RCy3: Network biology using Cytoscape from within R
[version 3; peer review: 3 approved]

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Abstract
RCy3 is an R package in Bioconductor that communicates with Cytoscape via its REST API, providing access to the full feature set of Cytoscape from within the R programming environment. RCy3 has been redesigned to streamline its usage and future development as part of a broader Cytoscape Automation effort. Over 100 new functions have been added, including dozens of helper functions specifically for intuitive data overlay operations. Over 40 Cytoscape apps have implemented automation support so far, making hundreds of additional operations accessible via RCy3. Two-way conversion with networks from igraph and graph ensures interoperability with existing network biology workflows and dozens of other Bioconductor packages. These capabilities are demonstrated in a series of use cases involving public databases, enrichment analysis pipelines, shortest path algorithms and more. With RCy3, bioinformaticians will be able to quickly deliver reproducible network biology workflows as integrations of Cytoscape functions, complex custom analyses and other R packages.

Keywords
Bioconductor, Cytoscape, Automation, Scripting, Network biology

This article is included in the Bioinformatics gateway.
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Author roles: Gustavsen JA: Methodology, Software, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Pai S: Conceptualization, Methodology, Software, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Isserlin R: Conceptualization, Methodology, Software, Validation, Visualization, Writing – Review & Editing; Demchak B: Conceptualization, Funding Acquisition, Project Administration, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Pico AR: Conceptualization, Funding Acquisition, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: AP, BD, RI and SP were supported by NIGMS P41GM103504 for the National Resource for Network Biology JAG was supported by Google Summer of Code.

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How to cite this article: Gustavsen JA, Pai S, Isserlin R et al. RCy3: Network biology using Cytoscape from within R [version 3; peer review: 3 approved] F1000Research 2019, 8:1774 https://doi.org/10.12688/f1000research.20887.3

First published: 18 Oct 2019, 8:1774 https://doi.org/10.12688/f1000research.20887.1
Introduction
In the domain of biology, network models serve as useful representations of interactions, whether social, neural or molecular. Since 2003, Cytoscape has provided a free, open-source software platform for network analysis and visualization that has been widely adopted in biological and biomedical research fields. The Cytoscape platform supports community-developed extensions, called apps, that can access third-party databases, offer new layouts, add analytical algorithms, support additional data types, and much more.

In 2011, the CytoscapeRPC app was created to enable R-based workflows to exercise Cytoscape v2 functionality via functions in the corresponding RCytoscape R package over XML-RPC communications protocols. In 2015, the CyREST app was created to enable R-based workflows to exercise Cytoscape v3 functionality. This was achieved by the first version of the RCy3 R package, which re-implemented much of RCytoscape’s organization, data structures and syntax over REST communications protocols.

Here, we describe version 2.0 of the RCy3 package, which is better aligned with Cytoscape’s CyREST API. We have rewritten every function, deprecated 43 functions and added over 100 new functions. This work provides a more intuitive and productive experience for Cytoscape users learning about the RCy3 package, and it positions RCy3 to take advantage of future Cytoscape Automation development and evolution. The goal of this paper is to describe the implementation and operation of the updated RCy3 package and to provide detailed use cases relevant to network biology applications in common and advanced bioinformatics workflows.

Methods
Design and Implementation
RCy3 is a component of Cytoscape Automation. At the core of Cytoscape Automation is CyREST, which implements an interface to the Cytoscape Java application via the REST protocol. A collection of GET, POST, PUT and DELETE operations practically cover the complete feature set of the Cytoscape desktop software. Additional features, including those provided by user-installed Cytoscape apps, are covered by a separate command-line interface called Commands (Figure 1). For version 2.0, we redesigned the RCy3 package to parallel CyREST and Commands APIs to standardize the syntax and organization of its functions and streamline its future development. RCy3 functions are grouped into categories to aid the parallel development with Cytoscape Automation APIs and to facilitate navigation and comprehension of the overall package (Table 1).

Figure 1. The Cytoscape Automation stack. The Java desktop version of Cytoscape (bottom) supports Commands (red) and CyREST (green) interfaces for scripting and automation either directly (blue) or via R and Python harmonization libraries (purple).
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<thead>
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<th>Category</th>
<th>Description</th>
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The basics

All RCy3 functions are ultimately implemented as calls to a small set of utility functions that execute the CyREST or Commands REST protocol (e.g., cyrestGET and commandsPOST). The internals of these functions handle the composition of operations and parameters to be sent via hitr functions to CyREST, as well as the processing of results from JSON to appropriate R objects using RJSONI.

In most RCy3 functions there is an optional argument for base.url. This is the URL used by RCy3 to connect to the Cytoscape desktop application via CyREST, and it defaults to "http://localhost:1234/v1". The default CyREST port is 1234, and it can be changed in Cytoscape through Edit/Preferences/Properties or by command-line (see CyREST setup guide). If you change the CyREST port, you should reflect the change in the base.url argument per function call or change each function’s default value using the default package.

The second most common argument in RCy3 functions is network. If left as NULL (default), the currently selected network in the Cytoscape application is referenced. Network name or SUID (session unique identifier) can thus be explicitly specified or inferred from the current state of the application. The current network can also be controlled and retrieved by setCurrentNetwork and getCurrentNetwork. Given a base.url and network (when needed), the majority of RCy3 functions simply validate parameters and construct arguments in order to call one of the cyrest* or commands* functions.

The commandsRun function is a special RCy3 function that allows users to directly issue commands via Cytoscape’s command-line syntax (e.g., "node list network=current"), including commands implemented by Cytoscape app developers (see Use cases). This single function can perform hundreds of operations made available by both Cytoscape and automation-enabled apps. Over 40 of these RCy3-supported apps are currently registered in the Cytoscape App Store. The cyrestAPI and commandsAPI open interactive Swagger documentation for the CyREST and Commands programmatic interfaces. Cytoscape Automation can be performed via these Swagger web pages. The same operations and parameters are supported by the cyrest* and commands* functions in RCy3. Command-line syntax can also be run from the Automation panel in Cytoscape, at manual.cytoscape.org/en/stable/Command_Tool.html.

Generic and specific

The primary goal of RCy3 is to provide wrappers for every feature made available by CyREST and Commands. However, we also have a secondary goal of providing useful and intuitive functions for common workflows in R. So, in addition to the generic functions implemented to parallel the CyREST and Commands APIs, we have also implemented sets of specific helper functions.

As an example, consider the common Cytoscape operation of mapping network data values to visual style properties. CyREST has a POST endpoint for /styles/{style name}/mappings that takes a JSON data structure defining the mapping. We implemented updateStyleMapping which takes a style.name and mapping arguments and sends them out via cyrestPOST. We also implemented mapVisualProperty to help construct the mapping argument. With these generic functions one can perform any of the hundreds of visual style mappings supported by Cytoscape, including new ones added in the future. However, these functions are not simple to use, requiring knowledge of specific property names, like "NODE_FILL_COLOR", and mapping data structures. To simplify usage for common situations, we therefore also implemented specific functions for over a dozen of the most commonly used mappings (e.g., setNodeColorMapping). With autocomplete in tools like RStudio, after just typing setNode... a script author is presented with a series of intuitively named functions with obvious arguments.

Networks in R

Networks are a popular visualisation option in R often implemented as graph models by igraph and Biocondutor’s graph (i.e., graphNEL). RCy3 can create networks in Cytoscape from either igraph, graphNEL or dataframe objects (createNetworkFrom*). Likewise, igraph and graphNEL objects can be created from networks (create*FromNetwork), and dataframes from node and edge tables in Cytoscape (getTableColumns).

In the case of createNetworkFromDataFrames, two dataframes are accepted as arguments, one for nodes and one for edges. The nodes dataframe must include a column named "id", and the edges
dataframe must include "source" and "target" columns. Additional columns are imported as node and edge attributes into Cytoscape. The function can also work with just one dataframe. If a dataframe of only edges is passed to createNetworkFromDataFrames, then a connected network will be created with all of the nodes. If a dataframe of only nodes is passed, then a network with no connections, only nodes, will be created.

RCy3 can also import network file formats supported by Cytoscape natively (e.g., SIF, xGMML and CX) and via user-installed apps (e.g., GPML and adjacency matrices). With these functions RCy3 can interoperate with any other Bioconductor packages that deal with networks in a standardized manner, providing advanced network visualization options and advanced network analytics from the Cytoscape ecosystem (see Table 2).

<table>
<thead>
<tr>
<th>Package</th>
<th>Description</th>
<th>Network model</th>
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<td>graph</td>
<td>Essential graph data structures</td>
<td>graphNEL</td>
<td>✔</td>
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<tr>
<td>RBGL</td>
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</tr>
<tr>
<td>Rgraphviz</td>
<td>Plotting with graphviz library</td>
<td>graphNEL</td>
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<tr>
<td>pathview</td>
<td>Data integration and visualization on pathways</td>
<td>graphNEL</td>
<td>✔</td>
</tr>
<tr>
<td>KEGGgraph</td>
<td>Interface between KEGG pathways and graphs</td>
<td>graphNEL</td>
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<tr>
<td>Gostats</td>
<td>Tools for interacting with Gene Ontology</td>
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<tr>
<td>TCGAbiolinks</td>
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<td>igraph</td>
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<tr>
<td>graphite</td>
<td>Graph objects from pathway databases</td>
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<tr>
<td>minet</td>
<td>Infers mutual information networks</td>
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<td>SPIA</td>
<td>Signaling pathway impact analysis</td>
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<tr>
<td>RDGAVIDWebService</td>
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<td>STRINGdb</td>
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<td>GENIE3</td>
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<td>BioNet</td>
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<td>GDCRNATools</td>
<td>Integrative analysis of IncRNA, mRNA and miRNA</td>
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<td>✔</td>
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<tr>
<td>CEMiTool</td>
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</tr>
<tr>
<td>Linnorm</td>
<td>Linear model and normality based transformation</td>
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<tr>
<td>RedeR</td>
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<td>RTN</td>
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<tr>
<td>rWikiPathways</td>
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<td>hypergraph</td>
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<tr>
<td>paxtoolsr</td>
<td>Access to BioPAX models in Pathway Commons</td>
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<td>netbiov</td>
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<td>igraph</td>
<td>✔</td>
</tr>
<tr>
<td>rsbml</td>
<td>Link to libsbml for SBML parsing</td>
<td>graphNEL</td>
<td>✔</td>
</tr>
<tr>
<td>ndexr</td>
<td>Interface to NDEx servers</td>
<td>igraph, CX</td>
<td>✔</td>
</tr>
</tbody>
</table>
Operation

In order to work with **RCy3** you must have Cytoscape v3.7 or later installed and running. Cytoscape can be installed from cytoscape.org. The RCy3 package can be installed from Bioconductor:

```r
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install("RCy3")
library(RCy3)
```

Launch Cytoscape and keep it running whenever using RCy3. Confirm that you have everything installed and that RCy3 is communicating with Cytoscape via CyREST:

```r
cytoscapePing()
# [1] "You are connected to Cytoscape!"
```

As with any R package, one can access the documentation and browse over a dozen vignettes included in the RCy3 package:

```r
help(package=RCy3)
browseVignettes("RCy3")
```

Use cases

The following sections demonstrate a variety of common and advanced network biology use cases as runnable R code snippets. The code for these use cases is also available as an online Rmd notebook and Rmd file in the Cytoscape Automation repository (see *Data availability*). The first set focuses on fundamental Cytoscape operations that are common to most use cases:

- Loading networks (from R objects, Cytoscape files and public databases)
- Visualizing network data
- Filtering by node degree or data
- Saving and exporting networks

Additionally, there are examples that demonstrate analytical workflows, relying not only on Cytoscape, but also on Cytoscape apps and other R packages:

- Building maps of enrichment analysis results using EnrichmentMap and AutoAnnotate
- Visualizing integrated network analysis using BioNet
- Performing advanced graph analytics using RBGL

**Loading Networks.** Networks come in all shapes and sizes, in multiple formats from multiple sources. The following code snippets demonstrate just a few of the myriad ways to load networks into Cytoscape using **RCy3**.

```r
# From graph objects (graphNEL)
g <- makeSimpleGraph()
createNetworkFromGraph(g)
## And round-trip back from Cytoscape to graph
g2 <- createGraphFromNetwork()

# From igraph objects
library(igraph)
ig <- igraph::make_graph("Zachary")
createNetworkFromIgraph(ig)
## And round-trip back from Cytoscape to igraph
ig2 <- createIgraphFromNetwork()
## Note that the Cytoscape model infers directionality
```
# From dataframes
nodes <- data.frame(id=c("node 0","node 1","node 2","node 3"),
group=c("A","A","B","B"), #categorical strings
score=as.integer(c(20,10,15,5)), #integers
stringsAsFactors=FALSE)

edges <- data.frame(source=c("node 0","node 0","node 0","node 2"),
target=c("node 1","node 2","node 3","node 3"),
interaction=c("inhibits","interacts","activates","interacts"), #optional
weight=c(5.1,3.0,5.2,9.9), #numeric
stringsAsFactors=FALSE)

createNetworkFromDataFrames(nodes, edges)

From Cytoscape-supported file formats...

# From Cytoscape session files
## Will erase and replace all data from current session!
openSession() # default file = galFiltered.cys

# From local network files
importNetworkFromFile() # default file = galFiltered.sif
## Supported file formats: SIF, GML, xGMML, graphML, CX, plus

# From NDEEx (https://ndexbio.org), the network database
importNetworkFromNDEEx("5be85817-1e5f-11e8-b939-0ac135e8bacf")
## Account information or accessKey are required arguments only
## when accessing private content

From public databases via Cytoscape apps...

# From STRING (https://string-db.org), starting with a list of genes/proteins
installApp("stringApp") # http://apps.cytoscape.org/apps/stringapp
gene.list <- c("T53","AKT1","CDKN1A")
gene.str <- paste(gene.list, collapse = ",")
string.cmd <- paste("string protein query cutoff=0.99 limit=40 query",
gene.str, sep = ",")
commandsRun(string.cmd)

# From WikiPathways (https://wikopathways.org), starting with a keyword
library(rWikiPathways) # install from Bioconductor
installApp("WikiPathways") # http://apps.cytoscape.org/apps/wikopathways
keyword <- "glioblastoma"
gbm.pathways <- findPathwaysByText(keyword)
gbm.wpids <- gbm.pathways[1]$id # let's just take the first one
wikopathways.cmd <- paste("wikopathways import-as-pathway id",
  gbm.wpids, sep = ",")
commandsRun(wikopathways.cmd)

Visualizing data on networks. Cytoscape excels at generating publication-quality network visualization with data overlays. This vignette demonstrates just one of the hundreds of visual style mapping options using RCy3.

# Load sample network
closeSession(FALSE) # clears all session data without saving
importNetworkFromFile() # default file = galFiltered.sif

# Load sample data
csv <- system.file("extdata","galExpData.csv", package="RCy3")
data <- read.csv(csv, stringsAsFactors = FALSE)
loadTableData(data, data.key.column="name")
# Prepare data-mapping points

gal80Rexp.min <- min(data$gal80Rexp, na.rm = TRUE)
gal80Rexp.max <- max(data$gal80Rexp, na.rm = TRUE)

## For a balanced color gradient, pick the largest absolute value

gal80Rexp.max.abs <- max(abs(gal80Rexp.min), abs(gal80Rexp.max))

# Set node color gradient from blue to white to red

setNodeColorMapping('gal80Rexp', c(-gal80Rexp.max.abs, 0, gal80Rexp.max.abs),
                     c('#5577FF', '#FFFFFF', '#FF7755'), default.color = '#999999')

Filtering networks by degree and by data. Network topology and associated node or edge data can be used
to make selections in Cytoscape that enable filtering and subnetworking. The filters are added to the Select tab in the Control
Panel of Cytoscape’s GUI and saved in session files.

# Load demo Cytoscape session file

openSession() # default file = galFiltered.cys

net.suid <- getNetworkSuid() # get SUID for future reference

# Filter for neighbors of high degree nodes

createDegreeFilter(filter.name = "degree filter",
criterion = c(0,9),
predicate = "IS_NOT_BETWEEN")

selectFirstNeighbors() # expand selection to first neighbors

createSubnetwork(subnetwork.name = "first neighbors of high degree nodes")

# Filter for high edge betweenness

createColumnFilter(filter.name = "edge betweenness",
type = "edges",
column = "EdgeBetweenness",
4000, "GREATER_THAN",
network = net.suid)

createSubnetwork(subnetwork.name = "high edge betweenness")

Saving and exporting networks. There are local and cloud-hosted options for saving and sharing network models and
images. The Cytoscape session file (CYS) includes all networks, collections, tables and styles. It retains every aspect
of your session, including the size of the application window. Network and image exports include only the currently
active network. Export to NDEx requires account information you can obtain from ndexbio.org. Files are saved to the
current working directory by default, unless a full path is provided.

# Saving sessions

saveSession("MySession") #.cys

## Leave filename blank to update previously saved session file

# Exporting images and networks

exportNetwork() #.sif

## Optionally specify filename, default is network name

## Optionally specify type: SIF(default), CX, cyjs, graphML, NNF, SIF, xGML

exportImage() #.png

## Optionally specify filename, default is network name

## Optionally specify type: PNG (default), JPEG, PDF, PostScript, SVG

# Exporting to NDEx, a.k.a. “Dropbox” for networks

exportNetworkToNDEx(username, password, TRUE)

## Account information (username and password) is required to upload

## Use updateNetworkInNDEx if the network has previously been uploaded

Building maps of enrichment analysis results. This workflow illustrates how to plot an annotated map of enrichment
results using the EnrichmentMap Pipeline Collection of apps in Cytoscape. An enrichment map is a network
visualization of related genesets in which nodes are gene sets (or pathways) and edge weight indicates the
overlap in member genes\textsuperscript{9}. Following the construction of the enrichment map, AutoAnnotate clusters redundant gene sets and uses WordCloud\textsuperscript{11} to label the resulting cluster (Figure 2). The code uses the Commands interface to invoke EnrichmentMap and AutoAnnotate apps. After installing apps, run commandsAPI() to open the live Swagger documentation to browse and execute command-line syntax.

\begin{verbatim}
installApp("EnrichmentMap Pipeline Collection") # installs 4 apps

# Download sample gmt file of human pathways
gmt.file <- "rcy3_enrichmentmap.gmt"
download.file(file.path("http://download.baderlab.org/EM_Genesets",
    "September_01_2019/Human/symbol/Pathways",
    "Human_WikPathways_Sepember_01_2019_symbol.gmt"),
    gmt.file)

# Run EnrichmentMap build command
em_command <- paste('enrichmentmap build analysisType="generic"
    "gmtFile="", paste(getwd(), gmt.file, sep="/"),
    "pvalue="", 1,
    "qvalue="", 1,
    "similaritycutoff="", 0.25,
    "coefficients="", "JACCARD")
print(em_command)
commandsGET(em_command)

# Run the AutoAnnotate command
aa_command <- paste("autoannotate annotate-clusterBoosted",
    "clusterAlgorithm=MCL",
    "labelColumn=EnrichmentMap::GS_DESCR",
    "maxWords=3")
print(aa_command)
commandsGET(aa_command)

# Annotate a subnetwork
createSubnetwork(c(1:4), "mclCluster")
commandsGET(aa_command)

Visualizing integrated network analysis using BioNet. The BioNet package implements analytical methods to perform integrated network analysis, for example, of gene expression data and clinical survival data in the context of protein-protein interaction networks. Partnered with RCy3, the analytical results from BioNet can
\end{verbatim}

\textbf{Figure 2.} Annotated enrichment map of pathways. A few of the largest clusters of pathways from WikiPathways are displayed as a network with WordCloud-based labels annotating groups (yellow areas). The size of the labels correspond to the size of the groups.
be visualized in Cytoscape with vastly more options for customization. Starting with the “Quick Start” tutorial from BioNet, we pass the results directly to Cytoscape for visualization:

```r
library(BioNet) # install from Bioconductor
library(DLBCL) # install from Bioconductor
data(dataLym)
data(interactome)

## The following steps are from BioNet's Quick Start tutorial:
pvals <- cbind(t = dataLym$t.pval, s = dataLym$s.pval)
rownames(pvals) <- dataLym$label
pval <- asgrPvals(pvals, order = 2, plot = FALSE)
subnet <- subNetwork(dataLym$label, interactome)
subnet <- rmSelfLoops(subnet)
fb <- fitBumModel(pval, plot = FALSE)
scores <- scoreNodes(subnet, fb, fdr = 0.001)
module <- runFastHeinz(subnet, scores)

logC <- dataLym$diff
names(logC) <- dataLym$label
plotModule(module, scores = scores, diff.expr = logC)

# Using RCy3 we can generate a custom visualization of the same output
## Create network from graphNEL object and load data calculated above
createNetworkFromGraph(module, "module", "BioNet")
loadTableData(as.data.frame(scores))
loadTableData(as.data.frame(logC))

## Set styles
setLabelMapping("geneSymbol")
setFontSizeDefault(18)
setBorderWidthDefault(3.0)
logC.max.abs <- max(abs(min(logC)), abs(max(logC)))
setColorMapping('logC', c(-logC.max.abs, 0, logC.max.abs),
                   c('#5577FF', '#FFFFFF', '#FF7755'), default.color = '#999999')
createColumnFilter("Positive scores", "scores", c(0, max(scores)), 'BETWEEN')
setShapeBypass(getSelectedNodes(), "ELLIPSE")
```

Performing advanced graph analytics using RBGL. As an interface to the BOOST library, the RBGL Bioconductor package offers an impressive array of analytical functions for graphs. Here we will start with a network in Cytoscape, load it into R as a graph object, perform shortest path calculation using RBGL and then visualize the results back in Cytoscape (Figure 3).

```r
library(RBGL) # install from Bioconductor
# Convert a sample Cytoscape network to a graph object
openSession()
g <- createGraphFromNetwork()

# Identify start and finish nodes (styling is optional)
start <- "YNL216W"
finish <- "YER040W"
setBorderWidthBypass(c(start,finish), 20)
setColorBypass(start,"#00CC33")
setColorBypass(finish,"#CC00CC")

# Use RBGL to perform shortest path calculation
shortest <- sp.between(g, start, finish)
shortest$"YNL216W:YER040W"$length

# Visualize results in Cytoscape
selectNodes(shortest.path, "name")
setBorderWidthBypass(shortest.path, 20)
createSubnetwork()
```
Discussion

Every operation exposed by Cytoscape’s REST API has now been implemented as a function in RCy3 2.0. Furthermore, RCy3 provides dozens of higher-level helper functions in support of common usage, such as setNodeColorMapping, to make script writing more intuitive and efficient. The issue trackers for CyREST and RCy3 are linked for functions pending implementation, such as mergeNetworks, as well as for bug fixes. Thus, RCy3 is expected to keep pace with future development of Cytoscape Automation. More broadly, RCy3 is an integral part of the Cytoscape ecosystem, which includes the network repository NDEx, Cytoscape apps and services, and web components like cytoscape.js. The ecosystem has also defined interfaces and standard formats to interoperate with interaction databases and annotation services. Adopting RCy3 for network analysis will establish a connection to the Cytoscape ecosystem and enable Cytoscape Automation workflows. Furthermore, RCy3 enables reproducible scripting of integrated network biology workflows that otherwise rely on transient interactions with Cytoscape’s GUI. As the sharing and publishing of analysis scripts and workflows become more routine (if not mandated), software tools designed to work in an open and accessible ecosystem are becoming essential.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

- RCy3 Rmd notebooks: https://cytoscape.org/cytoscape-automation/for-scripters/R/notebooks/
- RCy3 workshop presentations: http://tutorials.cytoscape.org/#automation
- Video demonstrations of RCy3: https://www.youtube.com/playlist
- Cytoscape Automation training: http://automation.cytoscape.org/
- Cytoscape Automation code repository: https://github.com/cytoscape/cytoscape-automation

Software availability


Source code available from: https://github.com/cytoscape/RCy3

Figure 3. Visualizing shortest path results. In Cytoscape, the start and finish nodes were colored green and magenta, respectively (left panel). The shortest path calculated by RBGL was then selected and nodes along the path were highlighted with thick borders (middle panel). Finally, a subnetwork was created from the selected path (right panel). Note that while common gene names are displayed as node labels, official yeast identifiers are the actual node names that are referenced in the script.
Acknowledgments
We would like to acknowledge contributions by other developers on the original implementation of RCy3 by Paul Shannon, Tanja Muetze and Georgi Kolishkovski. We also greatly appreciate the input from Mark Grimes (testing), Martin Morgan (Bioconductor), and the excellent work on CyREST and Cytoscape Commands by David Otasek and John “Scooter” Morris.

References

Open Peer Review

Current Peer Review Status: ✔ ✔ ✔

Version 2

Reviewer Report 28 November 2019

https://doi.org/10.5256/f1000research.23645.r56320

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Ludwig Geistlinger

Department of Epidemiology and Biostatistics, City University of New York (CUNY), Graduate School of Public Health and Health Policy, New York, NY, USA

Gustavsen and colleagues present a well-written software article that describes usage of RCy3, a R/Bioconductor package for using Cytoscape from within R.

The package is very well documented in both analysis vignettes on the Bioconductor landing page of the package and in the article itself.

The implemented functionality covers a range of useful features including network import/export, network layout, and integration with network and enrichment analysis packages.

I have 3 minor comments:

○ Table 2 is supposed to show the "Top 30 network-related Bioconductor packages ordered by rank" but misses the EnrichmentBrowser package (http://bioconductor.org/packages/EnrichmentBrowser) which is (and was in Sep 2019) highly ranked in the various corresponding BiocViews:
  Rank 2: https://bioconductor.org/packages/release/BiocViews.html#___NetworkEnrichment
  Rank 10: https://bioconductor.org/packages/release/BiocViews.html#___Network
  Rank 13: https://bioconductor.org/packages/release/BiocViews.html#___GraphAndNetwork

The relevant functions EnrichmentBrowser::compileGRN (compiles a gene regulatory network in a simple 3 column format regulator-target-effectType), EnrichmentBrowser::nbea (network-based enrichment analysis working on such GRNs), and EnrichmentBrowser::ggeaGraph (constructs a graphNEL object that annotates differential expression and edge consistency) produce network-representations that are compatible with the formats supported by RCy3. I tested that.

○ The lines
  gal80Rexp.min <- min(data$gal80Rexp, na.rm = T)
gal80Rexp.max <- max(data$gal80Rexp, na.rm = T)
should be replaced with

gal80Rexp.min <- min(data$gal80Rexp, na.rm = TRUE)
gal80Rexp.max <- max(data$gal80Rexp, na.rm = TRUE)

consistent with the remainder of the article and good R style.

- The lines
  
  # Run EnrichmentMap build command
  em_command <- paste('enrichmentmap build analysisType="generic",
                      "gmtFile=" paste(getwd(), gmt.file, sep="/"),
                      "pvalue="1,
                      "qvalue="1,
                      "similaritycutoff="0.25,
                      "coefficients="JACCARD")

  can be simplified to

  # Run EnrichmentMap build command
  em_command <- paste('enrichmentmap build analysisType="generic",
                      paste0("gmtFile=", file.path(getwd(), gmt.file)),
                      "pvalue=1",
                      "qvalue=1",
                      "similaritycutoff=0.25",
                      "coefficients=JACCARD")

Is the rationale for developing the new software tool clearly explained?
Yes

Is the description of the software tool technically sound?
Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Bioinformatics, Cancer genomics, Gene expression data analysis
I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 01 Dec 2019
Alexander Pico, Gladstone Institutes, San Francisco, USA

Thanks for the review and code style suggestions. Sorry we missed your package! I'll look into adding it to the table.

Competing Interests: No competing interests were disclosed.

Reviewer Report 27 November 2019
https://doi.org/10.5256/f1000research.23645.r57133

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James Denvir
Department of Biomedical Sciences, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, USA

I have no further comments.

Is the rationale for developing the new software tool clearly explained?
Yes

Is the description of the software tool technically sound?
Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.
**Reviewer Expertise:** Bioinformatics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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**Version 1**

Reviewer Report 21 November 2019

https://doi.org/10.5256/f1000research.22981.r55401

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**Krithika Bhuvaneshwar**

Innovation Center for Biomedical Informatics, Georgetown University Medical Center, Washington, DC, USA

This is a review of the manuscript titled "RCy3: Network biology using Cytoscape from within R". RCy3 is a Bioconductor package that communicates with Cytoscape via its REST API to build and visualize networks. This manuscript is about a re-designed/updated RCy3 package and its function and implementation.

My comments are below:

○ The authors say "We have re-written every function". Is it backward compatible? Should existing users of the older version of the package have to make changes in their code?

○ The authors say "Over 100 new functions have been added, including dozens of helper functions specifically for intuitive data overlay operations". This is a great achievement. In addition to this statement, perhaps it would be helpful for users to summarize/compare what the main improvements are in this newer version?

○ Does this newer version include more or better (or the same) interoperability with other major packages that use RCy3? For example, exporting and importing to/from Ndex. From the example code listed in the paper, it seems there is no change in the way the interoperability works?

Minor comment:

It is helpful to call the package RCy3 2.0 or something to note the version change? Would keeping the same name be confusing for users?

Overall great work! RCy3 is one of the major packages that work with Cytoscape so very important for the R/BioC Community. Not all authors of Bioconductor and R packages take effort to update their packages, so I commend this effort. I have used RCy3 in the past and am excited to try out this newest version of the package.
Is the rationale for developing the new software tool clearly explained?
Yes

Is the description of the software tool technically sound?
Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Bioinformatics, Genomic Data Science, Translational and Precision Health Informatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 21 Nov 2019

**Alexander Pico,** Gladstone Institutes, San Francisco, USA

Thank you for your careful review and questions. The package is not backward compatible. We in fact initially submitted it as a completely new package, but were encouraged by Bioconductor reviewers to coordinate with the original RCy3 authors and submit it as a new version of the same package, which we did. The version number is kept separate from the package name per Bioconductor and semantic versioning conventions, so we maintained the original package name. There is a vignette dedicated to upgrading scripts from the original to version 2: ([https://bioconductor.org/packages/release/bioc/vignettes/RCy3/inst/doc/Upgrading-existing-scripts.html](https://bioconductor.org/packages/release/bioc/vignettes/RCy3/inst/doc/Upgrading-existing-scripts.html)), which highlights the major differences and improvements.

The original version focused on graphNEL objects, so interoperability with other graphNEL-based packages perhaps hasn't changed. But I just now double checked the old code base and I can't find any NDEx, igraph, data frame or adjacency matrix related functions for network interoperability in the original version.

**Competing Interests:** No competing interests were disclosed.
This article presents an R package, RCy3, designed to control and automate the network visualization software application Cytoscape. The article describes both a low-level ("generic") and a high-level ("specific") API and presents use-cases with some code samples.

Overall this appears to be an API with great potential utility for the bioinformatics community, and the article presents the package clearly and with several useful and informative examples.

I have a couple of minor comments and suggestions, which I hope will serve to improve the article.

The abstract mentions the potential for RCy3 to deliver reproducible workflows. In my opinion, reproducibility is among the most important benefits of code/script-based workflows over workflows performed using "point and click" GUI-based applications. However, the article does not elaborate on this outside of the abstract. A couple of sentences in the discussion describing the potential for RCy3 to enhance reproducibility would be worthwhile.

The article mentions that the Cytoscape App store currently lists over 40 "RCy3-supported apps". A quick note on what is needed for a third party app to be "RCy3-supported" might be pertinent here. This may be already sufficiently covered in existing publications (e.g. reference 6), in which case a simple note to that effect would suffice.

The code block for the section "Building maps of enrichment analysis results" is not quite as clear as the other code blocks in the article. A comment indicating that commandsAPI() opens a web browser page (externally; at least in my environment) and comments after the print() statements showing the expected output, as in the other blocks of code, would be helpful. The uses of file.path and paste(... sep='/') are a little confusing here (to be honest, I was surprised to find these actually worked on Windows systems); brief comments added to the code might aid readability here.

Is the rationale for developing the new software tool clearly explained?
Yes

Is the description of the software tool technically sound?
Yes
Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Yes

Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioinformatics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 21 Nov 2019

Alexander Pico, Gladstone Institutes, San Francisco, USA

Thank you for your careful review and suggestions. We share your opinion regarding the importance of reproducibility and will add a sentence accordingly to the Discussion section. The support of automation by Cytoscape apps is indeed a major topic of reference 6; we will note that in the text as well. Regarding the code block, we will likely remove the commandsAPI() call to not surprise/distract users with a browser window opening. The use of file.path is simply to avoid a really long string, i.e., longer than the column width of this publication. The use of forward slash is in paths is a generic solution to avoid platform-specific conventions and avoid having to escape backslashes. Neither of these last two “tricks” are at all specific to RCy3, so we might just leave those as-is.

Competing Interests: No competing interests were disclosed.
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