

Getting value out of your Gene Regulatory Network



M. Lucas

IRD - UMR DIADE - Equipe CERES



NETBIO 2026

Getting value out of your Gene Regulatory Network



Or...



M. Lucas

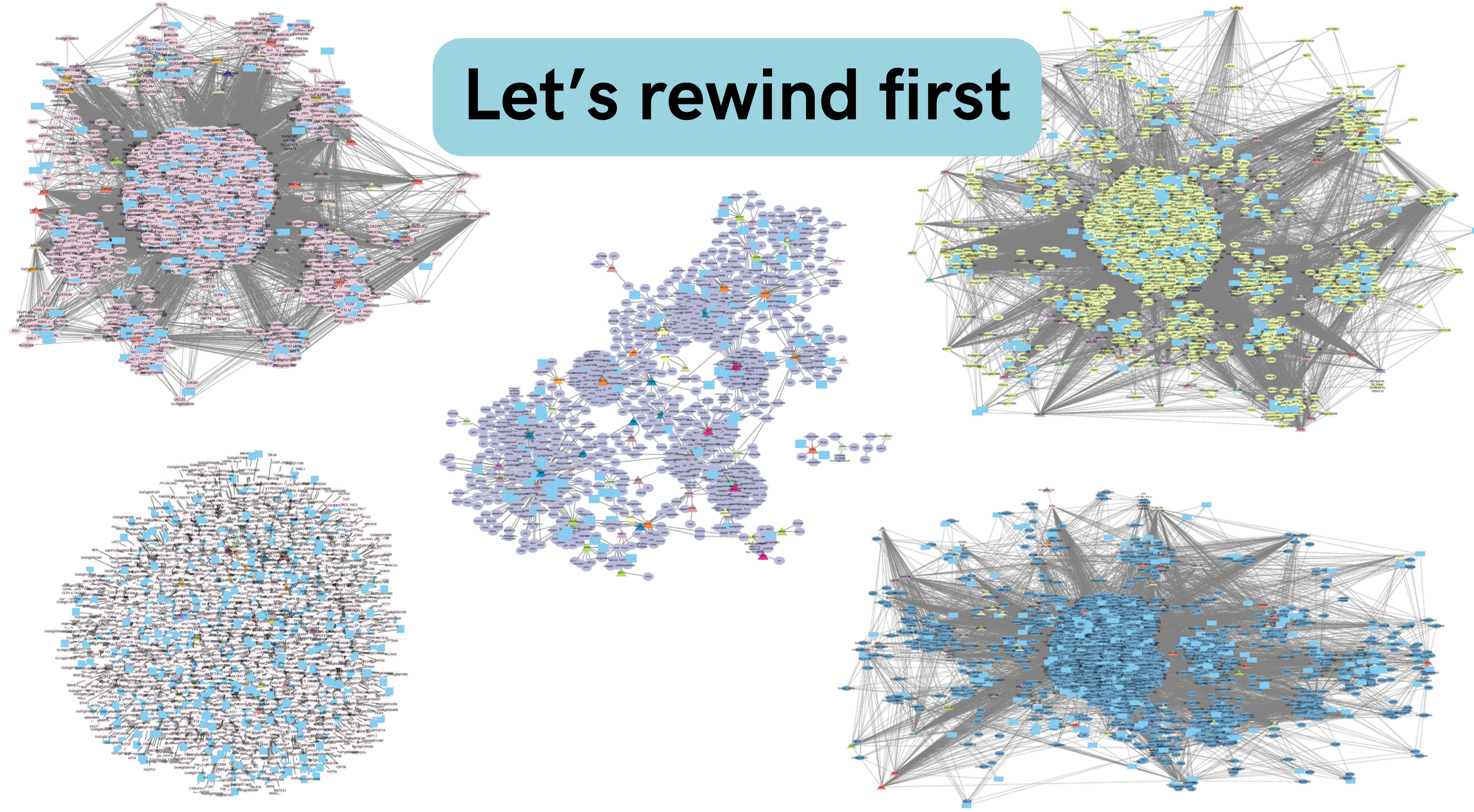
IRD - UMR DIADE - Equipe CERES

NETBIO 2026

“What do I do with this now ?”

“What do I do with these now ?”

Let's rewind first



You are a biologist (or you know a biologist) with an FBP



You are a biologist (or you know a biologist) with an FBP



“Favorite Biological Process”



“I got a great idea !”



**“Let’s do transcriptomics
on our FBP !”**



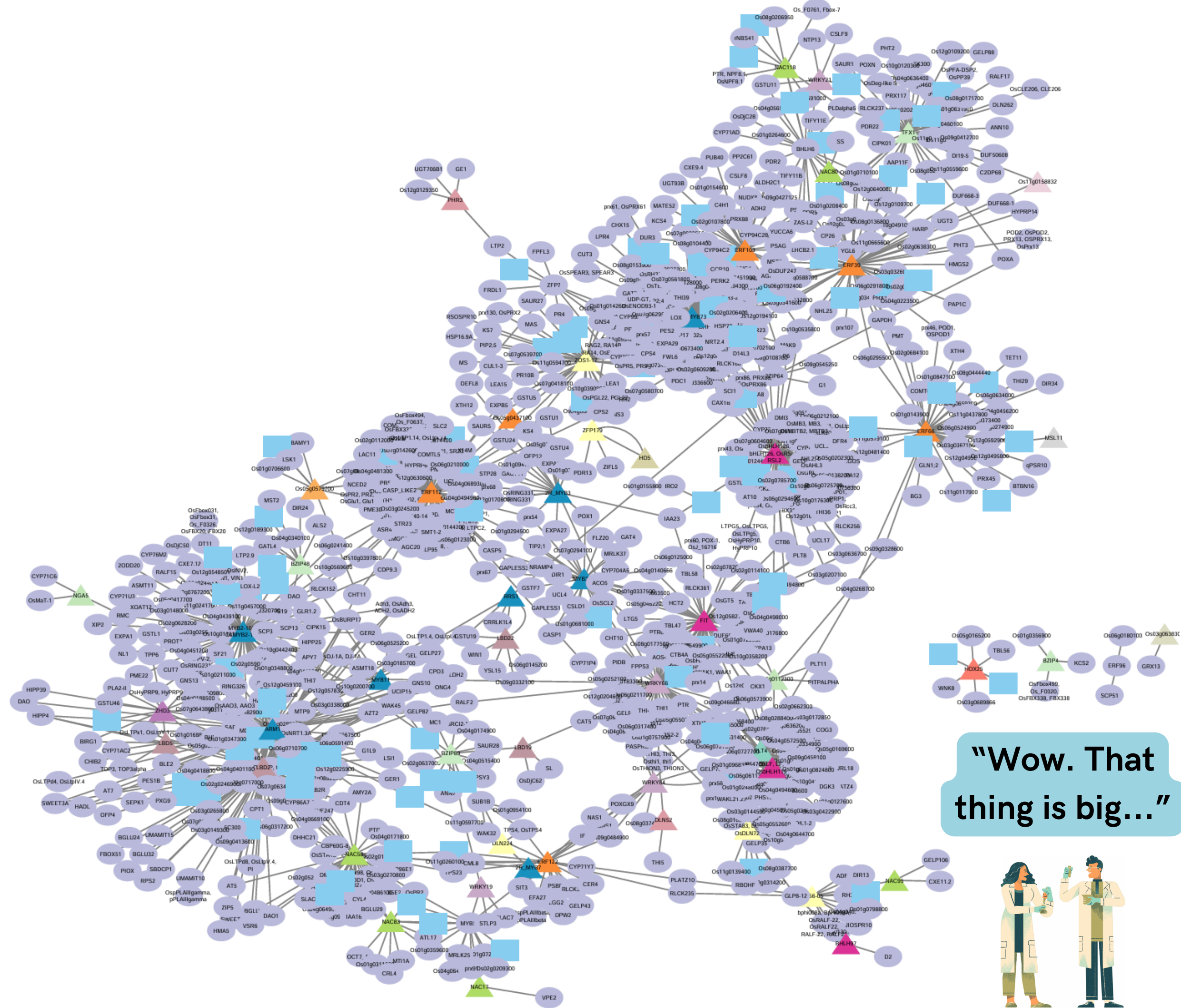
**“That way we can get a
gene network and use it !”**





**A FEW
MOMENTS LATER**

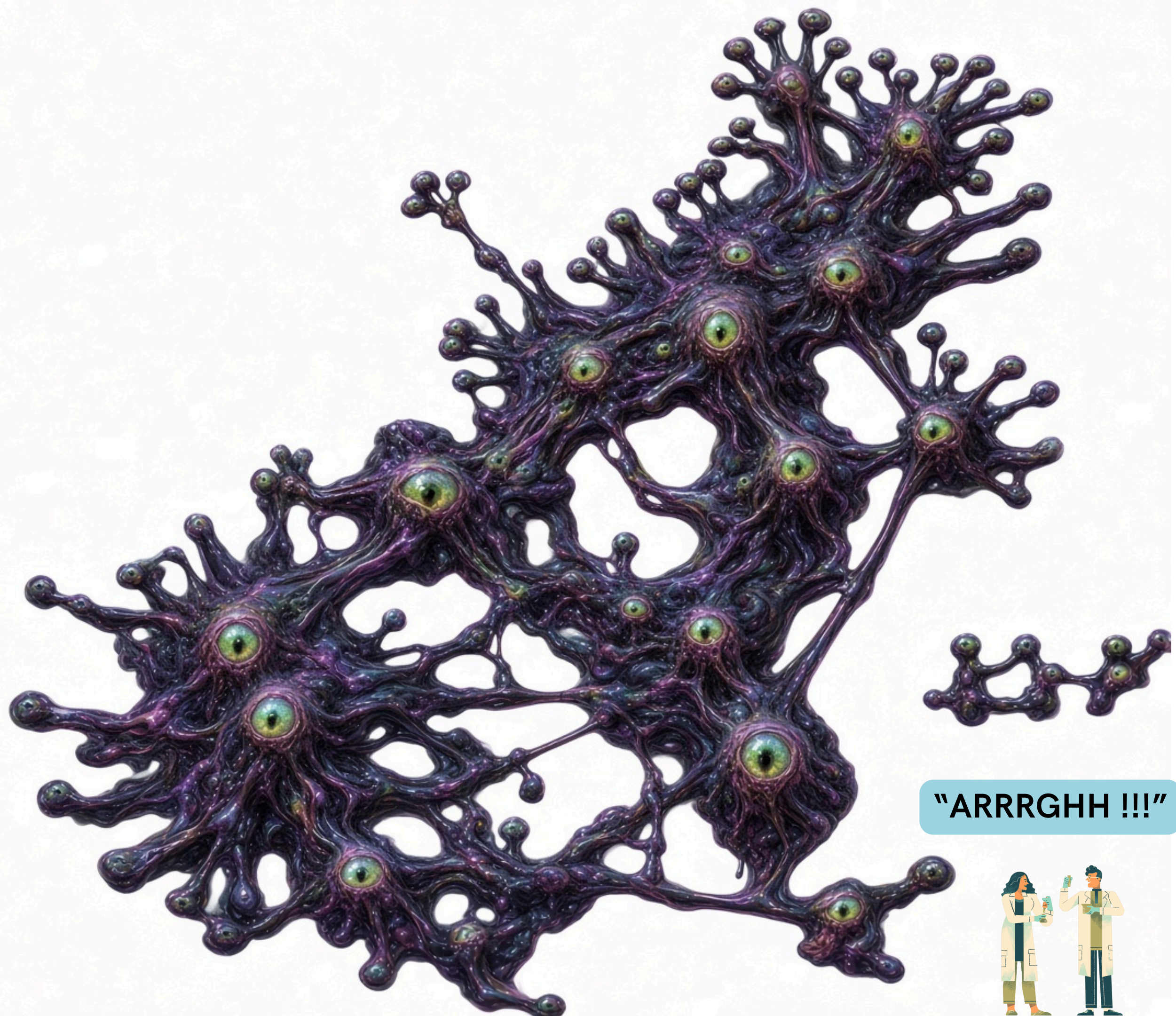
I present to you :
the regulatory
gene network



I present to you :
the regulatory
gene network

(or as I like to call it,
the Shoggoth)

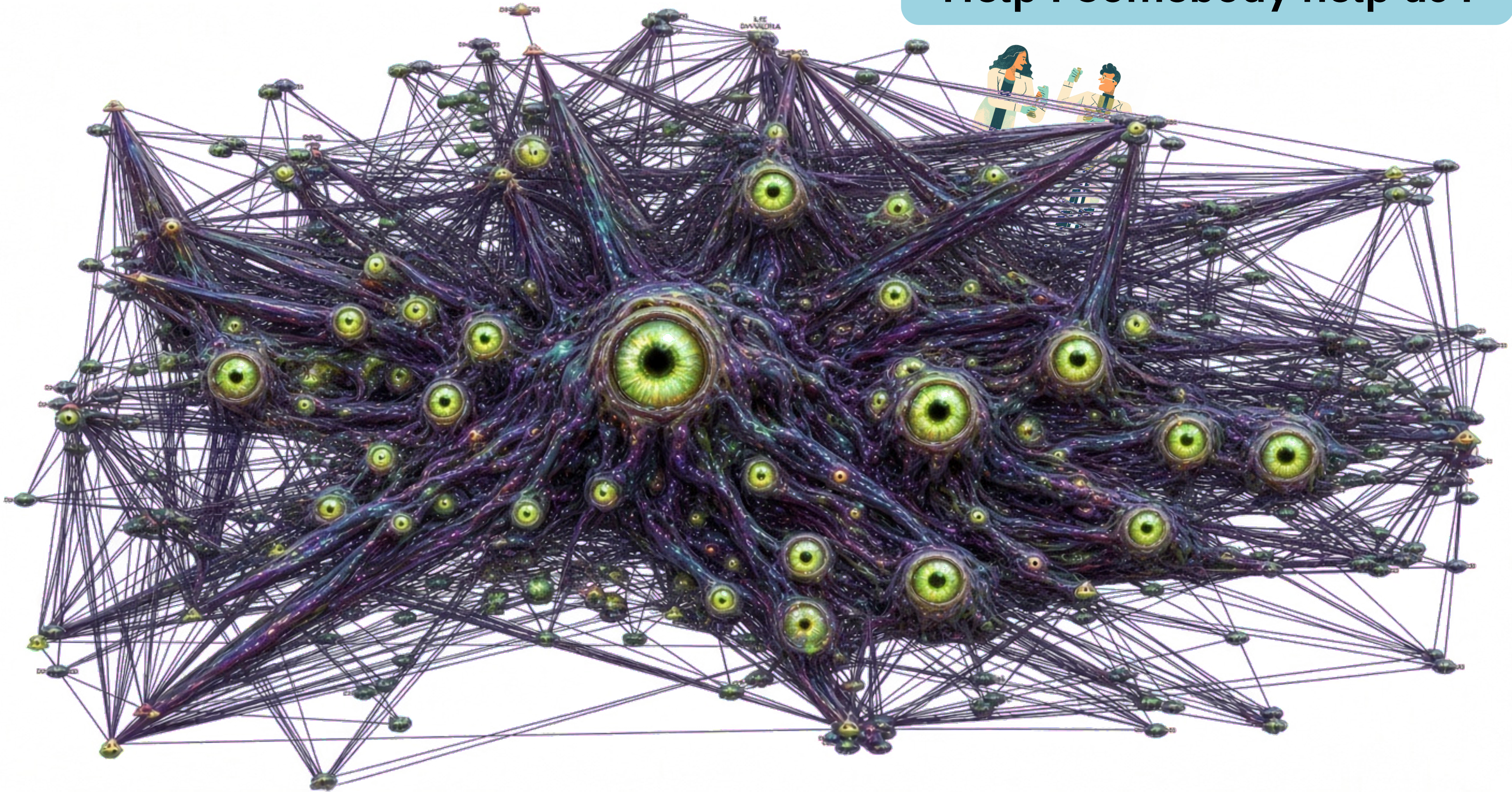
"It was a terrible, indescribable
thing [...] — a shapeless
congeries of protoplasmic
bubbles,[...] with myriads of
temporary eyes forming and un-
forming [...] that bore down upon
us" (H.P. Lovecrat, 1931, At the
Mountain of Madness)



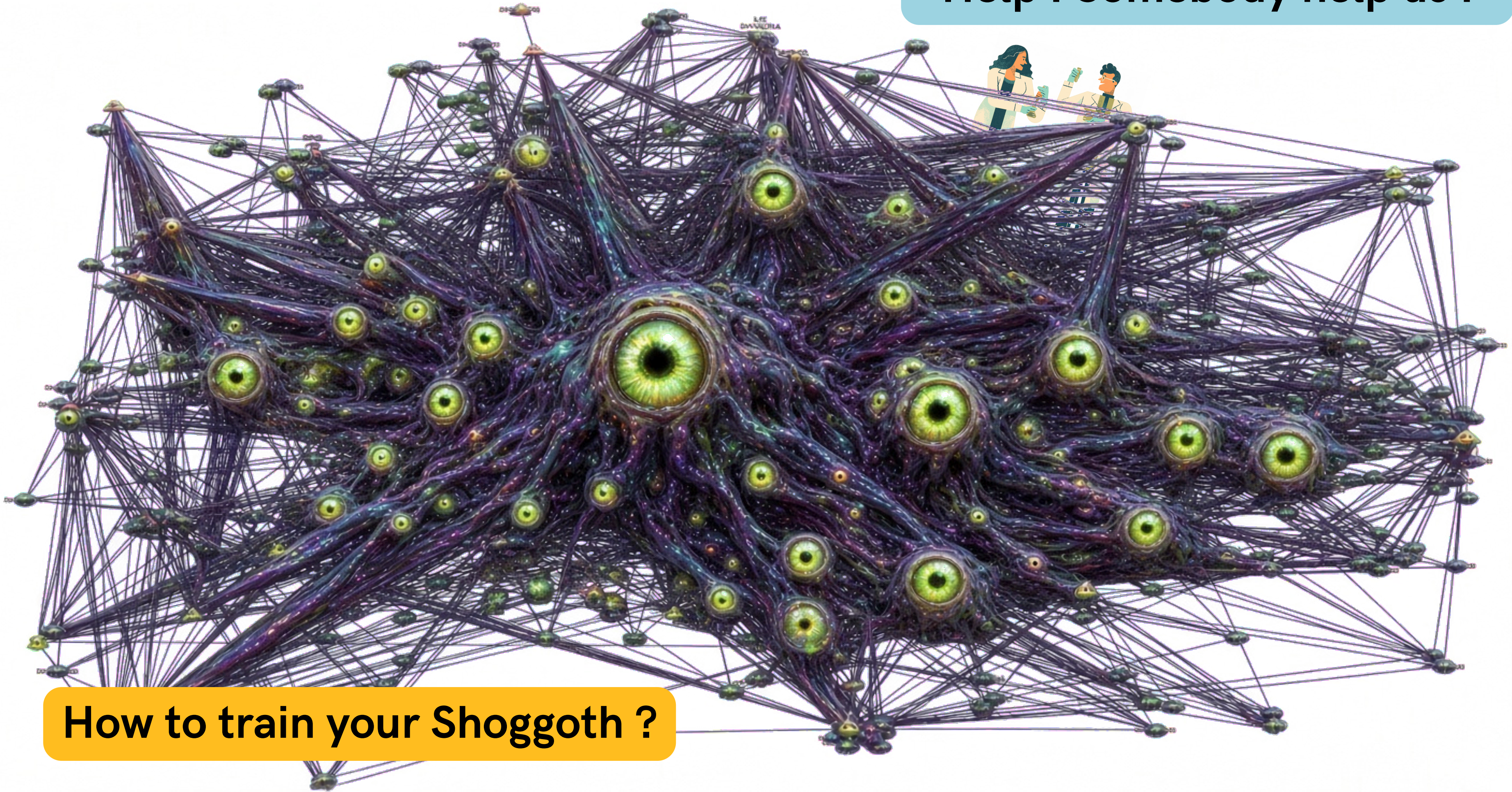
"ARRRGHH !!!"



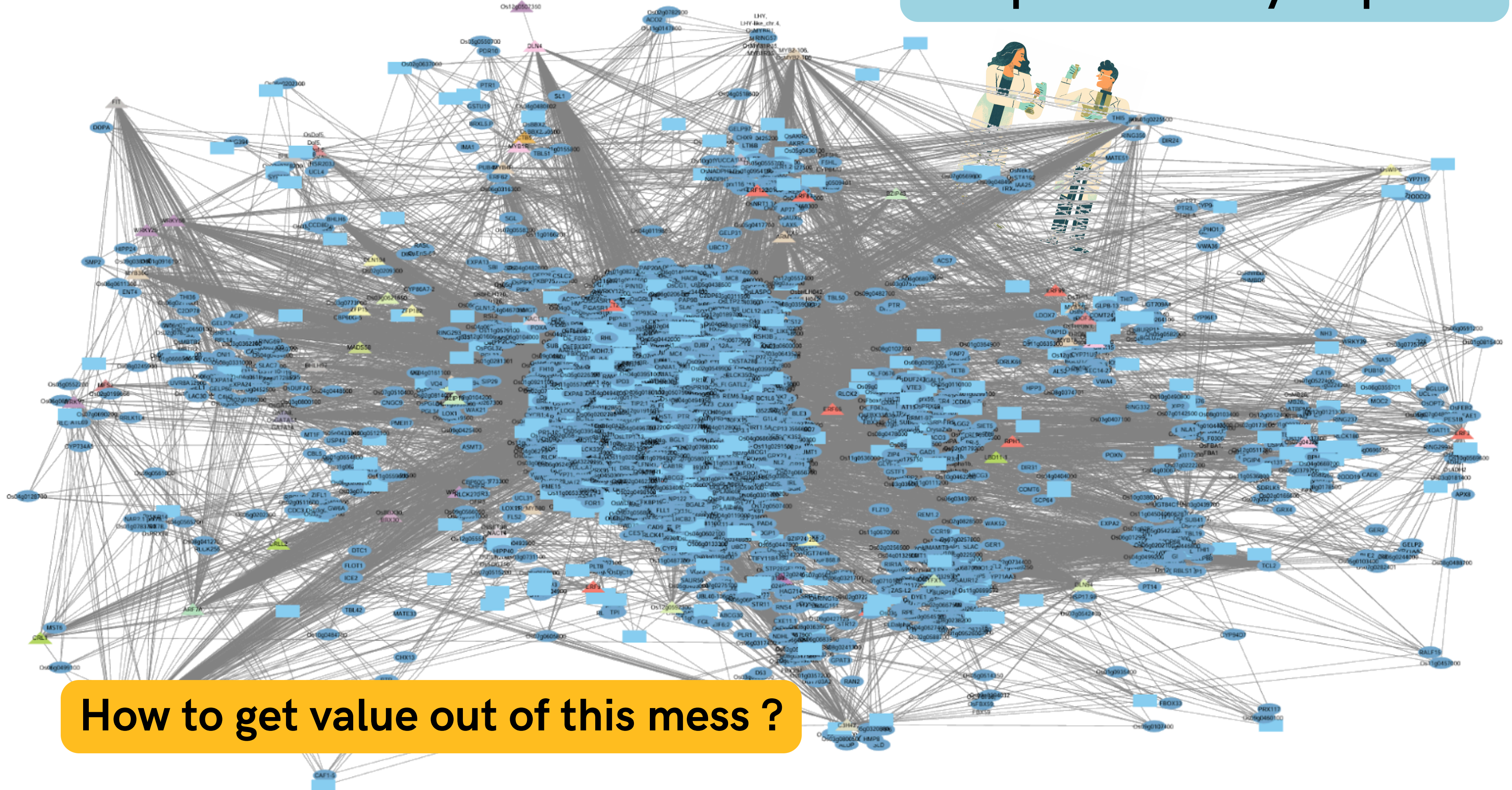
“Help ! Somebody help us !”



“Help ! Somebody help us !”



How to train your Shoggoth ?



How to get value out of this mess ?

To give some answers to that question, first let's get a closer look at two Shoggoth subspecies

"Oh God ! There is more of them !"



To give some answers to that question, first let's get a closer look at two Shoggoth subspecies

"Look ! Look !"



**The common
Smooth Shoggoth**



**The much rarer
Barbed Shoggoth**

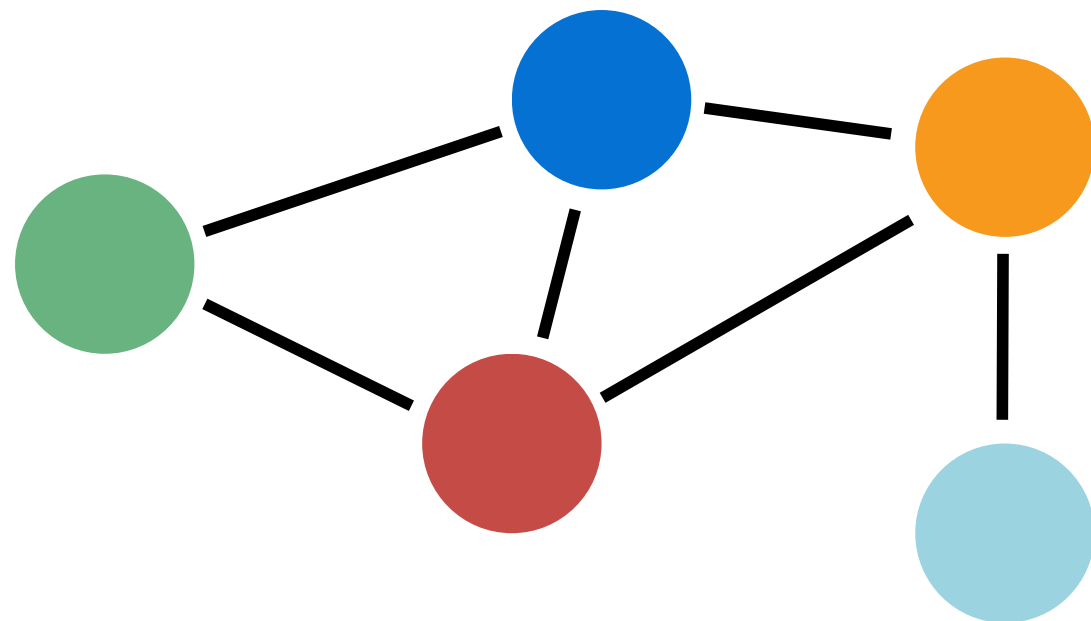


To give some answers to that question, first let's get a closer look at two sorts of networks

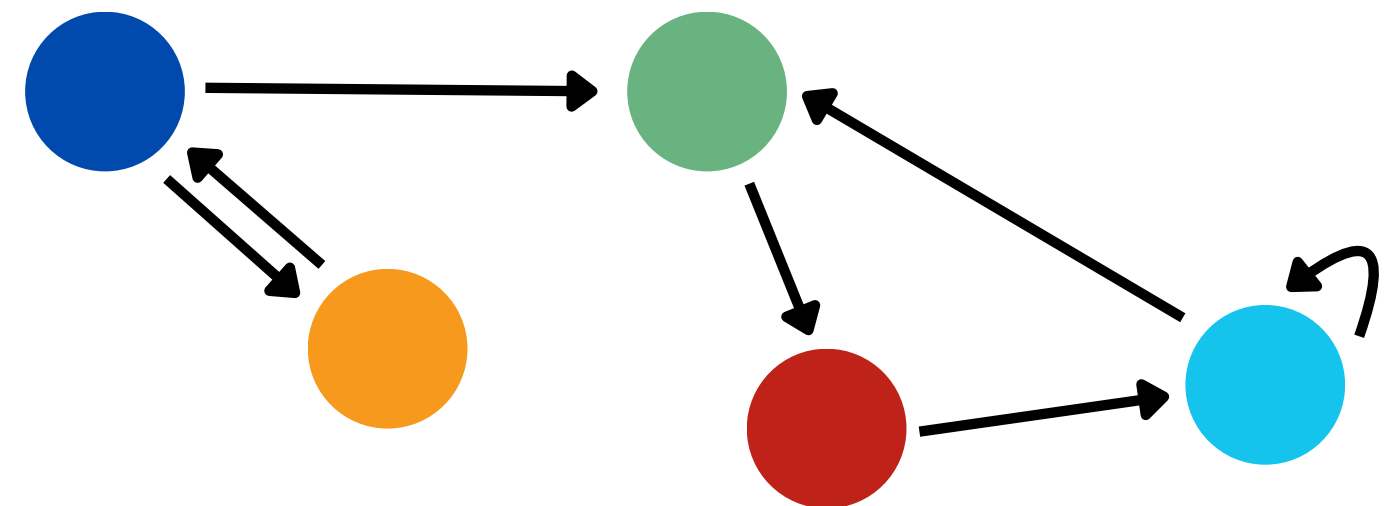
"It's just some networks."



a.k.a. undirected network



a.k.a. directed network

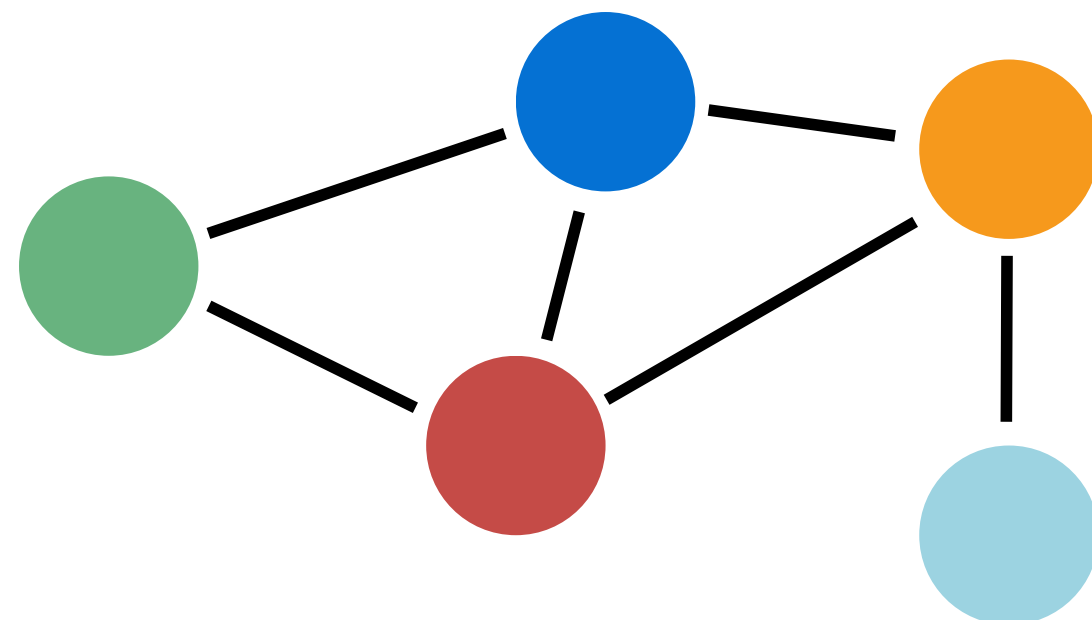


To give some answers to that question, first let's get a closer look at two sorts of networks

"You really need vacations..."

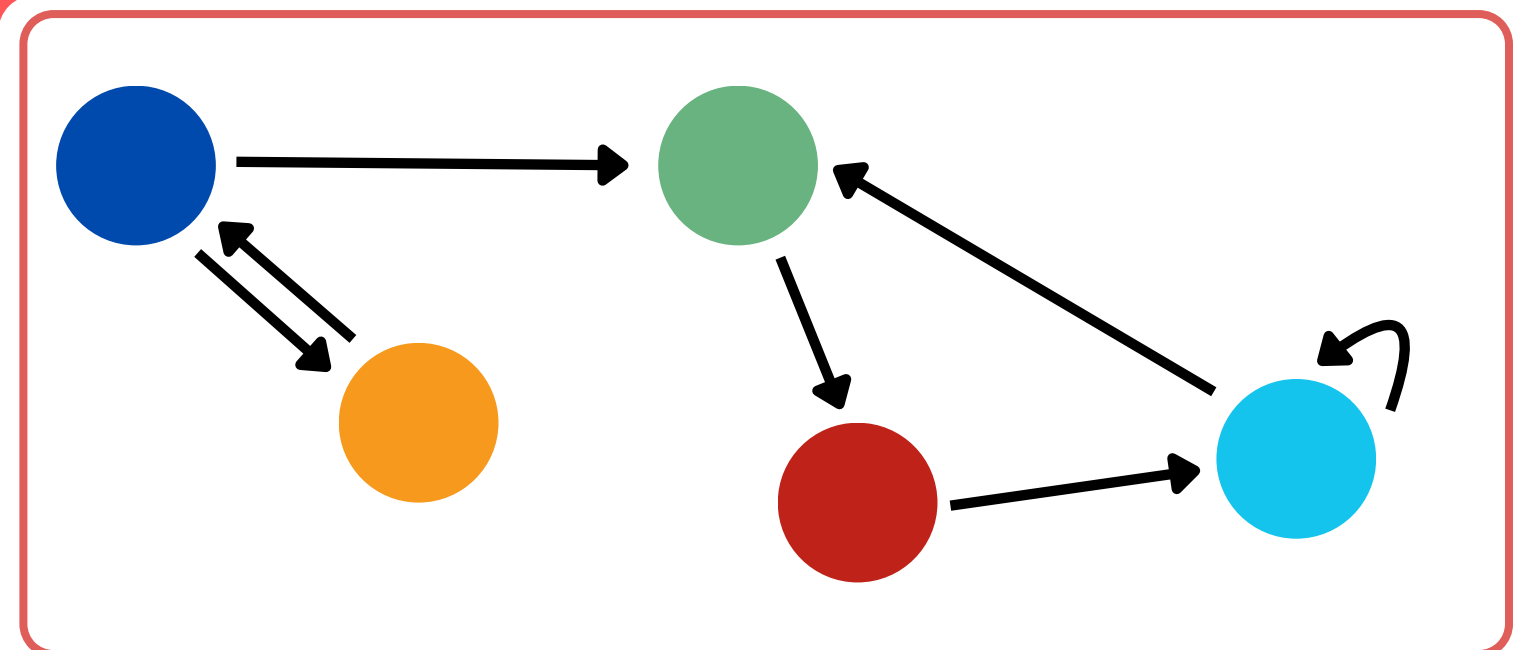


a.k.a. undirected network



Much harder to get, requires special type of data or in depth mechanistic knowledge

a.k.a. directed network

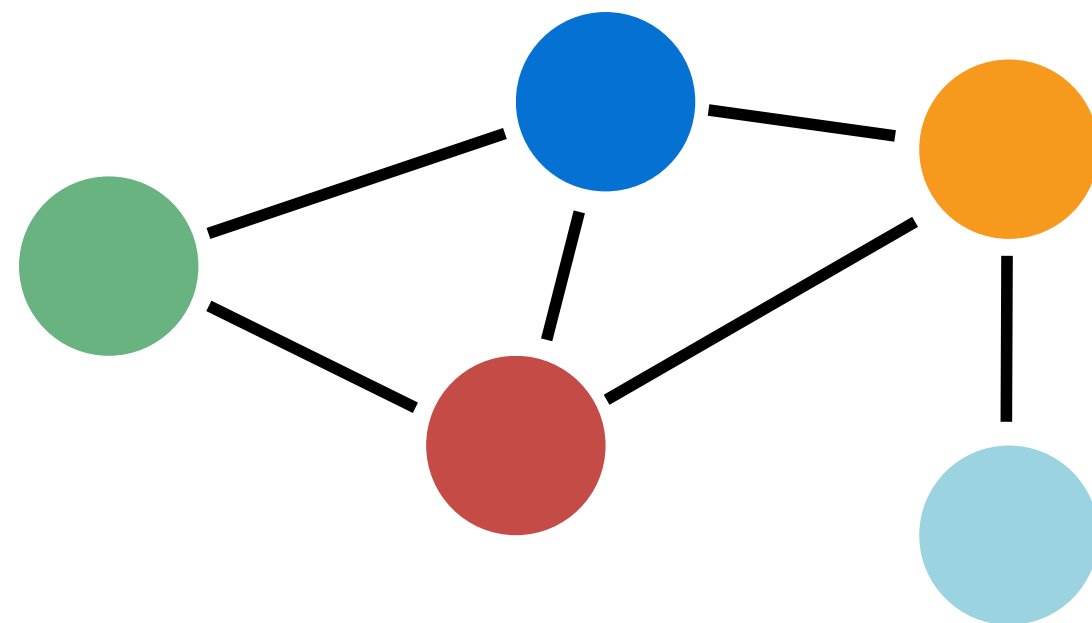


To give some answers to that question, first let's get a closer look at two sorts of networks

"Yeah, I guess..."



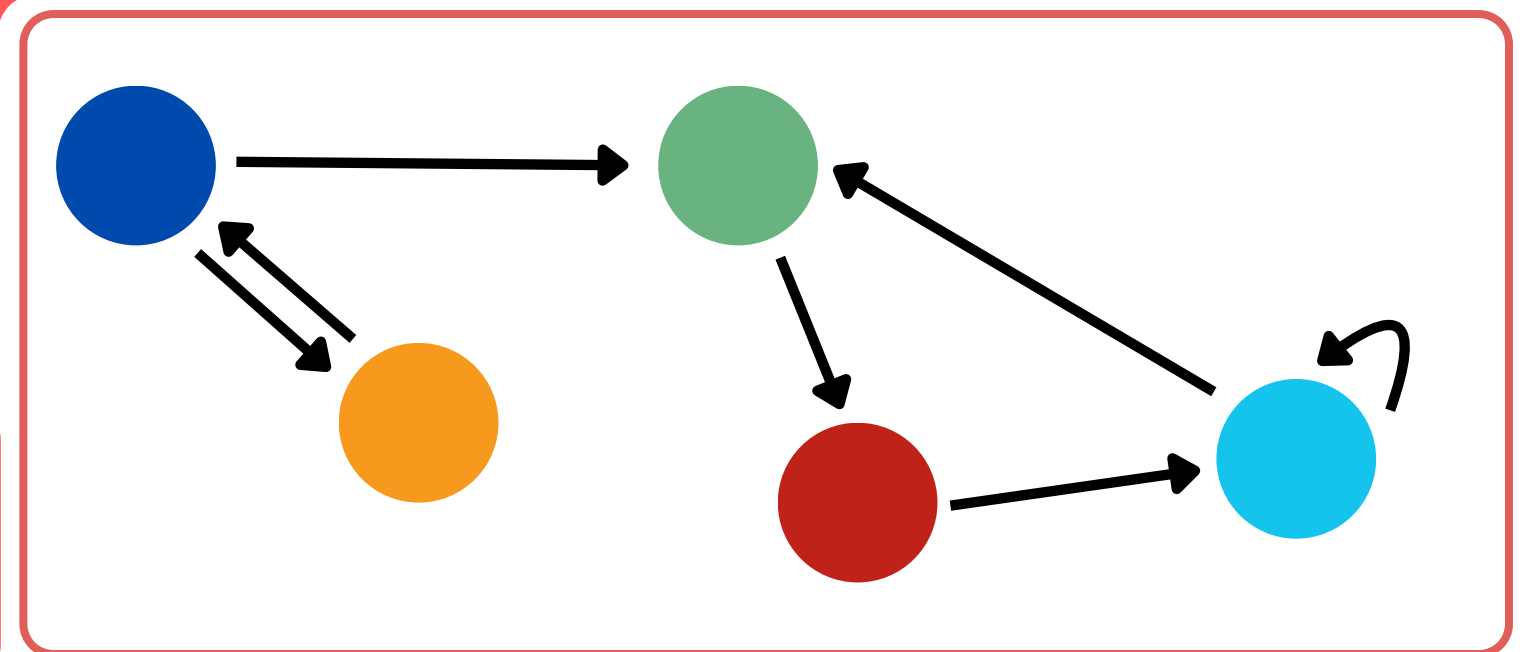
a.k.a. undirected network



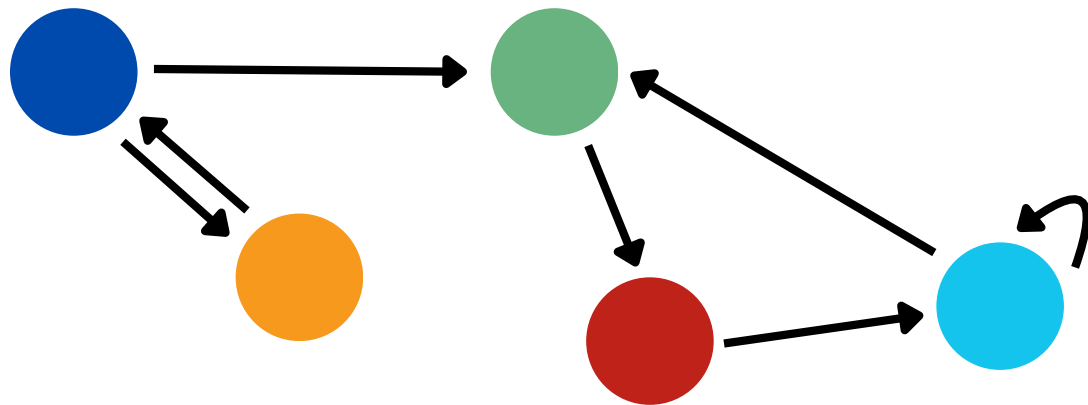
Much harder to get, requires special type of data or in depth mechanistic knowledge

But very fun to play with !

a.k.a. directed network

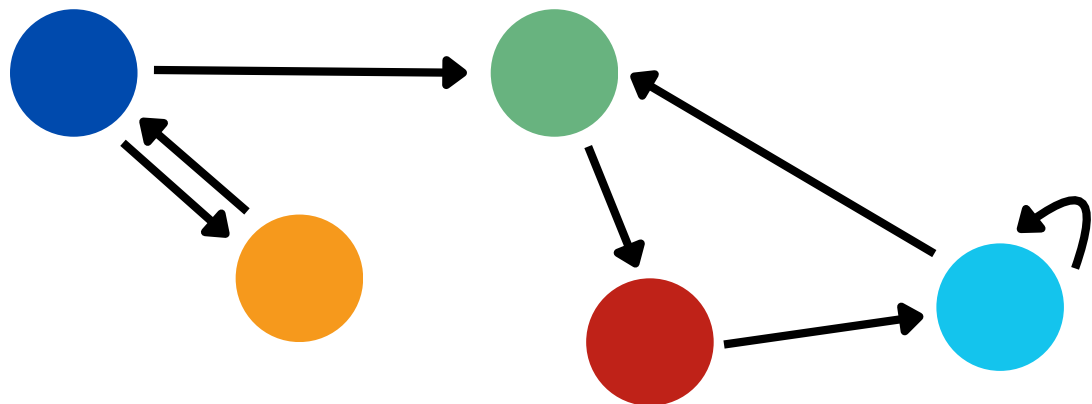


Getting value out of directed networks



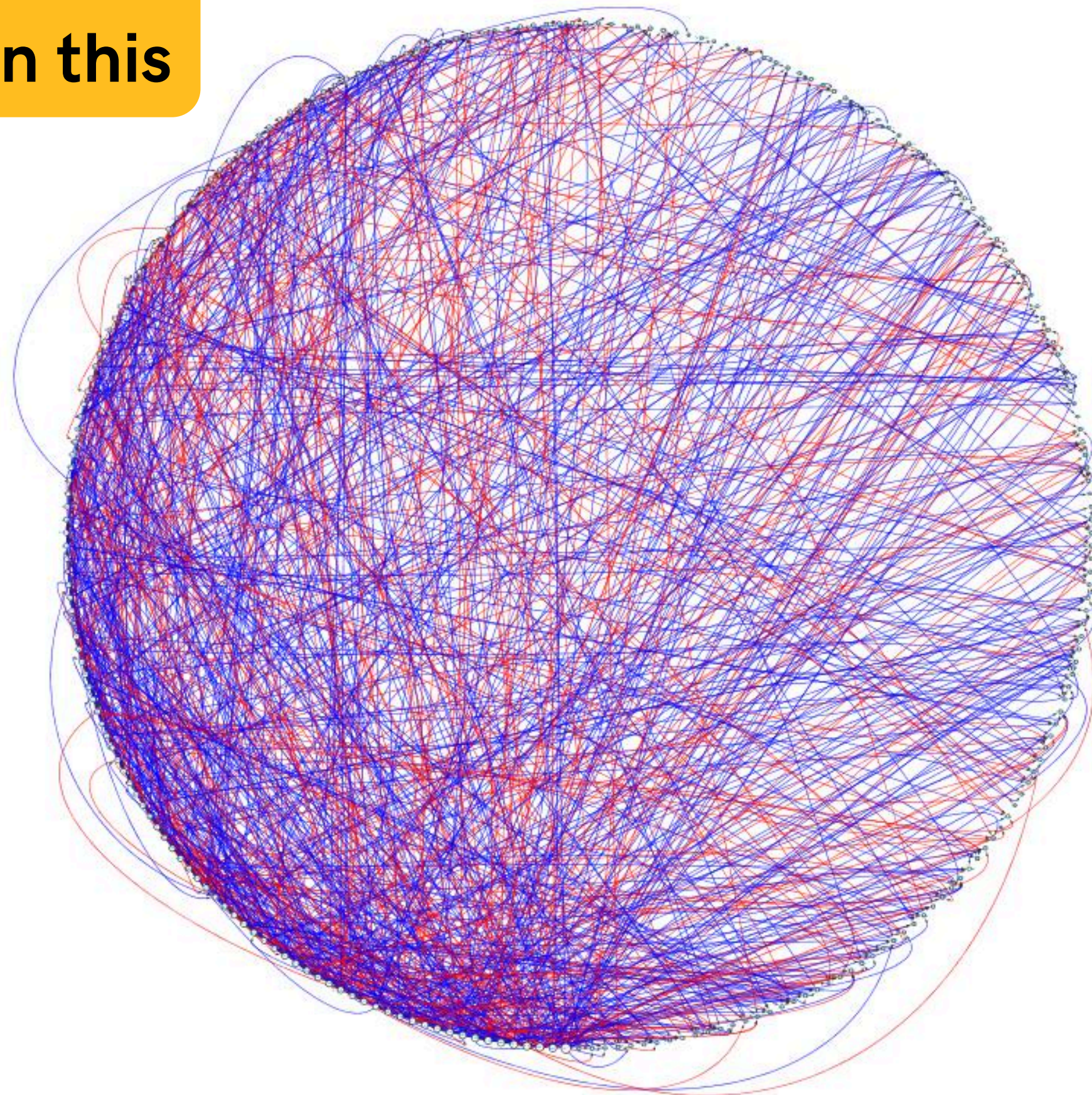
The theory:
model the activities
of the genes
mechanistically

**Getting value out of
directed networks**

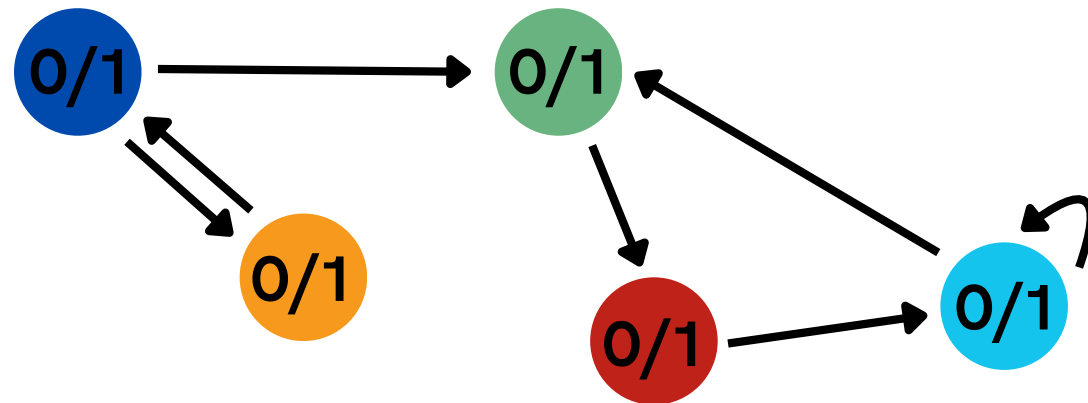


**The theory_:
model the activities
of the genes
mechanistically**

**The reality_:
Do it on this**

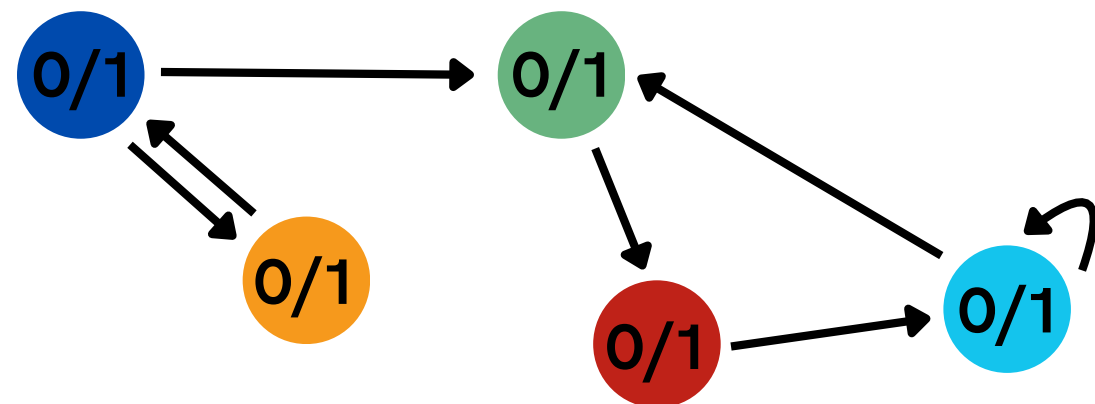


Getting value out of directed networks

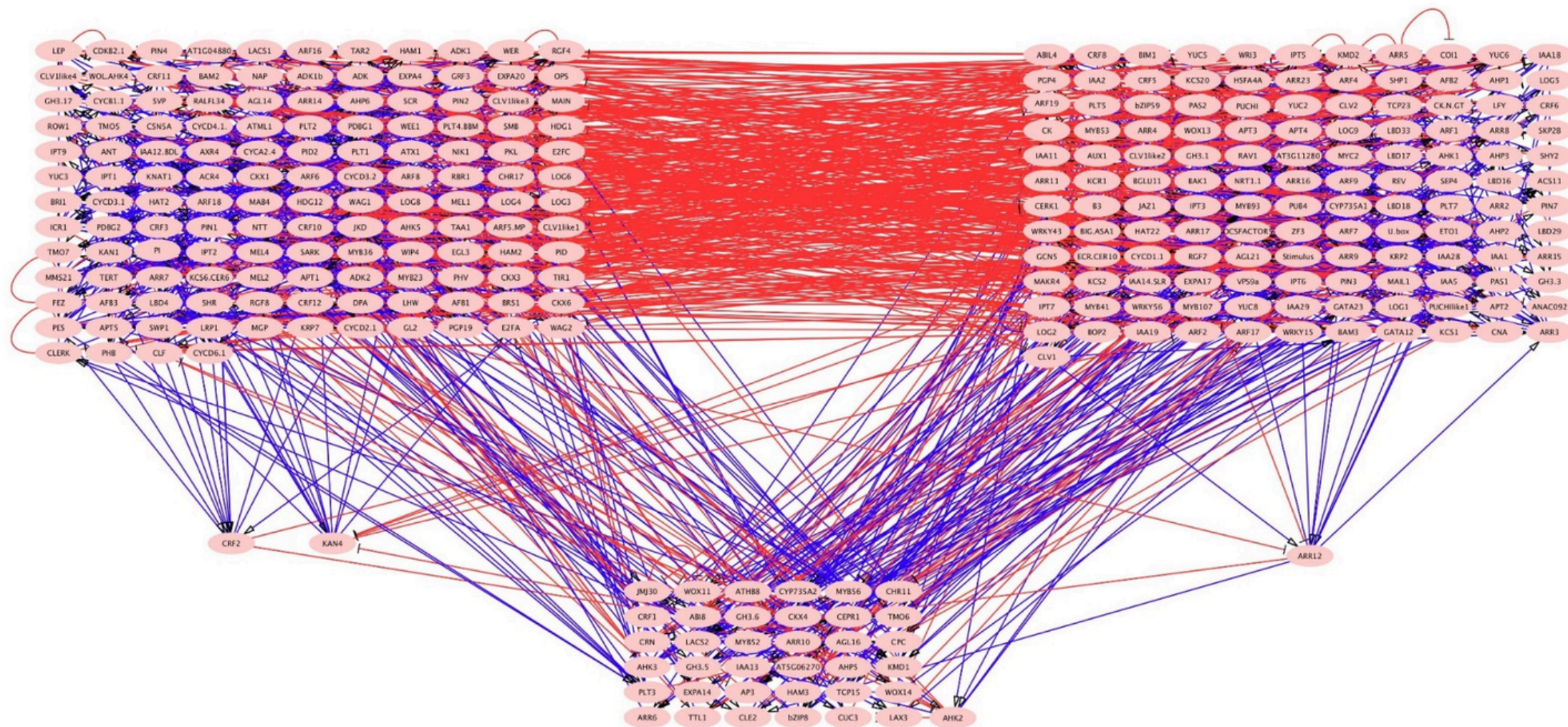


Our solution :
use boolean models
to simplify the system

Getting value out of directed networks



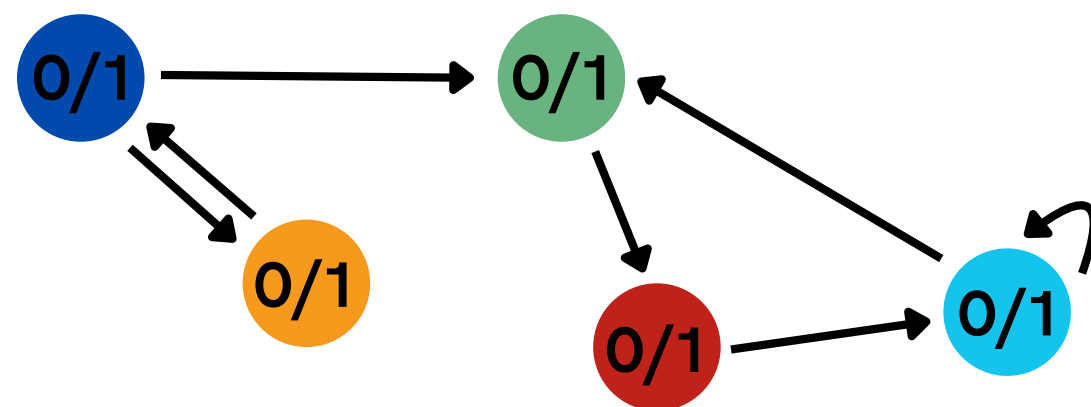
The principle :



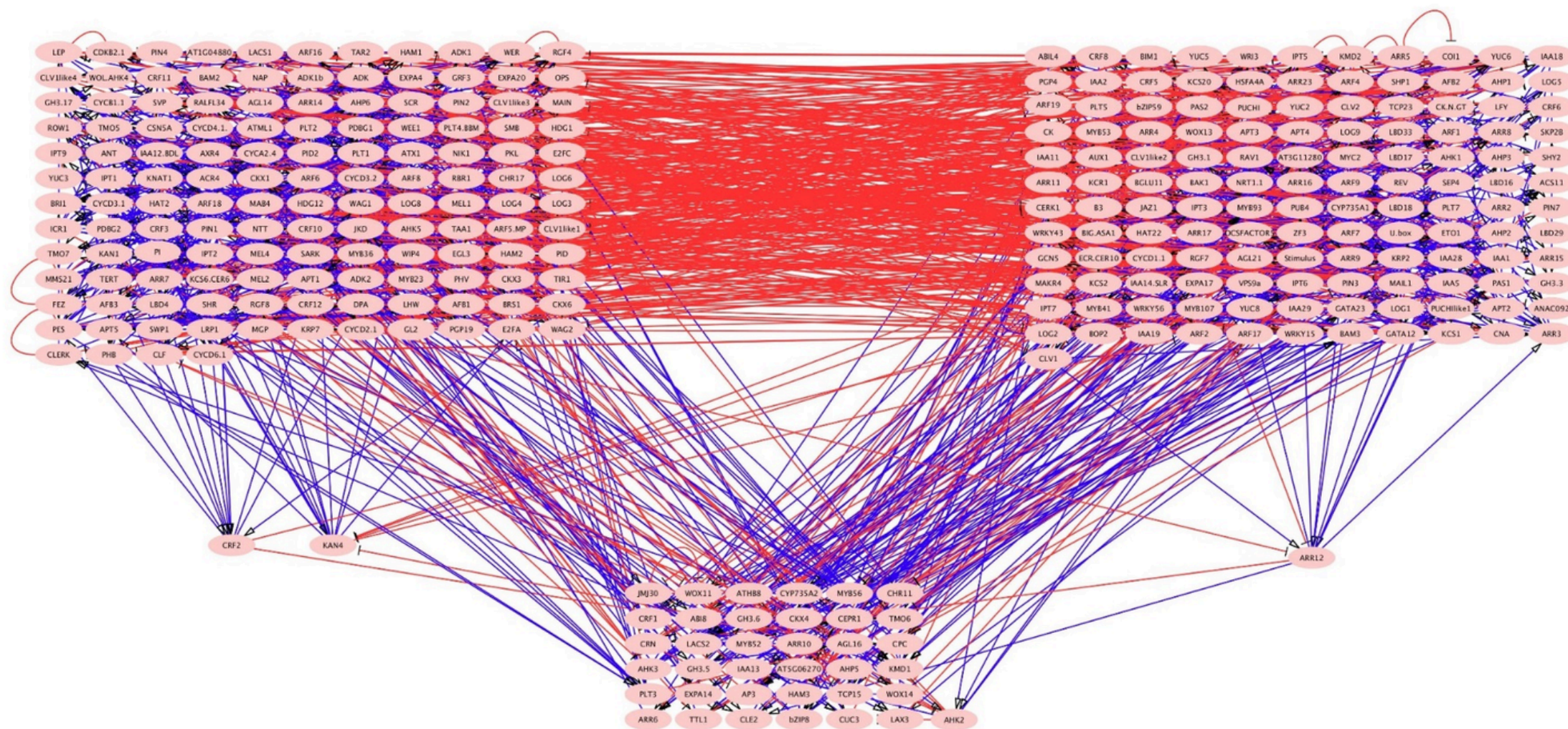
Encode biological state of the network as a boolean vector

[0 0 1 1 0 1 1 1 0 1 ... 0]

Getting value out of directed networks



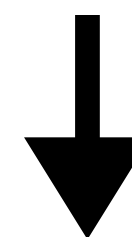
The principle :



Encode biological state of the network as a boolean vector

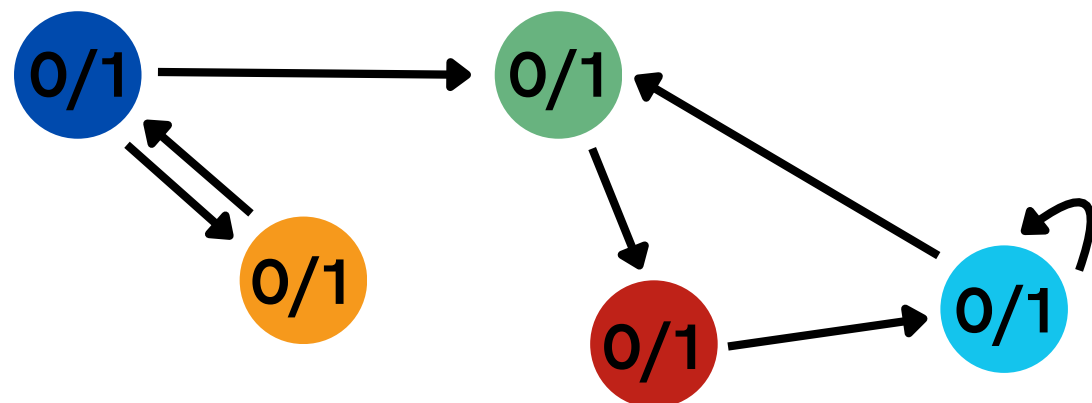
[0 0 1 1 0 1 1 1 0 1 ... 0]

Run the state flow to stability



[0 1 0 0 1 0 1 1 1 0 ... 0]

Getting value out of directed networks

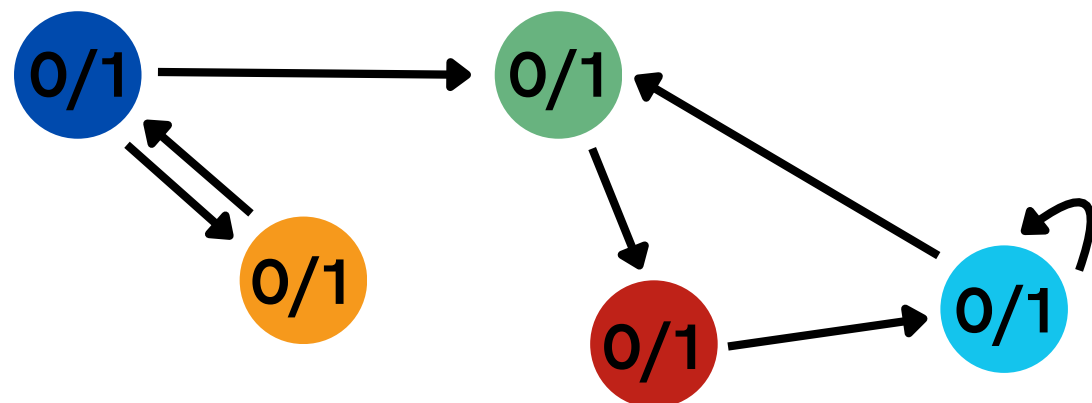


[0 0 1 1 0 1 1 1 0 1 ... 0]

How to use that to
"get value" ?

[0 1 0 0 1 0 1 1 1 0 ... 0]

Getting value out of
directed networks

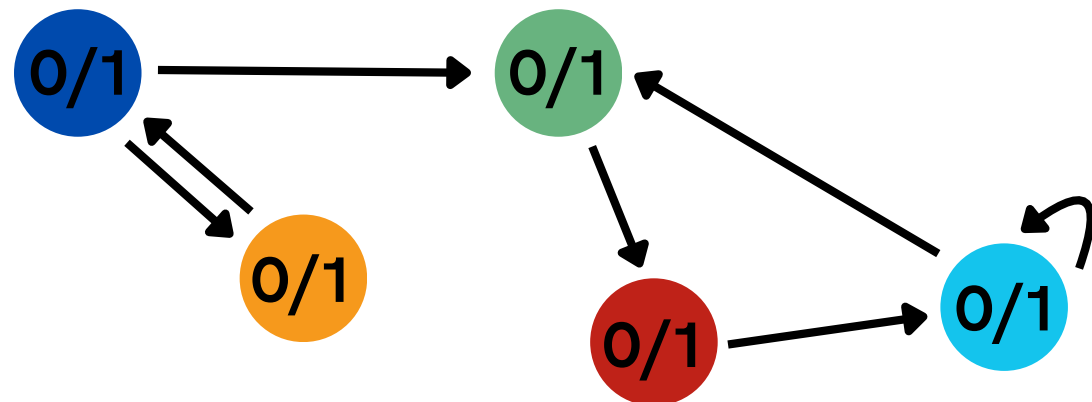


[0 0 1 1 0 1 1 1 0 1 ... 0]

Use systematic
in silico
mutagenesis

[0 1 0 0 1 0 1 1 1 0 ... 0]

Getting value out of
directed networks

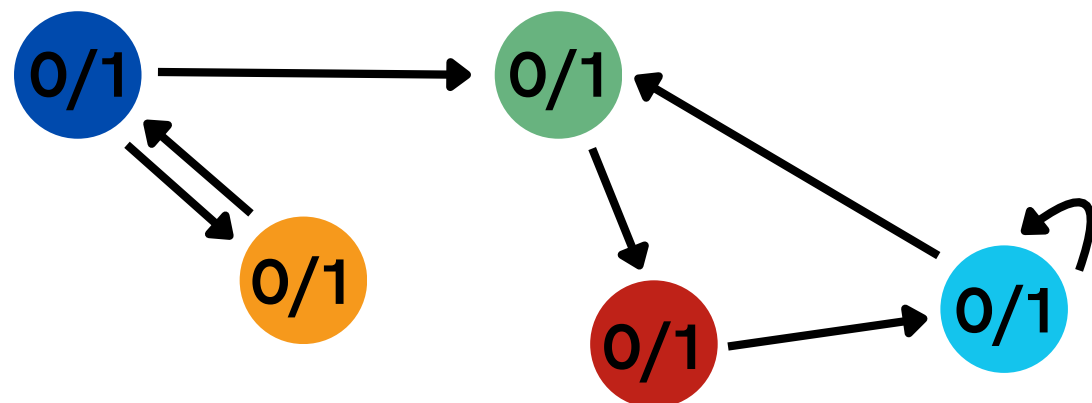


Mutate 1st gene
(KO or OE)

[**1** 0 1 1 0 1 1 1 0 1 ... 0]

[0 1 0 0 1 0 1 1 1 0 ... 0]

Getting value out of
directed networks



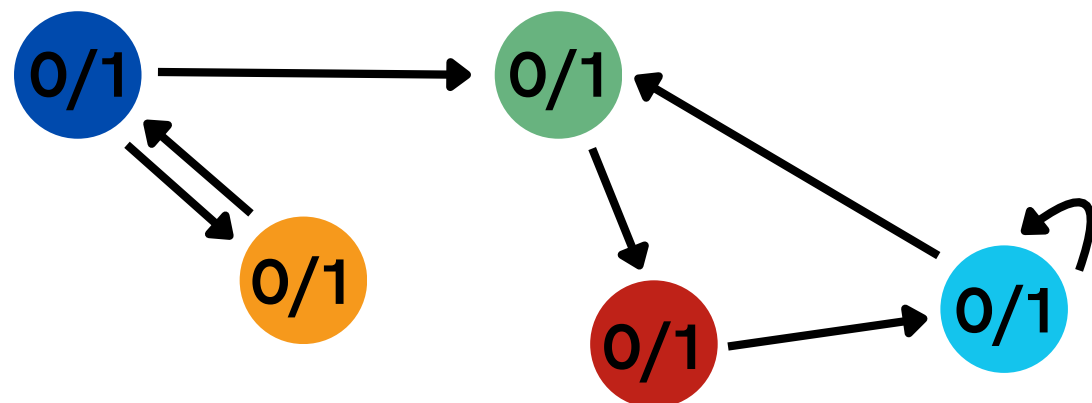
Mutate 1st gene
(KO or OE)

[**1** 0 1 1 0 1 1 1 0 1 ... 0]

This will change
the state flow

[0 1 0 0 1 0 1 1 1 0 ... 0]

Getting value out of
directed networks



Mutate 1st gene
(KO or OE)

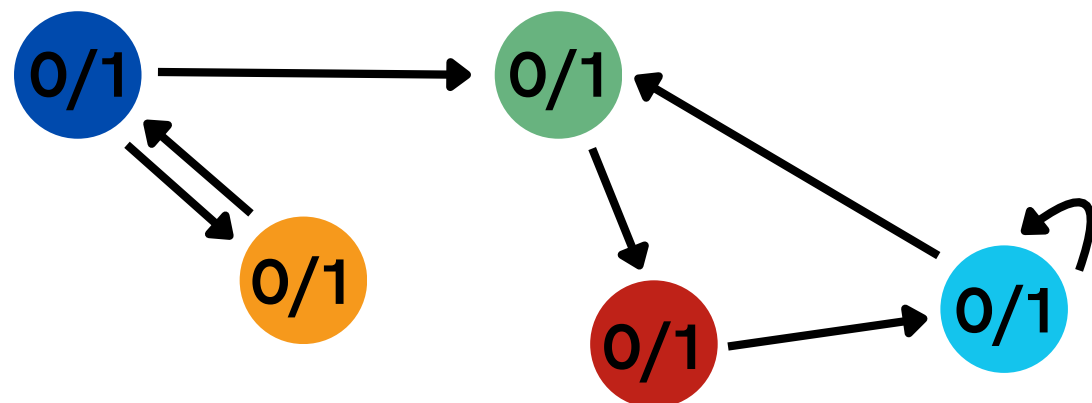
[**1** 0 1 1 0 1 1 1 0 1 ... 0]

This will change
the state flow

And the
final state

[**1** 1 0 **1** 1 0 1 1 **0** 0 ... 0]

Getting value out of
directed networks



Mutate 1st gene
(KO or OE)

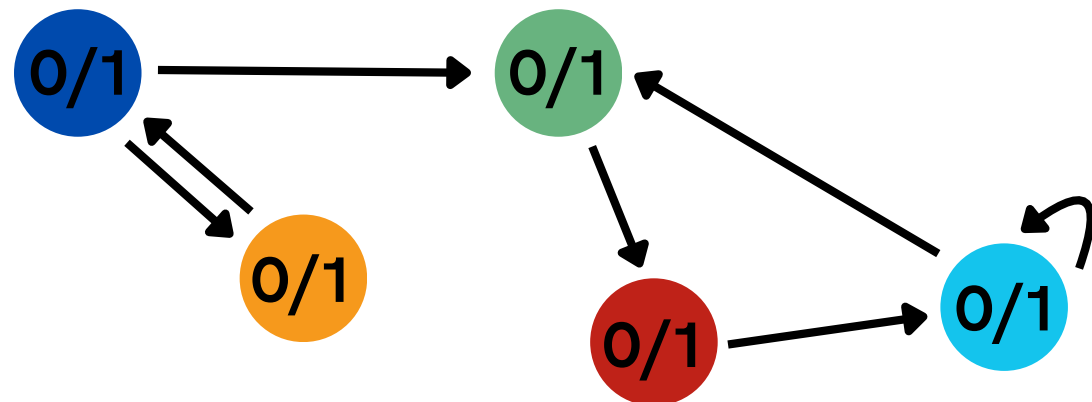
[**1** 0 1 1 0 1 1 1 0 1 ... 0]

This will change
the state flow

Compare the 'wild type'
and 'mutant' final states
(Hamming distance)

[0 1 0 0 1 0 1 1 1 0 ... 0] / [**1** 1 0 **1** 1 0 1 1 **0** 0 ... 0]

Getting value out of
directed networks



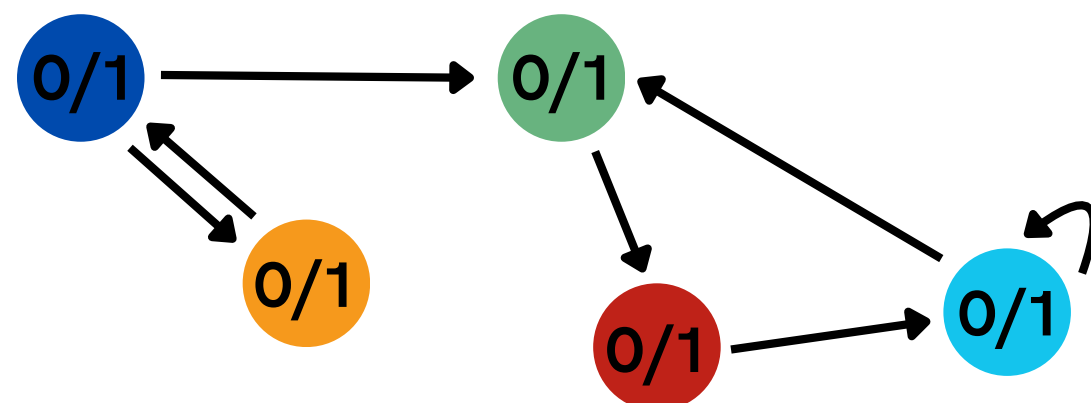
Mutate 1st gene
(KO or OE)

[**1** 0 1 1 0 1 1 1 0 1 ... 0]

Congratulation, you know
how much the 1st gene
impact the whole network

[0 1 0 0 1 0 1 1 1 0 ... 0] / [**1** 1 0 **1** 1 0 1 1 **0** 0 ... 0]

Getting value out of directed networks



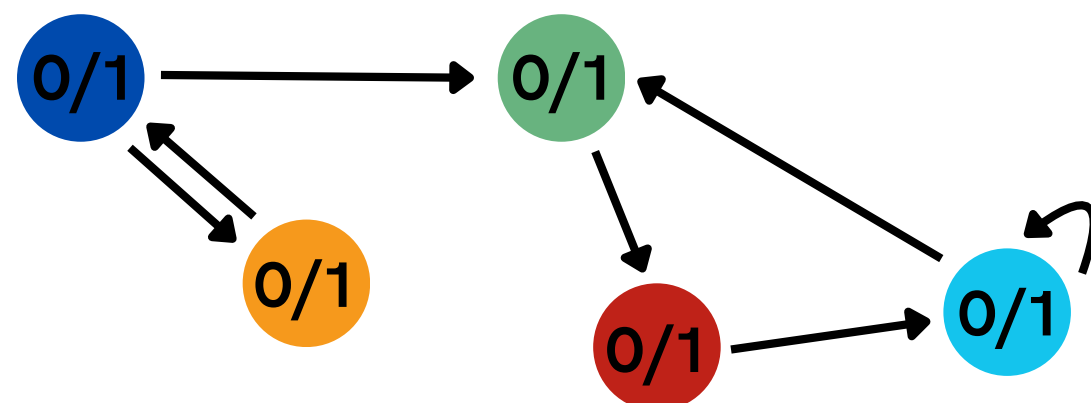
Mutate 1st gene
(KO or OE)

[**1** 0 1 1 0 1 1 1 0 1 ... 0]

Congratulation, you know
how much the 1st gene
impact the whole network
(well, from 1 initial state)

[0 1 0 0 1 0 1 1 1 0 ... 0] / [**1** 1 0 **1** 1 0 1 1 **0** 0 ... 0]

Getting value out of
directed networks



[1 0 0 1 1 0 1 0 0 1 ... 0]
[1 1 0 0 0 0 1 1 1 0 ... 1]
[1 0 1 1 0 1 1 1 0 1 ... 0]

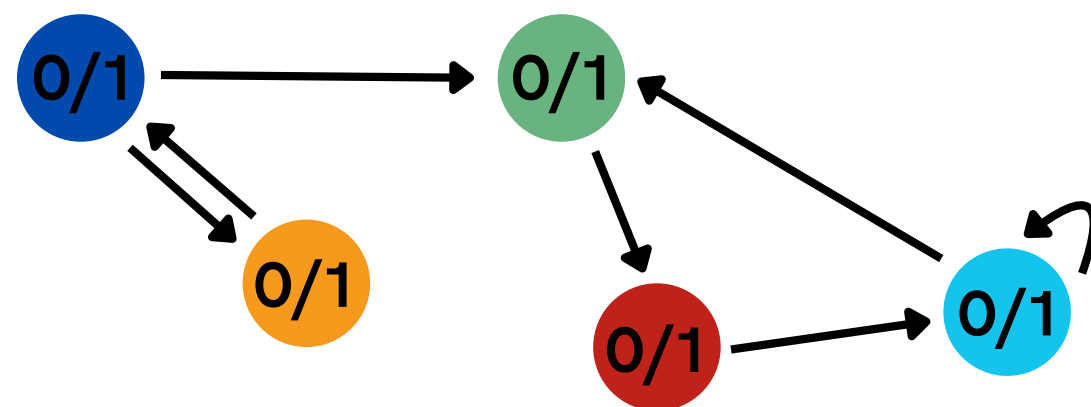
For a **large number**
of initial states

Mutate **all genes**
one by one
(KO and OE)

Score all the distances
and do a mean

[0 1 0 0 1 0 1 1 1 0 ... 0] / [1 1 0 1 1 0 1 1 0 0 ... 0]

Getting value out of
directed networks



[1 0 0 1 1 0 1 0 0 1 ... 0]
[1 1 0 0 0 0 1 1 1 0 ... 1]
[1 0 1 1 0 1 1 1 0 1 ... 0]

For a **large number**
of initial states

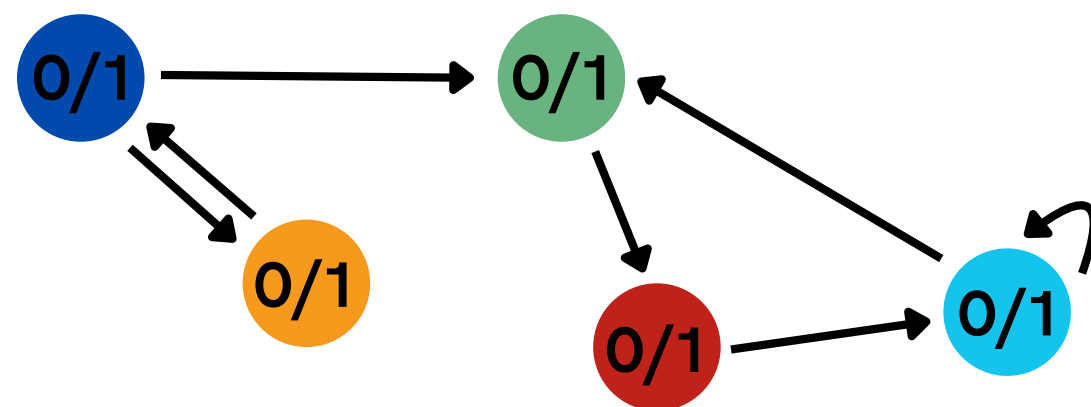
Mutate **all genes**
one by one
(KO and OE)

Score all the distances
and do a mean

Oh, and do that
for all possible
parameters of the
boolean model

[0 1 0 0 1 0 1 1 1 0 ... 0] / [1 1 0 1 1 0 1 1 0 0 ... 0]

Getting value out of
directed networks



[1 0 0 1 1 0 1 0 0 1 ... 0]
[1 1 0 0 0 0 1 1 1 0 ... 1]
[1 0 1 1 0 1 1 1 0 1 ... 0]

For a **large number**
of initial states

Mutate **all genes**
one by one
(KO and OE)

The ARGOS algorithm
(Automated Research of Genes Of Significance)

Score all the distances
and do a mean

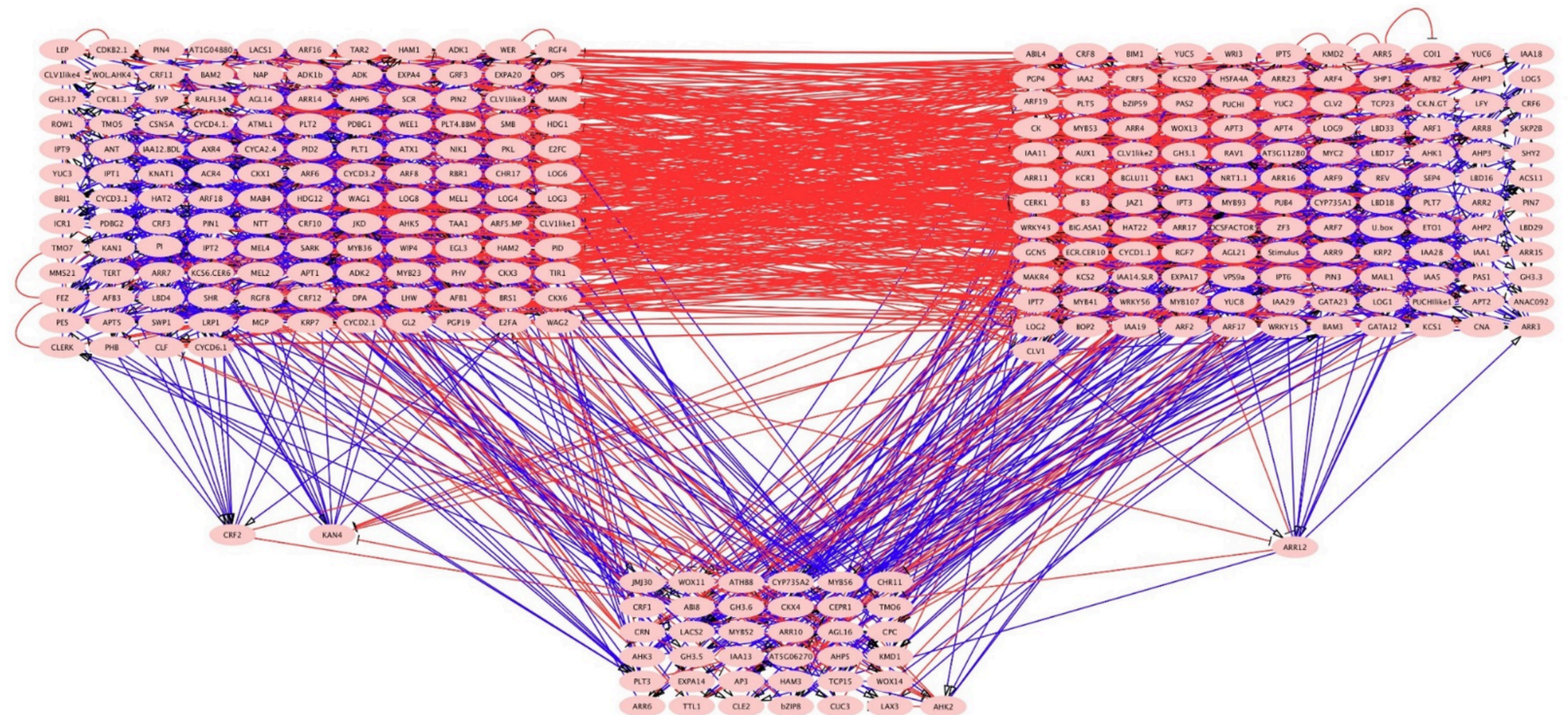
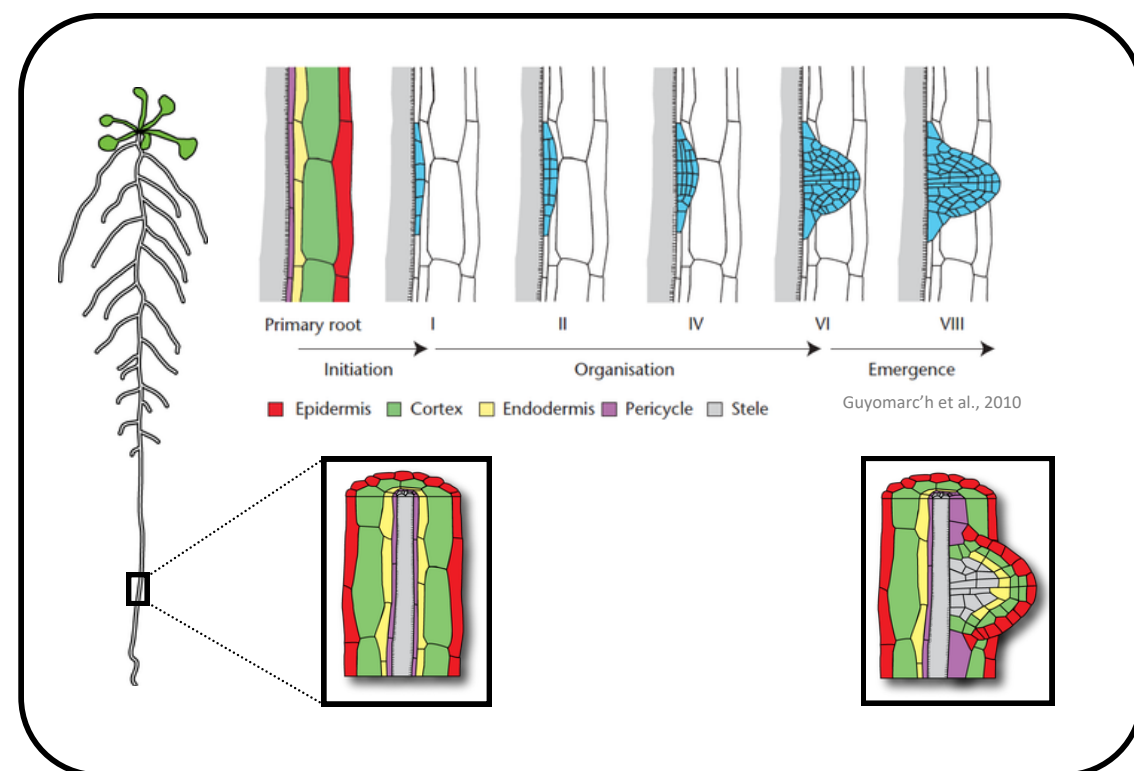
Oh, and do that
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[0 1 0 0 1 0 1 1 1 0 ... 0] / [1 1 0 1 1 0 1 1 0 0 ... 0]

Getting value out of
directed networks

The ARGOS algorithm
(Automated Research of Genes Of Significance)

Application to
Arabidopsis lateral root
organogenesis



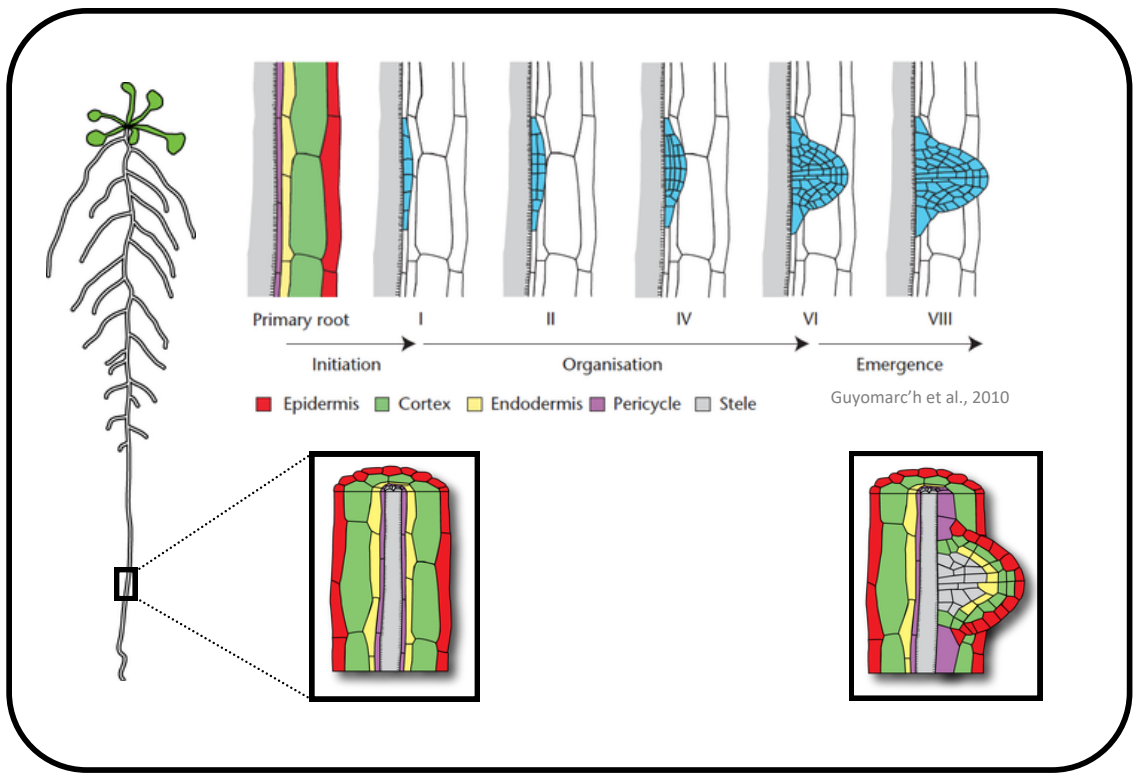
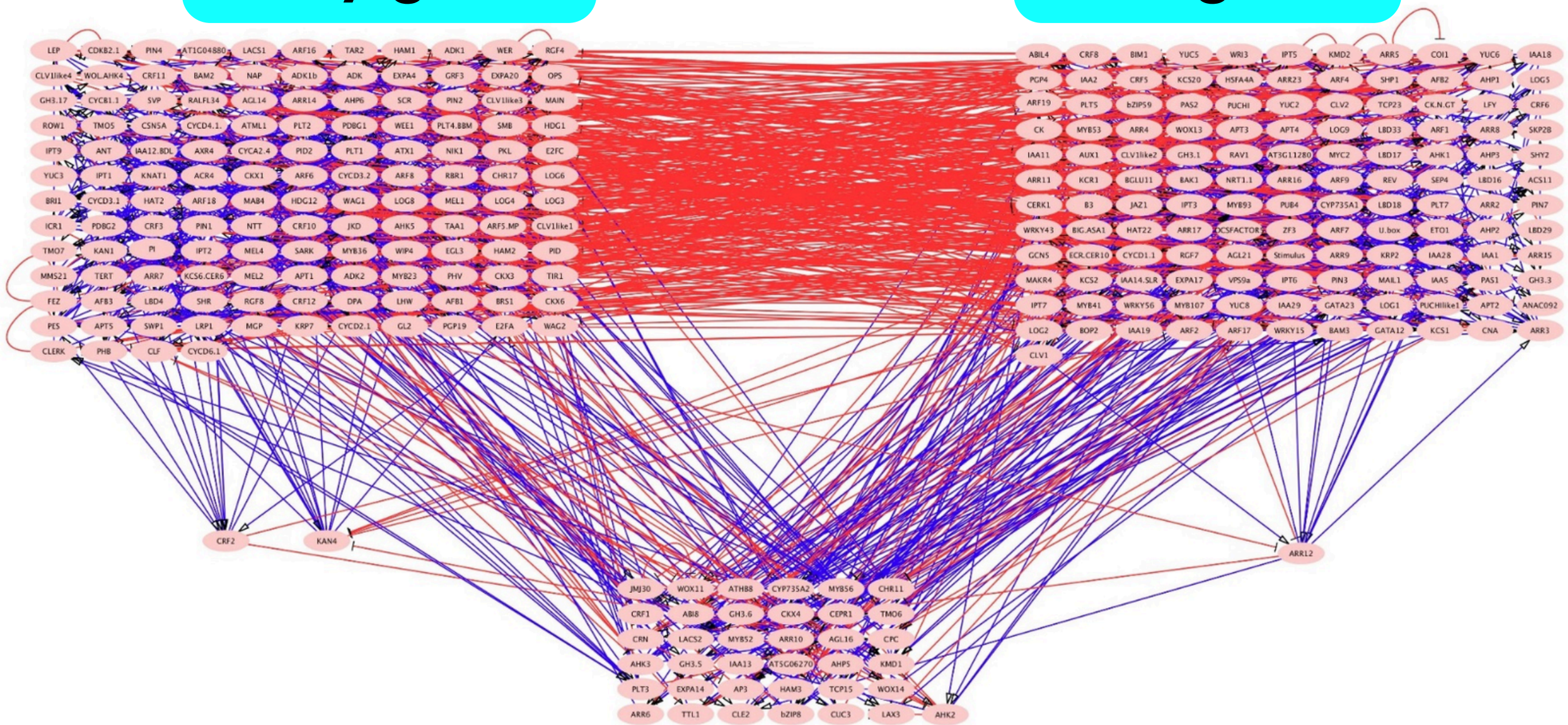
Getting value out of
directed networks

The ARGOS algorithm
(Automated Research of Genes Of Significance)

Application to
Arabidopsis lateral root
organogenesis

Early genes

Late genes



Getting value out of
directed networks

The ARGOS algorithm
(Automated Research of Genes Of Significance)

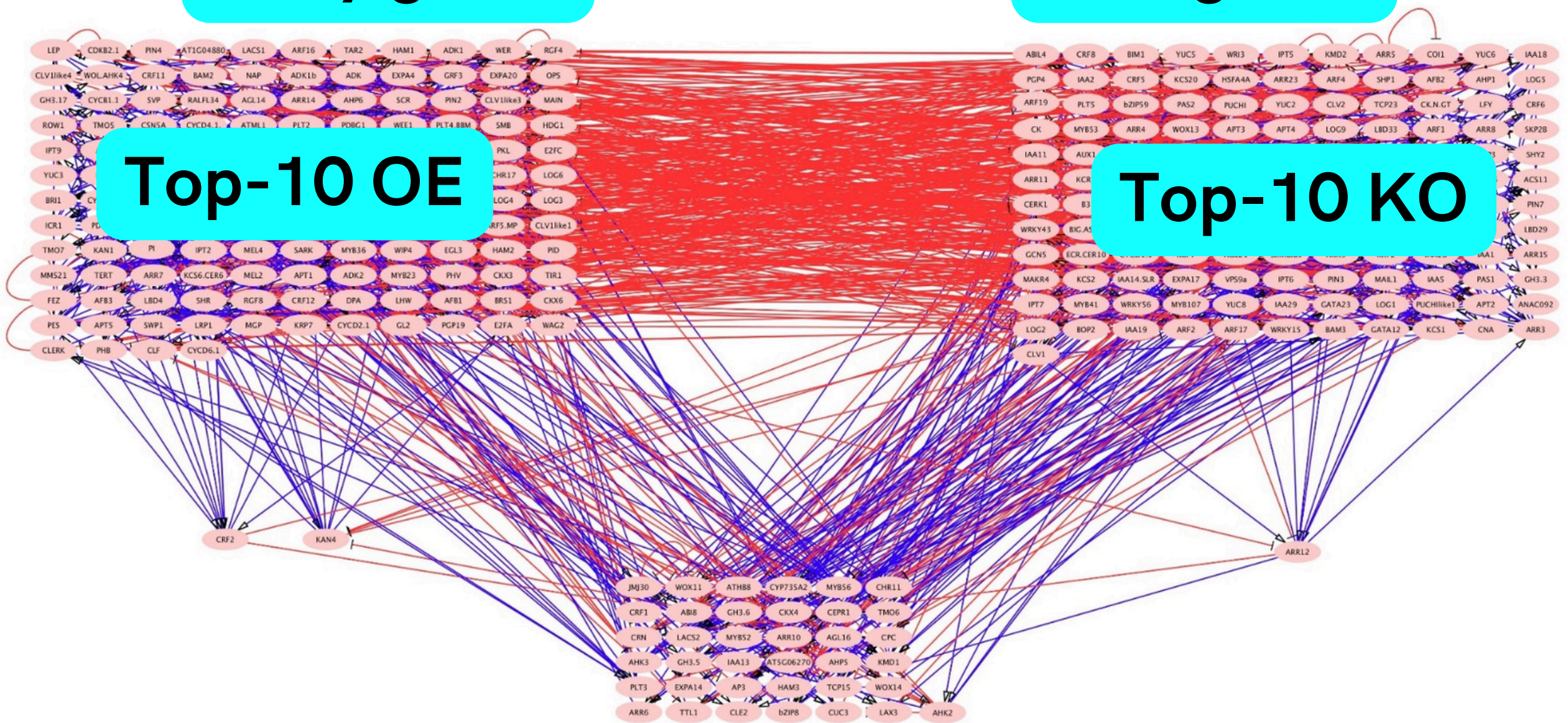
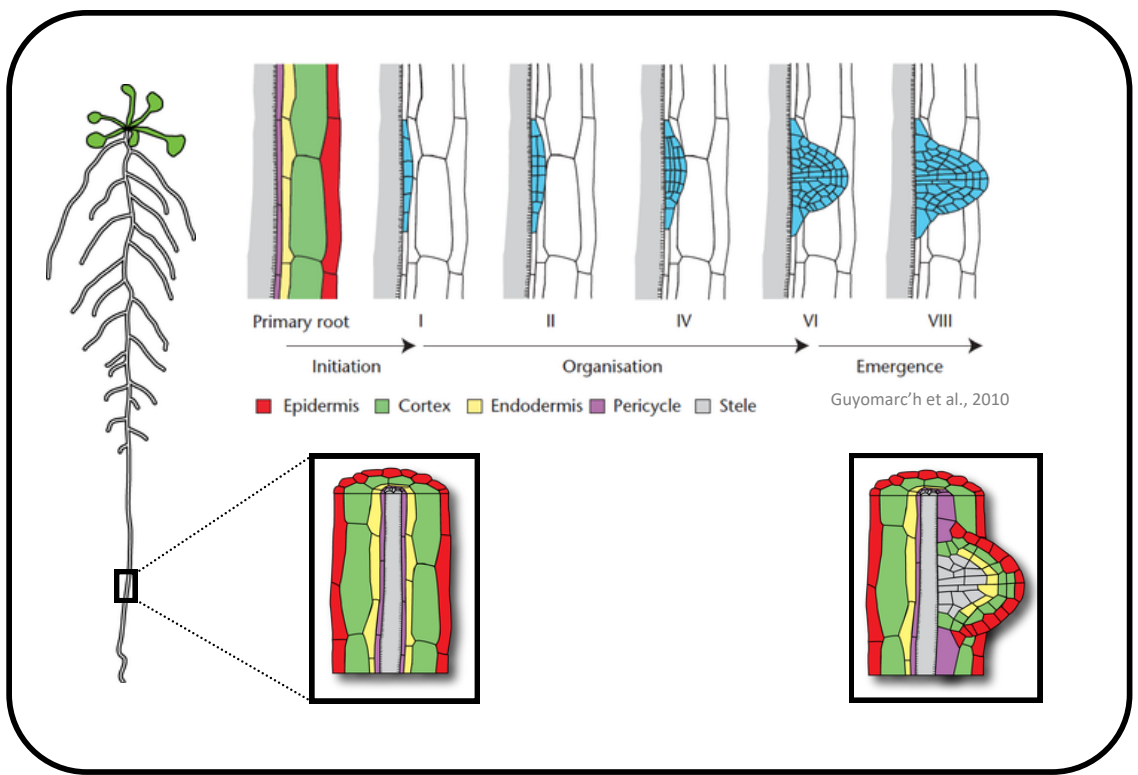
Application to
Arabidopsis lateral root
organogenesis

Early genes

Late genes

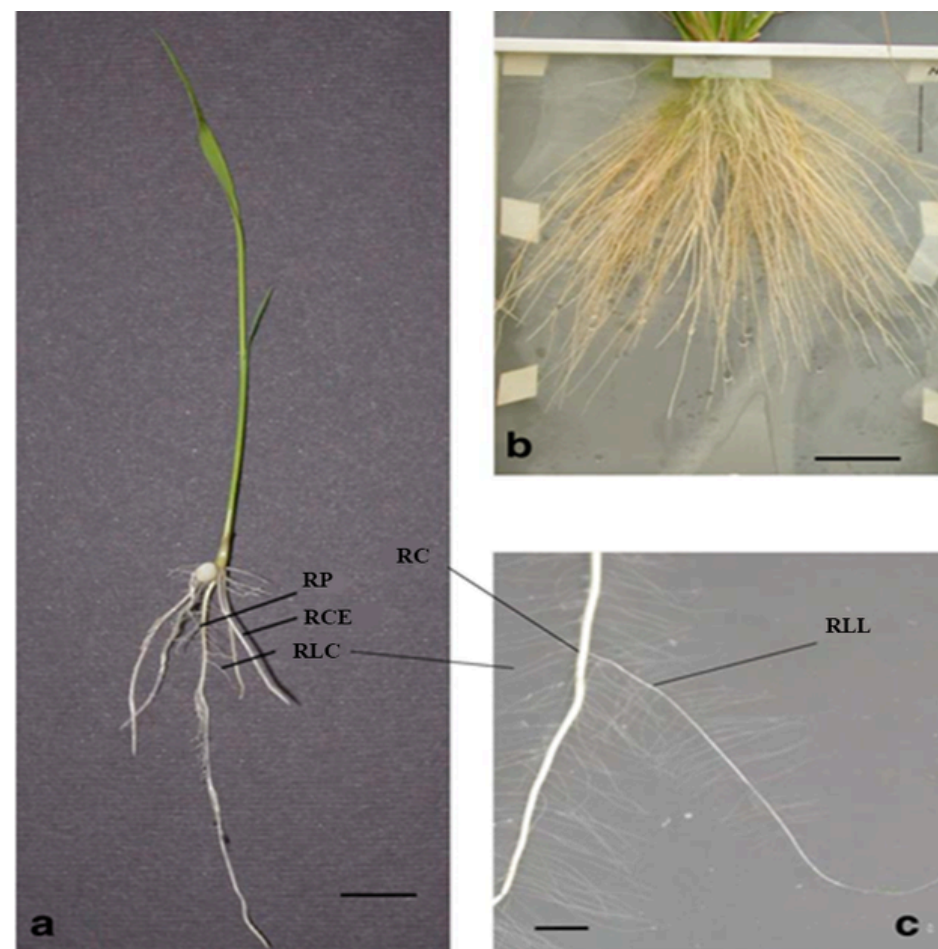
Top-10 OE

Top-10 KO

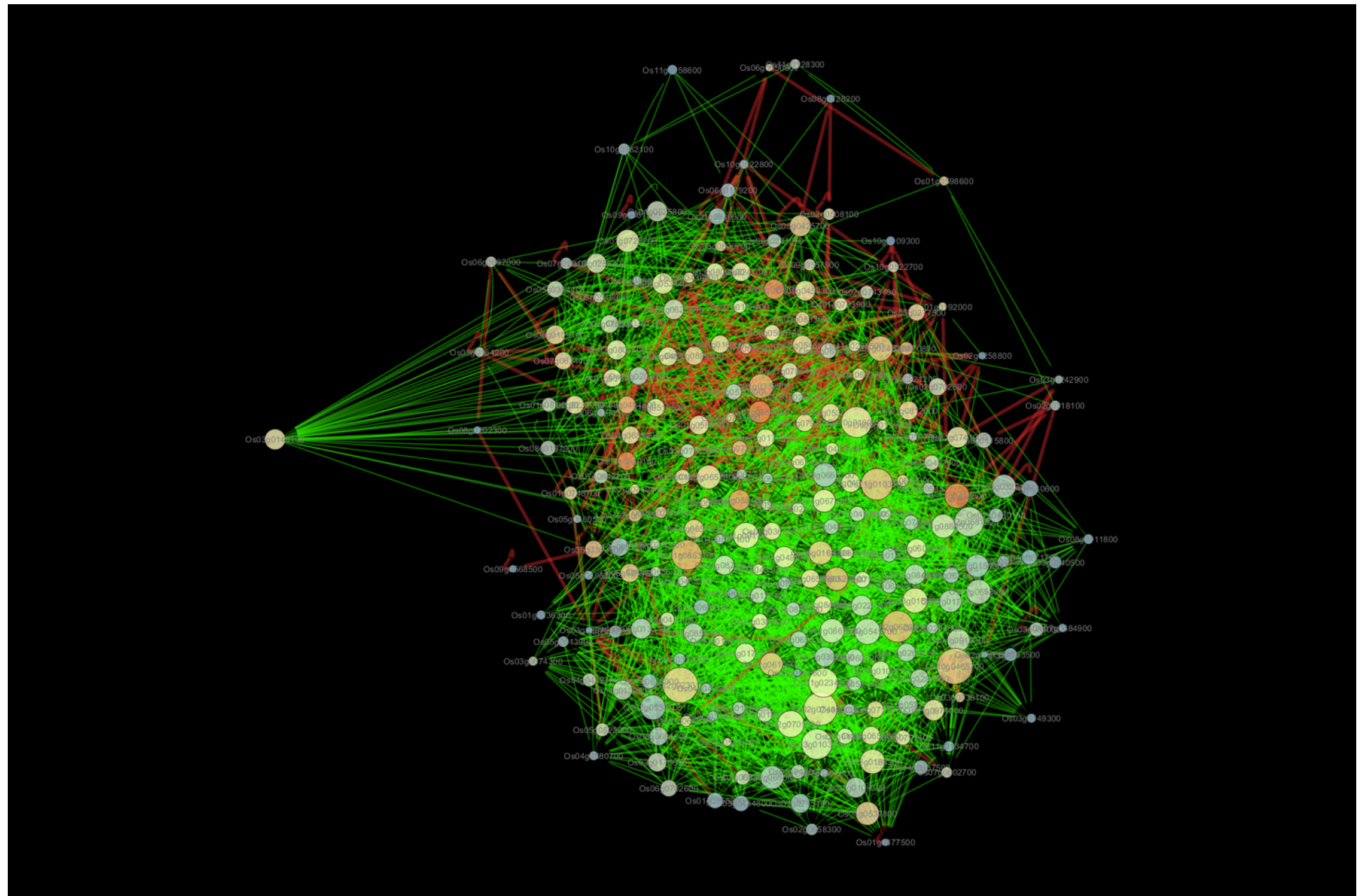


Getting value out of
directed networks

Application to Rice
crown root
organogenesis

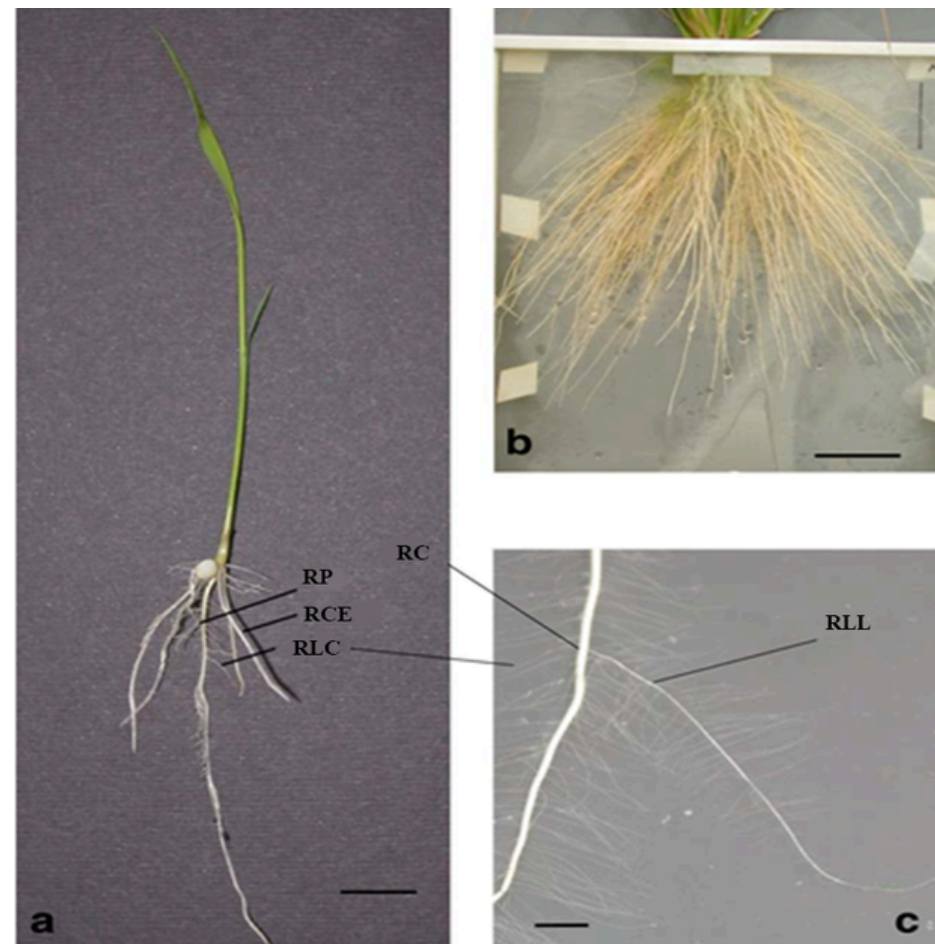


The ARGOS algorithm
(Automated Research of Genes Of Significance)



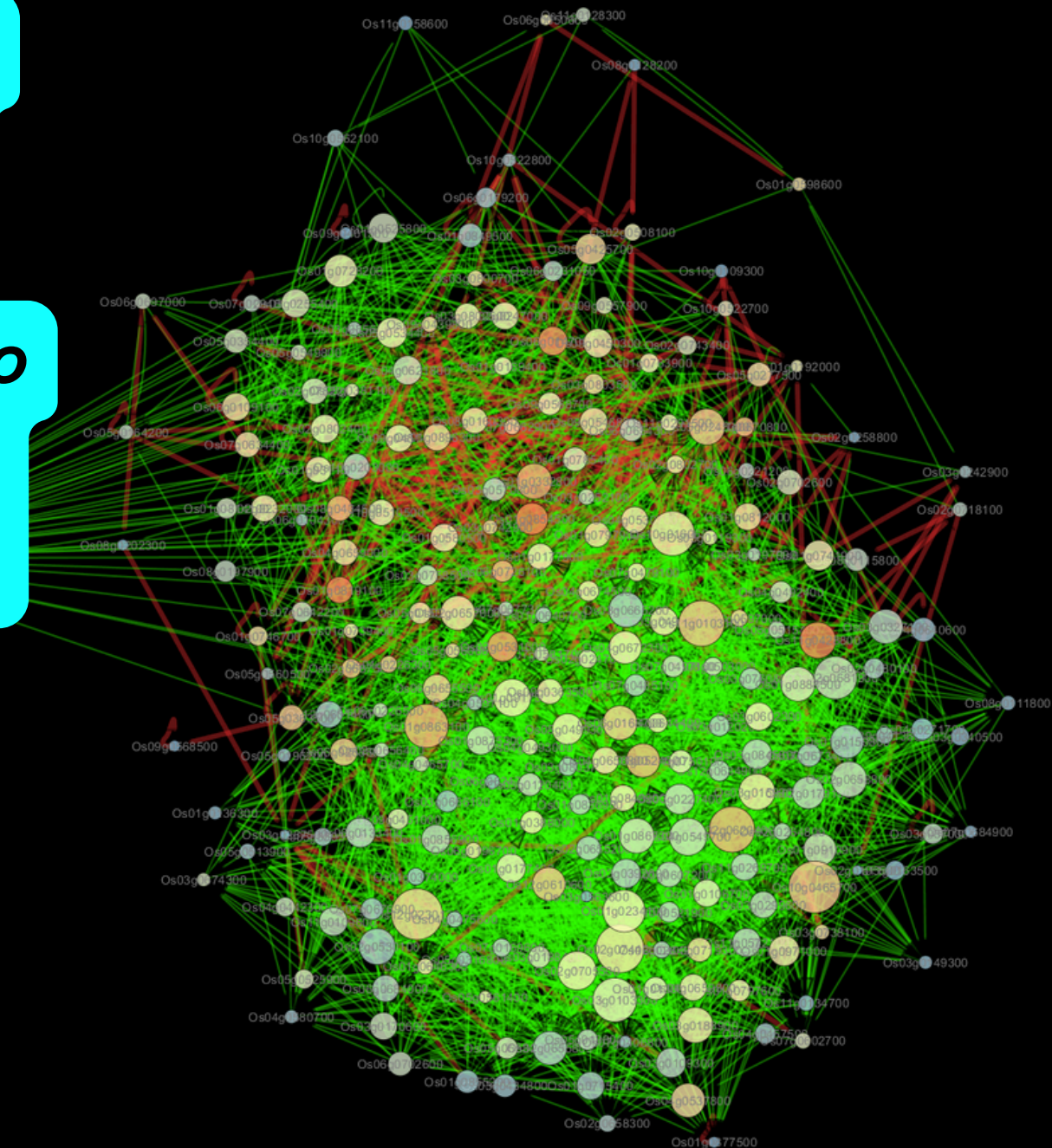
Getting value out of
directed networks

Application to Rice
crown root
organogenesis



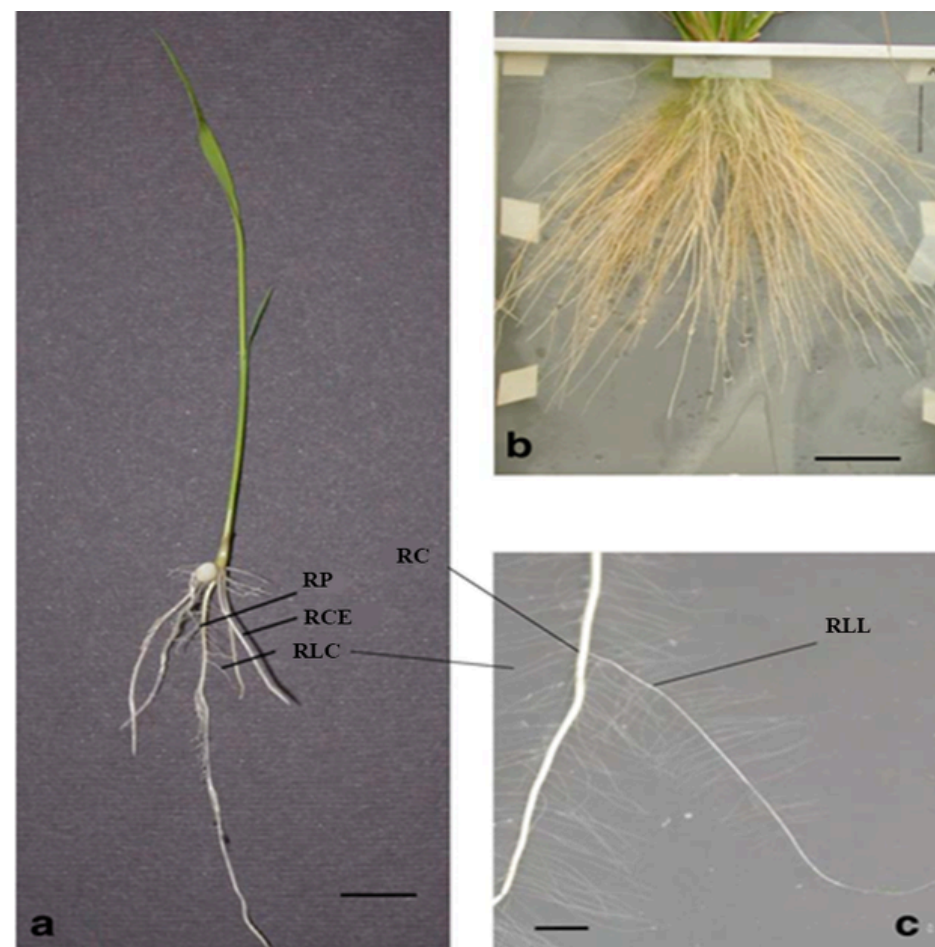
The ARGOS algorithm
(Automated Research of Genes Of Significance)

Top OE & KO
selected for
functional
analysis (*in vivo*
KO and OE) &
phenotyping

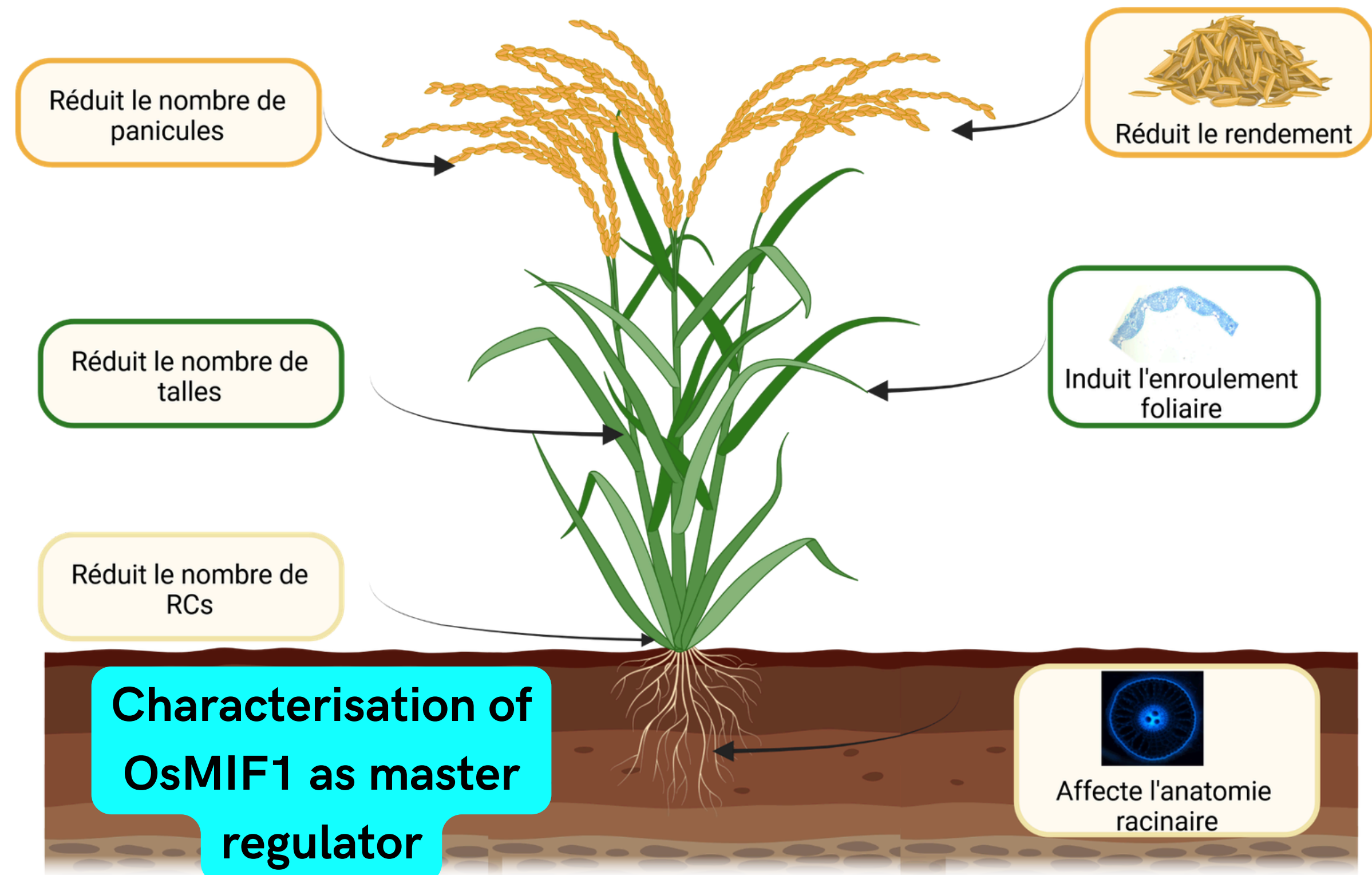


Getting value out of
directed networks

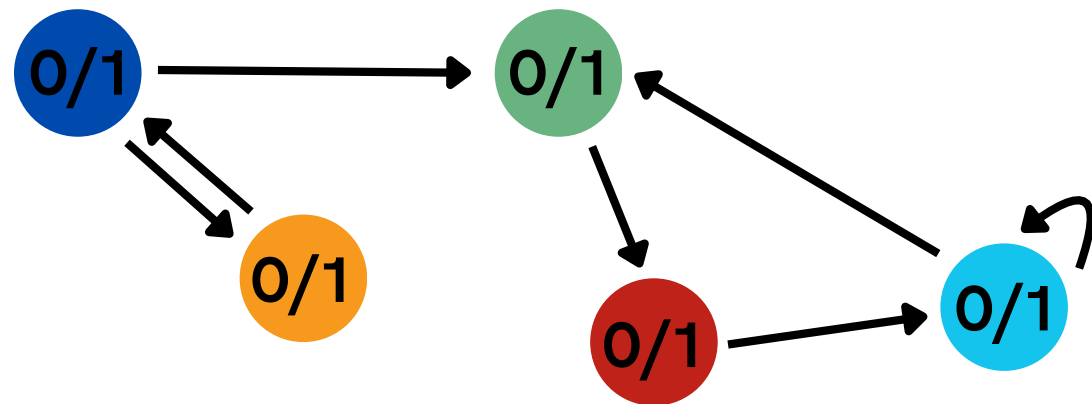
Application to Rice
crown root
organogenesis



The ARGOS algorithm
(Automated Research of Genes Of Significance)



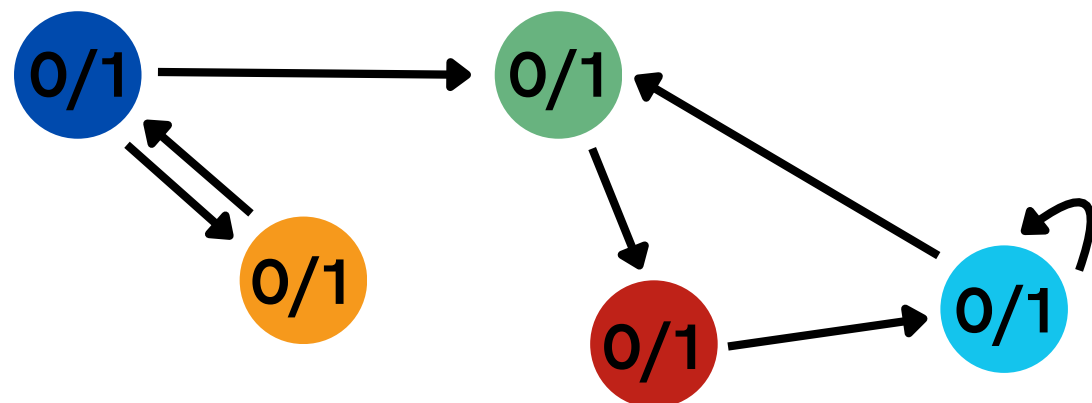
Getting value out of
directed networks



In summary, we have ways to
train that rare barbed Shoggoth
to get value out of it...



Getting value out of
directed networks



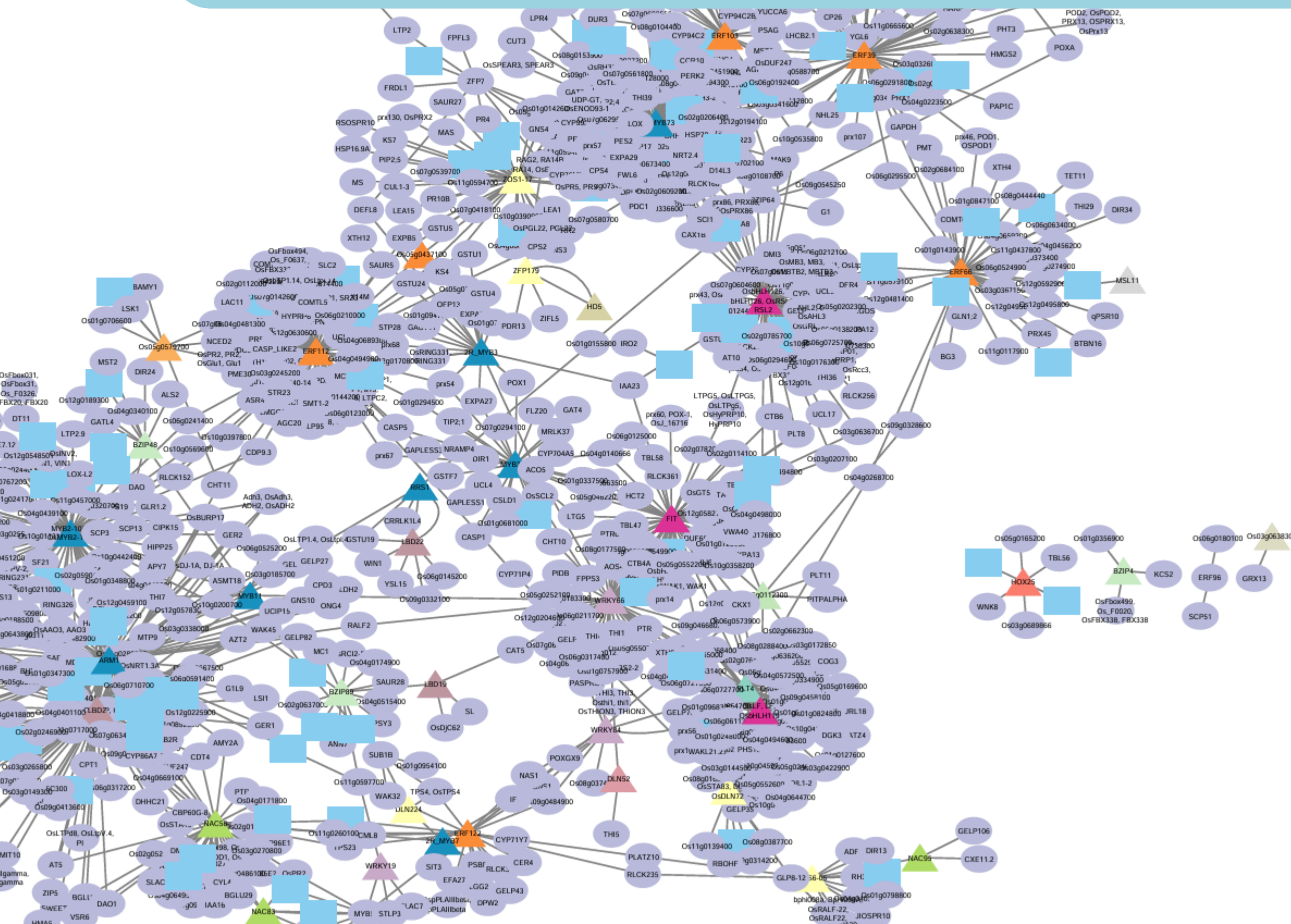
In summary, we have ways to
train that rare barbed Shoggoth
to get value out of it...

What about the poor
smooth Shoggoth ?
How to get some
value from it ?



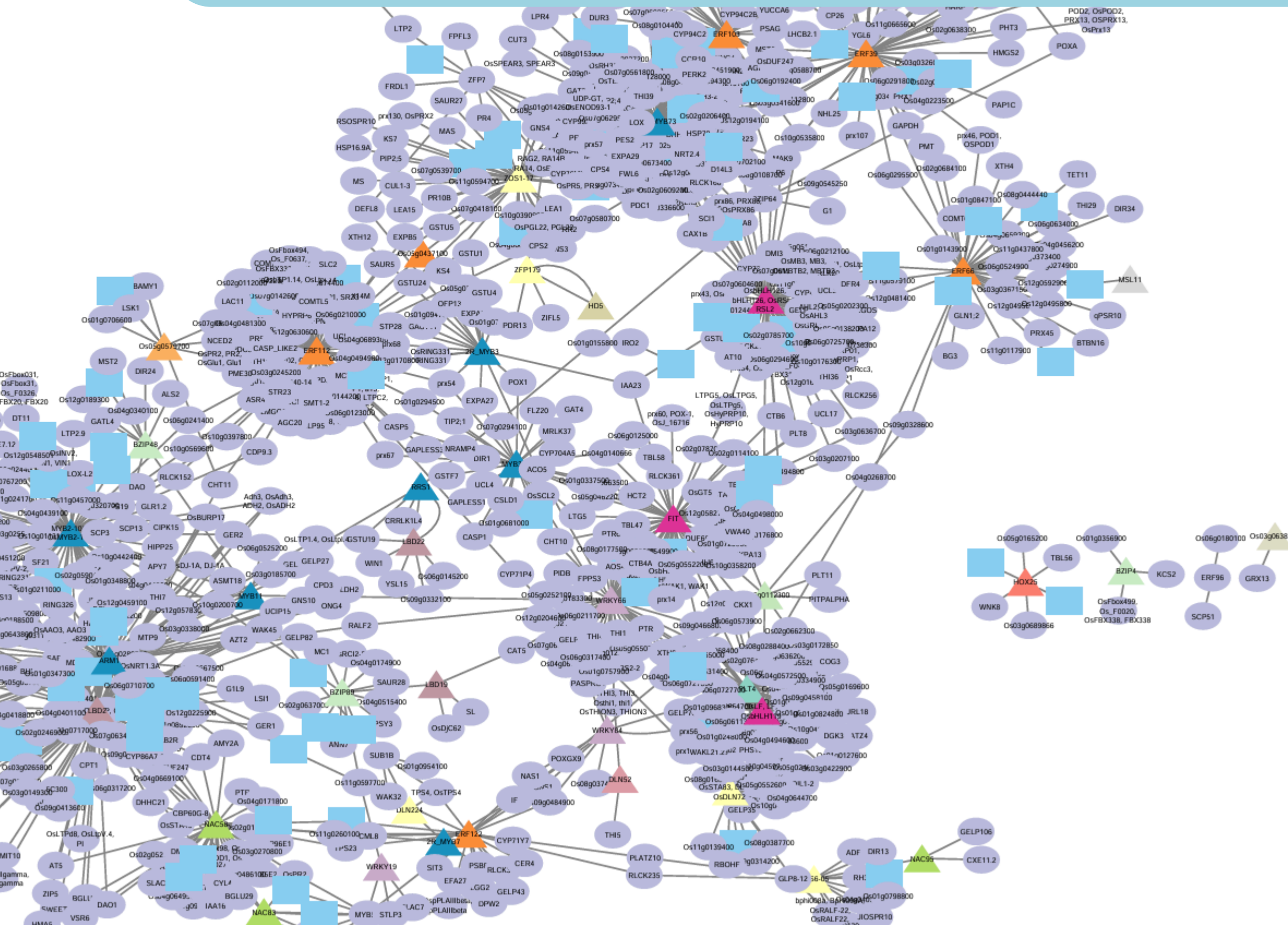
So you have a smooth Shoggoth.





So you have a big undirected network.

“I want to look at it and make it pretty.”



I want to be pretty.



So you have a big undirected network.

“I want to look at it and make it pretty.”

“And maybe do some Topological Analysis.”



So you have a big undirected network.

“I want to look at it and make it pretty.”

Gephy

Cytoscape

“And maybe do some Topological Analysis.”



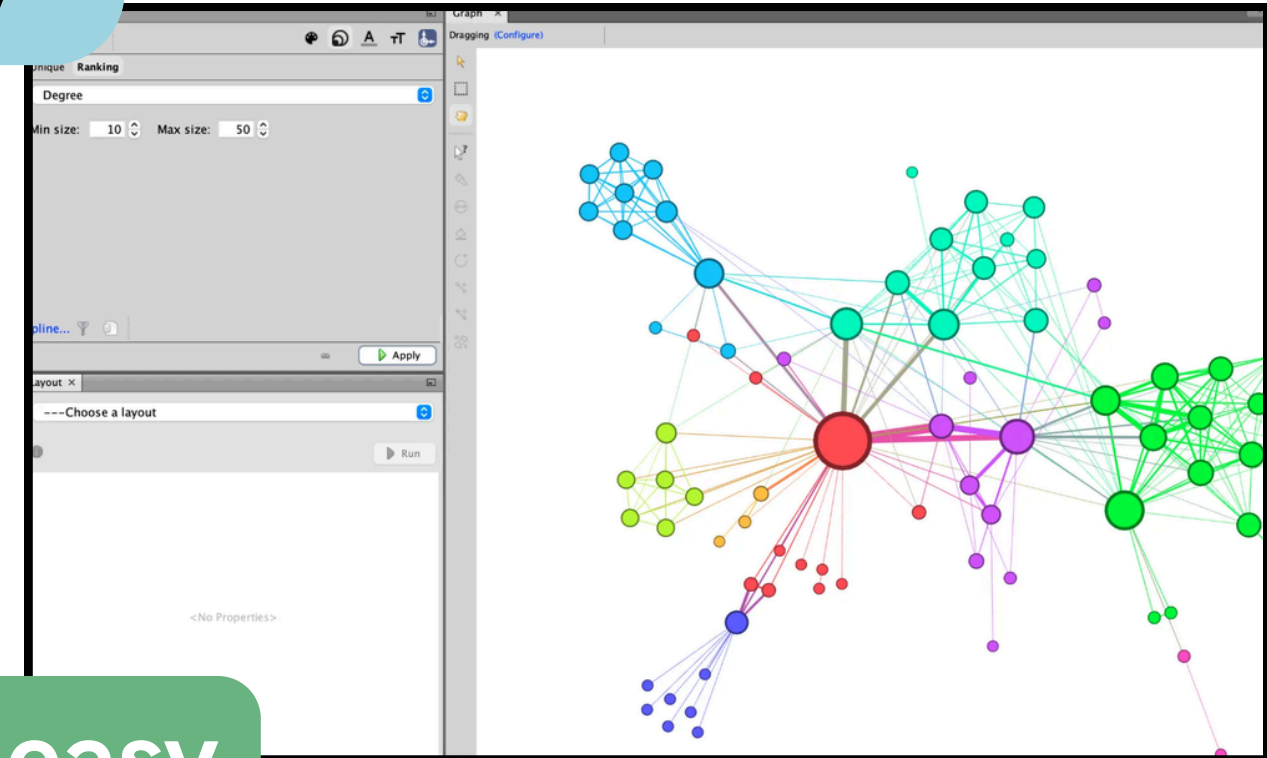


Gephy



<https://gephi.org/> → Installable

<https://lite.gephi.org/> → Online



Get appealing visual fast & easy
Get some metrics directly & Filter the graph
Available plugin library

“Statistics”

On the right side of the screen, the ‘Statistics’ window allows you to calculate certain properties of the graph. For example, it includes centrality metrics and calculations that provide information about the overall structure of the network, its clusters, and communities.

“Network Diameter”



- To calculate betweenness centrality, click on “Run” next to “Network Diameter”.
- This opens a small pop-up window in which you can specify whether the graph is directed or not (in this case, it is not).
- Click OK to close it and start the calculation.

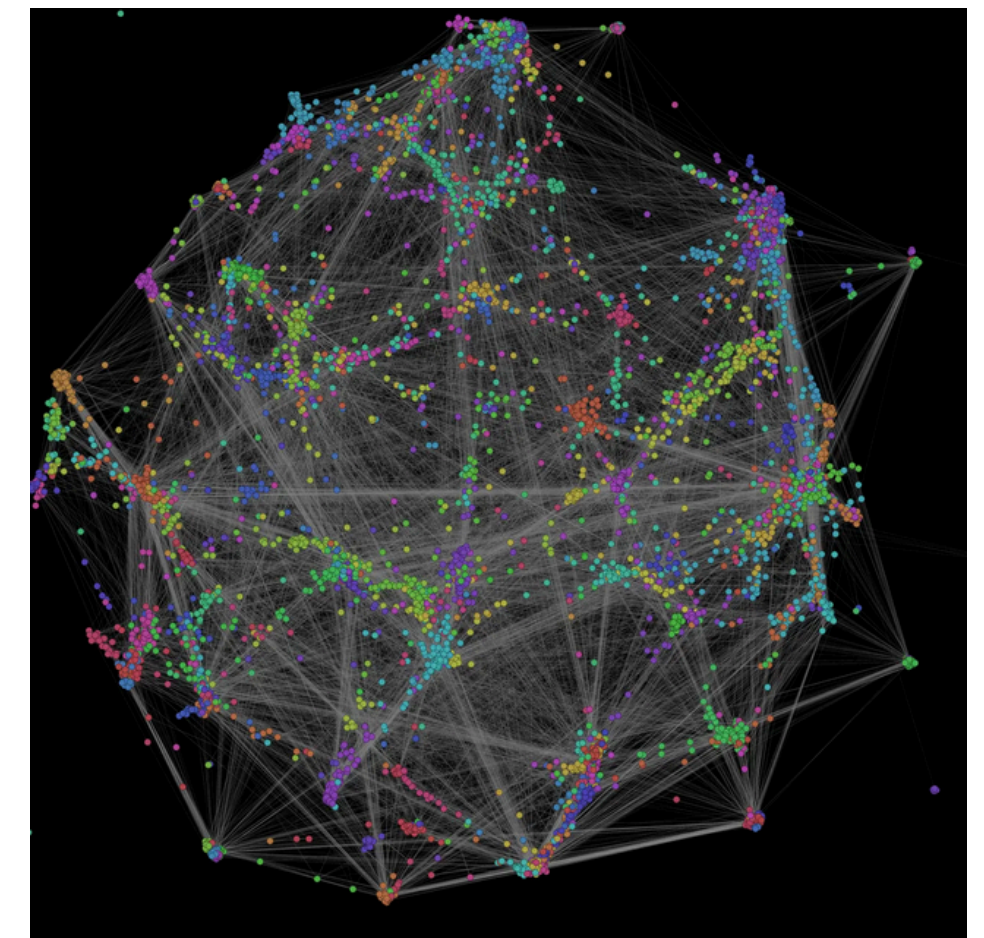
“Metrics”

The “Metrics” menu on the left side of the screen allows you to calculate certain properties of the graph. For example, it includes centrality metrics and calculations that provide information about the overall structure of the network, its clusters, and communities.

Betweenness centrality

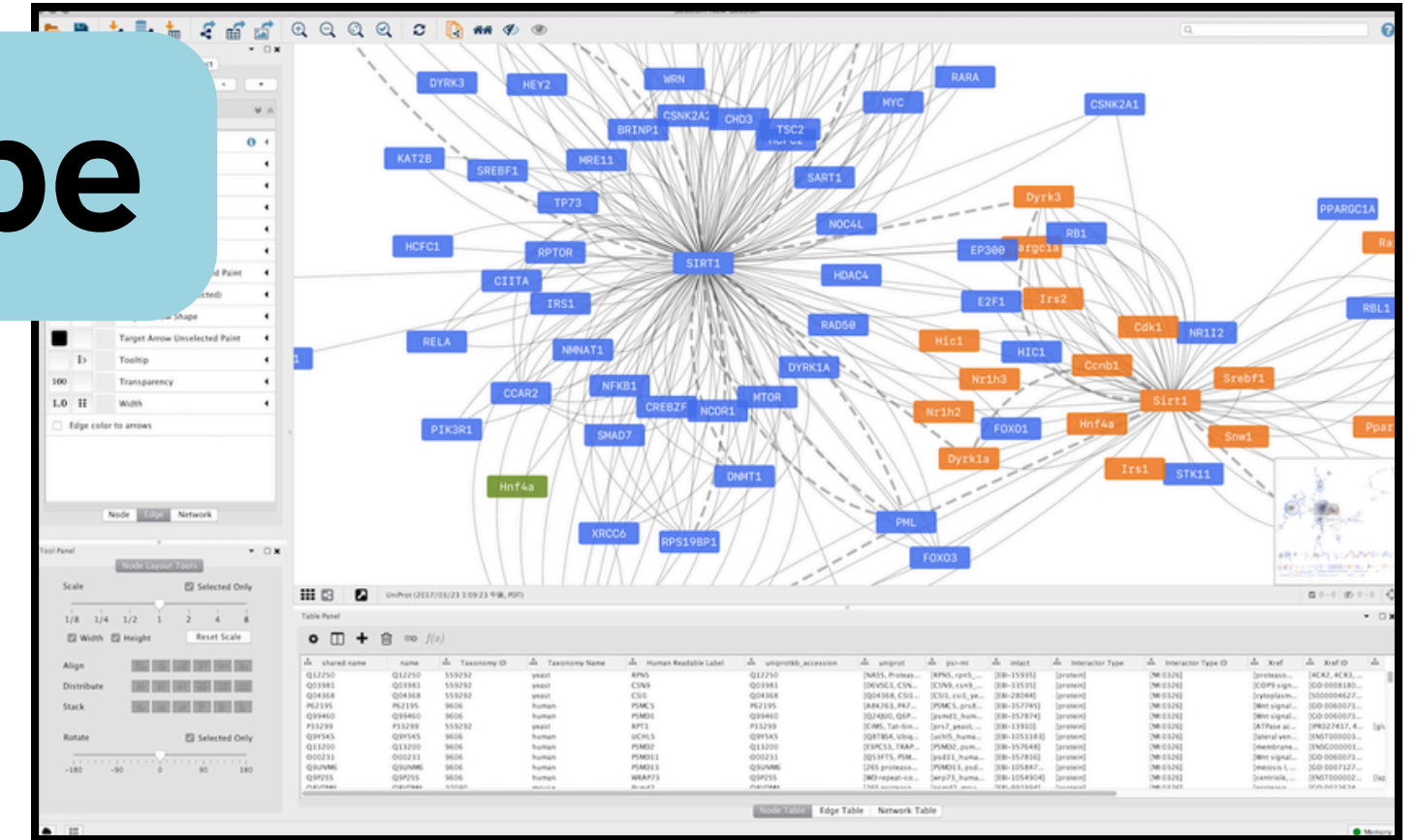


- Select “Betweenness centrality” under the “Metric” menu
- Click on “Compute metric” at the very bottom of the menu.
- The values for this metric are now recorded in the Nodes table of the “Data” table in a fresh new column labeled “BetweennessCentrality”.





Cytoscape



<https://cytoscape.org/> → Installable

<https://web.cytoscape.org/> → Online

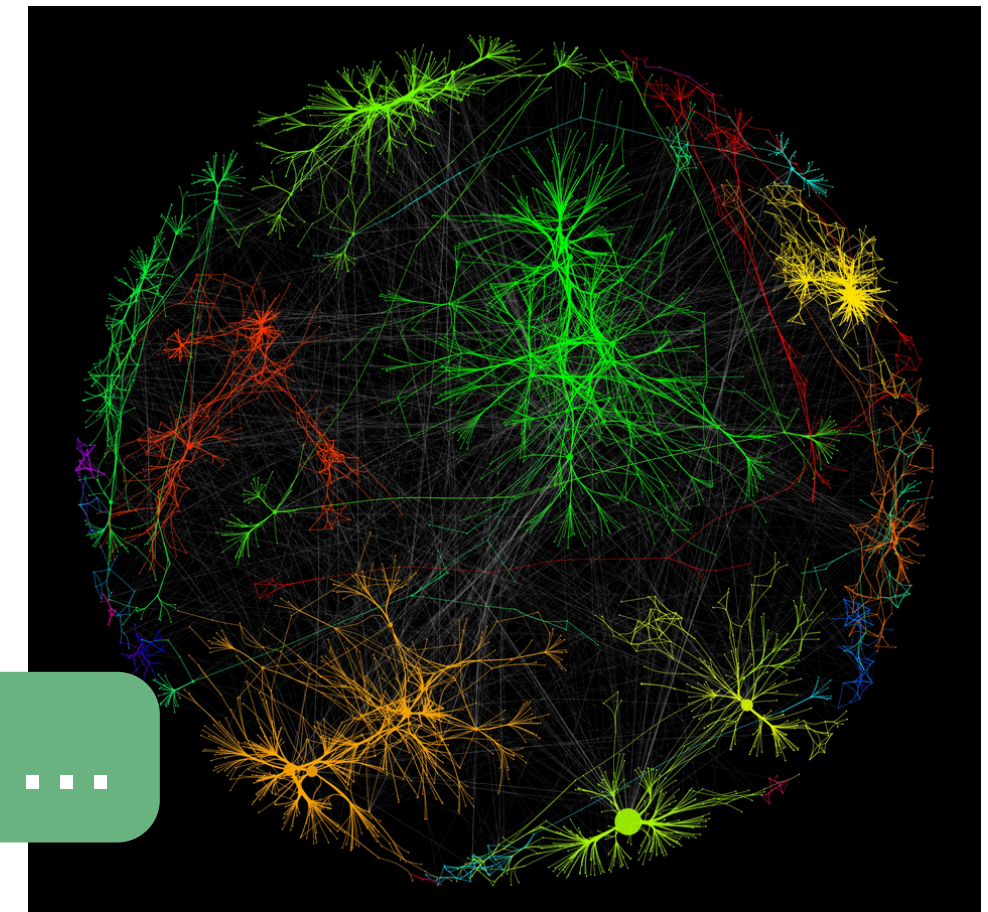
Much steeper learning curve to get beautiful graphs

& for general usage

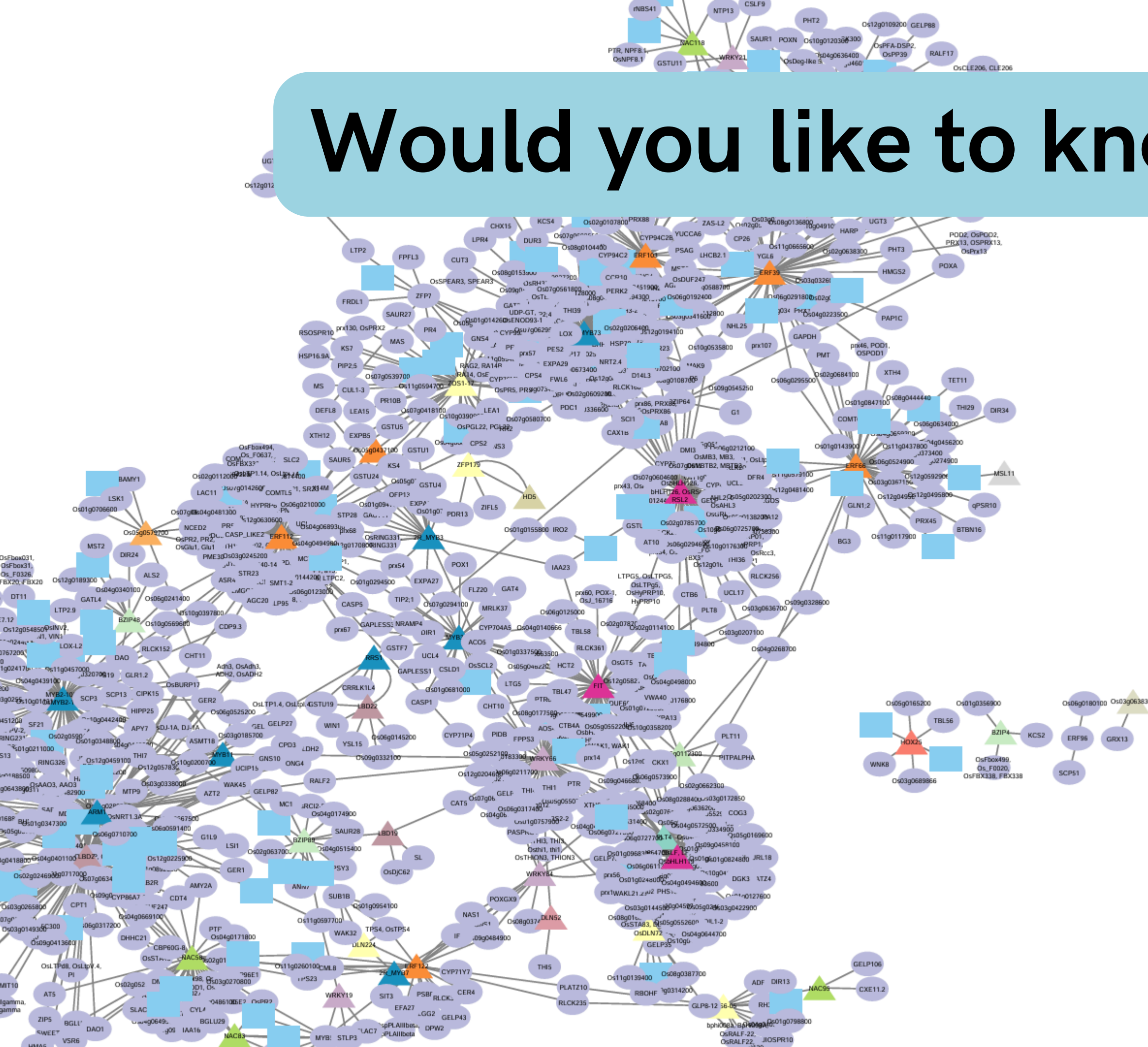
More extensive plugin (app) library

(except for the 2.x plugins never ported to 3.x...)

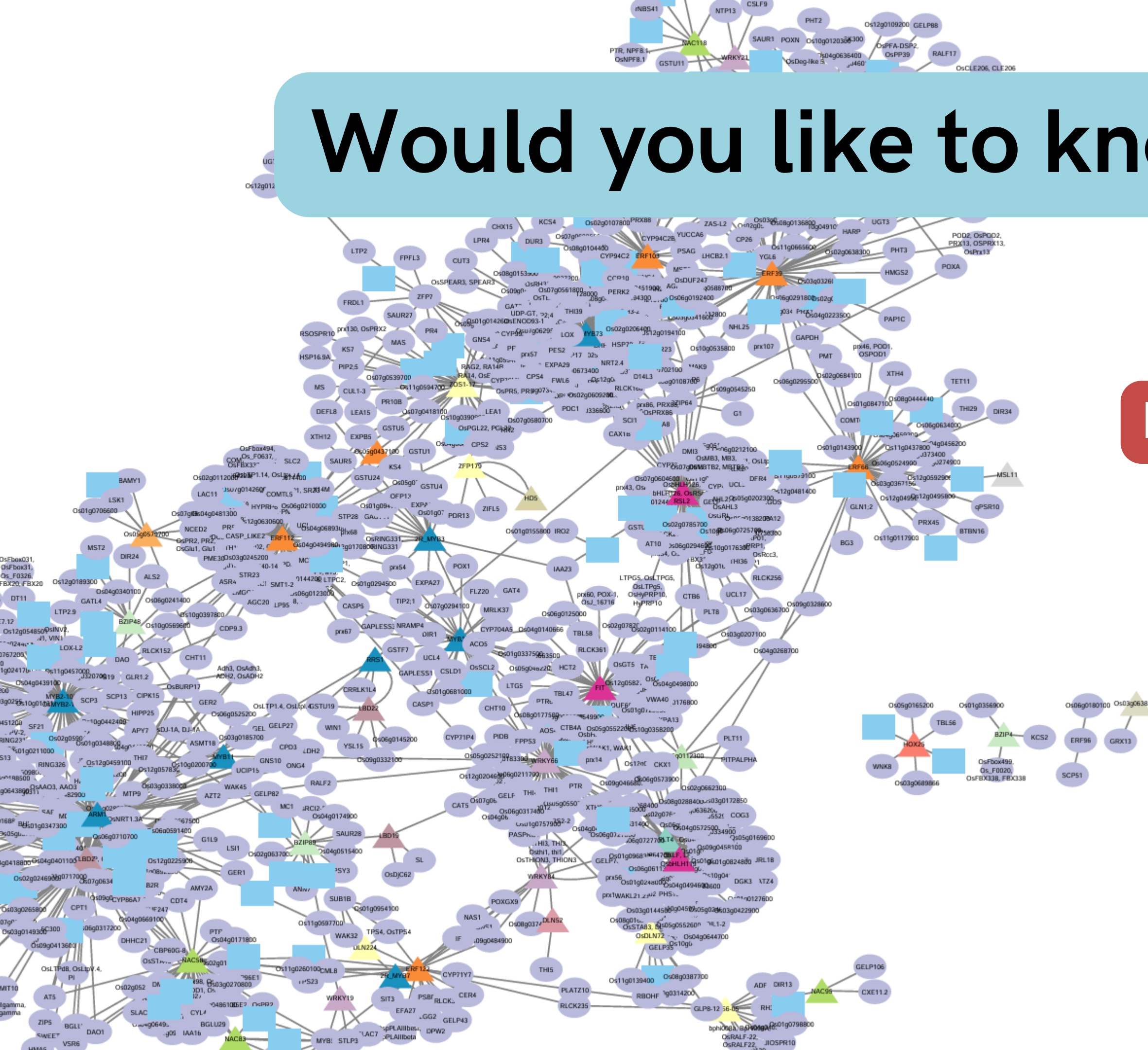
Cluster detection, merge networks, create subnetworks, ...



Would you like to know more ?



Would you like to know more ?



“This is not enough.
I want to really put my hands
into my network.”

Would you like to know more ?

“This is not enough.
I want to really put my hands
into my network.”

Welcome to
the dark side ...
(coding)

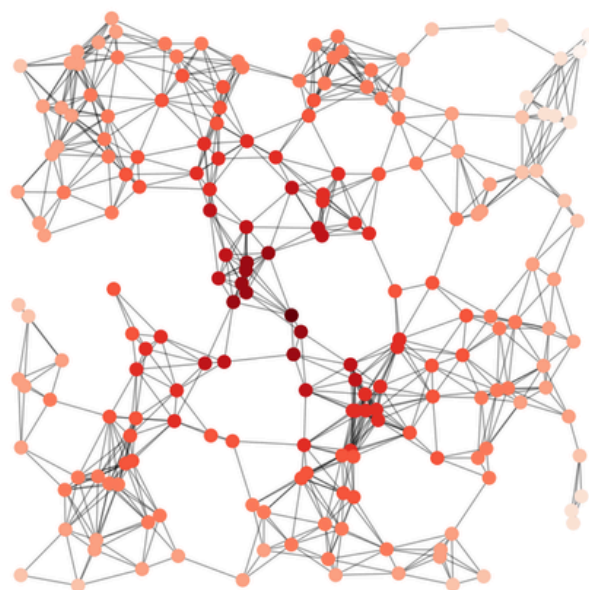
**When you need to explore the network structure,
use algorithms for network traversal, comparison
(isomorphism or graph edit distance), ...**



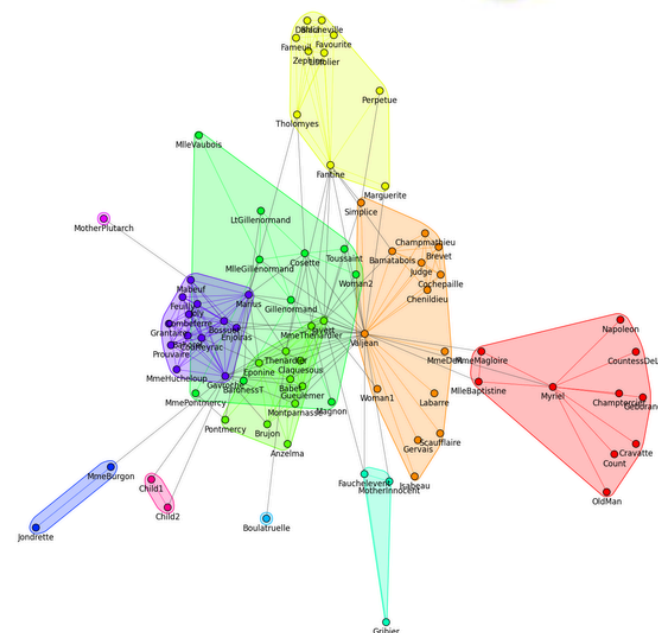
NetworkX

Network Analysis in Python

(Python)

**igraph.org**

(Python, R, C++)



The dark side (coding)

When you need to explore the network structure,
use algorithms for network traversal, comparison
(isomorphism or graph edit distance), ...



And want to make it extra pretty...



ipysigma (jupyter widget)

That's the best

Hub detection

Clusters/Modules detection

Centrality measurements

“But I want more from my Shoggoth...”



Ok. Then what you reaaaally need is ...



More smooth Shoggoths.



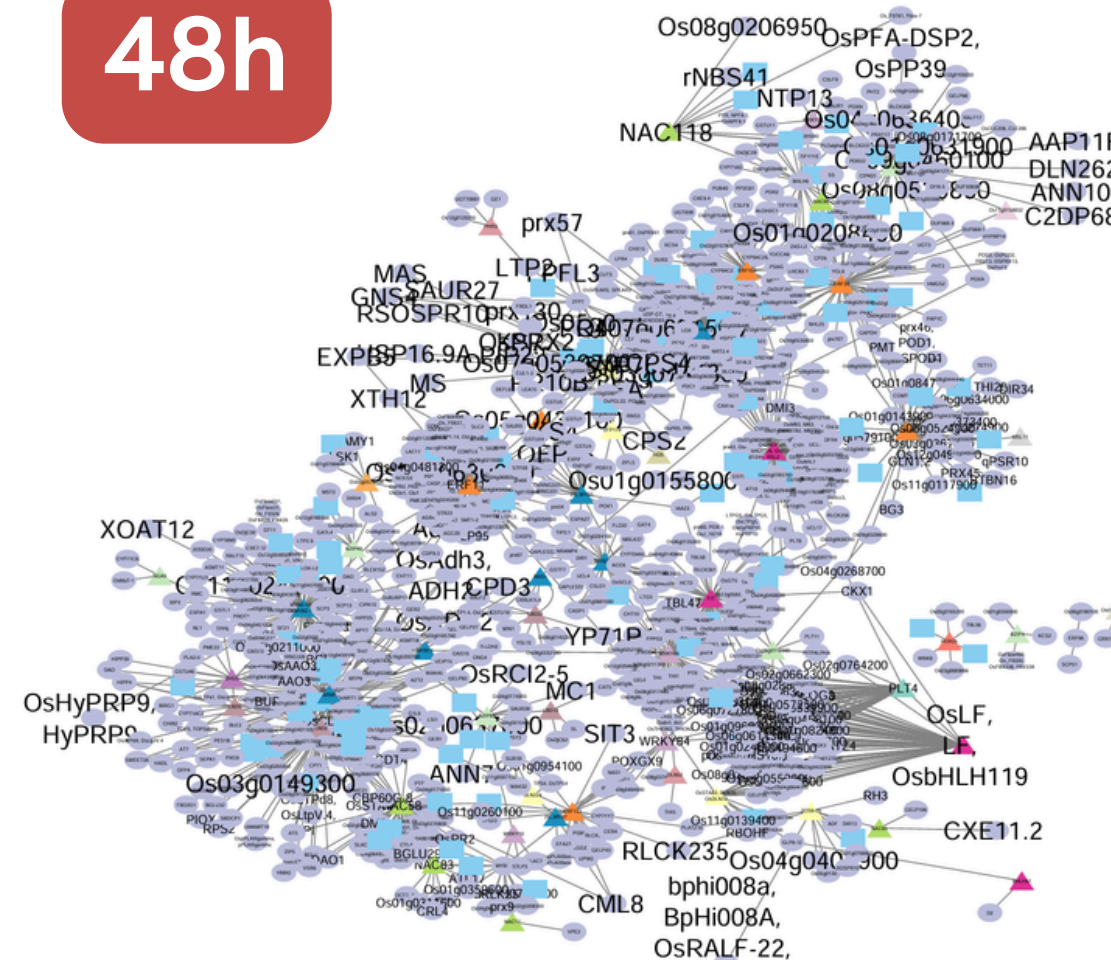
More undirected graphs.

Ok, do you have several graphs relating to the same biological event in different conditions (mutant, time series, treatments,...) ?

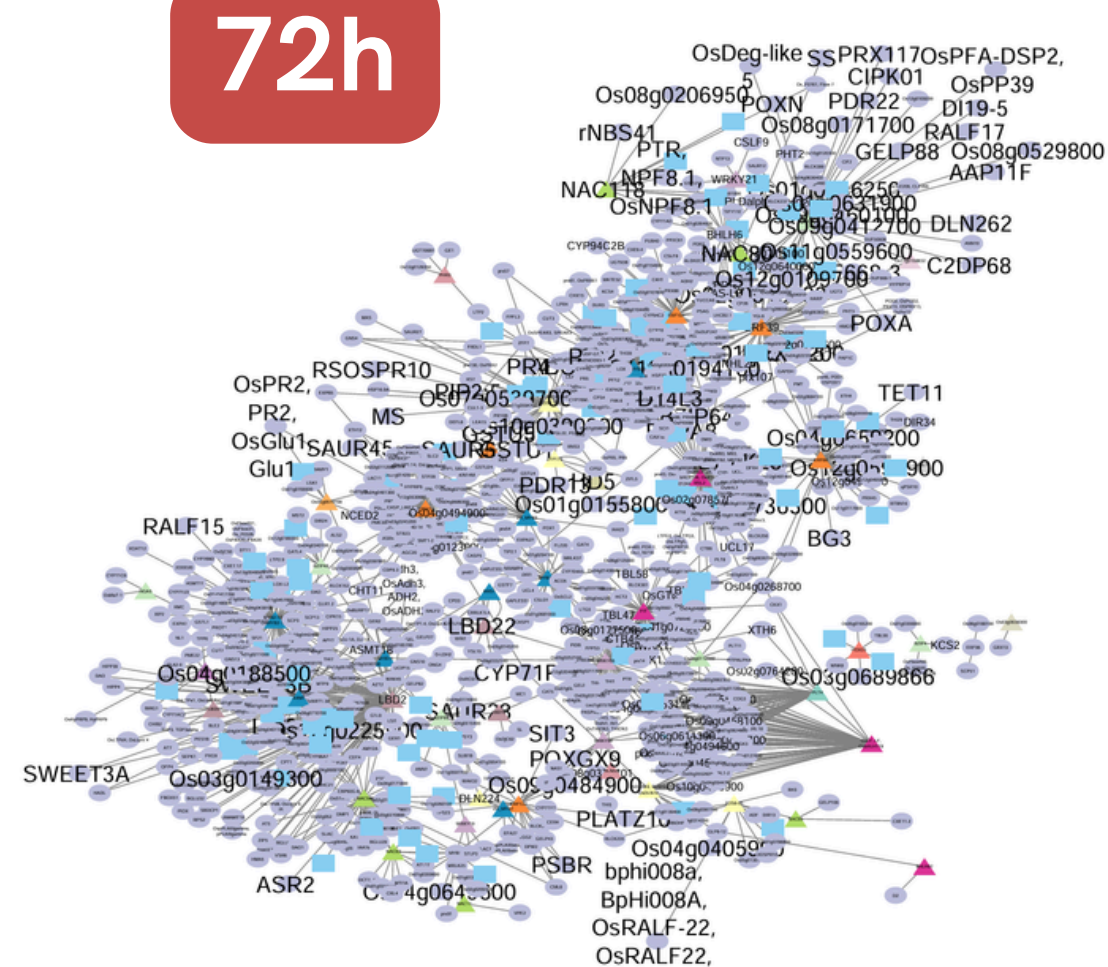
For example...

Rice CR network
crl5 vs WT

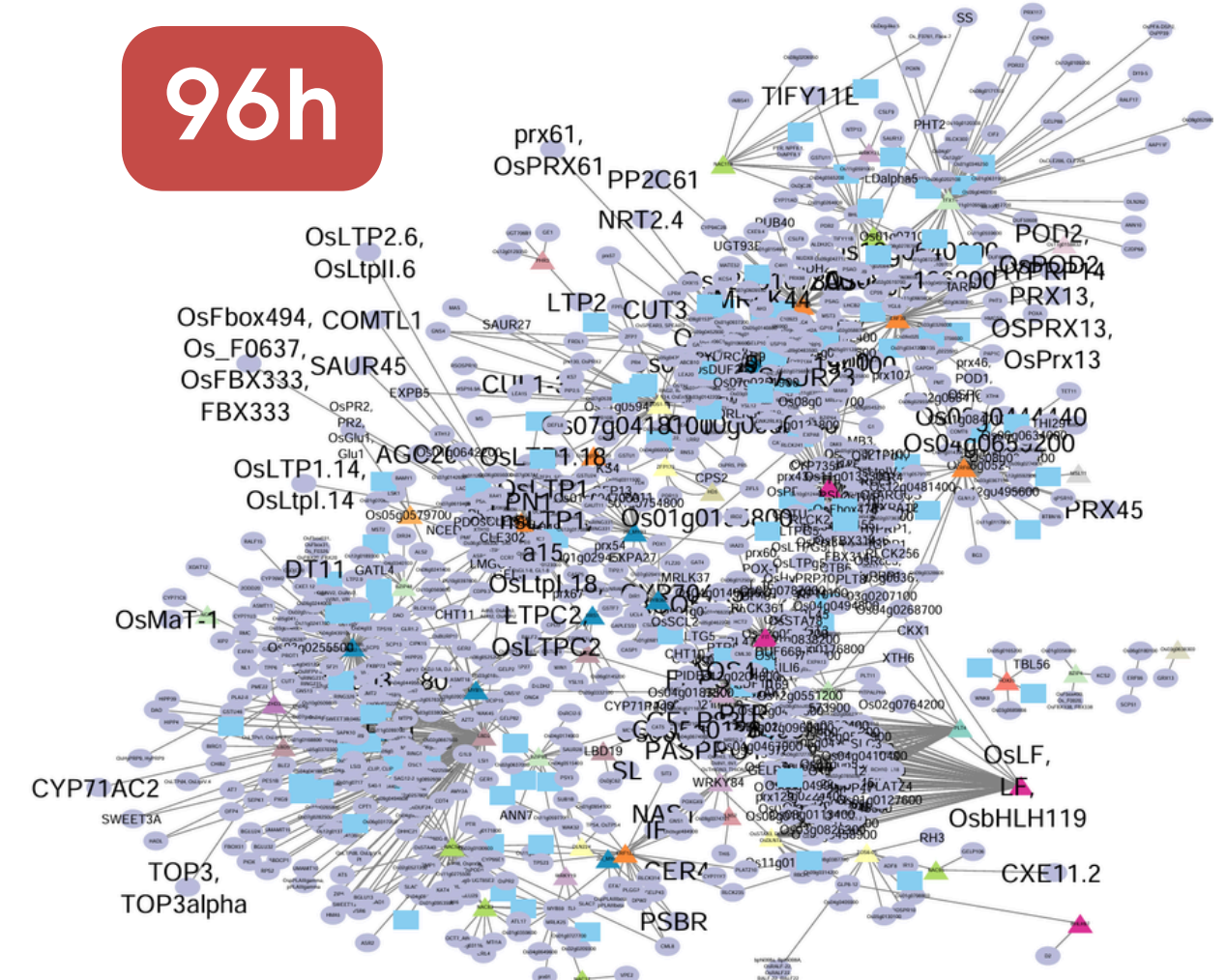
48h



72h



96h



Then you can do comparative graph analyses



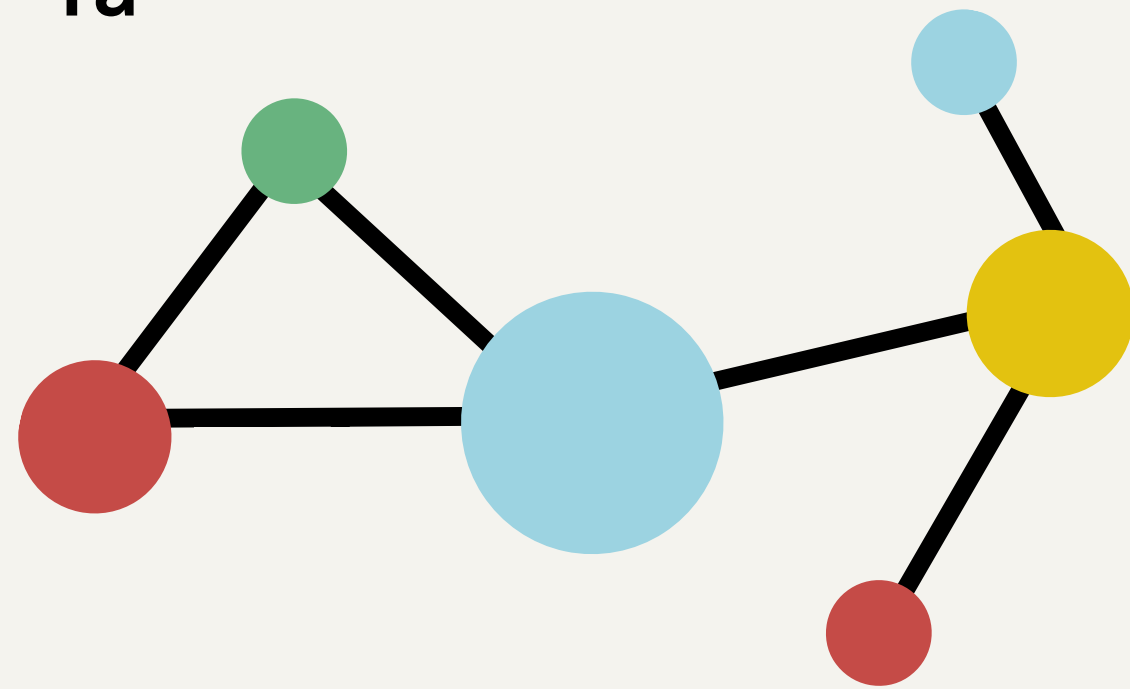
Then you can do
comparative graph analyses

Let's have a look at some
metrics for that

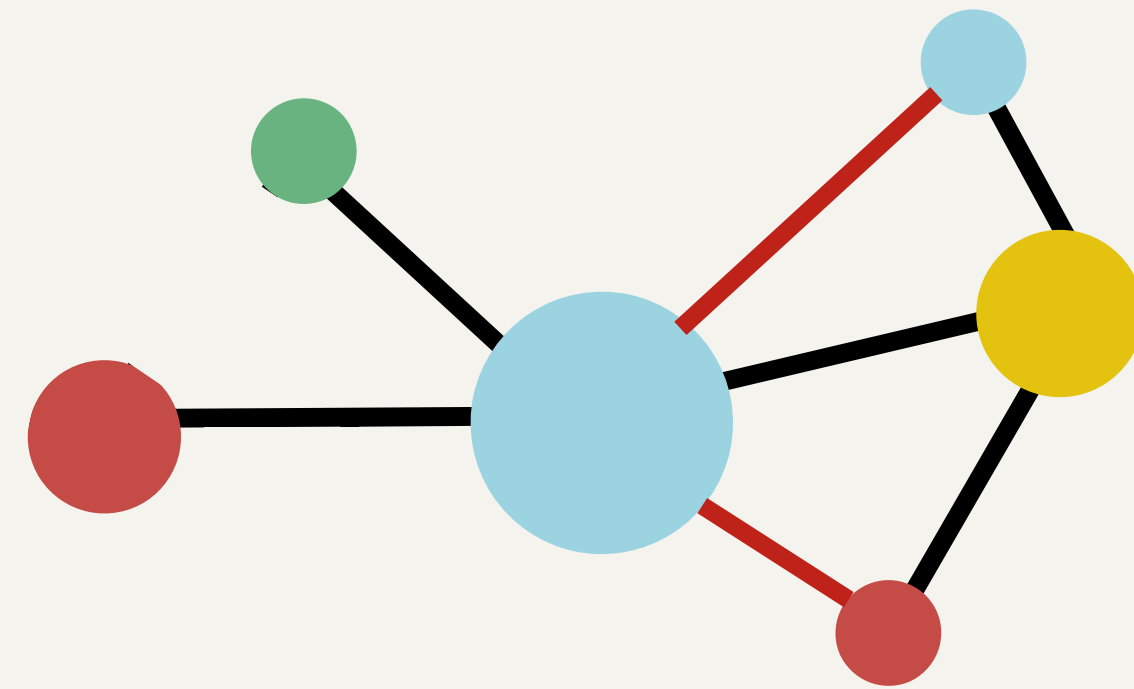


Let's take two related networks

GRN-1a

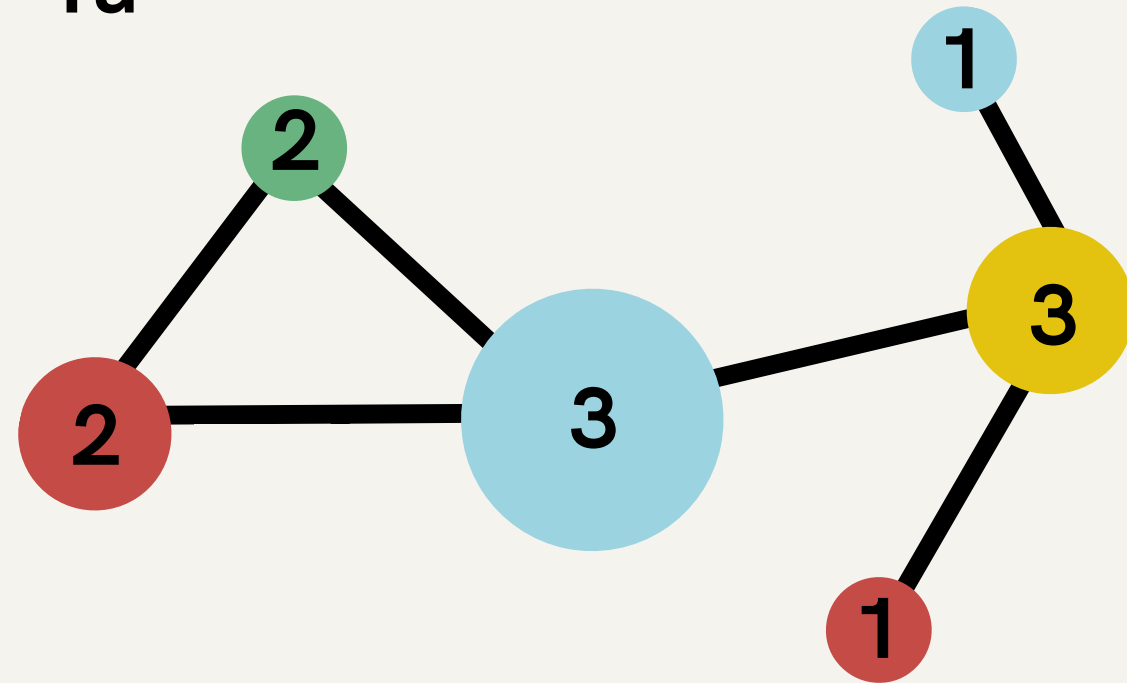


GRN-1b

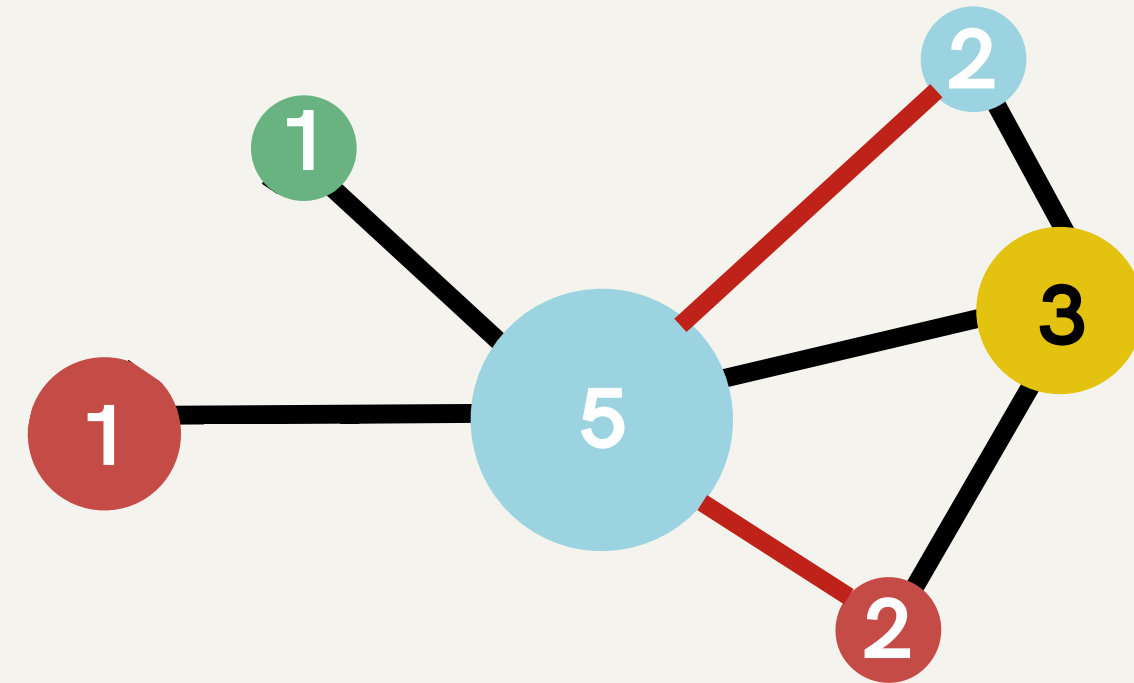


Let's take two related networks

GRN-1a

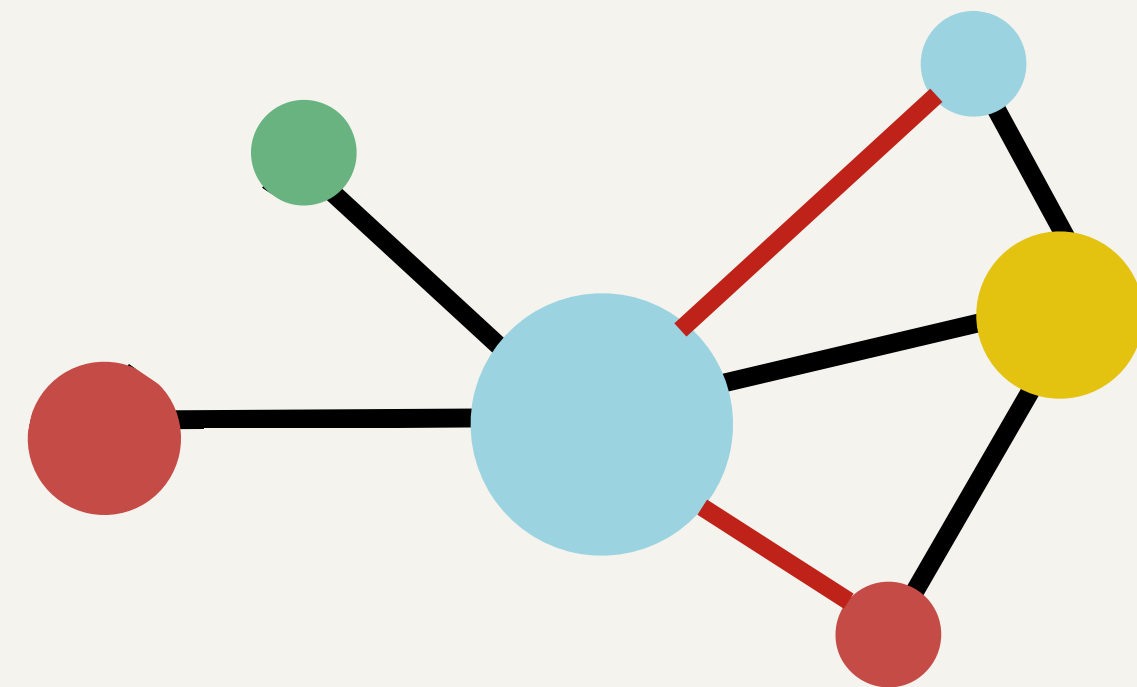
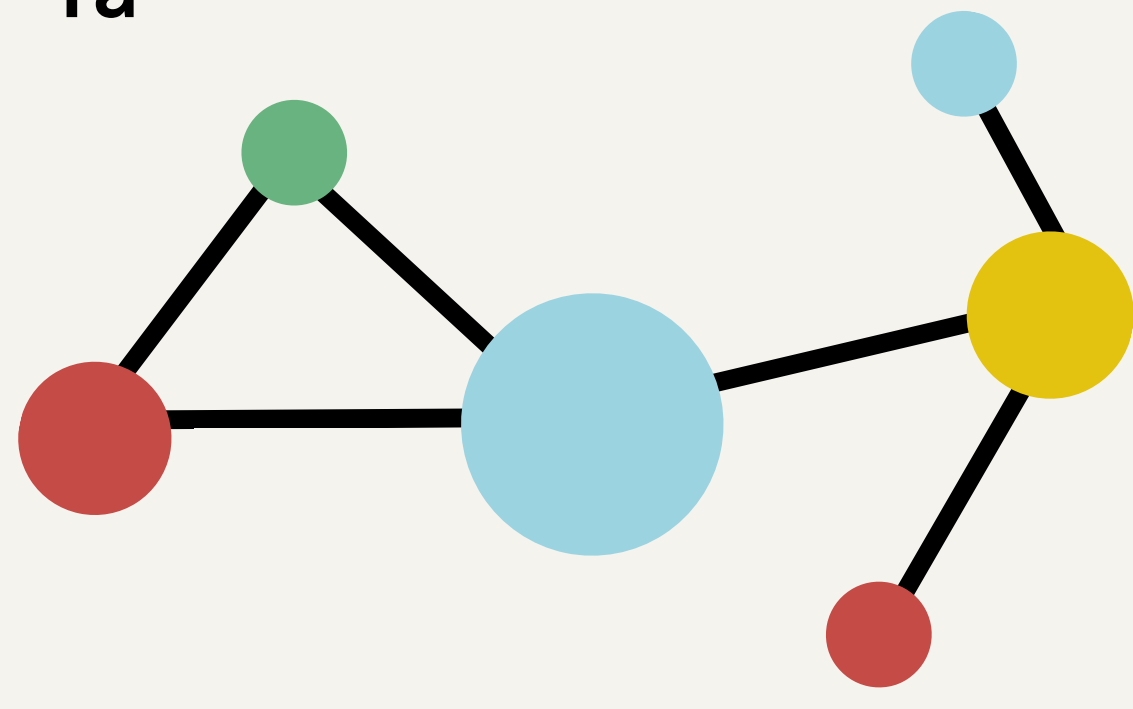


GRN-1b



We can compare their
degree distributions

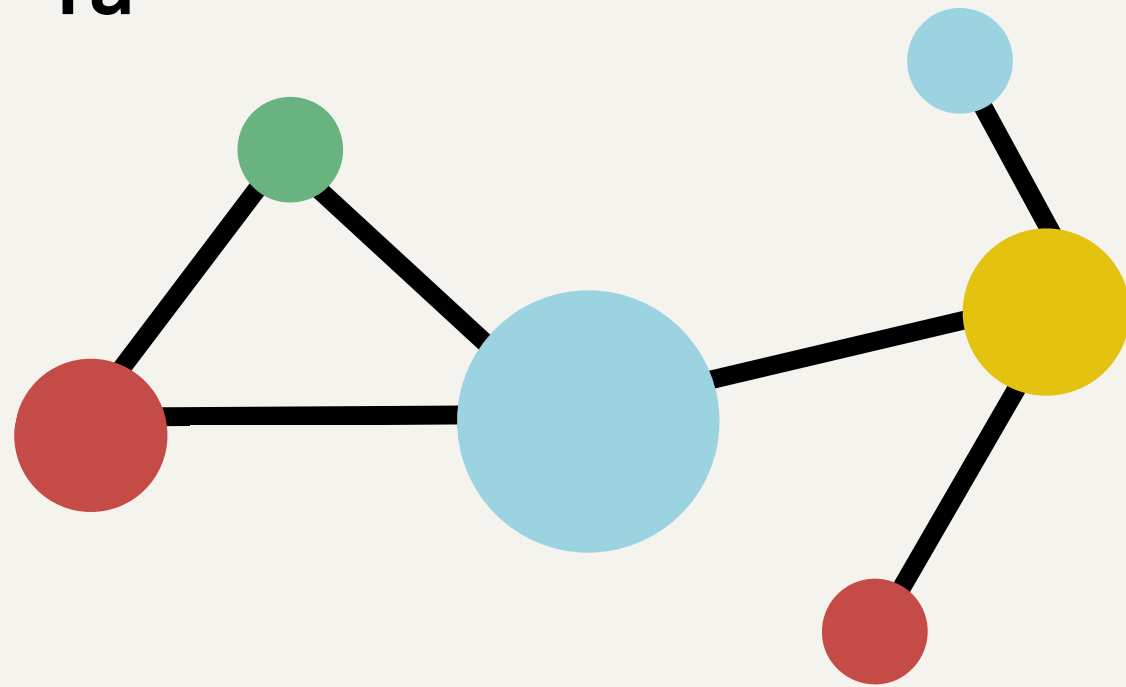
Let's take two related networks



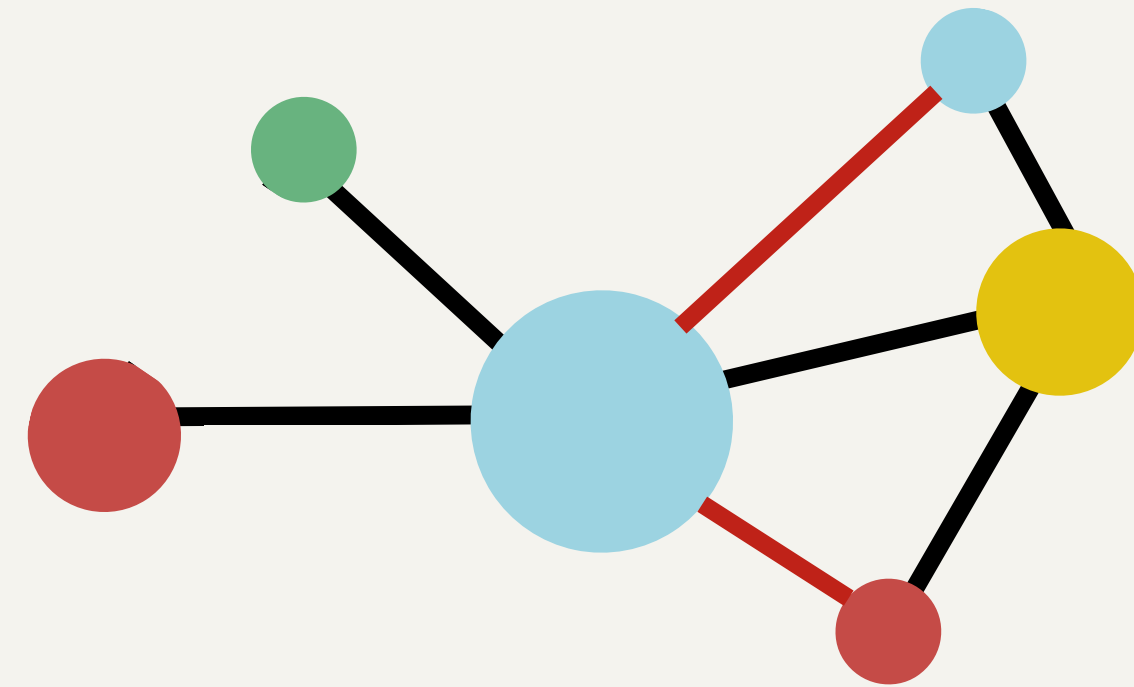
We can compare first-order global identity

Let's take two related networks

GRN-1a



GRN-1b

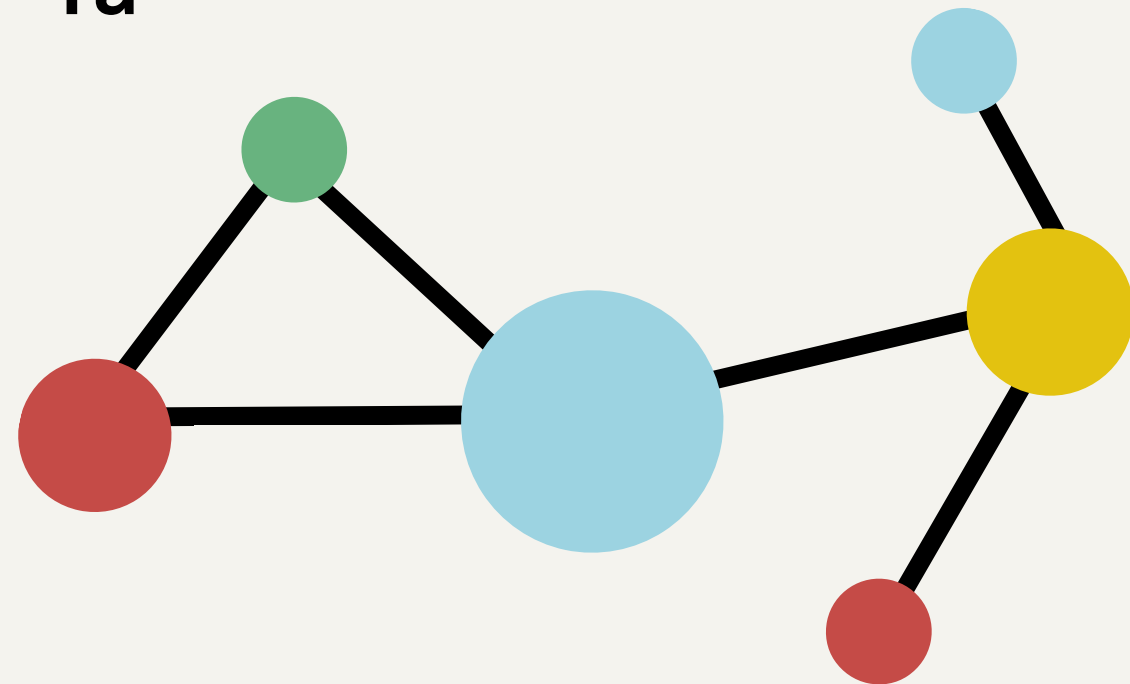


We can compare first-order global identity

How many common edges ?

Let's take two related networks

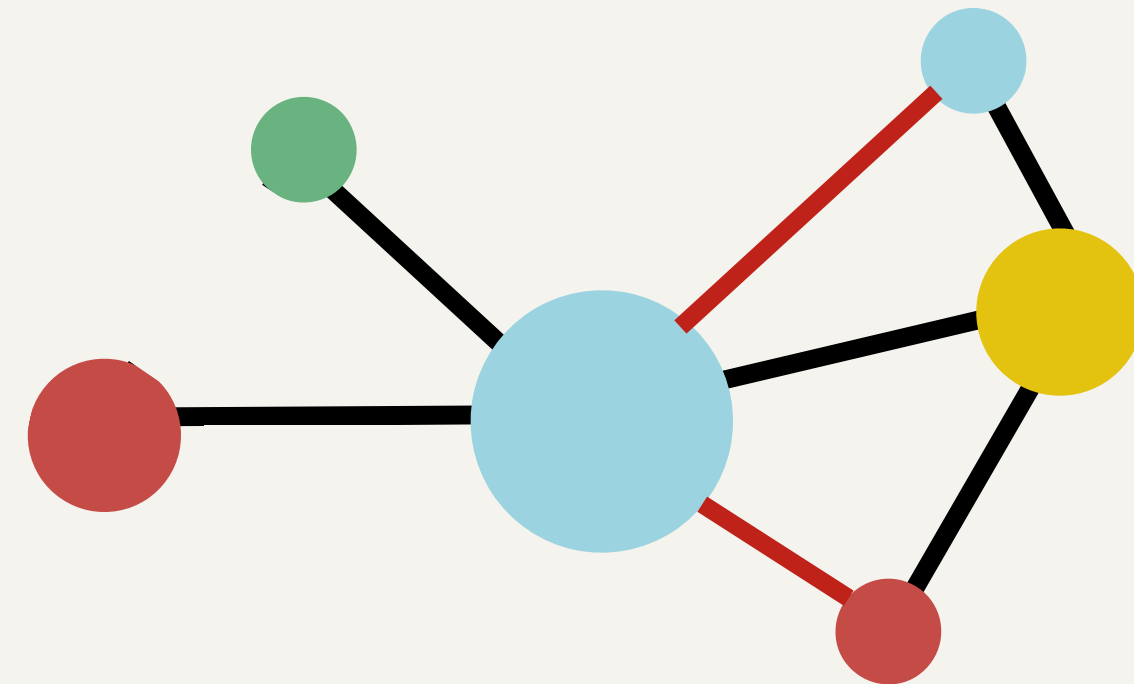
GRN-1a



5 of 6 edges in
common (83%)



GRN-1b



5 of 7 edges in
common (71%)

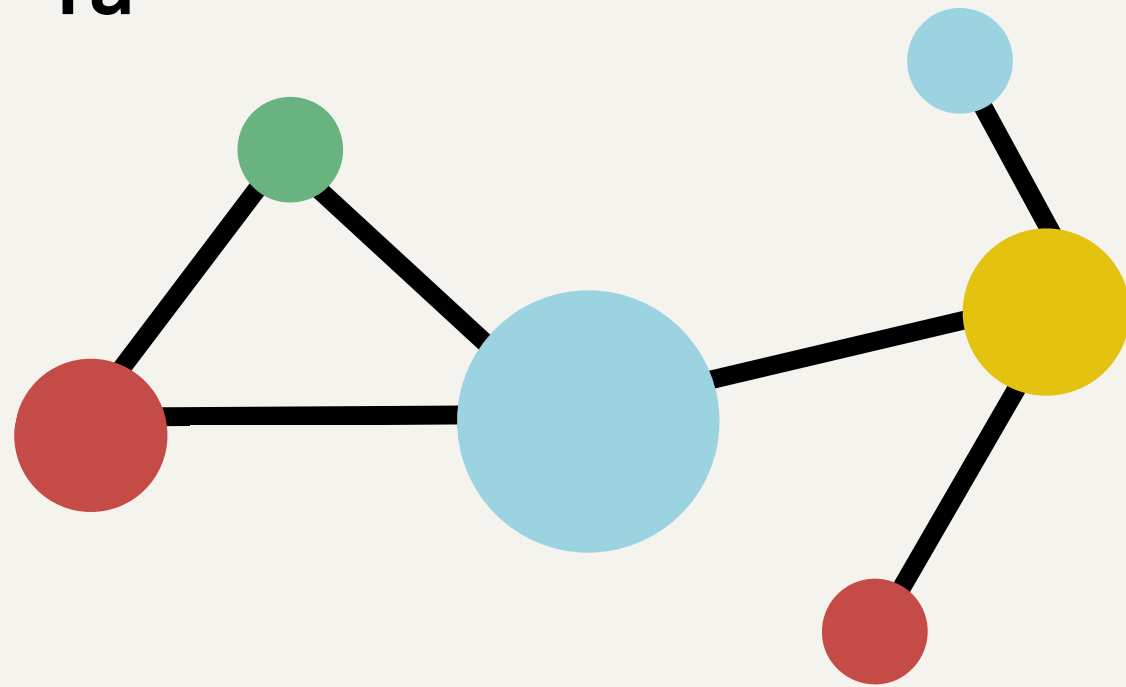


First-Order Global identity

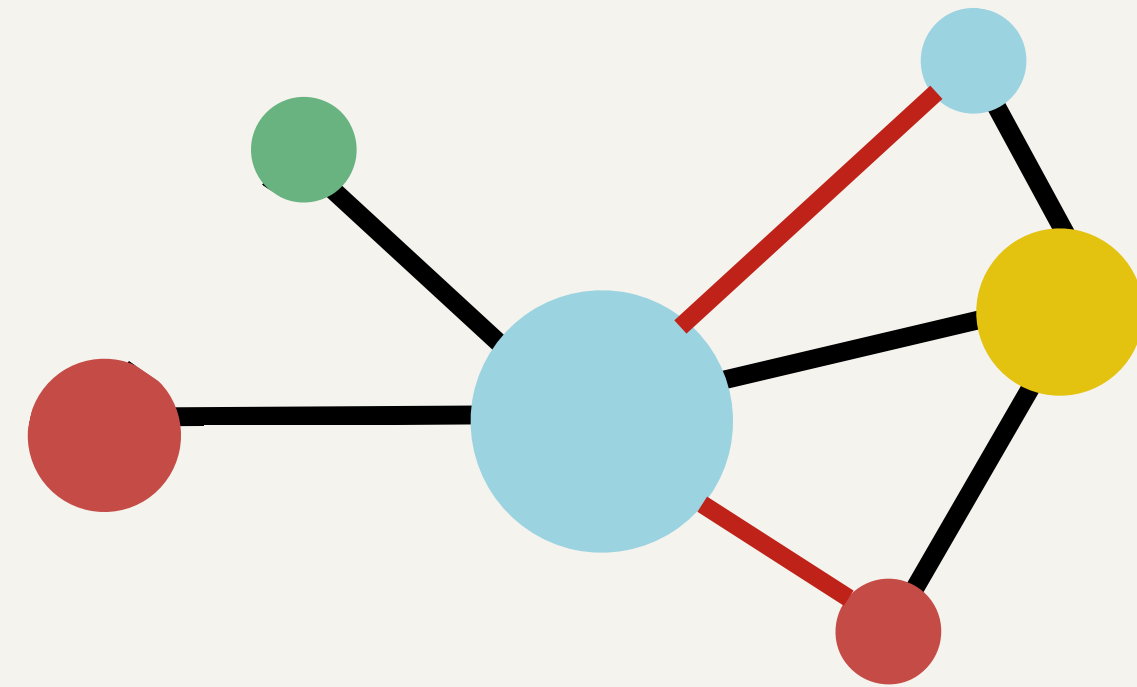
How many common edges ?

Let's take two related networks

GRN-1a



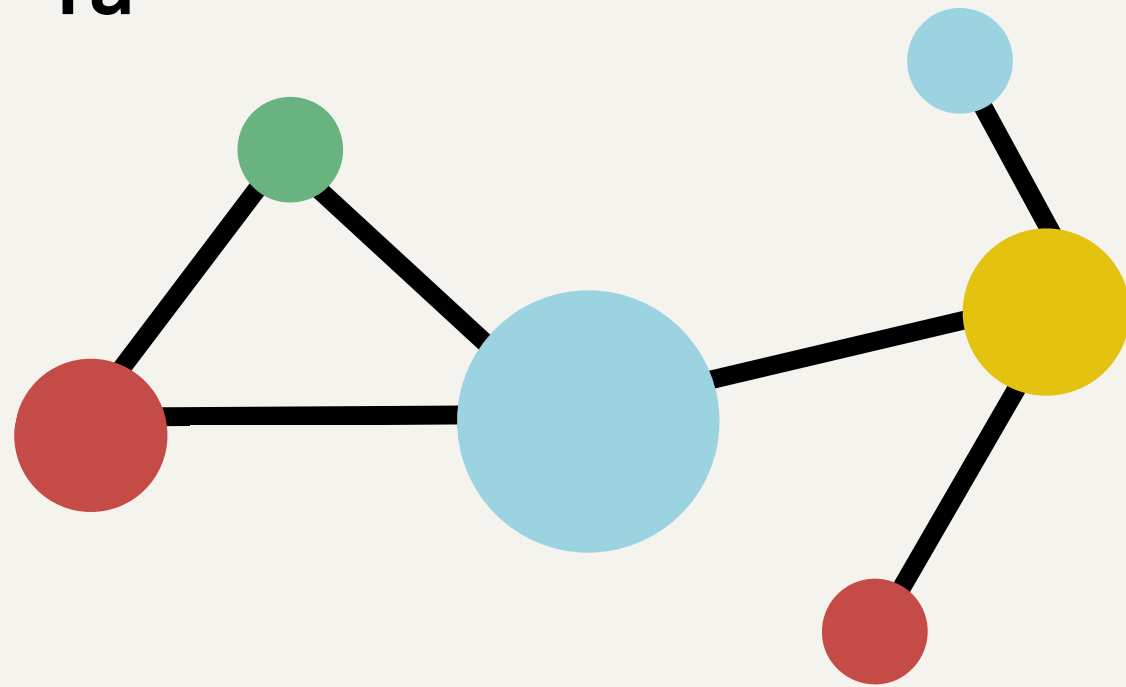
GRN-1b



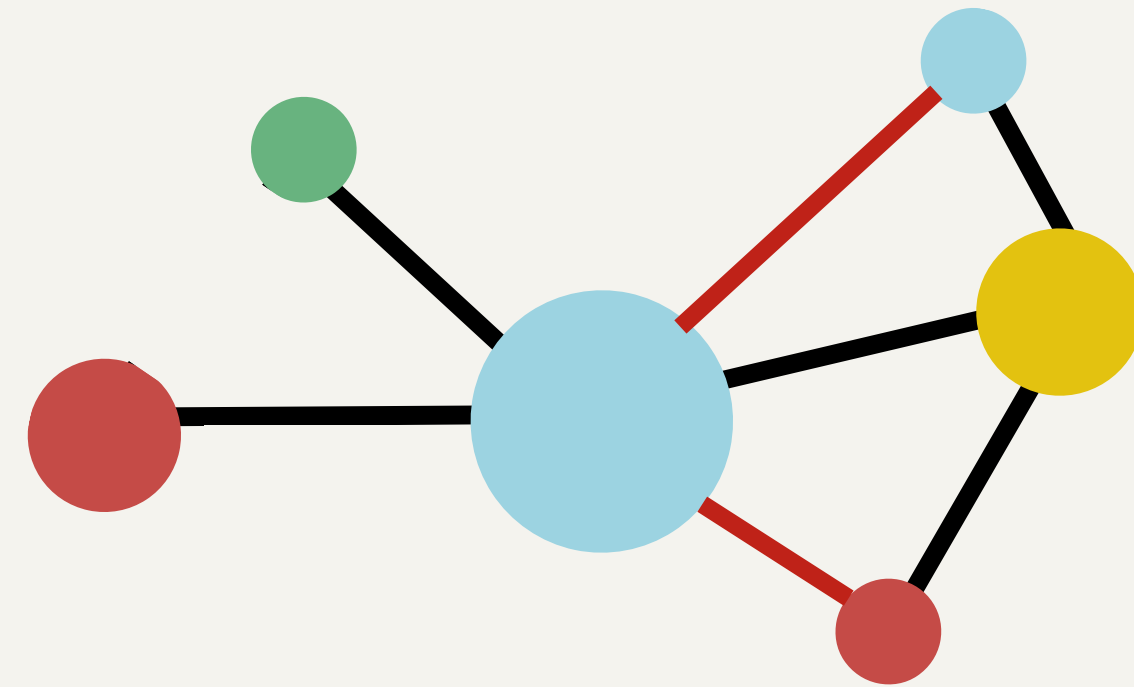
We can compare Nth-order global identity

Let's take two related networks

GRN-1a



GRN-1b

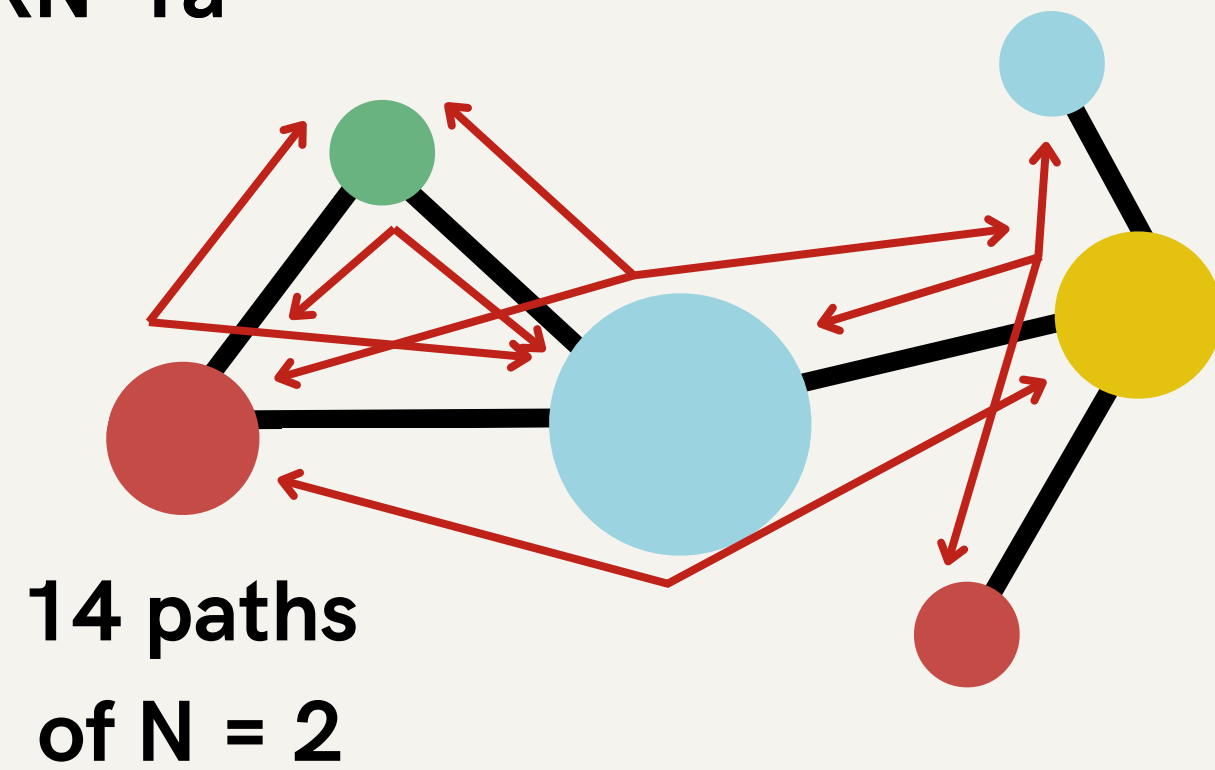


We can compare Nth-order global identity

How many common paths of length N ?

Let's take two related networks

GRN-1a



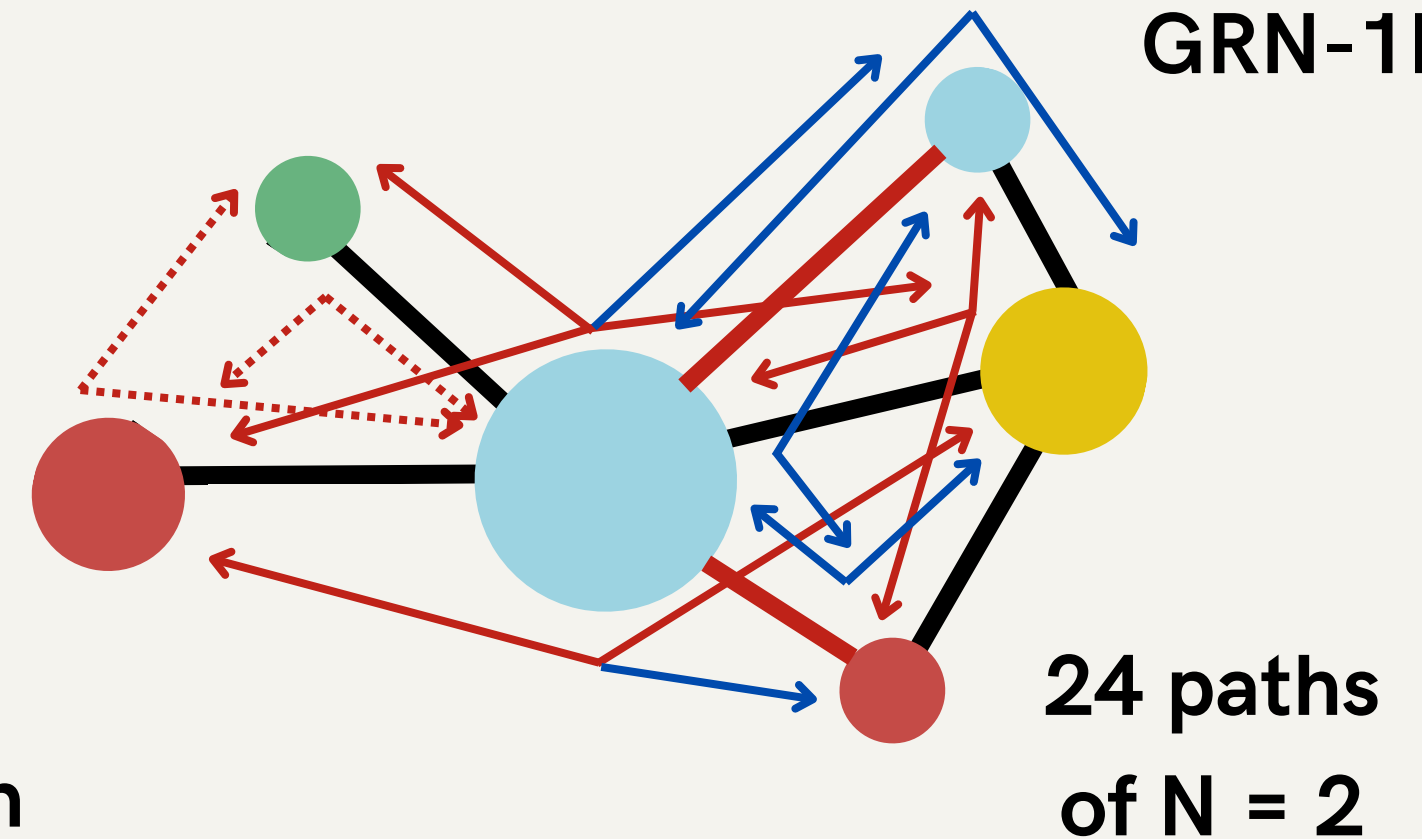
10 of 14 paths in
common (71%)

for
 $N=2$

12 of 24 paths in
common (50%)

Nth-Order Global identity

GRN-1b



How many common paths of length N ?

**“Okay so we have metrics for
graph comparison.**

How does that give me value ?”

So we focus on the **dynamics of topological changes** rather than the topology of a single network

i.e : look at where the network is changing and how qualitatively

Re-calibration for Priority Score System

Class A — Major gain or loss of control: Strong regulatory rewiring

Priority	Δ Degree	Δ Betweenness	Δ Stress	Biological interpretation	Score
I_MUT_ONLY	Present	Present	Present	Gene appears exclusively in the mutant GRN, indicating de novo regulatory activation or mutant-specific hub emergence	3
I	↓	↓	↓	Loss of connectivity, routing control, and traffic load in mutant → collapse of regulatory influence; WT-dominant regulator	3
II	↑	↑	↑	Coordinated gain in connectivity, control, and load → mutant acquires (strong, hub-like) regulatory dominance	3
III	↓	0/↑	↑/↓	Partial rewiring: loss of direct connections with redistribution of control → context-dependent regulatory reprogramming	3

Class B — Functional redistribution without structural rewiring

Priority	Δ Degree	Δ Betweenness	Δ Stress	Biological interpretation	Score
IV	0	↑	0	Connectivity preserved but routing importance increases → gain in regulatory leverage without new edges	2
V	0	0	↑	Stable topology with increased traffic → signal load amplification without centrality gain	2
VI	0	↑	↑	Fixed degree with increased routing and load → passive regulatory amplifier or bottleneck	2

Class C — GRN Structural alteration

Priority	Δ Degree	Δ Betweenness	Δ Stress	Biological interpretation	Score
VII	↓	↑/↓	0/↑/↓	Mutant gains edges but loses or redistributes routing → structural expansion with diluted control	1
VIII	0	↑/↓	↑/↓	No rewiring but altered flow patterns → passive redistribution	1
IX	↑	↑	↓	Mutant loses edges and routing but remaining paths overloaded → network compression / forced bypassing	1
X	↑/↓	0	0	Edge change without functional impact → structural noise	1

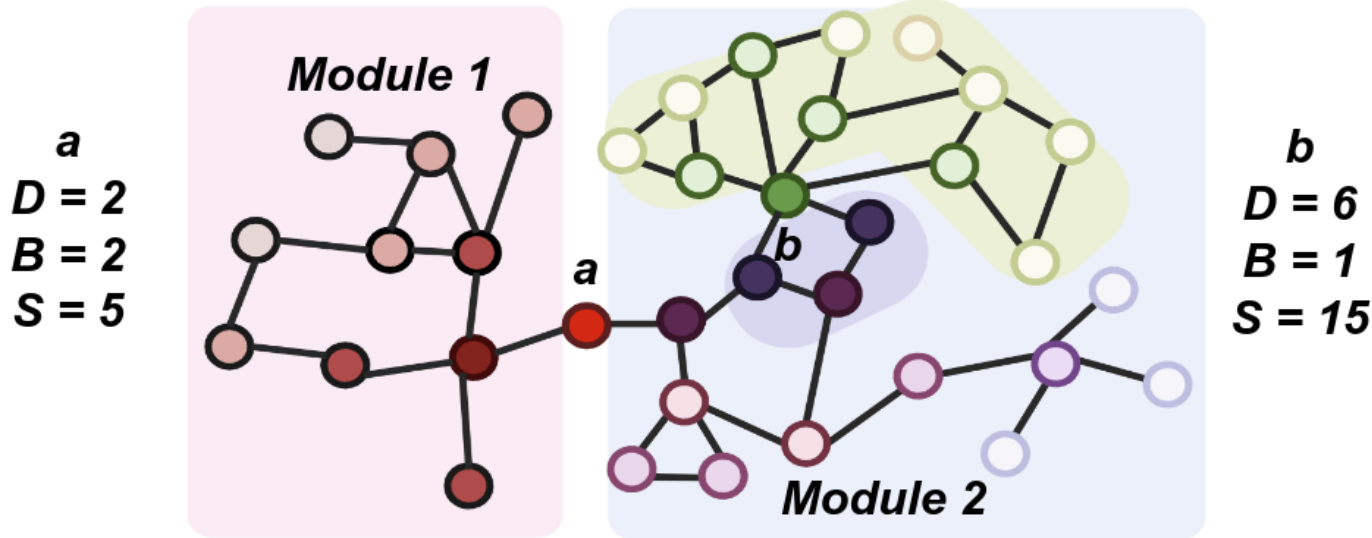
Class D — No detectable regulatory impact

Priority	Δ Degree	Δ Betweenness	Δ Stress	Biological interpretation	Score
XI	0	0	0	No topological or flow change → regulatory neutrality	0

So we focus on the **dynamics of topological changes** rather than the topology of a single network

Using subtractive networks, modules identification and integrated scoring

A Topological Parameters of a weighted GRN



B

WT	c_1	c_2	c_3	...	c_n
c_1				...	
c_2				...	
c_3				...	
...	...	...	...	...	...
c_n				...	

M	c_1	c_2	c_3	...	c_n
c_1				...	
c_2				...	
c_3				...	
...	...	...	...	...	...
c_n				...	

$$\Delta D_i^{(k)} = D_i^{WT(k)} - D_i^{MUT(k)}$$
$$\Delta B_i^{(k)} = B_i^{WT(k)} - B_i^{MUT(k)}$$
$$\Delta S_i^{(k)} = S_i^{WT(k)} - S_i^{MUT(k)}$$

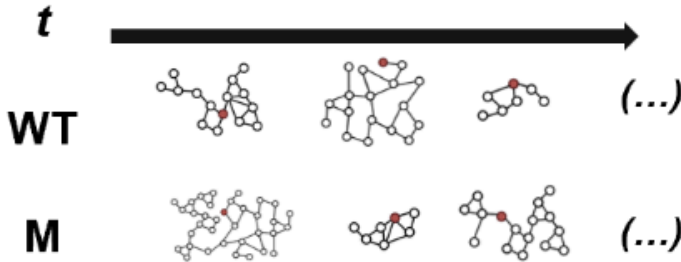
$$PS_i^{(k)} = \begin{cases} 3 & \text{if Class A} \\ 2 & \text{if Class B} \\ 1 & \text{if Class C} \\ 0 & \text{if Class D} \end{cases}$$

C

Priority	Δ Degree	Δ Betweenness	Δ Stress	Class	TPS
I_MUT_ONLY	Present	Present	Present	A	3
I	↓	↓	↓	A	3
II	↑	↑	↑	A	3
III	↓	0/↑	↑/↓	A	3
IV	0	↑	0	B	2
V	0	0	↑	B	2
VI	0	↑	↑	B	2
VII	↓	↑/↓	0/↑/↓	C	1
VIII	0	↑/↓	↑/↓	C	1
IX	↑	↑	↓	C	1
X	↑/↓	0	0	C	1
XI	0	0	0	D	0

D

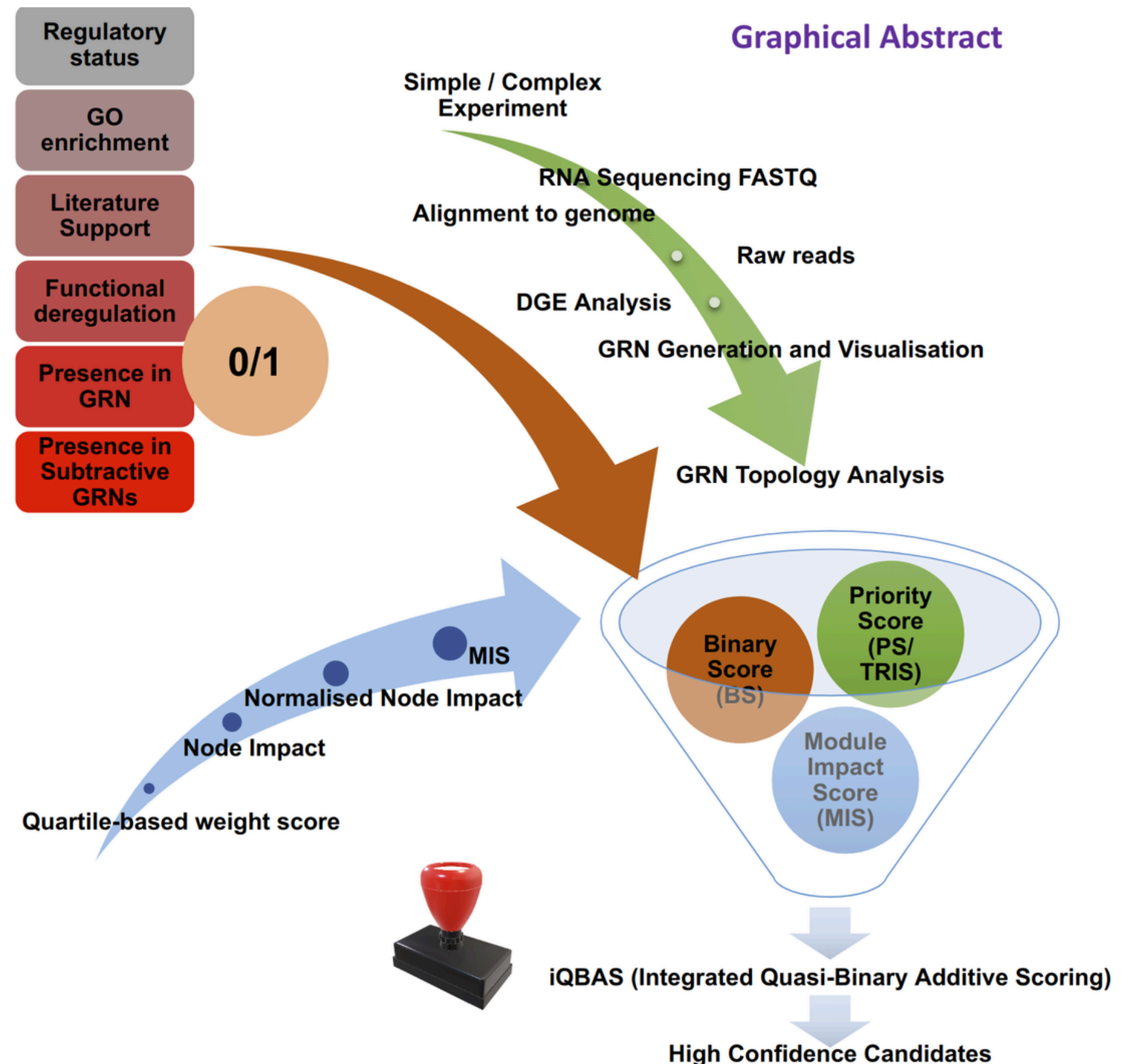
For gene i ,
In comparative temporal networks 1, 2, ..., k



$$TPS_i = \frac{1}{K} \sum_{k=1}^K PS_i^{(k)}$$

So we focus on the **dynamics of topological changes** rather than the topology of a single network

Definition of a scoring system to identify Genes of Interest



So we focus on the
**dynamics of topological
changes** rather than the
topology of a single
network

Definition of a scoring
system to identify
Genes of Interest

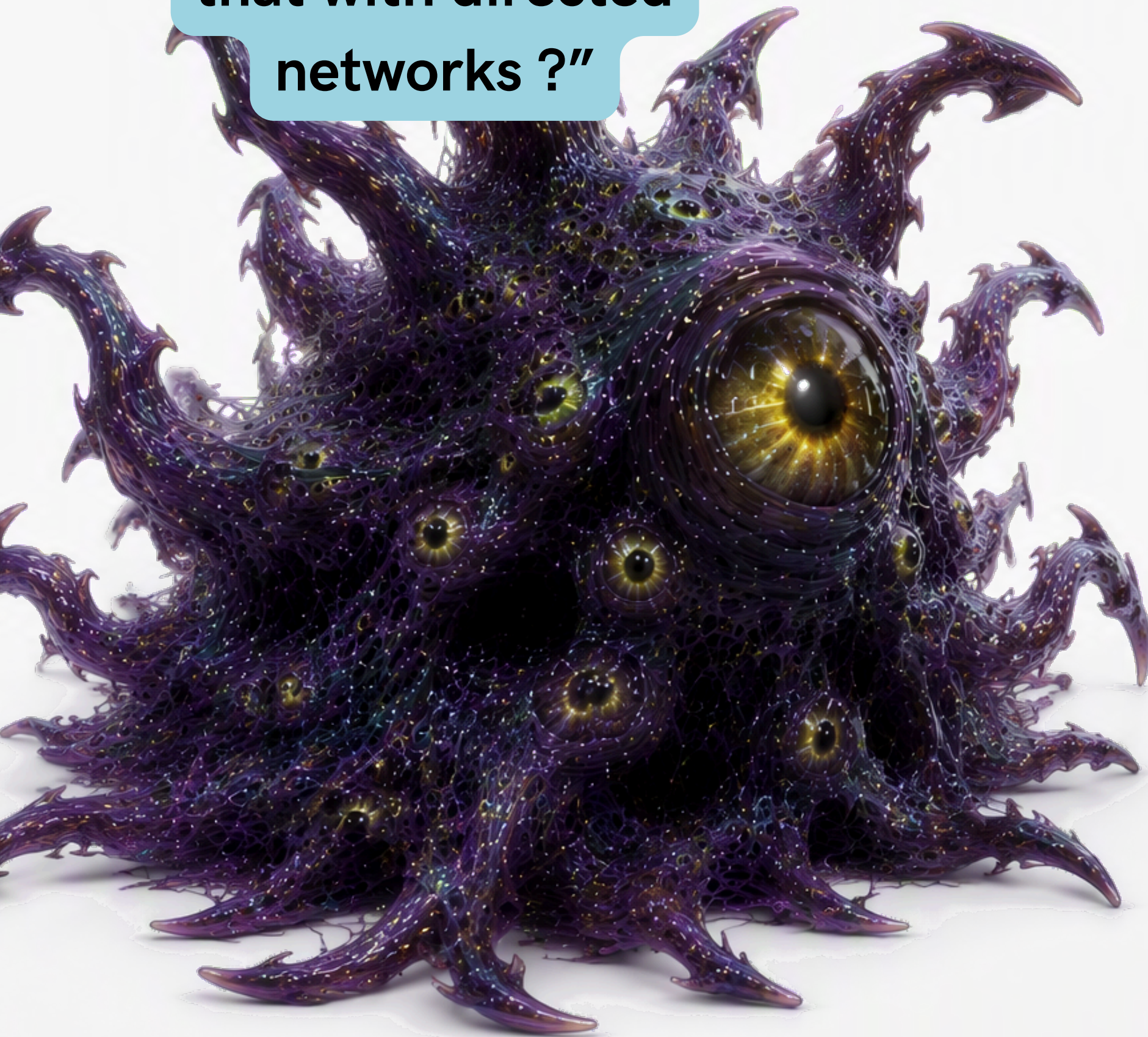
And get that
sweet smooth
Shoggoth juice.



So now you know how to
get some value out of
undirected networks too
with comparative
topological scoring.

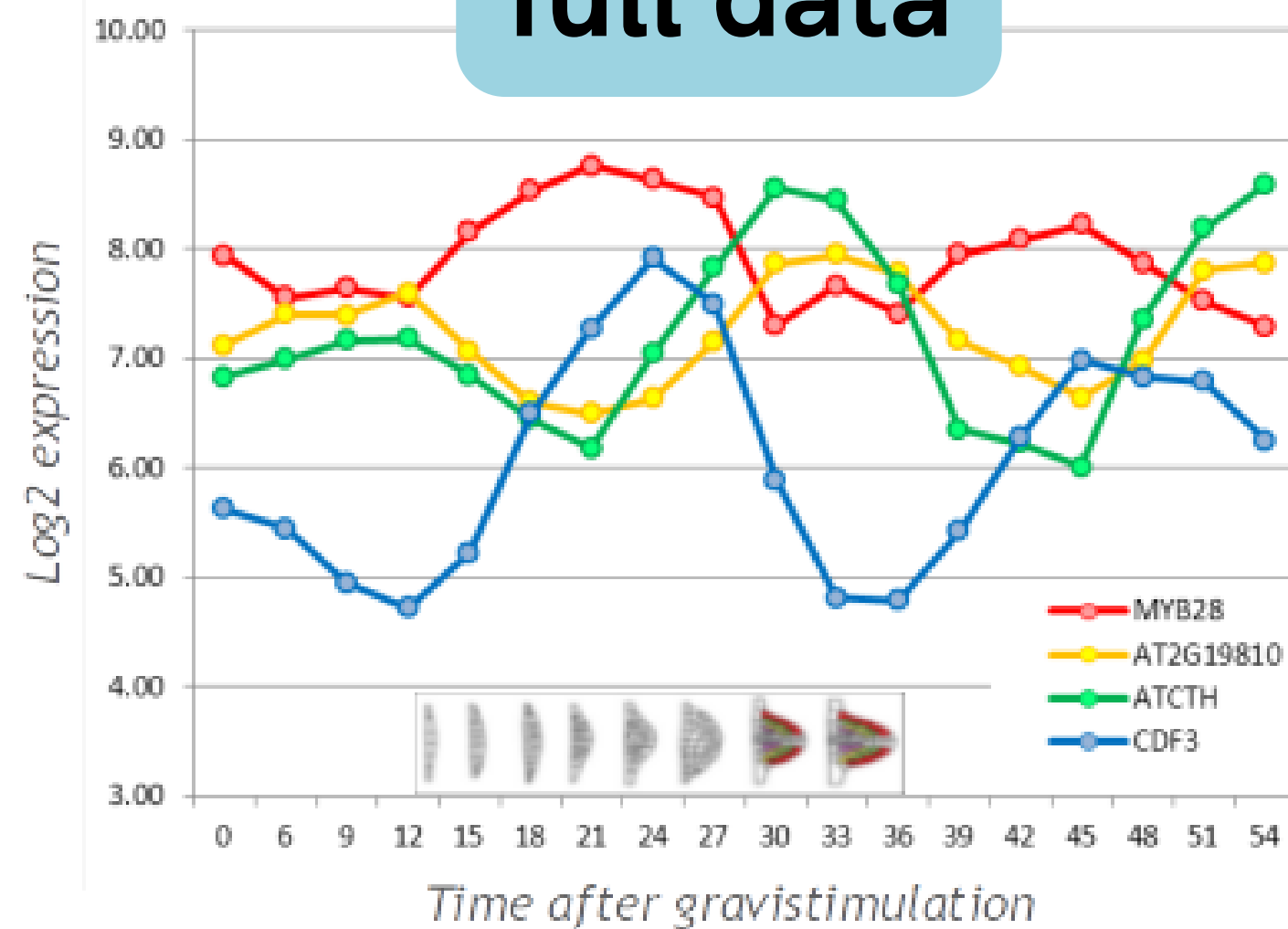


“But couldn’t we also use
that with directed
networks ?”

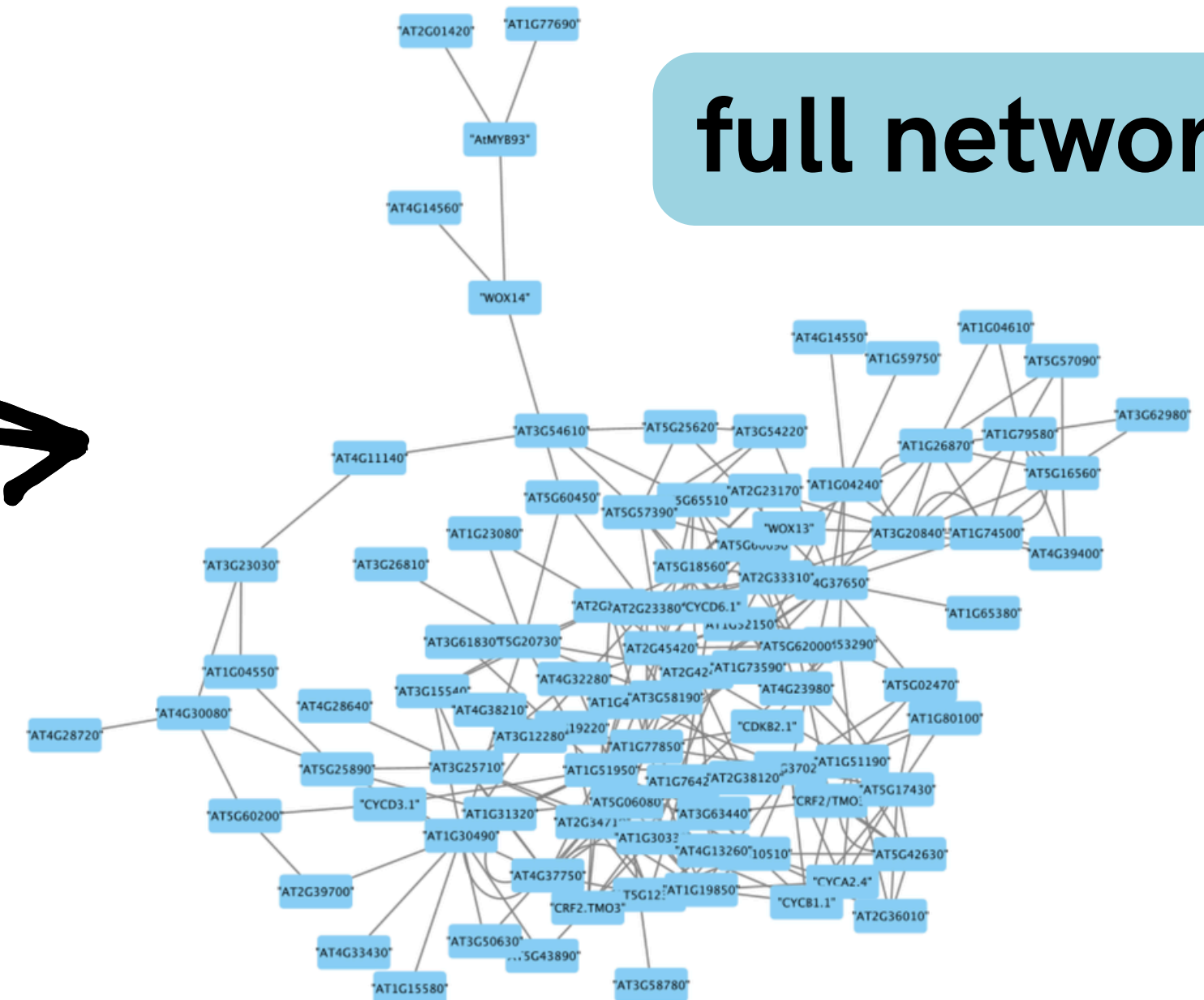


Well, we can do things
even funnier, like this :

full data

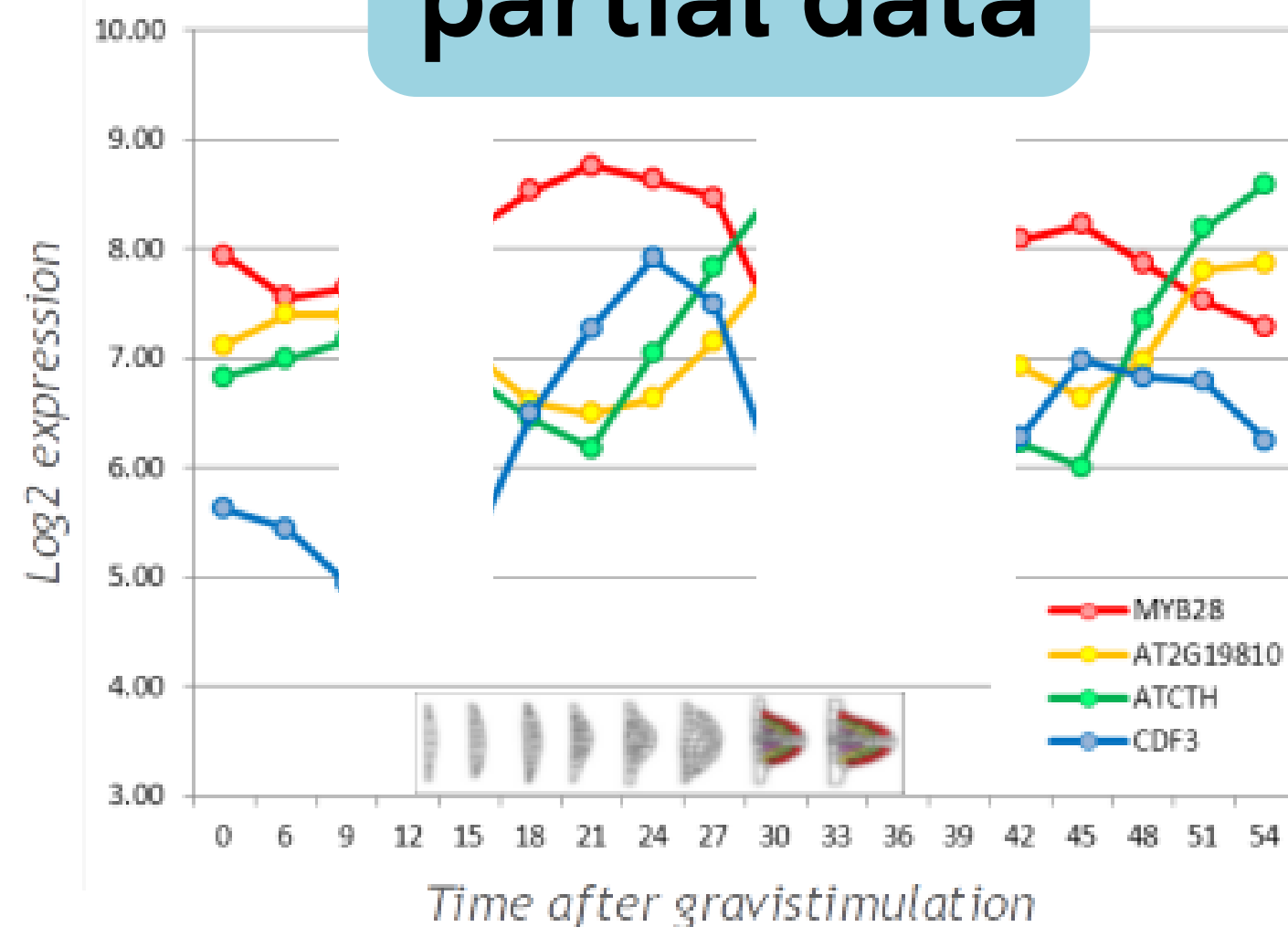


full network

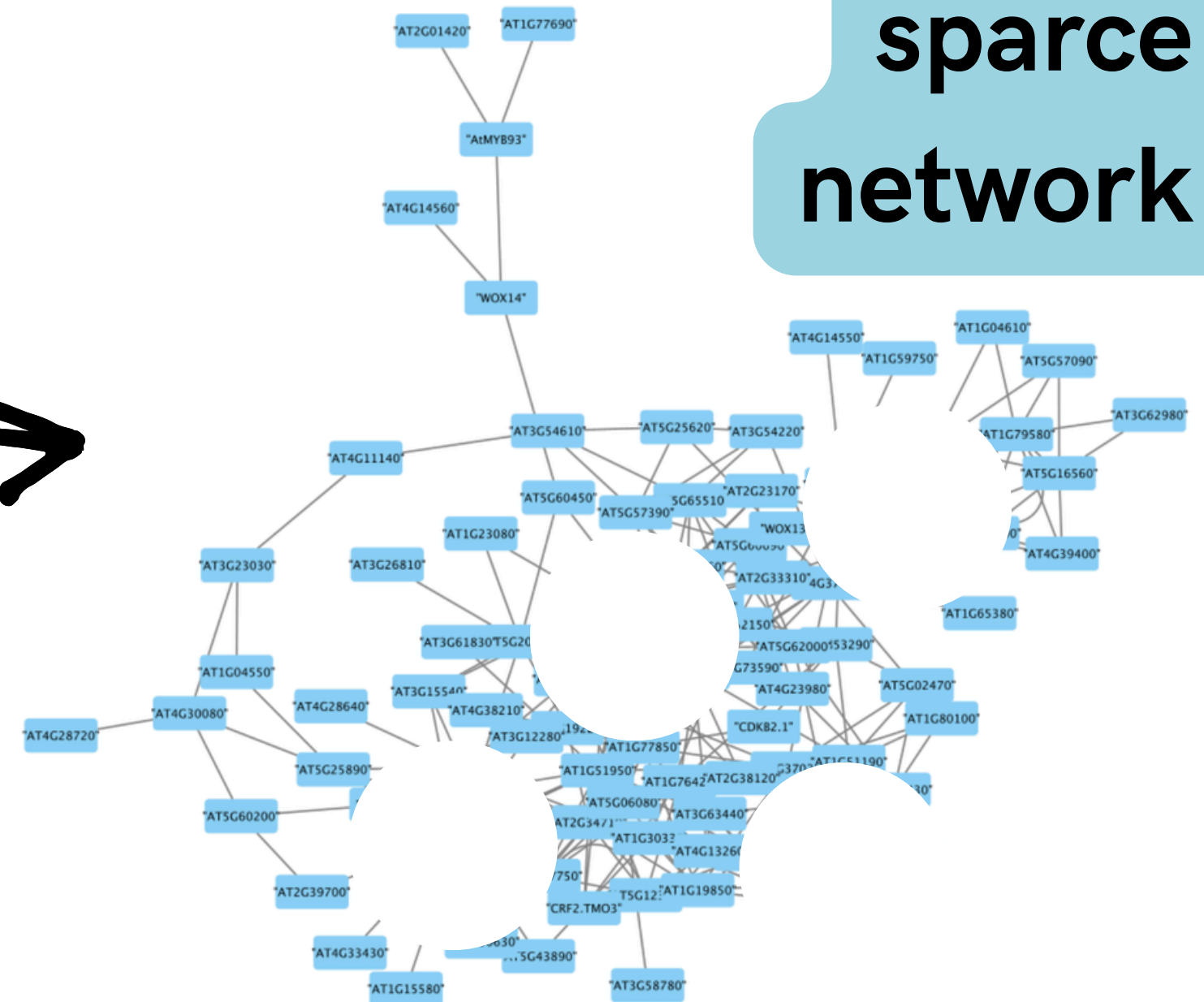


Well, we can do things
even funnier, like this :

partial data

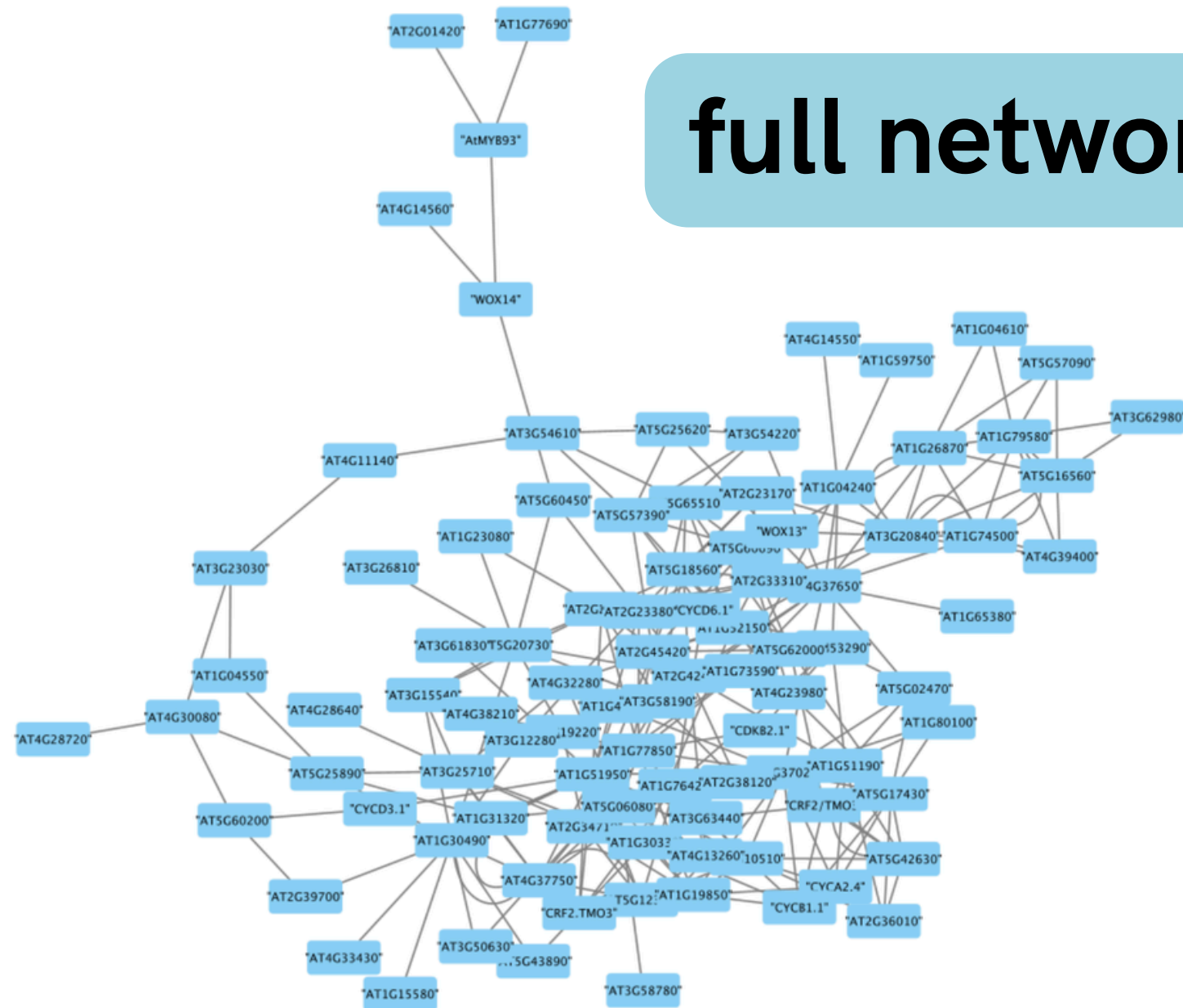


sparse
network ?

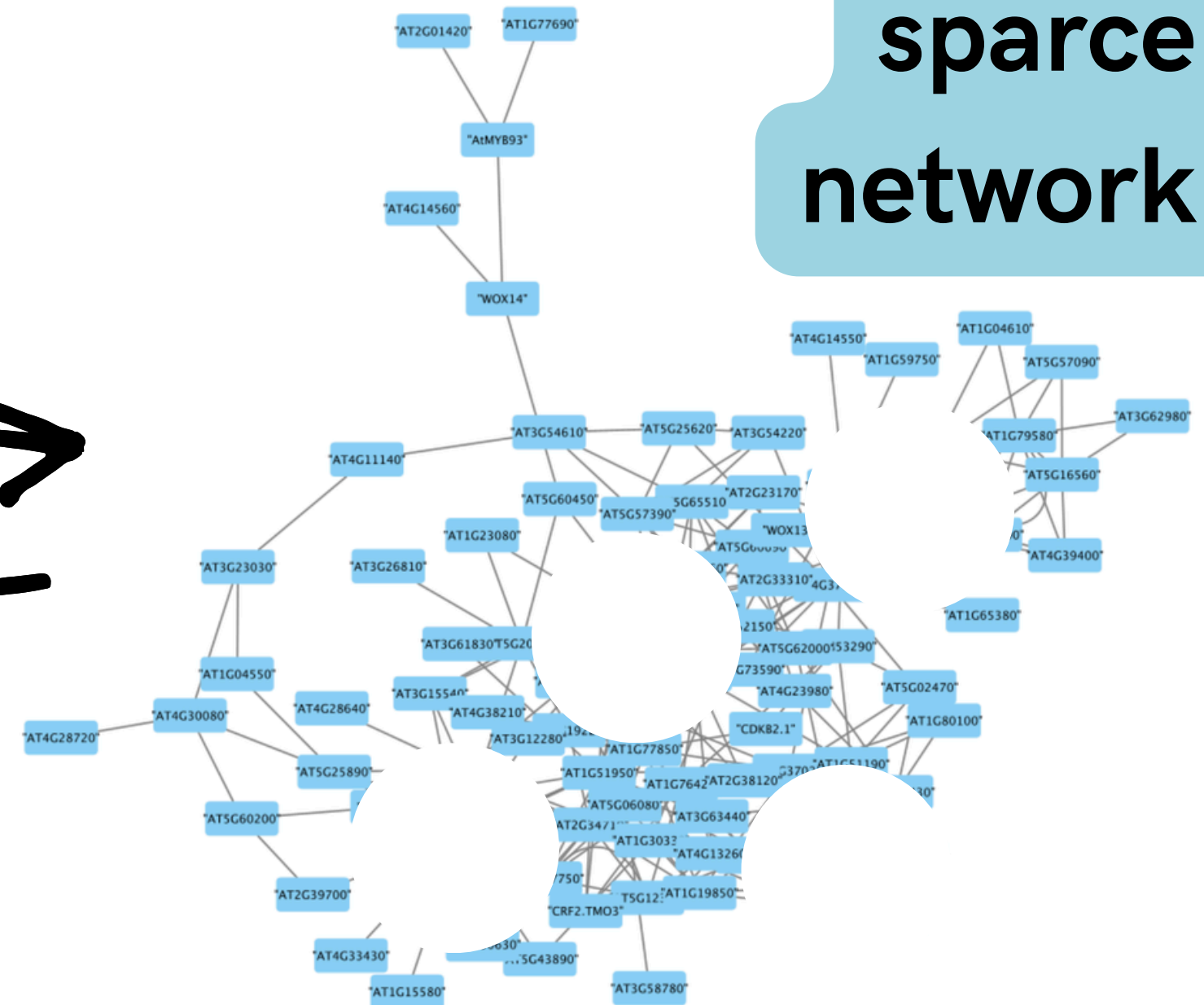


**Well, we can do things
even funnier, like this :**

full network



**sparse
network ?**



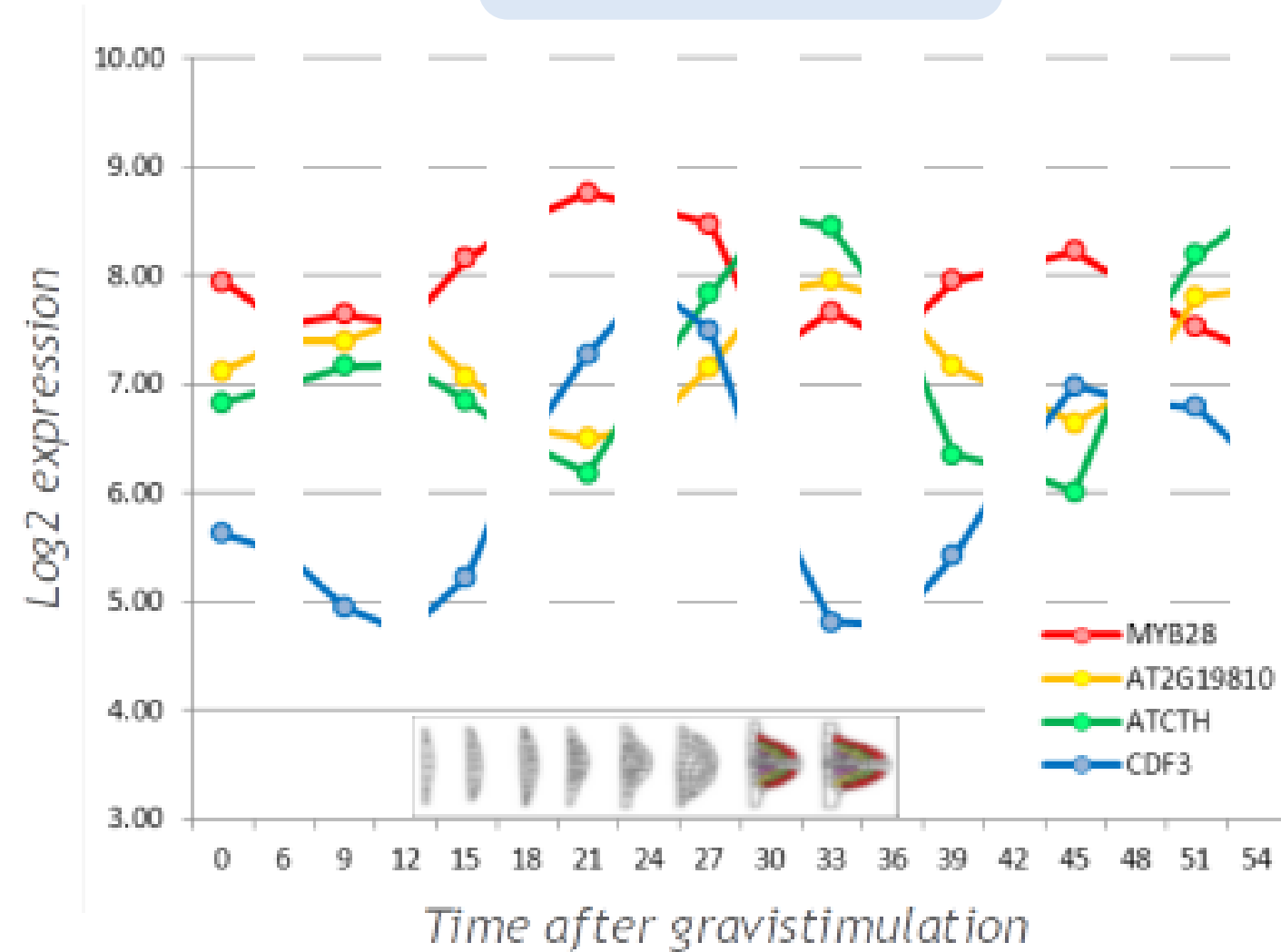
**Well, we can do things
even funnier, like this :**

**Test the impact of data
degradation on network
reconstruction**

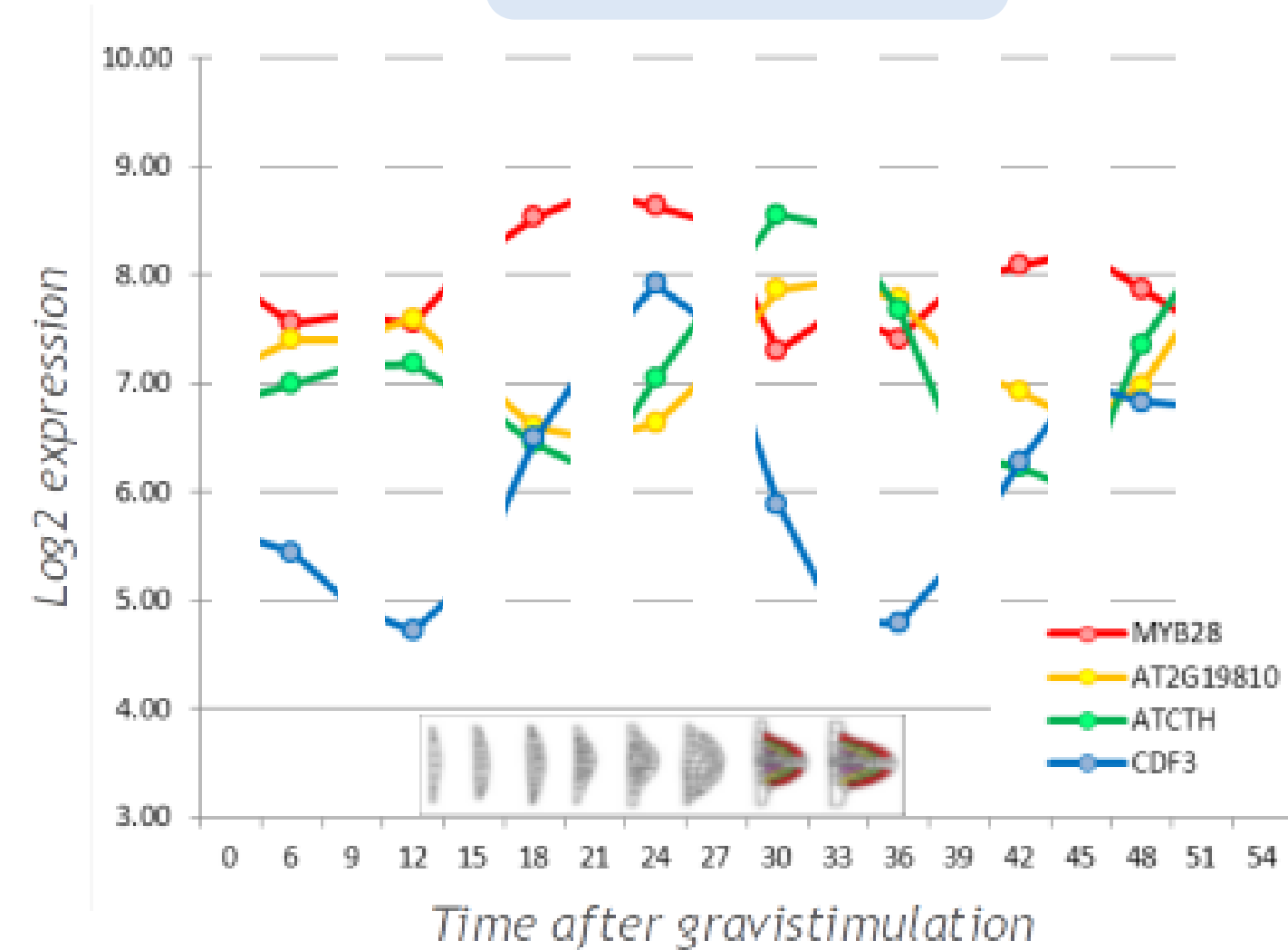


The data degradation we tested :

Keep only half

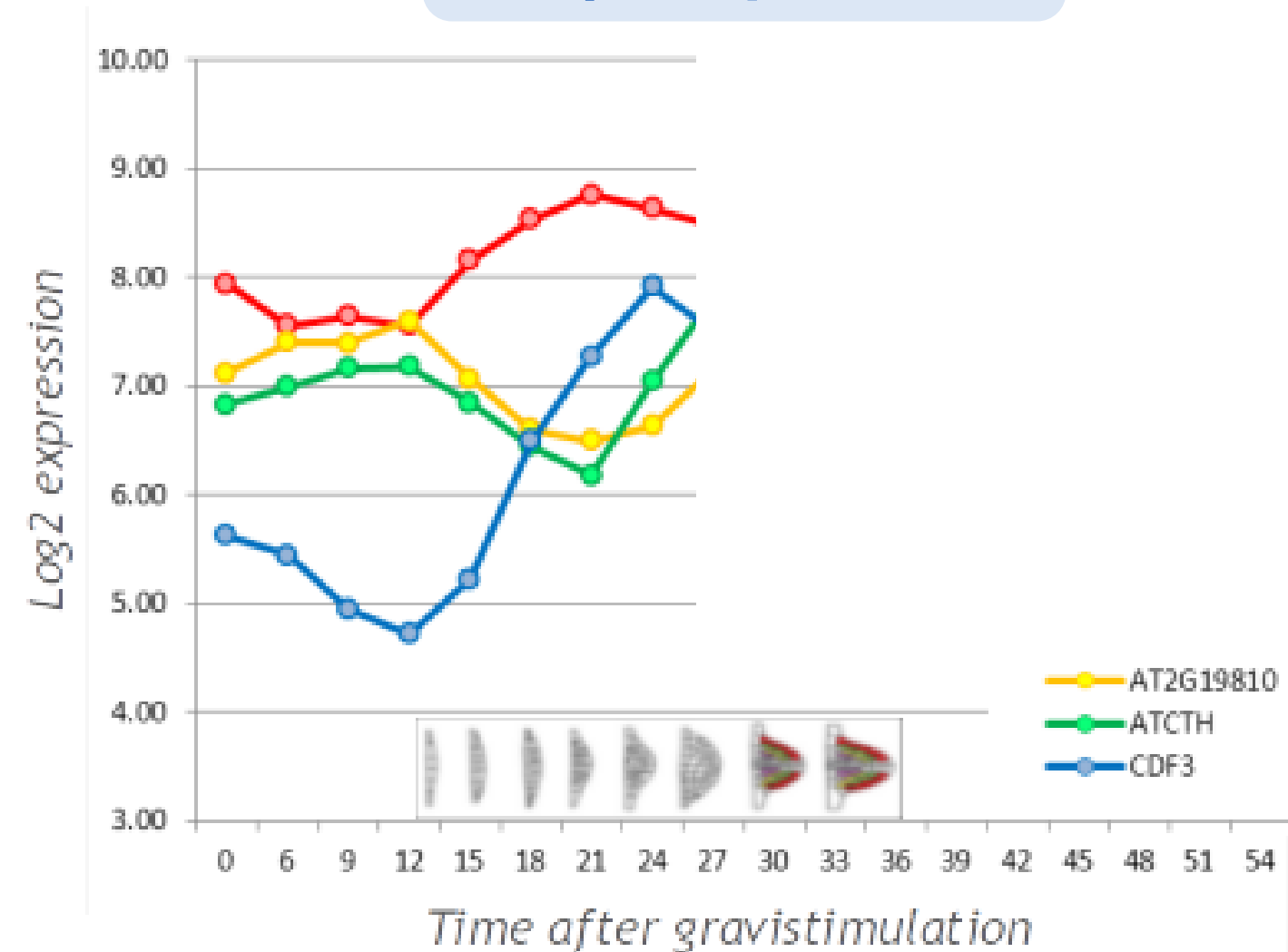


Keep only half

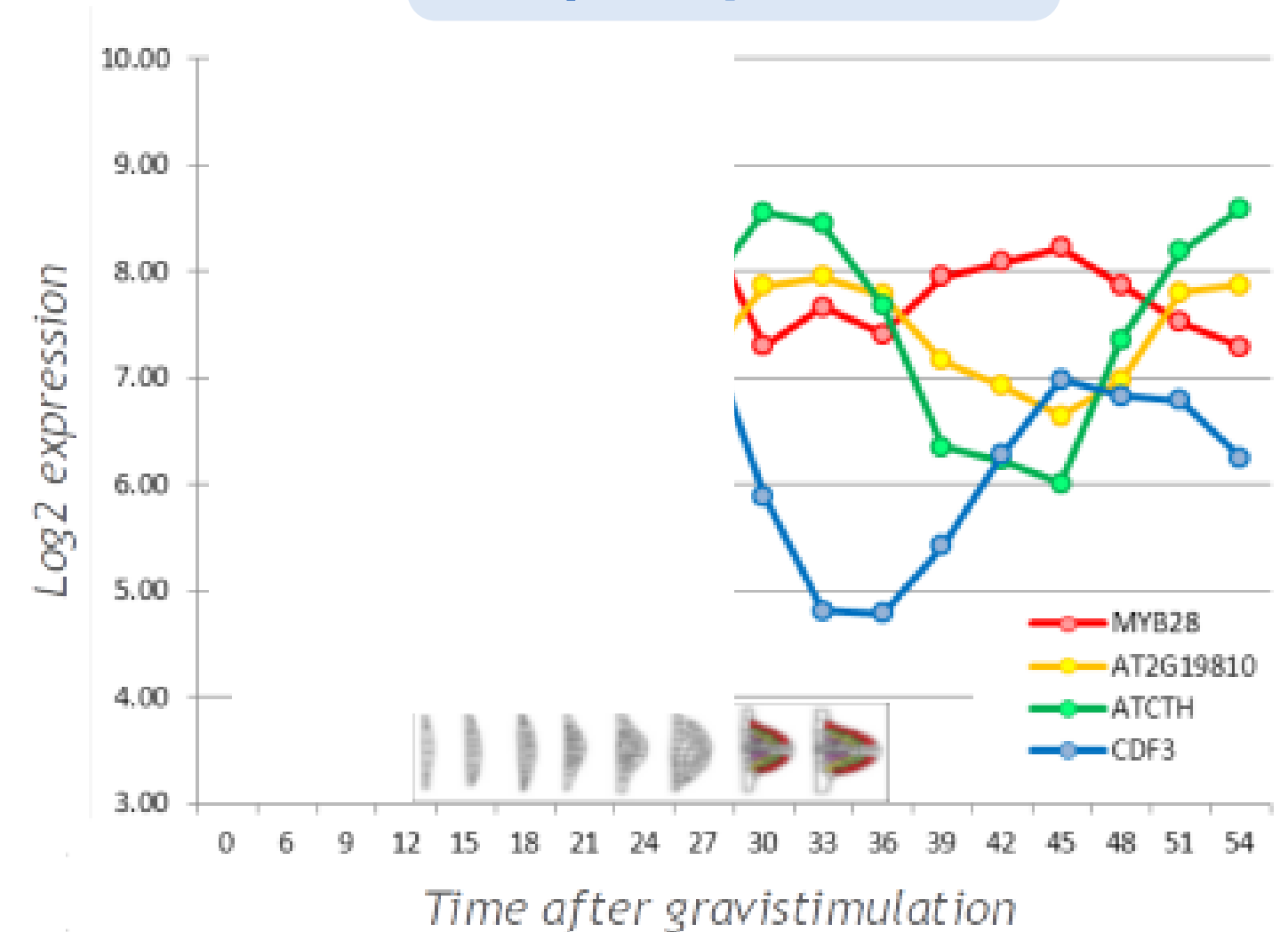


The data degradation we tested :

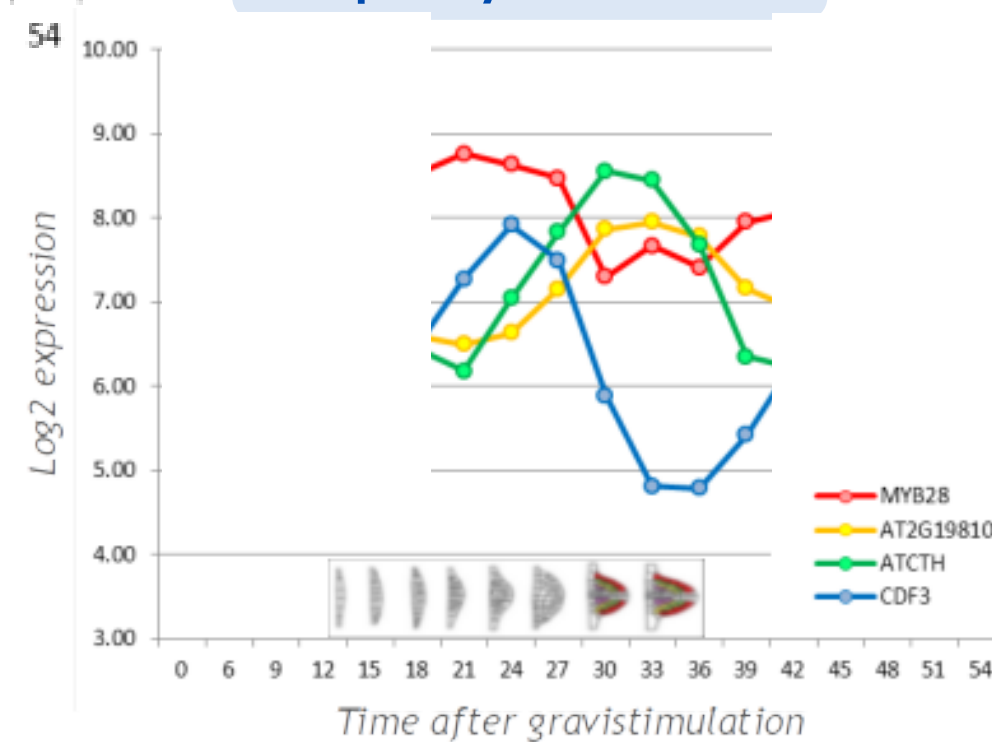
Keep only 1st half



Keep only 2nd half

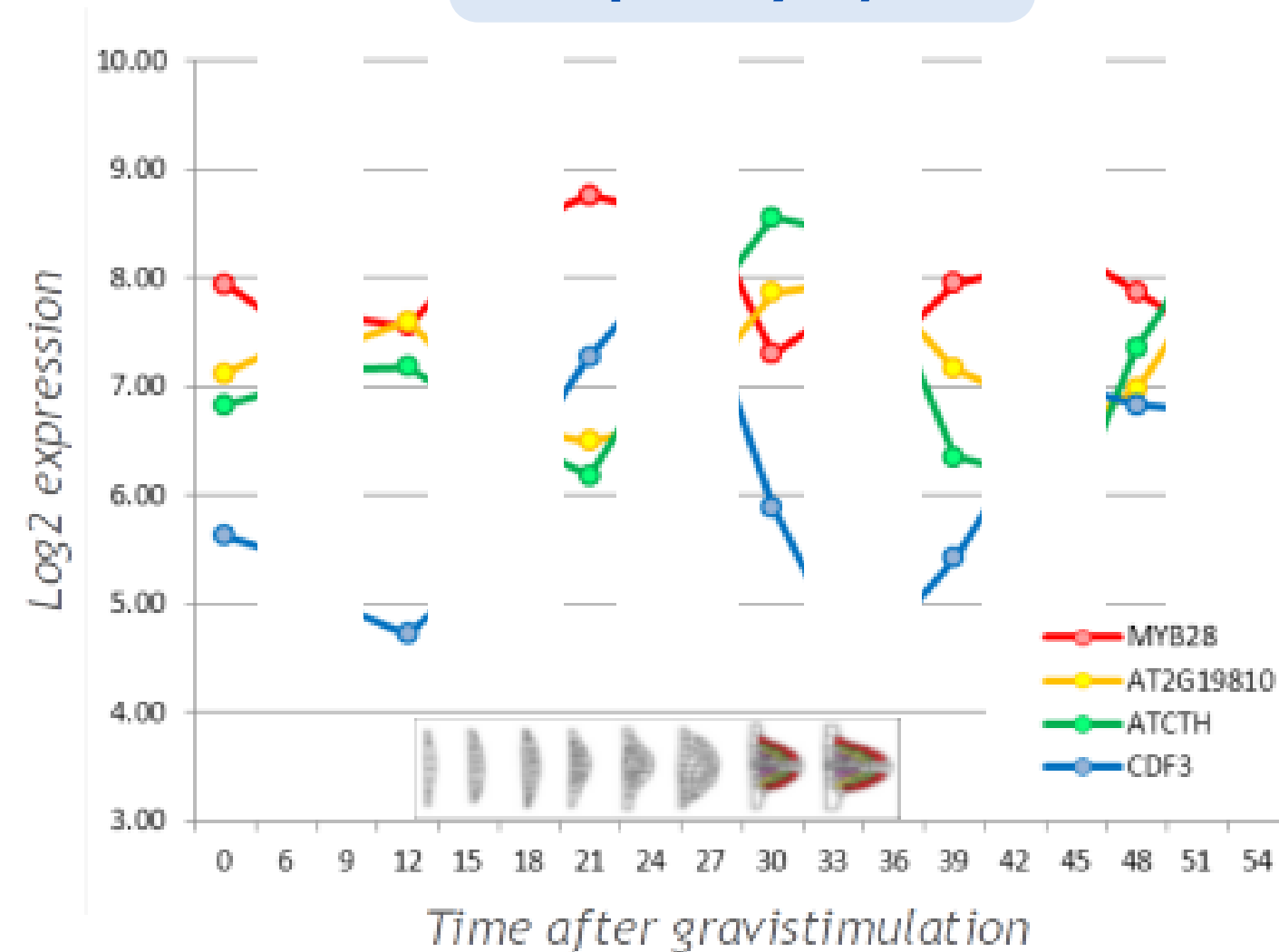


Keep only central half

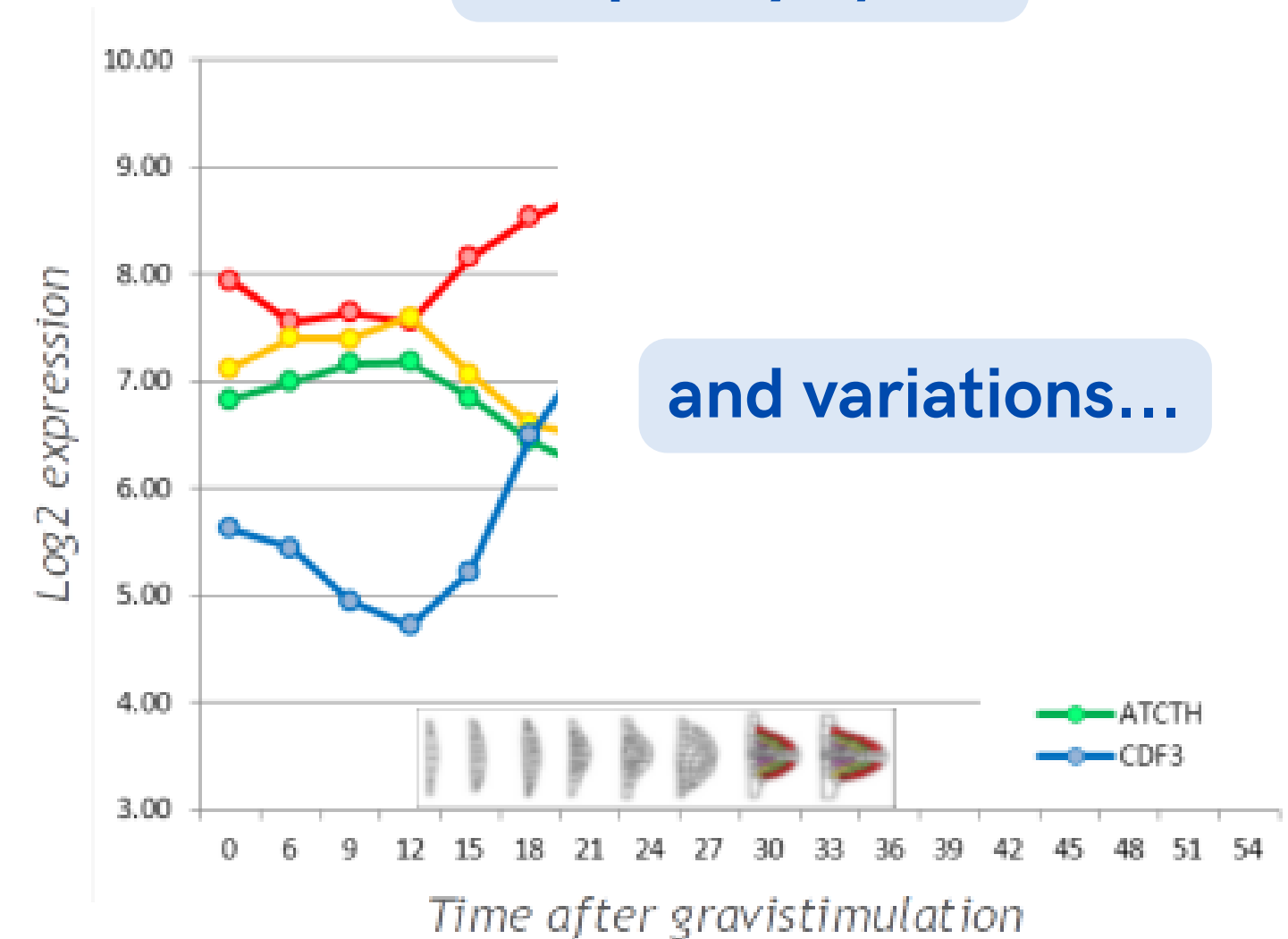


The data degradation we tested :

Keep only 1/3...



Keep only 1/3...



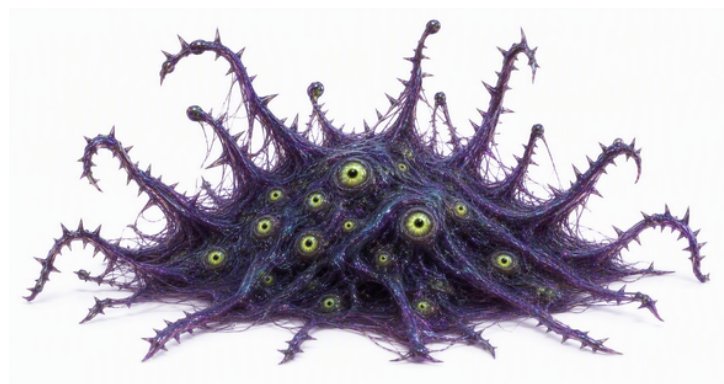
and variations...

The data degradation we tested :

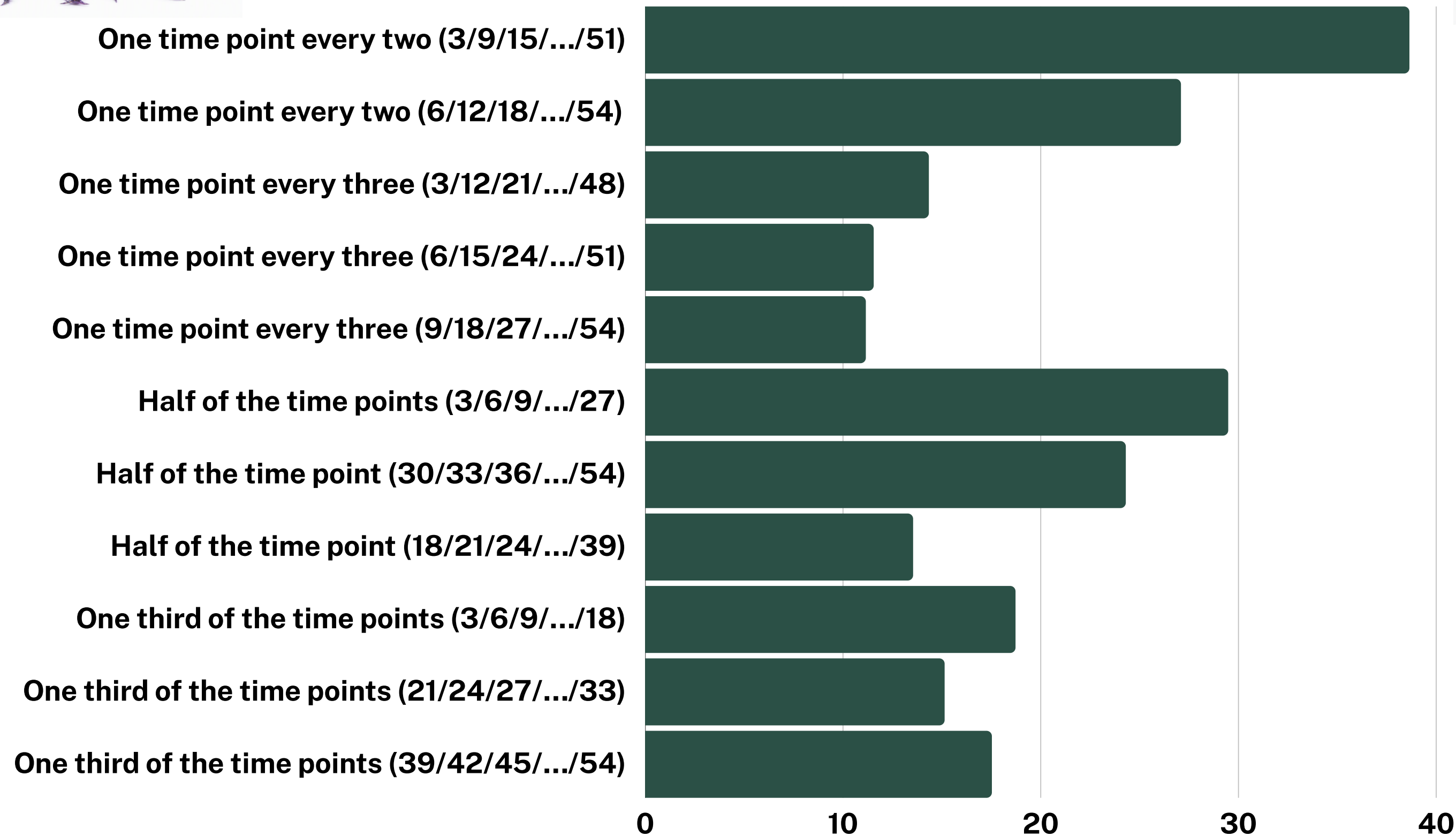
Type of deletion	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54
One time point every two	X		X		X		X		X		X		X		X		X	
		X		X		X		X		X		X		X		X		X
One time point every three	X			X			X			X			X			X		
		X			X			X			X			X			X	
			X			X			X			X			X			X
Half of the time point	X	X	X	X	X	X	X	X	X									
						X	X	X	X	X	X	X	X					
										X	X	X	X	X	X	X	X	X
One third of the time point	X	X	X	X	X	X												
						X	X	X	X	X	X							
												X	X	X	X	X	X	X

Data deletion schemes

Infer the network, run the comparison metrics...



The results:



Results of Metric No. 2 (Percentage of Identity at Degree 1)

Future things : test imputation of missing data

To what extent can we
repair the poor sparse
Shoggoth ?



Thanks to everyone involved...



CERES & F2F



CPIB



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Charlotte André

Johanna Saoud

Lilou Zulewski

Vedika Joshi



& Thanks for your attention