SOFTWARE TOOL ARTICLE

Exploring causal relationships in proteomic profiles in Cytoscape using the CausalPath App [version 1; peer review: 1 approved with reservations]

Pritam Saha¹, Özgun Babur², Chris Sander³⁻⁵, Augustin Luna³⁻⁵

¹Electrical Engineering Department, Jadavpur University, Kolkata West Bengal, India
²Computer Science Department, University of Massachusetts Boston, Boston, MA, USA
³Department of Cell Biology, Harvard Medical School, Boston, MA, USA
⁴Department of Data Science, Dana-Farber Cancer Institute, Boston, MA, USA
⁵Broad Institute of Harvard and MIT, Cambridge, MA, USA

Abstract

Introduction: CausalPath compares experimentally measured changes in molecular profiles against curated biological pathways and infers causality between changes in measured features from profiling experiments (e.g., RNA-seq or proteomics from total or phospho-protein levels).

Methods: We developed the CausalPath Cytoscape App, an app (i.e., plugin) for visualizing results from the CausalPath method within the Cytoscape Java-based desktop network analysis and visualization platform.

Use Cases: Users are given instruction that represents use cases in multiple cancer research areas through the visualization of CausalPath analysis results generated from data by the Clinical Proteomic Tumor Analysis Consortium.

Discussion: The CausalPath Cytoscape App visualizes the set of known interactions that are supported by molecular profiling data via the CausalPath method. This integration of CausalPath and Cytoscape benefits users interested in performing secondary analyses (e.g., module detection) on the sub-networks that result from CausalPath analysis by utilizing the many analytical features available in the Cytoscape software ecosystem.

Keywords:
proteomics, network analysis, Cytoscape app, Cytoscape, mechanistic hypotheses, curated signaling
Corresponding authors: Pritam Saha (science.pritam98@gmail.com), Özgun Babur (Ozgun.Babur@umb.edu), Augustin Luna (aluna@jimmy.harvard.edu)

Author roles: Saha P: Software, Visualization, Writing – Original Draft Preparation; Babur Ö: Conceptualization, Software, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; Sander C: Funding Acquisition, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; Luna A: Conceptualization, Funding Acquisition, Software, Supervision, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The authors received funding from the Google Summer of Code program and funding for the National Resource for Network Biology (NRNB) from the National Institute of General Medical Sciences (NIGMS P41 GM103504). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Saha P et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Saha P, Babur Ö, Sander C and Luna A. Exploring causal relationships in proteomic profiles in Cytoscape using the CausalPath App [version 1; peer review: 1 approved with reservations] F1000Research 2022, 11:458
https://doi.org/10.12688/f1000research.109027.1

First published: 25 Apr 2022, 11:458 https://doi.org/10.12688/f1000research.109027.1
Introduction

CausalPath is a recently developed software tool that allows users to map proteomic and other molecular profiles to pathway information from the Pathway Commons database (RRID: SCR_001749) and other resources. CausalPath focuses on providing users a means to assess causality among correlated measurements. CausalPath can generate a network model of molecular signal flow that is consistent with both the profiling data and the published literature (Figure 1A). Results in the generated network model are linked to Pathway Commons, an integrative database of molecular interactions, and literature connections, therein.

Several tools currently support the visualization of CausalPath, including causalpath.org, Newt, and ChiBE. A comparison of unique features of these tools with respect to usage with CausalPath has been previously discussed in one of our recent publications. The CausalPath Cytoscape app provides a tool for importing, visualization, and utilizing CausalPath results from within Cytoscape, thereby facilitating user access to additional analyses not found in the aforementioned CausalPath tools. Cytoscape is a widely used extensible bioinformatics environment for the analysis and visualization of biological networks with nearly 10 million downloads since 2014. By providing users with a Cytoscape-based interface for the visualization of CausalPath results, users have access to the Cytoscape ecosystem of functionality. Briefly, this allows CausalPath users to have access to Cytoscape features such as network layouts and sharing of CausalPath results through to Network Data Exchange (NDEX). Additionally, various secondary analyses are available for users to run including, for example, gene set analysis through tools (e.g., through NDEX Integrated Query), module detection, and various network propagation methods.

Methods

The work is implemented in the Java 16 programming language using the Cytoscape software platform. Multiple Cytoscape application programming interfaces (APIs) have been used to call the different functionalities. The CausalPath Cytoscape App is described in the three following sections:

User interface

The user interface consists of two panels (as seen in Figure 1):

1. Input Panel: A panel component that appears on the left that is built using Cytoscape swing-application-api where users select files from their local file storage. The Java Swing library is used to choose the appropriate file and the Cytoscape io-api to read the network file. The panel contains a progress bar to show the progress and a help button that uses Cytoscape service-api to redirect to the CausalPath project website.

2. Information Panel: A panel component appearing on the right where a node or edge-specific information is shown when a user selects a node or edge.

Network visualization

Data from the network and format files is stored using multiple data structures (e.g., HashMaps with different class types and Arrays). A network view is created using Cytoscape view-model-API for the network and different styles for each node and edge are configured using Cytoscape presentation-api. Finally, we used the Cytoscape work-api to show the network. By default, the app lays out the network using a force-directed layout.

CausalPath visualizations include annotations on nodes (i.e., small circles attached to nodes). These annotations provide information on post-translational modifications (i.e., phosphorylations) as well as information on non-proteomic data (e.g., gene expression data and copy number information). To provide these annotations, we have made use of the Cytoscape enhancedGraphics plugin and the vizmap-api to map the sites to each node and we have used the “Label” chart type. Annotations are placed on the border of nodes when they are present in the input data (e.g., the example in Figure 1). This app uses the Cytoscape vizmap-api to map the sites to each node and we have used the “Label” charts style from the enhanced Graphics plugin to design the site annotations for each node. The visual notation of CausalPath uses a notation outlined previously with one exception. Instead of using border colors to indicate the activatory or inhibitory nature of the site, we used the text color of the letter within the annotation circle. This change was done to Cytoscape as this was the representation available with vizmap-api labels chart.

Data formats

The CausalPath Cytoscape App requires two input files to visualize CausalPath result networks:

- Network file (with a .sif extension): The network data contains multiple rows where each row consists of tab-separated values where the first value refers to the source node, the second value refers to the edge type, and the third one refers to the target node.
**Format File (with a .format extension):** The .format file also has multiple rows where each row consists of space and pipe-separated values. Each row can contain one of the multiple possible categories of information. The categories include: 1) styling for a node (e.g., color, border color, border width), 2) styling information for each site-related annotation linked to a particular node, or 3) "tooltip" information that will appear in the right-hand information panel, such as specific measured node values. More details can be found on this [GitHub repository](https://github.com).

**Installation and usage**

The minimum system requirements for use of the CausalPath Cytoscape app include: Hardware: CPU: 1 GHz CPU, Memory: 512MB, Monitor: 1024×768 resolution; Software: Java 11.

Here, we provide installation and usage instructions for the CausalPath Cytoscape app for visualizing existing CausalPath results. Users that desire to additionally run a CausalPath analysis must do so separately. We have recently published a detailed protocol for how users can do this locally on their computers.[1](#)

Additionally, the protocol provides guidance on available options for conducting a CausalPath analysis. A collection of results from previous work is available on [Zenodo](https://zenodo.org) and will be used here as input files for the CausalPath Cytoscape app.[2](#)

To install the CausalPath Cytoscape app users must first install [Cytoscape](https://cytoscape.org). Next, users must install the enhanceGraphics app from the “Apps -> App Manager…” menu of Cytoscape. After this, users can then install the CausalPath Cytoscape app App Manager menu.

Once installed users can visualize the results using the CausalPath Cytoscape app and run any additional analyses available from Cytoscape. The steps to visualize CausalPath results in Cytoscape are as follows. First, with Cytoscape open, users enable the CausalPath by clicking the App menu and then CausalPath. Next, from the Cytoscape Control Panel menu that appears, users will load both their SIF network and .format file. Lastly, users need to click the “Submit” button to visualize the network.
Use case

Figure 1 (partial view of the network) is a visualization of the results generated by CausalPath for a dataset taken from the National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC) (RRID:SCR_017135) breast cancer study that collected proteomic and phosphoproteomic profiles for 105 of the original The Cancer Genome Atlas (TCGA) (RRID:SCR_003193) breast cancer samples with mass spectrometry; see data availability section for information on the input files used in this section. Specifically, Figure 1 shows luminal (luminal A and luminal B) samples compared to basal-like samples. Briefly, these results highlight overexpression of the ESR1 gene and genes directly downstream of ESR1 in luminal samples as compared to basal-like samples; a key characteristic of basal-like breast cancer is that it is hormone-receptor negative (estrogen-receptor and progesterone-receptor negative).

The causative.sif and causative.format input files (Figure 1C) from the LumAB-vs-Basal folder are loaded by using the clicking app "SIF File" and "Format File" buttons of the Input Panel shown in Figure 1B; the full path to these two input files within the Zenodo archive is CausalPath-data/CPTAC-BRCA/subtypes/LumAB-vs-Basal; see data availability section for information on the Zenodo archive and the data formats section for a description of the contents of .sif and .format files. After clicking the "Submit" button users will see the resulting output visualization as shown in Figure 1B. For node or edge-specific information, the user should select that node or edge. The information regarding that node or edge will be shown in the side panel as shown in Figure 1. Users can also visualize multiple networks at the same time by repeating the instructions to load networks in the "Installation and usage" section. Additionally, we note for readers that the Zenodo archive provided in the data availability section provides pre-computed results for many of the cancer types (e.g., ovarian, bladder, renal, thyroid, pancreatic, colon, etc.) for which there is TCGA proteomics data. These CausalPath results for each of these cancer types can be visualized in the same manner as described above.

Discussion

The CausalPath Cytoscape app is a software tool for visualizing network models with consistency with both the profiling data and the published literature. Use of CausalPath aids researchers who seek to understand the results of their experimental data and who would otherwise manually explore scientific literature to assess concordance with existing findings. By providing this tool as a Cytoscape app, we simplify the installation and use of CausalPath with existing analysis pipelines that combine other analyses available through Cytoscape that, for example, include a gene set and network modularity analyses.

Data availability

Source data

The source data used in Figure 1 comes from a previous study using CausalPath results for LuminalAB-vs-Basal-like breast cancer comparison. The CausalPath input data and analysis results are publicly available on Zenodo (https://doi.org/10.5281/zenodo.4477801).

Software availability

The CausalPath Cytoscape app is available from the Cytoscape App Store: https://apps.cytoscape.org/apps/causalpath_cytoscapeapp. The app is compatible with versions of Cytoscape 3.7 and above.

Source code available at: https://github.com/cannin/causalpath_cytoscape_app

Archived source code at the time of publication: https://doi.org/10.5281/zenodo.6081659

License: Apache License 2.0 license.

Acknowledgements

We thank Scooter Morris and Alexander Pico for their valuable feedback regarding Cytoscape during the development of the project. Additionally, we would like to thank the coordinators of the Google Summer of Code program.

References

1. Luna A, Siper MC, Korkut A, et al.: Analyzing causal relationships in proteomic profiles using CausalPath. STAR Protoc. 2021 Dec 17; 2(4): 100955.

Page 5 of 11
2. Babur Ö, Luna A, Korkut A, et al.: Causal interactions from proteomic profiles: Molecular data meet pathway knowledge. Patterns (N Y). 2021 Jun 11; 2(6): 100257. PubMed Abstract | Publisher Full Text

3. Rodchenkov I, Babur Ö, Luna A, et al.: Pathway Commons 2019 Update: integration, analysis and exploration of pathway data. Nucleic Acids Res. 2020 Jan 8; 48(1): D489-D497. PubMed Abstract | Publisher Full Text

4. Barsi S, Szalai B: Modeling in systems biology: Causal understanding before prediction?. Patterns (N Y). 2021 Jun 11; 2(6): 100280. PubMed Abstract | Publisher Full Text

5. Cerami EG, Gross BE, Demir E, et al.: Pathway Commons, a web resource for biological pathway data. Nucleic Acids Res. 2011 Jan; 39(Database issue): D685-D690. PubMed Abstract | Publisher Full Text

6. Balci H, Siper MC, Saleh N, et al.: Newt: a comprehensive web-based tool for viewing, constructing and analyzing biological maps. Bioinformatics. 2021 Jun 16; 37(10): 1475-1477. PubMed Abstract | Publisher Full Text

7. Babur Ö, Dogrusoz U, Demir E, et al.: ChiBE: interactive visualization and manipulation of BioPAX pathway models. Bioinformatics. 2010 Feb 1; 26(3): 429-431. PubMed Abstract | Publisher Full Text

8. Shannon P, Markiel A, Ozier O, et al.: Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003 Nov; 13(11): 2498-2504. PubMed Abstract | Publisher Full Text

9. Pillich RT, Chen J, Rynkov V, et al.: NDEx: A Community Resource for Sharing and Publishing of Biological Networks. Methods Mol. Biol. 2017; 1558: 271-301. PubMed Abstract | Publisher Full Text

10. Mertins P, Mani DR, Ruggles KV, et al.: Proteogenomics connects somatic mutations to signalling in breast cancer. Nature. 2016 Jun 2; 534(7605): 55-62. PubMed Abstract | Publisher Full Text

11. Badve S, Dabbs DJ, Schnitt SJ, et al.: Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Mod. Pathol. 2011 Feb; 24(2): 157-167. PubMed Abstract | Publisher Full Text

12. Babur Ö, Luna A, Korkut A, et al.: CausalPath Analysis Inputs and Outputs. Zenodo. 2021. Publisher Full Text

13. Pritam, Özgün, Chris, et al.: CausalPath Cytoscape App Source Code (1.0.2). Zenodo. 2021. Publisher Full Text
Open Peer Review

Current Peer Review Status: 🟢

Version 1

Reviewer Report 10 May 2022

https://doi.org/10.5256/f1000research.120481.r135821

© 2022 Isserlin R. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Ruth Isserlin

Donnelly Centre for Cellular and Biomolecular Research, Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada

The publication “Exploring causal relationships in proteomic profiles in Cytoscape using the CausalPath App” by Saha et al describes a new Cytoscape app developed for users of the Causalpath program to visualize their results in the Cytoscape ecosystem. This enables users of the Causalpath to have access to all the features present in Cytoscape when analyzing their results. On the initial read of the paper I had difficulty understanding the purpose and workings of the app. Only after reading both of the papers describing causalpath method and the detailed protocol did I begin to understand the current application note. I feel like this paper could benefit from either an expansion of the introduction and background section or a advisory to read both the previous papers prior to reading this paper.

Methods - User Interface: There are a lot of links in the user interface to the different methods and code. There is no link to the causalpath.org website. I think it would be beneficial to have a link to the site where they can generate the data needed to run in the app. If you are concerned with the number of links listed in the app maybe having the title Causal Path be a direct link to the website as possible suggestion.

In the methods section it would be useful to make a separate section called ‘data’ with a link to the data download on zenodo and a brief description of the analysis done to create the files contained in it. Additionally, a recommendation of which files to use to try out the app. There is mention of the zenodo download but I think it would be beneficial to have it in its own section, right at the start of the methods section so readers can easily grab the file and try out the app.

“To install the CausalPath Cytoscape app users must first install Cytoscape. Next, users must install the enhanceGraphics app from the “Apps -> App Manager ...” menu of Cytoscape. After this, users can then install the CausalPath Cytoscape app App Manager menu.” - maybe have a causalpath app collection available as well that installs both the required apps so there is no need to install the two different apps.
Feature - remembering the previous directory would be very helpful especially when using the dataset supplied on zenodo that consists of many different analyses embedded in a complicated file structure.

Bug - the requirement to unselect the network in network panel in order to create a new causalpath network should be fixed.

There is a specific example in the paper to get the figure that is shown in the paper but when you use any of the other data the networks are not coloured or even laid out using any of the cytoscape layouts. Why is this? Is all of that done manually by the user as opposed to being automated in the app? It seems that I can get coloured networks only when I choose files from the subtypes directory.

Minor issues:
“The CausalPath Cytoscape app provides a tool for importing, visualization, and utilizing CausalPath results from within” - mixture of tenses. Would be better as - “The CausalPath Cytoscape app provides a tool for importing, visualizing, and utilizing CausalPath results from within”.

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?
Partly

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

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

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, however I have significant reservations, as outlined above.
Augustin Luna, Harvard Medical School, Boston, USA

We thank the reviewer for their time. Our detailed responses are below and we have submitted a new manuscript version.

The publication “Exploring causal relationships in proteomic profiles in Cytoscape using the CausalPath App” by Saha et al describes a new Cytoscape app developed for users of the Causalpath program to visualize their results in the Cytoscape ecosystem. This enables users of the Causalpath to have access to all the features present in Cytoscape when analyzing their results. On the initial read of the paper I had difficulty understanding the purpose and workings of the app. Only after reading both of the papers describing causalpath method and the detailed protocol did I begin to understand the current application note. I feel like this paper could benefit from either an expansion of the introduction and background section or a advisory to read both the previous papers prior to reading this paper.

Methods - User Interface: There are a lot of links in the user interface to the different methods and code. There is no link to the causalpath.org website. I think it would be beneficial to have a link to the site where they can generate the data needed to run in the app. If you are concerned with the number of links listed in the app maybe having the title Causal Path be a direct link to the website as possible suggestion.

Response: We have added the link under the title to the CausalPath website.

In the methods section it would be useful to make a separate section called 'data' with a link to the data download on zenodo and a brief description of the analysis done to create the files contained in it. Additionally, a recommendation of which files to use to try out the app. There is mention of the zenodo download but I think it would be beneficial to have it in its own section, right at the start of the methods section so readers can easily grab the file and try out the app.

Response: We have edited the Methods section based on the comments. We now have a “Data and usage” section to better communicate with and instruct users. We use the phrasing "strongly advised":

"Users are strongly advised to read the two previous CausalPath publications"

to address this feedback and another comment by the reviewer to put an advisory notice for users. We have reordered the Methods to such so that this “Data” section is seen earlier by readers and follow the reviewers suggestion to advise users to download the archive of past results.

“To install the CausalPath Cytoscape app users must first install Cytoscape.8 Next, users must install the enhanceGraphics app from the “Apps -> App Manager ...” menu of Cytoscape. After this, users can then install the CausalPath Cytoscape app App Manager menu.” - maybe have a causalpath app collection available as well that installs both the required apps so there is no need to install the two different apps.
Response: We are grateful to the reviewer for highlighting this thing. As it is the structure of Cytoscape to download the enhanceGraphics app separately because it is the library that is provided by Cytoscape to provide advanced style algorithms. Additionally, we will continue to gauge interest by other users for alternative installation methods.

Feature - remembering the previous directory would be very helpful especially when using the dataset supplied on Zenodo that consists of many different analyses embedded in a complicated file structure.

Response: We appreciate the reviewer for mentioning this issue. We have added this feature to our app to remember the last browsed directory such that users can track the previously opened files.

Bug - the requirement to unselect the network in network panel in order to create a new causalPath network should be fixed.

Response: We appreciate the reviewer for finding this bug in the app. We have fixed the issue in our next version. Now, the user can create as many networks without any hindrance.

There is a specific example in the paper to get the figure that is shown in the paper but when you use any of the other data the networks are not coloured or even laid out using any of the cytoscape layouts. Why is this? Is all of that done manually by the user as opposed to being automated in the app? It seems that I can get coloured networks only when I choose files from the subtypes directory.

Response: In Figure 1D, we have included images of two additional networks from the collection available in the Zenodo archive; the causative .sif and .format files were loaded. This should help illustrate for users that not all networks will have all (or necessarily even many nodes) colored. This is entirely dependent on the CausalPath analysis conducted and its results.

Minor issues:
"The CausalPath Cytoscape app provides a tool for importing, visualization, and utilizing CausalPath results from within" - mixture of tenses. Would be better as - “The CausalPath Cytoscape app provides a tool for importing, visualizing, and utilizing CausalPath results from within”

Response: We have edited the text.

Competing Interests: None
The benefits of publishing with F1000Research:

• Your article is published within days, with no editorial bias
• You can publish traditional articles, null/negative results, case reports, data notes and more
• The peer review process is transparent and collaborative
• Your article is indexed in PubMed after passing peer review
• Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com