Cytoscape 2.0 Manual

Introduction

Cytoscape is an open-community software project for integrating biomolecular interaction networks with high-throughput expression data and other molecular states into a unified conceptual framework. Although applicable to any system of molecular components and interactions, Cytoscape is most powerful when used in conjunction with large databases of protein-protein, protein-DNA, and genetic interactions that are increasingly available for humans and model organisms. A software "Core" provides basic functionality to layout and query the network; to visually integrate the network with expression profiles, phenotypes, and other molecular states; and to link the network to databases of functional annotations. The Core is extensible through a straightforward plug-in architecture, allowing rapid development of additional computational analyses and features. **The central organizing metaphor of Cytoscape is a network graph, with genes, proteins, and molecules represented as nodes and interactions represented as links, i.e. edges, between nodes.**

Development

Cytoscape is a collaborative project between the Institute for Systems Biology (Dr. Benno Schwikowski), the University of California San Diego (Dr. Trey Ideker), and Memorial Sloan-Kettering Cancer Center (Dr. Chris Sander).

Schwikowski Lab: http://www.systemsbiology.org/personal/benno
Ideker Lab: http://www-bioeng.ucsd.edu/faculty/area/ideker_lab/
Sander Lab: http://www.cbio.mskcc.org/

License

Cytoscape is protected under the LGPL (Lesser Gnu Public License). The License is included as an appendix to this manual, but can also be found online: http://www.gnu.org/copyleft/lesser.txt

1. Launching Cytoscape

Currently, Cytoscape runs under Java on Linux, Windows, and Mac OS X. Although Cytoscape handles arbitrary types and sizes of interaction network, it is most powerful when used in conjunction with large interaction data sets such as are currently available for species such as the yeast S. cerevisiae.

(1) Download and unpack the distribution. Cytoscape is distributed as a compressed archive (tar.gz or zip) containing the following files and directories:

`cytoscape.jar`	Main Cytoscape application (Java archive)
`cytoscape.props`	User-configurable properties and preferences
`vizmap.props`	User-configurable visual mapping preferences
`cytoscape.sh`	Shell script used to run Cytoscape (Linux, Mac OS X)
`cytoscape.bat`	Shell script used to run Cytoscape (Windows)
`LICENSE.txt`	Cytoscape GNU License
`Cytoscape2_0Manual.pdf`	Cytoscape 2.0 Manual (the document you are reading now)
`sampleData/`	
`galFiltered.gml`	Sample molecular interaction network file *
`galFiltered.sif`	Identical network in Simple Interaction Format *
`galExpData.pvals`	Sample gene expression matrix file *
`BINDyeast.sif`	Network of all yeast protein-protein interactions in the BIND database as of February, 2002 **
`GO/`	Directory containing Gene Ontology database entries (currently for yeast only). Info in this directory is used to associate gene names with synonyms as well as process, function, and cellular location data.
`plugins/`	Directory containing cytoscape plug-ins, in .jar format.

* Sample data sets taken from Ideker et al, Science 292:929 (2001)
** Obtained from data hosted at http://www.bind.ca/

(2) If necessary, install Java. If not already installed on your computer, download and install the Java 2 Runtime Environment, version 1.4.1 or higher. It can be found at:

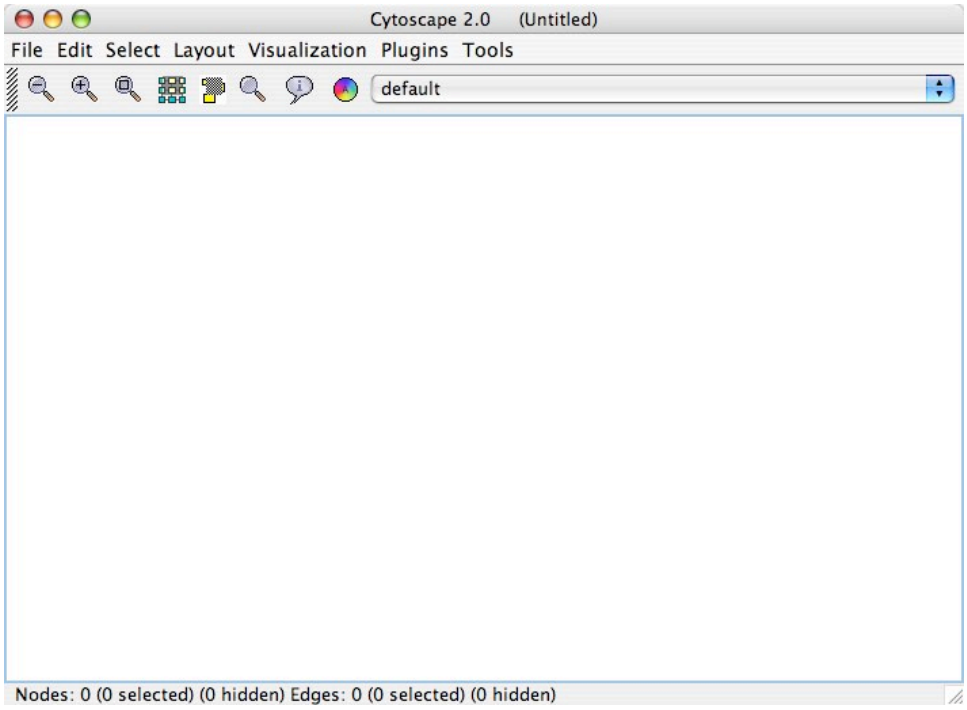
http://java.sun.com/j2se/index.jsp

(3) Launch the application by running "cytoscape.sh" from the command line (Linux or Mac OS X) or double-clicking "cytoscape.bat" (Windows). Alternatively, you can pass the .jar file to Java directly using the command "java -jar cytoscape.jar". In Windows, it is also possible to directly double-click the .jar file to launch it. However, this does not

allow specification of command-line arguments (such as the location of the GO data directory, see the *Command Line Arguments* section for details).

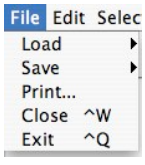
> **! Important Note:**
> For the application to work properly, ALL FILES MUST BE LEFT IN THE DIRECTORY IN WHICH THEY ARE UNPACKED. The core Cytoscape application assumes this directory structure when looking for certain files, such as cytoscape.props, vizmap.props, and the GO/ database].

Cytoscape Window
When you succeed in launching Cytoscape, a window will appear that looks like this:



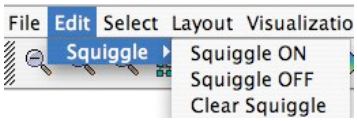
2. Quick Tour of Menus

File
The File menu contains most basic file functionality: File / Load for loading files; File / Save for saving; File / Print for printing to either a printer or a PostScript file. File / Close closes only this window of Cytoscape, leaving other Cytoscape windows open. If this is this is the last open Cytoscape window, File / Close also exits Cytoscape. File / Exit closes all windows of Cytoscape and exits the program. Details of the Load and Save sub-menus can be found in the *Building and Storing Networks* section.



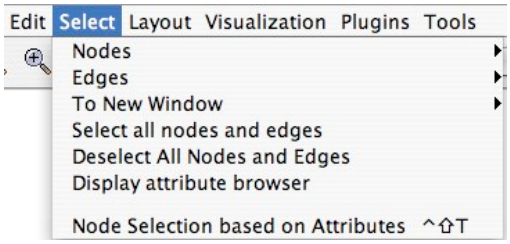
Edit

The Edit menu contains a Squiggle feature that enables you to mark up your network. This can be particularly useful during live presentations.



Select

The Select menu contains methods and operations for selecting nodes and edges, operating on existing selections, and displaying the attribute browser. More details about the Nodes, Edges, and To New Window sub-menus can be found in the *Selection and Filtering* section.

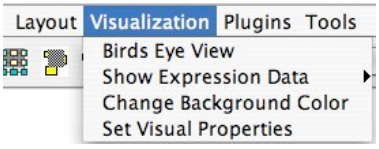


Layout

The Layout menu has an array of features for organizing the graph visually; these features are explored in-depth in the *Visualization* section. The main features include arranging the entire graph according to one of several algorithms; aligning and rotating groups of nodes; and adjusting the size of the graph.

Visualization

The Visualization menu provides options for changing the mapping from biological data to a visual representation: colors of nodes, thickness of edges, etc. These features are explored in-depth in the *Visualization* section. This menu also provides a Bird's Eye view of your entire graph, and multiple options for viewing expression data.



Plugins

The Plugins menu will contain all plug-ins that you have chosen to load in your cytoscape.props file.

Note: A complete list of Cytoscape PlugIns is available online at: http://cytoscape.org/plugins.html

3. Command Line Arguments

Cytoscape recognizes a number of optional command line arguments, including run-time specification of network files and expression data:

-g <GML network filename>	(xxx.gml) Loads a network file in GML format (see *4. Building and Storing Interaction* Networks)
-i <SIF interactions filename>	(yyy.sif) Loads a network file in SIF format (see *4. Building and Storing Interaction* Networks)
-b <bioData directory>	(e.g. GO/annotationsAndSynonyms) Specifies which directory to use for the BioDataServer annotations.
-e <expression filename>	(zzz.pvals) Loads an expression data file (see *5. Loading Gene Expression* Data)
-x	Prevents expression file from also loading into Cytoscape's *6. Node* and Edge Attributes.
-n <nodeAttributes filename>	(zero or more) Loads a node attributes file (see *6. Node and Edge* Attributes)
-j <edgeAttributes filename>	(zero or more) Loads an edge attributes file (see *6. Node and Edge* Attributes)
-h	help: display these command line arguments
-v	display version
--JLD	specifies a directory in which plug-in .jar's reside.

Most data sets may also be loaded after Cytoscape is running. See the sections on *5. Loading Gene Expression* Data and *6. Node and Edge* Attributes for details.

Additional command line arguments that are not recognized by the Cytoscape core are passed to the plug-in modules. Please refer to the documentation for each specific plug-in for more details.

4. Building and Storing Interaction Networks

Cytoscape reads an interaction network in two ways: from a simple interaction file (SIF or .sif format) or from a universal format known as Graph Markup Language (GML or .gml format). SIF specifies nodes and interactions only, while GML stores additional information about network layout and allows network data exchange with a variety of other network display programs. Typically, SIF is used to import interactions when building a network for the first time. Once the interactions have been loaded and layout has been performed, the network may be saved to and subsequently reloaded from GML format in future Cytoscape sessions. Both SIF and GML are implemented as ASCII text files.

SIF FORMAT:
Lines in the SIF file specify a source node, an interaction type, and one or more target nodes:

```
geneA <interaction type> geneB
geneC <interaction type> geneA
geneD <interaction type> geneE geneF geneB
geneG
...
geneY <interaction type> geneZ
```

In the network specified by this file, genes are represented by nodes, and interactions are represented by edges between nodes. For compactness, a gene also represents its corresponding protein. Nodes may also be used to represent compounds and reactions instead of genes, but this is non-standard, as yet. Note that it is possible to specify an isolated node with no interactions, as in the line "geneG" above.

Gene names must be unique. If the network is to be integrated with Gene Ontology (GO) or gene expression data, the gene names must exactly match the systematic ORF names specified in the other data files. We strongly encourage naming genes and proteins by their systematic ORF name; common names may be displayed on the screen for ease of interpretation, so long as these are available to the program in the bioData directory (Cytoscape ships with all yeast ORF-to-common name mappings in a synonym table within the GO/ directory).

The tag `<interaction type>` must be one of:
- `pp` ..... protein -- protein
- `pd` ..... protein -> DNA

Additional interaction types are also possible, but as yet, nonstandard:
- `pr` ..... protein -> reaction
- `rc` ..... reaction -> compound
- `cr` ..... compound -> reaction
- `gl` ..... genetic lethal relationship

Any text string will work, but these are the conventions that have been followed thus far.

GML FORMAT:

In contrast to SIF, GML is a rich graph format language supported by many other graph visualization packages. Its file format specification is available at:

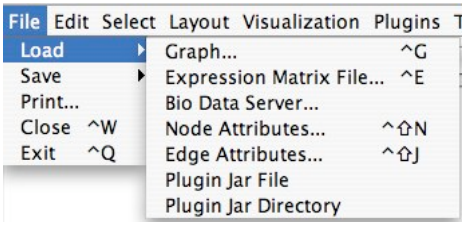
http://www.infosun.fmi.uni-passau.de/Graphlet/GML/

It is generally not necessary to modify the content of a GML file directly. Once a network is built in SIF format and then laid out, the layout is preserved by saving to and loading from GML.

COMMANDS:

Load and save network files using the File menu of Cytoscape. Network files may also be loaded directly from the command line using the –i (SIF format) or -g (GML format) options.

FOR EXAMPLE:



To load a sample molecular interaction network in SIF format, use the menu File / Load / Graph. In the resulting file dialog box, select the file "sampleData/galFiltered.sif". After a few seconds, a small network of 329 nodes should appear in the main window. To load the same interaction network as a GML, use the menu: File / Load / Graph again. In the resulting file dialog box, select the file "sampleData/galFiltered.gml". As of Cytoscape version 1.1, Plug-ins can also be loaded from the File / Load menu, as can node and edge attribute files.

5. Loading Gene Expression Data

Interaction networks are certainly useful as stand-alone models. However, they are most powerful when integrated with information about the biological states that are induced by the network, such as gene or protein expression levels. Once loaded, expression ratios/levels may be visually superimposed on the network, used in a filter to select a subset of nodes, or used to identify active modules and subsystems (see sections below). Expression data are only relevant once a network has been loaded.

FORMAT:

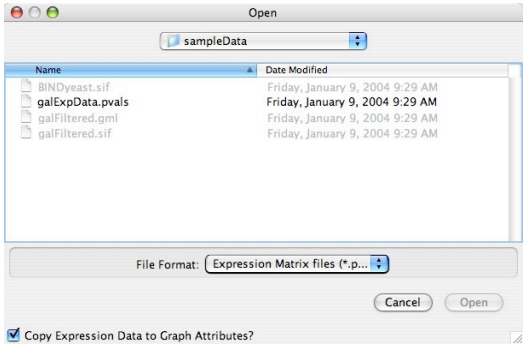
Gene expression ratios are specified over one or more experiments using an ASCII text file. The file consists of a number of space- or tab-delimited fields, one line per gene, with the following format:

```
GeneName [CommonName] ratio1 ratio2 ... ratioN [pval1 pval2 ... pvalN]
```

Brackets [] indicate fields that are optional. The first two fields are the systematic gene name followed by an optional common name. Expression ratios are provided for each experiment, optionally followed by a p-value per experiment or other measure of the significance of each ratio, i.e. whether the ratio represents a true change in expression. Significance values are generated by a variety of software packages for analyzing expression data generated by DNA microarrays, for instance our program VERA (http://www.systemsbiology.org/VERAandSAM). A list of other microarray analysis packages is available at: http://linkage.rockefeller.edu/wli/microarray/soft.html

COMMANDS:
Load an expression data file using the File menu of Cytoscape, or by specifying the filename using the -e option at the command line. The –x command line option indicates that the expression data should not be loaded into node attributes. This is an advanced option, and is typically only used when the number of expression conditions is sufficiently large that it becomes unwieldy in the normal user interface.

FOR EXAMPLE:
Load a sample gene expression data set using the menu: File / Load / Expression Matrix File. In the resulting file dialog box (shown at right), select the file "sampleData/galExpData.pvals". As described in the following sections, Cytoscape is now ready to integrate these data with the underlying molecular interaction network. **Note:** the checkbox in the lower left corner of the file dialog asks whether to "Copy Expression Data to Graph Attributes" – un-checking this box has the same effect is as the command line option -x, and it is left checked by default.



6. Node and Edge Attributes

Node and edge attribute files are very simply formatted: A node attribute file begins with the name of the attribute on the first line, and on each following line, has the name of the node, followed by an equals sign, followed by the value of that attribute. For example:

```
FunctionalCategory
YAL001C = metabolism
YAR002W = apoptosis
YBL007C = ribosome
```

An edge attribute file has much the same structure, except that the name of the edge is the source node name, followed by the interaction type in parentheses, followed by the target node name. Directionality counts, so switching the source and target will refer to a different (or perhaps non-existent) edge. Following is an example edge attributes file:

```
InteractionStrength
```

```
YAL001C (pp) YBR043W = 0.82
YMR022W (pd) YDL112C = 0.441
YDL112C (pd) YMR022W = 0.9013
```

Note that the second and third edge attribute values refer to two different edges (source and target are reversed, though the nodes involved are the same).

Node and edge attributes may be loaded at the command line using the –n and –j options, via the File / Load menu, or using Ctrl-Shift-N and Ctrl-Shift-J.

When expression data is loaded using an expression matrix file (*5. Loading* Gene Expression Data), it is automatically copied into the Node Attributes data structure unless explicitly specified not to.

Edge and Node attributes can be mapped to visual properties (colors, shapes, etc.) using the *7. Visualization* menu item.

7. Visualization

BASIC FEATURES:
Use the zooming buttons located on the toolbar to zoom in / out of the interaction network shown in the current network display. Zoom out and Zoom in are the two leftmost magnifying glass icons on the toolbar (below); the next magnifying icon is for zooming in to an area of selected nodes.

Navigate around the network by using the window sliders or by clicking with the right mouse button (with no nodes or edges selected) and dragging in the desired direction.

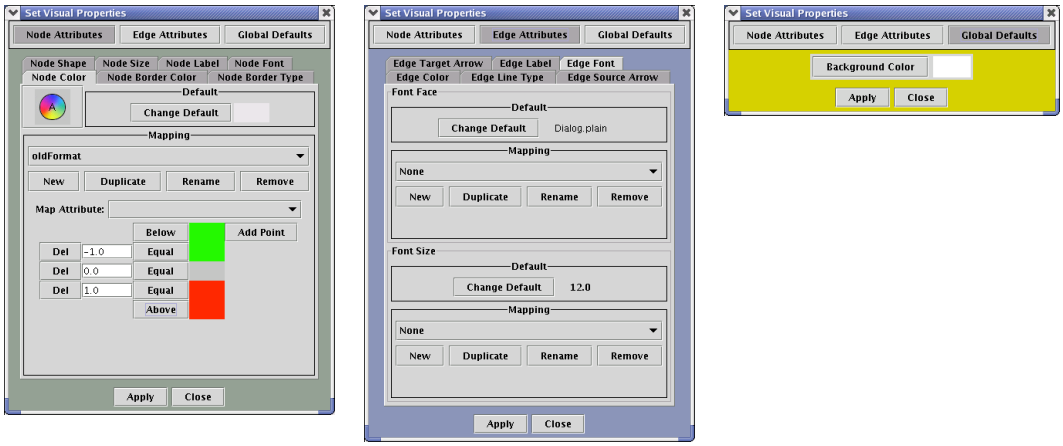
Use the left mouse button to select one or more nodes (genes). A right click then launches a node attributes panel providing detailed information about the selected nodes, including its corresponding annotation in the Gene Ontology (GO) database (if available and specified with the -b command line option). If a gene expression experiment has been selected (see below), the relevant expression data are also shown in the node attributes panel.

NETWORK LAYOUT:
Explore a variety of schemes for layout of the network in two dimensions using the "Layout" menu. First select the type of layout desired (Circular, Hierarchical, ...) and then choose "Whole graph" or "Current Selection" to perform the layout on either all nodes or only those that are currently selected. It is also possible to Align Selected Nodes vertically or horizontally, or to Rotate Selected Nodes a particular number of degrees. Saving a network in GML format (see section above) records the network

topology (nodes and links) and its current layout; saving in Interaction format (.sif) will not preserve layout.

CONFIGURING AND MAPPING VISUAL PROPERTIES:

Bring up the Visual Properties dialog (shown on the next page) by choosing Visualization / Set Visual Properties. Default node and edge visual properties such as color, size, and style are specified in the upper portion of the dialog. Defaults may also be configured at run-time by editing the vizmap.props and cytoscape.props file. Please follow the models provided in the .props files that are included with this distribution.



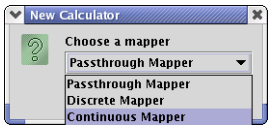
Above: The three faces of the *Set Visual Properties* dialog.

For every visual property of Cytoscape (the nodes' colors, the edges' label font, etc.), there is a menu in the *Set Visual Properties* dialog. For each property, there is a default, and a potential mapping. Mappings usually map an attribute of nodes or edges to visual properties; you can specify both *which attribute* to map and *how to map* the attribute to a visual property using the dialog.

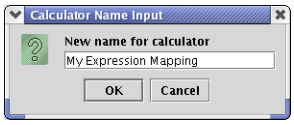
For instance, Node Label may be mapped to display the gene names (canonical or common) or current gene expression values. Edge color may be used to reflect the type of interaction each edge represents, e.g. protein-protein ('pp' tag) or protein->DNA ('pd' tag). As described next, one of the most important mappings is from node color to gene expression values.

SUPERPOSITION OF EXPRESSION DATA ON THE NETWORK:

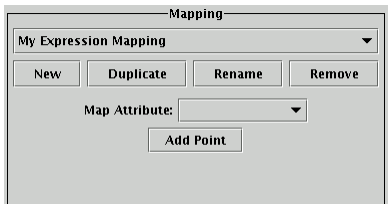
To visually map and superimpose expression data on the network, choose either **Node Color** or **Node Border Color** from the *Set Visual Properties* dialog. Assuming you have loaded an expression data set, or another node attribute, follow these steps:



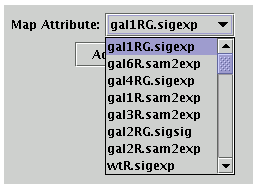
Create a new calculator by pressing the "New" button under Mapping. The New Calculator dialog will come up; select "Continuous Mapper."



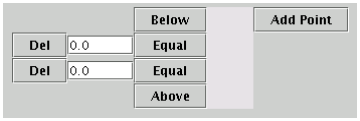
Choose a name for your new calculator.



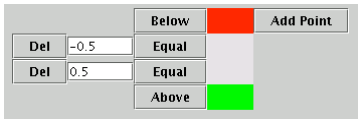
At this point, a new continuous mapping will be ready, except that it doesn't currently map any attribute, and even if it did, that mapping hasn't yet been defined. On to the next step.



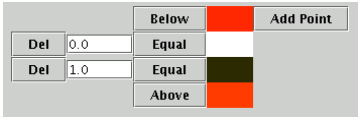
Select an attribute to map.



Click the "Add Point" button twice.



Choose colors and control points for the mapping. The mapping shown at left would color a node red whose expression level was less than -0.5 (presumably a log ratio); nodes with expression levels greater than +0.5 would be colored green; any node with an expression level between -0.5 and +0.5 would be colored gray. The continuous mapping will interpolate between colors you provide if the attribute value is between the control points you set on the left side of the dialog. For example, the mapping shown to the right will interpolate between white and black for attribute values ranging from zero to one. Numbers outside this range are colored red, in this mapping.



Once you have designed a mapping to your liking, press the Apply button at the bottom of the *Set Visual Properties* dialog, and see your expression data superimposed on the network!

The initial expression color mappings which Cytoscape load at run-time are specified in the vizmap.props file as a list of fields and their comma-separated RGB values; the choices of which mappings are actually used when you first bring up Cytoscape are

specified in the cytoscape.props file. Follow the model provided in the .props files included with this distribution.

VISUAL STYLES – NEW WITH VERSION 1.1

Though the screenshots above are from Cytoscape 1.0, Cytoscape 1.1 and Cytoscape 2.0 offer "Visual Styles," in which visual calculators are grouped together, so you can switch rapidly from one Cytoscape appearance to another. The choice of which style to use is saved in the cytoscape.props file as the edgeAppearanceCalculator and nodeAppearanceCalculator; the specification of all available styles is saved in the vizmap.props file.

Appendix: GNU Lesser General Public License

GNU LESSER GENERAL PUBLIC LICENSE
Version 2.1, February 1999

[This is the first released version of the Lesser GPL. It also counts as the successor of the GNU Library Public License, version 2, hence the version number 2.1.]

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For example, on rare occasions, there may be a special need to encourage the widest possible use of a certain library, so that it becomes a de-facto standard. To achieve this, non-free programs must be allowed to use the library. A more frequent case is that a free library does the same job as widely used non-free libraries. In this case, there is little to gain by limiting the free library to free software only, so we use the Lesser General Public License.

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(For example, a function in a library to compute square roots has a purpose that is entirely well-defined independent of the

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This option is useful when you wish to copy part of the code of the Library into a program that is not a library.

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If distribution of object code is made by offering access to copy from a designated place, then offering equivalent access to copy the source code from the same place satisfies the requirement to distribute the source code, even though third parties are not compelled to copy the source along with the object code.

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d) If distribution of the work is made by offering access to copy from a designated place, offer equivalent access to copy the above specified materials from the same place.

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