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Shapes of cell signaling

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Abstract

Cell signaling is a complex process organized in time and space. Signal transduction is constantly modulated by cell-intrinsic and cell-extrinsic input cues and the resulting phenotypic responses such as morphological can feed back into the system. This provides cells with a responsive, accurate, and rugged system to deal with changes in the surroundings or the genome. Whilst signaling networks (dynamic transient protein–protein interactions modulated by post-translational modifications in response to input cues) have been researched for decades, further analysis of their spatial organization is critical for both basic and disease biology and will benefit from recent advances in computational modeling and image analysis using deep/machine learning and in microscopy and imaging. Furthermore, mathematical modeling with reaction-diffusion approaches on time-varying geometries complements the investigations, allowing to conceptualize the organizational principles of signaling and information transduction in the four dimensions of time and space.

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Keywords

Signaling in context, Cell shape, Deep hidden physics, Reaction-diffusion systems, Cell morphology, Complex diseases.

Cell signaling is foundational to biological organization [1,2]. The morphology of eukaryotic cells and their organelles is closely associated with the biochemical states of these (sub)systems. The dynamical morphology and corresponding network states are the basis for the cells' ability to respond to external and internal cues and altering phenotypes or making complex integrative decisions [3,4]. Cell signaling does not occur by homogeneous mixing of signaling molecules, instead, it most often happens in localized transient complexes or even near solid-state chemical reactions on docking proteins involving very few single-digit numbers of proteins [2,5,6]. This way the cell can stringently and in a highly localized manner regulate its morphology whilst keeping the components being regulated close to the regulators. Cell and cellular signaling are not only dependent on time and space but also 'context' or perhaps better termed the biochemical network state of the cell, which in turn depends on the cell type, mutations, nutritional conditions, physicochemical parameters, embedding in a tissue or growth in a cell culture [7]. The network state of the cell is in a constant state of flux. Activation of a master controller such as a kinase in a specific network state can commit the cell to apoptosis, whereas activation of the same kinase in another network state can result in cell proliferation [8–10]. Thus, entirely opposite phenotypic states can be reached through the same regulator depending on context. This is a result of the multivariate nature of cell signaling networks and the fact that a cell is never in total isolation from other cells or external and internal cues. A dramatic consequence of this is that debates over whether a certain molecule is a tumor suppressor or oncogene are essentially flawed as both can be true, depending on the network state of a specific cancer or tumor cell [11]. It also means that classic pathway descriptions and a large fraction of the literature on cell signaling should be considered context-dependent intersections or snapshots that may not translate to other cell types or genetic backgrounds or are only valid under a specific set of biochemical perturbations.

These principles have profound implications for how we study biological systems and their response to cues. It first and foremost means that we must rely on multivariate perturbations when conducting stimulation-based experiments. It also means we must design our experiments to accept the multivariate nature of biological systems and stop making bold conclusions from single stimuli (e.g. with just a growth factor) or from extreme dose stimuli (e.g. 150 ng/ml EGF (epidermal growth factor) [12]) as these basically force the network states into (adaption to) a nonphysiological or artificial context which may not be relevant from a biological point of view or to the actual cell type being studied.

The eukaryotic cell's phenotype is basically composed of macromolecular assemblies in combination with small

molecules, lipids, water, and ions. Therefore, continuous imaging of macromolecules and these dynamic assemblies whilst sampling experimentally the corresponding network and cell signaling states would, from a theoretical point of view, provide the best basis for developing biological forecasting models. Such models would link signaling network states to the phenotype and enable to forecast how a perturbation or change in the network will alter the phenotype.

Recently, we demonstrated that in some sense, signaling network states and phenotypes are mirror images in the sense that it is possible to move bidirectionally between them [13].

Meanwhile, it is known that the development of drug resistance in cancer cells can lead to changes in cell morphology [14]. What we showed is that by deploying deep neural networks to analyze this relationship, complex cell morphologies can encode states of signaling networks and unravel cellular mechanisms otherwise hidden. From a very large imaging data set, we showed that from morphology (cell shape space) alone, we could predict whether a cell was resistant to a therapeutic antibody (ErbB-family) and predict the potential mechanism of resistance [13]. Our work raises many questions, for example, whether this is a specific feature of antibody treatments or whether it can also be observed with small compound treatments. Another open question is whether it will be possible to use this 'bridge' to study mechanisms of many other phenotypes and signaling networks and the underlying mechanisms. This should be investigated in a massive scale imaging experiment.

Here, we (i) emphasize the need for accounting for cell shape in signaling and systems biology more broadly; (ii) describe experimental approaches to quantify spatial and temporal cell behavior; (iii) provide an overview of mathematical modeling of cell shape and dynamics; and (iv) present a vision for robustly monitoring cell shape and dynamics.

Why is space important?

The cell with its morphological structures provides the environment in which signaling operates. A cell is exposed to hundreds of thousands of input cues at any given time point, for example, from mechanical stresses resulting from, for example, cell–cell contacts, fluid flows, osmotic changes, DNA damage, hormone and cytokine changes, or its own metabolic processes. These cues are picked up by the cell's receptors and other sensing molecules and translated into signals that propagate in highly nonlinear, transient, dynamic networks primarily composed of signaling proteins interacting through post-translational modifications. Receptors for external and internal cues are typically embedded in membrane structures and interact with other membrane-associated or diffusive (adaptor) proteins. When an external cue is processed, the information needs to get from the cell surface to the nucleus; internal signals also need to be conveyed between organelles (e.g. in plants from the nucleus to chloroplast and back). At the same time, organelles, cytoskeleton, or microtubules interfere with the diffusion of signaling molecules. When it comes to the cell shape, models show that the curvature of the membrane as well as the ratio between the surface and volume influence molecular diffusion and interaction and, hence, information transmission [15,16].

Signaling is inherently noisy, but cell fate depends on information being transmitted reliably. Concepts have been developed to quantify information transmission in cellular signaling networks [17–19] based on Shannon's information theory [20] and need to be extended into space and into spatial organization of near membrane-located protein complexes. Questions arise about how to integrate a variety of signals or how to sharpen the signal reaching its destination, for example, the nucleus? Suggested solutions include selected inactivation [21] or employment of scaffolds proteins (own studies).

Experimental techniques to investigate spatial composition and dynamics

Imaging techniques are very powerful methodologies to obtain data about spatial signaling in living cells. Different techniques are available, and image acquisition and image analysis are their own quickly developing fields of research. Computational algorithms and mathematical breakthroughs in, for example, machine learning [22], are a major part of this development.

A frequent approach is to tag proteins with a fluorescent dye and then follow its distribution in the cell. A recent example is an assay combining proximity ligation with chemical labeling of cysteine residues in the sulfenic acid state to visualize a protein-tyrosine phosphatase and resulting in spatially and temporally limited protein oxidation within cells contributing to cellular signaling [23]. In addition, optogenetic methods to visualize interesting components are currently gaining momentum [24–26] allowing to measure the spatiotemporal distribution of, for example, *Smad2* in transforming growth factor beta (TGF-β) signaling [26] or *KRas* in epidermal growth factor (EGF) signaling [25]. An example for the analysis of spatial receptor distribution is ratiometric G-protein coupled receptor (GPCR) signaling for directional sensing in yeast [27].

Imaging naturally comes with challenges: (i) despite impressive progress, the spatio-temporal resolution is still insufficient to precisely follow single molecules. Single-particle tracking, that is, assigning signals from different images to the same molecule to assess its spatial dynamics is still a hard problem (e.g. Ref. [28]). (ii) All methods of imaging somehow interfere with the

signaling process itself, and the aim is to minimize this. For example, tagging is often combined with over-expression of the protein, which affects the quantitative contribution to signaling. The tag may also change binding properties or activities. (iii) Moreover, most methods focus on a single or few selected molecules, which gives insight into this molecule's fate, but not yet provide the global dynamic overview finally wanted. (iv) The spatial organization of signaling in human cells cannot always be understood from single cells alone but refers to cells in their community.

Mathematical modeling of cell shape and signaling in four dimensions — from tradition to deep hidden physics

A frequent type of mathematical models describing signaling in space and time are reaction-diffusion models in the form of partial differential equations. Taking into account processes, both on or close to the surface (S) and in the volume of the cell (V), a general form of the reaction-diffusion equations reads

$$\frac{\partial x_i}{\partial t} = f_i(\vec{x}, \vec{y}) + D_S \Delta_S x_i + k_s,\ i = 1, \ldots n \tag{1}$$

$$\frac{\partial y_j}{\partial t} = f_j(\vec{x}, \vec{y}) + D_V \Delta_V y_j + k_v,\ j = 1, \ldots m \tag{2}$$

where, $\vec{x} = (x_1, .., x_n)^T$ denotes the amount of the compounds moving at the surface, $\vec{y} = (y_1, .., y_m)^T$ the compounds diffusing in the volume, f_i and $f_j$are the reactions those compounds can undergo, D_S and D_V are the diffusion coefficients in 2 and 3 dimensions, respectively, Δ_S and Δ_V are the respective Laplace operators, and k_s and k_v can be some external stimuli (Figure 1).

Historic attempts to explain growth and form of single cells using physical principles [29] suggested that turgor pressure and surface tension determine cell shape. The Helfrich potential [30] summarizes the energy contributions such as surface tension and bending energies of biological membranes and has been used to explain the shape of motile cells such as fish keratocytes [31] or curvature of red blood cells and vesicles [32,33]. However, in many cases, cell shape is not static and does not just follow from energy minimization. Instead, it results from a response to an external cue.

Polarization is an important process requiring spatial orientation of a cell, typically orchestrated by Rho-GTPases. A classic is the Gierer-Meinhardt model [34] which describes pattern formation of morphogens based on auto- and cross-catalysis combined with short-range activation, long-range inhibition, and a distinction between activator and inhibitor concentrations on one hand, and the densities of their sources on the other. Different biochemical mechanisms have been suggested for the necessary symmetry breaking, among them local excitation, global inhibition [35], Turing instability (e.g. Ref. [36]), and wave-pinning [37].

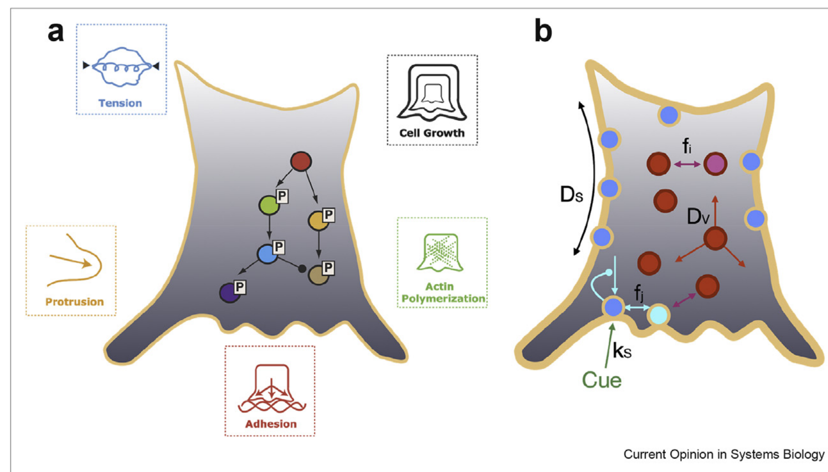
One extensively researched example is the mating of the haploid forms of Baker's yeast, where direction in space and polarization are critical for the choice of a mating partner. Because yeast cannot actively swim, they have to precisely detect the location and distance of the mating partner from gradients of pheromone, then establish cellular polarization and finally grow toward the mating partner, that is, form a protrusion called shmoo. Models have been presented for the establishment and sensing of the pheromone gradients [38,39], the actual polarization of the cell based on shuttling of Cdc42 [15,40–48], the resulting intracellular gradient [49] as well as the resulting shape changes [50]. In motile cells such as the fish keratocytes, F-actin dynamics contribute to the polarization [51,52].

The shape of the cell also modulates the signaling processes [53,40] via (**i**) the curvature of the relevant surfaces, (**ii**) the alternation of the ratio between the surface and volume, and (**iii**) the available volume. The other way around, intracellular gradients of signaling molecules are able to influence cellular growth and shape [54,55]. Shape changes and the task to develop predictive models for signaling networks in growing or deforming cells establish interesting mathematical challenges. On the one hand side, shape dynamics have to be extracted from images and be transformed into an adaptive surface. For example, Ma et al., 2020 [21] used soft X-ray tomographic imaging of cells for the reconstruction of the cell structure with surface and organelles.

On the other hand, models have to capture the shape changes leading to reaction-diffusion systems on evolving domains. Solutions for this problem beyond partial differential equations in one spatial dimension [56] are, for example, adaptive triangulations as used in Ref. [50]. The description of spatial configurations is an active field of research using different versions of finite element methods [57].

Despite exciting technical developments, it is still an important task to find sensible simplifications to keep the model tractable to learn more about the implications of spatial distribution of components and their tasks including receptors at the cell surface, large dynamically forming complexes at the cell surface, and signal 'pathways' or networks transmitting information to targets, with the aim to understand the temporal organization, signal integration, and which information is eventually transmitted.

Figure 1



(a) Eukaryotic cells and individual cell types not only contain many different organelles (not shown) but can often adopt many morphologies and dynamic phenotypes that, for example, relates to cell growth, adhesion to the extracellular matrix, protrusions, or actin formation. These are both regulated and composed of cell signaling network states. Thus, correlating these in a bidirectional manner is critical for understanding and forecasting biological systems from single cells to multicellular or complex tumor environments. **(b**) Signal states can be regulated by external (k_s) or internal cues, diffusion of molecules on surfaces (D_S) or in volumes (D_V), reactions (f_i, f_j), and changing gradients.

Genome-scale experimental design and the vision of a very large imaging array

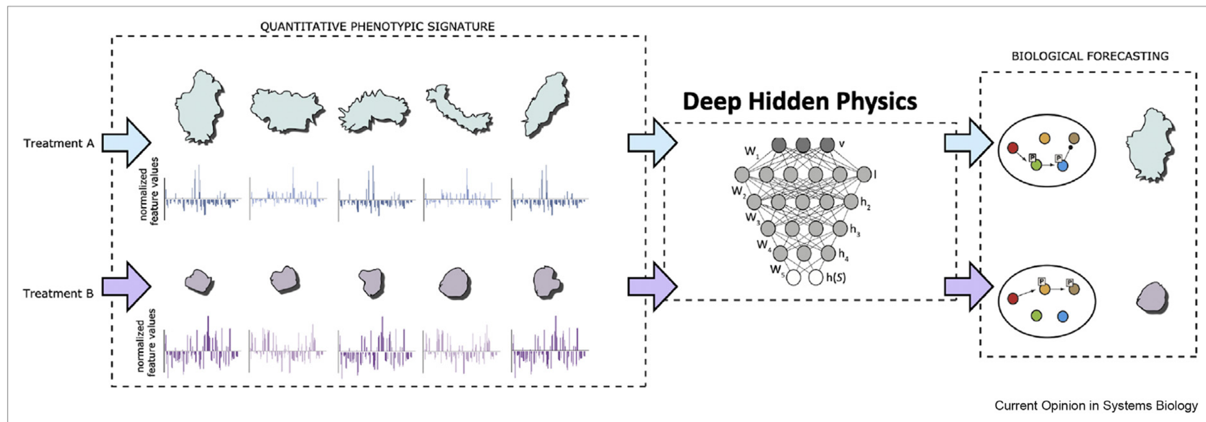
Critically, moving forward with deeper biological understanding is imaging **live cells** in their (*in situ*) context, for example, in tissues, 3D culture, and other types of cell culture, organs, and tumors all of varying biological complexities. Imaging of cell–cell contacts and everything from cell populations to single/individual cells is critical to derive multiscale models. Methods that can scale to genome-scale and facility analysis across 100.000s of perturbations (genetic/ clustered regularly interspaced short palindromic repeats (CRISPR) /RNAi, metabolic, chemical/drugs/small molecules, antibodies, and combinations thereof) in a fully automated mode is vital [58,59]. At the same time, it is important to be able to analyze cell shape at different scales: multicellular, cellular, and subcellular even down to the molecular assembly level (complexes) with new methods, for example, electron microscopy/tomography, *in vivo* NMR, multiphoton and non-invasive imaging, cytometry by time of flight (CyTOF) imaging or scanning (positron emission tomography (PET), continuous-spectrum emission tomography (CET), etc.) in complex tissues. In the future, ideally, it should be possible to conduct this to the degree where robust models of the molecular assemblies can be derived and how these create dynamic morphological changes and phenotypes, that is, multiscale models across time, space and at different scales and integrated with context.

It is clear we do not have all the tools currently to do this; however, it is relatively clear that things are moving in the right direction.

Moving forward what is required is perturbation studies, not only steady states. Signaling makes sense only as a dynamic process. We must activate or challenge the system to see its potential and dimensionality and to see how vulnerable or robust our models are. With models, we can test how robust our assumptions are but only in combination with diverse quantitative data. Thus, we clearly need more live-cell imaging. When we image different levels of the cell with many levels and precise quantifications, we can aim to build a whole cell and tumor or tissue models. Classic definition (spiky, long, elongated, round, etc.) of phenotypes does not provide enough description. Phenotypes are like a continuum, and morphological shape space contains a massive amount of information that we can use to understand complex and nonlinear molecular interaction network dynamics. Hence, shape must be confronted/correlated with signaling dynamics and network rewiring. Form and function are integrated and indeed two sides of the same coin. There is also spatial organization at other levels, which we need to be able to model. It's important for tumor evolution and other complex diseases. For the mammalian cell, shape is decisive for fate and cell–cell contact. Cancer is a disease driven by organization of signals in space-time and shape, thus no wonder the treatment of cancer also requires to embrace cell shape and complex phenotypes.

To this end, we propose that massively parallel imaging under hundreds of thousands of perturbations in combination with network sampling using from single cell to community-level mass spectrometry, CyTOF [60], single-cell proteomics with, for example, Single Cell

Figure 2



Quantitative phenotypic signatures (high-dimensional normalized feature vectors or autoencoders representing quantitative imaging space) are used in deeply hidden physics models [63,64] to generate predictive/forecasting models of corresponding signaling network states and morpho-/pheno-dynamics.

ProtEomics by MS (SCoPE-MS) [61,62], next-generation sequencing (NGS), phosphorylation mass-spectrometry (phospho-MS), RNAseq, etc. would enable development of cell forecasting models similar to climate/weather predictions. Multiscale studies are vital and thus a European **Very Large imaging Array** (VLiA) analogous to the Very Large Array (VLA) in New Mexico or Square Kilometre Array would also include structural methods such as electron microscopy (EM)/ electron tomography (ET)/ nuclear magnetic resonance (NMR), atomic force microscopy (AFM), and other similar tools to get information at different molecular and assembly scales. Crucially, sampling must be performed in live cells and/or in time series to optimize the likelihood of forecasting. The experimental design is critical, both hypothesis- and nonhypothesis-driven studies should be conducted, however, always in a manner so that the data generated can assist with answering a specific biological question. This is far less trivial than it may read and it is often ignored by large omics studies.

A platform like this could be used for several flagship studies, one could, for example, be of reverse engineering CRISPR based on cancer mutations in a cell line or primary cell to analyze the network adaptation and possibility of reversing malignant phenotypes.

We argue it is time to build such big quantitative live-cell imaging facilities to obtain massive amounts of perturbation-based data that can be used to train learning algorithms or different types of models. Biology research will have to embrace the model known from, for example, particle physics (Large Hadron Collider (LHC), CERN, and accelerators) in terms of infrastructure and collaboration. However, it is also clear that not just one technology can do the job. It has been evident for years that genomics and genome sequencing or mass spectrometry are not enough to understand complex biological issues such as cancer phenotypes or therapeutic responses. We now need to move systems biology away from its 'omics' or 'single-cell' era to fully embrace massive and multiscale complex nonlinear dynamics and perturbation-based studies that link biological behavior to the underlying network dynamics with the clear goal of forecasting and prediction of critical to know phenotypes/biological processes (Figure 2). Such a concept would encapsulate everything ongoing in previous systems biology from biomarkers to models of cell−cell communication to the guided reverse engineering of cells and tumor cells.

Conflict of interest statement

Nothing declared.

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