most fundamental things we can reliably count on after all are the numbers and the geometries. Statistics of the numbers (frequencies/probabilities) and the associated flows (fluxes) representing the correlations in between which have underlying geometry/topology structures [5, 6, 203] may be the basic building blocks of the fundamental and emergent laws at different scales [6]. Therefore, according to this logic, in practice the landscape and flux can be obtained from the statistics and correlations of the observational data. This gives to the force law for the dynamics and the corresponding thermodynamics at the scales of the observations. On the other hand, through the hidden Markovian chain analysis, the important states and their correlations can be further emerged from the real-time traces, leading to significant dimensional reduction. It can give rise again the force law governed by the landscape and the flux emerged from the statistics of the states and their correlations as well as the associated thermodynamics at this new emergent scale. This provides a quantitative foundation and realization of the entity and relationship determined world from the relational philosophy [6, 194, 195]. It is also important to realize that the statics is not the intrinsic nature of the world but the dynamics or evolution in time is. Thus, the basic building blocks based on the statistics of the

(experimentally observed) numbers/flows and the associated correlations in between both in time can lead to the dynamical evolution laws at the fundamental and emergent levels [6], giving rise to a quantitative basis for the processing philosophy [6, 194].

The probability and probability flux determining the dynamics can also be used to quantify the entropy and entropy flows, giving rise to the corresponding thermodynamics as discussed [5, 6]. One can turn the coin around and ask the question whether the laws for dynamics can be resulted or "derived" from the thermodynamics if one treats the thermodynamics as "fundamental" at current scale. In this perspective, the equation of state from the thermodynamics may be viewed as the law governing the dynamics. An example is from the expanding universe where the evolution dynamics can be viewed as the equation of state from the underlying thermodynamics [196]. More generally, Einstein equation for the space time dynamics can be seen as the equation of state and derived from the thermodynamics [197]. Therefore, gravity may have an entropic or thermodynamic origin [198]. From this perspective, various forms of the dynamics such as equation of motion [196, 197] and time dependent Hamilton–Jacobi equation [5, 6, 204] may be viewed as the equation (of state) for the evolution emerged from the landscape and flux based thermodynamics (first law and second law), either equilibrium or nonequilibrium.

On the other hand, the probability and probability flux can be used to represent the information and information flow [199–201]. This indicates that the information and information flow might also be used as the building blocks for the fundamental law. Some hints have already appeared in the link between the information and space time in the spirit of Wheeler's original proposal of it from bit [202]. The world maybe after all made of or originated from the information or entropy: the statistics of the numbers and their correlations as the fundamental entities of being. The information perspectives are thus expected to be important for the biological information processing such as in the cells and brain function [205] which requires more attentions and investigations.

In the future, the landscape and flux concepts and applications can be generalized to the spatial temporal dynamics [175, 176, 206–208] and reveal how emergent behaviors of the complex biological systems arise in space and time.

Another important aspect is to build bridge between the molecular level description and cellular description and the bridge between the cellular level description to the organism description so one can see how biological functions are realized at different scales [12, 151, 153, 209].

I have briefly reviewed some of the progresses made in recent years on using the concepts of the landscape and flux to understand the equilibrium and nonequilibrium biological systems. This is no doubt limited from the bias of the personal taste and also the finite space for discussions. I apologize to the parts neglected and the possible missing references.

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Declarations

Competing interests The authors declare that they have no conflict of interest.

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