NETWORK METHODS FOR IDENTIFYING REGULATORS OF INFLUENZA A VIRUS INFECTION

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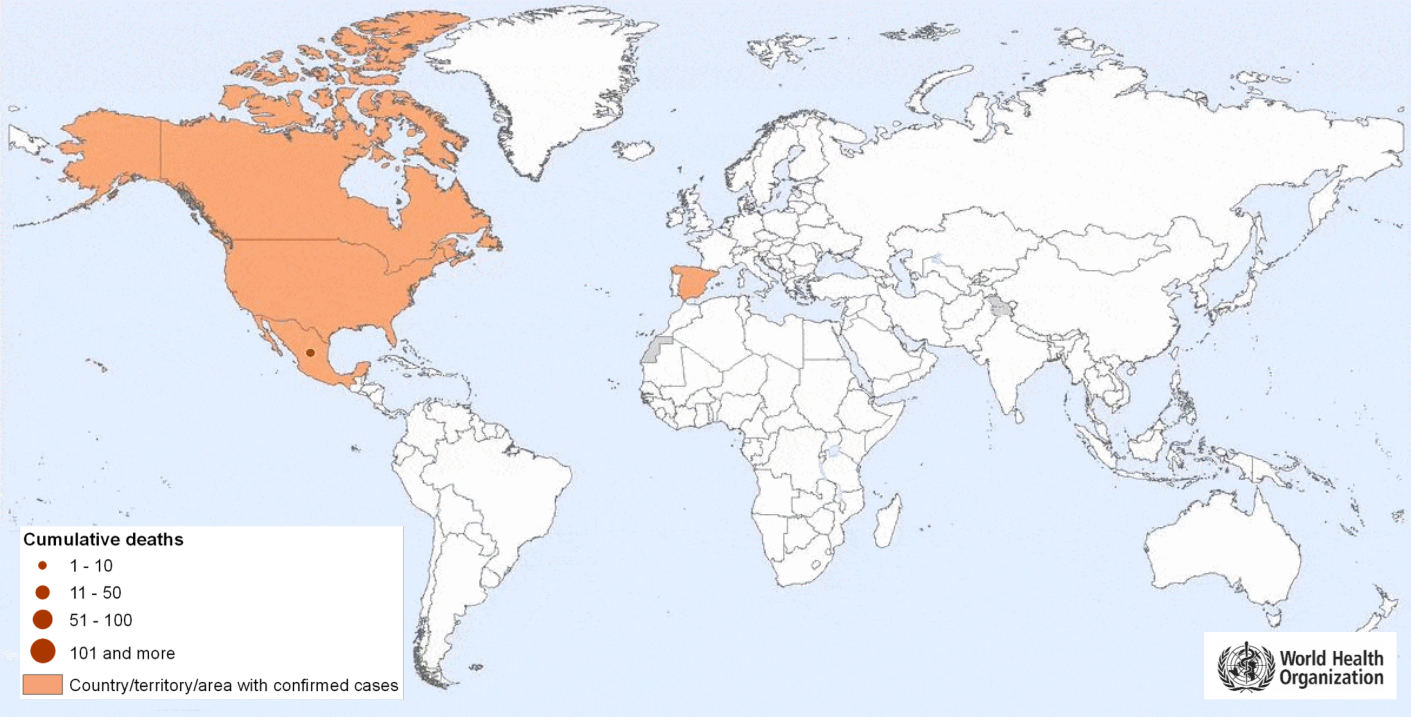
DRUG TARGET DEVELOPMENT IS NEEDED TO ADDRESS GLOBAL INFLUENZA INFECTION



Only **three** FDA-approved antiviral treatments available

- One is not recommended for children and people with breathing problems

Question: *Can existing protein-protein interaction data be used to* ***predict drug target candidates in a novel way?***

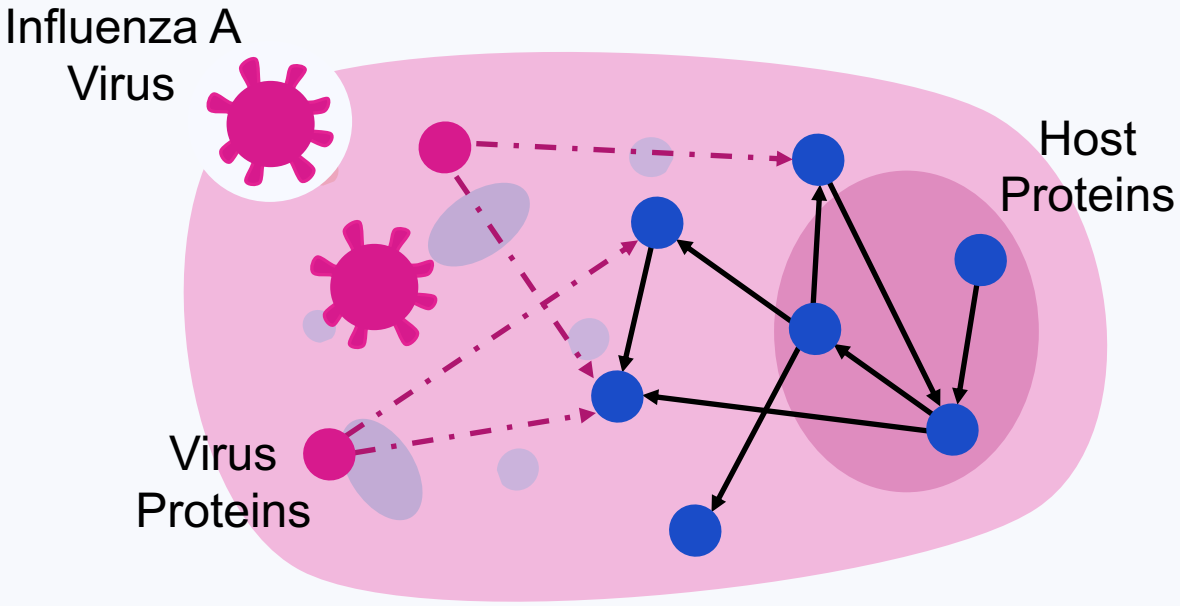
TWO NETWORK APPROACHES TO DRUG TARGET DISCOVERY

1

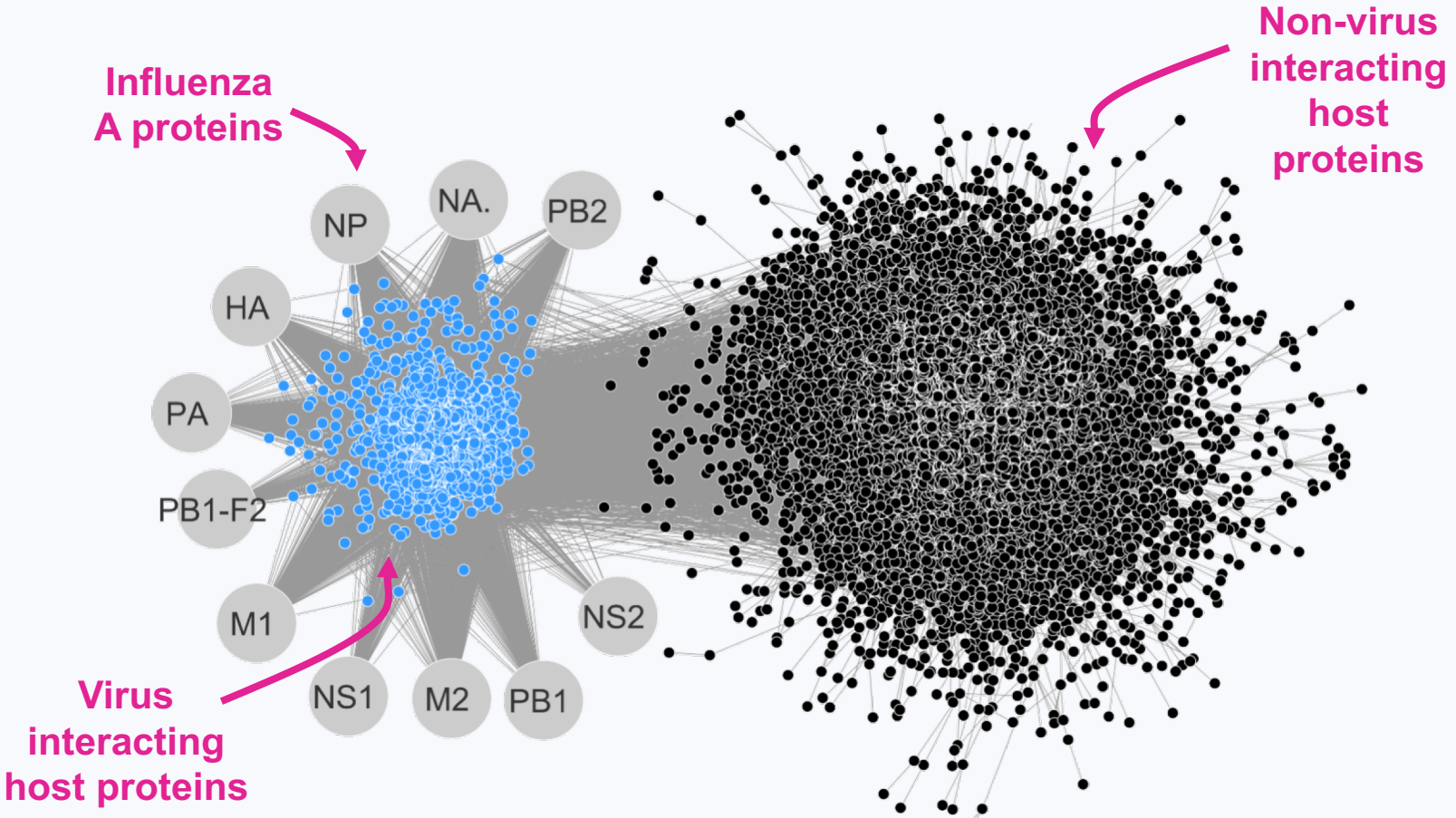
Disease
subnetwork

2

Cellular
controllability



PPI NETWORKS: A CRASH COURSE



Degree:

Number of interactions a protein is involved in

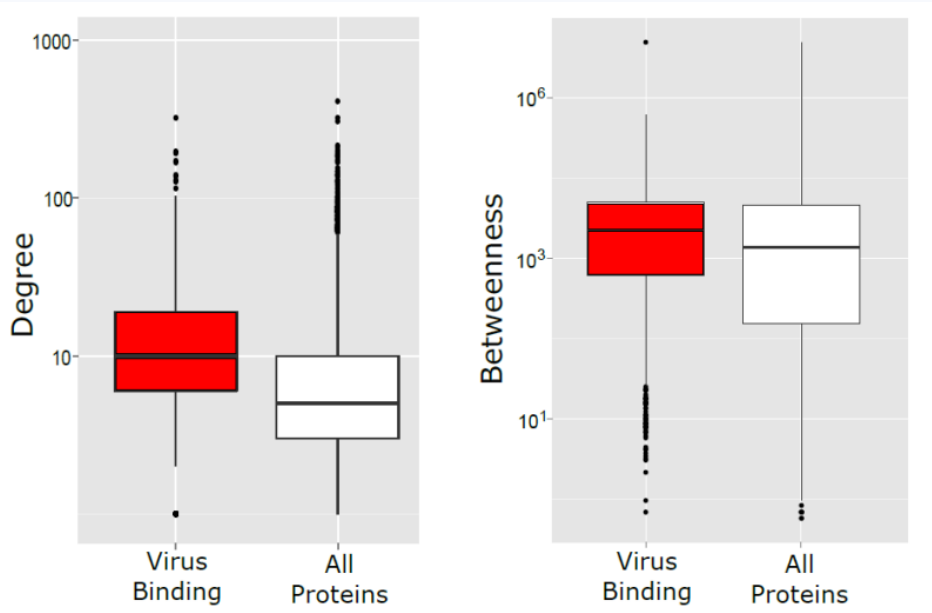
Betweenness:

Measure of network flow "bottleneckness"

PREVIOUS WORK USES NETWORK TOPOLOGY TO IDENTIFY DISEASE RELEVANT PROTEINS

Influenza proteins prefer to interact with proteins in significant network positions

Degree and betweenness p-values: $<10^{-16}$

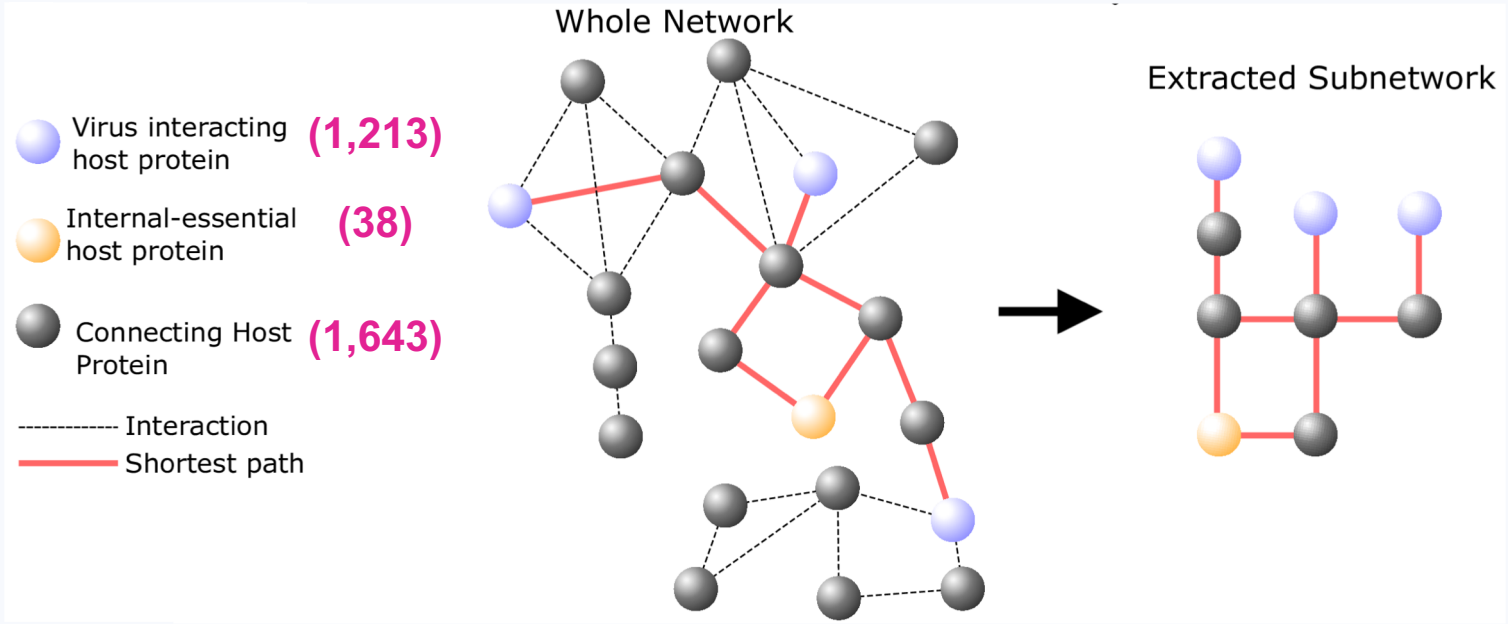


Problem:

Topology is not sufficient *as a guide for drug target discovery*

Little analysis of ***downstream proteins***

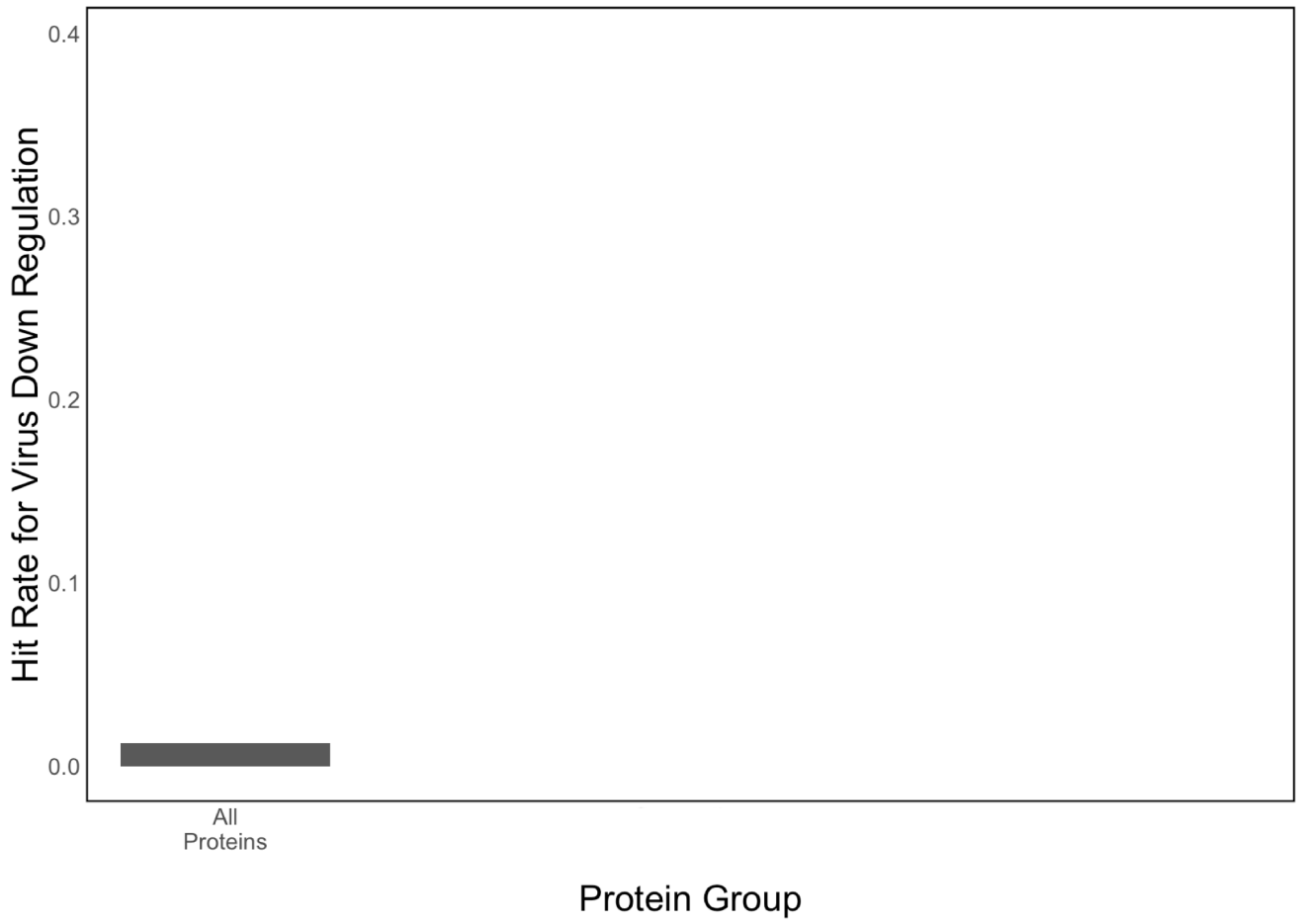
VIRUS-SPECIFIC SUBNETWORK METHOD FOR TARGET IDENTIFICATION



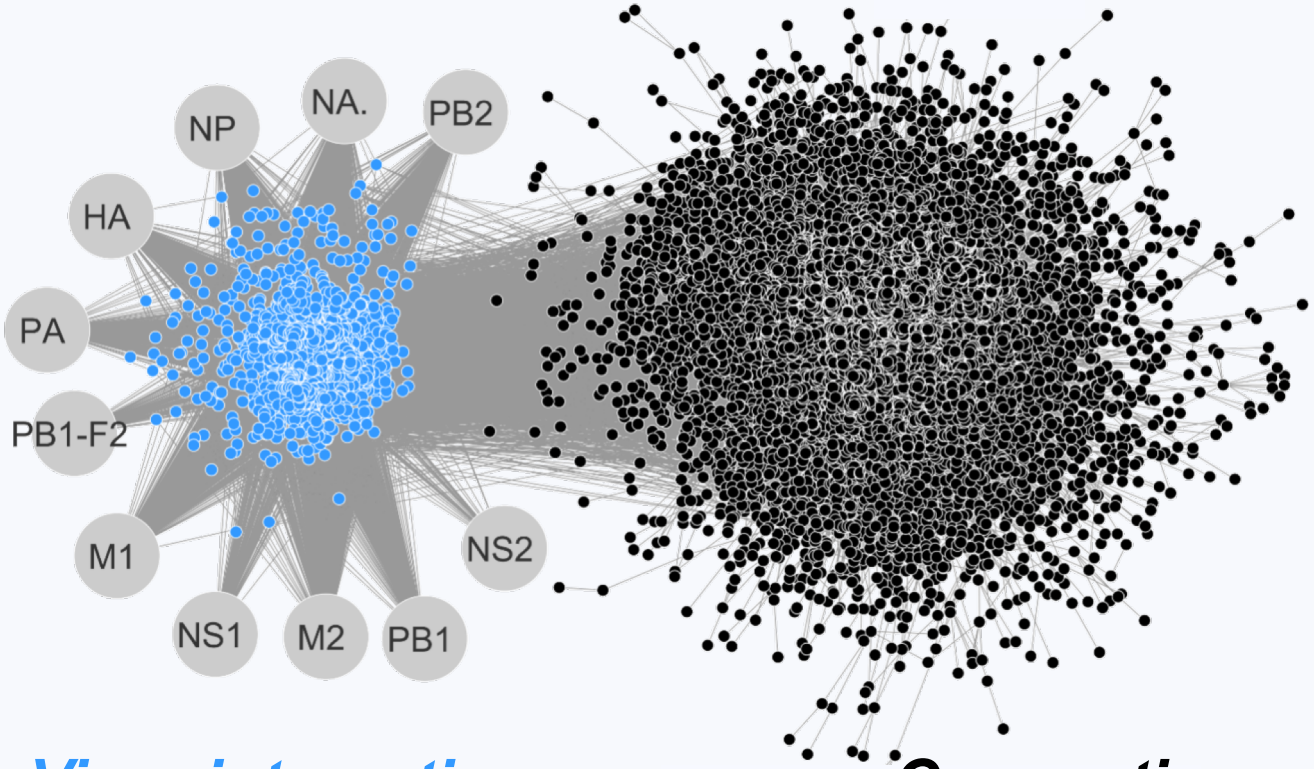
Connecting protein: Proteins between virus interacting proteins and proteins identified as relevant to virus replication in an siRNA screen

*Analyze subnetwork proteins for **potential as antiviral drug targets***

SUBNETWORK POSITION ACTS AS PREDICTOR OF ANTIVIRAL DRUG TARGET CANDIDACY



SUBNETWORK PROTEINS ARE FUNCTIONALLY DISTINCT FROM VIRUS-INTERACTING PROTEINS



Virus interacting:

- Virus replication
- RNA transcription
- Protein translation

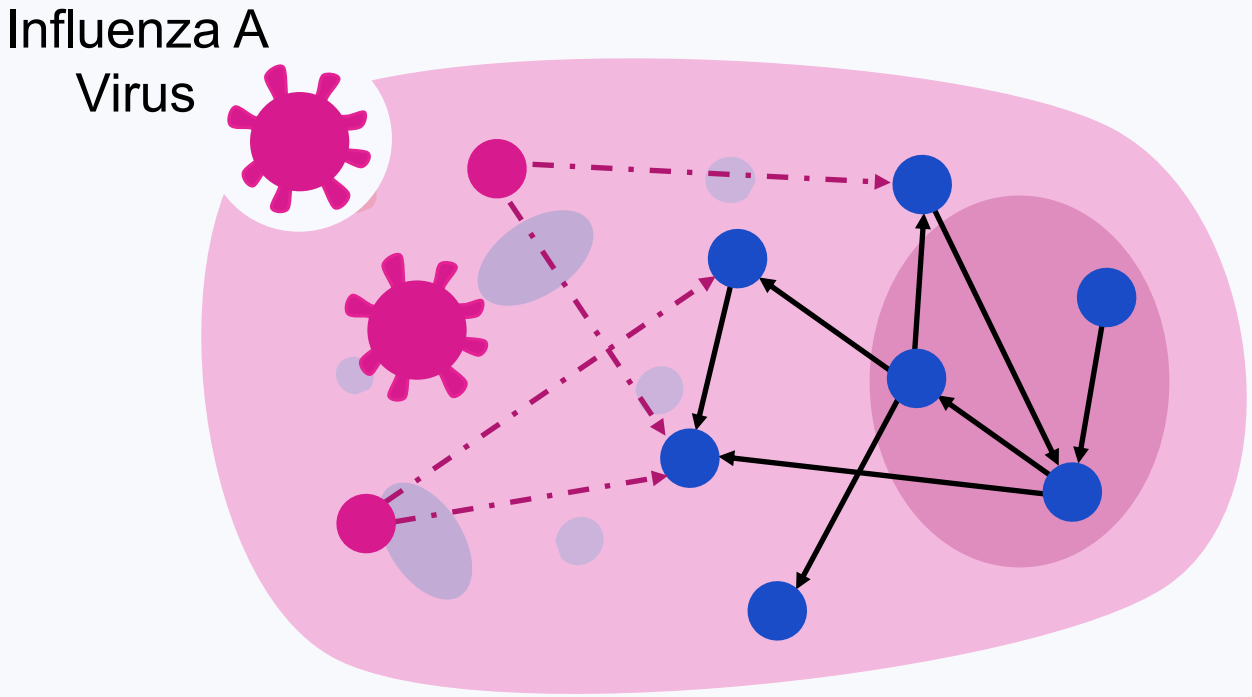
Connecting:

- Immune response
- NFkB pathway

SUMMARY: SUBNETWORK

- Integrating virus-host interactions, siRNA data, and network topology methods can improve antiviral drug target discovery
- The novel subnetwork method:
  - Isolates disease specific pathways that allow for the promotion of viral replication
  - Detects proteins that are traditionally unidentified by network methods

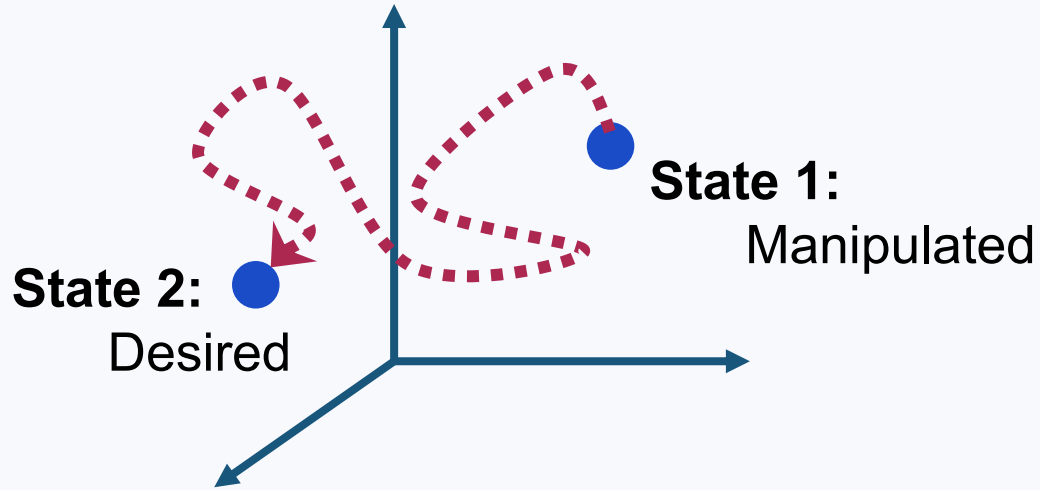
VIRUSES CONTROL CELLULAR NETWORKS TO PROMOTE REPLICATION



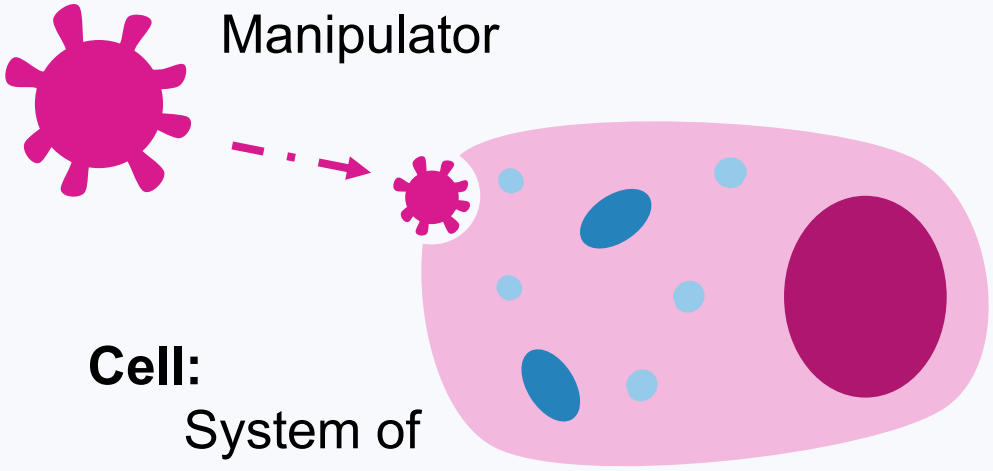
Question: *How does the virus* ***manipulate*** *the cell to influence specific biological pathways?*

ENGINEERING APPROACHES TO UNDERSTANDING CELLULAR CONTROL

To **control a system**, individual states must be driven to desired values



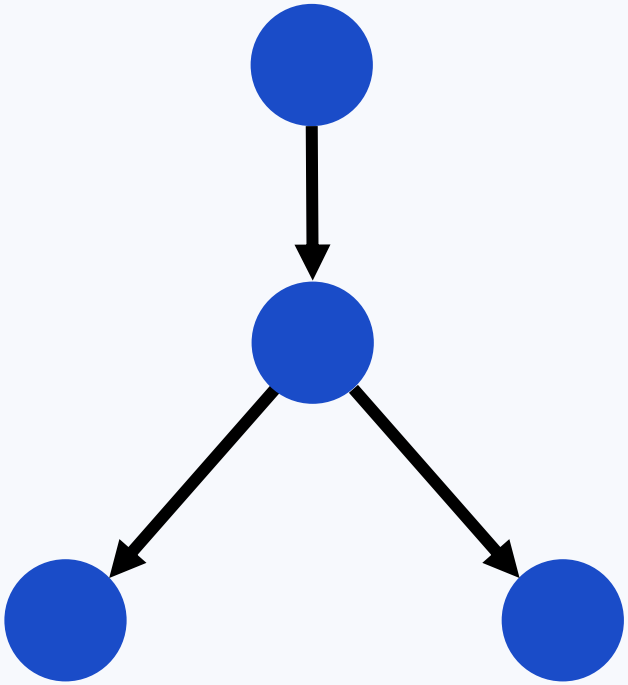
Virus:
Manipulator



Cell:
System of protein states

Viral infection can be modeled as a *controllability problem*

STEP 1: IDENTIFY MINIMUM CONTROL SET FOR CELLULAR CONTROL



● **Minimum control set: 2**

After infection:

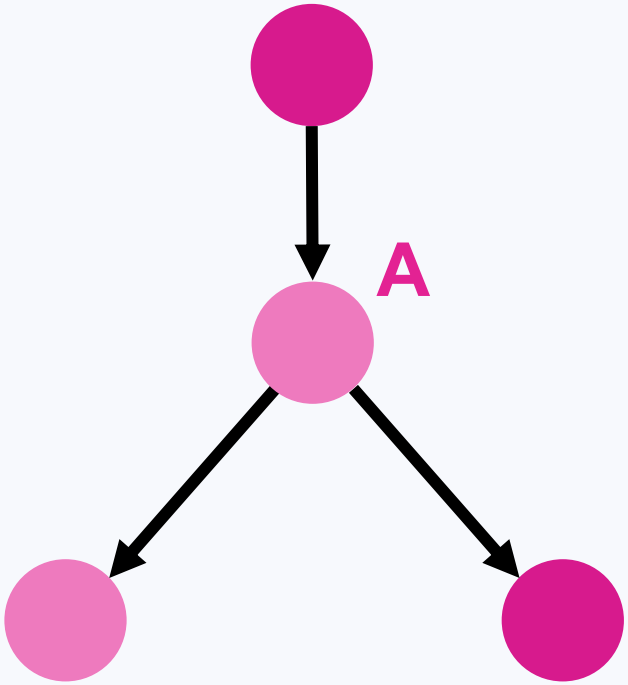
Same proteins with 11 exceptions (Viral proteins)

8.9% of minimum control set also interact with viral proteins

Significant betweenness compared to non-virus interacting minimum control proteins (p-value: 2.2×10^{-16})

Infection does not alter **magnitude** of cellular control

STEP 2: OBSERVE CHANGES TO CONTROL USING DEPLETION ANALYSIS



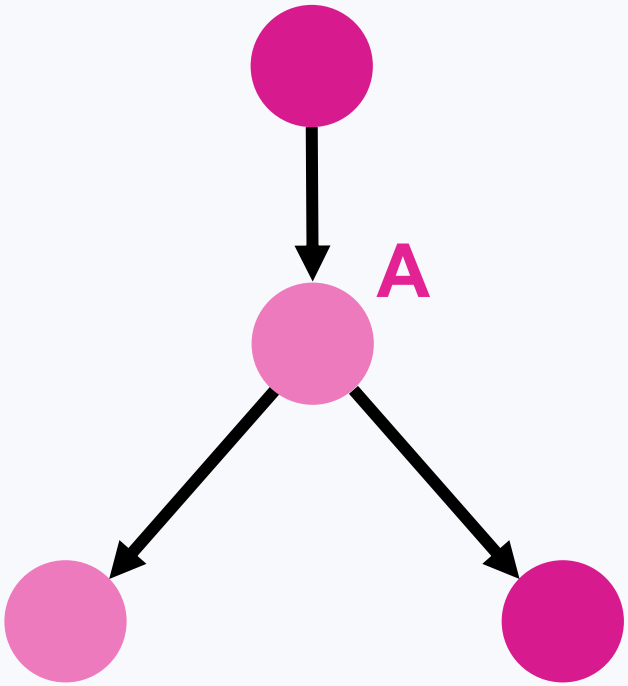
Remove each protein to detect differences in minimum control set

Remove protein **A**:

- **Minimum control set: 3**

Depletion analysis measures ***alterations in the ability to control the network***

STEP 2: OBSERVE CHANGES TO CONTROL USING DEPLETION ANALYSIS

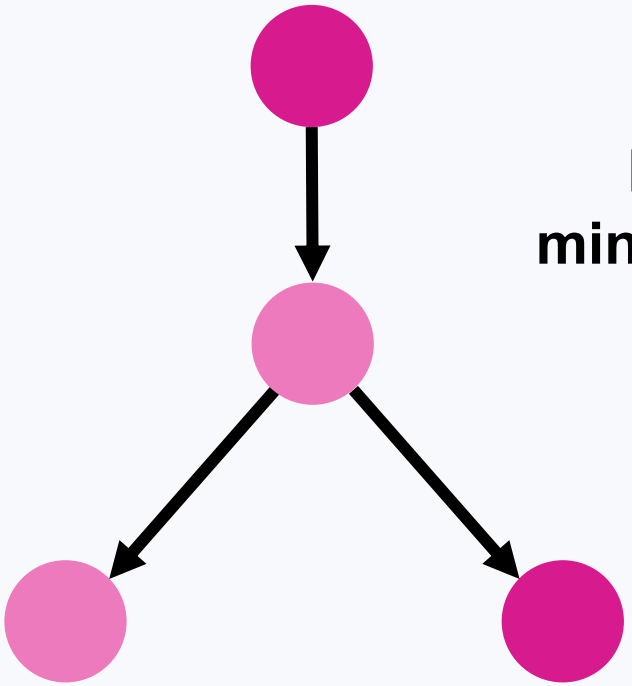


The absence of single proteins does **not alter** the control structure of the infected system

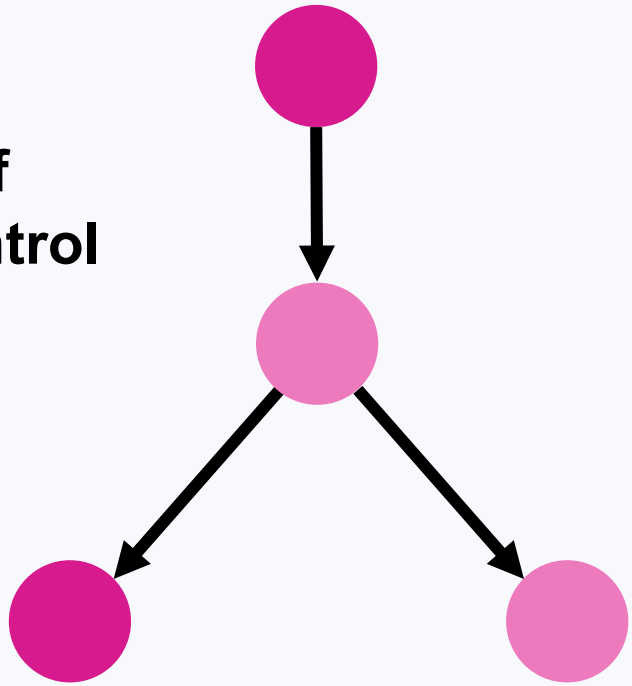
Only changes seen are a result of the **11** changing minimum control proteins

Fails to detect known changes in immune response and transcriptional processes

STEP 3: IDENTIFY KEY PROTEINS USING GLOBAL ANALYSIS

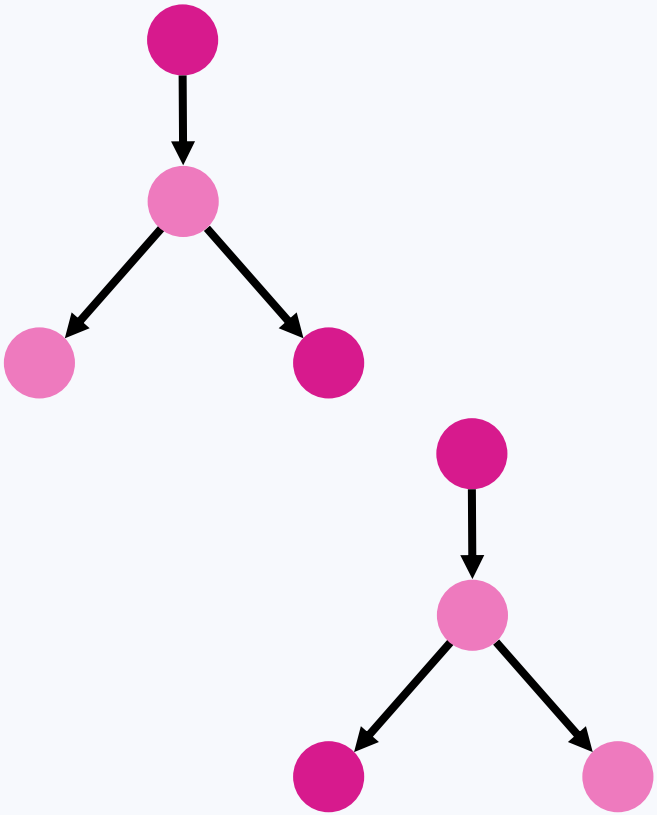


Number of minimum control sets: 2



Global analysis measures ***a protein's significance to all ways a network can be controlled***

STEP 3: IDENTIFY KEY PROTEINS USING GLOBAL ANALYSIS



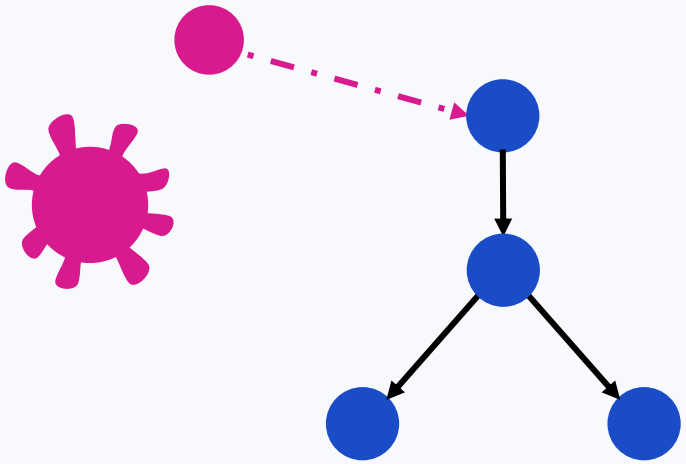
24 host proteins display a change in significance post-infection

All identified proteins are **both minimum control and virus interacting proteins**

(2% of all proteins)

Global analysis identifies **infection specific changes** to network behavior

VIRUS INTERACTING PROTEINS: GATEWAY TO CELL MANIPULATION



Network is more **difficult** to control in their absence

Often **globally significant** and involved in many ways to control the network

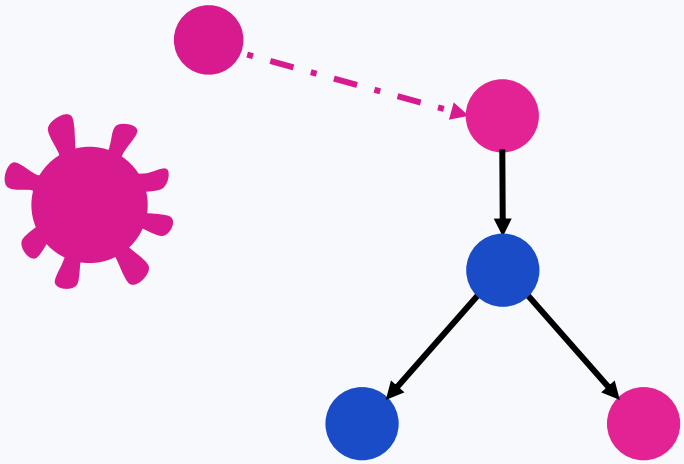
*Responsible for the **ease** and **propagation of control** through the system*

MINIMUM CONTROL SET: FUTURE OF DRUG DESIGN?

Network is easier to control in their absence

Weak point in host defense

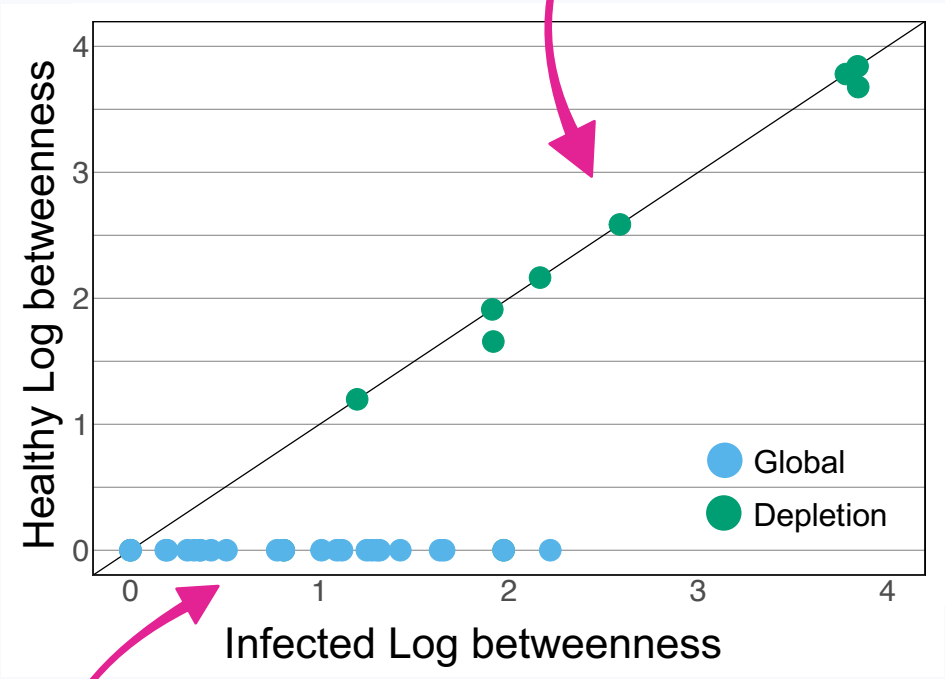
Not always involved with global significance



*One possible drug target development strategy is to promote the **protection of minimum control proteins***

TOPOLOGY OF GLOBAL ANALYSIS PROTEIN SET CHANGES POST INFECTION

Depletion method: no proteins with topological significance



Global method: high network significance during infection only

CONTROLLABILITY PREDICTED PROTEINS HOLD DISEASE-RELEVANT FUNCTIONAL ROLES

Protein set functions (IPA) :

Depletion (11 changing minimum set):

mRNA processing (*CELF1, **HNRNPA0**, SF384, and SRPK2*, p-value: 3.33×10^{-6})

Global (24 minimum set/virus interacting):

Protein synthesis, centered around **NF-kB**

Cell infection (*EPHA2, FBL, PFKM, PSMA5, SSR1,* and *TFRC*, p-value: 9.58×10^{-4})

Interferon regulated genes:

Depletion: 11/11

Global: 20/24

6 Global proteins identified in >10 studies

PROTEINS IDENTIFIED BY CONTROLLABILITY ARE NOT ENRICHED FOR VALIDATED HOST FACTORS

Validation data from 6 partial siRNA screens for host factors involved in influenza replication

Depletion: 2/11 validated (fisher test p: 0.685)

SF3B4 SRPK2

Global: 5/24 validated (fisher test p: 0.252)

OSMR PPA1 PSMA5 POLE4 GDI2

Genes of interest may be outside of partial genome screens

What should be screened next?

SUMMARY: CONTROLLABILITY

- A comparison of controllability analyses of healthy and infected cell networks reveals key regulators of cellular control
- 24 proteins are recommended for future drug target efforts based on:
  - Network characteristics
  - Controllability behavior
  - Biological relevance

THANK YOU

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