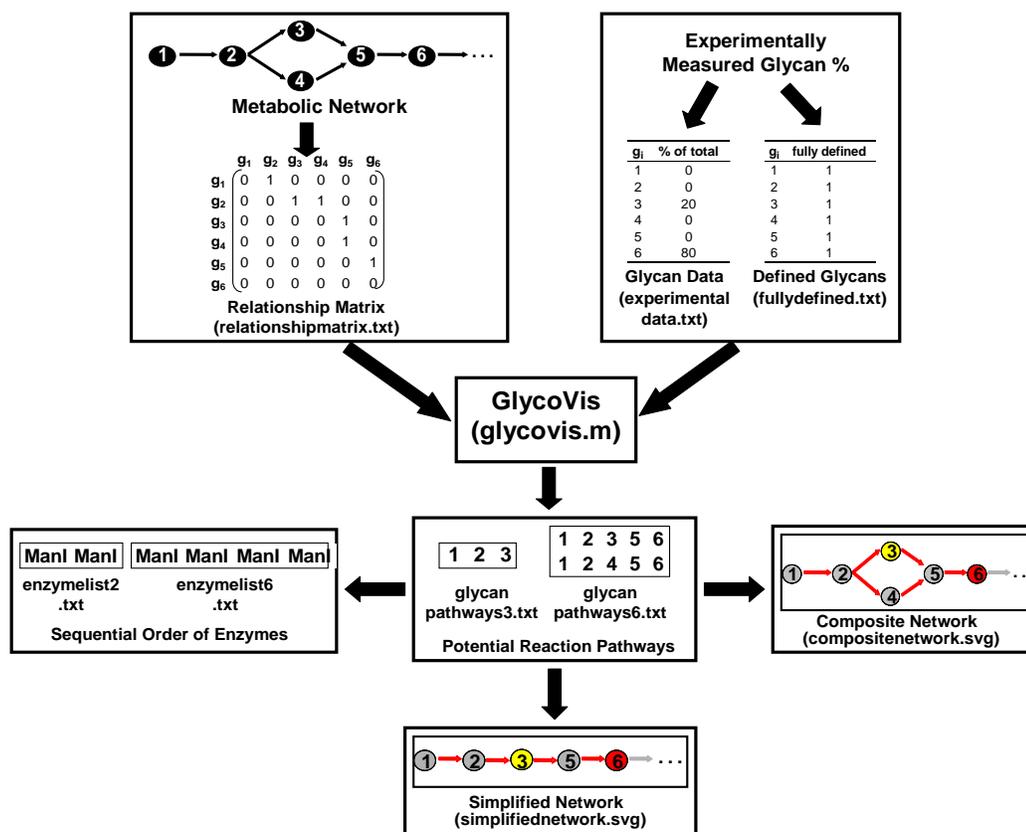


1. Main Contents

- **Installer:** this folder contains two executable files for Matlab Runtime environment installation. Users only need to run either one of these files:
 - “*GlycoVis Installer_mcr.exe*”: Installation does NOT require Internet connection
 - “*GlycoVis Installer_web.exe*”: Installation requires Internet connection
- **To be copied:** this folder contains all the files needed to run the program. See the notes below for detailed explanation.
- “*Publication.pdf*”: This file is the paper published by the authors describing the algorithm and main features of the program. Some examples were also included in the paper.
- “*GlycoVis Tutorial.pdf*”: This tutorial file.

2. Program Flowchart



3. Operating System Required

Microsoft Windows XP, Microsoft Windows 7

4. Programs Required

- Microsoft Excel
- Microsoft Internet Explorer, or Google Chrome, or Firefox

5. Introduction

GlycoVis is an automated visualization program to facilitate the visualization of metabolic networks. It runs on Windows based machines, and has been tested on both Windows XP and Windows 7 operating systems. An illustrative use of the program towards visualizing the protein

N-glycosylation pathway is shown below. The program is adaptable towards other enzymes and systems. This version of the program takes into consideration 10 protein glycosylation enzymes. (See below)

Enzyme Name	Name Used In This Program
mannosyl-oligosaccharide 1,2-alpha-mannosidase	Man I
mannosyl-oligosaccharide 1,3-1,6-alpha-mannosidase	Man II
alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	GnT I
alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	GnT II
beta-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase	GnT III
alpha-1,3-mannosylglycoprotein 4-beta-N-acetylglucosaminyltransferase	GnT IV
alpha-1,6-mannosyl-glycoprotein 6-beta-N-acetylglucosaminyltransferase	GnT V
glycoprotein 6-alpha-L-fucosyltransferase	FucT
beta-N-acetylglucosaminylglycopeptide beta-1,4-galactosyltransferase	GalT
beta-galactoside alpha-2,3/6-sialyltransferase	SiaT

6. Relationship Matrix

The enzymatic pathways were deduced using literature reported substrate specificities. There are multiple glycans that can potentially react with each enzyme, however not all of them do. The program assumes those reactions that are capable of occurring, to have an equal probability of doing so. This criterion can be easily changed and implemented using the program's relationship matrix as described below. The current release of the program considers 341 distinct N-glycans. To transform the network of 341 N-glycans into a workable, mathematical formulation, a matrix was constructed to describe the glycan product-substrate relationships, and the corresponding enzymes that facilitate them. This matrix is saved under the filename "*relationshipmatrix.txt*" in the "**instructions**" folder. Each row in the matrix corresponds to a glycan reactant, and each column corresponds to a potential glycan product. Those reactant-product pairs that are allowed are given a non-zero value in the matrix corresponding to the numerical abbreviation of the enzyme responsible for their reaction, and those that are prohibited are given a zero value. The relationship matrix can be readily modified to remove or include additional glycans, or glycan specificities. GlycoVis can also be readily modified to have additional levels of glycan specificities.

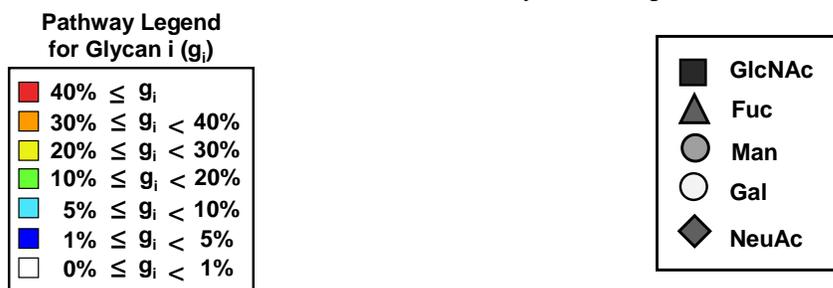
7. Glycan Data Entry

In the attached "*glycovisdataentry.xls*" worksheet in the "**instructions**" folder, insert your glycoform profile data into the appropriate rows corresponding to the N-glycan measured. For convenience, individual glycans can be found in the spreadsheet by filtering the glycans according to their sugar composition. The auto-filter feature of Excel has been enabled in the spreadsheet so that you can specify the total number of each of the sugars that comprise the N-glycan. An additional worksheet is provided in "*glycovisdataentry.xls*" that should be saved into a separate .txt file in the "**instructions**" folder. By default this file is "*experimentaldata.txt*". Typically, the exact sugar composition of the N-glycan is known, but the positional information of the terminal sugars on the glycan antennae is not. GlycoVis takes this into consideration by changing the shape of the nodes corresponding to these glycans. By default, those glycans that are measured exactly are given an oval node shape, whereas those that are not are given a box shaped node. You should specify in "*glycovisdataentry.xls*" whether or not each of the measured glycans were specifically measured. An additional worksheet is provided that should be saved into a separate .txt file in the "**instructions**" folder. By default this file is "*fullydefined.txt*".

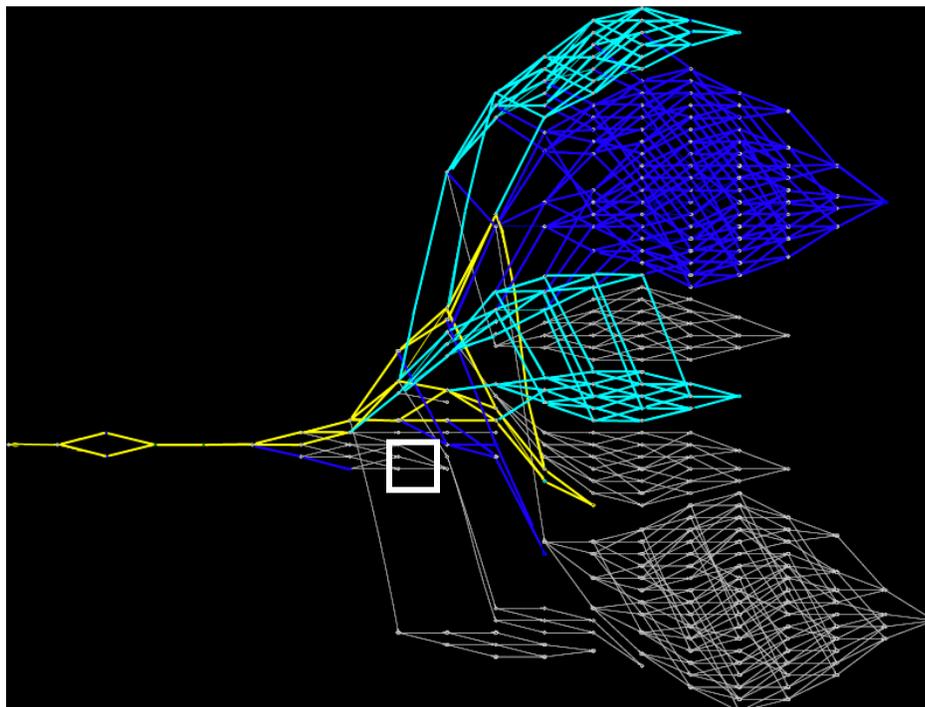
8. Potential Reaction Pathways and the Corresponding Enzymes

GlycoVis calculates all the potential reaction paths traversed for the synthesis of each prominent glycan. These potential reaction pathways are highlighted on a composite pathway map. GlycoVis interfaces with GraphViz (<http://www.graphviz.org>) for the illustration of these pathways. A copy of the appropriate Graphviz programs has already been included in GlycoVis. In the illustration, nodes symbolically represent the glycans, and edges represent the enzymatic reactions. The nodes are colored according to their corresponding glycan percentage of the total glycoform profile, and the edges are highlighted with the same color as the nodes towards which they are directed to. If the same edge is used for pathways directed towards two distinct nodes, then the color corresponding to the higher percentage node is used. However, this default criterion for highlighting the nodes and edges can be easily changed. The composite pathway map is constructed and saved in the folder "C:\glycovis\compositenetwork" in .svg format under the filename "compositenetwork.svg". The two "hot key" for .svg formatted files is CTRL. Holding down CTRL and scroll the mouse simultaneously allows one to create a zoom box that will bring various regions of the network into higher magnification. Mouse scrolling allows one to move the network around the screen space. The displayed composite network is a dynamic interface that allows you to zoom-in and click on the various nodes to see closer-up the glycan structures, and the pathways that traverse them. (See example below)

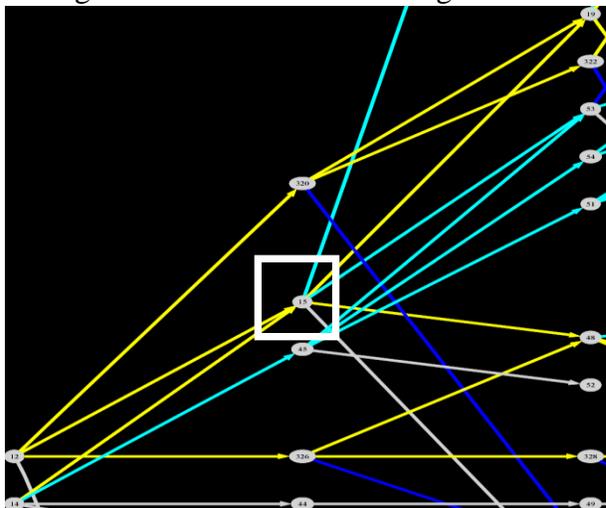
Node/edge color scheme used by default in GlycoVis Symbolic representation of monosaccharides



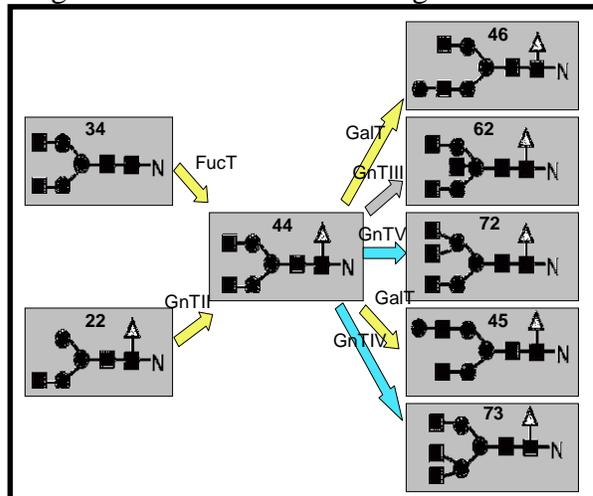
Representative GlycoVis Composite Network



Magnification view of boxed region above



Magnification view of boxed region to the left



All potential reaction pathways from each experimentally measured glycan are saved into separate .txt files. By default, the filenames are "glycanpathwaysX.txt", for prominent glycan X. In addition, the enzymes responsible for the reactions in each pathway are also saved into separate .txt files. By default, these filenames are "enzymesX.txt", for prominent glycan X. These files are tabbed-delimited files, and can be opened in Excel. A representative screenshot is shown below:

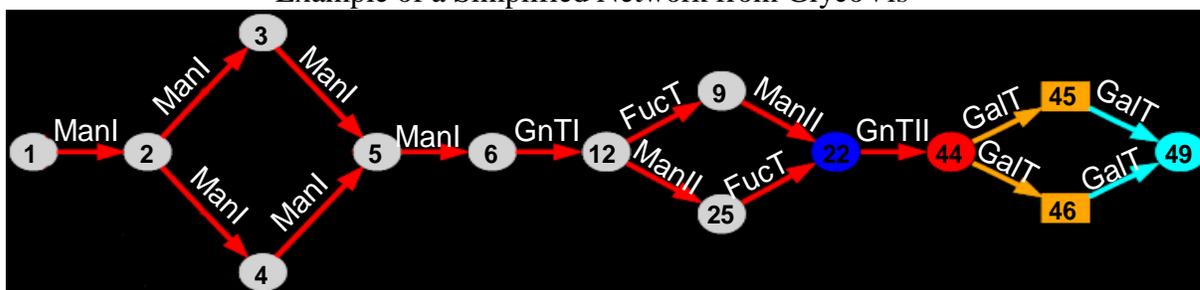
114	164	214	264	314	glycanpathways24
115	165	215	265	315	glycanpathways26
116	166	216	266	316	glycanpathways40
117	167	217	267	317	glycanpathways41
118	168	218	268	318	glycanpathways42
119	169	219	269	319	glycanpathways43
120	170	220	270	320	glycanpathways147
121	171	221	271	321	glycanpathways148
122	172	222	272	322	glycanpathways152
123	173	223	273	323	glycanpathways154
124	174	224	274	324	glycanpathways156
125	175	225	275	325	glycanpathways157
126	176	226	276	326	glycanpathways165
127	177	227	277	327	glycanpathways210
128	178	228	278	328	glycanpathways229
129	179	229	279	329	glycanpathways317
130	180	230	280	330	glycanpathways323
131	181	231	281	331	
132	182	232	282	332	
133	183	233	283	333	
134	184	234	284	334	
135	185	235	285	335	
136	186	236	286	336	
137	187	237	287	336	
138	188	238	288	compositenetwork	
139	189	239	289	enzymelist6	
140	190	240	290	enzymelist7	
141	191	241	291	enzymelist8	
142	192	242	292	enzymelist9	
143	193	243	293	enzymelist24	
144	194	244	294	enzymelist26	
145	195	245	295	enzymelist40	
146	196	246	296	enzymelist41	
147	197	247	297	enzymelist42	
148	198	248	298	enzymelist43	
149	199	249	299	enzymelist147	
150	200	250	300	enzymelist148	
151	201	251	301	enzymelist152	
152	202	252	302	enzymelist154	
153	203	253	303	enzymelist156	
154	204	254	304	enzymelist157	
155	205	255	305	enzymelist165	
156	206	256	306	enzymelist210	
157	207	257	307	enzymelist229	
158	208	258	308	enzymelist317	
159	209	259	309	enzymelist323	
160	210	260	310	enzymelist325	
161	211	261	311	glycanpathways5	
162	212	262	312	glycanpathways7	
163	213	263	313	glycanpathways8	
				glycanpathways9	

9. Simplified Reaction Path

Having a concise image of the glycan biosynthetic pathways facilitates quicker physiological interpretation. Towards this end an additional simplified reaction path is shown ("*simplifiednetwork.svg*") in the folder "**C:\glycovis\simplifiednetwork**" to more symbolically represent the potential pathways (see below). In these illustrations, those reaction paths that are not utilized by the prominent glycans are removed, leaving only the highlighted edges, and nodes.

Again, the resulting pathway map is a dynamic interface with clickable nodes that open additional windows showing the glycans and the reactions that traverse them.

Example of a Simplified Network from GlycoVis



10. Step-by-Step Instructions to Run GlycoVis:

- Step 1:** Install the Matlab Runtime environment for glycovis using either of these two methods:
- Execute the "GlycoVis Installer_mcr.exe" file. This method does **NOT** require Internet connection.
 - Execute the "GlycoVis Installer_web.exe" file. This method requires Internet connection.

Step 2: Copy all the files and folders inside the "To be copied" zipped folder onto the "C:\glycovis\" folder.

Step 3: Enter experimentally determined glycan data into "C:\glycovis\instructions\glycovisdataentry.xls"

Step 4: Save data from the "Percentages" worksheet within "glycovisdataentry.xls" into the file "experimentaldata.txt" in the "instructions" folder.

Step 5: Save data from the "Fully Defined" worksheet within "glycovisdataentry.xls" into the file "fullydefined.txt" in the "instructions" folder.

Step 6: Run glycovis by double clicking on "glycovis.exe" in the "C:\glycovis\" folder.

Step 7: The composite and simplified networks are returned back to the screen. These files are also saved in the "compositenetwork" and "simplifiednetwork" folders.

Note1:

As a security precaution, your browser may "restrict" these files from opening automatically. You may have to adjust browser settings to prevent "active content" from being blocked, or manually allowing it by clicking on the warning below the address toolbar.

Note2:

You may have to zoom-out on the compositenetwork.svg and/or simplifiednetwork.svg files to fit the entire image on the screen

11. Instructions on How to Modify GlycoVis to Include/Exclude Individual Glycans/Reactions

To introduce additional glycans into the program, one new row and column needs to be added to "relationshipmatrix.txt" for each glycan introduced. All glycans that are capable of forming the new glycan(s) should be given a nonzero value into the array element(s) whose row(s) corresponding to the glycan substrates capable of performing the reaction, and the column(s) corresponding to the newly introduced glycan(s). All glycans that are capable of being formed from the new glycan(s) should be given a nonzero value into the array element(s) whose row corresponds to the newly introduced glycan(s) and whose column corresponds to the glycan(s) that are capable of being formed. To include additional reactions into the existing system, entries

with a zero value in "newrelationshipmatrix.txt" corresponding to the desired glycan reactant-product pair should be changed to a nonzero value. To exclude individual reactions in the existing system, entries with a nonzero value in "relationshipmatrix.txt" corresponding to the glycan reactant-product pair should be changed to a zero.

12. Comments on GlycoVis

- You have the choice to visualize the individual N-glycans using the .gif files that incorporate geometric symbols, or the chemical structures. Both are provided in the glycovis package, however by default, the geometric symbols are used for both the composite network and simplified network. If you want to use the chemical structures instead, you must copy those figures from the "glycan figures with structural information" folder into the "compositenetwork" and "simplifiednetwork" folders.
- Unless modified, the program was designed under the assumption that the input and output files should be in the same folder (e.g "C:\glycovis\").
- There are a large number of files created in the "simplifiednetwork" and "compositenetwork" folders. It is easiest to find the file of interest by using the View -> Arrange Icons By ->Type feature in the menu bar
- The composite network images currently open into a larger window than necessary so as to preclude resolution loss of these larger network images. You must zoom out once the file is opened to fit the entire network image on the screen.
- Longer computational times can be expected for mapping those glycans at the termini of the biosynthetic pathway due to the larger number of potential pathways that can be traversed for their synthesis.
- It is perhaps easiest to find the files of interest by not viewing hidden files. You can accomplish this by selecting "Do not show hidden files and folders" in Tools -> Folder Options -> View tab, and setting "Do not show hidden files and folders".
- GlycoVis formatted images can be edited outside the program by using other programs released with Graphviz (see www.graphviz.org for additional information).