



HAL
open science

Action at a distance in transcriptional regulation

William Bialek, Thomas Gregor, Gašper Tkačik

► **To cite this version:**

William Bialek, Thomas Gregor, Gašper Tkačik. Action at a distance in transcriptional regulation. 2019. pasteur-03216303

HAL Id: pasteur-03216303

<https://pasteur.hal.science/pasteur-03216303v1>

Preprint submitted on 3 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Action at a distance in transcriptional regulation

William Bialek,^{a,b} Thomas Gregor,^{a,c} and Gašper Tkačik^d

^aJoseph Henry Laboratories of Physics, and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton NJ 08544 USA

^bInitiative for the Theoretical Sciences, The Graduate Center, City University of New York, 365 Fifth Ave, New York NY 10016

^cDepartment of Developmental and Stem Cell Biology UMR3738, Institut Pasteur, 75015 Paris, France

^dInstitute of Science and Technology Austria, Am Campus 1, A-3400 Klosterneuburg, Austria

(Dated: December 18, 2019)

There is increasing evidence that protein binding to specific sites along DNA can activate the reading out of genetic information without coming into direct physical contact with the gene. There also is evidence that these distant but interacting sites are embedded in a liquid droplet of proteins which condenses out of the surrounding solution. We argue that droplet-mediated interactions can account for crucial features of gene regulation only if the droplet is poised at a non-generic point in its phase diagram. We explore a minimal model that embodies this idea, show that this model has a natural mechanism for self-tuning, and suggest direct experimental tests.

In multicellular organisms, the transcription of genes into messenger RNA is controlled by the binding of transcription factor proteins to “enhancer” sites that can be separated from the gene by tens of thousands of base pairs along the DNA sequence [1–6]. Close approach of enhancers to their target promoters has been inferred from cross-linking experiments [7], and there is direct evidence that the action of the enhancer requires physical proximity to the promoter site where transcription is initiated [8]. But proximity is not contact: the most recent measurements indicate that the enhancers and their target promoters remain separated by 150 – 350 nm even during active transcription [8–12].

How is the apparent action at a distance possible? Interactions between the enhancer and promoter could be transmitted along the length of the DNA molecule, but it seems more plausible that this interaction is transmitted across the shorter three dimensional distance [13]. Recent observations indicate that there is a medium for this transmission, a condensed droplet of the protein “mediator” which surrounds the promoter [14]; these droplets also contain high concentrations of RNA polymerase [15], are associated with foci of active transcription [16], can form in vitro [17], and contain other co-activating factors [18]. We propose that the droplet acts as a larger scale version of an allosteric protein [19–21]: in the same way that the protein structure allows binding of small molecules at one site to influence binding or enzymatic activity at a distant site, the droplet would allow binding of transcription factors (TFs) at an enhancer site to influence activity at the distant promoter site (Fig 1). We will argue that this is possible only if the droplet is at a non-generic point in its phase diagram, and that the collective interactions among the enhancer sites can drive the system toward such points.

Two facts will be crucial to our discussion. First, to the extent that the mediator droplets are similar to other examples of intracellular phase separation [22], they will

be liquid-like [23], and thus in general will not transmit structural changes across hundreds of nanometers. Second, gene expression can be controlled in a quantitative, graded fashion in response to changing concentrations of transcription factors [24–27].

If we did not have the constraint of graded responses, we could imagine that binding of TFs to enhancer sites triggers droplet condensation, and that this is the essential mechanism of regulation, as proposed for “super-enhancers” [18, 28]. But this is an all-or-none mechanism, and it is difficult to harness the triggering of phase separation to generate a quantitatively graded response to changes in TF concentration. The existence of droplets by itself does not solve the problem.

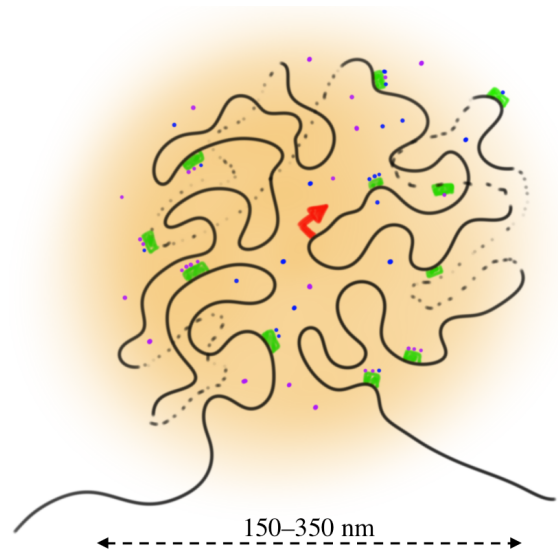


FIG. 1: The DNA strand (black) surrounds a condensed droplet (orange). Promoter site is marked by an arrow (red), enhancer sites as blocks (green) with transcription factors (magenta and blue) both bound to these sites and freely diffusing.

Even if transcription requires droplet condensation, there are pathways for regulation once the droplet has formed. In eukaryotes transcription involves a very large number of different proteins, and it is plausible that many of these components condense into the droplet. With multiple components the phase diagram is more complicated than just two phases [29, 30], so droplets can condense and still have additional degrees of freedom related to the addition or expulsion of different molecular species. Let us summarize these variables by an order parameter $\phi(\vec{r})$, which can vary with position \vec{r} inside the droplet. These are the degrees of freedom that can

propagate interactions through the droplet.

The simplest model envisions a set of K identical binding sites for a single class of transcription factors (at positions \vec{r}_i), plus one promoter site (at \vec{r}_a), all embedded in a droplet. These binding sites typically will be arrayed across multiple enhancers, all of which can contribute to regulating transcription. The relevant variables are $\sigma_i = \pm 1$ for empty and occupied binding sites, and $A = \{0, 1\}$ for the inactive and active states of the promoter. All of these variables couple to the order parameter, and it is important that these couplings are spatially local. The free energy is then

$$\mathcal{F}[\phi(\vec{r}); \{\sigma_i\}, A] = \mathcal{F}_0[\phi(\vec{r})] + E_0 A - \frac{k_B T}{2} \ln(c/c_0) \sum_{i=1}^K \sigma_i + \sum_{i=1}^K g_i \sigma_i \phi(\vec{r}_i) + g_a A \phi(\vec{r}_a), \quad (1)$$

where g_i is the interaction between the order parameter and binding of TFs to the enhancers, g_a is the interaction between the order parameter and the active vs inactive state of the promoter, E_0 is the free energy difference between the two states of the promoter in the absence of TFs, c is the concentration of these factors, and c_0 is the “bare” binding constant of the TF to the enhancer sites.

Let’s assume that, as in conventional models of allostery, the transmission of information can be described as an equilibrium thermodynamic effect [31–33]. Hence, we define an effective free energy by integrating out the fluctuations of the order parameter,

$$e^{-F_{\text{eff}}(\{\sigma_i\}, A)/k_B T} = \int \mathcal{D}\phi \exp\left(-\frac{\mathcal{F}[\phi(\vec{x}); \{\sigma_i\}, A]}{k_B T}\right), \quad (2)$$

where $\mathcal{D}\phi$ is the measure for integration over $\phi(\vec{r})$. F_{eff} is composed of independent and interacting parts, $F_{\text{eff}} = F_0 + F_{\text{int}}$, and to leading order in the couplings we have

$$F_0 = E_0 A - \frac{k_B T}{2} \ln(c/c_0) \sum_{i=1}^K \sigma_i \quad (3)$$

$$F_{\text{int}} = - \sum_{i,j=1}^K \frac{g_i g_j}{2k_B T} C(r_{ij}) \sigma_i \sigma_j - \sum_{i=1}^K \frac{g_a g_i}{k_B T} C(r_{ia}) \sigma_i A, \quad (4)$$

where we choose coordinates so that the average order parameter is zero in the Boltzmann distribution defined by \mathcal{F}_0 , and $C(r)$ is the correlation function of the order parameter fluctuations in this distribution,

$$\langle \phi(\vec{r}_i) \phi(\vec{r}_j) \rangle \equiv C(r_{ij}), \quad (5)$$

with $r_{ij} = |\vec{r}_i - \vec{r}_j|$. The question of whether the droplet transmits information from the enhancer to the promoter

becomes the question of whether fluctuations in the order parameter are correlated over these long distances [34].

In liquids at generic parameter values, density fluctuations have a short correlation length ξ , so that $C(r) \simeq e^{-r/\xi}$, with ξ on the nanometer scale, and thus these modes cannot support action at a distance. There can be additional degrees of freedom associated with the orientational ordering of molecules in the droplet, or with conformational changes of these molecules, but again with generic parameters we expect to find small ξ . The alternative is that the parameters describing the droplet are at a non-generic point in the phase diagram, where the correlation length can become long, and this is the “critical droplet” scenario we explore here.

Close to a first-order phase transition the free energy \mathcal{F}_0 has two nearly degenerate minima [34]. In a sufficiently small droplet, fluctuations in the order parameter are dominated by flickering between these minima, and there is an effective surface tension that keeps the entire droplet in one minimum, so that $C(r)$ becomes only weakly dependent on distance [35]. Close to a second-order phase transition the correlation length diverges, and $C(r)$ decays very slowly, as power of distance. Either of these scenarios seems to require some tuning of the droplet parameters, to which we return below.

If all the transcription factor binding sites couple to the droplet in the same way, then we should have all the $g_i = g$. We can capture the essential predictions of this model if all the distances r_{ij} and r_a are roughly equal to a typical R , in which case we can simplify to

$$F_{\text{int}} = -\frac{J}{2} \sum_{i,j=1}^K \sigma_i \sigma_j - J_a \sum_{i=1}^K \sigma_i A, \quad (6)$$

with two parameters $J = (g^2/k_B T)C(R)$ and $J_a =$

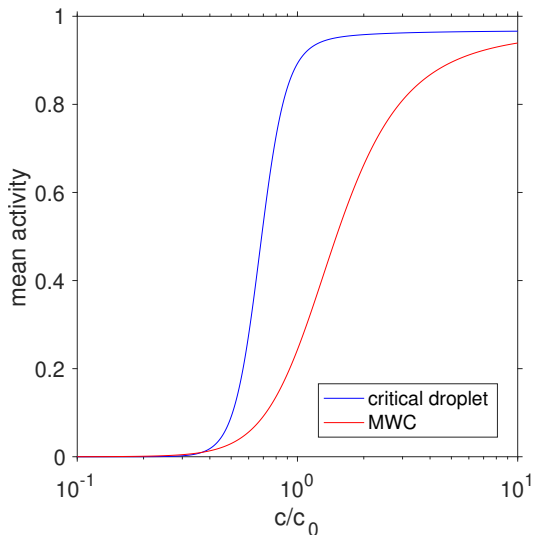


FIG. 2: Mean promoter activity as function of the TF concentration. Results from Eqs (3) and (6) with $K = 8$ sites, $J = 0.2k_B T$, $J_a = k_B T$, and $E_0 = k_B T \ln(100)$, compared with the corresponding MWC model ($J_a = 0.2k_B T$, $J = 0$). Note that interaction energies are on the order of $k_B T$ or less, but the droplet generates a very steep response to changing TF concentrations.

$(gg_a/k_B T)C(R)$. To gain intuition, we note that if $J = 0$ then Eq (6) becomes identical to the Monod–Wyman–Changeux (MWC) model for allosteric proteins [20, 32], with the $A = 1/0$ switch in promoter activation playing the role of the R/T conformational transition [20, 21]. A critical droplet thus generates cooperative interactions among distant sites that are similar, qualitatively, to more familiar examples of allostery (Fig 2).

Transcriptional activators become repressors just by changing the sign of the coupling g_i , which describes interaction of the TF with the surrounding droplet, and is determined by the face of the protein opposite from the DNA binding domain. A generalization is to imagine that we have K_a binding sites for activators at concentration c_a and K_r sites for repressors at concentration c_r . Then at low repressor concentrations there is cooperative activation, but at higher repressor concentration the system approximates a switch that depends on the ratio of powers of the concentrations, $c_a^{K_a}/c_r^{K_r}$.

A second generalization is to have multiple nearby binding sites within one enhancer interact more strongly, perhaps through additional degrees of freedom, and then let the emergent states of multiple enhancers couple to the droplet. The system could then approximate logical operations corresponding to combinations of ANDs within each enhancer and ORs among enhancers.

An important implication of this model is that transcription is regulated not by the binding of transcription factors to individual binding sites, but rather by an integrated signal from multiple binding sites that are dis-

tributed around the droplet of size R . If we think of the transcriptional output as a “measurement” of the TF concentration, then the accuracy of this measurement is limited by the random arrival of molecules [36–38]; the smallest concentration differences δc to which a system can respond reliably is given by

$$\frac{\delta c}{c} \simeq \frac{1}{\sqrt{D\ell c\tau}}. \quad (7)$$

where c is the background concentration, D is the diffusion constant, τ is the time over which the system can average, and ℓ is the linear size of the sensitive element. If the response is driven by a single binding site, then ℓ is the size of that site, but if the system integrates over many binding sites, then ℓ can approach the linear dimensions of the entire array of sites, in our case the size of the droplet, which is $\sim 100\times$ larger than individual binding sites. From Eq (7), responses which would require hours of integration at a single site thus become reliable in minutes. Transcription factor concentrations are so low that this difference can be crucial [37, 39].

Poising a condensed droplet near a critical point seems to require fine tuning of its parameters. Cells can exert exquisite control over protein and nucleic acid concentrations [39, 40], but matching the concentrations of crucial molecules to their critical values still seems difficult. In our case, however, there is a thermodynamic driving force that pushes the system toward conditions where correlation lengths are long. To estimate this effect, let’s assume that the droplet has a critical point when one of its components is at concentration x_0 . The chemical potential of the surrounding solution holds the concentration close to some mean concentration \bar{x} , and variations Δx around this mean cost a free energy $F_1 \simeq (\bar{n}k_B T/2)(\Delta x/\bar{x})^2$, where $\bar{n} \simeq R^3\bar{x}$ is the mean number of molecules in the droplet. But at a concentration x the correlation length will be $\xi = a|x_0/(x - x_0)|^\nu$ [34], where a is a microscopic length scale. Away from criticality, the gain in free energy from interaction among K binding sites is $F_2 \simeq -(J_0/2)K(K - 1)e^{-R/\xi}$, and in total we have

$$\frac{2F}{\bar{n}k_B T} \simeq (\Delta x/\bar{x})^2 - A \exp\left[-\frac{R}{a}\left|\frac{x_0}{\Delta x_0 + \Delta x}\right|^\nu\right], \quad (8)$$

with $\Delta x_0 = \bar{x} - x_0$ and $A = (J_0/k_B T)K(K - 1)/\bar{n}$. The dominant component of the droplet is present at only $\bar{n} \sim 100$ [14], so with $K \sim 10$ binding sites it is easy to have $A \sim 1$; to be conservative we consider $A = 0.25$. We could plausibly have $R \sim 150$ nm and the molecular scale $a \sim 5$ nm, but again to be conservative we choose $R/a = 5$. Assuming that the chemical potential alone sets $\bar{x} = 1.5x_0$, we see that the possibility of mediating interaction among binding sites creates a sharp minimum of the free energy at the critical point, sufficient to pull the system very close to criticality (Fig 3).

Taking this thermodynamic driving force seriously, we note that when transcription is active, enhancer bind-

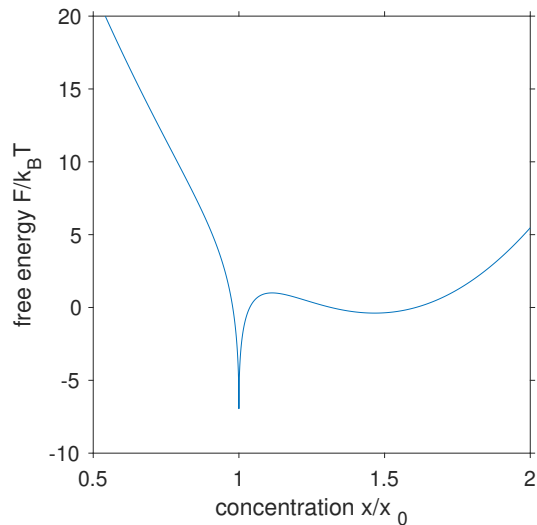


FIG. 3: Free energy as a function of concentration in the droplet, from Eq (8), with $\bar{n} = 100$, $\Delta x_0 = x_0/2$, $A = 0.25$, $R/a = 5$, and $\nu = 1/2$. Note the weak minimum at the $x = x_0 + \Delta x_0$, set by the chemical potential, which is dominated by the minimum at criticality, $x = x_0$.

ing sites with states that are “aligned” to this activation have a free energy that is lower by $J_a \propto C(R)$, and since $C(R)$ decreases with r this generates a small force pulling the enhancer toward the promoter. In contrast, enhancers in states that are not contributing to activation of transcription have a free energy that is higher by J_a and a force pushing enhancer and promoter apart. These small forces are balanced by a stiffness, which also determines the thermal fluctuations in the enhancer–promoter distance. The result is that aligned vs anti-aligned enhancers should be at different mean distances from the promoter site, and this displacement is $\Delta R/R \sim (J_a/k_B T)(\delta R/R)^2$, where δR is the standard deviation of the distance R , and we assume that $d \ln C(R)/d \ln R \sim 1$. These displacements should be directly observable, for example by measuring the positions of different enhancers for the pair-rule genes in the early fly embryo [41]. More generally this suggests that single-molecule observations of enhancer motions could be connected, quantitatively, to the energetics of cooperative transcriptional activation.

To summarize, a large number of transcription factor binding sites, embedded in a droplet that surrounds the promoter, will generate cooperative regulation if the droplet is poised near special points or lines in its phase diagram where correlation lengths become long. In this scenario the droplet functions much like an allosteric protein, but this is possible only because of the proximity to criticality. This is similar to the long-ranged interactions between proteins that we expect to see in a membrane [42] if lipid compositions are close to a critical point, as observed [43–45]; it has also been suggested that chro-

matin itself is close to a sol/gel phase boundary [46]. There is a much wider range of ideas about how criticality could play a role in biological function [47, 48], but what is special in our example is that we have identified, as an intrinsic part of the functional behavior, a mechanism that drives the system toward its critical point, and perhaps this is more general. Consequences of this thermodynamic driving force should be directly observable in the physical positions of enhancer and promoter sites.

We thank L Barinov, SA Kivelson, and MS Levine for helpful discussions. This work was supported in part by the US National Science Foundation, through the Center for the Physics of Biological Function (PHY-1734030) and Grant PHY-1607612; by National Institutes of Health Grants P50GM071508, R01GM077599, and R01GM097275; and by Austrian Science Fund grant FWF P28844.

-
- [1] W De Laat and D Duboule, Topology of mammalian developmental enhancers and their regulatory landscapes. *Nature* **502**, 499–506 (2013).
 - [2] M Levine, C Cattoglio, and R Tjian, Looping back to leap forward: Transcription enters a new era. *Cell* **157**, 13–25 (2014).
 - [3] EEM Furlong and M Levine, Developmental enhancers and chromosome topology. *Science* **361**, 1341–1345 (2018).
 - [4] R Stadhouders, GJ Filion, and T Graf, Transcription factors and 3D genome conformation in cell-fate decisions. *Nature* **559**, 345–354 (2019).
 - [5] B van Steensel and EEM Furlong, The role of transcription in shaping the spatial organization of the genome. *Nat Rev Mol Cell Biol* **20**, 327–337 (2019).
 - [6] S Schoenfelder and P Fraser, Long-range enhancer-promoter contacts in gene expression control. *Nat Rev Genet* **20**, 437–455 (2019).
 - [7] B Mifsud et al, Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. *Nature Genetics* **47**, 598–606 (2015).
 - [8] H Chen, M Levo, L Barinov, M Fujioka, JB Jaynes, and T Gregor, Dynamic interplay between enhancer–promoter topology and gene activity. *Nature Genetics* **50**, 1296–1303 (2018).
 - [9] LJ Mateo, SE Murphy, A Hafner, IS Cinquini, CA Walker, and AN Boettiger, Visualizing DNA folding and RNA in embryos at single-cell resolution. *Nature* **568**, 49–54 (2019).
 - [10] JM Alexander, J Guan, B Li, L Maliskova, M Song, Y Shen, B Huang, S Lomvardas, and OD Weiner, Live-cell imaging reveals enhancer-dependent *Sox2* transcription in the absence of enhancer proximity. *eLife* **8**, e41769 (2019).
 - [11] T Heist, T Fukaya, and M Levine, Large distances separate coregulated genes in living *Drosophila* embryos. *Proc Natl Acad Sci (USA)* **116**, 15062–15067 (2019).
 - [12] L Barinov, S Ryabichko, and T Gregor, unpublished.
 - [13] NS Benabdallah and WA Bickmore, Regulatory domains and their mechanisms. *Cold Spring Harb Symp Quant*

- Biol* **80**, 45–51 (2015).
- [14] W-K Cho, J-H Spille, M Hecht, C Lee, C Li, V Grube, and II Cissé, Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. *Science* **361**, 412–415 (2018).
- [15] II Cisse, I Izeddin, SZ Causse, L Boudarene, A Senecal, L Muresan, C Dugast-Darzacq, B Hajj, M Dahan, and X Darzacq, Real-time dynamics of RNA polymerase II clustering in live human cells. *Science* **341**, 664–667 (2013).
- [16] WK Cho, N Jayanth, BP English, T Inoue, JO Andrews, W Conway, JB Grimm, J-H Spille, LD Lavis, T Lionnet, and II Cisse, RNA Polymerase II cluster dynamics predict mRNA output in living cells. *eLife* **5**, e13617 (2016).
- [17] A Boija et al, Transcription factors activate genes through the phase-separation capacity of their activation domains. *Cell* **175**, 1842–1855. (2018).
- [18] BR Sabari et al, Coactivator condensation at super-enhancers links phase separation and gene control. *Science* **361**, eaar3958 (2018).
- [19] J Monod, J-P Changeux, and F Jacob, Allosteric proteins and cellular control systems. *J Mol Biol* **6**, 306–329 (1963).
- [20] J Monod, J Wyman, and J-P Changeux, On the nature of allosteric transitions: A plausible model. *J Mol Biol* **12**, 88–118 (1965).
- [21] MF Perutz, *Mechanisms of Cooperativity and Allosteric Regulation in Proteins* (Cambridge University Press, Cambridge, 1990).
- [22] S Banani, H Lee, A Hyman, and MK Rosen, Biomolecular condensates: organizers of cellular biochemistry. *Nat Rev Mol Cell Biol* **18**, 285–298 (2017)
- [23] Y Shin, YC Chang, DDW Lee, J Berry, DW Sanders, P Ronceray, NS Wingreen, M Haataja, and CP Brangwynne, Liquid nuclear condensates mechanically sense and restructure the genome. *Cell* **175**, 1481–1491 (2018).
- [24] A Colman-Lerner, A Gordon, E Serra, T Chin, O Resnekov, D Endy, CG Pesce, and R Brent, Regulated cell-to-cell variation in a cell-fate decision system. *Nature* **437**, 699–706 (2005).
- [25] S Takahashi and PM Pryciak, Membrane localization of scaffold proteins promotes graded signaling in the yeast MAP kinase cascade. *Current Biology* **18** 1184–1191 (2008).
- [26] L Giorgetti, T Siggers, G Tiana, G Caprara, S Notarbartolo, T Corona, M Pasparakis, P Milani, ML Bulyk, and G Natoli, Noncooperative interactions between transcription factors and clustered DNA binding sites enable graded transcriptional responses to environmental inputs. *Molecular Cell* **37**, 418–428 (2010).
- [27] KW Rogers and AF Schier, Morphogen gradients: from generation to interpretation. *Annu. Rev. Cell. Dev. Biol.* **27**, 377–407 (2011).
- [28] D Hnisz, K Shrinivas, RA Young, AK Chakraborty, and PA Sharp, A phase separation model for transcriptional control. *Cell* **169**, 13–23 (2017).
- [29] RP Sear and JA Cuesta, Instabilities in complex mixtures with a large number of components. *Phys Rev Lett* **91**, 245701 (2003).
- [30] S Mao, D Kuldinow, MP Haataja, and A Košmrlj, Phase behavior and morphology of multicomponent liquid mixtures. *Soft Matter* **15**, 1297–1311 (2019).
- [31] Many allosteric proteins are enzymes, and the non-equilibrium nature of an active catalyst could make an equilibrium description impossible. In fact the equilibrium description seems to work, so we start here.
- [32] S Marzen, HG Garcia, and R Phillips, Statistical mechanics of Monod–Wyman–Changeux (MWC) models. *J Mol Biol* **425**, 1433–1460 (2013).
- [33] T Einav, L Mazutis, and R Phillips, Statistical mechanics of allosteric enzymes. *J Phys Chem B* **120**, 6021–6037 (2016).
- [34] J Sethna, *Statistical Mechanics: Entropy, Order Parameters and Complexity* (Oxford University Press, 2006).
- [35] There is a length scale formed from the ratio of this surface tension to the free energy difference per unit volume between the two minima, which vanishes on the line of first-order transitions, so the length scale diverges. A problem in this scenario is to be sure that flickering between the minima is fast enough.
- [36] HC Berg and EM Purcell, Physics of chemoreception. *Biophys J* **20**, 193–219 (1977).
- [37] W Bialek and S Setayeshgar, Physical limits to biochemical signaling. *Proc Natl Acad Sci (USA)* **102**, 10040–10045 (2005).
- [38] JS van Zon, MJ Morelli, S Tănase-Nicola, and PR ten Wolde, Diffusion of transcription factors can drastically enhance the noise in gene expression. *Biophys J* **91**, 4350–4367 (2006).
- [39] T Gregor, DW Tank, EF Wieschaus, and W Bialek, Probing the limits to positional information. *Cell* **130**, 153–164 (2007).
- [40] MD Petkova, SC Little, F Liu, and T Gregor, Maternal origins of developmental reproducibility. *Curr Biol* **24**, 1283–1288 (2014).
- [41] Pair rule genes are expressed in a seven-striped pattern along the long axis of the *Drosophila* embryo, and different stripes are activated by different enhancers [JB Jaynes and M Fujioka, *Dev. Biol.* **269**, 609–622 (2004)]. By looking at the same enhancer in different stripes, or multiple enhancers within a single stripe, we can sample conditions in which the promoter is active but different enhancers are aligned or not with this activity [12].
- [42] BB Machta, SL Veatch, and JP Sethna, Critical Casimir forces in cellular membranes. *Phys Rev Lett* **109**, 138101 (2012).
- [43] SL Veatch, P Cicuta, P Sengupta, A Honerkamp-Smith, D Holowka, and B Baird, Critical fluctuations in plasma membrane vesicles. *ACS Chem Biol* **3**, 287–293 (2008).
- [44] AR Honerkamp-Smith, SL Veatch, and SL Keller, An introduction to critical points for biophysicists; observations of compositional heterogeneity in lipid membranes. *Biochim Biophys Acta* **1788**, 53–63 (2009).
- [45] SP Rayermann, GE Rayermann, CE Cornell, AJ Merz, and SL Keller, Hallmarks of reversible separation of living, unperturbed cell membranes into two liquid phases. *Biophys J* **113**, 2425–2432 (2017).
- [46] N Khanna, Y Zhang, JS Lucas, OK Dudko, and C Murre, Chromosome dynamics near the sol-gel phase transition dictate the timing of remote genomic interactions. *Nat Commun* **10**, 2771 (2019).
- [47] T Mora and W Bialek, Are biological systems poised at criticality? *J Stat Phys* **144**, 268–302 (2011).
- [48] MA Muñoz, Colloquium: Criticality and dynamical scaling in living systems. *Rev Mod Phys* **90**, 031001 (2018).