Control of protein-based pattern formation via guiding cues

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Abstract | Proteins control many vital functions in living cells, such as cell growth and cell division. Reliable coordination of these functions requires the spatial and temporal organization of proteins inside cells, which encodes information about the cell’s geometry and the cell-cycle stage. The study of such protein patterns has long focused around formation in uniform environments. However, in recent years, it has become evident that spatial heterogeneities are essential for protein patterning, and various guiding cues in the cell or at the cell boundary can be exploited to reliably control protein pattern formation. We review how protein patterns are guided by cell size and shape, by other protein patterns that act as templates, and by the mechanical properties of the cell. The basic mechanisms of guided pattern formation are elucidated with reference to observations in various biological model organisms. We posit that understanding the controlled formation of protein patterns in cells will be an essential part of understanding information processing in living systems.

To ensure their survival, cells must tightly regulate a wide range of functions, such as migration, growth, DNA synthesis and cell division. For example, to produce two viable daughter cells, a cell must precisely coordinate its growth with the duplication and segregation of DNA, and with subsequent cell division. Robust timing and reliable control of these functions requires cells to process spatiotemporal information, such as information about cell size and shape, cell-cycle state, the cell’s surroundings and the current state of other cellular processes. Such spatiotemporal information is encoded in protein patterns — inhomogeneous spatial distributions of proteins — that regulate these cellular functions, whereby each type of protein may perform distinct tasks. How then are proteins spatially and temporally organized in a cell? The idea that the collective organization of interacting chemicals (chemical reactions) in an initially homogeneous medium can give rise to spatial patterns dates back to Alan Turing’s work on spontaneous pattern formation in reaction–diffusion systems. This work greatly advanced the understanding of pattern formation in biological systems. However, many aspects of protein patterns, such as their positioning, timing, reliability and controllability — which are essential for the viability of living organisms — remain poorly understood. Protein patterns in cells serve a timed and targeted functional purpose, and to do so they are required to form in response to certain signals and control mechanisms rather than spontaneously emerging from an initially homogeneous distribution. Indeed, increasing numbers of theoretical and experimental studies find that protein distributions can respond and adapt to cell shape, size and mechanics, as well as to signals encoded in previously established protein patterns.

This response is, in fact, bidirectional. Cells are not static objects but rather an active material whose size, shape and mechanical properties are altered dynamically through protein interactions in response to the cell’s environment and the current state of the cell cycle. These dynamic interactions between protein patterns and cell architecture are the subject of a burgeoning field of study at the interface between cell biology and theoretical physics. The field benefits from constantly improving experimental techniques, and from insights from physics that allow one to model and understand the guided organization of proteins into patterns.

In this Review, we discuss how protein patterns are controlled by geometric, mechanical and biochemical cues. We only briefly summarize the basics of pattern formation, as other reviews provide a comprehensive introduction to this subject. We recommend an elementary course on the mathematical tools required to study the physics of protein interactions and pattern formation, in particular, ordinary differential equations (ODEs) and nonlinear dynamics. For an introduction to the theory of pattern-forming systems, we direct the reader to pertinent textbooks and to lecture notes for a review on quantitative modelling of pattern formation in mass-conserving systems. Other reviews have focused on the theory of two specific aspects of pattern formation, namely the role of bistability for polarity and the curvature-generating properties of proteins;
Key points
- Cells rely on spatial and temporal protein distributions to maintain their viability and biological function.
- Intracellular protein patterns are controlled, oriented and positioned by guiding cues that include cell size and shape, pre-existing protein patterns and the cell’s mechanical properties.
- A combination of theoretical models with experimental observations has shed new light on the mechanisms of protein pattern formation in cells.
- Further uncovering of mechanisms underlying pattern guidance is key to developing a fundamental understanding of living systems.

the relevance of protein patterns for cells (from a more biological perspective)\(^4\), in particular, with respect to midcell localization\(^6\); and current advances in understanding pattern formation at a molecular level\(^9\). We also highlight three reviews that emphasize the importance and role of modelling for understanding cell polarity\(^4,22,23\) and biological phenomena in general\(^44\).

In this Review, we discuss several theoretical models that have been developed with a view to reproducing and accounting for pattern guidance, together with examples of well-studied biological model organisms in which pattern guidance has been observed to play a critical role in cell viability. In particular, we discuss how biophysical theory has been instrumental in clarifying the underlying physical concepts of pattern guidance in living cells. We give an overview of the predominant types of protein transport and chemical reactions that are involved in the formation of patterns in cells. We also discuss how these factors can be affected by cell shape and size, pre-existing protein patterns and cell mechanics, and how these cues guide and control protein pattern formation.

Nucleoside triphosphate (NTP). Nucleotide molecules with three phosphate groups, typically based on guanine (GTP), adenosine (ATP) or cytosine (CTP), forming the main carriers of chemical energy in cells. Nucleoside diphosphate (NDP) instead has two phosphate groups.

NTPases
An NTPase is an enzyme that binds to NTP and hydrolyses it to NDP, thereby releasing energy.

Phosphorylation
Proteins can be phosphorylated by the addition of a phosphate group, as a means of storing chemical energy.

Dephosphorylation
Removal of a phosphate group from a protein, in order to release chemical energy.

Oligomers
An oligomer is a complex made up of a few proteins of the same type (homo-oligomer) or a different type (hetero-oligomer).

The formation of higher-order protein aggregates leads to a change in the Péclet number (as discussed below). Such an effect has been suggested to play a role in the...
transport of PAR-3 proteins in the C. elegans embryo. Diffusive transport may dominate for PAR-3 monomers (Pe < 1), whereas transport becomes dominated by flow (Pe > 1) upon cell-cycle-dependent aggregation of PAR-3 into complexes together with two other proteins, namely PAR-6 and atypical protein kinase C (aPKC)\(^5\).

Yet another process is the formation of higher-order oligomers, such as those observed for membrane-bound MinD\(^4\),\(^6\). Like the nonlinear attachment kinetics discussed above, cooperative reactions have also been suggested to participate in protein complex formation, potentially allowing for feedback loops\(^3\)\(^4\).

**Theory.** Mathematically, the dynamics of well-mixed protein reaction networks are described by sets of coupled nonlinear ODEs for the concentrations \(u_i(t)\) of each of the \(S\) different protein types and conformations \(i \in \{1, \ldots, S\}\),

\[
\dot{u}_i(t) = f_i(u_i(t)). \tag{1}
\]

In such chemical rate equations, the nonlinear reaction terms \(f_i\) (together with the reaction rates) must be inferred from the underlying reaction network by using the law of mass action. Such systems of coupled nonlinear ODEs can be analysed using dynamical systems theory. The basic idea of this theory, which goes back to the pioneering work of Henri Poincaré\(^5\)\(^4\), is to characterize the system dynamics in terms of certain geometric structures in the phase space spanned by the set of dynamical variables \(u_i(t)\) (REFS\(^1\)\(^3\))\(^4\).

Of particular interest are the asymptotic dynamics of the system over long timescales, which are characterized by the attractors in phase space within the framework of dynamical systems theory. These attractors include fixed points that correspond to reactive equilibria (BOX 1), limit cycles that correspond to nonlinear oscillators, and more intricate geometric objects\(^1\)\(^3\)\(^4\). Importantly, the local properties of the fixed points, in particular their stability, can be determined using ODEs linearized around these fixed points\(^1\)\(^3\)\(^4\).

**Protein transport**

Transport mechanisms play a crucial role in the control of spatial variations in protein concentration (FIG. 1b).

**Diffusion.** Perhaps the most basic means of protein transport is diffusion. It is a consequence of Brownian motion and is directed from regions of high to regions of low protein concentration \(u(x,t)\) with a diffusive current \(\nabla u(x,t)\) (Fick’s law). For spherical particles of radius \(r\), the diffusion constant is given by the Stokes–Einstein relation \(D = k_BT/(6\pi\eta r)\), where \(\eta\) is the viscosity of the surrounding cytosol\(^5\)\(^5\). A qualitatively similar relation holds for transmembrane proteins\(^5\)\(^6\)\(^7\). This relation implies that the diffusive transport of proteins depends on their...
Macromolecular organelles.

Cytoplasm
Heterogeneous material making up most of the volume of a cell (excluding the nucleus), consisting primarily of the cytosol and macromolecular organelles.

Box 1 | Methods of analysing pattern formation

Reactive equilibrium
Chemical reactions convert reactants into products and vice versa, thereby resulting in fluxes. An equilibrium state is reached if the sum of all fluxes equals zero; this condition determines the equilibrium concentrations of constituents. This equilibrium state is commonly referred to as a reactive equilibrium, and is generally distinct from a thermodynamic chemical equilibrium because fluxes can originate from non-equilibrium processes (broken detailed balance)\(^{11,22}\). For example, in NTPase cycles, proteins detach from the membrane and must undergo a conformational change before they can reattach. The reactive equilibrium in this case is given by a balance between reactive fluxes onto and off the membrane.

Mathematically, the reaction kinetics of a well-mixed system are expressed by ordinary differential equations (ODEs)

$$\frac{\partial u(t)}{\partial t} = f(u; \{a_i\}),$$

where \(f(u; \{a_i\})\) contains the (nonlinear) interactions between the components of \(u\) and therefore corresponds to the sum of individual reactive fluxes, and \(\{a_i\}\) denotes the set of control parameters of the system. Formally, a reactive equilibrium conforms to the steady-state solution \(\partial u = 0\) of Eq. (5) and is termed the fixed point of the ODE system; that is, \(f(u^*; \{a_i\}) = 0\) for steady-state solutions \(u^*\). Thus, the number and stability of fixed points depends on \(f(u; \{a_i\})\) (part a of the figure). For simplicity in the figure there is only one control parameter \(a\); stable fixed points are represented by filled circles and unstable fixed points by open circles. In general, the long-term dynamics are governed by attractors of the nonlinear system, the properties of which are the subject of the field of dynamical systems theory\(^{13}\).

Phase space analysis
To assess the qualitative dynamics of nonlinear dynamics systems, one must often resort to geometric phase space analysis (part b of the figure). In phase space, each point corresponds to a specific state of the system, with the phase space flow tracing out the time evolution of the system. Next to the flow lines, fixed points (\(fu^* = 0\)) and nullclines (\(f(u) = 0\)) are characteristic features that reflect the topology of phase space. In particular, this representation allows one to identify important features of the system, such as steady states or limit cycles.

As a characteristic example, consider the phase space diagram shown in part b of the figure, for a two-component system with dynamics given by \(\partial u_1 = -u_1 + u_2\) and \(\partial u_2 = u_1 - u_2\). Intersections of the nullclines (blue and orange) correspond to fixed points, the stability of which can be determined by visualizing the phase space flow. The system at hand possesses one stable fixed point (filled circle) and one saddle fixed point (open circle). The separatrices (black) divide the phase space into qualitatively distinct areas. Given a specific initial state (red square), the time evolution of this state can be determined by following the flow line (red), which provides qualitative information about the system’s dynamics.

Dispersion relation
In spatially extended systems, patterns typically form when a (spatially homogeneous) steady state is unstable against random spatial perturbations. The formal way to probe for instabilities is to perform a linear stability analysis. One first expands spatial perturbations in normal modes and then linearizes the dynamics around a spatially homogeneous steady state \(u^*\). From the linearized system, one can determine the dispersion relation \(\sigma(q)\), which relates the growth rate \(\sigma\) of perturbations to their respective wave number \(q\). A typical dispersion relation is shown in FIG. 2. Positive values of the growth rate indicate that spatial perturbations are amplified and grow exponentially. Because the critical mode \(q\) with the highest growth rate is expected to dominate near onset, this unstable mode sets the characteristic wavelength of the initial pattern. However, in general, the dispersion relation only informs about the characteristic length scale of the pattern in the vicinity of the homogeneous steady state\(^1\); the dominant length scale of the final pattern can be quite different.

Size and on the local properties of the surrounding medium.

Importantly, both the membrane and the cytoplasm are highly heterogeneous environments crowded with macromolecular structures that interact with proteins, for example, by temporarily binding or by taking up space\(^{20}\). To study pattern formation, however, one often disregards inhomogeneities and instead assumes an effective diffusion constant that subsumes the interactions that are not explicitly modelled. Hence, the diffusion constant is a mesoscopic quantity representing the mobility of proteins in a homogeneous, dilute fluid environment. In essence, for many applications in protein pattern formation, the complex cytoplasmic environment is reduced to an effective cytosol, and the heterogeneous membrane is considered as an effective (dilute) fluid\(^{20}\). This simplification is justified because the length scale of patterns is typically larger than that of heterogeneities in the cytoplasm or on the membrane. (We refer to the cytoplasm and membrane as substrates.) The diffusion coefficients of membrane-bound proteins \((D_m)\) are generically at least two orders of magnitude lower than those of their cytosolic counterparts \((D_c)\):

\[ D_m \approx 0.01 \mu \text{m}^2 \text{s}^{-1}, \text{ compared with } D_c \approx 10 \mu \text{m}^2 \text{s}^{-1} \text{ (Ref.10).} \]
Molecular motors
Enzymes that use energy released by NTP hydrolysis to perform mechanical work, and that are generally associated with cytoskeletal filaments.

Microtubules
Protein filaments composed of tubulin, which form an integral part of the cytoskeleton. Microtubules exhibit a polarity, with the ends denoted as plus and minus ends.

Actin filaments
Also known as microfilaments, these are polar filaments of actin proteins, which form an integral part of the cytoskeleton. Their ends are denoted as plus and minus ends.

Actin cortex
Thin and dynamic network that acts as a scaffold that determines the cell’s shape and which is comprised of actin filaments, motor proteins and other associated proteins.

Although the models discussed in this Review suggest that the heterogeneous character of the cellular substrates is of minor importance for protein pattern formation, it would be interesting to explicitly probe the robustness of these models against more realistic substrates, using models with time- and space-dependent diffusion constants, for example.

Active transport. Proteins can also be transported via active processes driven by the chemical energy of ATP, guanosine triphosphate (GTP) or cytidine triphosphate (CTP) at the molecular level. Of particular biological relevance are translational molecular motors\(^ {31-42}\). An important subclass of these motors consists of kinesins and dyneins that bind to and ‘walk’ on microtubules. As they walk, kinesins and dyneins can carry cargo, such as other proteins, along the microtubules\(^ {41,42}\). Depending on the type of motor and, in some cases, other factors such as external forces\(^ {43}\), this form of active transport is directed to either the plus or minus end of the microtubules\(^ {44}\). Certain classes of myosin motor perform similar tasks by transporting cargo along actin filaments. Such active cargo transport is involved in the polarization process of budding yeast, in which actin filaments are anchored to the polarity site and myosin motors deliver protein-coated vesicles towards the polarity site\(^ {42}\).

Another class of active transport processes is mediated by the directed polymerization of cytoskeletal filaments such as F-actin, driven by ATP hydrolysis\(^ {40}\), and microtubules, driven by GTP hydrolysis\(^ {40}\). For instance, tubulin-like FtsZ filaments are particularly important active structures in bacterial cell division. These filaments exhibit treadmilling dynamics (Fig. 1b), because FtsZ monomers can only bind to the plus end and detach from the minus end\(^ {45}\). By consuming GTP, this treadmilling allows FtsZ filaments to translocate directionally along the cell membrane, coordinating the activity of downstream cell-division processes\(^ {46}\). Similarly, treadmilling of actin filaments is important for cell migration, in particular, for the extrusion of lamellipodia\(^ {47}\).

Both in vivo and in vitro experiments have shown how important these active transport processes are for the polarization of cells\(^ {48-76}\). For example, during cell growth in fission yeast, microtubules are aligned along the long axis of the cell and direct the active transport of the tip factors Tea1 and Tea4 towards the cell poles in two waves\(^ {49-81}\). The kinesin-like motor Tea2 mediates the transport of Tea1–Tea4 complexes along microtubules that emanate from the nucleus\(^ {78}\). In addition, these complexes bind to microtubule tips assisted by Mal3, a tip-binding protein. Therefore, owing to the directed microtubule polymerization along the long cell axis, the tip factors are transported to the cell poles\(^ {81}\). At the poles, they serve as a spatial cue for cell growth, thereby helping the cell to elongate along its long axis\(^ {81}\).

Advective transport. In the fluid environment of a cell, proteins are also transported by cytoplasmic\(^ {82,83}\), cortical\(^ {81}\) and membrane flows\(^ {84,85}\). The effect of these flows on protein transport through friction strongly depends — like diffusion — on the viscosity of the environment.

One means of inducing flows is cortical contractions, which are caused by the interplay of actin filaments, cross-linker proteins and myosin motors in the actin cortex\(^ {86-88}\). For example, in the C. elegans zygote, local depletion of the concentration of the motor protein myosin at the cell cortex leads to a gradient of contractile stress, such that the cell cortex flows from the anterior to the posterior pole\(^ {87}\). Cortical contractions can also lead to flows in the cytoplasm or membrane that are mediated by hydrodynamic coupling between membrane, cortex and cytoplasm\(^ {82}\). In addition, they can induce cell-shape changes that lead to flows in the cytoplasm. For example, surface contraction waves during the maturation of starfish oocytes induce such flows\(^ {84,85}\). Similarly, shape changes resulting from blebbing incidents coincide with intracellular flows\(^ {86}\).

The Péclet number. The relative impact of diffusion and flow on protein transport is quantified by the Péclet number \( \text{Pe} = \xi v / D \), where \( v \) is the typical protein advection velocity and \( \xi \) a characteristic length scale. Large values of the Péclet number correspond to protein transport that is dominated by flow rather than diffusion. Small proteins with large diffusion constants are less affected by flow than large proteins or protein assemblies. In addition, the detailed chemical interactions of proteins with other biomolecules and cellular structures can affect the effective diffusivity and advection velocity\(^ {89}\). As in the case of diffusive transport, the advection velocity — and hence the Péclet number — is a mesoscopic quantity that disregards the heterogeneous structure of the environment. This approximation is justified because variations in the mobility coefficients within a given substrate are usually much smaller than the variations between different substrates, such as the cytoplasm and the membrane. In general, a protein is less affected by flows when diffusing in the cytoplasm than when bound to the more viscous membrane.

Theory. The spatiotemporal transport of, and reactions between, proteins are mathematically described by nonlinear partial differential equations (PDEs)\(^ {90}\). The protein dynamics in terms of their cytosolic (volume) concentrations \( c(r, t) \) and membrane (area) concentrations \( m(\sigma, t) \) typically take the form of transport equations with flux and source terms

\[
\frac{\partial c}{\partial t} = - \nabla \cdot J + f_{\text{cyt}}(c),
\]

\[
\frac{\partial m}{\partial t} = - \nabla_{S} \cdot J_{m} + f_{\text{mem}}(m, c|_{S}),
\]

which represent a broad and general class of dynamical systems far from thermodynamic equilibrium. The divergences of the cytosolic flux \( J_{c} \) and membrane flux \( J_{m} \) account for the (mass-conserving) spatial transport of proteins, and generally contains both diffusive and advective contributions. Here \( \nabla_{S} \) denotes the covariant derivative for the curvilinear coordinates \( \sigma \in S \) on the membrane surface \( S \). For simplicity, the membrane is often considered a static object, but models can be extended to dynamic surfaces. Doing so requires extending the dynamics by an explicit expression for
the time evolution of the membrane geometry, \( S \rightarrow S(t) \) [REFS. 186–189]. The source terms \( f_m \) and \( f_{\text{mem}} \) result from the chemical reactions of the underlying protein networks, as discussed above. Note that not only do membrane-bound proteins react with each other, but membrane reactions also involve interactions with cytosolic proteins in close proximity to the membrane (\( c_{\text{LS}} \)).

The set of nonlinear PDEs (Eqs. (2) and (3)) is closed by reactive boundary conditions at the membrane
\[
\mathbf{J} \cdot \mathbf{n} |_{S} = \mathbf{g}(m, c_{\text{LS}}),
\]
which ensures local mass conservation: cytosolic fluxes normal to the membrane (\( \mathbf{n} \) denotes the outward normal vector) must be balanced by reactive fluxes \( \mathbf{g}(m, c_{\text{LS}}) \) at the membrane\(^{19}\). An additional constraint for many models of protein pattern formation is the global conservation of protein mass, that is, the assumption that no proteins are produced or degraded on the timescale of pattern formation. This assumption is violated on longer timescales, over which protein production and degradation processes — in particular gene expression — need to be taken into account\(^{20}\).

**Lateral instabilities and trigger waves**

This set of general transport equations provides the theoretical framework for studying the spatiotemporal dynamics of protein patterns. A more general and detailed introduction can be found elsewhere\(^{3}\). To conclude our introduction to the basic principles of pattern formation, we briefly introduce two particularly interesting phenomena: pattern-forming instabilities and trigger waves.

A pattern-forming instability arises when a spatially uniform steady state becomes unstable against spatially inhomogeneous perturbations (FIG. 2d). One example of such a pattern-forming instability is a mass-redistribution instability (BOX 1), which amplifies spatial variations in protein number, thus leading to a protein concentration pattern\(^{20}\). The dynamics and length scale of these patterns on short timescales are determined by the growth rate and wavelength of the unstable modes (BOX 1). The growth rate of the unstable modes depends on the specific reaction kinetics and transport properties of the dynamics. The wavelength of the fastest growing unstable mode determines the characteristic length scale of the initially growing pattern. Whereas the initial pattern is dominated by the dynamics of the unstable modes, the dynamics on longer timescales may be dominated by other processes, such as coarsening\(^{20}\) and nonlinear interactions of the unstable modes far away from the linear regime.

In addition, nonlinear protein reaction kinetics can lead to the existence of several reactive equilibria at the same total protein concentration, which is a necessary requirement for trigger waves. This phenomenon is best exemplified by systems that show bistability\(^{20}\) (BOX 2), for which the system can be at different reactive equilibria at different regions in the cell, giving rise to front-like protein activity patterns. Such front-like patterns propagate with a finite velocity, whose magnitude and sign depend on the details of the reaction kinetics\(^{20,21}\). This propagation is constrained by the limited abundance of proteins, which can result in localized wavefronts in cells\(^{187–189}\). Moreover, unstable reactive equilibria can give rise to spatially homogeneous oscillations and travelling spiral waves\(^{181,182}\).

The spatiotemporal properties of patterns, such as the orientation of static patterns or the direction of propagating wavefronts, need to be controlled tightly by the cell. This is achieved with the aid of guiding cues. In the following sections, we discuss the most prominent types of guiding cues observed to play a role in pattern formation processes in cells.

**Geometric guiding cues**

On the largest scales, cells are characterized by their size and shape, which together confine protein transport and protein reaction kinetics.

**Cell size**

Experiments show that protein patterns are affected not only by the reaction and transport properties of the cell, but also by its size. Examples include the transition from pole-to-pole oscillatory patterns to stripe patterns of MinD in filamentous *E. coli* cells\(^{12,113}\) and the failure of PAR proteins in *C. elegans* to polarize in small cells\(^{8}\).

**Bulk–boundary ratio.** On the timescale of pattern formation and dynamics, the total concentration of proteins remains constant. Because of these resource limitations, protein concentrations on the membrane and in the cytosol generally depend on the ratio of membrane area to cell volume. Moreover, the total concentrations of proteins control pattern-forming instabilities and the number and stability of reactive equilibria (BOX 1), thus variations in cell size can qualitatively affect protein patterns.

To understand the underlying idea, for simplicity consider uniformly distributed concentrations of cytosolic proteins \( c \) and membrane-bound proteins \( m \). The total number of proteins \( N \) is thus \( N = S m + V c \), where \( S \) denotes the membrane (surface) area and \( V \) denotes the cytosolic (bulk) volume (FIG. 2a). Rewriting this mass-conservation relation in terms of the total protein density \( \rho = N/V \) yields \( \rho = mS/V + c \). Thus, the protein concentrations on the membrane and in the cytosol depend on the ratio of membrane to volume \( S/V \). For example, for a spherical cell with radius \( R \), \( \rho = 3m/R + c \).

**Cytosolic protein gradients.** Because the proteins of interest for probing cell size and geometry are not permanently fixed to either the membrane or the cytosol but circulate between these compartments owing to various chemical processes such as membrane detachment, attachment and recruitment, the cell membrane effectively acts both as a source and a sink for cytosolic proteins. These chemical reactions must be balanced by diffusive fluxes in the cytosol, to ensure local mass conservation. Hence, on these very general grounds, spatial gradients in the cytosolic protein density must be assumed\(^{184,185}\). Strikingly, these gradients generally do not equilibrate over time, but are maintained by interplay between diffusion and non-equilibrium reaction kinetics (BOX 2).
An example is the case in which proteins in the cytosol have two different conformations, an inactive and an active state. Only proteins in the active state are able to bind to the membrane, and they typically undergo a conformational change to the inactive state upon detachment from the membrane (Fig. 2b). In the cytosol, inactive proteins switch back to the active state at rate $\lambda$. This reactivation step requires the consumption of energy and is a generic feature in NTPase or phosphorylation/dephosphorylation cycles. Because detached proteins cannot immediately bind to the membrane again, a growing unstable mode is constrained by the lateral length scale $\ell$. If the cell size is much smaller than $\ell$, no pattern-forming instability arises as no unstable mode can fit.

**Finite size effects.** In addition, cell size can affect pattern-forming instabilities. The largest possible wavelength for a growing unstable mode is constrained by the lateral length of the cell. Thus, a reaction network can lead to a pattern-forming instability in large cells but result in a stable and spatially uniform steady state or a weak gradient in small cells (Fig. 2c,d). This size effect has been observed for the polarity pattern of PAR proteins in *C. elegans* (Fig. 2c). Similarly, cell size may limit not only the existence of a pattern but also the type of protein pattern that can be established.

**Cell shape and curvature sensing.** For a variety of cells, including bacteria, migrating fibroblasts, unincellular eukaryotes and large zygotes, cell shape and local membrane curvature serve as important guiding cues for protein attachment to the membrane. The mechanisms underlying such curvature detection are based on the interaction of proteins with the membrane, in particular, on the membrane binding affinity of a protein (in curvature-sensing proteins) and on the probability that a protein makes contact with the membrane in a process of collective curvature sensing. Both factors can be affected by cell shape (membrane curvature).
Box 2 | Nonlinear feedback in protein pattern formation

**Bistability and propagation of bistable fronts**
Feedback loops are ubiquitous in biological systems and essential for many cellular processes. For instance, the calcium waves that follow fertilization of an egg are the result of a positive feedback loop in which cytosolic calcium promotes the flow of additional calcium into the cytoplasm. In general, feedback loops lead to nonlinear dynamics that exhibit multiple (linearly stable) reactive equilibria.

A common case is bistability, in which the dynamics $\partial u = f(u)$ has three reactive equilibria, two of which are (linearly) stable ($u_s$) and one of which is (linearly) unstable ($u_u$). Consider a spatially extended bistable system with spatially uniform reaction kinetics $f(u)$, described by the reaction–diffusion equation

$$\partial_t u(x, t) = D \partial_x^2 u(x, t) + f(u(x, t)).$$

In such a system, a front-like profile, in which an interface connects two plateaus at the two linearly stable fixed points $u_s$ and $u_u$ (part a of the figure), will propagate and one plateau invades the other with a constant velocity $v = \int_{u_u}^{u_s} du f(u)$. These fronts come to a halt only for a certain choice of parameters, namely when the areas enclosed by $f(u)$ in the intervals $[u_s, u_u]$ and $[u_u, u_s]$ are equal. For noisy initial conditions interpolating between the two stable plateaus $u_s$, the reaction kinetics first lead to a smoothing of the perturbation and then result in directed front propagation at velocity $v$ (part b of the figure).

**Mass-redistribution instability**
A general design feature of biochemical networks underlying protein self-organization is that their dynamics approximately preserve the mass of each protein species. In other words, on the timescale of pattern formation, both protein production and protein degradation can be neglected. Some key features of the patterning dynamics can already be seen with a two-component, mass-conserving system consisting of a cytosolic and a membrane species (whose concentrations are denoted as $c$ and $m$, respectively) in one spatial dimension:

$$\partial_t m(x, t) = D_m \partial_x^2 m + f(m, c),$$
$$\partial_t c(x, t) = D_c \partial_x^2 c - f(m, c).$$

It is instructive to consider the system's dynamics in $(m,c)$ phase space, as shown in part c of the figure for the biologically relevant limit $D_c \gg D_m$. The reactive nullcline ($f(m, c) = 0$) typically shows an N-shape. Since the reaction kinetics are mass-conserving, reactive flows tend to remain within the corresponding local phase spaces $(m(x,t) = m + c)$, and point towards the reactive equilibria determined by the intersection points of these local phase spaces with the reactive nullcline. Now consider a homogeneous steady state $\pi$ in phase space that intersects the nullcline in a region of negative slope (open circle). Spatial perturbations $\delta n$ around the homogeneous steady state lead to a shift of the local reactive equilibria. In phase space, the perturbation is represented by local phase spaces (thin blue lines) that contain masses that differ from the homogeneous state and therefore lead to reactive fluxes (red arrows) towards the reactive equilibria (orange filled circles). Because of the resulting reactive currents, an upward shift $\delta n$ in total density leads to a decrease in cytosolic density and vice versa. The reactive fluxes lead to a growing inhomogeneous density distribution in real space (inset), which is further amplified by diffusive fluxes (pink arrows in the phase space plot) that arise from the cytosolic concentration gradients, creating a positive feedback loop. (Note that, as cytosolic diffusion is much faster than membrane diffusion $D_c \gg D_m$, diffusive fluxes must point along the vertical direction.) Eventually, a steady-state pattern is reached when the diffusion currents at the membrane and in the cytosol balance out. In phase space, the steady state is represented by a flux-balance subspace (thick grey line) given by $\partial_x (x) + D_m/D_c \cdot n(x) = 0$ where $n_\eta$ is a constant that can be interpreted as the (spatial) average cytosolic density. In summary, this pattern formation mechanism involves an intricate coupling between mass-redistribution and local reaction kinetics.

**Curvature-sensing proteins.** One prominent set of proteins that can individually sense membrane curvature are proteins containing a curved BAR domain. These proteins preferentially bind to membrane regions that have a curvature comparable to that of the BAR domain itself (Fig. 2c). For example, during persistent cell motion,
the curvature-sensitive protein BAIAP2, which contains such a BAR domain, accumulates at curved membrane patches at the cell front, inducing the formation of lamellipodia\(^{4,22}\). Because BAR domains have a length of about 20 nm, the sensitivity of individual proteins to weakly curved surfaces is limited\(^{4,23,128}\). However, membrane curvature can facilitate the oligomerization of proteins into extended curved structures that are capable of sensing membrane curvature on length scales larger than that of the individual protein\(^{19,4,136}\). Other important examples for such joint curvature sensing are dynamin, which forms helical collars around the thin neck during budding in yeast\(^{31,132}\), and MreB, which assembles into filaments that orient along the highest membrane curvature\(^{133,134}\).

Furthermore, some proteins recognize membrane curvature through defects in membrane structure. This mechanism is exemplified by proteins with ‘ALPS’ motifs. ALPS motifs do not have a defined structure in solution but insert into lipid bilayers by folding into an \(\alpha\)-helix. ALPS motifs bind preferably to regions with low lipid packing density\(^{135}\). Such low-density packing can arise from membrane curvature, as one sheet of the lipid bilayer is stretched compared with a flat membrane. In experiments, ALPS motifs were found to bind strongly to liposomes with sufficiently strong positive curvature (\(R < 50\) nm), and to weakly curved liposomes with a high concentration of conically shaped lipids\(^{135}\). Thus, curvature-dependent binding affinity can lead to predominant accumulation of proteins at curved membrane regions.

It has been reported that proteins that sense curvature can also deform the membrane: the helical structure of dynamin oligomers induces membrane curvature during scission of the yeast bud\(^{131,136,137}\). Proteins with BAR domains play a curvature-sensing role at low concentrations but stabilize membrane curvature at high protein concentrations\(^{24,129}\). Such a dual role can lead to a positive feedback loop, when a slightly curved membrane leads to the accumulation of curvature-sensitive proteins. Interestingly, transmembrane protein complexes can also sense membrane curvature, as was shown for the methyl-accepting chemotaxis protein TlpA of \(B.\) \(subtilis\)\(^{138}\). These proteins, in turn, deform the membrane, leading to a further increase in the binding affinity. This feedback has been proposed as a general mechanochemical mechanism for protein recruitment. However, the formulation of a mechanistic theory for such curvature-regulating feedback loops remains an open problem.

**Collective curvature sensing.** The distribution of proteins on the membrane and in the cytosol can depend on the cell geometry, even when the binding affinity of proteins is independent of membrane curvature\(^{4,139}\). The underlying mechanism is based on the aforementioned cytosolic gradients of proteins that switch between an inactive and an active state in the cytosol. As the required reactivation step is a non-equilibrium process that consumes energy, these gradients are maintained by a constant cycling of such proteins between the membrane and the cytosol, and therefore do not equilibrate by cytosolic diffusion. Because cytosolic gradients from opposing membrane points overlap at curved regions, one generally expects accumulation of inactive proteins in regions of high curvature (such as near the cell poles of elongated cells, including the rod-shaped \(E.\) \(coli\), the \(C.\) \(elegans\) zygote and \(B.\) \(subtilis\)\(^{4,18}\) and a corresponding depletion of active proteins (Fig. 2f). Moreover, the effect of such a cytosolic gradient on the protein distributions in curved geometries depends in particular on the characteristic length of the cytosolic gradient relative to the local membrane curvature\(^{4,134}\).

Although this reasoning explains where proteins are most likely to encounter the membrane, its effect on the ensuing protein pattern depends on the protein reaction kinetics. For proteins that exhibit simple attachment–detachment dynamics with the membrane, the increased encounter probability leads directly to an increase in protein concentration at the poles, which is further enhanced if the protein autocatalytically promotes its own binding\(^{4,134}\). In contrast, if two proteins mutually inhibit each other’s binding, an increased encounter probability leads to the formation of an interface between two protein domains on the membrane\(^{4,134}\).

**Biochemical guiding cues**

For spatially homogeneous systems, several theoretical and experimental studies have identified biochemical circuits that can perform logic operations\(^{136}\), generate pulses\(^{141,142}\), act as noise-reduction filters\(^{143}\) or process biochemical signals in other ways\(^{144,145}\). In these circuits, the information from an input signal — typically encoded in the concentration of a protein — is processed and an output signal is generated.

In general, however, protein concentrations tend to be spatially inhomogeneous, so that a locally varying input can lead to a locally varying output protein concentration in the cell. In this way, an input pattern can serve as a template or biochemical guiding cue for the formation of an output protein pattern. Such biochemical guidance has been observed in many biological processes and over widely varying scales, ranging from tissue development\(^{139,140}\), growth\(^{141,142}\) and development\(^{143,144}\) to the positioning of the cell-division site\(^{145,146,147,148}\). In all these cases, the input patterns encode positional information, because each concentration marks a specific location or region in space\(^{135}\).

In fact, there are several known instances in which protein patterns (input) control the formation of other patterns (output)\(^{115,156,159}\). However, the physical mechanisms responsible for the processing of the positional information encoded in patterns, and the generation of a qualitatively different output pattern (such as a gradient or step profile), are still largely unclear.

Such input–output relations are found, for example, in the polarity mechanism of budding yeast. Several ‘landmark proteins’ mark specific locations in the cell, such as the previous bud site. These landmark proteins (input) alter the kinetics of nucleotide exchange in the polarity factor Cdc42 (output), and thus contribute to the control of cell polarity in a symmetry-breaking manner\(^{160,161}\). Another example is provided by the mid-cell localization machinery of \(Caulobacter\) \(crescentus\). In these elongated cells, ParB–\(parS\) (input) complexes

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[^4]: Helix

Prevalent helical-like protein structure, which is highly stable owing to hydrogen bonds.
In the following, we focus on systems in which the concentration profile of one (input) protein controls the reaction kinetics of another (output) protein, such that one or more reaction rates become spatially inhomogeneous. This inhomogeneity can result in an output protein pattern that is qualitatively different from the input pattern, a concept that has been termed spatial network computations.

Spatially varying reaction kinetics

Because protein reaction kinetics can depend on the concentration of other proteins, a spatially varying input protein concentration can lead to locally varying reactive equilibria of the output protein. In particular, not only can the protein concentration at each local reactive equilibrium be altered, but also the number and the stability of these equilibria can change in response to a varying input concentration (BOX 1). Heuristically, this means that space itself serves as a control parameter for the protein reaction kinetics. Hence, the input protein pattern encodes positional information.

The dynamics of the output protein depend crucially on its explicit biochemical interactions with the input proteins. For example, for a particular interaction between proteins, this dependence can lead to bistability of the output protein concentration over a limited range of input concentrations, as observed in starfish oocytes. Then, because the input protein concentration is spatially varying, there exists a parameter range of protein concentrations that maps to a region in space in which the output protein reactions are bistable, thereby making space a control parameter. We refer to this phenomenon as regional bistability. Likewise, a protein pattern can cause a pattern-forming instability in a specific spatial region, a phenomenon termed regional instability. Thus, an input pattern can lead to a qualitatively different spatial concentration profile of the output protein, with the explicit output pattern strongly depending on the reaction kinetics (FIG. 5a). This fundamental property of protein interactions is likely to represent the mechanism that underlies many of the biochemically guided pattern-forming systems observed in experiments.

Wave localization by protein gradients

Biochemical trigger waves, consisting of a travelling front or pulse of biomolecule concentration, are a common means of long-ranged signal transmission in cells. Examples include calcium waves, the propagation of mitosis and apoptosis in Xenopus eggs, actin polymerization waves in Dictyostelium and neutrophils, and intracellular signalling. A key component of models for trigger waves, such as the FitzHugh–Nagumo model, are bistable reaction kinetics (BOX 2). These bistable reaction kinetics result in information transmission and also allow trigger waves to serve as a readout for positional information encoded in other protein patterns.

We illustrate how spatially varying reactive equilibria allow proteins to read out this positional information with the example of a protein gradient leading to a bistable front being localized to a specific position.

**Control parameter**
A parameter that alters the qualitative dynamics when it is changed, also referred to as a bifurcation parameter in nonlinear dynamics.

**Mitosis**
Stage of the cell cycle during which chromosomes are segregated into the two daughter cells.

**Fig. 3 | Principles of biochemical pattern guidance.** a | Left: characteristic bifurcation diagram for pattern-forming systems, in terms of concentration of the input protein on the membrane (x-axis) and concentration of the output protein on the membrane and in the cytosol (y-axis). For reaction kinetics with the concentration of the input protein as a control parameter, a spatially varying input protein concentration can serve as a map between space and varying reaction kinetics. Top right: an input protein concentration gradient corresponds to a cutline through the bifurcation diagram (grey line) laid out in space, which divides the cell into regions of distinct stability. Bottom right: for a system in which the input concentration gradient connects two monostable regions via a bistable region, the resulting front pattern (red line) is pinned to a threshold concentration value of the input concentration. Fixed points of the protein reaction kinetics are indicated by filled (stable) and open (unstable) circles. b | Edge detection. An input pattern (blue) spatially alters the reaction kinetics of the output protein, resulting in a regional instability of the output protein close to the input edge (grey filled area). This instability leads to a peak pattern of the output protein concentration (orange) that marks the position of the input edge. Insets show a possible realization of this edge-sensing process, which leads to a ring around a template patch. The plots depict the concentration profiles along the black cutline. c | Diffusiophoresis. Diffusive fluxes of pattern-forming proteins (carrier particles, orange) are established at pattern interfaces. Carrier particles transport cargo particles (dark blue) via frictional interactions, resulting in a complementary pattern of cargo particles.
in the cell. In general, in a system with homogeneous bistable reaction kinetics forming a front pattern (Box 2), the front propagates through the system at a speed and direction that depend on, among other factors, the concentration of the input protein\textsuperscript{103,172}. In the presence of an input pattern, the reaction kinetics are no longer homogeneous, hence, a regional bistability can emerge. Because the front only propagates in a bistable parameter range, propagation is constrained to this regional bistability. In particular, because the direction of propagation depends on the input concentration, the front is pinned at a threshold input concentration\textsuperscript{195} (Fig. 5a). Because of the correspondence between input concentration and space, this means that the front is localized to a specific position within the regional bistability. Thus, the position of the front interface marks the location of the input threshold concentration, allowing the positional information encoded in the input pattern to be read out. Such a threshold-sensing mechanism has been proposed to play a role in the propagation of surface contraction waves during meiosis in starfish oocytes\textsuperscript{94} and during chemotaxis in eukaryotes\textsuperscript{174}.

**Edge-sensing and ring formation**

Proteins can also localize at the edges of spatial domains that exhibit a high concentration of other proteins or macromolecules. For example, during cellular wound healing, the Rho-GTPase Cdc42 and an associated GTPase regulator, Abr, accumulate locally to form two concentric rings\textsuperscript{196}. Experimental evidence suggests that this structure is hierarchically organized, with the outer Cdc42 ring being dependent on the presence of an inner Abr zone. Although it is not particularly surprising that a given spatial protein profile serves as a template for creating another protein profile with a similar shape, it is striking that the downstream profile assumes a qualitatively different shape, with a peak localized at the edge of the upstream profile (inner Abr ring).

A suggested explanation of such edge-sensing is a regional instability\textsuperscript{97,166}. The step-like Abr profile, acting as an input protein pattern, defines two spatial domains with qualitatively different reaction kinetics for Cdc42, which takes the role of the output protein. The outer domain may effectively act as a stimulus that induces a lateral mass-redistribution instability in the inner domain, which leads to a concentration peak of the output protein at the template edge (Fig. 3c). Moreover, the formation of this output concentration ring can be controlled by both the magnitude of the input pattern step and the total amount of output protein. Thus, edge sensing is enabled by a regional mass-redistribution instability in a downstream protein pattern, which is itself triggered by an upstream protein pattern that acts as a step-like template.

Beyond the specific example discussed above, there are other biologically important processes that involve edge sensing. As in the case of wound healing, in Xenopus oocytes a ring of Rho forms around a patch of high Cdc42 concentration prior to polar body emission\textsuperscript{176}. Another biological process in which protein templates seem to play an essential role is that of macropinocytosis, a form of endocytosis associated with cell surface ruffling. In this process, actin-recruiting proteins co-localize to high-density patches of PIP3 (a charged phospholipid) and a Ras-GTPase. The actin-recruiting proteins form a ring around the edge of the PIP3 domain, leading to the assembly of a contractile actomyosin ring\textsuperscript{177}. This whole process is invariably linked to the presence of PIP3 and Ras patches, suggesting that these biomolecules serve as a biochemical guiding cue for the actin-recruiting proteins. The specific physical mechanisms responsible for each of these edge-sensing processes have not yet been uncovered.

**Tracking of moving patterns**

In addition to varying in space, the input protein concentration can vary in time at a fixed location in the cell. Temporal changes of the input concentration can lead to sudden changes of the reactive equilibrium, which, in turn, results in transient dynamics of the output concentration before the new reactive equilibrium is established — a phenomenon referred to as excitability in the field of nonlinear dynamics\textsuperscript{14,173}. Such transient dynamics can mark the position of local changes in the input concentration. For example, in the case of a travelling front pattern, the input concentration varies in time at a fixed position as the front passes by. Owing to the transient output dynamics, this variation can lead to a travelling output concentration peak that closely follows the moving front. This phenomenon has been observed in starfish oocytes, in which a travelling front pattern leads to a moving concentration peak that is ultimately responsible for the surface contraction waves observed during meiosis\textsuperscript{103,174,179}. Similar observations have been made in vitro for an artificial cortex based on frog egg extracts\textsuperscript{196}.

**Phoretic transport**

A more intricate mechanism by which spatiotemporal protein patterns could serve as cues for the development of subsequent protein patterns involves various types of phoretic transport processes. Phoretic transport, in general, is the result of an external field gradient acting on the protein\textsuperscript{181,182}. Examples include concentration gradients of carrier particles (diffusiophoresis)\textsuperscript{183,184}, chemical potential gradients (chemophoresis)\textsuperscript{185,186}, electrical potential gradients (electrophoresis)\textsuperscript{187} or temperature gradients (thermophoresis)\textsuperscript{188}, along which cargo can be transported. Thus, cargo particles can form a pattern guided by such gradients\textsuperscript{181}. Notably, in phoretic transport mechanisms, energy is consumed to maintain the gradient, resulting in a flux of cargo particles. By contrast, in other transport mechanisms such as active transport, energy is consumed to fuel molecular motors that move cargo particles.

In the field of phoretic transport, research has long focused on colloidal particles\textsuperscript{181–183,189}. Experimental evidence for phoretic transport in biological systems related to protein organization and pattern formation has only recently been discovered\textsuperscript{181,185}. For example, in-vitro experiments have shown that diffusiophoresis can result in the spatial organization of DNA origami nanostructures in a concentration gradient of MinD\textsuperscript{181}.

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**Apoptosis**
Cellular process leading to actively induced cell death.

**Meiosis**
A type of cell-division process that generates daughter cells that contain half as many chromosomes as the parent cell.

**Chemotaxis**
Directed locomotion of cells along chemical gradients.

**Endocytosis**
Cellular process that enables the uptake of biomolecules into the interior of the cell.
In this example, the Min proteins self-organize into a stationary pattern\(^{186}\), resulting in diffusive fluxes at the domain edges (Fig. 5c). These diffusive fluxes are transferred to the DNA nanostructures via friction, leading to diffusiophoretic transport of the latter along the Min gradients. Thus, the movement of the DNA nanostructures mimics the movement of the Min proteins, resulting in the formation of an anticorrelated pattern of the DNA nanostructures. Such diffusiophoretic transport may play an important role in the distribution of large particles in cells in general\(^{191}\).

In the context of plasmid segregation, chemophoresis has been suggested as a mechanism that drives the movement of plasmids on the nucleoid\(^{185}\). ParA proteins on the nucleoid surface are thought to bind to large cargo, such as plasmids. On unbinding, ParA proteins are released from the nucleoid, resulting in local depletion of ParA at the position of the cargo. The ParA concentration gradient at the edge of this depletion zone creates a chemical potential gradient for the cargo, which tends to bind more strongly at regions of high ParA concentration. Thus, the cargo moves along the chemical potential gradient away from the depletion zone\(^{185,186}\). This chemophoretic movement is suggested to be sufficient to ensure a balanced distribution of plasmids on the nucleoid\(^{185}\).

**Mechanical guiding cues**

In addition to biochemical guiding and guidance by cell size and shape, the mechanical properties of a cell can also affect protein pattern formation by altering the transport and reaction kinetics of proteins.

Flows generally arise from stress gradients. In cells, such gradients can be generated via shape deformations (Fig. 6a). For example, flows are generated in the cytoplasm by shape deformations in starfish oocytes\(^{84}\). In these cells, a surface contraction wave travels across the membrane from the animal to the vegetal pole, which locally increases the pressure in the cytosol, resulting in cytoplasmic flows along the oocyte’s animal–vegetal axis. Likewise, in *Drosophila* embryos, apical constrictions (rather than surface contraction waves) lead to cytoplasmic flows\(^{182}\), and in *Drosophila* neuroblasts, cortical contractions induce flows in the cortex\(^{182}\).

Besides deforming the cell shape, contractions of the actomyosin cortex can also lead to cortical flows, as a consequence of either spatially inhomogeneous actomyosin activity\(^{4}\) or anisotropic cortical tension\(^{194}\). For example, cortical flows in *C. elegans* zygotes before PAR polarization arise because of non-uniform actomyosin activity\(^{3}\). Through hydrodynamic coupling, such flows may also induce cytoplasmic flows\(^{53,92}\).

**Upcoming challenges**

In this Review, we focus on guidance mechanisms in model biological organisms that have been studied experimentally, and for which theoretical models exist. However, a much larger number of cellular processes rely on guiding cues whose underlying biophysical mechanisms are still unknown.

**Robustness against guiding cues**

Guiding cues can vary over time — for example, cell size and shape change throughout the cell cycle. Moreover, these changes can affect the process of protein pattern formation in quite different ways: protein patterns can either adapt to the changing guiding cues, as discussed in this Review, or they can be impervious to variations in geometric, mechanical and biochemical factors. Pattern-forming mechanisms that are robust to changes...
in cell geometry or mechanics have been identified\(^\text{[140,146]}\), but a general understanding of robustness in pattern formation is still lacking.

**Mechanochemical feedback loops**

We discussed above how protein patterns can flexibly adjust to changes in the physical properties of cells. However, proteins can also actively modify the mechanical properties of the cell, resulting in a feedback loop between cell mechanics and protein patterns. Theoretical studies have shown that the coupling to cell mechanics in such mechanochemical feedback loops can lead to the formation of protein patterns\(^{[100,195–200]}\). For example, coupling of a contractility-regulating chemical agent to an active fluid surface can result in shape deformations of axisymmetrical surfaces, accompanied by polarization of the chemical agent\(^{[101]}\). This phenomenon shows similarities to the aforementioned self-reinforcing polarity mechanism of *C. elegans*, in which cortical flows are created by asymmetrical actomyosin activity\(^{[194]}\). In addition, a recent experimental study showed that the spatiotemporal patterning of the Min protein system can induce substantial shape deformations in giant unilamellar vesicles (GUVs)\(^{[201,202]}\). This observation suggests a generic interplay between reaction–diffusion dynamics and membrane mechanics. We hypothesize that membrane properties, such as spontaneous curvature, may influence the kinetics of protein binding, and vice versa\(^{[196,197]}\). In combination with the hydrodynamic coupling of the cell membrane to the cortex and the cytosol, this interplay can lead to a mutual feedback between the dynamics of protein patterns and cell shape.

A theoretical characterization of this two-way coupling between biochemical processes and cell mechanics is a promising avenue for future research\(^{[200]}\). Because such mechanochemical models need to account for protein reaction–diffusion dynamics as well as a dynamically varying 3D cell shape, they are challenging to study both analytically and numerically\(^{[195,199,203]}\). In future research, it will be important to further develop methods and, in particular, biologically realistic 3D models, such that they can be compared with quantitative experimental data and contribute to the interpretation of experimental results in mechanochemical model systems.

Mechanochemical feedback loops are a special case of a general phenomenon that can be observed in many pattern-forming systems: many patterns in cells are not the result of a single guiding cue, but are the products of multiple interacting cues and processes\(^{[53,195,206–208]}\). However, it is often difficult to separate all the processes involved in the robust formation of functional protein patterns in living cells, as the example of *C. elegans* polarization shows\(^{[34,203]}\). Recognizing and incorporating such interacting processes into the theoretical analysis of pattern-forming systems will therefore be a major task for future research on pattern formation.

**Perspectives for pattern guidance**

At the conceptual level, there are three main challenges in the context of understanding the biophysical basis of pattern guidance. These relate to progress in the study of fundamental aspects of processes in living systems far from thermal equilibrium; finding the right level of simplification for a given complex biological system; and improving both computational and experimental tools.

**Frontiers in non-equilibrium physics.** Several interesting physics questions arise from the biological model systems that we discuss in this Review. A central issue concerns how the dynamics of pattern-forming systems are mechanistically controlled by spatial and temporal gradients. Such gradients lead to a variety of fascinating phenomena including information processing\(^{[149]}\), templating\(^{[166]}\) and hierarchies of different patterns\(^{[214]}\). Because gradients can form for different physical quantities, they can influence the formation of patterns in many ways. Any gradient in an intensive thermodynamic variable, such as a chemical potential, can give rise to corresponding particle currents, as described by non-equilibrium thermodynamics\(^{[209]}\). Transport properties are also strongly influenced by spatial variations in kinetic coefficients (such as diffusion constants). These processes lead to additional advection currents, which we have not addressed in this Review. Moreover, owing to dynamic feedback between these particle currents and protein patterns, the gradients themselves may become part of the dynamics rather than acting solely as external guiding cues. This can lead to intricate pattern-forming dynamics on multiple spatial and temporal scales\(^{[210,211]}\), which expands the possibilities for future research. In particular, to gain insight into the underlying principles of such multiscale systems, new theoretical approaches are required that allow the dynamics to be reduced to the essential degrees of freedom at the relevant length and timescales.

**Levels of biological and geometric complexity.** Another crucial and general challenge is how to deal with the different levels of complexity in biological systems. For example, the full extent of most interaction networks of proteins is unknown, and it is often unclear whether integrating all possible interaction pathways into a theoretical model is actually necessary to explain a particular phenomenon\(^{[100,212]}\). Even when networks are fully characterized, the information flow through the reaction network can be difficult to understand. For well-mixed reaction systems, methods to analyse such information flows have been developed, such as modular response analysis\(^{[212]}\). For spatially extended systems, in which information is stored and processed by patterns, such methods have yet to be developed.

In addition, temporal regulatory mechanisms, such as cell-cycle-induced gene regulation, are often excluded from models of pattern-forming systems, even though the relevance of such regulatory mechanisms for pattern formation is not yet fully understood\(^{[145,143]}\). Whenever such mechanisms are in place, global mass conservation — which is a cornerstone in many models of protein pattern formation — no longer applies, allowing for additional paradigms for pattern formation\(^{[104]}\).

Other difficulties for theory arise because it is difficult both to avoid overfitting models, and to separate important components of interaction networks from...
irrelevant interactions (on the timescale of interest). From a physics point of view, there is a need to develop theoretical frameworks that allow for systematic coarse-graining and that show how the manifold components of a biological system can be reduced to its core elements.

Similarly, the question of how theory should deal with the dimensionality and geometric form of biological systems needs careful consideration. For example, reducing the dimension of a specific system — to simplified 1D models, for example — may help to obtain a representation that is analytically more accessible. Although such a simplification can be useful for gaining insight into the underlying dynamics and for guiding experiments, it may also obscure important aspects of pattern guidance. As pointed out in this Review, certain phenomena, such as curvature sensing, only occur in realistic geometries and would therefore be erased in simplified models, such as curvature sensing in a 1D system. The challenge for models is to find the appropriate level of simplification without loss of crucial features, such as geometric effects and cytosolic gradients, as discussed in this Review.

Improving experimental and computational methods. Another roadblock that impedes progress is the limited availability of experimental, analytical and computational tools. On the experimental side, the current challenges include improving the spatial and temporal resolution of the quantities of interest (such as proteins), and accessing quantitative information such as local densities, reaction rates, transport properties and forces. In addition, conducting experiments under well-controlled conditions, in which only one or a few parameters are adjusted at a time, is often difficult. For instance, altering intrinsic quantities in experiments, such as temperature, affects not only kinetic rates but also the transport properties of proteins (diffusion). Hence, one relies on new experimental techniques and methods to conduct controlled experiments that will help to guide and test theory in the future.

In addition, many experimental results indicate that pattern formation, and pattern guidance in particular, are the result of a tight interplay between biochemical interactions, hydrodynamics of cellular substrates and membrane mechanics. Although numerous theoretical advances have been made in each of these areas (including reaction–diffusion dynamics and non-equilibrium physics), there is so far no unified theoretical and computational approach that would allow a thorough analysis of such multiphysics problems. Therefore, to gain a deeper understanding of pattern guidance in realistic biological systems, a comprehensive theoretical framework that allows the study of the interplay between these different fields of physics must be developed.

In particular, an important task for future research is the numerical implementation of bulk–boundary coupled reaction–diffusion systems in combination with hydrodynamics and deformable, time-evolving membranes. The primary difficulties lie in the development of an efficient and stable numerical approach that allows one to solve multiphysics problems in which the numerical domain itself is part of the solution. In the case of reaction–diffusion dynamics on dynamic membranes without coupling to a bulk volume, this difficulty can be addressed by deriving the time evolution of the surface from the (normal) variation of a free energy functional that describes the mechanical properties of the membrane. However, this approach does not account for dynamics in the bulk, such as intracellular flows and bulk–boundary coupling of protein reactions. Promising approaches that can cope with these problems in the future are the level-set and the phase-field methods. These strategies allow one to segregate the computational domain into different regions (such as the interior and exterior of a cell), with the interface between these regions corresponding to a (smooth) boundary (which could represent the cell membrane, for example). In this way, one can define and solve a coupled set of PDEs between different regions, including the interface, and at the same time allow these regions to evolve over time by solving the level-set or phase-field equation. Most notably, the phase-field method is used to model cell migration, with applications to reaction–diffusion systems arising only in the past few years. At the same time, new methods are being developed.

In the long run, it will be a challenge to not only model a deformable domain, but also incorporate the biochemical and mechanical details of membranes in computational approaches.

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This study uses confocal microscopy to demonstrate spatiotemporal patterning of Min proteins in GUVs accompanied by changes of the vesicle shape and its mechanical properties, proposing a mechanism for shape control based entirely on self-organized protein patterns.

Using a cell-free reconstitution of ATP-driven cargo transport without motor proteins. This study combines theoretical analysis with in vitro experiments on Min proteins and DNA origami nanostructures to propose diffusophoresis, guided by protein patterns, as a motor-free directional transport mechanism of non-specific cargo.

Using a cell-free reconstitution of ATP-driven cargo transport, this study provides evidence of chemotropic motion of surface-confined plasmid cargo guided by a protein gradient.

This study, combined with theoretical studies of the interaction of Min proteins and DNA origami nanostructures to propose diffusophoresis, guided by protein patterns, as a motor-free directional transport mechanism of non-specific cargo.

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