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There's (still) plenty of room at the bottom

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Motifs, circuits, and networks are core conceptual elements in modern systems and synthetic biology. While there are still undoubtedly more fascinating computations to discover at network level, there are also rich computations that we are only beginning to uncover within the diverse molecules that constitute the networks. Here we explore some work, both new and old, that showcases the incredible computational capacity of seemingly simple molecular mechanisms. A more sophisticated understanding of computations at the molecular level will inspire the development of a more nuanced toolbox for future biological engineering.

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Exploring the molecular toolbox

Almost 60 years ago, Richard Feynman gave a now-famous lecture titled 'There's Plenty of Room at the Bottom' [1•], where he argued that we had only just begun to understand the extent to which the physical world can be manipulated at the molecular scale. He expressed wonderment at processes like photosynthesis and the translation of proteins. At the time, relatively little was known about the structure, function, and organization of the molecules that underly these phenomena, but it was clear from their design that evolution is a resourceful engineer (Figure 1).

In the last few decades, Feynman's vision has largely become a reality. We now understand, in a much deeper way, that single cells have an astonishing capacity to sense and make decisions about their environment. From quorum sensing in bacteria to embryonic development in animals, a great deal of information must be processed using DNA, RNA, and proteins. When studying information processing in biology, we often focus on the computational capacity of circuits and motifs of a few components [2] and networks of tens to hundreds of elements [3–5]. While there is certainly still much to be explored at the level of circuits and networks, this perspective often coarse-grains the finer molecular details of these systems.

Our viewpoint here will zoom in on these often-overlooked molecular details, and highlight several case studies where molecules perform an impressive range of computation. For instance, while a membrane receptor can simply be viewed as serving to transduce signal from outside the cell, a closer examination reveals that it can be an adaptive component in signaling, performing a wide range of nonlinear computation. Similarly, mRNA is a template for protein translation, but a closer look reveals it to be a powerful regulatory hub, integrating a variety of chemical and environmental stimuli.

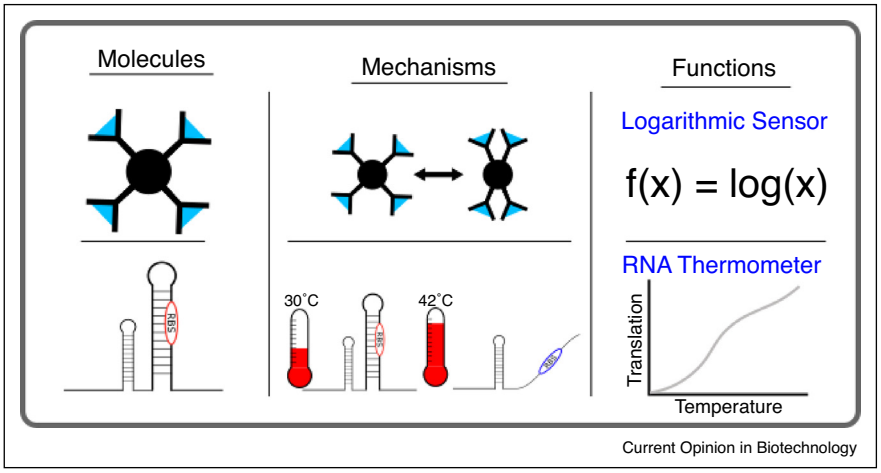
An enzymatic proofreader

We begin with a seminal paper by John Hopfield, published in 1973 [6], that asked a simple question: how is it that the synthesis of biological molecules has such a low rate of error? Hopfield considered a simple model of protein translation. Protein translation proceeds by an mRNA template being processed by a ribosome, which recruits tRNA that ultimately attach amino acids to the nascent chain. A key step is when a ligase attaches an amino acid to a transfer RNA (tRNA). If the ligase attaches the wrong amino acid then, even if the correct tRNA recruited, there will be an error in protein translation.

Hopfield first analyzed the expected error rate due to the ligation of incorrect amino acids to tRNA. Take, for example, equal concentrations of the amino acids isoleucine and valine. The binding affinity of the isoleucine ligase for the former is about 100× that of the latter, implying an approximate error rate of 10^{-2}. With more types of amino acids, one would expect the error rate to only get worse. These results are in stark contrast to the real error rate in protein translation, which is closer to 10^{-4} [7].

The key to resolving this discrepancy is the phenomenon that Hopfield referred to as kinetic proofreading. To motivate this idea, imagine a point during the assembly of a protein where amino acid • is required, however there is an equal amount of amino acid • available, described by the reactions in Figure 2a. Let us assume that the

Figure 1



Molecules, mechanisms, and functions. A primary goal of systems and synthetic biology is to gain a deep understanding of the connection between the molecules that make up biological processes and their associated functions. While we often focus on large biomolecular networks, we can often gain insight from studying the quantitative functional properties of individual molecular mechanisms. In the row, we see a protein with multiple subunits, the mechanism of conformational switching, and the function of responding to signal in a logarithmic fashion. On the bottom row, we see an RNA with a particular secondary structure, the mechanism of temperature regulating the availability of the ribosome binding site, and the function of protein translation being a function of temperature.

ligase $L_{\bullet}$ has a relative dissociation rate $\frac{K_{\bullet}}{K_{\bullet}} = 100$. This would yield the 10^{-2} error rate mentioned earlier.

Now imagine adding a second step that we will refer to as *thermodynamic* proofreading. Instead of directly adding an amino acid, we will instead have an intermediary stage where some form of post-translational modification takes place. Ideally this intermediate stage would allow for additional ligand specificity, giving the ligase an extra opportunity to ameliorate erroneous binding (Figure 2b). Unfortunately, at equilibrium this cannot be the case. Hopfield showed explicitly that due to the constraints of thermodynamic equilibrium, while the second step does preferentially reverse off target binding, the nature of the first step implies that there will be far more $L_{\bullet}-\bullet$ complexes than $L_{\bullet}-\bullet$ complexes. These rates *must* balance in such a way that the rate of erroneous binding is never less than the original 10^{-2}.

Since the impediment comes from thermodynamic constraints, it makes sense to ask if we can somehow sidestep them. One way to do this is to pump energy into the system, by some ATP-consuming enzymatic process, which means we are in the regime of chemical *kinetics* (Figure 2c). This frees us of thermodynamic equilibrium constraints and makes it possible to reduce error rates by a factor of the previous rate squared, or 10^{-4}. The addition of more successive energy-intensive steps would continue to drop the error rate. Over the last 40 years kinetic proofreading has emerged as a pervasive mechanism in various biological pathways, from T-cell receptor signal transduction [8] to chromatin remodeling [9,10]. On the theoretical side, recent work has vastly expanded our understanding of how kinetic proofreading works from the perspective of non-equilibrium statistical mechanics [11•]. While there is no free lunch in life, sometimes it is worth paying a bit extra to get a better meal.

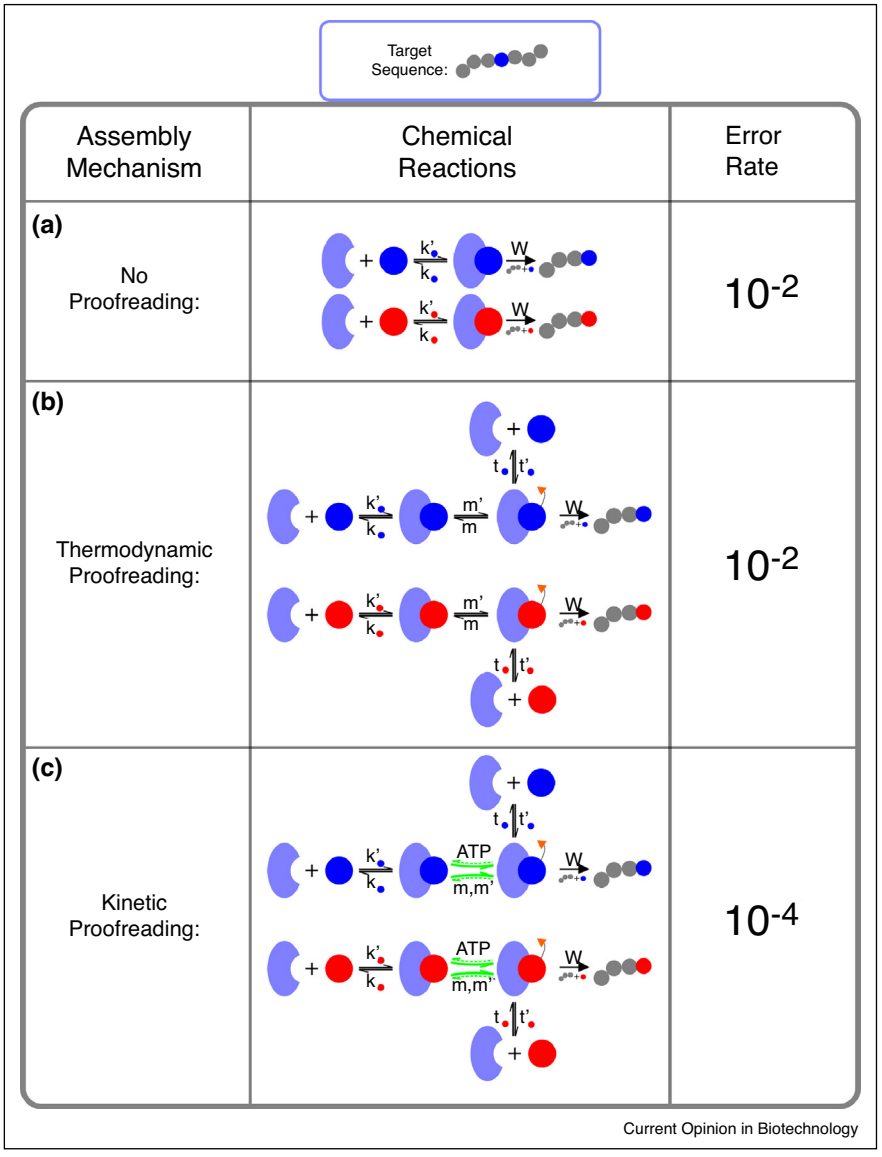
An RNA thermometer

Next we shift focus from preventing errors to preventing catastrophes. Every known organism on Earth has some mechanism for responding to a sudden increase in temperature, referred to as heat shock response (HSR) [12]. One reason heat shock is dangerous is that it can cause proteins to become misfolded, which can be lethal for a cell. To avoid this, the HSR system senses temperature change, and synthesizes chaperone proteins whose job is it is to refold proteins [13].

How does a cell sense when it needs to make chaperones? One way is to sense misfolded proteins (i.e. feedback response), the other is to sense temperature changes so that chaperone synthesis can begin before misfolded proteins have already accumulated (i.e. feedforward response). While both sensing mechanisms exist [14,15], we focus here on the latter feedforward response that is mediated by messenger RNA.

Since heat shock response needs to be fast, it is reasonable to posit that any temperature sensing mechanism must be directly linked to the synthesis of chaperones. To solve this, *Escherichia coli* have evolved an ingenious

Figure 2

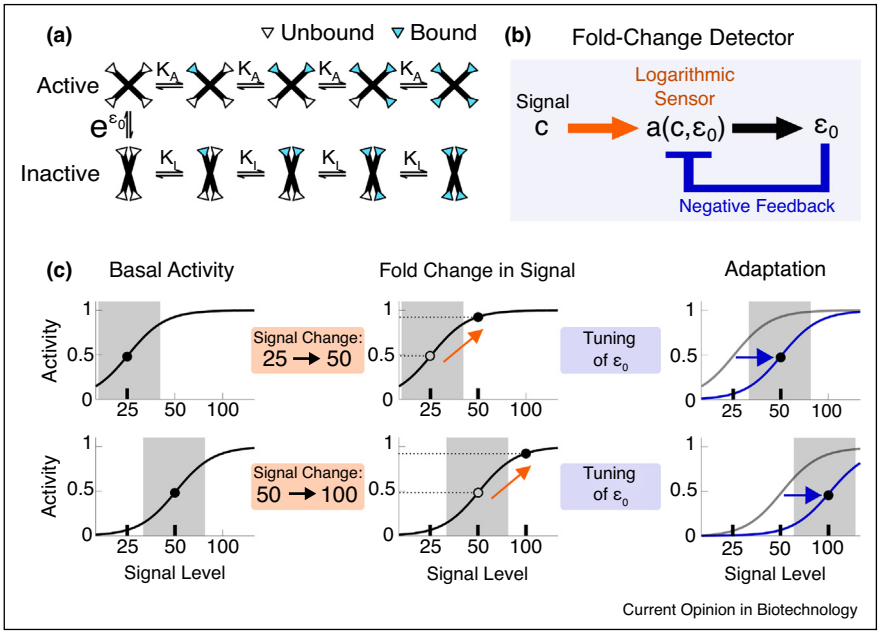


Proofreading through enzyme kinetics. **(a)** A naive approach to protein translation. Amino acids bind to corresponding ligases with various specific dissociation constants $K_D = \frac{k}{k'}$. Differing values of K_D provide the only source of specificity in terms of amino acid ligation (which occurs at the non-specific rate W). **(b)** A slightly more sophisticated approach to proofreading, where there is now a non-specific step mediated by rates m and m' which modifies the amino acids and a specific step mediated by t and t'. If all reactions are purely thermodynamic, the error rate for the setting is the same as in (a). **(c)** Here we see Hopfield's kinetic proofreading. The only difference here is that the non-specific modification at rates m and m' uses external energy. This kinetic process will reduce the error rate below the thermodynamic boundary that constrained the first two processes.

system. Their HSR is mediated by a single sigma factor σ^{32}, which regulates the production of dozens of HSR-related proteins [16]. In order to make σ^{32} expression temperature dependent, the σ^{32} mRNA has a hairpin structure which blocks the ribosome binding site at nominal temperatures [17,18]. At higher temperatures, the hairpin becomes unstable and consequently allows for initiation of translation. Moreover, because of the thermodynamic nature of this transition, the increase in translation exhibits a graded response over a range of temperatures.

Therefore, the σ^{32} mRNA acts as a thermometer, and is an example of the many ways biology regulates mRNA secondary structure to quantitatively control protein levels. An analogous mechanism is the riboswitch, where

Figure 3



Logarithmic computation through allostery. **(a)** A schematic diagram of the possible states for an allosteric protein. This protein has four subunits, each of which can either be bound or unbound. The active and inactive states have different K_D values, and there exists a mechanism for the states to switch back and forth on a concerted way at a rate determined by the allosteric parameter e^{ε_0}. **(b)** The general architecture of a circuit that can use an allosteric protein to implement fold-change detection. **(c)** The top row shows an allosteric protein at a basal level of activation. A signal change then occurs from 25 to 50, increasing the activity. In the final step, the activity of the protein is allosteric regulated such that its activation returns to basal. Because the allosteric regulation effectively moves the response curve in log space, we see exactly the same dynamics in the bottom row when the signal change is instead from 50 to 100. This figure is adapted from Olsman and Goentoro 2016 [20••].

small-molecule binding regulates RNA secondary structure and protein synthesis. The modular nature of RNA allows flexible implementation of post-transcriptional regulation, and has generated much interest in developing synthetic riboswitches and RNA thermometers [19••].

Logarithmic sensing from protein allostery

From protein regulation, we now consider the information processing capacity of proteins themselves. In the early 1960s, shortly after winning the Nobel Prize [21], the French scientist Jacques Monod, along with François Jacob, Jeffries Wymann and Jean-Pierre Changeux, developed an influential theoretical framework with which certain classes of protein regulation can be studied. Their work focused on describing the regulation of metabolic proteins, through a mechanism they called allostery [22,23]. Allostery has since been discovered in a wide range of biological processes including enzymes, ion channels, membrane receptors, and transcription factors [24,25•,26].

An allosteric protein is one with a binding site where ligand binds and regulates the protein's biological activity, and at least one other site. This secondary site, which binds an allosteric effector, regulates the activity of the protein without directly interacting with the primary site. The model of allostery proposed by Monod, Wyman, and Changeux (MWC) describes proteins with two (or more) distinct conformational states, each functionally different. The MWC model,

$$a(c,\varepsilon_0) = \frac{\left(1+\frac{c}{K_A}\right)^N}{\left(1+\frac{c}{K_A}\right)^N + e^{\varepsilon_0}\left(1+\frac{c}{K_I}\right)^N},$$

assumes that the allosteric effectors alters the conformational equilibrium of the protein (as seen in the diagram in Figure 3a). In this equation, a is the fraction of protein in the active state, c is the concentration of ligand, ε_0 is the effective free energy between the active an inactive states, N is the total number of subunits, and K_A and K_I are dissociation constants for the active and inactive states, respectively. For example, hemoglobin has four subunits ($N = 4$) that each can bind oxygen (c). Allosteric effectors, such as carbon dioxide, alters the free energy ε_0 of hemoglobin and alters the equilibrium between the active (high affinity for oxygen, K_A) and inactive (low affinity for oxygen, K_I) states.

Recent work [20••] shows how allosteric proteins can, in theory, act as logarithmic sensors. Stated mathematically, this work showed that, under certain parametric

constraints, the architecture in Figure 3b implements the relationship

$$\frac{da}{dt} \propto \frac{d \quad \ln \quad (c)}{dt}.$$

The work further shows that published biophysical measurements support that known allosteric proteins operate within this constraint and tunes its activity on log scale.

It is remarkable that a seemingly complex logarithmic computation is encoded simply in the structural regulation of a protein. Therefore, beyond acting as a transport molecule (e.g. hemoglobin) or enzyme (e.g. in metabolism), the widespread allosteric proteins may also act as a quantitative sensor mediating sensing on logarithmic scale. Logarithmic sensing may offer the advantage of facilitating response to signal across many orders of magnitudes. In hemoglobin, for example, given the wide range of oxygen levels and demand throughout the body, it makes sense that hemoglobin may have evolved to respond dynamically to relative, as opposed to absolute, changes in oxygen demand. Finally, when coupled to negative feedback, logarithmic sensing may mediate an emerging recurrent phenomenon in biological signaling, where cells respond to fold changes, rather than absolute change, in signal level (as seen in Figure 3c) [27–30].

A sequestration-based integrator

As a final example, we move on from the problem of sensing to the problem of control. Broadly speaking, the goal of control is to get the state of a system to match a desired set point. Feedback control does this in two steps: first by measuring the error between the actual and desired output, and second by adjusting the input of the system to reduce the error.

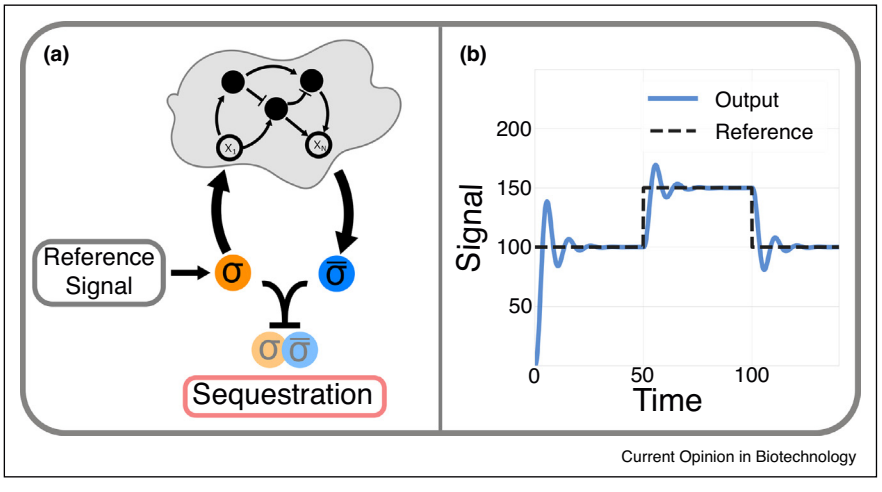
A common strategy in biological regulation is molecular sequestration. For instance, in RNA, a common regulation is sense-antisense pairing, where a given mRNA has a complementary RNA or DNA strand that has no direct biological function, but serves to regulate its biologically active partner [32,33]. In bacterial transcriptional regulation via sigma factors, there exist antisigma factors, whose primary role appears to be as a sequestration partner [34] (Figure 4a). Recent work by Briat *et al.* [31••] derived a fundamental connection between sequestration mechanism and a special class of feedback, integral feedback control [35].

This insight is most concisely illustrated by mathematics. First, let us consider what an integral control is. Let $x = (x_1, \ldots, x_N)$ be a state vector, which could represent the concentration of N species in a network. The dynamics of the network can be described as

$$\dot{x} = f(x, u),$$

where $f(x, u)$ is a function of the internal state x and a control input u, each perhaps characterized by production and degradation rates of different species in the network. It does not matter much what the details of $f(x, u)$ are, just that the closed-loop system is stable. Our goal is to set the concentration of the species in x to a particular level x_f. The task of feedback control is to find a $u(x)$ such that $\lim_{t\to\infty} x(t) = x_f$. Thus, we could define the error $e(t) = x_f - x(t)$, and $e(t) \to 0$.

Figure 4



Integration through molecular sequestration. **(a)** A cartoon of the sequestration feedback network from [31••]. Here we see a sigma factor (denoted σ) which that responds to a reference signal by inducing the production of an input to the network x_1. The output of the network x_N then induces the production of an anti-sigma factor (denoted $\bar{\sigma}$) that subsequently sequesters σ. **(b)** Reference tracking behavior in the sequestration feedback network. Here we see how the output (blue line) tracks changes in the reference signal (dashed line). The two match exactly at steady state because of integral control.

The question then becomes how we pick u. An effective control strategy is to set u as the integral of the error function:

$$u(t) = \int_0^t e(t')\,dt' = \int_0^t (x_f - x(t'))\,dt'.$$

This is known as integral feedback [35]. Integral feedback always adjusts the system to the desired state x_f, since at steady state, if the system is stable, then the following must hold:

$$\frac{d}{dt}u(t) = e(t) = 0$$
$$\Rightarrow x(t) = x_f.$$

Briat *et al.* derived that such an integral feedback control can be implemented through molecular sequestration that is common in cellular processes [31••] (Figure 4a). Let us introduce two control molecules u_1 and u_2, where the full system has the dynamics

$$\dot{x} = f(x,u) \quad (1)$$

$$\dot{u}_1 = \mu - \eta u_1 u_2 \quad (2)$$

$$\dot{u}_2 = \theta x_N - \eta u_1 u_2. \quad (3)$$

Here μ can be taken as some external reference (i.e. an inducer of u_1), θ as the rate of production of u_2, and η as the rate of sequestration of u_1 and u_2. Our goal is to set the steady-state level of $x_N \propto \mu$, independent of any parameters that are internal to $f(x,u)$. The sequestration reaction implies that u_1 and u_2 are removed from the system at the rate $\eta u_1 u_2$. Because sequestration affects both molecules equally (and in this simple model, irreversibly), the rate of sequestration for both control molecules must be exactly equal. It follows that (see more detailed derivation in [31••]),

$$u(t) = u_1(t) - u_2(t) = \theta \int_0^t \left(\frac{\mu}{\theta} - x_N(t')\right) dt'.$$

If we define the desired reference point $x_f = \frac{\mu}{\theta}$ and the error function $e(t) = x_f - x_N(t)$, we find

$$\dot{u} = 0 \Rightarrow x_N = \frac{\mu}{\theta},$$

implementing integral feedback control. We can think of the sequestration system in Eqn 1 as being composed of an actuator (u_1) that affects a circuit and a sensor (u_2) that measures the output (x_N). Thus, a seemingly simple molecular sequestration allows for a complex feedback control.

What's next?

If Feynman were still alive today, we imagine that he would support our endeavor to keep pushing the ability to engineer synthetic molecular and cellular networks comparable to what is found in nature. Modern molecular biology has made rapid progress toward accomplishing his initial goal of interrogating the precise structure and mechanics of cellular machineries. The time is now ripe for bioengineers and synthetic biologists to apply this vast array of knowledge, and start building.

To do so, we argue that it is important not only to push our understanding of what molecules do together, but also to appreciate diverse molecular machinery as complex computational devices. Just as a sentence is made up of words and grammar, biological circuits are made up of molecules and their associated interactions. We might view the circuits shaped by evolution as our Rosetta Stone, and current progress in engineering synthetic circuits as our first sentences spoken in this new language. In this analogy, to be able to express more complex ideas, we will not only need to develop a more sophisticated grammar (e.g. molecular interactions), but also importantly, a richer vocabulary (e.g. molecular functions). The examples we highlight here demonstrate that there is still a treasure trove from which we can learn about the vocabulary of biological circuits.

Beyond the work discussed here, there has been exploration of the computational capacity of scaffold proteins acting as signaling hubs in synthetic networks [42] and of the combinatorial capacity of receptors to multiplex different signaling sources [43]. Table 1 shows a variety of other molecular mechanism/function pairs from both synthetic and natural contexts. What is becoming increasingly apparent is that biology is full of fascinating quantitative behavior encoded in relatively simple molecular mechanisms. For example, it was recently shown by Amodeo *et al.* [39] that the timing of the mid-blastula transition in *Xenopus laevis* embryos is implemented through a clever histone titration mechanism. Early on in development, transcription is heavily repressed by a large number of histones. As cells divide the total number of histones stays constant, so the number of histones per cell is halved at each division. At a threshold concentration, there are few enough histones that transcription initiates and cells rapidly undergo differentiation and reorganization. This timing is incredibly consistent, occurring approximately 7 hours post fertilization in a synchronized manner across all cells in the embryo. In this setting, we can think of the histone titration mechanism serving the function of a developmental timer.

Table 1

Additional molecular mechanism/function pairs. Here we list a few other interesting molecular mechanisms that give rise to sophisticated quantitative behavior. These arise from both synthetic and natural contexts, and serve as a good reminder that biology is full of cleverly engineered behavior

Molecular mechanism	Function	Context	Source
Inducible caspase-9	Synthetic apoptosis	Synthetic	Gargett *et al.* [36]
Scaffold/anti-scaffold binding	Biomolecular concentration tracker	Synthetic	Hsiao *et al.* [37]
Dynamic instability of microtubules	Exploratory behavior	Natural	Tanaka *et al.* [38]
Histone titration	Developmental timing	Natural	Amodeo *et al.* [39]
Actin-like cytoskeleton localization	Cell wall curvature sensing	Natural	Ursell *et al.* [40]
RNA editing	Regulation of protein kinetics	Natural	Liscovitch-Brauer *et al.* [41]

60 years ago, when Feynman gave his lecture, we were just beginning to understand the human capacity to manipulate and interact with digital information. Today, digital computing is so routine that we barely even notice it in our everyday life. Perhaps 60 years from now, we will have the same relationship with biological programs that we have with digital ones.

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