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Cell-Cell Communication Inference and Analysis: Biological Mechanisms, Computational Approaches, and Future Opportunities

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Abstract. In multicellular organisms, cells coordinate their activities through cell-cell communication (CCC), which is crucial for development, tissue homeostasis, and disease progression. Recent advances in single-cell and spatial omics technologies provide unprecedented opportunities to systematically infer and analyze CCC from these omics data, either by integrating prior knowledge of ligand-receptor interactions or through de novo approaches. A variety of computational methods have been developed, focusing on methodological innovations, accurate modeling of complex signaling mechanisms, and investigation of broader biological questions. These advances have greatly enhanced our ability to analyze CCC and generate biological hypotheses. Here, we introduce the biological mechanisms and modeling strategies of CCC, and provide a focused overview of more than 140 computational methods for inferring CCC from single-cell and spatial transcriptomic data, emphasizing the diversity in methodological frameworks and biological questions. Finally, we discuss the current challenges and future opportunities in this rapidly evolving field, and summarize available methods in an interactive online resource (`https://cellchat.whu.edu.cn`) to facilitate more efficient method comparison and selection.

AMS subject classifications: 92-08, 92-04, 92C42, 92C37, 68T09, 62P10

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1 Introduction

Cell-cell communication refers to the process by which cells exchange signals to coordinate their activities through soluble signaling molecules or direct physical contact. It forms the fundamental basis for maintaining homeostasis and achieving functional integration in multicellular organisms. This process involves a series of highly specific molecular mechanisms, requiring the coordinated participation of various molecules – such as ligands, receptors, ions, and metabolites – as well as membrane structures, including tight junctions, gap junctions, and immunological synapses [3,15,39,73,109]. Elucidating the mechanisms underlying CCC not only deepens our understanding of development and disease, but also identifies potential targets for therapeutic interventions [153,160].

Traditionally, the study of CCC has relied heavily on labor-intensive experimental approaches, such as histological tissue section analysis, in vitro co-culture systems, and in vivo genetic manipulations. These methods are often time-consuming and technically demanding, typically permitting the investigation of communication between only a limited number of cell types and signals, thereby hindering a comprehensive elucidation of CCC networks. The rapid advancement of single-cell RNA sequencing (scRNA-seq) technologies has revolutionized CCC research [13,153]. The scRNA-seq data capture gene expression patterns relevant to CCC, providing indirect insights into the abundance of proteins involved in signal transduction. Building on this foundation, numerous computational tools have been developed to infer CCC by integrating scRNA-seq data with prior knowledge of ligand-receptor interactions (LRIs) [4, 9, 13, 31, 89, 153, 160, 179], thus yielding biologically interpretable insights. However, spatial information on cells is inherently lost in scRNA-seq data. Given that most CCC events occur within a short spatial distance, the emerging spatial transcriptomics brings new opportunities to improve CCC analysis [49,117]. The ongoing technological progress, such as single-cell and spatial multi-omics [22, 171], and increasingly complex biological questions continue to drive the evolution of computational approaches, enabling more comprehensive and in-depth analyses.

This review aims to equip biologically focused researchers with practical guidance on when and how to apply existing CCC tools, while providing computational biologists with the biological foundations and methodological advances of CCC to facilitate the development of novel CCC analysis tools. While related reviews exist [4, 9, 13, 31, 89, 153, 160, 179], here we emphasize the biological mechanisms and modeling strategies that underpin CCC, and critically examine the most recent and innovative computational approaches. This review begins with a systematic overview of the biological foundations of CCC, including various types of intercellular and intracellular signaling mechanisms. We then present a general modeling framework for CCC inference, and review a range of computational methods developed in recent years, with an emphasis on their methodological diversification and the specific biological questions they aim to address. Finally, we discuss key challenges and emerging opportunities in this rapidly advancing field. To aid the research community, we provide an interactive, web-based summary of all reviewed computational methods (available at `https://cellchat.whu.edu.cn`).

This resource details each method's overview, computational principles, and addressed biological questions to facilitate efficient method comparison and selection. It features interactive filtering based on methodological characteristics and includes a submission portal for researchers to contribute new tools, ensuring the resource remains current.

2 Biological mechanisms of cell-cell communication

2.1 Types of cell-cell communication

Precise, reliable, and reproducible mechanisms of CCC have evolved to coordinate biological activities among cells within multicellular organisms. Distinct communication systems operate across various tissues and organs to transmit information from signaling cells to their target cells [3,15,109]. The chemical signals used by signaling cells for information transmission are known as ligands, while the corresponding binding molecules on target cells are called receptors. Ligands comprise diverse molecules – including proteins (e.g. growth factors, cytokines), peptides, small molecules (e.g. metabolites, neurotransmitters, hormones), and lipids (e.g. prostaglandins, eicosanoids) – that bind to cell-surface receptors. A separate class of hydrophobic ligands (e.g. steroids, gases) diffuses across the plasma membrane to bind intracellular receptors. Receptors are broadly classified by localization and function: cell-surface (transmembrane) receptors and intracellular (cytoplasmic or nuclear) receptors. Cell-surface receptors are further categorized as ion channel-linked, G-protein-coupled, or enzyme-linked [73]. Upon ligand binding, receptors undergo conformational changes that activate intracellular signaling molecules within target cells, and further initiate signal transduction cascades.

CCC can be broadly classified into five types based on underlying principles: paracrine, autocrine, contact-dependent, synaptic, and endocrine signaling (Fig. 1). These categories are primarily distinguished by the distance over which ligands act to reach their target cells [15,39,109].

Paracrine signaling is a short-range mechanism of CCC in which ligand-producing cells secrete signaling molecules that diffuse locally and act exclusively on neighboring target cells, without requiring direct physical contact, as illustrated in Fig. 1. In this mode of signaling, ligands released by signaling cells traverse the extracellular matrix (ECM) and bind specifically to receptors on adjacent target cells. The spatial distribution of ligands within the ECM forms a concentration gradient that is essential for effective signal transmission, enabling target cells to generate differential responses depending on ligand concentration. After signal transduction, ligands are normally quickly degraded by enzymes or removed by neighboring cells. These regulatory processes help maintain ligand concentration homeostasis within the ECM, thereby minimizing interference with subsequent paracrine signaling. Prompt removal of ligands following signal activation also limits their spatial spread, reducing the likelihood of erroneous interactions with non-cognate target cells outside the intended signaling domain.

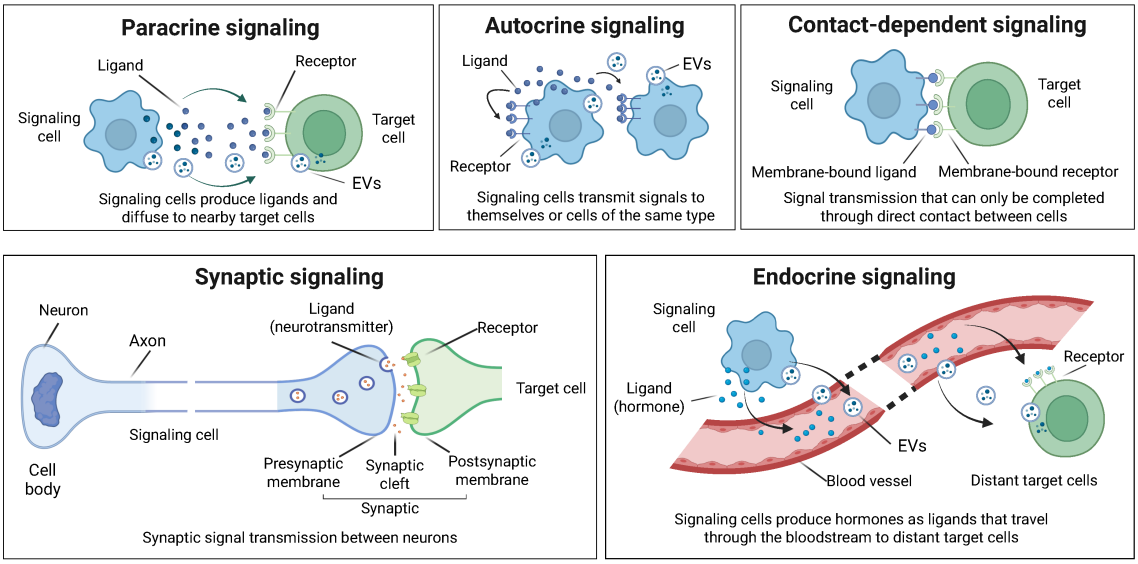

Figure 1: Diverse types of cell-cell communication. Paracrine signaling involves signaling molecules that act on neighboring cells within a localized area by diffusing through the extracellular space. Autocrine signaling refers to signals that act on the same cell that produces them or on a population of identical cell types. Contact-dependent signaling relies on direct physical contact between neighboring cells, such as direct membrane contact or gap junctions. Synaptic signaling enables long-distance communication through the specialized synaptic structures of neurons. Endocrine signaling involves signaling molecules produced by signaling cells that reach distant target cells via extracellular fluids, such as blood. Additionally, extracellular vesicles (EVs) also mediate intercellular signaling by delivering a range of bioactive molecules (e.g. proteins, lipids, nucleic acids) through paracrine, autocrine, and endocrine mechanisms. This figure is inspired by a previous work [3]. Created in BioRender. S. Jin, 2026, `https://BioRender.com/trsm0kl`.

Autocrine signaling is a specialized form of paracrine signaling in which cells secrete signaling molecules that act on themselves or on other cells of the same type (Fig. 1). In a population of genetically identical cells, the collective signal intensity produced by the group inherently exceeds that of a single cell, thereby enhancing coordinated population-level behavior. During early embryonic development, autocrine signaling plays a critical role in promoting cellular proliferation and differentiation, as well as maintaining a supportive pregnancy-associated microenvironment. In contrast, dysregulated autocrine signaling is strongly associated with the uncontrolled proliferation of cancer cells. Excessive autocrine production of growth factors can drive pathological hyperproliferation, thereby contributing to oncogenesis.

Contact-dependent signaling, also known as juxtacrine signaling, occurs through direct physical contact between the signaling and target cells [52] (Fig. 1). This form of communication can be classified into three major types. The first involves membrane-bound signaling molecules on the surface of the signaling cell that specifically bind to receptors on the plasma membrane of an adjacent target cell. The second type entails the direct delivery of ligands from the signaling cell into the intracellular space of the tar-

get cell, where they subsequently interact with receptors. The third mechanism involves the formation of intercellular channels that facilitate the direct transfer of small signaling molecules between the cytoplasms of neighboring cells. Well-characterized examples include gap junctions in animal cells and plasmodesmata in plant cells. Notably, large macromolecules such as proteins and nucleic acids are generally unable to pass through these channels.

The signal transduction mechanisms described above primarily involve short-range communication. However, long-distance CCC is essential for coordinating organism-wide biological activities, as illustrated in Fig. 1. The human nervous system represents the most sophisticated form of long-distance signaling, comprising vast networks of neurons that communicate via specialized synaptic structures. Synaptic signaling enables rapid, long-distance, yet contact-dependent communication between neurons [98]. Although the cell bodies of communicating neurons may be distant, their elongated axons and dendrites transport signals directly to specialized sites of cell-cell contact. This architecture ensures efficient, spatiotemporally precise information transfer regardless of the distances between the cell bodies of contacting cells. A synapse is a highly specialized structure for information transfer, comprising a presynaptic membrane, a synaptic cleft, and a postsynaptic membrane (Fig. 1). Upon arrival of an electrical impulse at the synaptic terminal, neurotransmitters are released from synaptic vesicles into the cleft. These diffusing neurotransmitters then bind to receptors on the postsynaptic membrane, triggering a new electrical signal in the target cell. This process mediates a critical bidirectional conversion between electrical and chemical signaling.

Another major form of long-distance signaling is endocrine signaling (Fig. 1). In this process, endocrine cells secrete hormones that are transported through the bloodstream to distant target cells to convey regulatory information. Unlike synaptic signaling, which transmits biological information within milliseconds via direct neuron-to-neuron communication, endocrine signaling depends on circulatory distribution, resulting in significantly slower transmission speeds [45]. The signaling molecules involved in this pathway – collectively known as hormones – are typically characterized by low water solubility, a property that facilitates their long-distance transport in the blood. In contrast, water-soluble signaling molecules are generally involved in paracrine signaling. Despite its slower onset, endocrine signaling exerts more prolonged effects on target cells. Even a small quantity of hormone molecules can elicit sustained regulatory responses, playing a vital role in the modulation of diverse physiological processes.

Notably, beyond classical ligand-receptor interactions, extracellular vesicle (EV)-mediated CCC has garnered significant interest for its potential applications as novel disease biomarkers and drug delivery systems. EVs are membrane-bound vesicles released by cells via plasma membrane budding and fission or multivesicular body formation, encapsulating diverse bioactive molecules such as proteins, lipids, and nucleic acids. Once released into the extracellular space, EVs interact with the extracellular and pericellular matrix, facilitating their transport to recipient cells through autocrine, paracrine, or endocrine routes [169]. Upon reaching a target cell, EVs exert their influence through three

primary mechanisms: direct binding to cell-surface receptors, fusion with plasma membrane to deliver cargo, or internalization into the cytoplasm [66]. Unlike conventional signaling, EV-mediated communication enables the coordinated delivery of multiple signaling molecules, potentially eliciting more complex, pleiotropic responses in recipient cells [115]. This multifaceted interaction repertoire introduces an additional layer of complexity to CCC networks.

2.2 Signal transduction in cell-cell communication

Upon ligand binding, a receptor transduces information from the signaling cell by initiating an intracellular signaling cascade that ultimately regulates gene expression in the target cell. This regulatory process, known as signal transduction, operates through distinct mechanisms depending on the receptor type. For signaling molecules that bind cell-surface receptors, the receptor typically orchestrates a cytoplasmic cascade of relay proteins. This cascade modulates target gene expression by activating specific transcription factors (TFs) within the nucleus (Fig. 2(a)). In contrast, intracellular receptors – often TFs themselves – are located in the cytoplasm or nucleus and are activated by hydrophobic ligands that are able to diffuse across the plasma membrane (Figs. 2(b) and 2(c)). Following ligand binding, the ligand-receptor complex directly interacts with specific DNA regulatory sequences to activate or repress transcription.

The signaling cascade involves intracellular signal propagation. Rather than proceeding as a simple linear chain, intracellular signaling operates as a complex regulatory network composed of various signaling molecules. These molecules can be broadly classified into second messengers and signaling proteins. Second messengers are small intracellular molecules that mediate rapid and broad signal dissemination. In contrast, signaling proteins are large macromolecules primarily responsible for highly specific signal transduction events. Upon receptor activation, second messengers transmit signals by rapidly altering their intracellular concentrations, thereby modulating the activity of downstream effectors. Signaling proteins, due to their large molecular size, undergo minimal concentration changes but exert their effects by activating downstream signaling components or modulating second messenger levels [131].

Furthermore, during intracellular signal transduction, the addition or removal of phosphate groups plays a critical role in regulating most signaling processes. As a result, signaling proteins often function as molecular switches. Two major classes of these switches are proteins modified through phosphorylation of specific amino acid residues by upstream kinases, and G proteins, which function by cycling between an active GTP-bound state and an inactive GDP-bound state. In addition, specific interactions among signaling proteins further facilitate the transmission and modulation of intracellular signals. Ultimately, the signaling cascade regulates the expression of target genes via activated TFs, thereby inducing a range of biological responses, including cell proliferation, differentiation, and metabolic reprogramming [132].

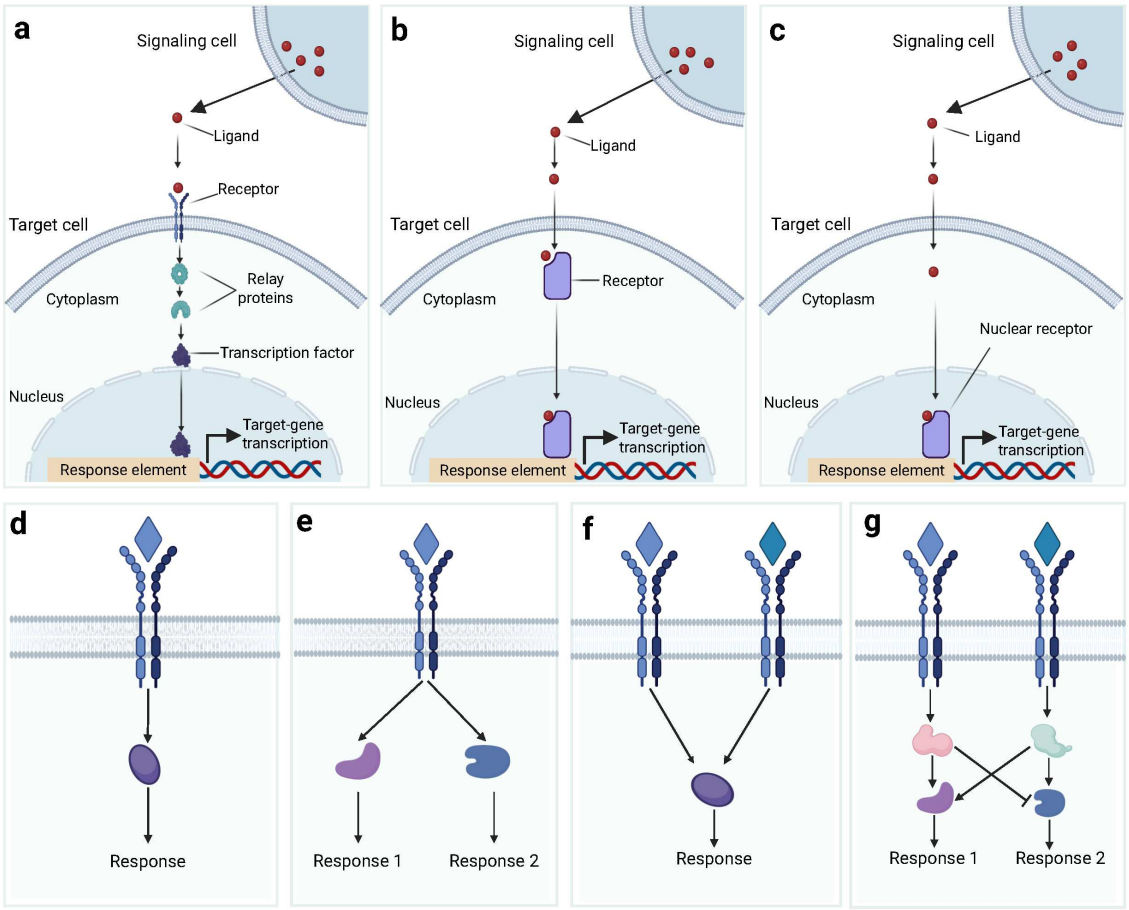

Figure 2: Cellular signal transduction mediated by different receptor types and major modes of intracellular signaling crosstalk. (a) Cell-surface receptor signaling: Ligand binding activates a cascade of cytoplasmic relay proteins and transcription factors, resulting in the nuclear translocation of transcription factors to regulate target gene expression. (b) Cytoplasmic receptor signaling: The ligand diffuses across the plasma membrane and binds its receptor in the cytoplasm. The resulting ligand-receptor complex then translocates to the nucleus, where it binds specific DNA regulatory sequences to direct transcriptional regulation. (c) Nuclear receptor signaling: The ligand diffuses across the plasma membrane and into the nucleus, where it binds a nuclear receptor. The ligand-receptor complex then directly modulates gene transcription. (d) Single-pathway activation: A lone intracellular signaling pathway is initiated by ligand-receptor binding. (e) Multiple-pathway activation: A single ligand-receptor pair triggers several distinct signaling pathways. (f) Signal convergence: Multiple, distinct ligand-receptor pairs activate a common downstream signaling pathway. (g) Pathway crosstalk: Interdependent signaling pathways, triggered by different ligand-receptor pairs, mutually influence one another. This figure is inspired by a previous work [16] and Pearson Education, Inc. Created in BioRender. S. Jin, 2026, `https://BioRender.com/xa3ut41`.

2.3 Signal crosstalk in cell-cell communication

Signaling pathways do not operate in isolation but function within an integrated network of protein–protein interactions. The signaling mode in which intracellular pathways influence one another is referred to as signaling crosstalk, which helps to integrate

signals from multiple inputs in different ways, giving rise to the vast range of cellular responses. Signaling crosstalk typically manifests in several distinct patterns [74, 123, 150] (Figs. 2(d)-2(g)). The first pattern is single signal response, wherein ligand-receptor binding activates only one intracellular signaling pathway (Fig. 2(d)). The second pattern is signal bifurcation, in which a single ligand-receptor pair simultaneously activates multiple signaling pathways (Fig. 2(e)). For instance, fibroblast growth factors (FGFs) can activate the RTK pathway, the STAT pathway, and an additional pathway involving lipid metabolism remodeling and increased intracellular calcium levels [64]. The third pattern is convergent signaling, where distinct ligand-receptor pairs converge to activate a shared intracellular pathway, such as during T lymphocyte differentiation [64] (Fig. 2(f)). The fourth pattern is modulatory crosstalk, where separate pathways initiated by different ligand-receptor pairs interact such that one pathway modulates – either enhances or inhibits – the transmission of another (Fig. 2(g)). For example, Hh signaling can potentiate Wnt pathway activity, while Wnt signaling, in turn, modulates Hh effectors – a dynamic interplay essential in tissue regeneration and cancer progression [135].

3 Modeling strategies for cell-cell communication

Based on the biological principles of cellular signal transduction, computational strategies have been developed to systematically investigate and characterize CCC from single-cell and spatial omics data. These computational approaches typically require at least two types of input data, as illustrated in Fig. 3(a). The first is the gene expression profile obtained from single-cell transcriptomics or spatial transcriptomics, and the second is prior knowledge of LRIs curated from experimental literature and public databases. Gene expression levels serve as indirect proxies for protein abundance, while the prior knowledge is utilized to identify and extract genes encoding interacting proteins from the expression data, thereby narrowing the analysis to protein activities that are potentially involved in mediating CCC.

To infer the CCC between cells, a commonly adopted assumption is that the likelihood of communication between a sender and a receiver cell is positively correlated with the expression levels of the ligand and its receptor in the corresponding cell. A scoring function is thus constructed to quantify the communication probability or strength mediated by each ligand-receptor pair between different cells or cell types (Fig. 3(a)). To enhance the robustness of the inference and reduce the impact of background noise, statistical methods such as permutation testing are subsequently employed to identify statistically significant CCC.

Moreover, sophisticated computational methods can be developed by considering diverse biological mechanisms and experimental designs. First, many ligand-receptor interactions operate in a multi-subunit architecture, and therefore their heteromeric complexes should be accurately represented. Second, cofactors, including soluble agonists and antagonists, and co-stimulatory and co-inhibitory membrane-bound receptors, in-

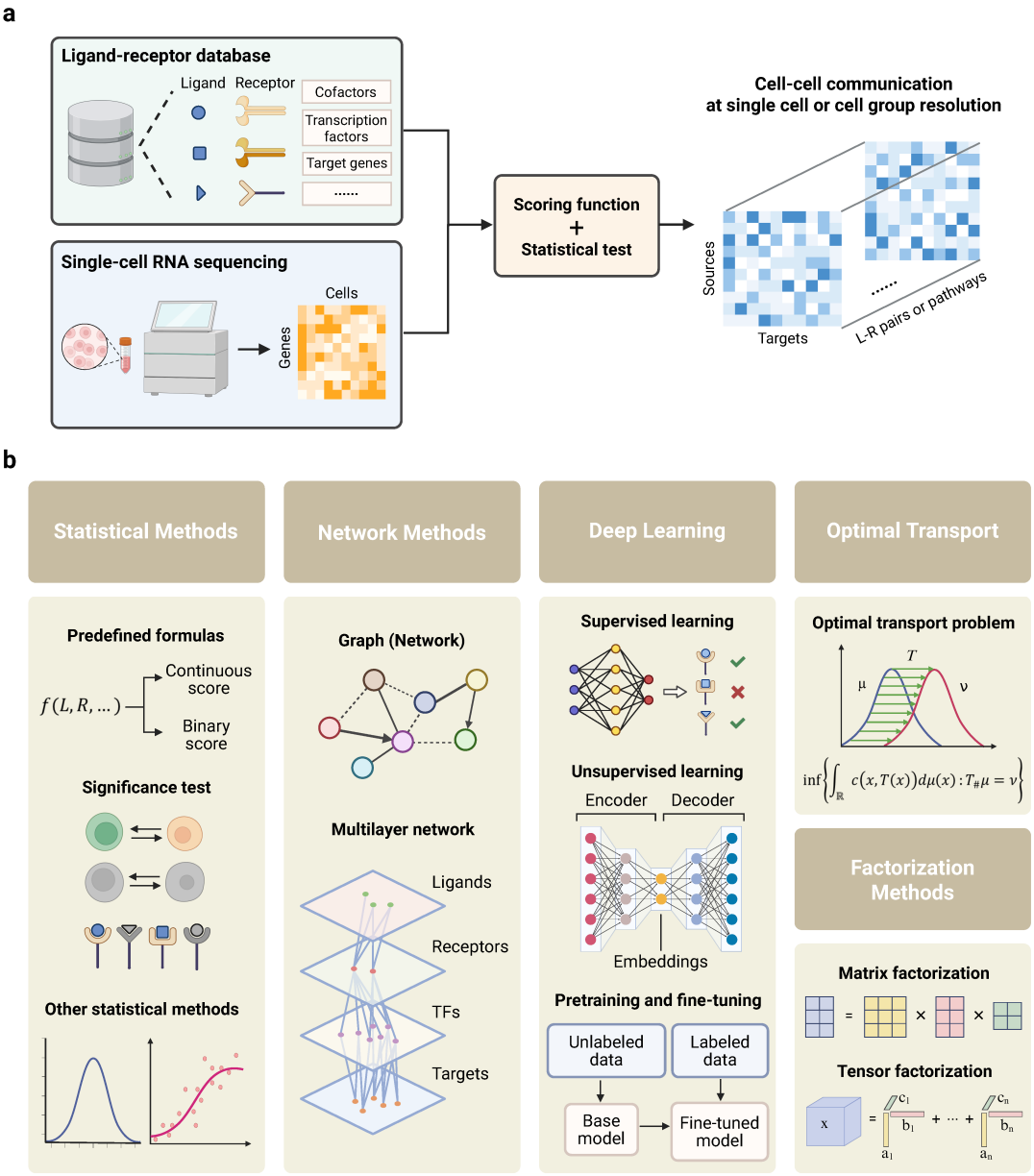

Figure 3: General strategies and computational methodologies for inferring CCC from gene expression data. (a) A typical workflow for CCC inference. Gene expression profiles from single-cell or spatial transcriptomics serve as proxies for protein abundance. These data are integrated with prior knowledge, including genes encoding interacting ligands and receptors, co-factors, transcription factors and target genes, to infer CCC. A scoring function quantifies the likelihood or strength of potential CCC events, followed by statistical testing to assess significance. The final output predicts CCC at single-cell or cell-group resolution. (b) Common computational methodologies. The five primary strategies for CCC inference include statistical methods, network methods, deep learning, optimal transport, and factorization methods. Created in BioRender. S. Jin, 2026, `https://BioRender.com/llyvm5g`.

troduce additional effects on the core interaction between ligands and receptors, which can be incorporated into CCC modeling. Indeed, many signaling pathways, such as BMP, WNT and TGF-β, are prominently modulated by their cofactors, both positively and negatively [73]. Third, downstream signaling response is often a key indicator of whether a cell has genuinely received a signal, greatly helping to mitigate false positives and establish causal relationships in signal transduction. Fourth, since secreted signaling and contact-dependent signaling operate over different spatial ranges, spatial distance should also be taken into account. Finally, comparative analysis across complex experimental designs – including multiple biological replicates and conditions – is essential. For example, analyzing temporal data can reveal the dynamic evolution of CCC, while cross-condition comparisons enable the systematic identification of altered signaling mechanisms and potential therapeutic targets.

4 Computational methods for inferring cell-cell communication

The advent of single-cell and spatially resolved omics technologies, especially transcriptomics, has driven an abundance of computational tools for modeling CCC (Fig. 4). These tools exhibit growing methodological diversity and vary in the biological features they are designed to investigate (Fig. 5). This diversity reflects ongoing efforts and innovations by researchers to explore CCC mechanisms from multiple perspectives and to develop analytical approaches tailored to specific biological questions. Existing methods for CCC analysis can be broadly classified into five categories based on their core inference strategies (Fig. 3(b)): (1) statistical methods, (2) network methods, (3) deep learning methods, (4) optimal transport methods, and (5) factorization methods. For each category, we first outline the core modeling principles and then evaluate how current tools address key biological questions. This evaluation is structured around five analytical aspects: spatial constraints, cellular resolution (e.g. single-cell level or spot-level resolution), intracellular signaling, temporal dynamics, and cross-condition comparison.

4.1 Statistical methods

Statistical methods can be broadly classified into two major strategies (Fig. 3(b)). The first and most prevalent strategy employs customized scoring functions to quantify the interaction strength between cell pairs, followed by statistical hypothesis testing or thresholding to evaluate significance. The second strategy utilizes more sophisticated statistical models to characterize CCC patterns.

Tools implementing the first strategy primarily diverge in their design of CCC scores and statistical tests. Some apply explicit rules to constrain signaling gene expression, producing binary interaction scores [37, 62, 176]. More commonly, tools generate continuous scores by calculating the mean, product, or correlation of ligand and receptor expression [26, 51, 78, 81, 91, 99, 111, 113, 133, 136, 154, 168, 188, 208]. The significance of these scores is typically assessed against a null distribution by randomly permuting cell labels.

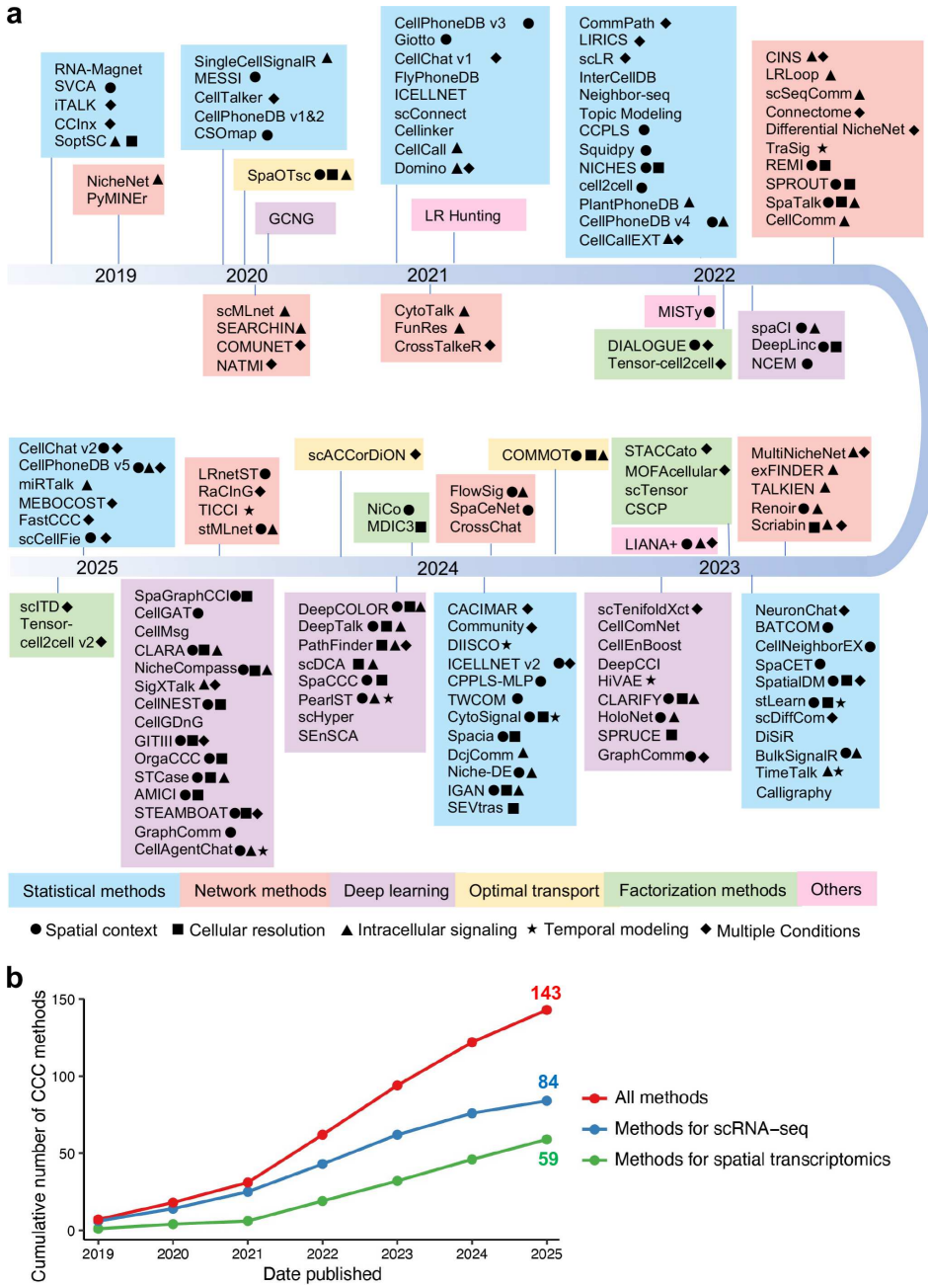

Figure 4: Timeline and growth of CCC inference methods. (a) The timeline of 143 CCC inference methods organized by publication or preprint year. Tools based on different methodological principles are distinguished using colored boxes, including statistical methods, network methods, deep learning methods, optimal transport methods, and factorization methods. Symbols denote the specific biological questions addressed. Methods without any symbols are general tools for inferring CCC between cell groups from scRNA-seq data. (b) Cumulative growth of CCC methods. The plot shows the cumulative number of CCC tools developed for scRNA-seq and spatial transcriptomics data over time. The number of methods published up to October 2025 is indicated.

Specific scoring functions reflect distinct biological considerations. CellPhoneDB [51], for instance, defines a communication score as the mean of the average ligand expression in one cluster and the average receptor expression in another. To account for protein complexes, it uses the minimum average expression among subunits of ligands or receptors. Other tools incorporate more refined biological models. Given the additional modulation of co-factors (e.g. agonists, antagonists, co-receptors) on the core interaction between ligands and receptors, CellChat [87] estimates the level of multimeric ligands and receptors by the geometric mean of their subunits, and designs its scoring function based on the law of mass action to integrate all known molecular interactions. Specifically, the communication probability $P_{i,j}$ from cell groups i to j for a ligand-receptor pair k is modeled as

$$\begin{aligned} P_{i,j}^k &= \frac{L_i R_j}{K_h + L_i R_j} \times \left(1 + \frac{AG_i}{K_h + AG_i}\right) \times \left(1 + \frac{AG_j}{K_h + AG_j}\right) \\ &\quad \times \frac{K_h}{K_h + AN_i} \times \frac{K_h}{K_h + AN_j} \times \frac{n_i n_j}{n^2}, \\ L_i &= \sqrt[m_1]{L_{i,1} \dots L_{i,m_1}}, \\ R_j &= \sqrt[m_2]{R_{j,1} \dots R_{j,m_2}} \times \frac{1 + RA_j}{1 + RI_j}. \end{aligned} \tag{4.1}$$

Here, L_i represents the expression level of ligand L in cell group i, and $L_{i,1}, \dots, L_{i,m_1}$ denote the expression levels of the m_1 subunits of ligand L in cell group i. Receptor R and its m_2 subunits in cell group j are defined analogously. RA_j and RI_j represent the average expression levels of co-stimulatory and co-inhibitory receptors in cell group j, respectively, while AG and AN denote the average expression levels of soluble agonists and antagonists. n is the total number of cells in the dataset, while n_i and n_j denote the numbers of cells in cell groups i and j, respectively. K_h denotes the parameter in the Hill function.

The second strategy employs more advanced statistical methods to model CCC [11, 78, 101, 137, 165, 172, 185]. Several tools within this category estimate communication strength through formal statistical frameworks. For instance, CCPLS [165] applies partial least squares (PLS) regression, interpreting regression coefficients as the influence of neighboring cell types – mediated by CCC – on highly variable gene expression. FastCCC [78] offers a highly scalable, permutation-free approach that uses fast Fourier transformation (FFT)-based convolution for rapid p-value calculation and a modular scoring system. Other tools utilize statistical methods primarily for the interpretation of inferred interactions. For example, after evaluating the significance of LRIs using null distributions of Spearman correlation coefficients between ligands and receptors, as well as between receptors and their downstream target genes, BulkSignalR [172] associates LRIs with specific cell types by constructing a LASSO regression model. Building upon communication score computation, cell2cell [11] employs a genetic algorithm to prioritize ligand-receptor pairs that best correlate with physical distance. Additionally, MESSI [101] identifies both intracellular and intercellular signaling genes using a Mixture of Experts (MoE) model

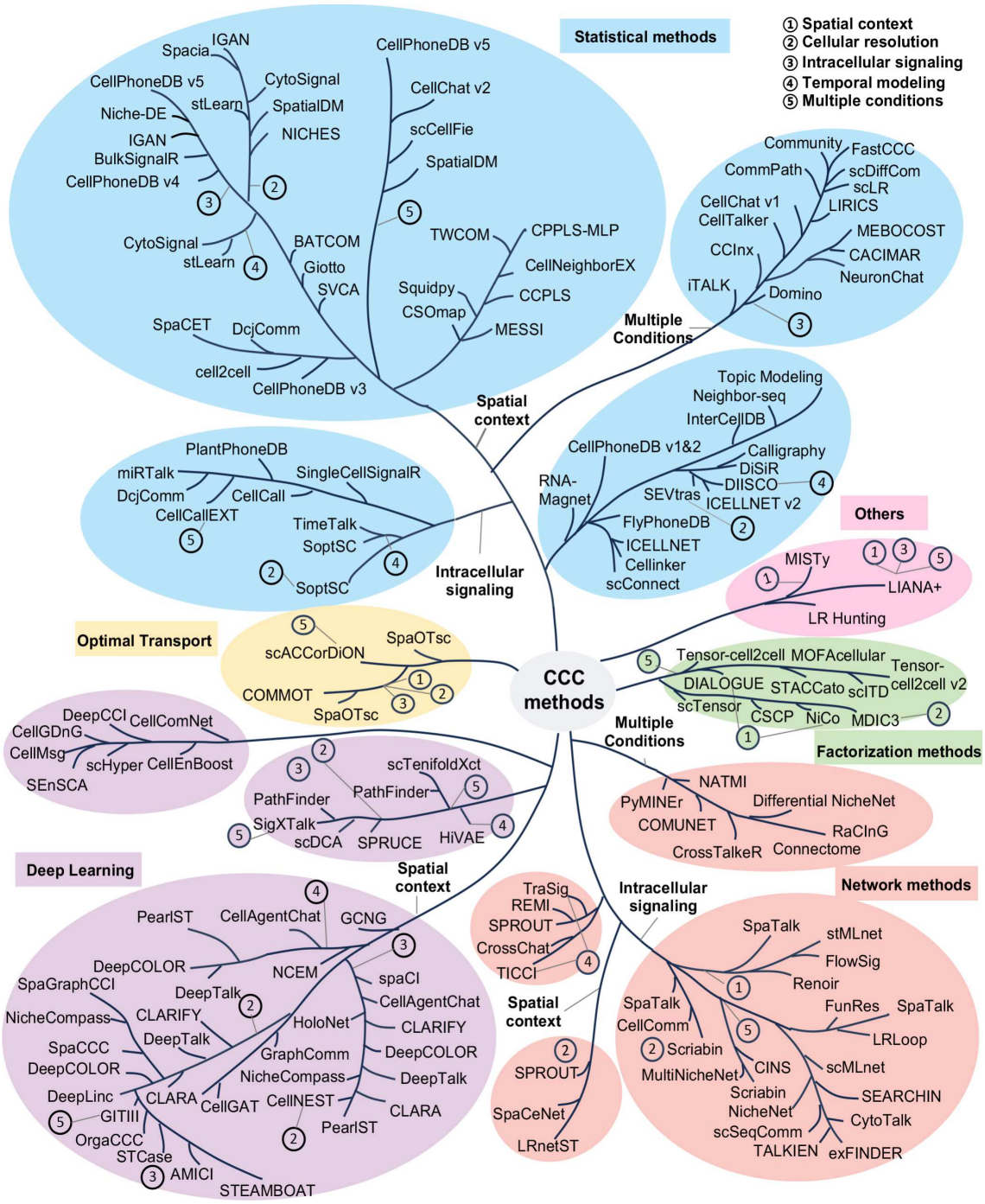

Figure 5: Methodological phylogeny of CCC tools. The evolutionary tree classifies 143 computational methods by their core computational strategies (main branches, colored) and the specific biological questions they address (sub-branches). This visualization, inspired by a previous review [9], illustrates the evolutionary relationships and functional diversity of CCC tools.

with multi-task learning. Topic Modeling [137] infers genes affected by intercellular interactions through latent Dirichlet allocation (LDA). Collectively, these tools highlight the diversity of statistical approaches used in CCC analysis.

Spatial omics provides an unprecedented opportunity to systematically investigate CCC on native tissues. Incorporating spatial context into CCC analysis allows the calculation of communication scores to be constrained within a short distance range, aligning with the intuitive assumption that cells in close proximity are more likely to interact. Giotto [50] identifies significantly interacting cell type pairs by randomly permuting cell type labels within a predefined spatial neighborhood network. Communication scores are then calculated only for cell pairs deemed spatially proximal and likely to interact based on cell type. CellNeighborEX [94] leverages spatial transcriptomics data to identify neighbor-dependent gene expression by comparing transcriptomes in heterotypic versus homotypic cellular contexts, providing an unbiased approach to uncover genes regulated by direct cell contact and the immediate microenvironment, thereby revealing a layer of intercellular communication that complements and extends beyond canonical ligand-receptor analysis. SpaCET [148] deconvolves immune and stromal cell lineage fractions in tumors by integrating cancer-specific genomic patterns and hierarchical regression, and it infers CCC by requiring both spatial colocalization and significant ligand-receptor co-expression within spots, providing a spatially grounded framework to analyze interactions in the tumor microenvironment. Niche-DE [122] is a regression-based statistical framework that identifies cell-type-specific genes whose expression is modulated by the local cellular context, and reveals LRIs that drive niche-differential expression patterns from spatial transcriptomics data.

Most tools compute communication scores using gene expression profiles aggregated at the cell group level. Although this strategy helps mitigate the sparsity of scRNA-seq data, it fails to fully exploit the advantages of single-cell resolution. Consequently, it cannot capture cell-specific interactions within cell groups and may introduce bias due to predefined cell clusters. To address these limitations, several recent methods have been developed to infer interactions directly between individual cell (or spot) pairs [110, 147, 211]. stLearn [142] integrates spatial information to compute communication scores in both "within-spot" and "between-spot" modes, while also accounting for cell type diversity to facilitate analyses across diverse biological contexts. Spacia [211] directly models CCC at single-cell resolution using a multi-instance learning framework and explicitly captures complex many-to-one relationships between sender and receiver cells. Conceptually different from other CCC inference methods, SpatialDM [107] explicitly incorporates spatial distance through a spatial weight matrix. It identifies significant ligand-receptor pairs and their local interaction hotspots using a bivariate Moran's I statistic, bypassing the need for permutation tests via an analytical null distribution. This makes SpatialDM both statistically rigorous and highly scalable to datasets comprising millions of cells. CytoSignal [110] predicts signaling locations and activity at single-cell resolution by computing a weighted average of ligand expression across a cell's neighborhood and then multiplying by the receptor expression in the target cell.

Based on the established importance of analyzing CCC, understanding the subsequent intracellular signaling cascades and transcriptional regulation is crucial for deciphering the complete cellular response mechanism. To bridge this gap, computational methods increasingly leverage curated pathway databases, such as KEGG [93] and Reactome [40], to connect LRIs to potential downstream genes through hypothesis testing or correlation analysis [27, 61, 177, 190]. Among these tools, SingleCellSignalR [27] infers CCC from scRNA-seq data using a curated ligand-receptor database and a regularized score that provides a stable threshold to control false positives, facilitating the identification of high-confidence interactions for downstream validation. A more integrated approach is exemplified by CellPhoneDB v5 [164]. It extends beyond LRI identification with its CellSign module [61], which is designed to connect interactions to intracellular signaling and transcriptional outcomes. This module utilizes curated knowledge of receptor-TF relationships and downstream TF activity data to model signal propagation. DcjComm [48] is a versatile computational tool that employs an NMF-based joint learning model to simultaneously identify functional gene modules, perform dimension reduction, and cluster cells, followed by a statistical model to infer CCC by integrating ligand-receptor pairs with downstream TF-target gene activities. Other tools incorporate the expression of downstream genes directly into the calculation of communication scores to provide a more holistic inference of CCC, such as SoptSC [178], CellCall [201], BulkSignalR [172] and IGAN [210]. The recent method BulkSignalR [172] addresses the challenge of inferring CCC from bulk and spatial multi-cellular data by statistically integrating LRIs with downstream pathway activity, providing a robust and functionally contextualized approach to decipher cellular networks from these prevalent data types. IGAN [210] leverages spatial transcriptomics to construct intercellular gene association networks between adjacent cells by performing a non-parametric statistical model. It further elucidates the full upstream and downstream pathway context and thereby reveals communication heterogeneity and pathway-level mechanisms.

Incorporating temporal dynamics into CCC analysis enables a more nuanced understanding of cell state transitions and extends the analytical scope of computational tools, which is particularly valuable in contexts such as embryonic development, cancer progression, and other temporally evolving biological processes. stLearn [142] introduces a framework that integrates gene expression with spatial information to reconstruct spatiotemporal trajectories, thereby uncovering spatial branching events that occur over time within tissues. Beyond trajectory-focused tools [142, 178], TimeTalk [177] combines trajectory inference with causal testing to identify regulatory relationships between ligand-receptor pairs and temporally relevant TFs during early embryogenesis. DIISCO [184], on the other hand, utilizes Gaussian process regression models to characterize dynamic changes in cell type proportions and to predict time-resolved intercellular communication patterns.

Advances in experimental technologies have increased the availability of multi-condition datasets, spurring demand for computational methods that compare CCC across biological contexts. Such comparative analysis is crucial for identifying altered signaling

mechanisms that drive cell fate decisions and phenotypic transitions. Common strategies for this task involve coupling differential CCC network analysis with differential gene expression profiling [34, 37, 206, 208], or applying statistical tests (e.g. Fisher's exact test, permutation tests) to communication scores [99, 111, 156, 176]. Beyond these approaches, CellChat [87, 88] employs network topology analysis, using centrality measures to identify key signaling sources, targets, mediators, and influencers within a network, and further groups pathways based on functional and topological similarity. Several tools are specifically designed for differential CCC analysis. scDiffCom [99] provides a statistically rigorous framework to identify dysregulated LRIs and cell-type pairs between conditions (e.g. aging or disease), powering the scAgeCom atlas of age-related communication changes in mouse tissues. Community [156] uniquely integrates cell type abundance, active fraction, and expression level to pinpoint altered interactions and disentangle compensatory mechanisms in case-control studies. CellCallEXT [60] extends its precursor CellCall [201] by incorporating transcription factor activity alterations between conditions to infer disease-associated changes in both intercellular communication and intracellular signaling. To address scalability in large-scale studies, FastCCC [78] introduces a permutation-free framework for analytical p-value computation. Its key innovation is a reference-based paradigm that leverages a large human atlas, providing a healthy baseline to robustly identify dysregulated communications in disease. CACIMAR [85] proposes a conservation score to identify CCC events that are conserved across different species. For spatial transcriptomics, SpatialDM [107] uses generalized linear models to test if experimental conditions significantly influence interaction density across multiple samples.

4.2 Network methods

Network methods exploit the connectivity structure of CCC by representing cells (or cell types) and genes as nodes and depicting interactions as directed edges, with edge weights corresponding to interaction strengths (Fig. 3(b)). These methods elucidate patterns of intercellular communication by analyzing the topological and statistical properties of nodes and edges using graph theory, network analysis, and probabilistic graphical models.

The properties of graphs and networks enable intuitive and precise modeling of gene regulatory processes, which is particularly beneficial for tools that integrate downstream signaling and represent genes as nodes. Some tools [18, 92, 118, 152] focus on analyzing intracellular signaling while other tools build comprehensive signaling networks that integrate both intercellular and intracellular communication [24, 33, 35, 70, 126, 149, 191]. These tools infer directed regulatory networks from ligands to downstream genes by integrating prior biological knowledge with gene expression data to identify specifically expressed genes or significant signaling pathways, thereby enabling network pruning. For example, to predict ligand-target links between interacting cell types, NicheNet [24, 149] employs the Personalized PageRank (PPR) algorithm to compute a regulatory potential

score for all ligand-target gene pairs from prior knowledge sources. This approach prioritizes ligands and their potential target genes by using the ligand of interest as the seed node and calculating a signaling importance score for each gene within the ligand-signaling network. The core approach PPR is formulated as

$$v = (1-d) \times u + d \times W \times v. \tag{4.2}$$

Here, v denotes the vector of importance scores for all nodes in the network, derived from the steady-state distribution of the random walker; u represents the personal preference for each node, with a value of 1 assigned to the ligand of interest and 0 to all other genes; W is the normalized adjacency matrix of the weighted ligand-signaling network. By applying PPR to each ligand and performing a cutoff, the $n \times m$ ligand-gene signaling importance score matrix is obtained. This matrix is then multiplied by the $m \times m$ weighted adjacency matrix of the gene regulatory network, yielding a $n \times m$ ligand-target regulatory potential score matrix L, where n is the number of ligands considered and m is the number of possible target genes. Let l_{ij} denote the regulatory potential score of ligand i for target gene j, which can be computed as follows:

$$l_{ij} = \sum_{k=1}^{m} (PPR_{ik} \times GRN_{kj}) \tag{4.3}$$

with PPR_{ik} the importance of gene k in the signaling of ligand i and GRN_{kj} the edge weight from gene k to target gene j.

To account for spatial constraints of CCC, methods have been developed to emphasize CCC analysis within proximal cells or spots [5, 36, 118, 146, 151, 152, 175, 180, 191]. For example, SpaTalk [152] scores the ligand-receptor-target signaling network between spatially proximal cells by constructing a k-nearest neighbor (KNN) cell graph network and a prior ligand-receptor-TF knowledge graph and estimating the probability of downstream TF activation via a random walk algorithm. Different from SVCA [14] that models the expression of genes independently of each other, SpaCeNet [151] employs a Gaussian graphical model to decompose the observed cellular profiles into contributions arising from cellular variability and cellular interactions, which captures complex multivariate relationships between genes, and disentangles the intracellular from intercellular gene correlations using spatial conditional independence. Renoir [146] maps spatially resolved ligand-target activities and identifies communication niches by integrating spatial transcriptomics with scRNA-seq data, using a comprehensive neighborhood scoring system that accounts for cellular composition, receptor expression, and spatial proximity to reveal context-specific signaling microenvironments. Building on the earlier method scMLnet [33], which constructs a multilayer signaling network comprising three subnetworks including ligand-receptor, receptor-TF and TF-target gene, stMLnet [191] models the spatial-temporal distribution of ligand concentration $u(x,y,z)$ during the diffusion process using a partial differential equation (PDE), and then computes the signaling strength of ligand-receptor pairs between specific cell pairs. The PDE used to model the

ligand diffusion process is formulated as follows:

$$\frac{\partial u(x,y,z)}{\partial t} = D\Delta u(x,y,z), \quad (x,y,z) \in \mathbb{R}^3 \setminus B_1, \tag{4.4}$$

where Δ is the Laplace operator, D is the diffusion coefficient, B_1 represents a unit ball indicating the sender cell. Incorporating the law of mass action, the signaling strength LRS_j^k of the ligand-receptor pair k at the receiver cell j is defined as follows:

$$LRS_j^k = \sum_{i=1}^{n} \left(\frac{1}{d_{ij}} L_i^k R_j^k \right). \tag{4.5}$$

Here L_i^k and R_j^k are the corresponding ligand expression in the sender cell i and receptor expression in the receiver cell j, respectively. d_{ij} represents the relative distance between the cell i and the cell j. Subsequently, the nonlinear regulatory relationship between ligand-receptor signaling activity and target gene expression is modeled using random forest regression. Suppose the inferred multilayer network contains m target genes, and each target gene TG_t is linked to n_t ligand-receptor pairs. Accordingly, m random forest regression models are constructed – one for each of the m target genes – as follows:

$$TG_t = f_t\left(LRS^{1_t}, LRS^{2_t}, \ldots, LRS^{n_t}\right), \quad t = 1,2,\ldots,m. \tag{4.6}$$

The signaling activities $LRS^{1_t}, LRS^{2_t}, \ldots, LRS^{n_t}$ across the receiver cells are used as input to random forest regression model f_t to predict the expression of TG_t.

Unlike methods that rely on prior pathway annotations, CytoTalk [79] performs de novo prediction of complete signal transduction pathways mediated by LRIs between two cell types from single-cell transcriptomics. It constructs two intracellular networks based on the mutual information of gene pairs – one for the sender and one for the receiver cells – and connects them via known LRIs. An optimal signaling network that considers genes with high cell type specificity and close connection to high-scoring ligand-receptor pairs is then identified by solving a network propagation-based prize-collecting Steiner forest problem. In addition, LRLoop [189] detects feedback signaling loops formed by pairs of LRIs through a network propagation algorithm. Collectively, these methods highlight the increasing diversity and sophistication of modeling strategies tailored to specific biological questions.

Leveraging network-based frameworks, TraSig [102] and TICCI [58] address intercellular interactions among dynamically transitioning cells during differentiation and development. TraSig first employs a probabilistic graphical model to infer the pseudotemporal ordering of cells, and subsequently applies a sliding window approach to reconstruct gene expression profiles along each trajectory edge, thereby enabling the inference of LRIs between cell clusters that overlap in pseudotime. In contrast, TICCI integrates intercellular association probabilities and communication probabilities to construct a class k-nearest neighbor graph with weighted edges, which is then used to assess cellular differentiation states and identify branching trajectories.

For datasets derived from different conditions, network topological metrics can be applied to compare differences between CCC networks [77, 129, 157, 170]. CINS [197] is a multi-step framework that uniquely leverages cell-type proportion changes across conditions in scRNA-seq data to first infer a Bayesian network of differential cell-type interactions and then uses a constrained regression model to identify the key ligand-receptor pairs mechanistically responsible for these interactions, providing a powerful tool for hypothesis generation in case-control studies. Among these, COMUNET [157] introduces a dissimilarity metric that accounts not only for the presence or absence of edges but also their weights and directions when comparing edge sets between two layers representing ligand-receptor pairs in a multilayer CCC network. When multiple samples are available, Scriabin [182] is a computational framework that performs comparative CCC analysis at true single-cell resolution by constructing cell-cell interaction matrices, employing a binning strategy for large-scale comparisons, and identifying co-expressed interaction programs that are inferred using the weighted gene correlation network analysis (WGCNA) framework. Scriabin powerfully reveals heterogeneous and rare communication events obscured by agglomerative methods. MultiNicheNet [23] prioritizes differentially expressed and active ligand-receptor pairs across multi-condition and multi-sample datasets by aggregating multiple criteria – such as differential expression, cell type specificity, and sample-level expression – into a final weighted score.

4.3 Deep learning methods

Deep learning has demonstrated remarkable effectiveness across a wide range of applications in the field of single-cell and spatial omics, with recent increasing efforts of developing computational methods to characterize complex nonlinear relationships in CCC (Fig. 3(b)). These methods excel at learning latent representations of cells and genes, potentially uncovering CCC patterns overlooked by traditional approaches. Particularly notable is the growing application of graph neural networks (GNNs), which naturally model structural relationships and dependencies in graphs representing cellular or gene interactions.

Spatial transcriptomics enables construction of cell-cell spatial graphs that provide contextual information for training neural networks [19, 56, 103, 144, 155, 163, 187, 196, 200, 212]. For instance, CellGAT [200] employs graph attention networks to integrate scRNA-seq data with protein interaction knowledge, predicting context-specific LRIs with multi-omics evidence. In contrast, DeepCOLOR [97] uses a variational autoencoder to recover cell-cell colocalization networks at single-cell resolution by integrating single-cell and spatial transcriptomes.

Training neural networks to predict gene expression in receptor cells enables the inference of CCC and the understanding of how LRIs affect downstream targets. HoloNet [103] and scDCA [84] identify CCC events influencing transcriptional responses through multi-view networks that predict target gene expression and interpret the relative contribution of each view via attention scores. In HoloNet, each view corresponds to a CCC

network defined by a specific ligand-receptor pair, whereas in scDCA, each view represents the interaction network between a specific pair of cell types. HoloNet represents the first deep learning method to incorporate detailed signaling mechanisms – including molecular diffusion, cofactors, and LRI-specific interaction ranges – building upon traditional approaches like CellChat. CellAgentChat [145] predicts gene expression profiles from ligand-receptor gene expression and infers regulatory relationships through receptor perturbation analysis. Other tools focus on reconstructing intracellular gene regulatory networks [17,19,54,163,174]. For example, spaCI [163] maps genes into a latent space using adaptive graph models with attention mechanisms, and constrains interacting gene pairs to be positioned closer together, thereby facilitating the prediction of LRIs and their upstream regulators from the learned representations. CLARIFY [17] reconstructs intracellular interaction networks using pre-inferred gene regulatory networks and provides downstream regulatory information for the inference of intercellular communication networks. CLARA [173] is a spatially-aware, transformer-inspired method that infers CCC at the resolution of individual cell pairs by modeling contextual relationships between ligands and receptors within local cellular neighborhoods, providing a highly granular view of the cellular interactome. By integrating gene expression profiles with spatial locations, CCC networks can be directly reconstructed without prior knowledge of ligand-receptor pairs or restriction to immediate neighborhoods [17, 55, 105, 193, 199]. For example, DeepLinc [105] can reveal potential long-range interactions by using variational graph autoencoder with adversarial learning. NicheCompass [19] interprets cellular interactions through decoder weights, and GITIII [187] characterizes the influence of neighboring cells on the central cell using a single-layer graph transformer model.

To investigate the temporal dynamics of CCC, HiVAE [112] and CellAgentChat [145] incorporate pseudotime trajectories inferred by existing tools to characterize cellular interactions. HiVAE quantifies information flow between different cell types by computing transfer entropy along the pseudotemporal ordering of cells from scRNA-seq data. CellAgentChat calculates communication scores only between cells within the same pseudotime bin or in adjacent bins, thereby reflecting the temporal proximity of interacting cells. It further trains a neural network to predict gene expression levels of each cell at the next time point, conditioned on the effects of cellular interactions.

Currently, relatively few deep learning methods have been developed to identify differential CCC across biological conditions. Among existing approaches for scRNA-seq data, scTenifoldXct [194] formulates CCC inference as a manifold alignment problem. It uses neural networks to learn low-dimensional representations of gene expression from two cell types – optionally incorporating known ligand-receptor pairs – and projects a coupled gene-pair similarity matrix from two samples into a unified latent space. Differential LRIs are then identified by computing Euclidean distances between these gene-pair representations. In a different strategy, PathFinder [54] employs a graph transformer, taking gene expression data and predefined gene-gene interaction paths as input, and is trained to predict the condition label of individual cells. The resulting path weights indicate the relative importance of each interaction in distinguishing between conditions.

Finally, we focus on the technical implementation of deep learning methods, highlighting architectural designs and training strategies. Current approaches employ diverse frameworks – including unsupervised and supervised learning strategies – implemented through variational autoencoders (VAEs) [96], graph neural networks (GNNs), and transformers. When applied to spatial transcriptomics, these architectures capture nonlinear dependencies, spatial organization, and interaction ranges inherent to CCC. In unsupervised learning-based tools, most methods employ autoencoders – particularly variational autoencoders (VAEs) [96] – to learn feature representations that capture potential CCC patterns [17, 19, 55, 97, 105, 112, 144, 161, 174, 199]. These models are typically optimized to minimize the discrepancy between the input and reconstructed data, and use the learned latent representations and reconstructed data to infer CCC. For instance, OrgaCCC [55] leverages cellular gene expression profiles, cell-cell spatial graph, and known LRIs-derived gene-gene graph to reconstruct both the intercellular interaction network and the LRI network through two orthogonal graph autoencoders operating at the cell and gene levels, respectively. SPRUCE [161] captures interaction topics for each cell-cell pair and identifies ligand and receptor genes enriched within each topic by employing an embedded topic model built upon a variational autoencoder framework, which achieves unbiased identification of interpretable cell states across multiple datasets by characterizing CCC patterns. Beyond autoencoders, scHyper [106] is a hypergraph neural network-based method that models CCC as a global, high-order network, leveraging the discrepancy between nodes' intrinsic and contextual embeddings to reconstruct non-linear interaction scores and provide a systems-level view of cellular crosstalk. CellNEST [212] adopts graph attention networks (GATs) along with contrastive learning to integrate ligand–receptor information with spatial position at single cell or spot resolution, where unsupervised training is performed by maximizing the Jensen-Shannon divergence between the original network and its corrupted counterpart. Finally, attention scores are used to quantify the probability of CCC, which is computed as follows:

$$\alpha_{i,j} = \mathrm{Tanh}\left(a^{\mathsf{T}}[W_v h_i + W_v h_j + W_e e_{i,j}]\right). \tag{4.7}$$

Here, h_i, h_j are vertex feature vectors for vertices i and j of the input graph, where a vertex corresponds to a cell or spot; $e_{i,j}$ represents the edge feature vector from j to i; W_v is a learnable weight matrix, while W_e is the equivalent matrix for edge features; the attention a is a learnable parameter.

In addition to CellNEST, attention mechanisms have been effectively leveraged by several recent methods such as GITIII [187], AMICI [75] and Steamboat [108]. GITIII [187] infers spatially-resolved CCC at single-cell resolution from imaging-based spatial transcriptomics data by leveraging a single-layer graph transformer model. It models how a cell's transcriptional state is shaped by the cell type compositions and expression profiles of its spatial neighbors, providing a powerful solution for datasets with sparse ligand-receptor coverage. Building upon this concept, AMICI [75] utilizes a sparsely regularized, multi-headed attention module to adaptively identify interactions across multiple spatial scales, delineate spatially dependent cellular sub-populations, and con-

nect these interactions to downstream functional consequences in receiver cells. Furthermore, Steamboat [108] applies a multi-head attention model to dissect cellular interactions across scales by decomposing a cell's gene expression into distinct components: intrinsic programs, local communication from neighbors, and long-range interactions. A distinctive feature of Steamboat is its capacity for in silico spatial perturbation, enabling the prediction of cellular responses to dynamic microenvironmental changes.

A fundamental challenge in applying supervised learning to CCC inference is the scarcity of gold-standard datasets with experimentally validated interactions, making reliable label definition difficult. To address this, several methods [138–140, 155, 186, 196, 200, 209] curate positive labels from experimentally documented LRIs, treating all other pairs as negatives. The trained models are subsequently used to infer previously unannotated LRIs. For example, CellMsg [186] leverages graph convolutional networks on multimodal protein features to de novo predict a high-confidence ligand-receptor interactome, which it then integrates with scRNA-seq data via a three-point estimation method to quantify communication strength, offering an enhanced, feature-driven approach to decipher CCC networks. Furthermore, SEnSCA [209] constructs negative samples of LRIs by replacing the common practice of randomly selecting negative samples from unlabeled ligand-receptor pairs with K-means clustering, thereby reducing label noise and sample distribution bias that could weaken model predictive performance. Other tools adopt alternative strategies, such as thresholding communication scores or aggregating outputs from established statistical methods, to predefine interaction labels as ground truth [106, 163, 192]. Another strategy shifts the prediction target from interactions themselves to downstream effects. In this approach, some models are trained to predict gene expression [56, 103, 145] or cell type classifications [54, 84], with signaling activity subsequently assessed post-hoc through analysis of model weights or sensitivity analyses.

Recent advances have produced sophisticated hybrid models that integrate multiple deep learning architectures to address the complexity of CCC. Unlike single-architecture approaches, these hybrid frameworks offer modular and flexible designs that can be tailored to specific biological questions. For instance, DeepTalk [193] employs a hybrid training strategy combining self-supervised pretraining with supervised fine-tuning to infer CCC at single-cell resolution. The model first learns global connectivity patterns through masked node prediction on large-scale cell graphs, then fine-tunes on specific datasets for binary edge classification, significantly enhancing generalization capability. GraphComm [155] represents another hybrid approach, constructing directed graphs from scRNA-seq data and applying GATs to integrate protein complex and pathway information. The method leverages a comprehensive knowledge base of over 30,000 protein interaction pairs to capture detailed cellular localization and intracellular signaling patterns, substantially improving CCC inference accuracy. The integration of large language models (LLMs) represents a particularly promising direction. SpaCCC [83] fine-tunes pretrained LLMs on gene expression prediction tasks, enabling the embedding of ligands and receptors into a shared latent space. This unified embedding facilitates the identification of potential LRIs and subsequent CCC inference.

4.4 Optimal transport methods

Methods based on optimal transport conceptualize CCC as a resource allocation problem (Fig. 3(b)). These methods typically infer the most probable communication paths between cells by minimizing the transportation cost of signaling molecules across spatial locations. SpaOTsc [28] is a pioneering approach that formulates two sequential optimal transport problems to decode spatial cellular organization and communication. The method first aligns scRNA-seq data (source distribution) with spatial transcriptomics data (target distribution) by computing an optimal transport plan that minimizes the cost based on gene expression dissimilarity. The resulting optimal transport distance quantifies spatial proximity between cell pairs. Subsequently, SpaOTsc infers CCC networks by solving the second optimal transport problem where sender and receiver cells are modeled as source and target distributions, with intercellular distances defining the transport cost. The optimal transport plan directly yields the likelihood of communication for each cell pair, integrating both spatial constraints and expression levels of ligands, receptors, and downstream genes. In addition, SpaOTsc trains a random forest model to predict downstream gene expression, thereby estimating the spatial range over which specific signaling pathways operate. To account for the competition between different ligand and receptor species as well as spatial distances between cells, COMMOT [29] develops a collective optimal transport method to handle complex molecular interactions and spatial constraints. Mathematically, the collective optimal transport problem is formulated as follows:

$$\begin{aligned}
&\min_{P\in T}\sum_{(i,j)\in I}\langle P_{i,j,\cdot,\cdot},C_{(i,j)}\rangle_F+\sum_i F(\mu_i)+\sum_j F(\nu_j),\\
&T=\left\{P\in\mathbb{R}_+^{n_l\times n_r\times n_s\times n_s}:P_{i,j,\cdot,\cdot}=0 \text{ for } (i,j)\notin I,\sum_{j,l}P_{i,j,k,l}\le X^L_{i,k},\sum_{i,k}P_{i,j,k,l}\le X^R_{j,l}\right\},\\
&\mu_i(k)=X^L_{i,k}-\sum_{j,l}P_{i,j,k,l},\quad \nu_j(l)=X^R_{j,l}-\sum_{i,k}P_{i,j,k,l},
\end{aligned}\tag{4.8}$$

where $X^L_{i,k}$ and $X^R_{j,l}$ represent the expression level of ligand i on spot k and the expression level of receptor j on spot l. F penalizes the untransported mass μ_i and ν_j. $P_{i,j,k,l}$ is the coupling matrix scoring the signaling strength from spot k to spot l through the pair consisting of the ligand i and receptor j, while I denotes the index set of bindable ligand-receptor pairs. $C_{(i,j)}$ is the cost matrix based on the thresholded distance matrix, designed to impose spatial distance constraints. The collective optimal transport problem determines a collection of optimal transport plans for all pairs of ligand and receptor species that can be coupled simultaneously, thereby enabling the consideration of competition among different species.

Apart from modeling CCC via optimal transport, ScACCorDION [130] focuses on characterizing changes in intercellular communication across multiple biological conditions. It represents the communication network between cell types within each sample as a directed weighted graph, and applies optimal transport to compute the Wasserstein

distance between these graphs. Additionally, ScACCorDION identifies communication events that differ between sample groups by estimating the barycenters of a collection of CCC networks.

4.5 Factorization methods

These methods infer CCC and their latent patterns using matrix or tensor factorization, capable of simultaneously analyzing multiple factors influencing CCC (Fig. 3(b)). Among matrix factorization-based tools [1, 114, 198], CSCP [198] aims to reveal communication patterns between cell types at a fine-grained level. It identifies pairs of cell subgroups with strong and similar intercellular crosstalk signals by solving a coupled non-negative matrix factorization problem, which minimizes differences in ligand or receptor expression between cells while maximizing the activity scores of crosstalk signals mediated by ligand–receptor pairs. By integrating scRNA-seq data with spatial transcriptomics data, NiCo [1] captures intra-cell type variability through a non-negative matrix factorization framework based on the concept of niche composition, leading to the identification of latent factors for each cell type. These latent factors are then used to infer relevant signaling mediators and cell type pairs with covarying factors, thereby enabling the construction of intercellular interaction networks between cell types.

Methods like DIALOGUE [82], MOFAcellular [57] and scITD [125] uncover latent multicellular programs from single-cell gene expression data by applying penalized matrix decomposition [183], multi-omics factor analysis (MOFA) [6,7] and Tucker tensor decomposition [167], respectively. Potential signaling is then identified based on the ligands and receptors enriched in the multicellular programs. Notably, MOFAcellular is a flexible multi-view integration framework, which enables the inclusion of additional tissue-level descriptions in the model, such as cell type compositions, spatial relationships, and CCC scores.

By organizing the inferred CCC between cell types into a tensor, several methods, including scTensor [166], Tensor-cell2cell [10] and STACCato [43], have been proposed to uncover CCC patterns as well as their unique combinations of cell types and ligand-receptor pairs by using non-negative Tucker decomposition [95], CANDECOMP/PARAFAC (CP) decomposition [30,68] and Tucker decomposition, respectively. Tensor-cell2cell is the pioneering method for uncovering context-driven CCC patterns by constructing a 4D-communication tensor. Mathematically, Tensor-cell2cell constructs a $C \times P \times S \times T$ fourth-order tensor χ, where C, P, S, T correspond to the number of contexts, ligand-receptor pairs, sender cells and receiver cells respectively. Tensor factorization is then performed by iteratively optimizing the following objective function until convergence:

$$\min_{c,p,s,t} \left\| \chi - \sum_{r=1}^{R} c^r \otimes p^r \otimes s^r \otimes t^r \right\|_F^2, \tag{4.9}$$

where $\otimes$ denotes the outer product and c^r, p^r, s^r and t^r are vectors of the factor r, each containing the loadings of the respective elements along a specific dimension of the ten-

sor, enabling the further identification and interpretation of context-dependent communication patterns. Following this idea, STACCato identifies condition-related CCC events while also explicitly adjusting for sample-level confounding variables (e.g. batch, age and gender). More recently, Tensor-cell2cell v2 [12] performs integrative analysis of protein- and metabo-litemediated CCC by using coupled tensor component analysis, thereby facilitating the joint interpretation of CCC patterns across multiple modalities.

5 Intuitive visualization and systems analysis of cell-cell communication

While most computational tools focus primarily on inferring CCC, several methods provide sophisticated visualization and systems-level analysis capabilities to help interpret complex communication networks (Fig. 6).

For network visualization, tools employ diverse graphical representations: CellChat [87, 88] utilizes circle plots, hierarchical diagrams, chord diagrams, heatmaps, bubble plots, and word clouds to effectively illustrate intercellular communication patterns and highlight signaling variations across cell types (Fig. 6(a)). Similarly, CellCall [201] employs alluvial plots to trace cascading relationships from ligands through receptors to downstream target genes, while NicheNet [24,149] provides heatmaps displaying ligand-target regulatory potential and predicted ligand activity (Fig. 6(b)). In spatial contexts, CellChat visualizes communication links directly on tissue architecture, and COMMOT [29] maps signaling activity to individual cellular locations (Fig. 6(c)).

Systems-level analysis applies quantitative approaches to decode complexity within CCC networks and identify emergent properties. CellChat employs network centrality metrics to identify key signaling sources, targets, mediators, and influencers within communication networks, revealing critical components of cellular microenvironments (Fig. 6(d)). Pattern recognition through matrix factorization enables prediction of primary incoming/outgoing signals for specific cell types and uncovers coordinated responses across diverse populations. Furthermore, CellChat clusters signaling pathways by defining similarity measures and applying manifold learning from both functional and topological perspectives, facilitating identification of signaling groups with shared architectures and biological interpretation of less-characterized pathways.

Spatial analysis reveals distinctive organizational patterns of CCC (Fig. 6(e)). COMMOT interpolates communication patterns into vector fields to visualize spatial directionality of signal transmission and reception. CytoSignal [110] introduces signaling velocity by leveraging RNA velocity of ligands and receptors, enabling identification and visualization of dynamic signal intensity changes across tissue space. Meanwhile, MintFlow [2] and GITIII [187] generate microenvironment-induced embeddings that enable fine-grained cell type separation based on communication-driven cellular states.

Comparative analysis across biological conditions detects complex CCC changes under varying contexts. CellChat identifies altered signaling pathways and ligand-receptor

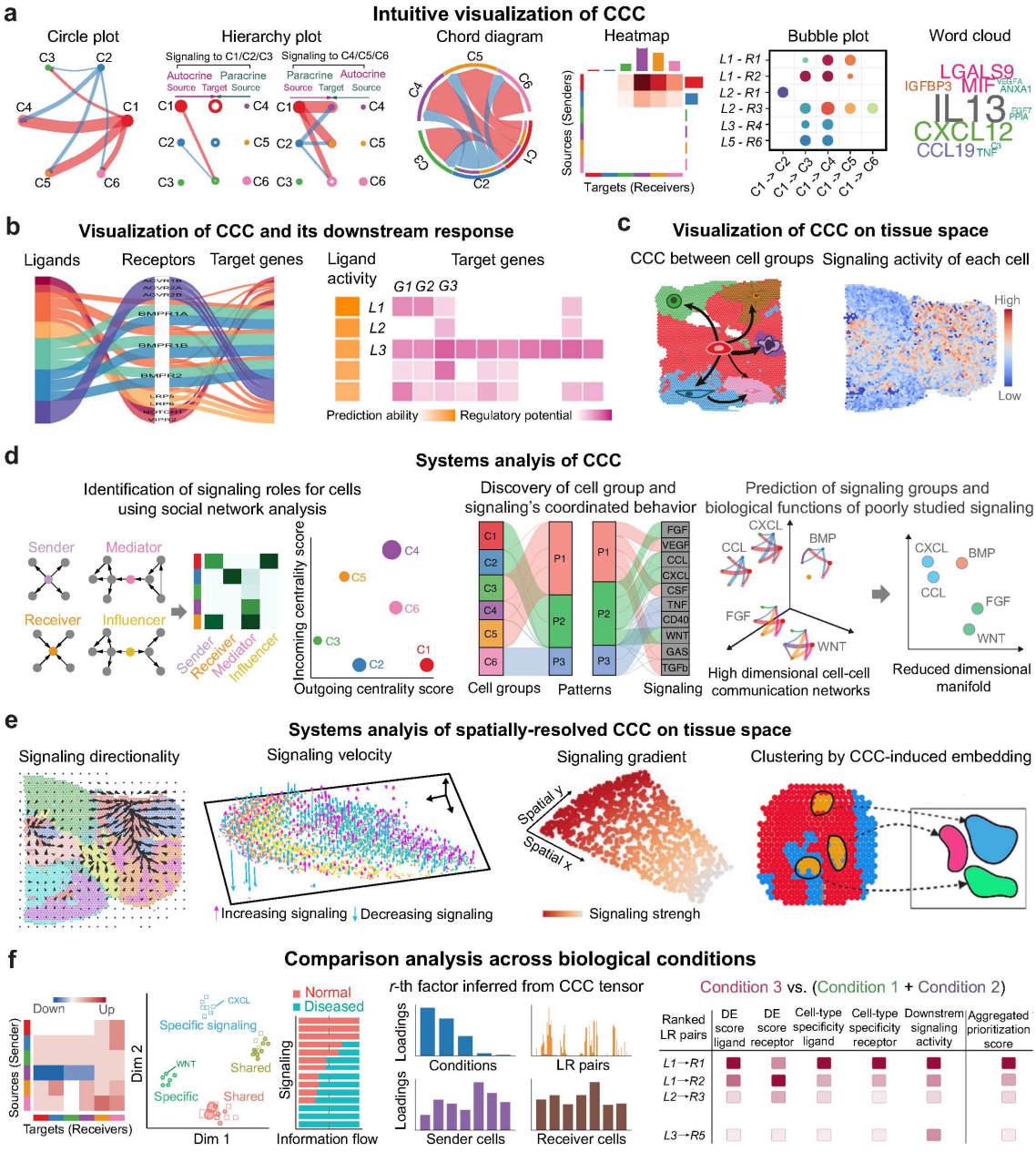

Figure 6: Visualization and analysis of CCC. (a) Common intuitive visualizations of inferred CCC networks. (b) The alluvial plot showing ligand-receptor-target gene relationships. (c) Spatial communication patterns depicting signaling directionality and strength on spatial tissues. (d) Examples of analysis techniques of CCC for scRNA-seq data, including identification of key signaling roles using network centrality analysis, pattern recognition to uncover coordinated cellular behaviors, and manifold learning to group functionally similar signaling pathways. (e) Examples of analysis techniques of CCC for spatial transcriptomics data, including inference of signaling directionality, mapping of signaling velocity and spatial gradients, and identification of microenvironment-induced cell states. (f) Comparative CCC analysis across biological conditions, highlighting differential communication patterns and context-specific interactions.

pairs by analyzing differences in network architecture, information flow, and through joint manifold learning of intrinsic CCC structures (Fig. 6(f)). Beyond the pairwise comparison, Tensor-cell2cell [10] employs tensor decomposition to uncover context-driven communication patterns with unique combinations of cell types and ligand-receptor pairs, visualizing context loadings, ligand-receptor pair loadings, and sender/receiver cell loadings to facilitate pattern interpretation across diverse conditions (Fig. 6(f)). STACCato [43] identifies condition-related CCC while adjusting for sample-level variables (e.g. batch, age, gender) using supervised tensor regression. MultiNicheNet [23] addresses differential CCC analysis in complex multifactorial designs, accommodating inter-sample heterogeneity while correcting for batch effects and covariates through ranking-based prioritization integrated into an aggregated score that highlights significantly altered interactions for downstream interpretation (Fig. 6(f)).

6 Challenges and opportunities

We have systematically surveyed over 143 computational tools for CCC analysis, categorizing them according to their underlying computational principles. While the field has diversified and advanced considerably in recent years, significant challenges remain alongside emerging opportunities.

6.1 Resources, validation and benchmarking

The development of CCC analysis tools relies on diverse prior knowledge resources and methodological frameworks. Systematic comparisons reveal that choices of resources and methods substantially impact inference results, thereby influencing downstream biological interpretations [46].

Accurate recapitulation of molecular interactions is essential for biologically meaningful CCC prediction. However, ligand-receptor databases vary considerably in content – including multisubunit complex annotation, functional classifications (e.g. secreted signaling or contact-dependent signaling, molecular function and subcellular location), inclusion of intracellular signaling events, and the total number of curated interactions. Detailed comparisons of existing databases are available in a previous review [31]. In addition to the protein-mediated ligand-receptor databases, resources are emerging for metabolite–protein interactions (e.g. MACC [59], MetalinksDB [53], MRCLinkdb [202]) and EV-mediated interactions (e.g. EV-COMM [32], miRTalkDB [154]). Since most tools rely on their own or referenced databases – and results are highly sensitive to database quality – researchers may draw different conclusions using different methods. A unified, well-annotated, high-quality interaction repository is urgently needed to standardize and enhance the reliability of CCC inference.

A major challenge remains the scarcity of experimental ground truth for evaluating computational predictions (Fig. 7(a)). Recent experimental advances – including barcode-based (BRICseq [80], RABID-seq [38]) and droplet-based (ProximID [21], PIC-seq [63])

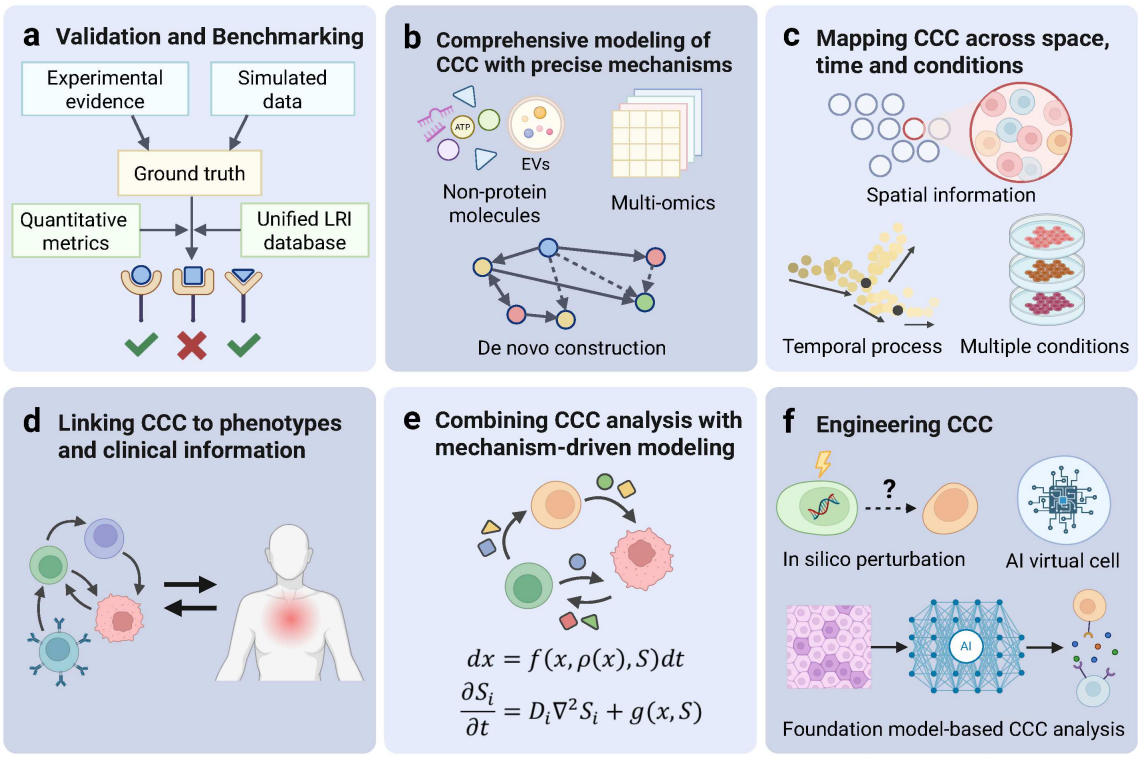

Figure 7: Challenges and opportunities in CCC analysis. (a) Validation and benchmarking challenges. Inferred CCC results are influenced by the choice of prior knowledge resources. However, the absence of experimental ground truth and standardized prior knowledge resources hinders systematic tool evaluation. (b) Modeling CCC with precise mechanisms and other modalities. Most CCC studies focus on protein-mediated ligand-receptor interactions, overlooking non-protein signaling and complex regulatory mechanisms. Advances in multi-omics technologies and de novo network reconstruction now enable more comprehensive CCC inference and the discovery of previously uncharacterized CCC. (c) Spatiotemporal and cross-condition analysis. Integrating spatial and temporal context, along with comparative studies across conditions, can reveal deeper biological insights – yet these approaches face considerable technical and analytical hurdles. (d) Clinical and phenotypic integration. Linking phenotypic and clinical data to CCC helps identify key CCC in disease progression, supporting the development of precision medicine. (e) Mechanistic-informed modeling. Further refinement to incorporate communication more explicitly will not only improve the accuracy of the learned cellular dynamics but also offer new insights into temporal CCC dynamics and cellular responses to altered cellular communication.Mathematical models of signaling mechanisms allow researchers to probe regulatory dynamics under experimentally inaccessible conditions. (f) Engineering and intervention. Emerging computational approaches – including in silico perturbation, foundation models, and virtual cells – open new avenues for predictive and engineering-based CCC research, though model interpretability remains a critical challenge. Created in BioRender. S. Jin, 2026, `https://BioRender.com/y1iwqii`.

technologies – now enable tracing of sender-receiver contacts at single-cell resolution, thereby facilitating validation of predicted CCC. For instance, PIC-seq identifies context-specific cell interactions and differentially regulated genes, while SPEAC-seq [181] captures soluble ligands to link sender-cell perturbations with receiver-cell responses. Although these methods improve measurement throughput and accuracy, most remain lim-

ited to direct cellular contacts and cannot simultaneously profile multiple ligand-receptor interactions or cell pairs. Further innovation in experimental techniques and curation of ground-truth datasets are essential for systematic validation of computational predictions.

Computational benchmarking using simulated data with known ground truth has been attempted in several studies [29,104,162,191]. However, accurately simulating CCC remains challenging due to the complexity and redundancy of signaling in multicellular systems. Systematic tool assessment is further complicated by the diversity of underlying databases, biological assumptions, data compatibility, and parameter settings. Although recent benchmarking efforts have established more systematic evaluation frameworks – particularly for scRNA-seq-based methods [116, 119] – development of objective and standardized quantitative metrics remains crucial. Accessible and unified platforms such as LIANA+ [47] are emerging to address this need. With over 50 computational methods now available for spatial transcriptomics data, systematic benchmarking of these spatially aware tools is urgently required to guide future methodological development.

6.2 Comprehensive modeling of CCC with precise mechanisms

While most CCC inference methods focus on protein-mediated LRIs, non-protein signaling mechanisms, including those mediated by small molecules such as metabolites and neurotransmitters, also play vital roles in biological systems and require systematic investigation (Fig. 7(b)). For instance, profiling enzymes and transporters involved in non-protein signaling enables inference of metabolite-mediated CCC [208] or neuron – neuron communication [81,206] from scRNA-seq data. A central challenge in metabolite-mediated CCC analysis lies in accurately estimating metabolic activity. Recently, a scalable tool called scCellFie [8] has been developed to infer metabolic activity per single cell or spatial spot from transcriptomic data. Non-protein signaling molecules exhibit greater diversity and more complex regulatory mechanisms than proteins, necessitating careful consideration in modeling.

Beyond metabolite signaling, EVs are increasingly recognized as key mediators of CCC in both prokaryotic and eukaryotic organisms, with considerable clinical potential as biomarkers and drug delivery vehicles [169]. Recent years have seen the emergence of databases, technologies, and computational tools to elucidate EV-mediated communication dynamics. For example, EV-COMM [32] compiles literature-curated EV-mediated interactions across cells and species, linking EV cargo to downstream pathways and functional outcomes. Similarly, miRTalkDB [154] catalogs experimentally supported EV-derived miRNAs and their target interactions in humans, mice, and rats, providing a foundation for inferring EV-miRNA-mediated CCC. Functional RNAs within EVs – such as mRNAs and miRNAs – can modulate recipient cell behavior and are critical to intercellular signaling. However, standard scRNA-seq protocols are optimized for cellular RNAs and often fail to capture EV RNAs effectively [124]. Bridging this technical gap requires specialized experimental and computational workflows that account for the low

abundance, heterogeneity, and fragmented nature of EV transcripts. Recent advances enable characterization of EV transcriptomic features, estimation of cellular secretion activity, and related analyses [71, 120, 207]. For instance, SEVtras [71] identifies droplets containing small extracellular vesicles and infers a vesicle signal score to estimate single-cell secretion activity from scRNA-seq data. More recently, miRTalk [154] has emerged as the first method to systematically infer EV-derived miRNA-mediated CCC by integrating module scores for EV biogenesis, secretion signatures, RNA-induced silencing complex–related genes, and miRNA-target expression. Despite these advances, the mechanisms and applications of EV-mediated CCC remain incompletely understood. Further investigation – particularly into vesicle heterogeneity in spatial contexts – will deepen insights into EV-driven communication.

Most current CCC studies rely on mRNA expression as a proxy for protein abundance, an assumption often undermined by post-transcriptional regulation and complex protein trafficking processes. The advent of single-cell and spatial multi-omics technologies – including proteomics [141, 159], epigenomics [42], and metabolomics [100, 121] – enables more comprehensive and granular characterization of CCC. Integrating single-cell ATAC-seq data, for example, can illuminate transcriptional regulation dynamics [90]. Moreover, intracellular post-translational modifications (PTMs) play central roles in signal integration and gene regulation. Emerging multimodal profiling technologies such as Phospho-seq [20] and SIGNAL-seq [134] allow measurement of PTMs, offering deep insights into cell signaling and transcriptional responses. SIGNAL-seq, in particular, enables simultaneous profiling of mRNA ligand-receptor pairs, intracellular PTMs, and transcriptional states in thousands of single cells. Expanding analytical tools to incorporate such multi-omics data will improve both inference and validation of CCC and support more effective benchmarking.

Advances in computational methods now enable more accurate modeling of complex CCC mechanisms, including signaling competition and cooperation, feedback regulation, and higher-order interactions. For example, COMMOT [29] accounts for competition among ligands and receptors; SigXTalk [76] quantifies signaling crosstalk fidelity and specificity using scRNA-seq data; and LRLoop [189] identifies intercellular feedback loops. A remaining challenge is the integration of diverse mechanisms to evaluate intercellular communication potential at a global scale. With continued research, dynamic and mechanistic models of CCC are expected to become feasible, promoting a systems-level understanding of cellular behavior.

Beyond conventional CCC inference, new computational approaches identify external signals not captured in the data, detect hierarchical communication structures, and reveal intercellular signaling flows. exFINDER [70] infers external signals and their downstream networks by leveraging prior knowledge of ligand–receptor–transcription factor pathways and scRNA-seq data, addressing signals originating outside the profiled cellular population. CrossChat [180] detects hierarchical structures in CCC through two complementary strategies: CrossChatH performs multi-resolution cell clustering to reveal global communicative hierarchies, and CrossChatT identifies local tree structures among

ligands and receptors based on inclusive or disjoint expression patterns. To identify LRIs where intracellular processes mediate signal inflow and trigger outflow of other intercellular signals, FlowSig [5] uses graphical causal modeling with conditional independence tests to infer a completed partially directed acyclic graph (CPDAG) representing intercellular flows.

6.3 De novo construction of CCC

De novo construction of cellular signaling networks represents an important frontier to infer CCC directly from single-cell and spatial omics data without reliance on prior knowledge of ligand-receptor pairs. This approach offers potential to uncover novel signaling pathways and intercellular interactions mediated by complex mechanisms, thereby providing fresh biological insights. CytoTalk [79] pioneered this paradigm by reconstructing signaling pathways from scRNA-seq data without using known pathway annotations. Its algorithm formulates this as a prize-collecting Steiner forest (PCSF) problem, identifying context-specific, parsimonious subnetworks that connect salient genes within and between cell types. The method operates on the rationale that cell-type-specific signals are encoded in highly expressed genes that are topologically proximate to active ligand-receptor pairs within a background protein-protein interaction network. While this approach reveals context-specific signaling cascades absent from canonical pathways, its utility is constrained by the completeness of the background network and computational demands for large datasets. For spatial transcriptomics with limited gene panels and inadequate ligand-receptor coverage, de novo construction offers distinct advantages. Methods including DeepLinc [105], Spacia [211], GITIII [187], AMICI [75] and Steamboat [108] infer CCC by integrating gene expression with spatial proximity without preselecting genes based on prior knowledge. However, omitting ligand-receptor information presents a fundamental challenge: establishing causal relationships for predicted interactions and distinguishing whether associated genes function as signal mediators or downstream targets.

6.4 Mapping CCC across space, time and conditions

Spatial context is crucial for accurate CCC inference (Fig. 7(c)), yet current spatial transcriptomics technologies vary substantially in cellular resolution, gene coverage, and signal-to-noise ratio, creating significant challenges for single-cell resolution analysis. Low-resolution methods like Visium [158] capture mixed cell populations within individual spots, complicating heterogeneity studies, while high-resolution in situ techniques such as Xenium and CosMx [72] offer single-cell resolution but suffer from limited transcript detection. The large scale, technical noise, and sparsity of high-resolution spatial data impose stringent requirements on CCC method design.

Temporal dynamics represent another critical dimension, enabling elucidation of communication mechanism evolution. Common approaches integrate cell lineage tra-

jectory analysis with CCC inference to identify stage-specific LRIs along developmental trajectories [102,177], while other models leverage multi-timepoint data to capture interaction dynamics [184]. However, transcriptomic data alone provides limited temporal resolution, as ligand and receptor expression may not coincide temporally with functional communication events, underscoring the need for multi-omics integration.

CCC events occur across diverse temporal scales, with different processes – ligand-receptor binding, signal transduction, transcription factor activation, and downstream responses – operating at distinct rates that reflect biological function and pathway specificity. Paracrine signaling, relying on molecular diffusion, proceeds slowly: a typical protein requires hours to diffuse 1 millimeter in aqueous environments. In contrast, endocrine signaling molecules transported via fluid flow in the circulatory system can cover the same distance in approximately 3 seconds [127]. Synaptic signaling operates even faster, with neuronal electrical impulses exceeding 100 m/s and neurotransmitter diffusion across synaptic clefts occurring within milliseconds [143]. Receptor activation is typically rapid – G protein-coupled receptors activate within milliseconds to microseconds [65]. Cellular responses also vary considerably: protein modification-based responses (cell motility, secretion, metabolism) occur within seconds to minutes, while gene expression changes requiring new protein synthesis generally demand minutes to hours [3]. Integrating dynamic equations that capture these temporal behaviors would enable more precise modeling of communication dynamics and enhance biological interpretation.

Comparative CCC analysis across conditions reveals both conserved and context-specific signaling patterns, but batch effects and technical covariates from multi-individual, multi-tissue, or multi-condition datasets can obscure genuine biological variation. Consequently, developing robust batch correction strategies, modeling inter-individual variability, and accurately identifying biologically meaningful CCC changes remain substantial challenges.

6.5 Linking CCC to phenotypes and clinical information

Disease progression involves dynamic tissue alterations and complex clinical manifestations. Integrating phenotypic and clinical data with CCC analysis can reveal interactions that play crucial roles in disease initiation and progression, offering new opportunities for phenotype manipulation, prognostic biomarker discovery, and therapeutic targeting [160]. Such integration is essential for elucidating disease mechanisms and advancing precision medicine (Fig. 7(d)). While some studies have identified phenotype-associated signaling pathways [54] or used ligand-receptor interactions for prognosis prediction [195], a key challenge lies in the heterogeneity of phenotypic and clinical data, and how to effectively integrate such data with molecular-level communication information within computational models. Furthermore, since phenotypic changes often arise from coordinated activity across multiple pathways – and vice versa – methods capable of disentangling these complex regulatory relationships are needed.

6.6 Combining CCC analysis with mechanism-driven modeling

Mathematical modeling enables exploration of biological processes across parameter ranges often inaccessible experimentally, facilitating evaluation of system dynamics under diverse conditions [44] (Fig. 7(e)). For instance, modeling revealed how IL-2's dual role in T-cell proliferation and apoptosis contributes to immune homeostasis [69]. Combining mechanistic models with CCC analysis can help interpret inferred signaling patterns, validate pathway functionality, and uncover how signaling dynamics influence system homeostasis or disease progression [128]. This approach requires accurate representation of biological mechanisms and dynamic relationships, along with appropriate model assumptions – challenges that intensify in multi-scale systems involving diverse spatial and temporal dimensions.

Traditional mathematical modeling is typically limited to few cell types and pathways. Integrating genome-wide CCC analysis with mechanistic modeling remains challenging, though recent advances in learning cellular dynamics from single-cell or spatial transcriptomics show promise [203]. Current methods for inferring RNA velocity or reconstructing vector fields from snapshot data, however, often overlook intercellular communication. Emerging approaches address this gap: one study incorporated a transformer module to capture CCC while learning cellular vector fields [86], and another developed CytoBridge, an unbalanced mean-field Schrödinger bridge method, to model cellular dynamics and interactions from time-series single-cell data [204, 205]. These examples use neural networks to learn CCC without prior ligand-receptor information. Further refinement to incorporate communication more explicitly will not only improve the accuracy of the learned cellular dynamics but also offer new insights into temporal CCC dynamics and cellular responses to altered cellular communication.

6.7 Engineering CCC

Rapid advances in single-cell omics and artificial intelligence are fostering goal-directed, intervention-based approaches in CCC research, enabling deeper mechanistic insight and improved prediction. Key emerging directions include in silico perturbation, foundation model–based analysis, and virtual cell modeling (Fig. 7(f)). In silico perturbation allows rapid assessment of genetic or chemical interventions on signaling and cell behavior, overcoming experimental limitations. For example, CellAgentChat uses an agent-based model to simulate perturbations via rule modifications, supporting novel intervention design [145]. In addition, foundation models pretrained on large-scale single-cell and spatial datasets excel at tasks like cell annotation, multi-omic integration, and perturbation prediction [41, 67], yet adapting them for CCC analysis remains difficult due to ground-truth scarcity and pathway complexity. Virtual cells – computational models simulating cellular behavior and interactions – can leverage AI to replicate molecular, cellular, and tissue dynamics across conditions [25]. However, robust evaluation frameworks and attention to interpretability are essential to ensure these methods reliably address biological questions.

7 Conclusion

Cell-cell communication encompasses diverse, finely regulated mechanisms whose investigation holds significant promise for biomedicine and precision health. Transcriptomics-based computational methods for inferring CCC have advanced substantially, evolving from generic approaches to specialized tools addressing distinct biological questions through varied methodological principles. These developments support a more integrated and biologically meaningful understanding of communication networks. Despite this progress, important challenges and emerging opportunities remain, particularly given rapid innovations in spatial omics technologies and artificial intelligence, which are poised to offer transformative perspectives for future CCC analysis.

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