



33rd CONFERENCE ON
**Intelligent Systems
for Molecular
Biology**

DATES: July 20 – 24

AND THE 24th
**European Conference
on Computational
Biology**

Computational approaches for deciphering cell-cell communication from single-cell transcriptomics and spatial transcriptomics data

Giullia Cesaro, James Nagai, Mayra Ruiz,
Barbara Di Camillo, Ivan G. Costa, Giacomo Baruzzo

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July 15th 2025



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Who we are



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PostDoc Researcher



James Nagai
PhD Student



Barbara Di Camillo
Full Professor



Mayra Ruiz
PostDoc Researcher



Giacomo Baruzzo
Assistant Professor



Ivan G. Costa
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Behind the scene



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PhD Student



Gaia Tussardi
PhD Student



Thiago Maié
PostDoc Researcher



Kai Peng
PhD Student



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Outline

TIME	CONTENT
09:00-09:30	Introduction to computational inference of intercellular and intracellular cell-cell communication Overview of cell-cell communication inference, assumptions and challenges, a priori biological knowledge, current approaches for intercellular and intracellular signaling <i>Barbara Di Camillo, Giacomo Baruzzo</i>
09:30-10:30	Hands-on analysis of Myocardial Infarction scRNA-seq Presentation of case study: human myocardial infarction, tutorial on cell-cell communication analysis from single cell transcriptomics data <i>Giulia Cesaro (Helpers: Baldan Matteo and Tussardi Gaia)</i>
10:30-10:45	Coffee Break

Outline

10:45-11:15	Introduction to comparative analysis of cell-cell communication and spatial analysis Overview of cell-cell communication comparative analysis and computational approaches, spatial transcriptomics technologies and platforms: pro and cons <i>Ivan Costa</i>
11:15-11:45	Hands on analysis of Myocardial Infarction ST and scRNA-seq (part 1) Tutorial on comparative analysis from single-cell transcriptomics and spatial transcriptomics data using myocardial infarction case study <i>James Nagai, Mayra Ruiz (Helpers: Thiago Maié and Kai Peng)</i>
11:45-12:00	Coffee Break
12:00-12:45	Hands on analysis of Myocardial Infarction ST and scRNA-seq (part 2) Tutorial on comparative analysis from single-cell transcriptomics and spatial transcriptomics data using myocardial infarction case study <i>James Nagai, Mayra Ruiz (Helpers: Thiago Maié and Kai Peng)</i>
12:45-13:00	Q&A Conclusion

Learning objectives

- Understanding and identifying the **key theoretical concepts** of cell-cell communication analysis
- Lay the **foundations** of **main computational approaches** for cell-cell communication inference
- Develop **critical thinking** skills to choose and apply the most **appropriate tools** tailored to the specific research questions and analysis contents
- Gain **hands-on experience** in applying these computational methods to **real-world data**
- Learn how to **interpret** and **evaluate** the **results** in the context of biological systems



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Introduction to computational inference of intercellular and intracellular cell-cell communication

Barbara Di Camillo & Giacomo Baruzzo
University of Padova, Italy
July 15th 2025

Multi-cells systems

Tissues and organs are **complex multi-cells systems made of multiple subpopulation of cells**

Tissues and organs are built up by **single, autonomous cells, without a full global view** of the system, and **no designated controlling cell**

Cells are **spatially and temporally organized** and able to **communicate and interact** with each other to orchestrate **self-assembly and response to stimuli as a whole**.

New technologies, new questions

- How is information processed within the single cell?
- How do cells communicate among them and with the environment?
- How do cells cooperate?
- How do tissues / organs manifest self-organization and renewal ?
- What new properties may result from collective cell behavior?

Cells processing information and exchanging information might be a central aspect to answer these questions

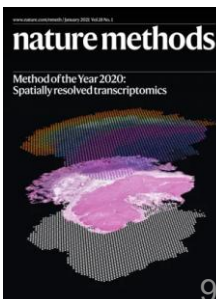
“scRNA-seq on cell development”
Breakthrough of the Year 2018
Science



“Single-cell multimodal omics”
Methods of the Year 2019
Nature Methods



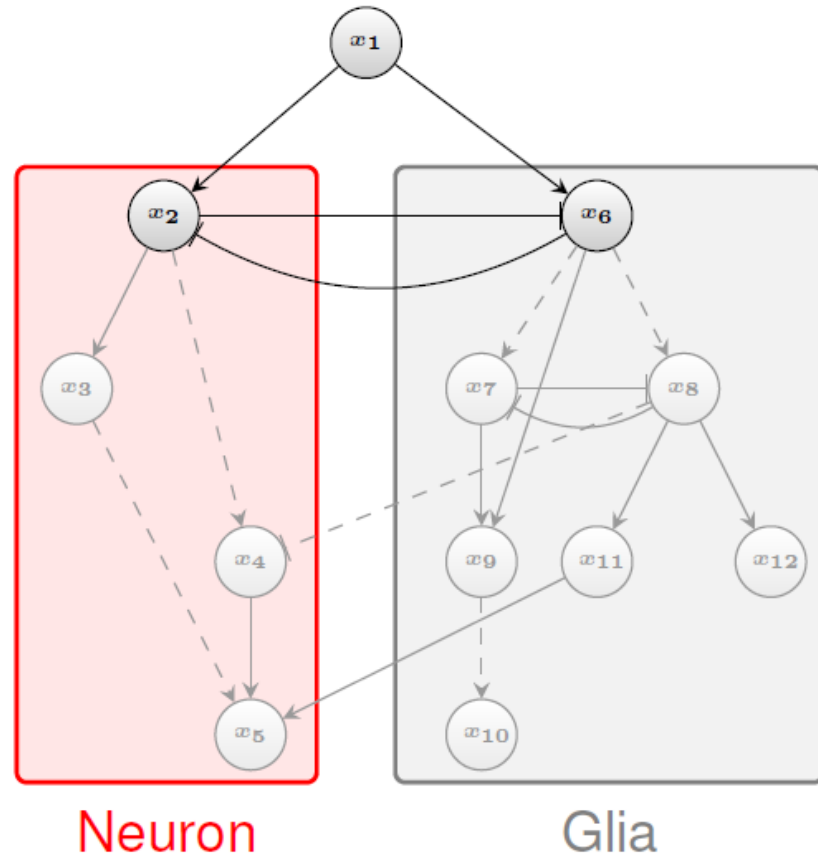
“Single-cell spatial transcriptomics”
Methods of the Year 2020
Nature Methods



*A naïve but hopefully inspiring
example:*

*Analysis of a minimal
gene regulatory network
of cell differentiation*

A two-stage differentiation process



First step switch

$$\text{Pax 6: } \dot{x}_1 = \gamma x_1(t) + \xi_1(t)$$

$$\text{Mash 1: } \dot{x}_2 = a \frac{x_1^n}{1 + x_1^n + x_6^n} - kx_2 + \xi_2(t)$$

$$\text{Hes 5: } \dot{x}_6 = a \frac{x_1^n}{1 + x_1^n + x_2^n} - kx_6 + \xi_6(t)$$

OPEN ACCESS Freely available online

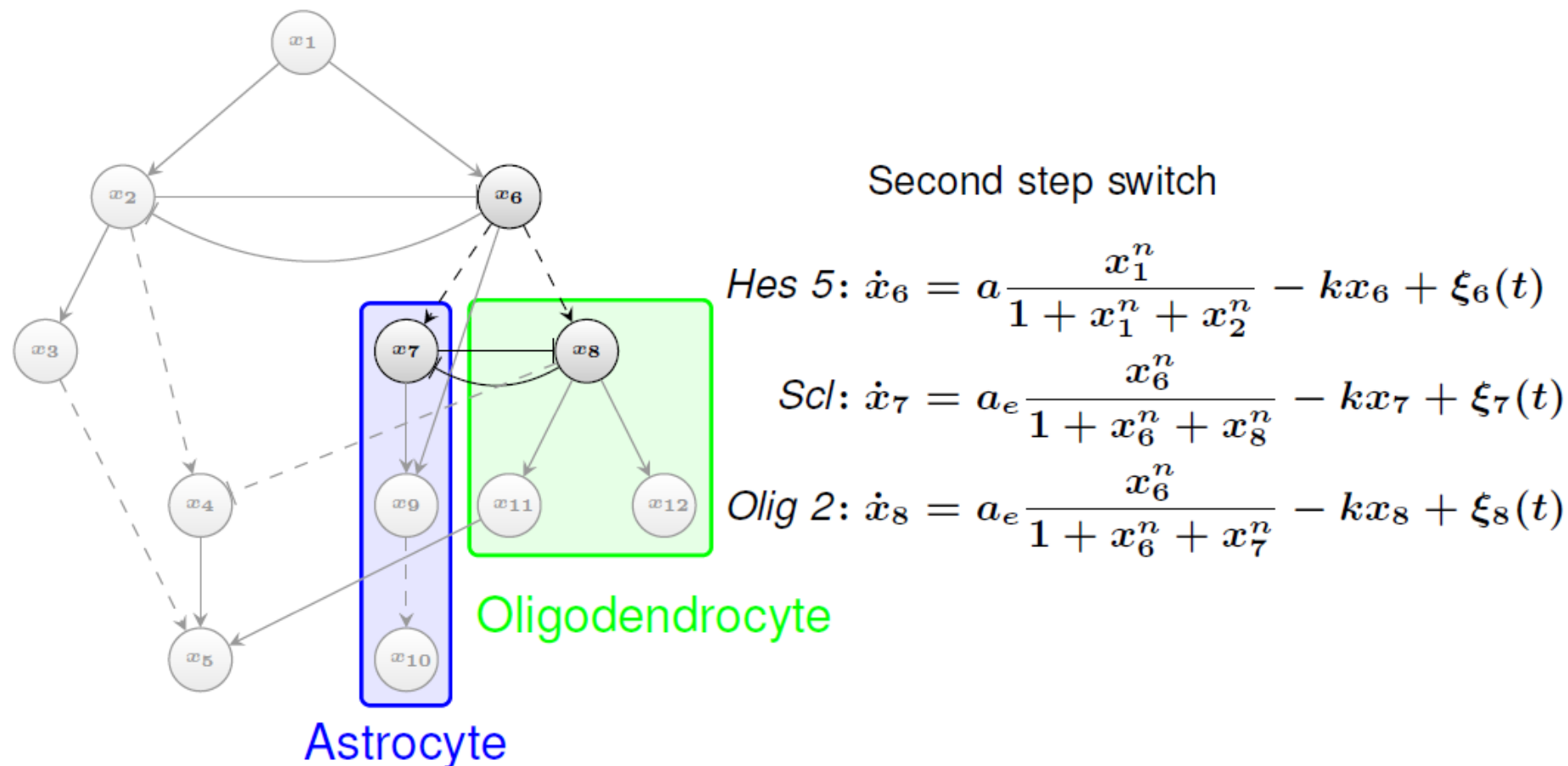
PLOS ONE

From Understanding the Development Landscape of the Canonical Fate-Switch Pair to Constructing a Dynamic Landscape for Two-Step Neural Differentiation

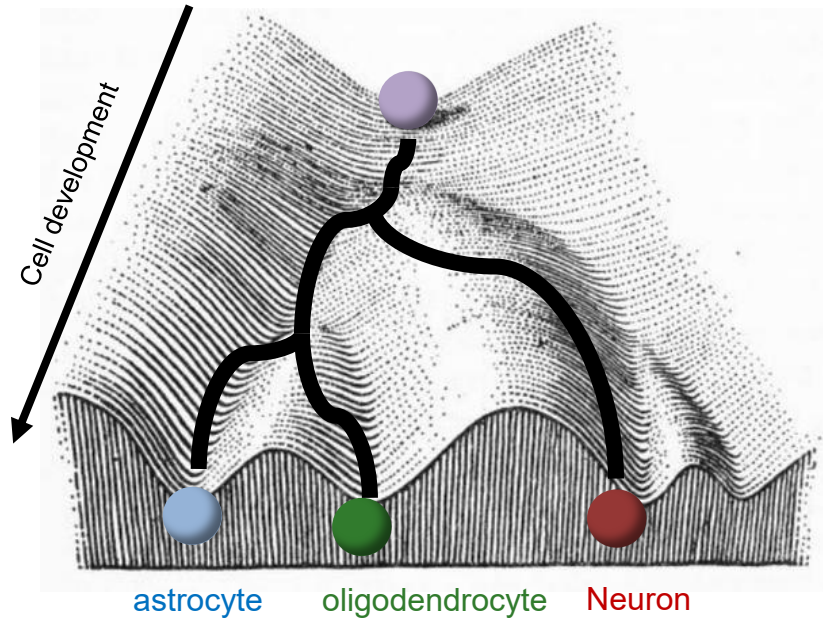
Xiaojie Qiu[‡], Shanshan Ding[‡], Tielu Shi^{*}

Center for Bioinformatics and Computational Biology, and The Institute of Biomedical Sciences, School of Life Sciences, East China Normal University, Shanghai, China

A two-stage differentiation process

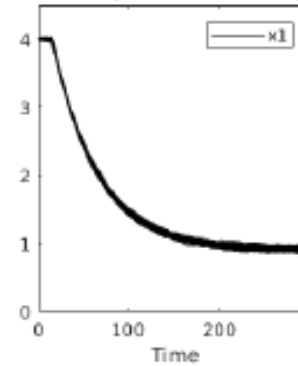


Waddington's landscape

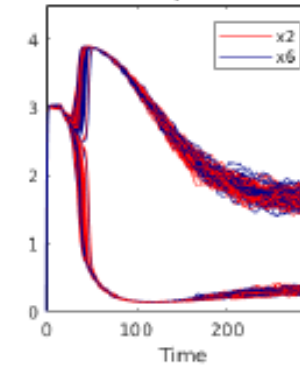


Multiple simulations, with fixed parameters and initial conditions, result in precursor cells differentiating to nervous cells of different types.

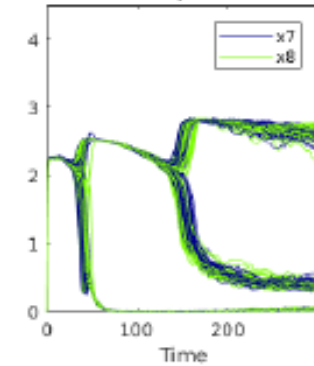
Pax 6 (100 simulations)



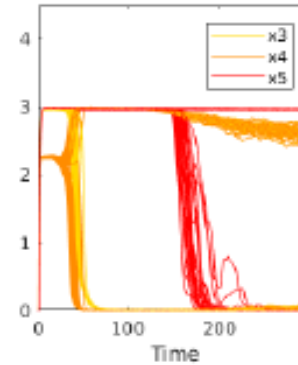
1st step switch



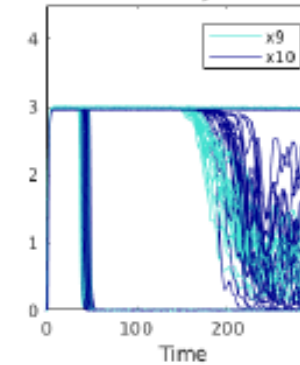
2nd step switch



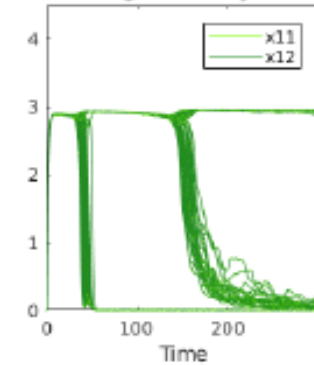
Neuron



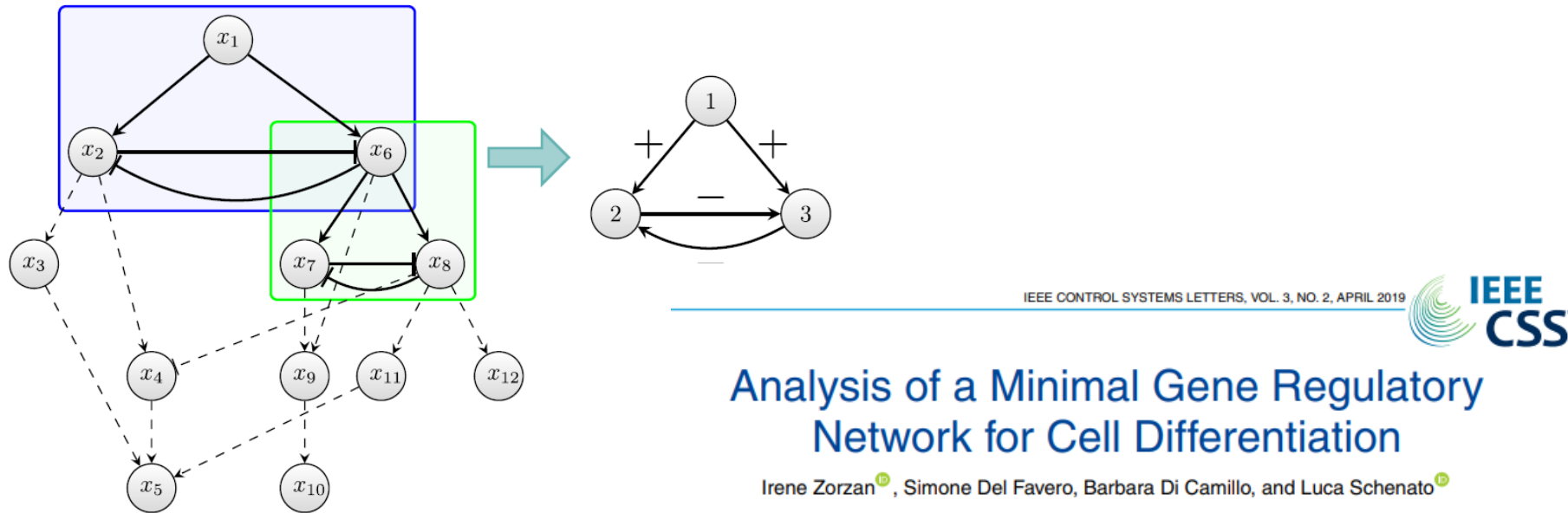
Astrocyte



Oligodendrocyte



A minimal cell regulatory module for cell differentiation

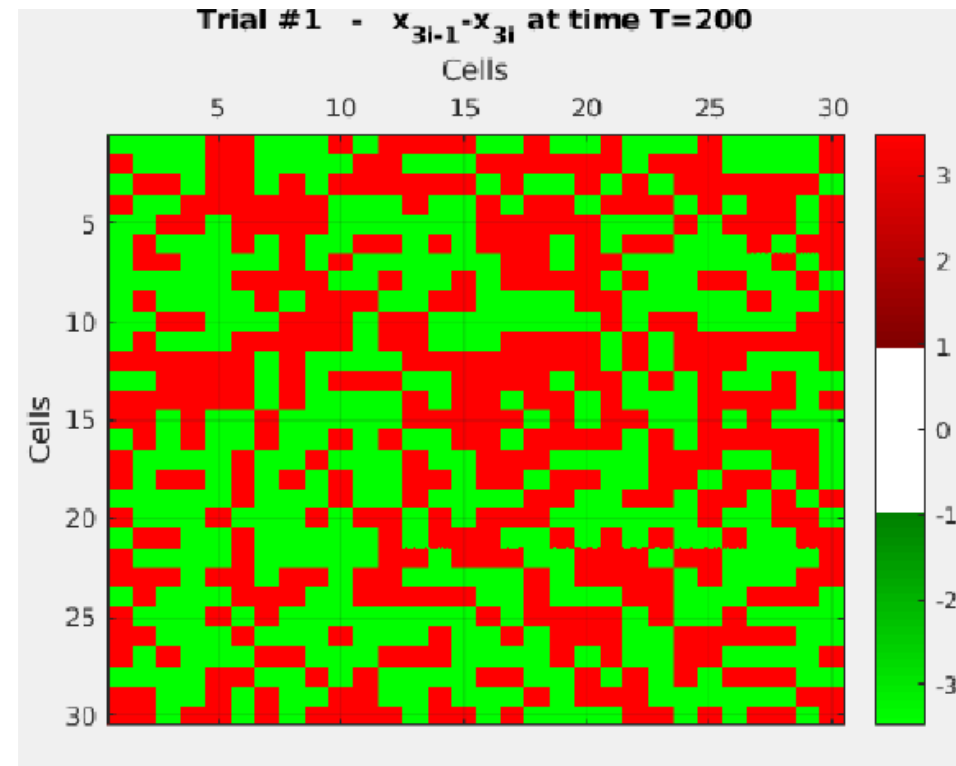
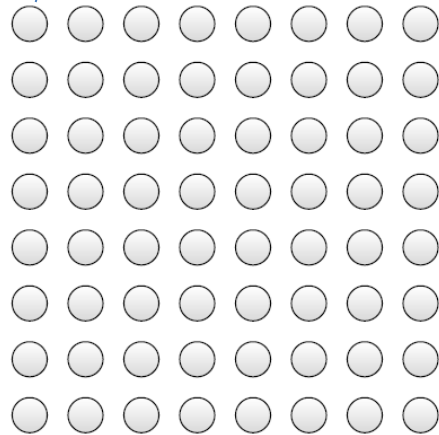
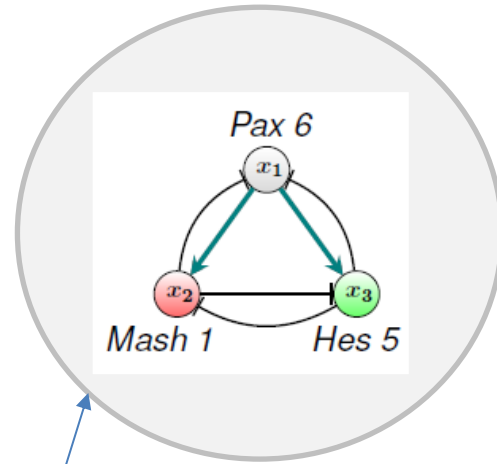


A key role is played by the expression level of Gene 1

- If it is sufficiently low or sufficiently high, the cell belongs to an **undifferentiated stage** where Gene 2 and Gene 3 are equally expressed
- If it is within a specific range of values (that depend on the system parameters), then Gene 2 is over-expressed wrt to Gene 3 or vice-versa (**differentiated stage**)

Simulation of the 1st step on a 2D lattice

Let's depict the fate of different cells in space

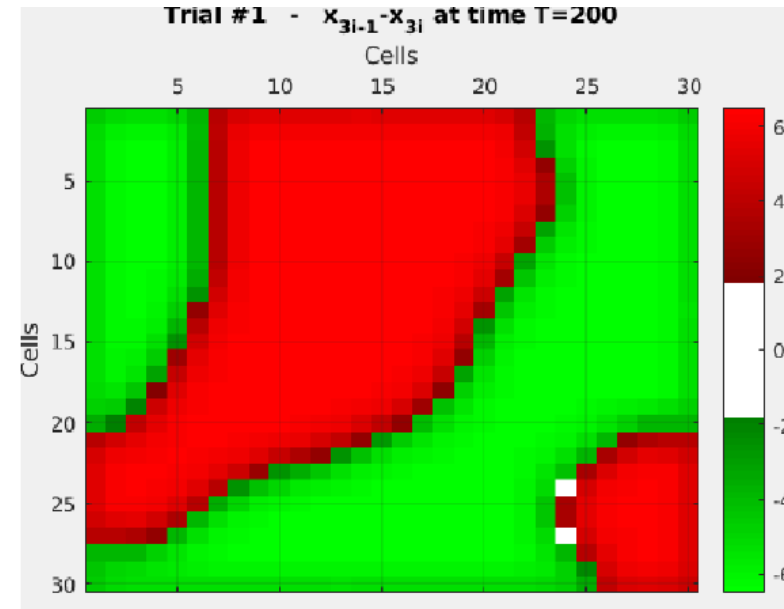
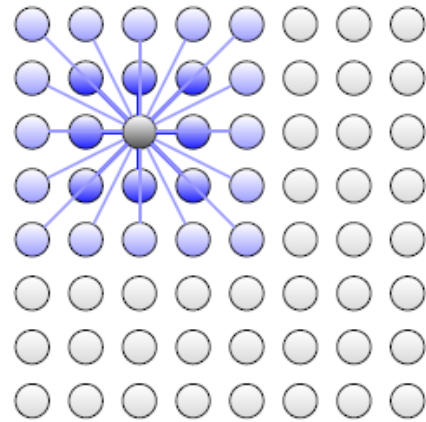


Simulating cell-cell interaction

We might think about an additional mechanism that allows driving the differentiation in a specific direction

Imagine **cells sensing their neighbours**:

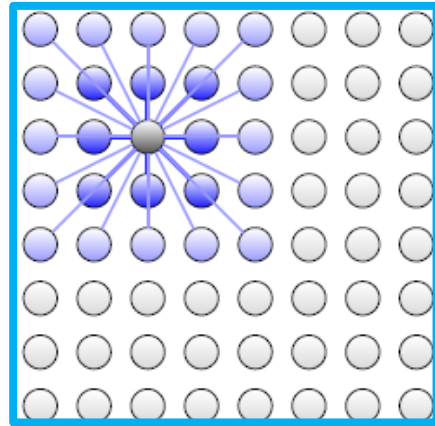
- Positive feedback among type-specific genes of the same type
- Negative feedback among type-specific genes of different type



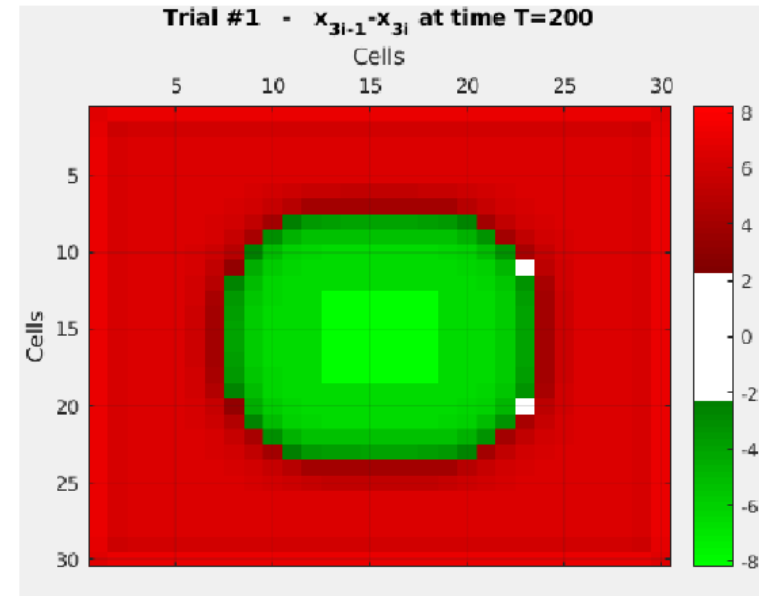
Simulating cell-cell and environment interaction

Cells sensing their neighbours and the environment:

- Positive feedback among type-specific genes of the same time
- Negative feedback among type-specific genes of different type
- Signal from the border of the lattice



Adding border effect...

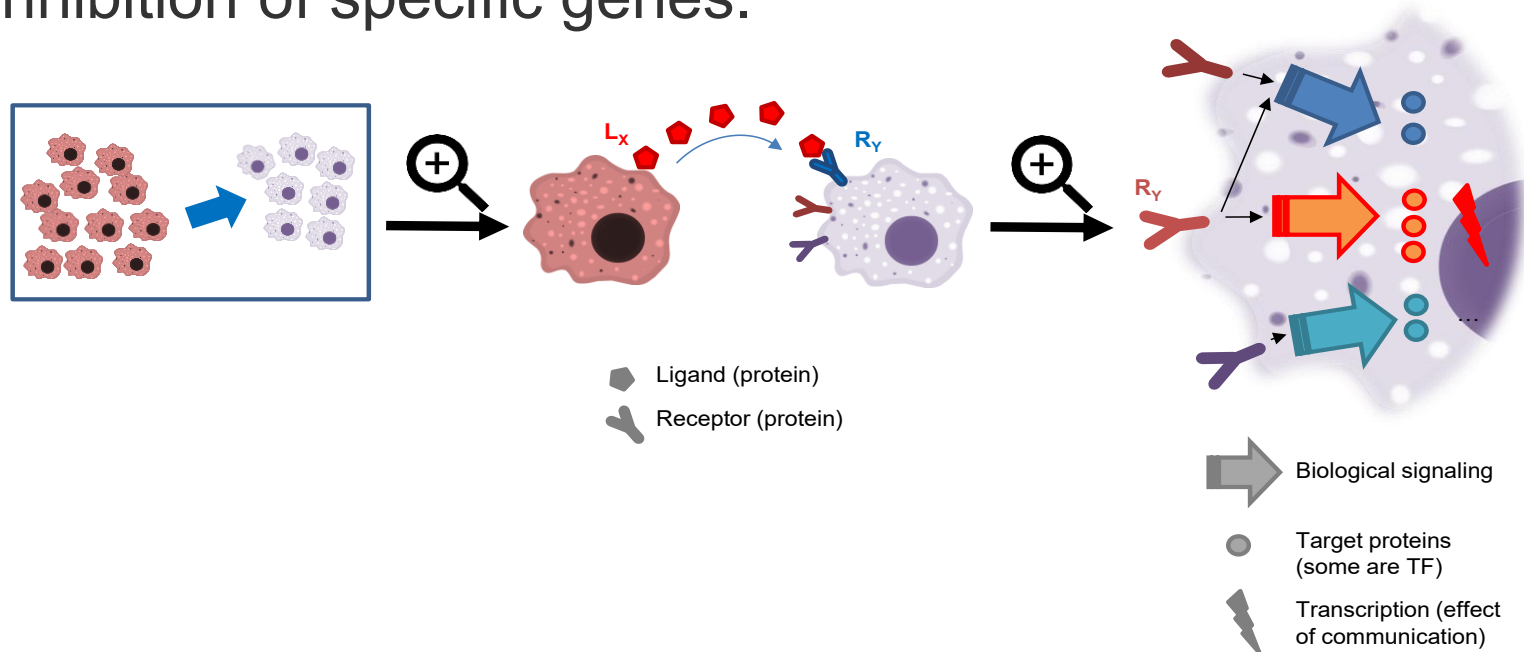


*How do we model cell
communication*

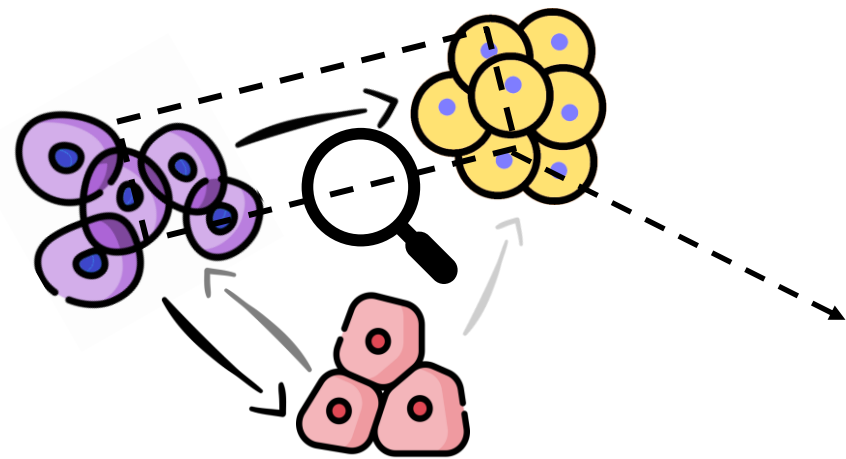
Single cells exchanging information

A widely used mechanism of cellular communication is based on ligand–receptor interactions:

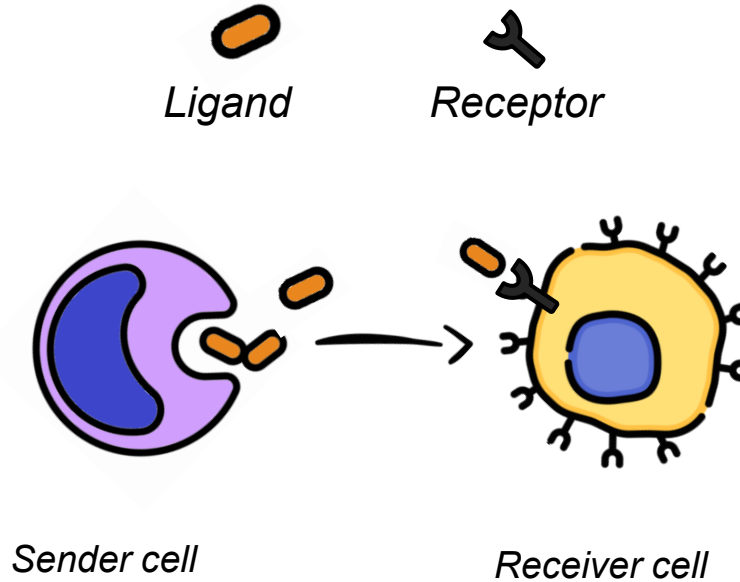
- A sender cell secretes a signaling protein, i.e. a ligand
- The ligand binds the receptor, of another cell (receiver cell).
- As a response the receptor activates a signaling cascade inside the cell that triggers a modification and in turns, might lead to transcriptional activation/inhibition of specific genes.



INTERcellular communication

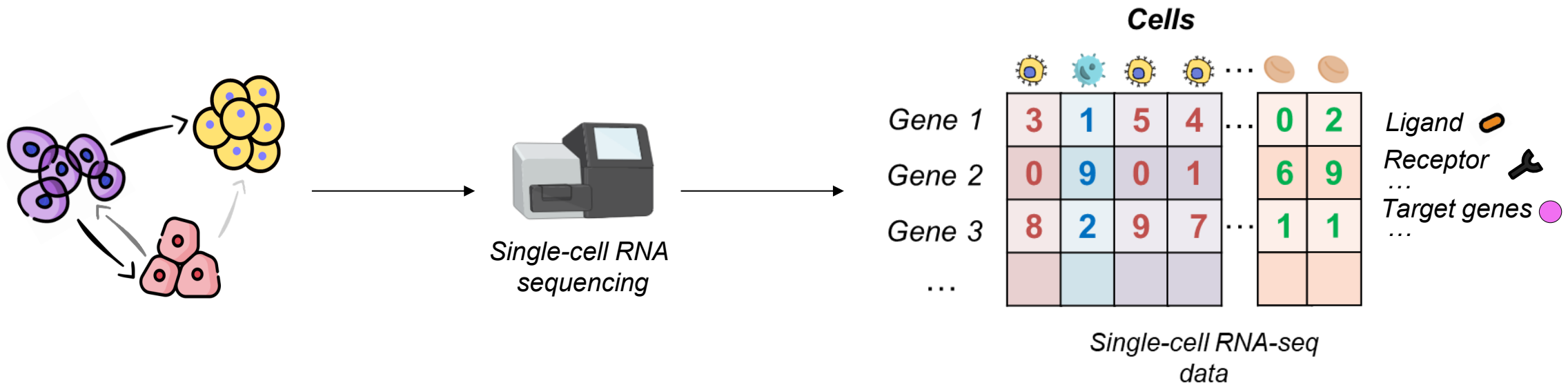


Intercellular signaling

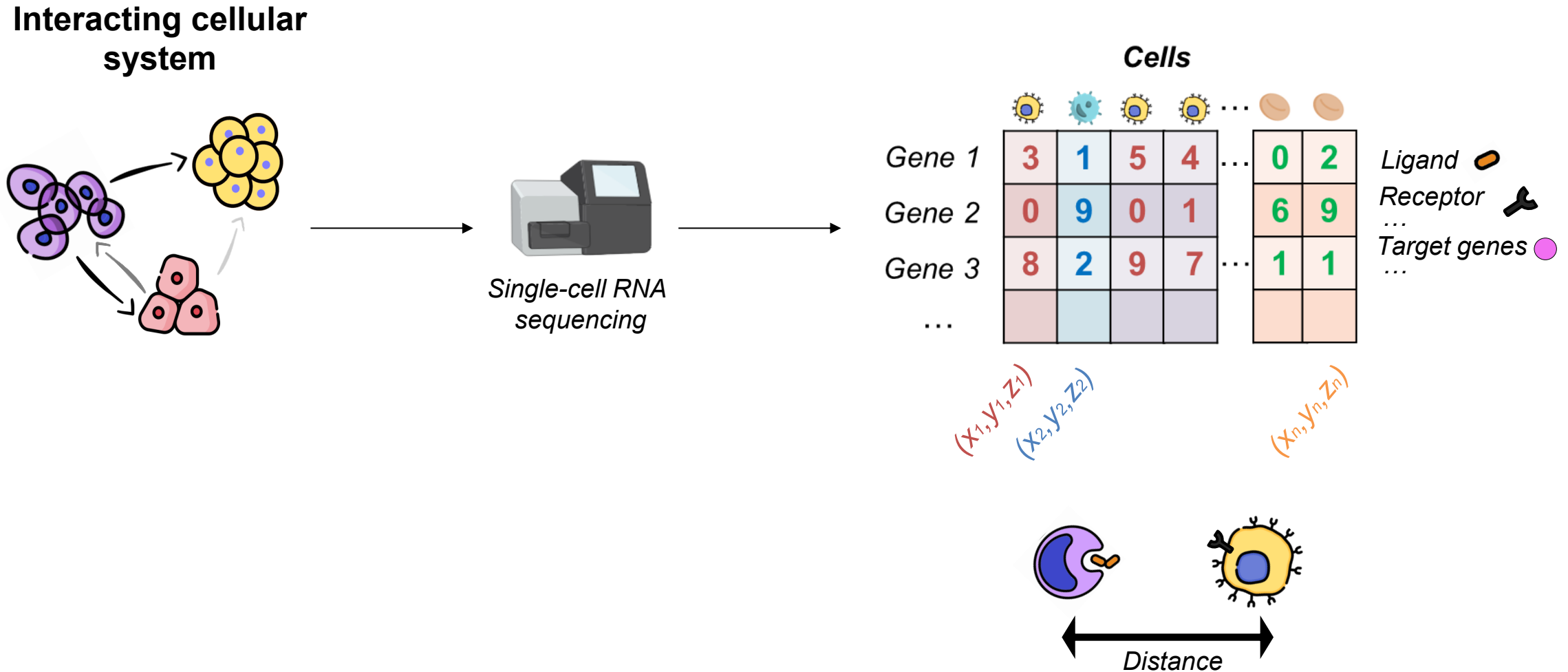


RNA expression as proxy for protein activity

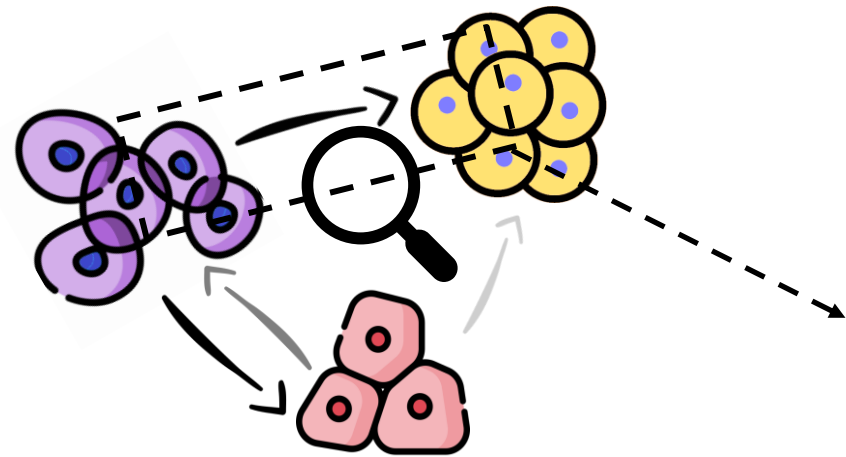
- Cell communication is primarily mediated by proteins: ligands and receptors
- Direct measurement of proteins at single-cell resolution is technically challenging and resource-intensive.
- We assume mRNA expression reflects the presence and activity of signaling proteins



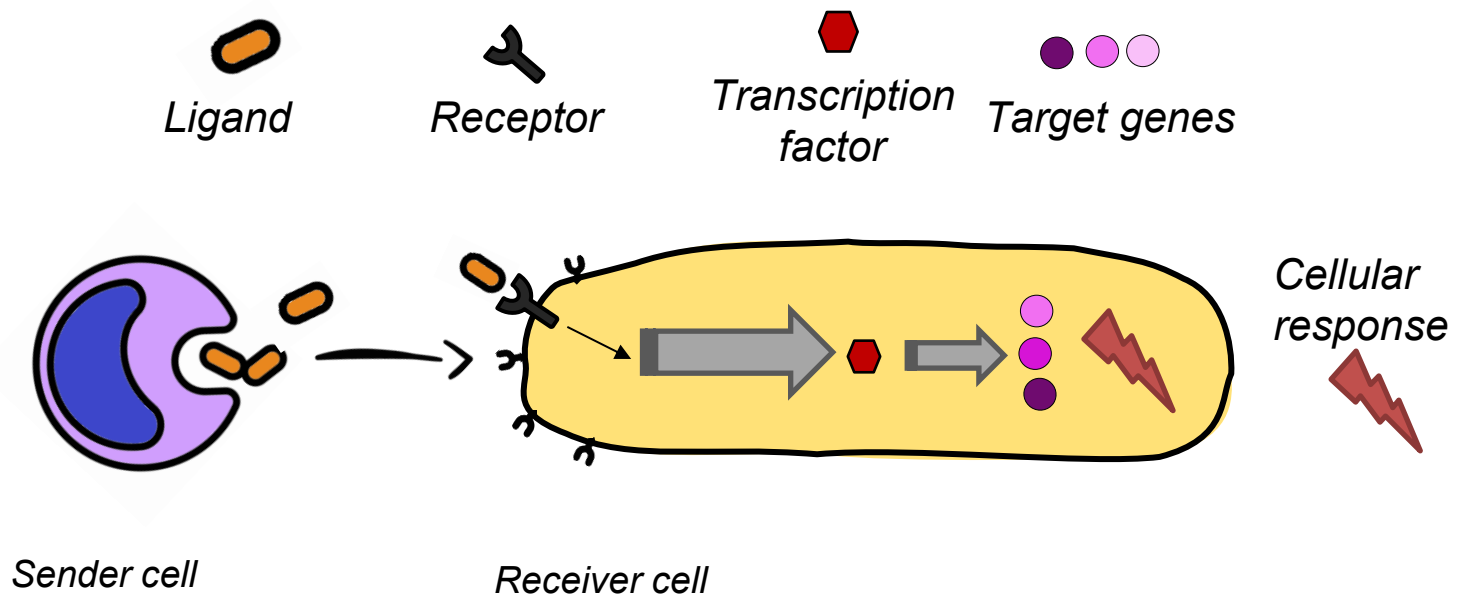
The added value of spatial transcriptomics



INTRAcellular communication

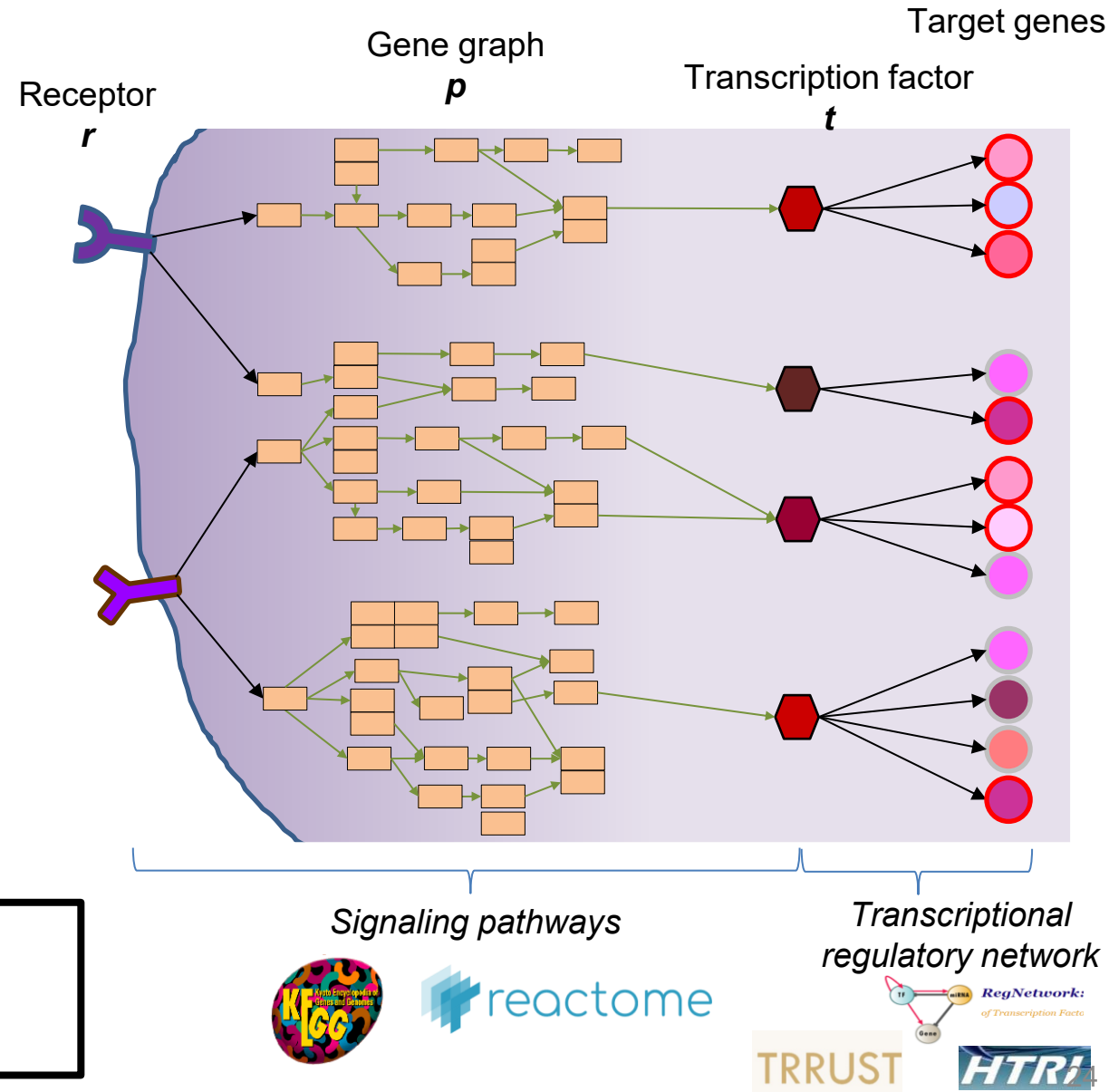
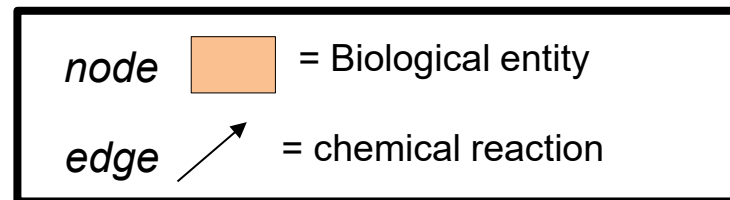


Intercellular signaling + Intracellular signaling



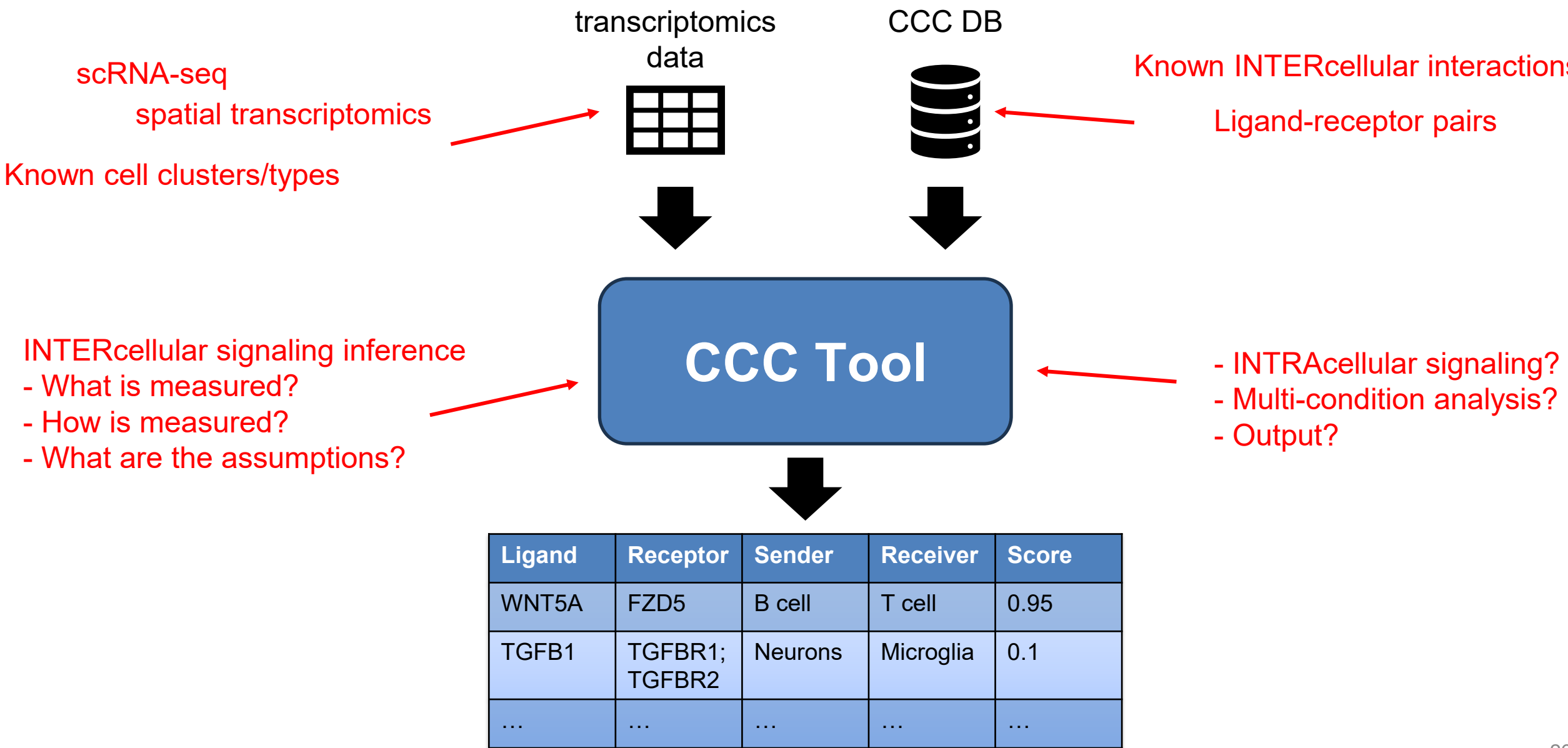
Zooming In: Intracellular Communication

- Once a ligand binds to its receptor, the signal is transmitted inside the cell through a cascade of signaling proteins.
- This intracellular signaling ultimately regulates target gene expression in the nucleus.
- By analyzing the expression of downstream target genes, we can infer the activation state of specific pathways.
- This also helps us model how extracellular signals shape cellular behavior.

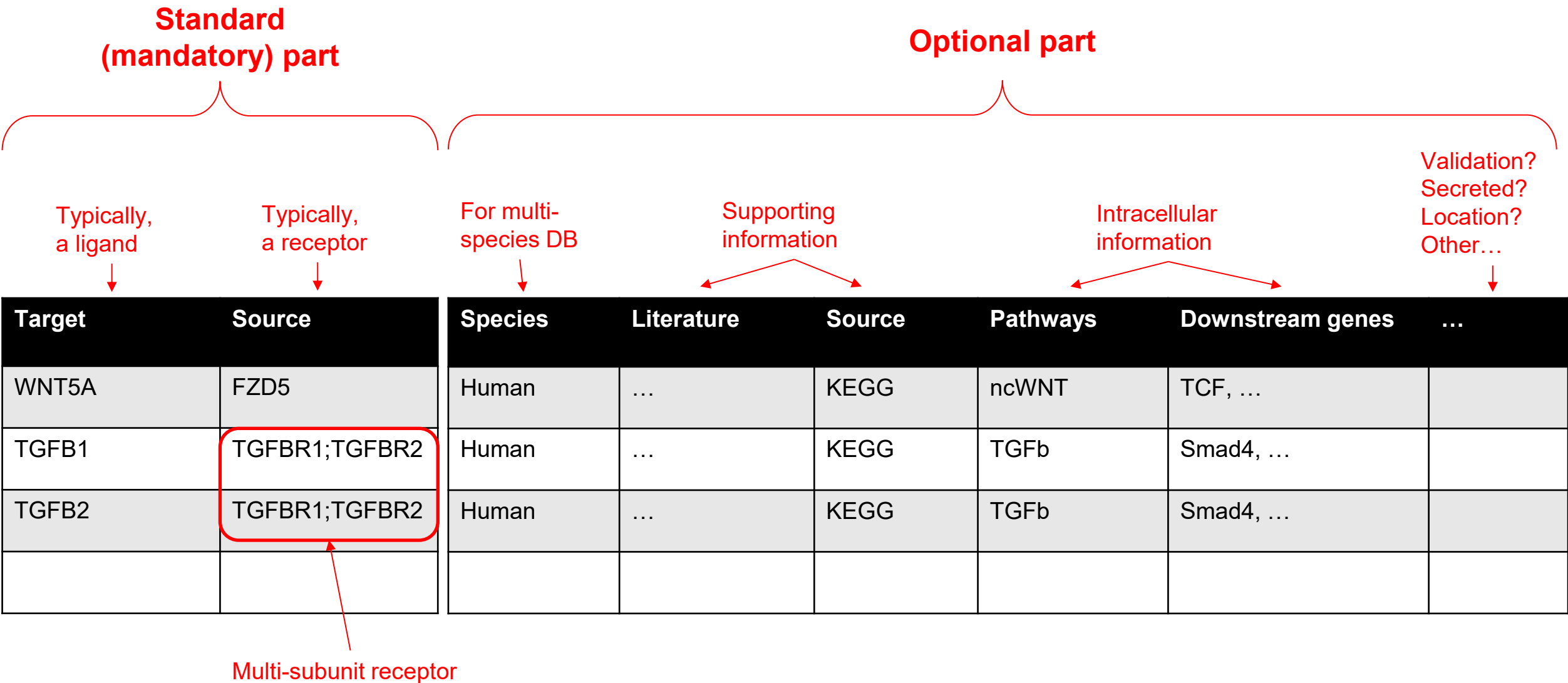


*Bioinformatics analysis
of cell communication
from transcriptomics data*

Bioinformatics analysis of CCC



CCC DB

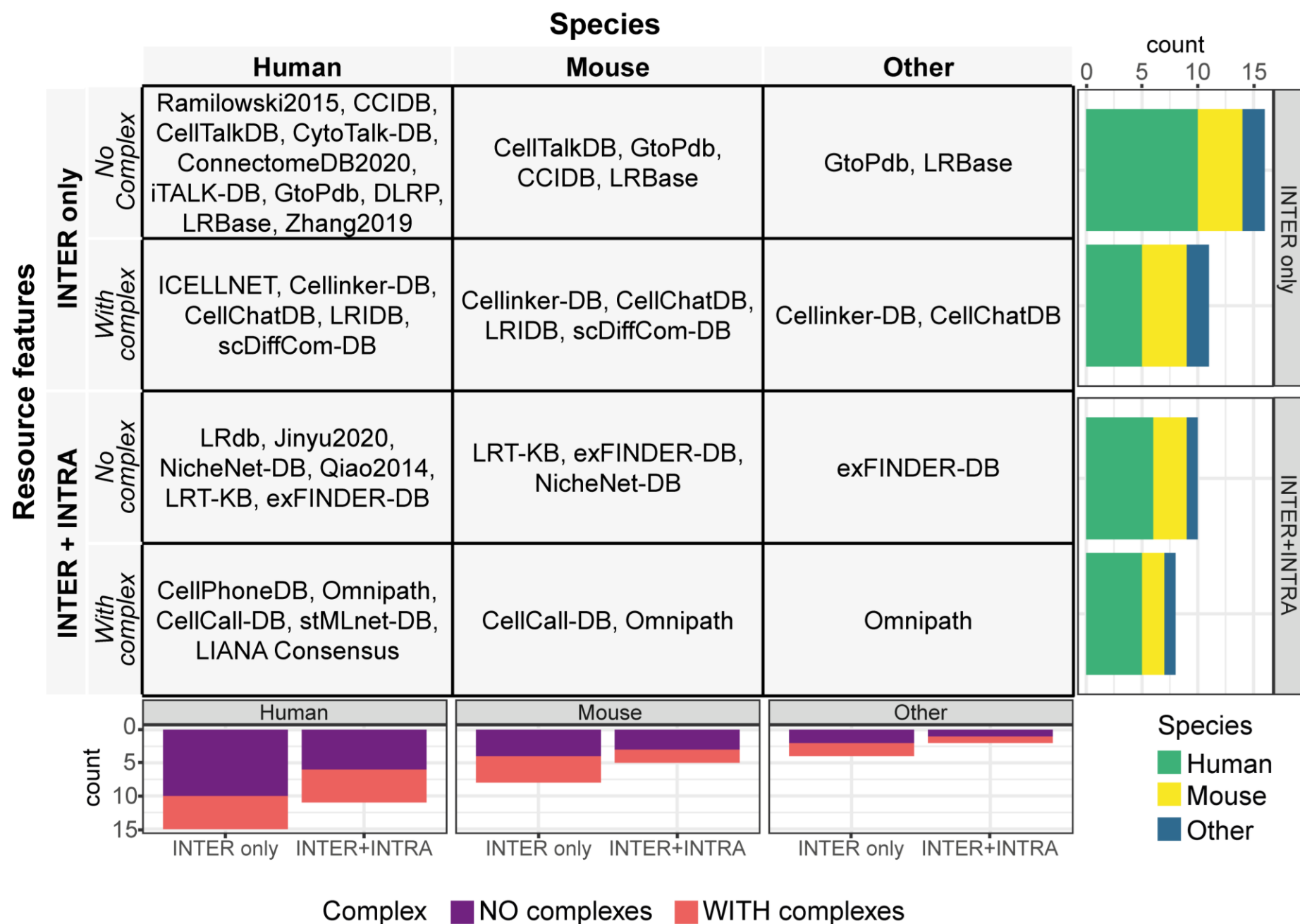


CCC DB

~ 50 CCC DBs, half of them include intercellular signaling on Human data

DBs differ in terms of:

- Species: Human, Mouse, Rat, Zebrafish, etc.
- Scope: generalist vs specific (e.g. tissue, disease)
- Sources: other DBs, literature, mining, etc.
- Num. LR pairs: from hundreds to thousands
- Information about protein complexes (multi-subunit): included or omitted
- Annotated signaling: INTER only (i.e. ligand-receptor) or INTER+INTRA
- ...



CCC - Catalog

- Web resources containing CCC DBs and CCC tools
- Detailed catalog of 26 CCC DBs
- Interactive filtering and statistics

CCC - Catalog Home Resources Tools Literature Contribute Team							
CCC resources							
Available resources for CCC analysis.							
Column visibility ▾							
10 ▾ entries per page							
Search: <input type="text"/>							
DB of interactions	Species	N of interactions Human	Intercellular interactions	Intracellular interactions	Use of protein complexes	CCC Tool using the DB	
CCIDB	Human; Mouse; Chicken	423	Yes	No	No		
CellCall-DB	Human; Mouse	19144	Yes	Yes	Yes	LIANA+ (Nature Cell Biology, 2024) ; CellCall (Nucleic Acids Res, 2021)	
CellChatDB	Human; Mouse; Zebrafish	3233	Yes	No	Yes	CellChat v2 (Nature Protocols, 2024) ; Tensor-cell2cell (Nature Communications, 2022) ; scSeqComm (Bioinformatics, 2022) ; HoloNet (Briefings in Bioinformatics, 2023) ; CellCallEXT (Cancers, 2022) ; SpatialDM (Nature Communications 2023) ; LIANA+ (Nature Cell Biology, 2024)	
Cellinker-DB	Human; Mouse; CoV_human	3744	Yes	No	Yes	Cellinker (Bioinformatics, 2021) ; CellCallEXT (Cancers, 2022) ; LIANA+ (Nature Cell Biology, 2024)	
CellPhoneDB	Human	2912	Yes	Yes	Yes	CellPhoneDB (Nature, 2018) ; Domino (Nature Biomedical Engineering, 2021) ; scSeqComm (Bioinformatics, 2022) ; CommPath (Comput Struct Biotechnol J, 2022) ; LIANA+ (Nature Cell Biology, 2024)	
CellTalkDB	Human; Mouse	3398	Yes	No	No	CellCallEXT (Cancers, 2022) ; CellAgentChat (bioRxiv, 2024) ; LIANA+ (Nature Cell Biology, 2024)	
ConnectomeDB2020	Human	2293	Yes	No	No	NATMI (Nature Communications, 2020) ; scSeqComm (Bioinformatics, 2022) ; stLearn (Nature Communications, 2023) ; LIANA+ (Nature Cell Biology, 2024)	
CytoTalk-DB	Human	1942	Yes	No	No	CytoTalk (Science Advances, 2021) ; scSeqComm (Bioinformatics, 2022)	
DLRP	Human	451	Yes	No	No		
exFINDER-DB	Human; Mouse; Zebrafish	6869	Yes	Yes	No	exFINDER (NAR, 2023)	

Review/comparison of available CCC DBs

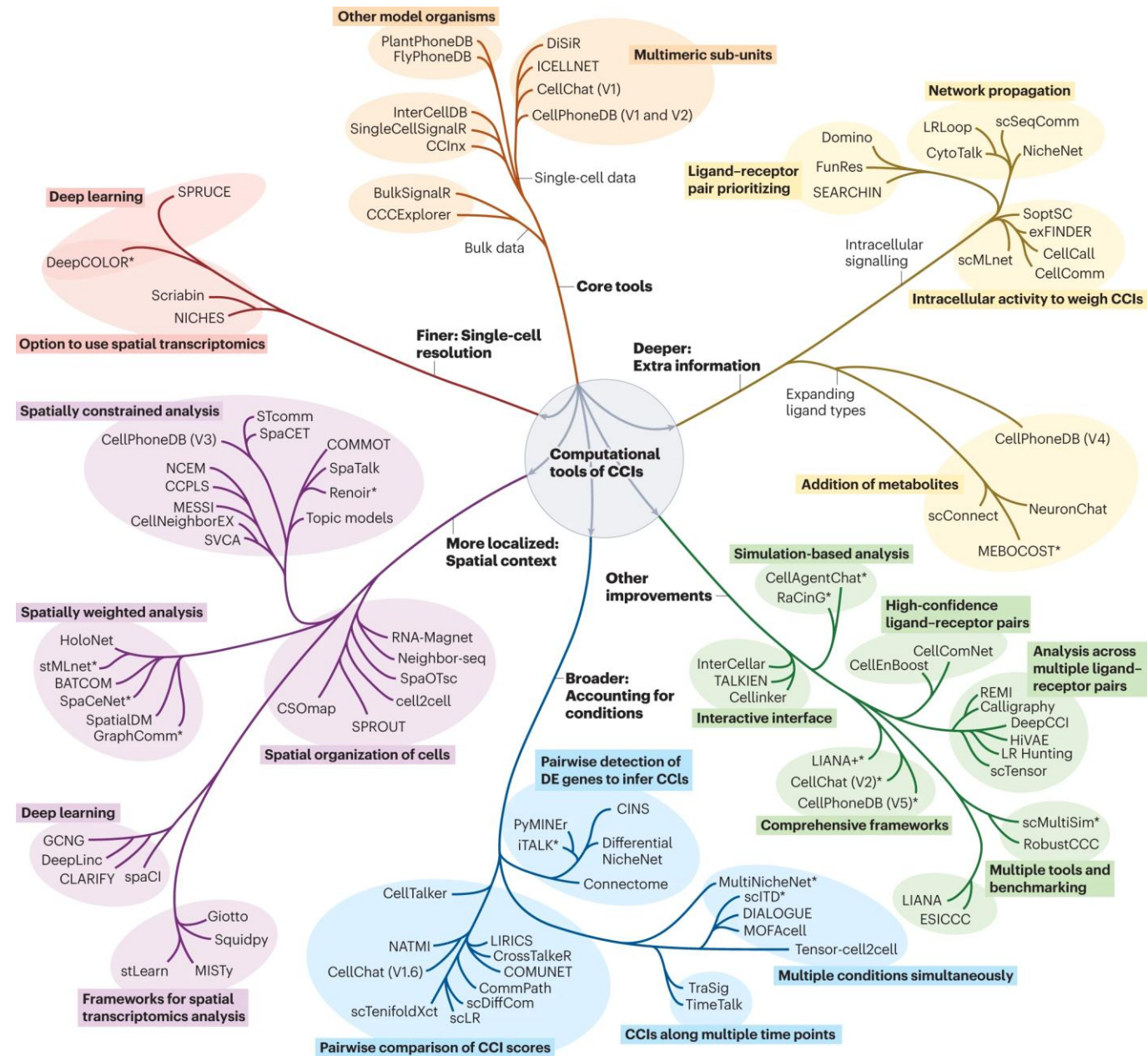
- Ma et al. (2021) “Applications and analytical tools of cell communication based on ligand-receptor interactions at single cell level”, Cell & Bioscience [[Link](#)]
- Armingol et al. (2021) “Deciphering cell-cell interactions and communication from gene expression”, Nature Reviews Genetics [[Link](#)]
- Peng et al. (2022) “Cell–cell communication inference and analysis in the tumour microenvironments from single-cell transcriptomics: data resources and computational strategies”, Briefings in Bioinformatics [[Link](#)]
- Dimitrov et al. (2022) “Comparison of methods and resources for cell-cell communication inference from single-cell RNA-Seq data”, Nature Communications [[Link](#)]
- Wang et al. (2022) “A systematic evaluation of the computational tools for ligand-receptor-based cell–cell interaction inference”, Briefings in Functional Genomics [[Link](#)]
- Cesaro et al. (2025) “Advances and Challenges in Cell-Cell Communication Inference: A Comprehensive Review of Tools, Resources, and Future Directions”, Briefings in Bioinformatics [[Link](#)]

CCC DBs used in this tutorial

Database	Species	Number of annotated LR pairs (Human only)	Protein complex (multi-subunit)	Intracellular signaling	URL	Sources
CellChatDB	Human; Mouse; Zebrafish	3233	Yes	No	Link	KEGG; CellPhoneDB; NeuronChatDB; Other literature
CellPhoneDB	Human	2912	Yes	Yes	Link	Uniprot; RCSB protein bank data; Reactome; HMPR; Other literature
ConnectomeDB	Human	2293	No	No	Link	Ramilowski2015; CellPhoneDB[v2]; STRING; Baccin2020; LRdb; ICELLNET; Other Literature
Liana Consensus	Human	4624	Yes	Yes	Link	Cellinker-DB; CellTalkDB; ConnectomeDB2020; iTALK-DB; Ramilowski2015; Kirouac2010; Baccin2020; CellPhoneDB; CellChatDB; EMBRACE; CellCall-DB; ICELLNET; GtoPdb; HPMR; OmniPath; KEGG; Reactome

CCC tools

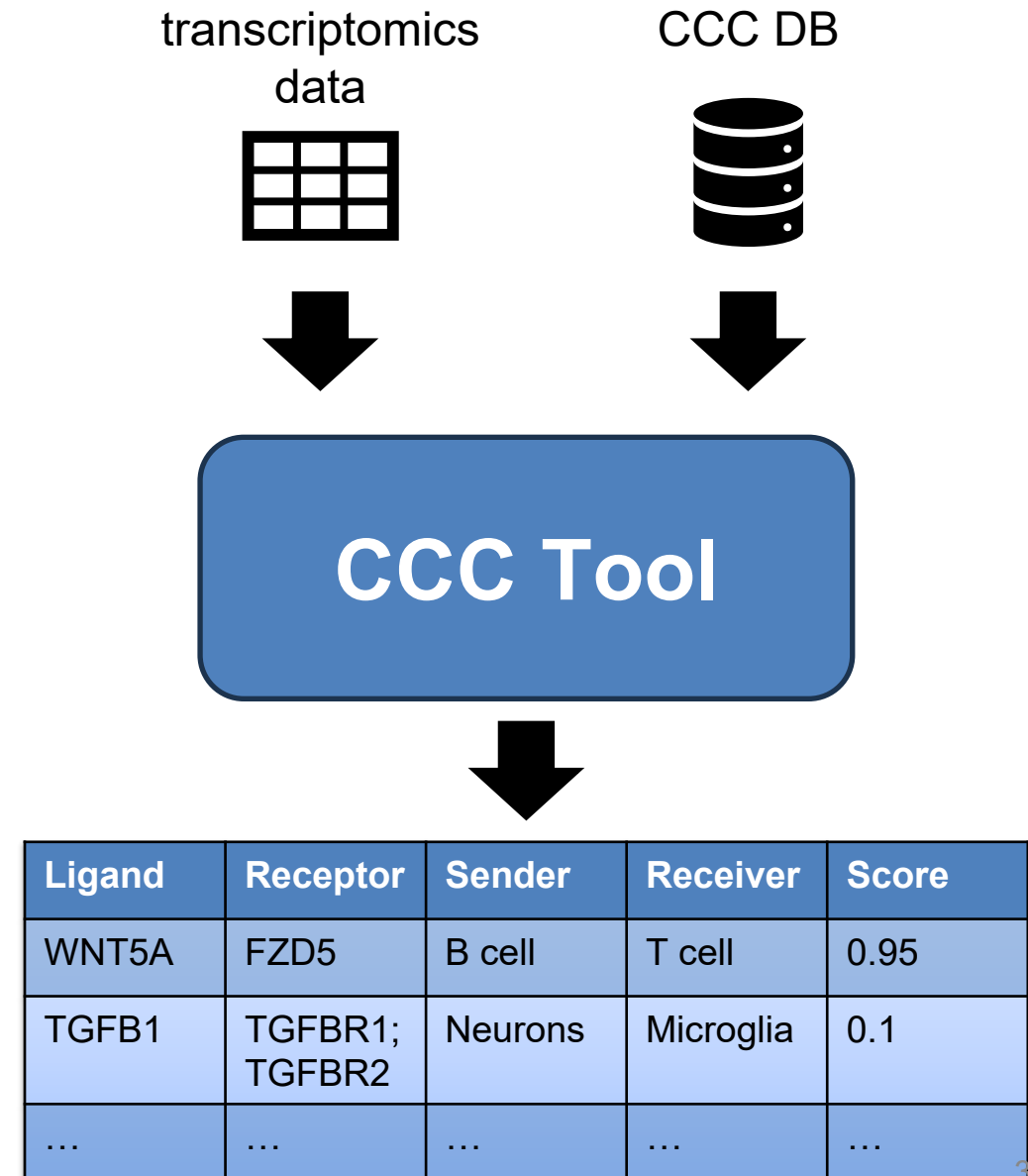
- More than 100 CCC tools!
- Almost half of them
 - support scRNA-seq or spatial transcriptomics (ST)
 - are “general purpose” tools
- Each tool has its own characteristics, pros and cons



CCC tools

CCC tools mainly differ in terms of:

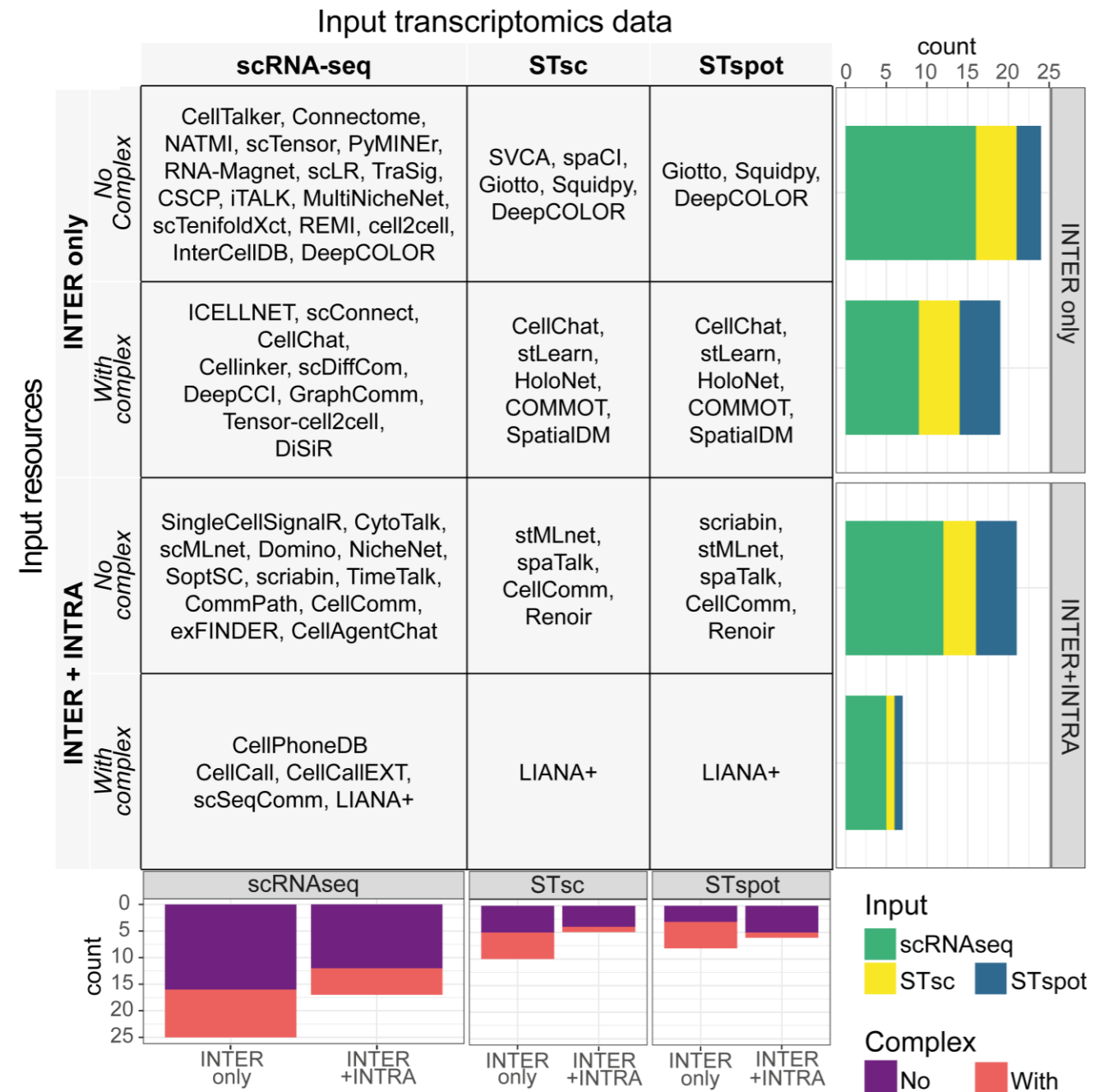
- Input
 - What type of transcriptomics data?
 - What CCC DBs?
- Intercellular inference
 - What they measure?
 - How they measure its?
 - What are the assumptions?
- Advanced analyses/features



CCC tools - Input

CCC tools differ in terms of input:

- Transcriptomics data:
 - scRNA-seq
 - single cell ST (STsc)
 - spot-based ST (STspot)
- CCC DBs:
 - built-in, user-specified, none
 - INTER only, INTER+INTRA
 - protein complexes information



CCC tools – INTERcellular inference

CCC tools differ in how they infer intercellular signaling:

- Score value/range:
 - binary (yes/no) -> easy to understand, but no ranking
 - continuous -> allows results prioritization, but need a threshold
 - other
- Score meaning:
 - intensity -> emphasizes “strong” CCC interactions
 - specificity -> emphasizes unique or cell-type-specific CCC interactions
 - other
- Methodology: machine learning, correlation, network algorithms, etc.

CCC tools – Other features

CCC tools differ in additional features for end users:

- Inference of INTRAcellular signaling: yes | no
- Support multi-condition analysis: yes | no
- Support time-series analysis: yes | no
- Built-in visualization: circus plot, dot plot, etc.
- ...

Why is important?

- Interpretability of results
- Level of details
- Handle complex design

And technical details:

- Programming languages: R, Python, etc.
- Release format: web app, R package, cmd line, etc.
- Support to parallel execution: yes | no
- Quality of life: tutorial, test dataset, container, etc.

Why is important?

- Integration with existing pipelines
- Usability
- Computational burden

CCC - Catalog

- Web resources containing CCC DBs and CCC tools
- Detailed catalog of 58 CCC tools
- Interactive filtering and statistics

CCC - Catalog Home Resources Tools Literature Contribute Team								
CCC tools								
Available bioinformatics tools for CCC analysis.								
Column visibility ▼								
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Search: <input type="text"/>								
Tool	LR DB - Source	INPUT - Type of data	INTER - Multisubunit	INTER - Score meaning	INTRA - Intracellular	OTHER - Visualization	SOFTWARE - Programming language	SOFTWARE - Code/ Software URL
cell2cell	built-in; user-specified	scRNAseq; bulk	No	Specificity, Intensity	No	Heatmap; CircosPlot; DotPlot	Python	Link
CellAgentChat	built-in; user-specified	scRNAseq	No	Specificity, Intensity	Yes	WebApp	R; Python	Link
CellCall	built-in	scRNAseq	Yes	Intensity	Yes	CircosPlot; Heatmap; SankeyPlot; SankeyPlot	R	Link
CellCallEXT	built-in	scRNAseq	Yes	Specificity, Intensity	Yes	DotPlot; SankeyPlot; CircosPlot; Heatmap	R	Link
CellChat	built-in; user-specified; built-in + user-specified	scRNAseq; STall	Yes	Specificity	No	NetworkPlot; CircosPlot; Heatmap; DotPlot; SankeyPlot; SpatialPlot; WebApp	R; C++	Link
CellComm	user-specified	scRNAseq; STall	No	Specificity, Intensity	Yes	DotPlot; NetworkPlot; BarPlot; Heatmap	R	Link
Cellinker	built-in	scRNAseq	Yes	Specificity, Intensity	No	WebApp	HTML; PHP	Link
CellPhoneDB (v4.1)	built-in; user-specified	scRNAseq	Yes	Specificity	Yes		Python	Link
CellTalker	built-in; user-specified	scRNAseq	No	Intensity	No	CircosPlot; ViolinBoxPlot	R	Link
COMMOT	user-specified	STall	Yes	Specificity, Intensity	No	NetworkPlot; Heatmap	Python	Link

<https://sysbiobig.gitlab.io/ccc-catalog/tools.html>

Literature about CCC tools

Review studies

Review studies focus on listing, classifying, and describing existing tools and resources. They do not typically involve extensive quantitative comparisons, agreement assessments, or tool rankings based on accuracy/robustness metrics derived from a ground truth.

- Shao et al. (2020) “New avenues for systematically inferring cell-cell communication: through single-cell transcriptomics data”, *Proteins & Cell* [[Link](#)]
- Ma et al. (2021) “Applications and analytical tools of cell communication based on ligand-receptor interactions at single cell level”, *Cell & Bioscience* [[Link](#)]
- Almet et al. (2021) “The landscape of cell-cell communication through single-cell transcriptomics”, *Current Opinion in Systems Biology* [[Link](#)]
- Armingol et al. (2021) “Deciphering cell-cell interactions and communication from gene expression”, *Nature Reviews Genetics*, 2021 [[Link](#)]
- Bridges et al. (2022) “Mapping and Validation of scRNA-Seq-Derived Cell-Cell Communication Networks in the Tumor Microenvironment”, *Frontiers in Immunology* [[Link](#)]
- Jin et al. (2022) “Computational exploration of cellular communication in skin from emerging single-cell and spatial transcriptomic data”, *Biochemical Society Transaction* [[Link](#)]
- Peng et al. (2022) “Cell-cell communication inference and analysis in the tumour microenvironments from single-cell transcriptomics: data resources and computational strategies”, *Briefings in Bioinformatics* [[Link](#)]
- Wang et al. (2023) “The promising application of cell-cell interaction analysis in cancer from single-cell and spatial transcriptomics”, *Seminars in Cancer Biology* [[Link](#)]
- Armingol et al. (2024) “The diversification of methods for studying cell-cell interactions and communication”, *Nature Review Genetics* [[Link](#)]
- Cesaro et al. (2025) “Advances and Challenges in Cell-Cell Communication Inference: A Comprehensive Review of Tools, Resources, and Future Directions”, *Briefings in Bioinformatics* [[Link](#)]

Literature about CCC tools

Comparison studies

Comparison studies mainly measure and quantify the agreement between CCC tools or CCC resources, often by comparing predictions and annotated interactions. These studies do not have the main goal of ranking tools/resources based on a ground truth.

- Dimitrov et al. (2022) "Comparison of methods and resources for cell-cell communication inference from single-cell RNA-Seq data", Nature Communications [[Link](#)]
- Wang et al. (2022) "A systematic evaluation of the computational tools for ligand-receptor-based cell-cell interaction inference", Briefings in Functional Genomics [[Link](#)]

Benchmarking studies

Benchmarking studies have the primary goal to rank CCC tools using quantitative metrics and a ground truth, assessing accuracy, robustness, or other performance aspects.

- Luo et al. (2023) "ESICCC as a systematic computational framework for evaluation, selection, and integration of cell-cell communication inference methods", Genome Research [[Link](#)]
- Liu et al. (2022) "Evaluation of cell-cell interaction methods by integrating single-cell RNA sequencing data with spatial information" Genome Biology [[Link](#)]
- Zhang et al. (2023) "RobustCCC: a robustness evaluation tool for cell-cell communication methods", Frontiers in Genetics [[Link](#)]
- Xie et al. (2023) "A Comparison of Cell-Cell Interaction Prediction Tools Based on scRNA-seq Data", Biomolecules [[Link](#)]
- Open Problems in Single cell (<https://openproblems.bio>)

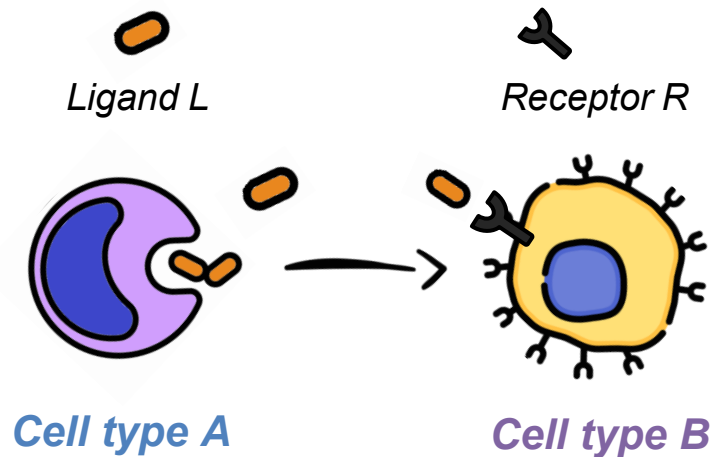
CCC tools used in this tutorial

Tool	RNA-seq data	CCC DBs	INTER score meaning	INTRAcellular inference	Multi-condition analysis	Programming Language	URL
CellPhoneDB (4.1)	scRNAseq	built-in OR user-specified	Specificity	Yes	Yes	Python	Link
scSeqComm	scRNAseq	built-in OR user-specified	Intensity	Yes	Yes	R	Link
LIANA+	scRNAseq OR ST	built-in OR user-specified	Specificity, Intensity	Yes	Yes	Python	Link
CellChat	scRNAseq OR ST	built-in OR user-specified	Specificity	No	Yes	R	Link

scSeqComm - INTERcellular signaling

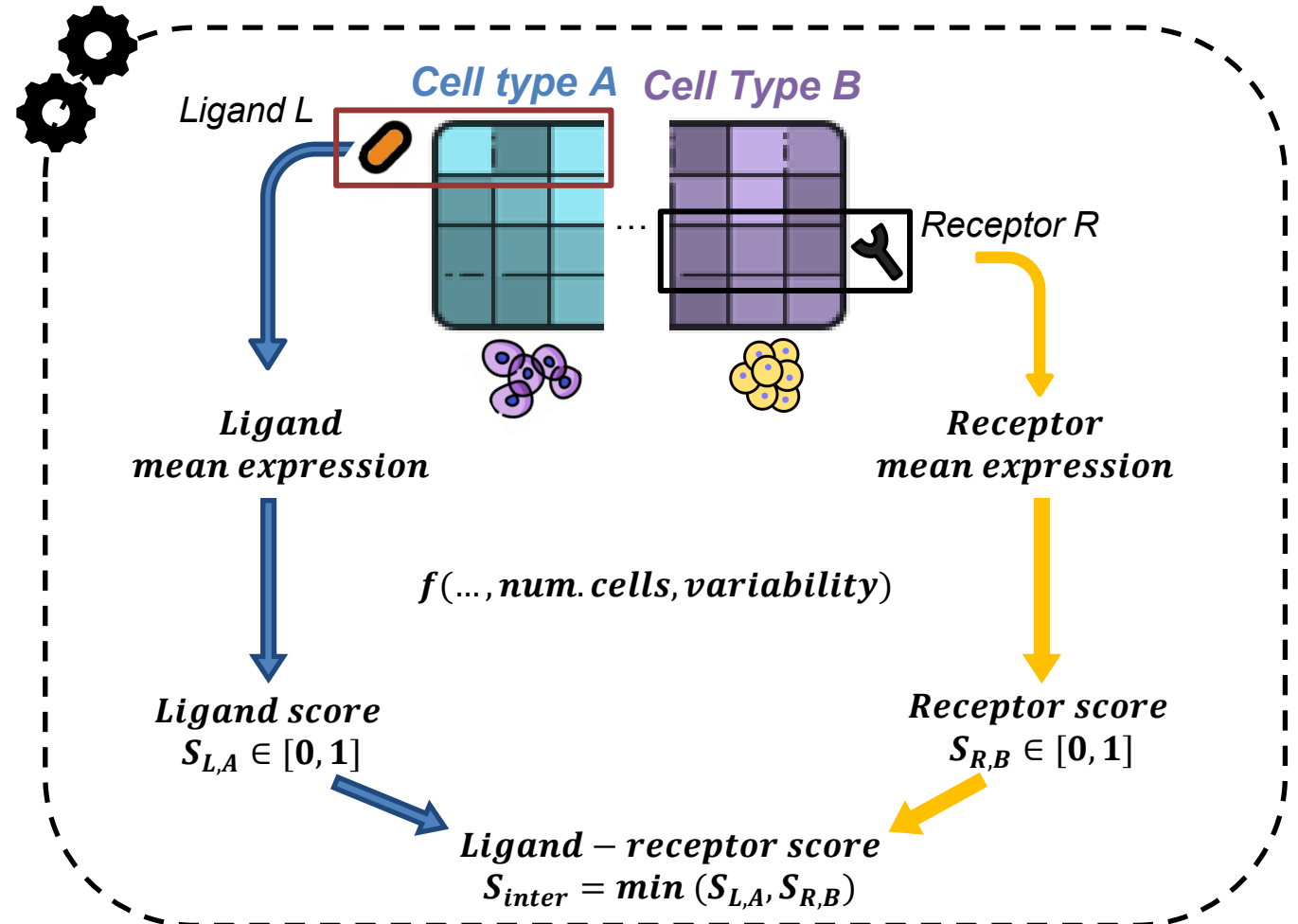
Quantify intercellular score

Strength/Intensity of Ligand-Receptor interaction across cell type A and B

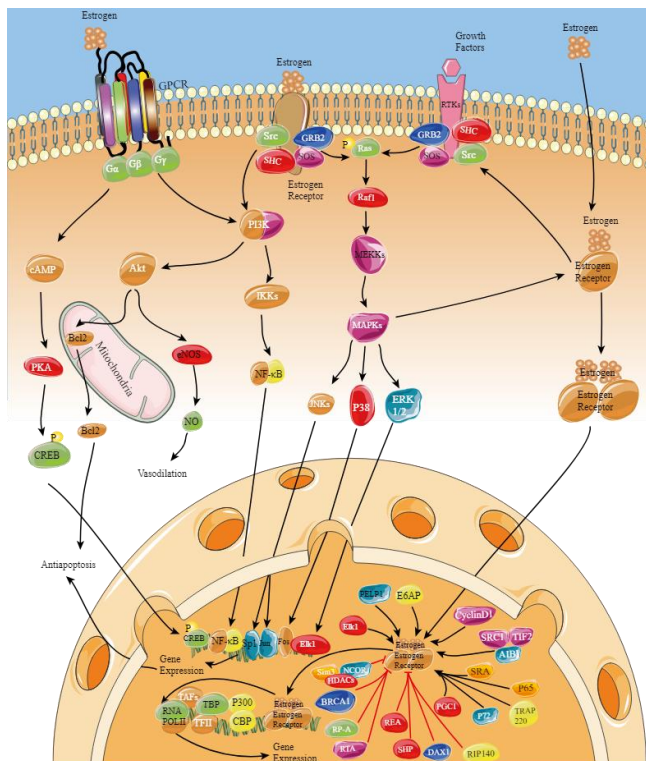


S_{inter} characteristics:

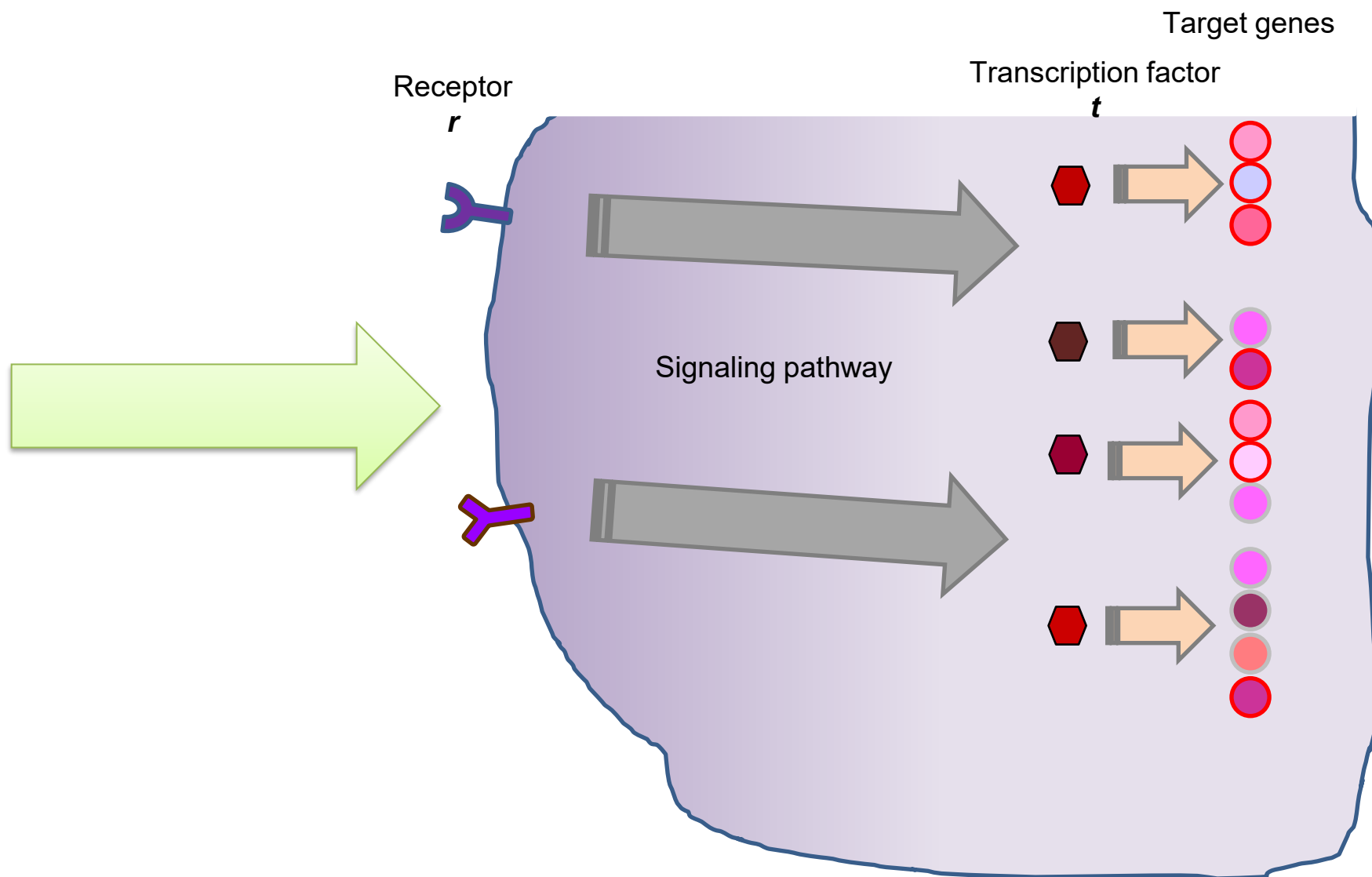
- Range $[0,1]$
- Minimum as fuzzy AND logical operator
- Measures intensity of intercellular signaling as functions of gene expression level...
- as well as the number of cells and cells variability



scSeqComm - INTRAcellular signaling

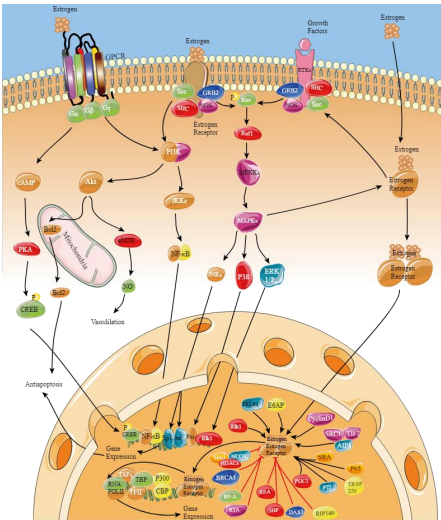


Example of intracellular signaling pathway



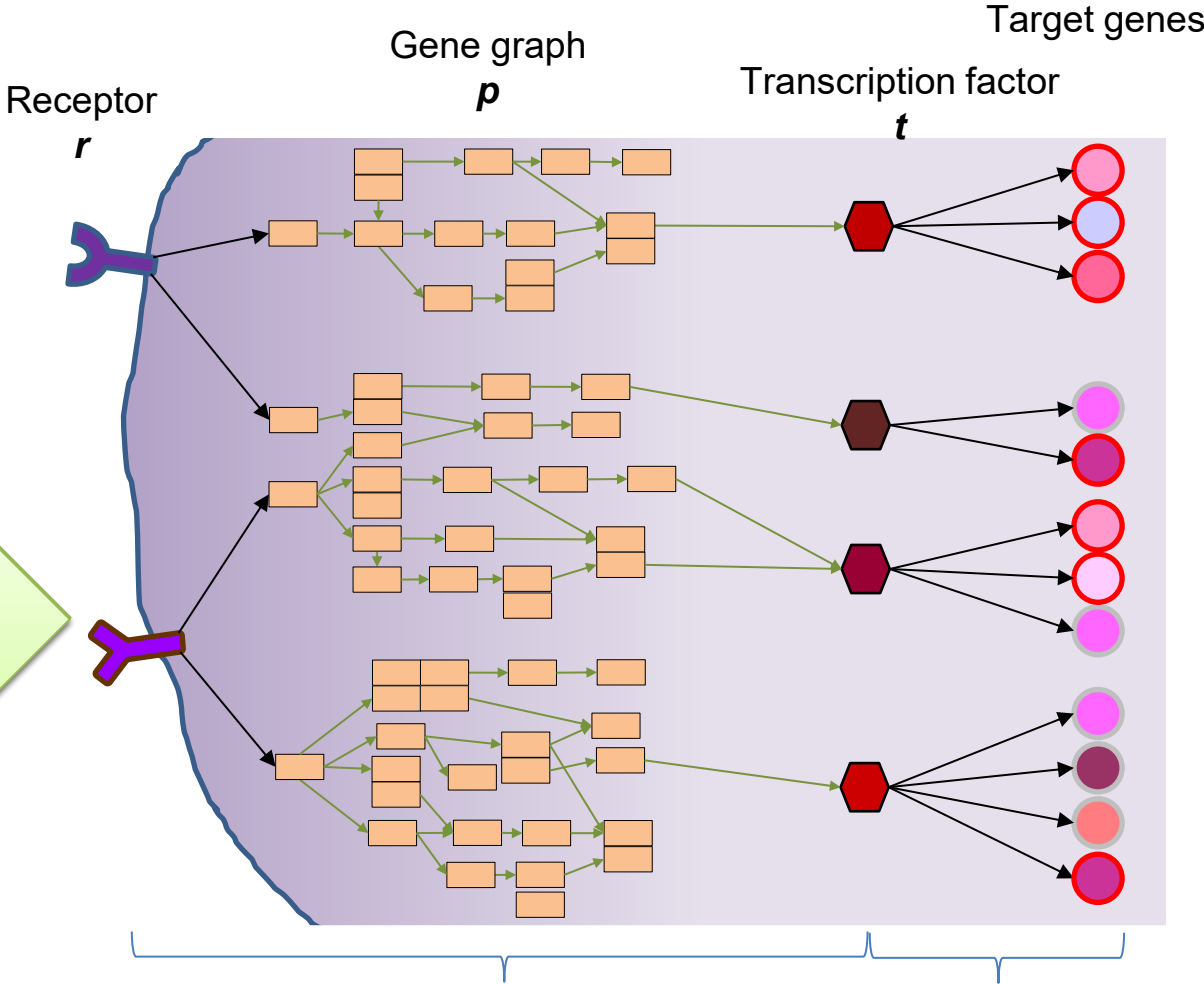
scSeqComm - INTRAcellular signaling


Conversion of biological knowledge in directed **gene graph**




Example of intracellular signaling pathway

DEFINITION OF BIOLOGICAL-DRIVEN RULES








node  = Biological entity

edge  = chemical reaction

Signaling pathways

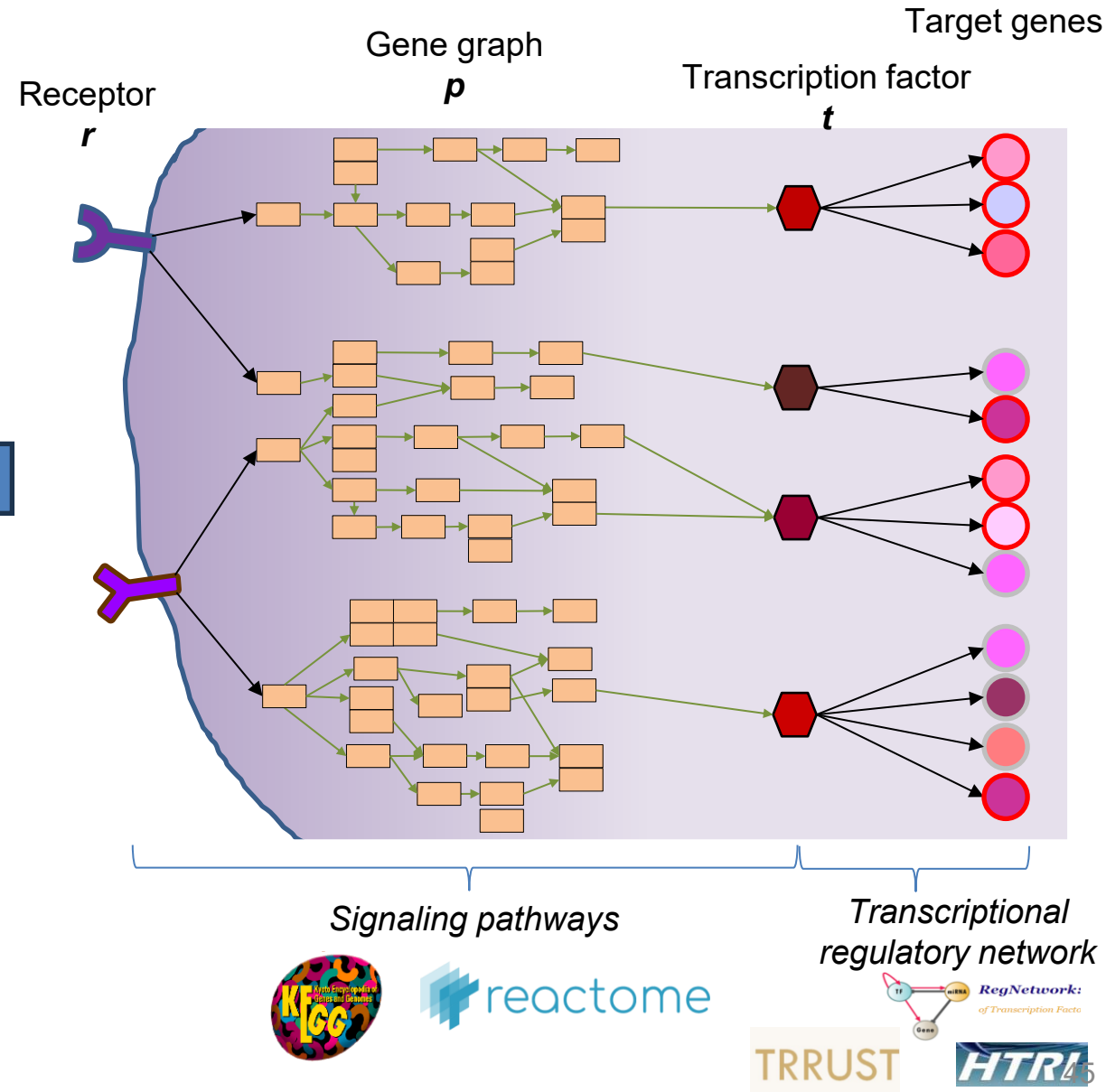
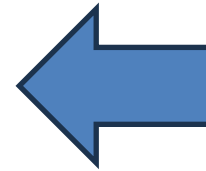
Transcriptional regulatory network



scSeqComm - INTRAcellular signaling

S_{intra} characteristics:

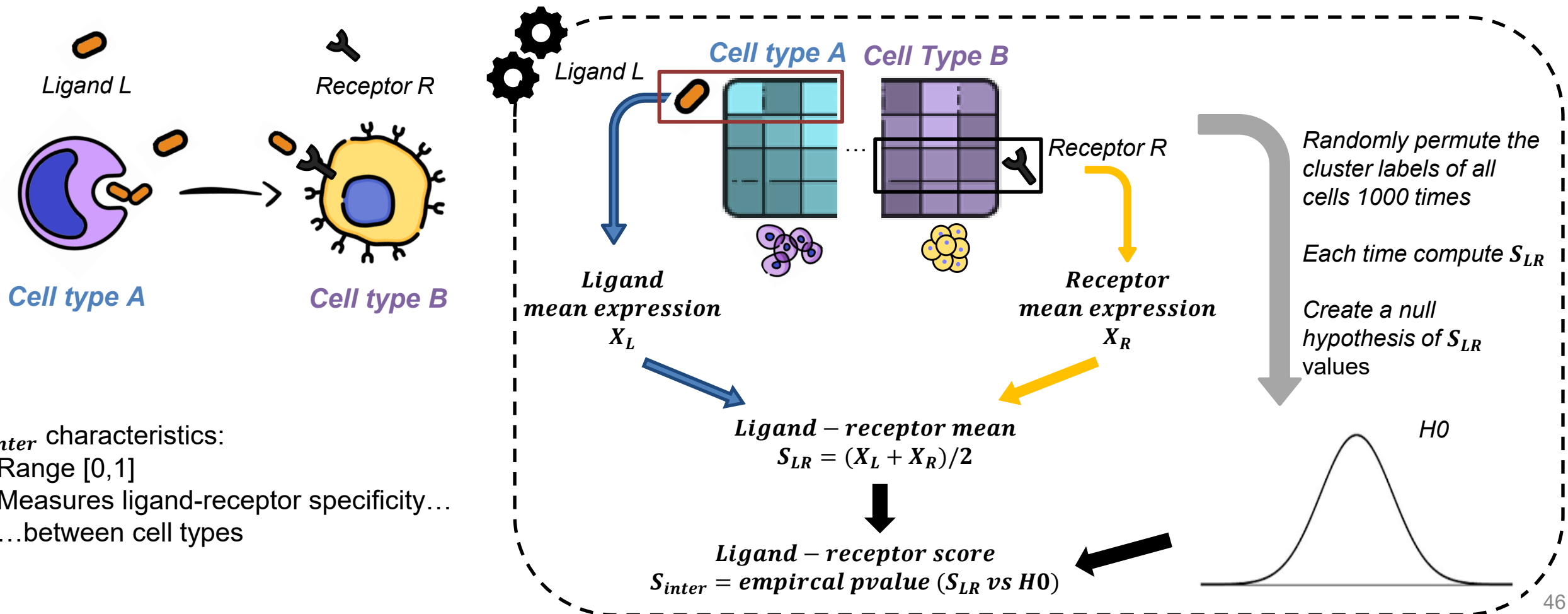
- Range [0,1]
- Function of **gene graph topology** (Personalized Page rank)...
- ...and **transcription factor / target gene activity** (statistical test on gene expression)



CellPhoneDB - INTERcellular signaling

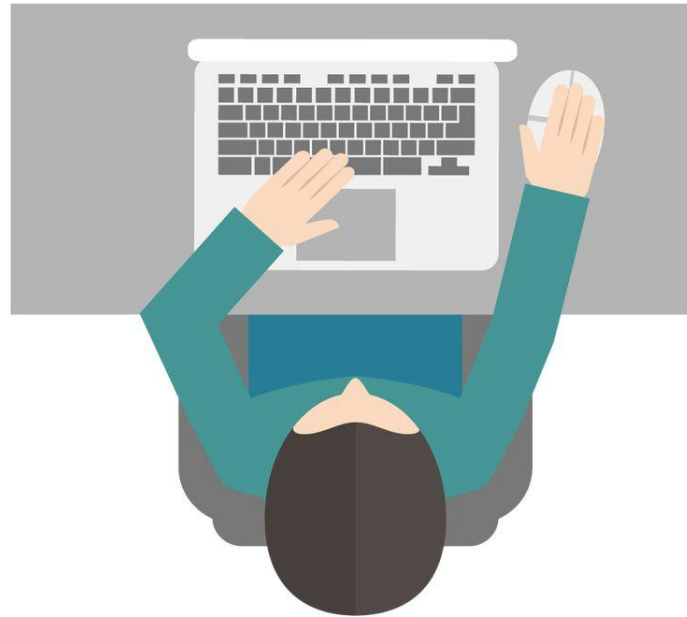
Quantify intercellular score

Specificity of Ligand-Receptor interaction across cell type A and B

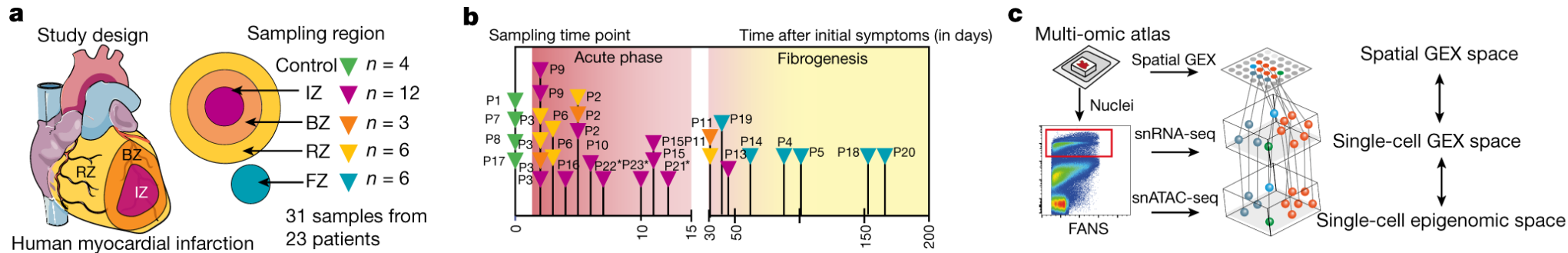


Hands on analysis of Myocardial Infarction scRNA-seq

Cesaro



Case study: Human Myocardial Infarction

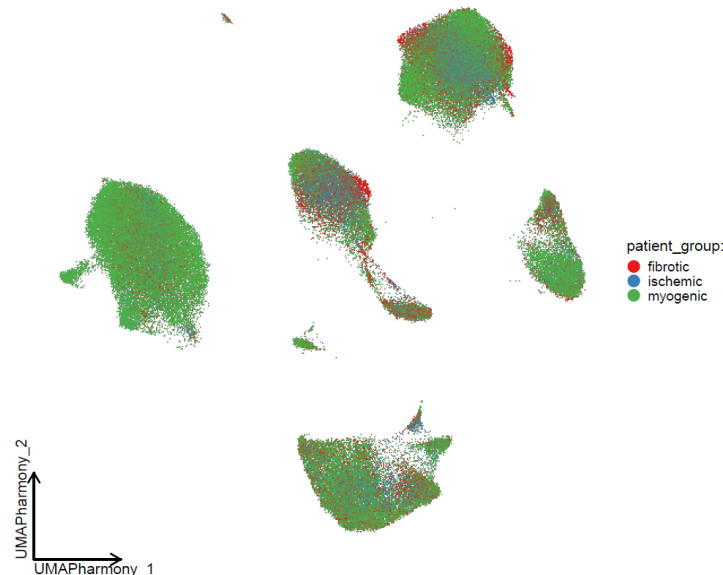


High-resolution map of human cardiac remodelling after myocardial infarction

- single-nucleus RNA sequencing (snRNA-seq)
- single-nucleus assay for transposase-accessible chromatin sequencing (scATAC-seq)
- spatial transcriptomics (ST)

23 samples

- Ischemic ($n=5$)
- Myogenic ($n=13$)
- Fibrotic ($n=5$)



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Spatial multi-omic map of human myocardial infarction

[Christoph Kuppe](#), [Ricardo O. Ramirez Flores](#), [Zhijian Li](#), [Sikander Hayat](#), [Rebecca T. Levinson](#), [Xian Liao](#), [Monica T. Hannani](#), [Jovan Tanevski](#), [Florian Wünnemann](#), [James S. Nagai](#), [Maurice Halder](#), [David Schumacher](#), [Sylvia Menzel](#), [Gideon Schäfer](#), [Konrad Hoelt](#), [Mingbo Cheng](#), [Susanne Ziegler](#), [Xiaoting Zhang](#), [Fabian Peisker](#), [Nadine Kaesler](#), [Turgay Saritas](#), [Yaoxian Xu](#), [Astrid Kassner](#), [Jan Gummert](#), ... [Rafael Kramann](#)  [+ Show authors](#)

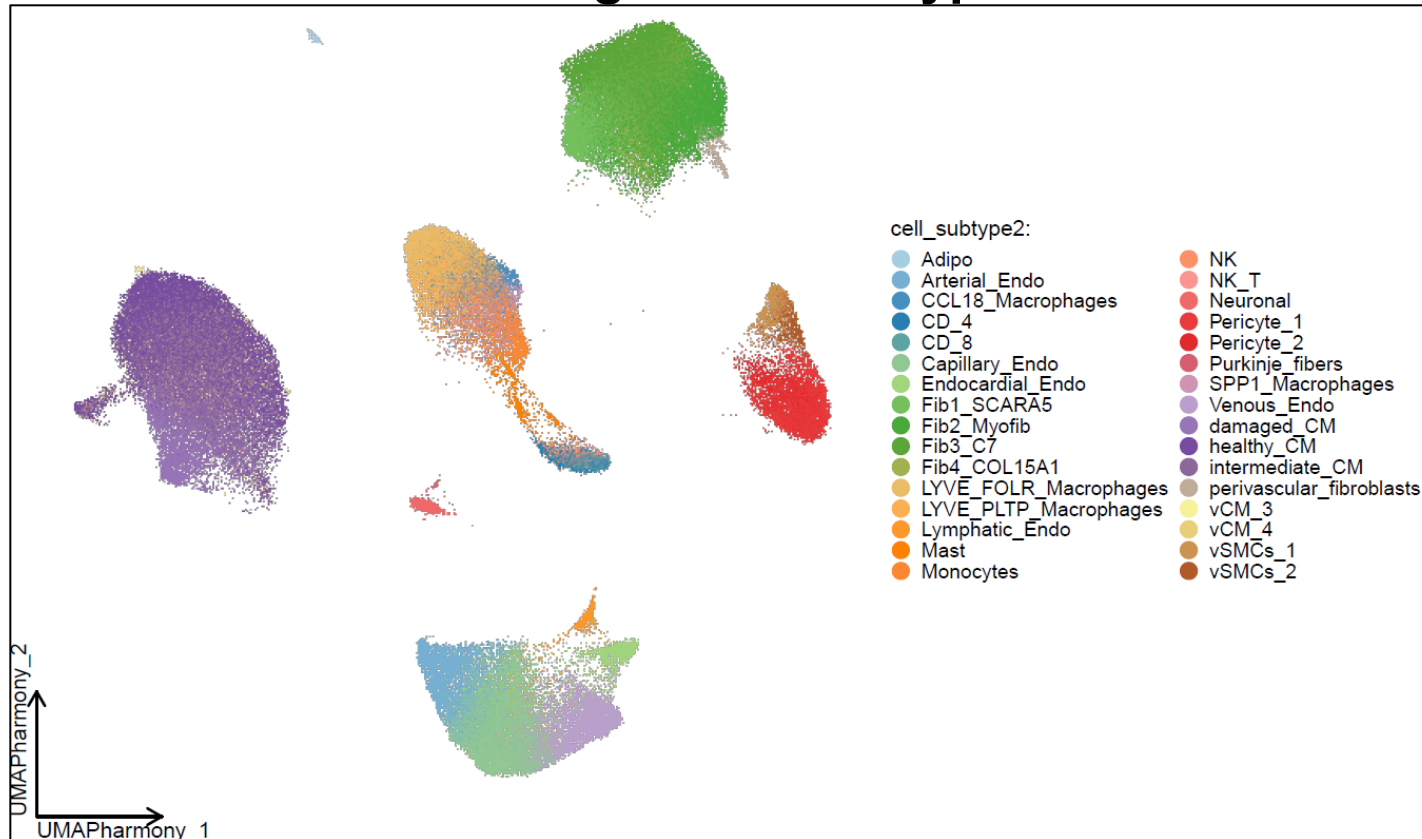
[Nature](#) 608, 766–777 (2022) | [Cite this article](#)

Case study: Human Myocardial Infarction

The dataset includes two levels of annotations:

- major cell type labels
- finer-grained cell type labels

Finer-grained cell types



Major cell types

