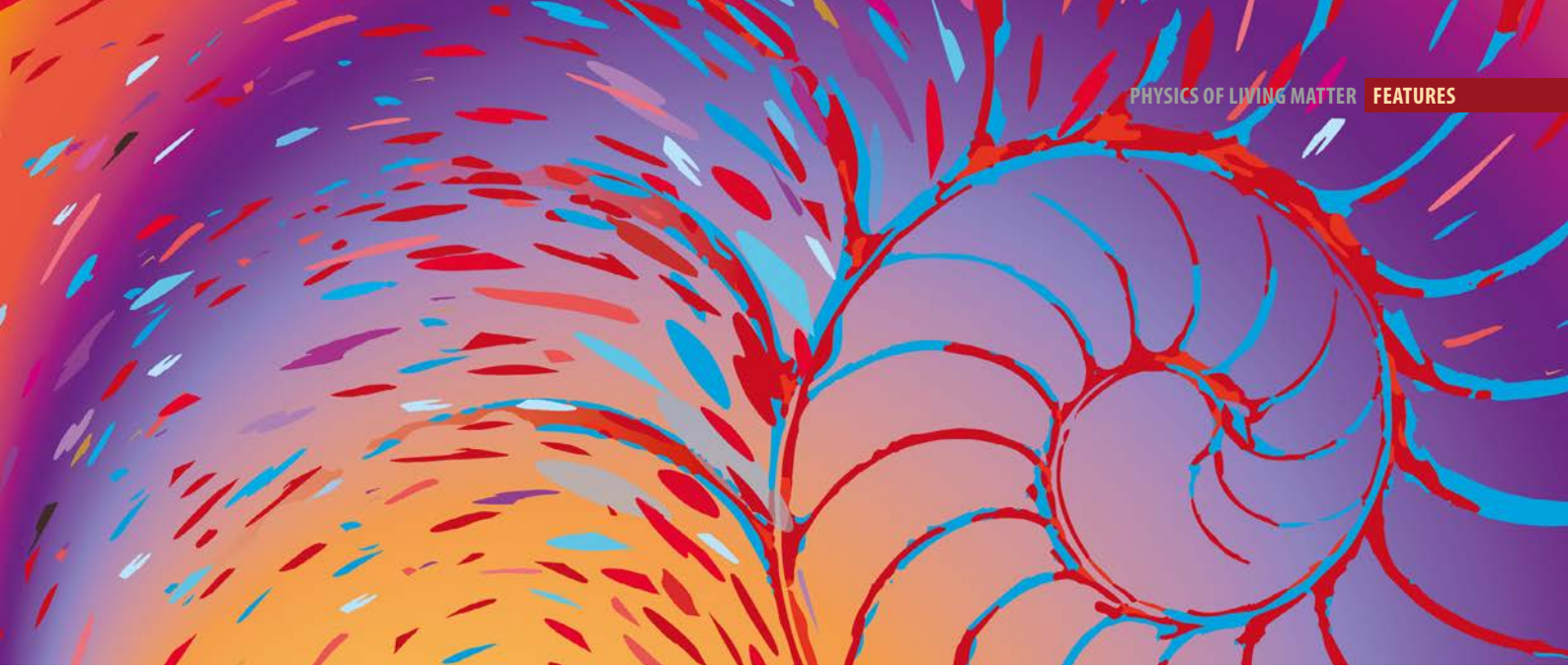

THE NONLINEARITY OF LIFE

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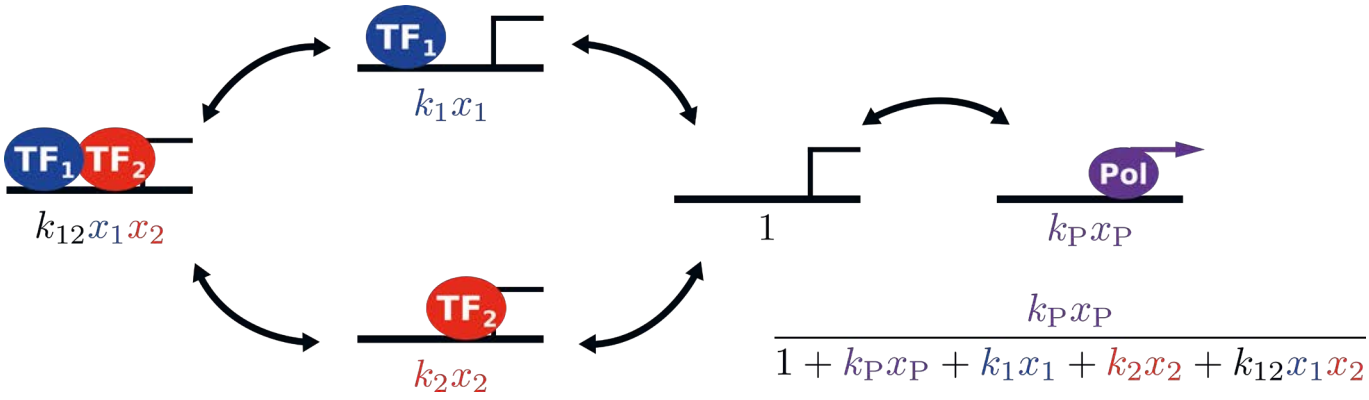
Statistical physics was originally developed to understand the behaviour of materials like gases, liquids or crystalline solids and the phase transitions between them. But in recent decades, concepts from statistical physics have been applied much more widely, in particular to biological systems.

Here they have provided insights into the mechanisms behind some profoundly nonlinear phenomena. One of these is the emergence of patterning in tissues during embryonic development, where cells differentiate from a common initial state into specialised states that are arranged in a highly reproducible fashion. This is achieved in spite of the significant inherent stochasticity in the molecular processes that underpin this behaviour, such as protein production and degradation. In this article we outline how methods from statistical physics can help us understand how biological systems realise this remarkable feat.

Gene regulatory networks and thermodynamic models

Developing organisms need to specify different cell types in an organised manner to produce the different tissues that form an organism, with the appropriate size and in the correct position. Each cell type is ultimately specified by the proteins that are present within that cell, so the system must ensure that the cells express the right proteins in the right place. This regulation is achieved by Transcription Factors (TFs), which are proteins that control the production of other proteins, including other transcription factors. TFs therefore interact with each other and can form complex Gene Regulatory Networks (GRNs).

A mathematical description of gene regulation is challenging because of the many nonlinearities involved: TFs can repress or activate the production of other proteins, often with a switch-like concentration dependence, and different TFs can act cooperatively, reinforcing each other's effects, or may compete with each other. Ideas from statistical physics already feature in the construction of models that can incorporate these effects, the so-called thermodynamic models of gene regulation [1]. These models assign thermodynamic weights to the binding states of a piece of DNA. As in mass-action kinetics for chemical reactions, these weights are proportional to the relevant TF concentrations and a binding state-dependent affinity. The protein production rate is then proportional to the relative weight of the states that do produce protein, typically because they have polymerase bound, as illustrated in Fig. 1. The denominator of this fractional weight is the total weight of all states and plays the role of a partition function, a key quantity in statistical physics.

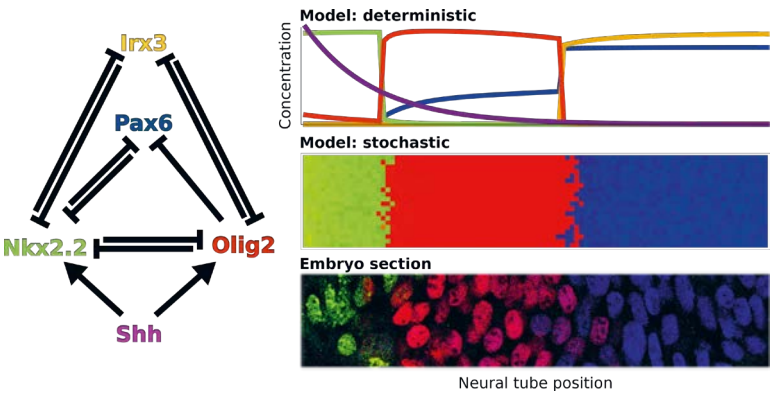


▲ **FIG. 1:** Sketch of different binding states of a stretch of DNA and transitions between them. Protein will be produced only from the state on the right, where polymerase is bound. When one or both of the transcription factors TF_1 and TF_2 are bound, polymerase cannot bind so these TFs act as repressors. The expressions below each state give its thermodynamic weight in terms of the TF and polymerase concentrations x_1, x_2 and x_p. The protein production rate is proportional to the relative weight of the relevant states (bottom right). If the affinity k_{12} is low compared to k_1k_2, the transcription factors bind anti-cooperatively: once one is bound the other is unlikely to bind as well.

Patterning as switching between fixed points

The patterning of the neural tube in vertebrates is a well characterised example of a GRN driving the spatial organisation of a system. A gradient (spatially varying concentration) of the protein Sonic-Hedgehog (Shh, a so-called morphogen) is "interpreted" by cells and this leads to different ultimate cell fates. These fate decisions are made by a GRN consisting of cross-repressive interactions with input from Shh (Fig. 2). The GRN dynamics can be modelled using thermodynamic models of protein production rates as explained above; with appropriate parameters one can then reproduce the experimentally observed timing of protein concentration changes and the final positioning of the cell identity boundaries (Fig. 2) [2]. In more physical language, what this analysis shows is that patterning can be viewed as the result of cells switching from one gene regulation fixed point to another in response to an external signal, here given by the spatial variation of morphogen concentration.

▼ **FIG. 2:** (Left) GRN responsible for patterning the ventral vertebrate neural tube. TFs repress each other (lines with bars at end), and input comes in the form of a gradient of Shh (arrows). Colouring of TFs is consistent throughout. (Right) Characteristic concentration pattern of a neural tube, showing discrete spatial domains of gene expression that ultimately lead to distinct choices of neuronal subtypes. Patterns are from deterministic simulation (top), stochastic simulation (middle) and in the mouse embryo (bottom).



Subnetwork dynamics and memory functions

We have seen that thermodynamic models of GRNs can capture experimentally observed patterning. But can we understand more *qualitatively* how the switching behaviour is generated by the GRN as the pattern is established over time? For this it is helpful to focus on the part of the network driving the dynamics. In the neural tube example this is the pair of TFs Nkx2.2--Olig2, which repress each other and so form a *bistable switch* between two steady states where either one of the TFs has a high concentration and the other a low one. If we want to describe the dynamics of such a subnetwork, we need to account for memory effects: the subnetwork can affect the rest ("bulk") of the network and these effects then feed back at a later time, meaning the subnetwork effectively remembers its past.

The Zwanzig-Mori projection method from statistical physics allows such memory effects to be determined, and we have developed two versions of this to extract information about the mechanisms that lead to emergent behaviour in GRNs.

Focussing firstly on the GRN dynamics near a steady state, the memory effects can tell us about the importance of interactions between TFs. *E.g.* in the neural tube system they identify one such interaction link as ensuring robustness to changes in initial conditions [3], which is important in the noisy world of biological development. We subsequently developed a versatile method that can predict nonlinear memory effects even far from steady states [4]. These are obtained systematically as corrections to a memory-less scenario called quasi-steady state (QSS), where the bulk is taken as adapting immediately to any change in the subnetwork. The approach can correctly capture the choice of cell fate using a subnetwork of just two subspecies, with much greater accuracy than the memory-less QSS approximation (Fig. 3). The memory effects are important also in accounting correctly for the

time delay in final patterning that is characteristic of neural tube patterning (Fig. 3). Importantly, for both of these phenomena one can deduce which specific parts of the network play the biggest role. Nonlinear memory functions thus reveal hidden information that cannot be extracted from *e.g.* directly simulating the GRN dynamics.

Boundary precision

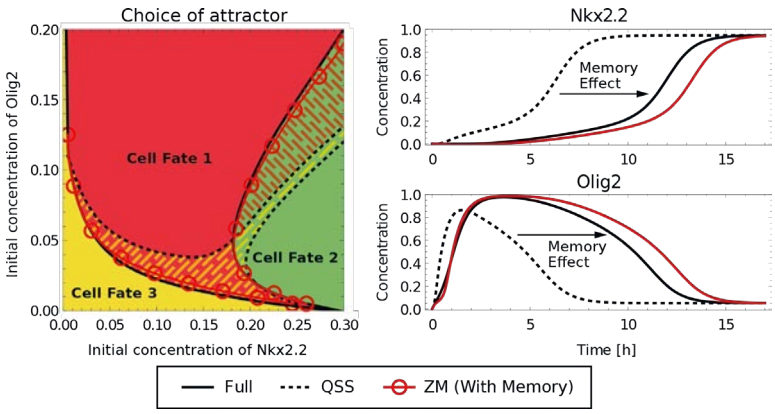
So far we have ignored fluctuations. But in biology as in physics there is a great amount of randomness, so how can tissue boundaries evolve so precisely? To understand this we can extend the description of GRN dynamics by explicitly including noise, *e.g.* from protein number fluctuations. This gives us Langevin equations, similar to those used in statistical physics to describe the Brownian motion of particles jiggled randomly by a surrounding liquid or gas. Such a stochastic model can then capture the timing, position and, importantly, precision of tissue patterning [5]. We can study in particular how boundary precision is reduced by changes in the GRN such as deleting nodes (TFs) or edges (interactions), and the predicted effects compare well with experiments. An analysis of the model provides an explanation (Fig. 4): boundaries are blurred when cells change stochastically from one fate to another, on the timescale where a tissue pattern is established. Much faster or slower transitions effectively happen always or never, so boundaries stay sharp when transition rates change rapidly from fast to slow as morphogen levels vary across a tissue. These transition rates can be worked out from our stochastic model by generalising Kramers' approach to the thermally activated escape of a particle from a potential well, and this then allows to predict boundary sharpness and its main drivers. We can even perform a systematic screen across a broad class of GRNs, to establish which network structures tend to produce sharp boundaries by the above mechanism [5].

Concluding remarks

We have only been able to cover a few examples of nonlinear physics in biology in this article, yet even so we have encountered a broad range of physical concepts, from thermodynamic weights and reaction kinetics to projection methods, Brownian motion and thermal activation. This suggests to us that there is much potential for future progress in this exciting interdisciplinary effort. ■

About the Authors

Edgar Herrera-Delgado is a postdoctoral researcher in the Mechanics of Mammalian Development group at Institut Curie, having obtained his PhD from King's College London and the Francis Crick Institute. His research focuses on understanding emergent behaviour in biology through modelling, analysis and experiments.



▲ **FIG. 3:** (Left) Basins of attraction for the neural tube network, shown against the initial concentration of the two key TFs. A description of this subnetwork including memory (red circles) captures the true boundaries (black) very well; these mark where cell fate decisions change. Ignoring memory (dotted lines) gives qualitatively wrong predictions (hatched areas). (Right) The timing of patterning is also accurately captured in a subnetwork description with memory, while the memory-less QSS approximation misses the characteristic transient.

Peter Sollich is professor of Non-Equilibrium Statistical Physics at the University of Göttingen and at King's College London, having worked previously at the University of Edinburgh.

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▼ **FIG. 4:** Effect of noise on boundary sharpness. When two cell fates are possible (red/green lines in 3d plot), cells can transition stochastically from "red" to "green"; the rate varies across the tissue. Where transitions are rapid on the developmental timescale they happen always (left on position axis), where they are very slow (right), never. In between the tissue boundary is blurred, so sharp boundaries are produced when transition times change strongly with position.