**METHOD**

Uncovering hypergraphs of cell-cell interaction from single cell RNA-sequencing data

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**Abstract**  
Complex biological systems can be described as a multitude of cell-cell interactions (CCIs). Recent single-cell RNA-sequencing technologies have enabled the detection of CCIs and related ligand-receptor (L-R) gene expression simultaneously. However, previous data analysis methods have focused on only one-to-one CCIs between two cell types. To also detect many-to-many CCIs, we propose scTensor, a novel method for extracting representative triadic relationships (hypergraphs), which include (i) ligand-expression, (ii) receptor-expression, and (iii) L-R pairs. When applied to simulated and empirical datasets, scTensor was able to detect some hypergraphs including paracrine/autocrine CCI patterns, which cannot be detected by previous methods.

**Keywords:** Single-cell RNA-sequencing; Cell-cell interaction; Hypergraph; Dimension Reduction; Tensor Decomposition; Non-negative Tucker Decomposition; R/Bioconductor

**Background**  
Complex biological systems such as tissue homeostasis \cite{1, 2}, neurotransmission \cite{3, 4}, immune response \cite{5}, ontogenesis \cite{6}, and stem cells niche \cite{7, 8} are composed by cell-cell interaction (CCI). Many molecular biology studies have been decomposed the system into constituent parts (e.g., genes, proteins, and metabolites) to clarify
their functions. Nevertheless, more sophisticated methodologies are still required, because CCI is the difference between the whole system and sum of their parts.

Previous studies have investigated CCIs using technologies such as fluorescence microscopy [9–13], microdevise-based methods such as microwells, micropatterns, single-cell traps, droplet microfluidics, and micropillars [14–22], and transcriptome-based method [23–53]. In particular, the recent development of single-cell RNA-sequencing (scRNA-seq) technologies has enabled the detection of exhaustive cell-type-level CCIs based on ligand and receptor (L-R) coexpression.

To assess the coexpression of known L-R genes, circle plots [24, 33, 36, 44, 45, 50], bigraph/Sankey diagrams [29, 30, 34, 49, 52, 53], network diagrams [26–28, 31, 37, 40, 41, 44, 46, 54] and heatmaps [30, 32, 34, 39, 46, 47, 49] are often drawn. Some studies have also introduced more systematical approaches for quantifying the degree of CCIs based on the L-R coexpression, such as the number of coexpressed L-R pairs [23, 24, 26, 27], Spearman correlation coefficients between L-R expression profiles [26, 31], original interaction scores between L-R coexpression [39], or hypothetical test based on random cell-type label permutation [29, 32, 37, 55].

All the approaches described above implicitly suppose that CCIs are the one-to-one relationships between two cell types and that the corresponding L-R coexpression is observed as the cell-type-specific manner. In real empirical data, however, the situation can be more complex; CCIs often exhibit many-to-many relationships involving many cell types, and an particular L-R pair can also function across multiple cell-type pairs. Therefore, in this work, we propose scTensor, which is a novel method based on a tensor decomposition algorithm. Our method regards CCIs as hypergraphs and extracts some representative triadic relationship from the data tensor, which includes (i) ligand-expressing cell types, (ii) receptor-expressing cell types, and (iii) L-R pairs.
CCI as hypergraph (CaH) and CCI-tensor

The simplest CCI representation is perhaps a directed graph, where each node represents a cell type and each edge represents the coexpression of all L-R pairs (Figure 1a, left). The direction of the edge is set as ligand → receptor. Such a data structure can also be described as an asymmetric adjacency matrix, in which each row and column represents a ligand and receptor, respectively. If some combinations of cell types are regarded as interacting, corresponding elements of the matrix are filled with 1 and otherwise 0. If the degree of CCI is not a binary relationship, weighted graphs and corresponding weighted adjacent matrices may also be used. The previous analytical methods are categorized within this approach [23, 24, 26–34, 36, 37, 39–41, 44–47, 49, 50, 52, 53, 55].

In contrast, this work describes CCIs as directed hypergraphs (CCI as hypergraph; CaH), where each node is a cell type but the edges are distinguished from each other by the different related L-R pair sets (Figure 1a, right). Such a context-aware edge is called a hyperedge and is described as multiple different adjacency matrices and the set of matrices is called a higher-order matrix or tensor. In contrast with the simple adjacency matrix, tensor contains considerable high-resolution information owing to its higher-order.

Prediction of many-to-many CCIs using tensor decomposition

Tensor data are constructed through the following steps (Figure 1b). Here, a scRNA-seq matrix and the cellular label specifying cell types are supposed to be provided by users. Firstly, Freeman-Tukey transformation [56] (FTT, $\sqrt{x} + \sqrt{x+1}$), which is a variance-stabilizing transformation, is performed to the data matrix. Next, the matrix is converted to a cell-type-level average matrix according to the label. Combined with a L-R database, two corresponding row-vectors of an L-R pair are extracted from the matrix. We originally developed the databases for 12 organisms (for more details, see Additional Files 1, 2, Table 2 and 3). The outer product (direct
product) of the two vectors is then calculated, and a matrix is generated. The matrix can be considered as the similarity matrix of all possible cell-type combinations using the L-R pair. Finally, for each L-R pair, the matrix is calculated, and a tensor is generated as the combined matrices. In this work, this is called the CCI-tensor.

After the construction of a CCI-tensor, we perform non-negative Tucker decomposition (NTD [57, 58]), which is known as a tensor decomposition algorithm. We originally implemented this algorithm and confirmed its convergence within a realistic number of iterations (for more details, see Additional File 3). NTD assumes that the CCI-tensor can be approximated by the summation of some representative CaHs. NTD has the three rank parameters \((R_1, R_2, and R_3)\) and a CaH is calculated as the outer product of the column vectors of three factor matrices \(A^{(1)} \in \mathbb{R}^{J \times R_1}\), \(A^{(2)} \in \mathbb{R}^{J \times R_2}\), and \(A^{(3)} \in \mathbb{R}^{K \times R_3}\) calculated by NTD (Figure 1c). Each CaH-strength is calculated by the core tensor \(G(r_1, r_2, r_3) \in \mathbb{R}^{R_1 \times R_2 \times R_3}\) of NTD. In this work, each CaH is termed \(CaH(r_1, r_2, r_3) = A^{(1)}_{r_1} \circ A^{(2)}_{r_2} \circ A^{(3)}_{r_3} \in \mathbb{R}^{J \times J \times K}\), where \(r_1, r_2,\) and \(r_3\) are the indexes of the columns of three factor matrices. All CaHs are ordered by the size of elements of the core tensor, and the patterns explaining the top 40\% of cumulative core tensor value are reserved as representative CaHs. For more details on CaH, see the Materials and Methods. The CaHs are extracted in a data-driven way without the assumption of one-to-one CCIs. Therefore, it can also detect many-to-many CCIs according to the data complexity.

**Results and discussion**

**Evaluation of multiple CCI prediction**

**Accuracy of the detection of CCIs and the related L-R pairs**

Here, we demonstrate the efficacy of scTensor by using the two simulation datasets (Figure 2a). Three different cell types are indicated as "A", "B", and "C". In the case I dataset, all CCIs represent the one-to-one relationships between two cell types. CCIs corresponding to A \(\rightarrow\) B, B \(\rightarrow\) C, and C \(\rightarrow\) A are colored by red, blue, and
green, respectively. In contrast, the CCIs in the case II dataset represent many-to-
many relationships involving many cell types such as $A \rightarrow B/C$ (red), $C \rightarrow A/B/C$
(blue), and $A/B/C \rightarrow B/C$ (green). To evaluate whether such ground truth combi-
nation of cell types and their related L-R pairs are enriched by \texttt{scTensor}, receiver
operating characteristic (ROC) curves and their corresponding area under the curve
(AUC) values were calculated (Figure 2b). In the analysis of each L-R set, only the
\textit{CaH}s with the maximum AUC value for each ground truth L-R set were regarded
as the corresponding \textit{CaH} being accurately detected by \texttt{scTensor} (Figure 2c, for
more details on the simulation datasets, see the Materials and Methods).

Again, note that the \textit{CaH}s detected by \texttt{scTensor} are not just CCIs, but sets of
CCIs and their related L-R pairs. To the best of our knowledge, the label permu-
tation method implemented in CellPhoneDB [37] is the only previous method that
can detect CCIs and their related L-R pairs simultaneously. To demonstrate the
efficacy of \texttt{scTensor} in terms of the detection of many-to-many CCIs, we also orig-
inally implemented this method and compared it with \texttt{scTensor} (for more details
on the algorithm, see the Materials and Methods). Note that each combination of
a CCI and its related L-R pair is separately extracted by NTD of \texttt{scTensor}, but
under the label permutation method, the combinations are not separated and are
just sorted in ascending order of their $P$-values. Combinations with low $P$-values
indicate significant triadic relationships.

In the case I dataset, ground truth L-R sets are highly enriched according to the
measures of both methods, and the AUC values show that there is no difference
in their performance (Figure 2b and c, left). On the other hand, in the case II
dataset, the label permutation method cannot correctly detect blue or green L-R
sets, and the AUC value becomes lower (Figure 2b, right). In the \texttt{scTensor} analysis,
red, blue, and green L-R sets are separately extracted as three \textit{CaH}s (Figure 2c,
right), and the AUC values are still high. This is because, the label permutation
is implicitly hypothesized the CCI as a one-to-one relationship. Therefore, in the case II dataset, many-to-many CCIs such as the CCIs corresponding to green L-R sets, are hard to detect by the method. This is because for each L-R pair, mean values for any combination of cell types are basically high in such situations, and a $P$-value corresponding to a one-to-one CCI tends to be large (i.e., not significant); accordingly, the observed L-R coexpression and the null distribution calculated are hard to distinguish. In the analysis of real datasets presented later, however, the L-R gene expression pairs are not always the cell-type specific, and it is more natural that the CCI corresponding to the L-R has a many-to-many relationship. This simulation shows that scTensor is a more general method for detecting CCIs and their related L-R pairs at once, irrespective of whether a particular CCI is one-to-one or many-to-many.

**Biological interpretation of real datasets**

To demonstrate the efficacy of scTensor in the analysis of empirical datasets, we applied scTensor to four real scRNA-seq datasets (Table 1). First, we used the scRNA-seq data derived from fetal germ cells (FGCs (♀)) and their gonadal niche cells (Soma) from female human embryos (Germline_Female [25]). As a known CaH pattern, CCIs of Soma with FGCs (♀) involving the BMP signaling pathway are reported, and scTensor accurately detects the CCIs and CCI-related L-R pairs as CaH(4,2,5) (Figure 3). Moreover, scTensor was able to extract some putative CaHs such as CaH(3,4,14) and CaH(1,4,22). CaH(3,4,14) is the autocrine-type CCI within FGCs (♀) and L-R pairs such as Wnt (WNT5A/WNT6) and some growth factor genes (NGF/IGF/FGFR/VEGF), suggesting that this CaH is related to the proliferation and differentiation of Soma. CaH(1,4,22) is the CCI corresponding to FGCs (♀) and Soma, and most of the receptors are related to G protein-coupled receptor (GPCR). The conjugated ligands are some neuropeptides, and this suggests...
that the peptides are related to the activation of GPCR in FGCs (♀) by some mechanism.

We also used the scRNA-seq data derived from FGCs (♂) and their gonadal niche cells from male human embryos (Germline_Male [25]). In contrast to the Germline_Female data, the original study reported that FGCs (♂) interact with Soma through AMH-BMPR interactions, and scTensor also detected the triadic relationship as CaH(1,1,10) (Figure 4). The conjugate receptor AMHR2 is also detected in this CaH. In this dataset, like in Germline_Female, in this data, autocrine-type CCIs such as CaH(3,3,9) and CCIs corresponding to FGCs (♂) and Soma (CaH(1,3,16)) were detected.

Next, we used the scRNA-seq data derived from immune cells isolated from the metastatic melanoma patients (Melanoma [59]). The meta-analysis of scRNA-seq and TCGA datasets in the original published study claimed that T cell abundance and the expression of complement system-related genes in cancer-associated fibroblasts (CAFs) are correlated, and CCIs between T cells and CAFs were therefore inferred. scTensor detected these CCIs as CaH(1,1,3)/CaH(1,1,9) and complement system-related genes such as C3, CXCL12, CFB, and C4A were also detected (Figure 5). scTensor was also able to capture a well-known CCI between T cells and B cells as CaH(2,2,6), although this was not a focus of the original study; this CCI is the antigen-presenting from B cells to T cells by class II major histocompatibility complex (MHC) through the coexpression of CD8 (T cell receptor) and human leukocyte antigen (HLA) genes. scTensor also detected CaH(4,1,13) and CaH(1,4,8), which are the CCIs of macrophages with T cells and an autocrine-type CCI of known chemokine ligands with their receptors.

Finally, we used data derived from non-myocyte cells isolated from mouse heart (NonMyocyte [27]). The original study focused on the CCIs of pericytes/fibroblasts with macrophages through the L-R pairs Il34/Csf1 and Csf1r, and the correspond-
ing CCIs were detected as $CaH(2,2,19)$ by scTensor (Figure 6). scTensor also
detected $CaH(1,2,17)$ and $CaH(3,2,21)$, which are the autocrine-type CCI among
macrophages and the CCI of NK cells with macrophages by known chemokine lig-
ands and their receptors.

Application to minor organism

To demonstrate the applicability of using scTensor in the species that is not
mouse or human, we used a scRNA-seq data derived from zebrafish habenular
neurons (Habenular_Larva [60]). Although the original study did not focus on
the CCIs among the neuronal cell types, scTensor detected some triadic relation-
ships as $CaH(3,3,4)$ (La_Hb01/03/07 with La_Hb02/08), $CaH(2,1,3)$ (La_Hb09 with
La_Hb02/08), and $CaH(1,3,1)$ (La_Hb02/04-06/08/11-15/Olf with La_Hb02/08)
(Figure 7). The spatial distribution of the cell types measured by RNA-fluorescence
in situ hybridization (FISH) shows that the cell-type pairs detected as $CaH(3,3,4)$
and $CaH(2,1,3)$ are dorsally located and proximal to each other in the habenula.
However, $CaH(1,3,1)$ was a more global interaction related to the entire habenula
regions. Although the spatial distribution of rare cell types La_Hb03/05/14 could
not be determined by RNA-FISH in the original study, scTensor was able to as-
sign the conjugated cell types of La_Hb03 as La_Hb02/08 in the dorsal region. This
result suggests that scTensor may also be useful in spatial transcriptomics [61, 62].

scTensor and L-R database implementations as R/Bioconductor packages

All the algorithms and L-R lists are available as R/Bioconductor packages and a
web application described below.

nnTensor and scTensor packages

NTD is implemented as the function of nnTensor R/CRAN package and internally
imported in scTensor. scTensor constructs the CCI-tensor, decomposes the tensor
by NTD, and generates an HTML report. scTensor is assumed to be used with
LRBase.XXX.eg.db, which are the L-R databases for multiple organisms. To enhance the biological interpretation of CaHs, a wide variety of gene information is assigned to the L-R lists through the other R/Bioconductor packages (Figure 8). For example, gene annotation is assigned by biomaRt [63] (Gene Name, Description, Gene Ontology (GO), STRING, and UniProtKB), reactome.db [64] (Reactome) and MeSH.XXX.eg.db [65] (Medical Subject Headings; MeSH), while the enrichment analysis (also known as over-representative analysis; ORA) is performed by GOstats [66] (GO-ORA), meshr [65] (MeSH-ORA), ReactomePA [67] (Reactome-ORA), and DOSE [68] (Disease Ontology; DO, Network of Cancer Gene; NCG, and DisGeNET-ORA). To validate that the detected gene expression of L-R gene pair is also consistently detected in the other data with tissue- or cell-type-level transcriptome data, the hyperlinks to RefEx [69], Expression Atlas [70], Single-Cell Expression Atlas [71], scRNASeqDB [72], PanglaoDB [73] are embedded in the HTML report, facilitating comparisons of the L-R expression with the data from large-scale genomics projects such as GTEx [74], FANTOM5 [75], NIH Epigenomics Roadmap [76], ENCODE [77], and Human Protein Atlas [78]. Additionally, in consideration of users who might want to experimentally investigate detected CCIs, we embedded the hyperlinks to Connectivity Map (CMap [79]), which provides the relationships between perturbation by the addition of particular chemical compounds/genetic reagents and succeeding gene expression change.

LRBase.XXX.eg.db-type packages

For data sustainability and the extension to the wide range of organisms, in this work, we originally constructed L-R databases as R/Bioconductor packages named LRBase.XXX.eg.db (where XXX represents the abbreviation for an organism, such as “Hsa” for Homo sapiens). LRBase.XXX.eg.db currently provides the L-R databases for 12 organisms (Table 2 and 3). The data process pipeline is almost the same as that of the FANTOM5 project for constructing the putative L-R lists.
Precise differences between LRBase.XXX.eg.db and FANTOM5 are summarized in Table S4 in Additional File 1.

**LRBaseDbi package**

All the LRBase.XXX.eg.db packages are generated by LRBaseDbi, which is another R/Bioconductor package. LRBaseDbi generates the LRBase.XXX.eg.db packages from the CSV files, in which NCBI Gene IDs are saved as two columns describing the L-R relationship (we call this function as “meta”-packaging).

In addition to the LRBase.XXX.eg.db packages we summarized, the users may want to specify the user’s original L-R list. For example, there are some other L-R databases such as IUPHAR [80], DLRP [81], FANTOM5 [75], CellPhoneDB [28], and Cell-Cell Interaction Database summarized by Gary Bader et. al. (http://baderlab.org/CellCellInteractions) (Additional File 1). Besides, if the users want to apply the scTensor to minor species, the L-R list may be constructed by the orthologous relationship with major species (e.g., human) [27]. The corresponding LRBase.XXX.eg.db is easily generated from the original L-R list by LRBaseDbi and can be used with scTensor.

**CellCelldb**

All analytical results scTensor are outputted as HTML reports. Hence, combined with a cloud web service such as Amazon Simple Storage Service (Amazon S3), reports can be used as simple web applications, enabling the user to share their results with collaborators or to develop an exhaustive CCI database.

We have already performed scTensor analyses using a wide-variety of scRNA-seq datasets, including the five empirical datasets examined in this study (https://q-brain2.riken.jp/CellCelldb/).
Conclusions

In this work, CCIs were regarded as CaHs, which are hypergraphs that represent triadic relationships, and a novel algorithm scTensor for detecting such CaH was developed. In evaluations with empirical datasets from previous CCI studies, previously reported CCIs were also detected by scTensor. Moreover, some CCIs were detected only by scTensor, suggesting that the previous studies may have overlooked some CCIs. To extend the use of scTensor to a wide range of organisms, we also developed multiple L-R databases as LRBase.XXX.eg.db-type packages. When combined with LRBase.XXX.eg.db, scTensor can currently be applied to 12 organisms.

There are still some plans for improving both LRBase.XXX.eg.db and scTensor to build on the advantages of this current framework. For example, the range of corresponding organisms and the L-R lists can be extended with the spread of genome-wide researches. Additionally, the algorithm can be improved, for example, by utilizing acceleration techniques such as randomized algorithm/sketching methods [82], incremental algorithm/stochastic optimization [83, 84], or distributed computing with MapReduce/Hadoop on large-scale memory machines [85] for NTD, which is now available. Tensors are a very flexible way to represent heterogeneous biological data [86], and easily integrate the side information about genes or cell types with semi-supervised manner. Such information will improve the accuracy and extend the scope of the data.

We aim to tackle such problems and develop the framework further through updates of the R/Bioconductor packages. In the package registration process for R/Bioconductor, package source code is peer-reviewed via the Bioconductor single package builder system and assigned to a curator (https://github.com/Bioconductor/packagebuilder), and even after the package is accepted, the daily package builder tests the source code every day (https://www.bioconductor.org/
checkResults/). Furthermore, biannual updates of Bioconductor require that the internal data of all data packages are updated (https://www.bioconductor.org/developers/release-schedule/). Such strict check systems for source code and internal data improve the sustainability and usability of packages. Our team has maintained over one hundred R/Bioconductor packages since 2015 [65], and we are still organizing a system for the maintenance of the combined LRBase.XXX.eg.db and scTensor framework.

Materials and methods

Construction of L-R list

Public databases

To compare our database with other databases (Additional File 1 and 2), the data from FANTOM5 (http://fantom.gsc.riken.jp/5/suppl/Ramilowski$et$al$_2015/_data/PairsLigRec.txt), DLRP (http://dip.doe-mbi.ucla.edu/dip/dlrp/dlrp.txt), IUPHAR (http://www.guidetopharmacology.org/DATA/interactions.csv), and HPRD (ftp://ftp.ebi.ac.uk/pub/databases/genenames/new/tsv/locus$groups/protein-coding$gene.txt) were downloaded. The subcellular localization data from SWISSPROT and TrEMBL were downloaded from UniProtKB (https://www.uniprot.org/downloads). Protein-protein interaction (PPI) data of STRING (v-10.5) were downloaded from https://stringdb-static.org/cgi/download.pl.

To unify the gene identifier as NCBI Gene ID, we retrieved the corresponding table from Biomart (Ensembl release 92). All the data were downloaded by RESTful access using the wget command and query.xml (http://www.biomart.org/martservice.html).

Simulation datasets

The simulated single-cell gene expression data were sampled from the negative binomial distribution $NB(FC_{gc}m_g,\phi_g)$, where $FC_{gc}$ is the fold-change (FC) for gene $g$ and cell type $c$, and $m_g$ and $\phi_g$ are the average gene expression and the dispersion.
parameter of gene $g$, respectively. For the setting of differentially expressed genes (DEGs) and non-DEGs, $FC_{gc}$ values were calculated based on the non-linear relationship of FC and the gene expression level $\log_{10} FC_{gc} = a \exp(-b \log_{10} (m_g + 1))$ as follows:

$$FC_{gc} = \begin{cases} 10^{4.42 \exp(-0.81 \log_{10} (m_g+1))} & \text{(DEG)} \\ 1 & \text{(non-DEG)} \end{cases}$$

The $m_g$ and gene-wise variance $v_g$ were calculated from the scRNA-seq dataset of human embryonic stem cells (hESCs) measured by Quartz-Seq [87], and the gene-wise dispersion parameter $\phi_g$ was estimated as $\phi_g = (v_g - m_g)/m_g^2$. The NB distribution reduces to Poisson when $\phi_g = 0$. To simulate the “dropout” phenomena of scRNA-seq experiments, we introduced the dropout probability $p_{\text{dropout}_{gc}} = \exp(-cFC_{gc}m_g^2)$, which is used in ZIFA [88] (default: $c=1$), and the expression values were randomly converted to 0 based on this dropout probability.

For the setting of the case I datasets, $150 \times 150 \times 500$ CCI-tensor was constructed. For each cell type, 50 cells were established, and in total, three cell types were set. For L-R set 1 (red), 50 L-R pairs were established, and the cell-type-wise ligand and receptor patterns were (1,0,0) and (0,1,0), respectively (0, non-DEG; 1, DEG). For L-R set 2 (blue), 50 L-R pairs were established, and the cell-type-wise ligand and receptor patterns were (0,1,0) and (0,0,1), respectively. For L-R set 3 (green), 50 L-R pairs were established, and the cell-type-wise ligand and receptor patterns were (0,0,1) and (1,0,0), respectively. The other 350 L-R pairs were sampled randomly as non-DEGs.

For the setting of the case II datasets, $150 \times 150 \times 500$ CCI-tensor were constructed. For each cell type, 50 cells were established, and in total, three cell types were established. For L-R set 1 (red), 50 L-R pairs were established, and the cell-
type-wise ligand and receptor patterns were (1,0,0) and (0,1,1), respectively. For
L-R set 2 (blue), 50 L-R pairs were established, and the cell-type-wise ligand and
receptor patterns were (0,0,1) and (1,1,1), respectively. For L-R set 3 (green), 50
L-R pairs were established, and the cell-type-wise ligand and receptor patterns were
(1,1,1) and (1,0,1), respectively. The other 350 L-R pairs were sampled randomly
as non-DEGs.

Real datasets

The gene expression matrix and cellular labels for Germline_Female and Germline_Male
scRNA-seq data were retrieved from the GEO database (GSE86146), and only
highly variable genes (HVGs: http://pklab.med.harvard.edu/scw2014/subpop_tutorial.html)
with low \( P \)-values (\( \leq 1E-7 \)) were extracted. The gene expression matrix and cel-
lar label of Melanoma scRNA-seq data were retrieved from the GEO database
(GSE72056), and only HVGs with low \( P \)-values (\( \leq 1E-10 \)) were extracted. The gene
symbols were converted to NCBI GeneIDs using the R/Bioconductor Homo.sapiens
package. The gene expression matrix and cellular labels for NonMyocyte scRNA-
seq data were retrieved from the ArrayExpress database (E-MTAB-6173), and only
HVGs with low \( P \)-values (\( \leq 1E-10 \)) were extracted. The gene symbols are con-
verted to NCBI GeneIDs using the R/Bioconductor Mus.musculus package. For
each dataset, t-Distributed Stochastic Neighbor Embedding (t-SNE) with 40 per-
plexity was performed using the Rtsne R package.

scTensor algorithm details

Construction of CCI-tensor

Here we assume that a data matrix \( \mathbf{Y} \in \mathbb{R}^{I \times H} \) is the gene expression matrix of
scRNA-seq, where \( I \) is the number of genes and \( H \) is the number of cells. Next, the
matrix \( \mathbf{Y} \) is converted to cell-type mean matrix \( \mathbf{X} \in \mathbb{R}^{I \times J} \), where \( J \) is the number
of mean vectors for each cell type. The cell-type label is supposed to be specified by
user’s prior analysis such as clustering or confirmation of marker gene expression.
The relationship between the $X$ and $Y$ is described as below:

$$X = YA,$$  \hfill (1) 

where the matrix $A \in \mathbb{R}^{H \times J}$ converts cellular-level matrix $Y$ to cell-type-level matrix $X$ and each element of $A$ is

$$A_{hj} = \begin{cases} 1/n_j & (h\text{-th cell belongs to } j\text{-th cell type}) \\ 0 & \text{(otherwise)}, \end{cases}$$

where $n_j$ is the number of cells belonging to $j$’s cell type.

Next, NCBI gene IDs of each L-R pair stored in LRBase.XXX.gene.db are searched in the row names of matrix $X$ and if both IDs are found, corresponding $J$-length row-vectors of the ligand and receptor genes ($x_L$ and $x_R$) are extracted.

Finally, a $J \times J$ matrix is calculated as the outer product of $x_L$ and $x_R$ and incrementally stored as a sub-tensor (frontal slice) of the CCI-tensor $\chi \in \mathbb{R}^{J \times J \times K}$ as below:

$$\chi::k = x_{L(k)} \circ x_{R(k)}$$  \hfill (2) 

where $K$ is the number of L-R pairs found in the row names of matrix $X$.

**CANDECOMP/PARAFAC and Tucker decomposition**

Here, we suppose that the CCI-tensor has some representative triadic relationship. To extract the triadic relationships from a CCI-tensor, here we consider performing some tensor decomposition algorithms. There are two typical decomposition methods; CANDECOMP/PARAFAC (CP) and Tucker decomposition [57, 58].
In CP decomposition, CCI-tensor $\chi$ is decomposed as follows:

$$\chi = \Lambda \times_1 A^{(1)} \times_2 A^{(2)} \times_3 A^{(3)}$$

$$= \sum_{r=1}^{R} \lambda_r A^{(1)}_r \odot A^{(2)}_r \odot A^{(3)}_r$$

subject to $\|A^{(1)}_r\| = \|A^{(2)}_r\| = \|A^{(3)}_r\| = 1,$

where $\times_n$ is mode-$n$ product, $R$ is the rank of $\chi$, and $\Lambda$ is diagonal cubical tensor, in which the element $\lambda_r$ on the superdiagonal can be non-zero. $A^{(1)} \in \mathbb{R}^{J \times R}$, $A^{(2)} \in \mathbb{R}^{J \times R}$, and $A^{(3)} \in \mathbb{R}^{K \times R}$ are factor matrices. $a_r \odot b_r \odot c_r$ is rank-1 tensor, and the scalar $\lambda_r$ is the size of rank-1 tensor. The rank-1 tensor indicates the triadic relationship described above, and CP model suppose that CCI-tensor is approximated by the summation of $R$ rank-1 tensor. There are some algorithms for optimizing CP decomposition problem such as alternative least squares (ALS) or power method [58]. Despite its wide use, CP decomposition has a drawback when using the problem in this work; the number of columns of three factor matrices must be a common number $R$, and the correspondence of $A^{(1)}_{r_1}$, $A^{(2)}_{r_2}$, and $A^{(3)}_{r_3}$ in each $r$ is one-to-one. This constraint is sometimes too strict and unnatural for biological applications. For example, in the CCI-tensor case, the number of ligand expression, receptor expression, and L-R-pair patterns are commonly $R$, and all of them must correspond to each other in each $r$. Thus, if an L-R pair assigned in a CCI, this L-R pair cannot be part of other CCIs.

To deal with this problem, the application of Tucker decomposition can be considered next. In Tucker decomposition, a CCI-tensor is decomposed as follows:

$$\chi = G \times_1 A^{(1)} \times_2 A^{(2)} \times_3 A^{(3)}$$

$$= \sum_{r_1=1}^{R_1} \sum_{r_2=1}^{R_2} \sum_{r_3=1}^{R_3} G(r_1, r_2, r_3) A^{(1)}_{r_1} \odot A^{(2)}_{r_2} \odot A^{(3)}_{r_3}$$

subject to $\|A^{(1)}_{r_1}\| = \|A^{(2)}_{r_2}\| = \|A^{(3)}_{r_3}\| = 1,$
where \( R_1, R_2, \) and \( R_3 \) are the rank of mode-1, 2, and 3, respectively. Unlike the CP model, the constraint conditions of the Tucker model are relaxed, that is, three factor matrices \( A^{(1)} \in \mathbb{R}^{J \times R_1}, A^{(2)} \in \mathbb{R}^{J \times R_2}, \) and \( A^{(3)} \in \mathbb{R}^{K \times R_3} \) can differ in their numbers of columns and any combination of \( A^{(1)}_{r_1}, A^{(2)}_{r_2}, \) and \( A^{(3)}_{r_3} \) can be considered. This is because \( G \in \mathbb{R}^{R_1 \times R_2 \times R_3} \) is a dense core tensor and any element, including non-diagonal elements, can have a non-zero value. There are some algorithms for optimizing Tucker decomposition, such as higher order singular value decomposition (HOSVD) or higher orthogonal iteration of tensors (HOOI) [58].

**Non-negative Tucker decomposition**

Despite its effectiveness, Tucker decomposition cannot be directly applied to the extraction of CaHs. This is because the factor matrices of the Tucker model can have negative value elements, and these make interpretation difficult. For example, if \( A^{(1)}_{r_1} \) contains very large positive elements and very small negative elements, we cannot determine which cell type is highly related to a ligand expression pattern and which cell type is not.

For the above reason, here we utilize NTD. Unlike Tucker decomposition based on singular value decomposition (SVD), NTD is based on non-negative matrix factorization (NMF), which is another matrix decomposition method. NMF is formalized as follows:

\[
X = WH
\]

subject to \( W \geq 0, \ H \geq 0. \) (5)

The typical algorithm for optimizing the NMF problem is multiplicative updating (MU) [58]. Two widely used forms are considered. The first form is minimization problem of Euclidean distance (min\(\|X - WH\|_{Euclid}\)), where \( H \) and \( W \) are iter-
ately updated by considering Gaussian noise:

\[
H \leftarrow H \ast \frac{W^T X}{W^T W H} \quad \text{and} \quad W \leftarrow W \ast \frac{X H^T}{W H H^T},
\]

where \( \ast \) is the element-wise (Hadamard) product. The second form is a minimization problem of Kullback-Leibler (KL) divergence \((\min \|X - WH\|_{KL})\), where \(H\) and \(W\) are iteratively updated by considering Poisson noise:

\[
H \leftarrow H \ast \frac{W^T X}{W^T W H} \quad \text{and} \quad W \leftarrow W \ast \frac{X H^T}{W H H^T}.
\]

These update rules are derived from the element-wise gradient descent with the spatial form of the learning rate [58]. Starting with a random non-negative initial value, update of \(W\) and \(H\) are updated iteratively until convergence. In this work, MU with the KL-form, which shows a stable convergence with simulation data, is used for following initialization step of NTD (for more details, see Additional File 3).

To extend the KL form of MU to NTD, we consider iterative updating \(A^{(n)} \circ_n A^{(-n)T}\), which is the matricized expression of Tucker decomposition. Here \(A^{(-n)}\) represents the factor matrices without \(A^{(n)}\). For example, if \(n=1\), this part becomes \(A^{(2)T} A^{(3)T}\). By considering a part of \(A^{(n)} \circ_n A^{(-n)T}\) as a variable and fixing other parts as constants, the KL form of MU can be performed to the matricized tensor, such as \(A^{(1)} \rightarrow A^{(2)} \rightarrow A^{(3)} \rightarrow \mathcal{G} \rightarrow \cdots\). Each updating rule for \(A^{(n)}\) is as follows:

\[
A^{(n)} \leftarrow A^{(n)} \ast \frac{X^{(n)} \circ_n G^{(n)T}}{12_z^T}.
\]

(8)
Additionally, the updating rule for core tensor $G$ is:

$$
G^{(n)} \leftarrow \max \{ \chi \times _1 A^{(1)T} \times _2 A^{(2)T} \times _3 A^{(3)T}, \epsilon \}
$$

$$
G^{(n)} \leftarrow \frac{\chi \times _1 A^{(1)T} \times _2 A^{(2)T} \times _3 A^{(3)T} \times _1 A^{(1)} \times _2 A^{(2)} \times _3 A^{(3)} A^{(3)} A^{(2)} A^{(1)} }{G \times _1 A^{(1)T} A^{(1)} \times _2 A^{(2)T} A^{(2)} \times _3 A^{(3)T} A^{(3)} }.
$$

where $\epsilon$ is a small value included to avoid generating negative values. In the

nnTensor, $1e-10$ is used.

**Extraction of CCIs as hypergraphs**

To extract the representative CaHs, scTensor estimates the NTD ranks by SVD performed for each matricized CCI-tensor ($X^{(n)}$, n=1,2, and 3). The eigenvalues and eigenvectors that explain the top 80% to 90% of variance are selected. With the estimated ranks of NTD ($\hat{R}_1, \hat{R}_2, \hat{R}_3$), NTD is performed, and only the triads (r1,r2,r3) with large core tensor values are selected as representative CaHs. In its default mode, scTensor selects the CaHs that explain the top 40% of cumulative core tensor values. For each CaH, corresponding column vectors of factor matrices were selected as $CaH(r_1, r_2, r_3) = A^{(1)}_{r_1} \circ A^{(2)}_{r_2} \circ A^{(3)}_{r_3}$.

To enhance the interpretation, each column vector is binarized in advance by two-class hierarchical clustering using Ward’s minimum variance method, and only large values are converted to 1, with other values becoming 0.

CCI-strength (cf. Figure 3, 4, 5, 6, 7) is calculated as the summation of mode-3 of reconstructed tensor from all CaHs. With the selected indexes in each mode $(r_1' \in \{1..\hat{R}_1\}, r_2' \in \{1..\hat{R}_2\}, r_3' \in \{1..\hat{R}_3\})$, CCI-strength is defined as follows:

$$
\text{CCI-strength}(i,j) = \sum_{k=1}^{K} \left[ \sum_{r_1=1}^{\hat{R}_1} \sum_{r_2=1}^{\hat{R}_2} \sum_{r_3=1}^{\hat{R}_3} G(r_1, r_2, r_3) A^{(1)}_{r_1} \circ A^{(2)}_{r_2} \circ A^{(3)}_{r_3} \right]_{i,j,k}
$$

(10)
Label permutation method

In this method, the cluster labels of all cells are randomly permuted 1000 times, and the average ligand expression level of a cluster and the average receptor expression level of a cluster are calculated [37]. For each L-R pair, the mean values of the averaged L-R expression level are calculated in all possible combinations of the cell types. This process generates 1,000 of synthetic L-R coexpression matrices and these are used to generate the null distribution, that is, in a combination of cell types, the proportion of the means which are “as or more extreme” than the observed mean is the calculated as $P$-value.

Availability and requirements

- scTensor: https://bioconductor.org/packages/devel/bioc/html/scTensor.html
- nnTensor: https://cran.r-project.org/web/packages/nnTensor/index.html
- LRBase.Hsa.eg.db: https://bioconductor.org/packages/release/bioc/html/LRBase.Hsa.eg.db.html
- LRBase.Mmu.eg.db: https://bioconductor.org/packages/release/bioc/html/LRBase.Mmu.eg.db.html
- LRBase.Ath.eg.db: https://bioconductor.org/packages/release/bioc/html/LRBase.Ath.eg.db.html
- LRBase.Rno.eg.db: https://bioconductor.org/packages/release/bioc/html/LRBase.Rno.eg.db.html
- LRBase.Bta.eg.db: https://bioconductor.org/packages/release/bioc/html/LRBase.Bta.eg.db.html
- LRBase.Cel.eg.db: https://bioconductor.org/packages/release/bioc/html/LRBase.Cel.eg.db.html
- LRBase.Dme.eg.db: https://bioconductor.org/packages/release/bioc/html/LRBase.Dme.eg.db.html
• LRBase.Dre.eg.db: [https://bioconductor.org/packages/release/bioc/html/LRBase.Dre.eg.db.html](https://bioconductor.org/packages/release/bioc/html/LRBase.Dre.eg.db.html)

• LRBase.Gga.eg.db: [https://bioconductor.org/packages/release/bioc/html/LRBase.Gga.eg.db.html](https://bioconductor.org/packages/release/bioc/html/LRBase.Gga.eg.db.html)

• LRBase.Pab.eg.db: [https://bioconductor.org/packages/release/bioc/html/LRBase.Pab.eg.db.html](https://bioconductor.org/packages/release/bioc/html/LRBase.Pab.eg.db.html)

• LRBase.Xtr.eg.db: [https://bioconductor.org/packages/release/bioc/html/LRBase.Xtr.eg.db.html](https://bioconductor.org/packages/release/bioc/html/LRBase.Xtr.eg.db.html)

• LRBase.Ssc.eg.db: [https://bioconductor.org/packages/release/bioc/html/LRBase.Ssc.eg.db.html](https://bioconductor.org/packages/release/bioc/html/LRBase.Ssc.eg.db.html)

• LRBaseDbi: [https://bioconductor.org/packages/release/bioc/html/LRBaseDbi.html](https://bioconductor.org/packages/release/bioc/html/LRBaseDbi.html)

• Operating system: Linux, Mac OS X, Windows

• Programming language: R (v-3.5.0 or higher)

• License: Artistic-2.0

• Any restrictions to use by non-academics: For non-profit use only

Abbreviations

CCI: cell-cell interaction; scRNA-seq: single-cell RNA sequencing; L-R: ligand and receptor; CaH: CCI as hypergraph; FTT: Freeman-Tukey transformation; NTD: non-negative Tucker decomposition; ROC: receiver operating characteristic; AUC: area under the curve; FGCs: fetal germ cells; Soma: gonadal niche cells; GPCR: G protein-coupled receptor; CAFs: cancer-associated fibroblasts; MHC: major histocompatibility complex; HLA: human leukocyte antigen; RNA-FISH: RNA-fluorescence in situ hybridization; PM: plasma membrane; PPI: protein-protein interaction; GO: Gene Ontology; MeSH: medical subject headings; ORA: over-representative analysis; DO: Disease Ontology; NCG: network of cancer gene; CMap: Connectivity Map; Amazon S3: Amazon simple storage service; FC: fold-change; DEGs: differentially expressed genes; human embryonic stem cells: hESCs; HVGs: highly variable genes; t-SNE: t-distributed stochastic neighbor embedding; CP: CANDECOMP/PARAFAC; ALS: alternative least squares; HOSVD: higher order singular value decomposition; HOOI: higher orthogonal iteration of tensors; NMF: non-negative matrix factorization; MU: multiplicative updating; KL: Kullback-Leibler

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KT and IN designed the study. KT designed the algorithm and benchmark, retrieved and preprocessed the test data to evaluate the proposed method, implemented the source code, and performed all analyses. MI implemented the pipeline for bi-annual automatic update of the R/Bioconductor packages. All authors have read and approved the manuscript.

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Figure 1  CCI as a hypergraph  

(a) Previous studies have regarded CCIs as graphs, and the corresponding data structure is expressed as an adjacency matrix (left). In this work, CCIs are regarded as hypergraphs, and the corresponding data structure is a tensor.  

(b) The CCI-tensor is generated by users’ scRNA-Seq matrices, cell-type labels, and L-R databases. NTD is used to extract CaHs from the CCI-tensor.  

(c) Each CaH$(r_1,r_2,r_3)$ is equal to the outer product of three vectors. $A_{r_1}^{(1)}$ represents the ligand expression pattern, $A_{r_2}^{(2)}$ represents the receptor expression pattern, and $A_{r_3}^{(3)}$ represents the related L-R pairs pattern.
Figure 2 Comparison between the label permutation method and scTensor using simulated datasets (a) Two datasets simulating two cases were constructed. In the first case, all CCIs consisted of one-to-one relationships between two cell types. In the second case, CCIs consisted of many-to-many relationships involving many cell types. (b) The ROC curve for the label permutation method. (c) The ROC curves of the CaHs detected by scTensor.
Figure 3  Results of scTensor for an empirical dataset (Germline_Female) (a) Detected CaHs. (b) Overview of the L-R patterns.
Figure 4 Results of scTensor for an empirical dataset (Germline_Male) (a) Detected CaHs. (b) Overview of the L-R patterns.
Figure 5 Results of scTensor for an empirical dataset (Melanoma) (a) Detected CaHs. (b) Overview of the L-R patterns.
Figure 6 Results of scTensor for an empirical dataset (NonMyocyte) (a) Detected CaHs. (b) Overview of the L-R patterns.
Figure 7 Results scTensor for an empirical dataset (Habenular_Larva) (a) Detected CaHs. (b) Overview of the L-R patterns.
Figure 8 Correspondence table containing the available L-R gene information for each organism. For each detected CaH, gene annotation, enrichment analysis, tissue- or cell-type-specific gene expression, and chemical-gene expression relationships are assigned.
### Tables

#### Table 1 Empirical datasets

| Name                      | Organisms      | ID         | # Gene | # Cell | # Cell type | Unit   |
|---------------------------|----------------|------------|--------|--------|-------------|--------|
| Germline_Female [25]     | *Homo sapiens* | GSE86146   | 2717   | 992    | 8           | TPM    |
| Germline_Male [25]       | *Homo sapiens* | GSE86146   | 2790   | 852    | 7           | TPM    |
| Melanoma [59]            | *Homo sapiens* | GSE72056   | 6603   | 2250   | 6           | TPM    |
| NonMyocyte [27]          | *Mus musculus* | E-MTAB-6173 | 2587  | 10519  | 12          | UMI count |
| Habenular_Larva [60]     | *Danio rerio*  | GSE109158  | 15206  | 4233   | 16          | UMI count |

#### Table 2 Summary of LBBase.XXX.eg.db for 12 organisms (1/2)

| XXX | Organisms                  | SWISSPROT (Secreted / Membrane) | TrEMBL (Secreted / Membrane) | STRING (PPI) |
|-----|----------------------------|---------------------------------|------------------------------|---------------|
| Hsa | *Homo sapiens*             | 1592 / 2269                     | 176 / 334                    | 18838         |
| Mmu | *Mus musculus*             | 1309 / 1806                     | 325 / 1555                   | 19715         |
| Ath | *Arabidopsis thaliana*     | 1260 / 1001                     | 244 / 80                     | 24174         |
| Rno | *Rattus norvegicus*        | 643 / 983                       | 232 / 1229                   | 19963         |
| Bta | *Bos taurus*               | 517 / 448                       | 192 / 390                    | 18349         |
| Cel | *Caenorhabditis elegans*   | 198 / 247                       | 28 / 60                      | 13545         |
| Dme | *Drosophila melanogaster*  | 249 / 333                       | 89 / 148                     | 11903         |
| Dre | *Danio rerio*              | 119 / 169                       | 318 / 376                    | 21746         |
| Gga | *Gallus gallus*            | 175 / 173                       | 185 / 154                    | 13084         |
| Pab | *Pongo abelii*             | 80 / 134                        | 212 / 211                    | 16691         |
| Xtr | *Xenopus Silurana tropicalis* | 57 / 83                     | 141 / 114                    | 15338         |
| Ssc | *Sus scrofa*               | 223 / 153                       | 202 / 445                    | 18683         |

#### Table 3 Summary of LBBase.XXX.eg.db for 12 organisms (2/2)

| XXX | # L-R Pairs (SWISSPROT × STRING) | # L-R Pairs (TrEMBL × STRING) |
|-----|---------------------------------|-------------------------------|
| Hsa | 21882                           | 472                           |
| Mmu | 16386                           | 476                           |
| Ath | 8697                            | 94                            |
| Rno | 5270                            | 68                            |
| Bta | 2220                            | 237                           |
| Cel | 106                             | 1                             |
| Dme | 384                             | 9                             |
| Dre | 99                              | 432                           |
| Gga | 140                             | 105                           |
| Pab | 34                              | 184                           |
| Xtr | 19                              | 107                           |
| Ssc | 277                             | 130                           |
Additional Files

1. Additional file 1 — Development of L-R databases for multiple organisms (PDF 2.2 MB)
2. Additional file 2 — Distributions of and correlations among 8 STRING-scores (ZIP 18.5 MB)
3. Additional file 3 — Convergence of NTD with toy model and empirical data (PDF 9.5 MB)