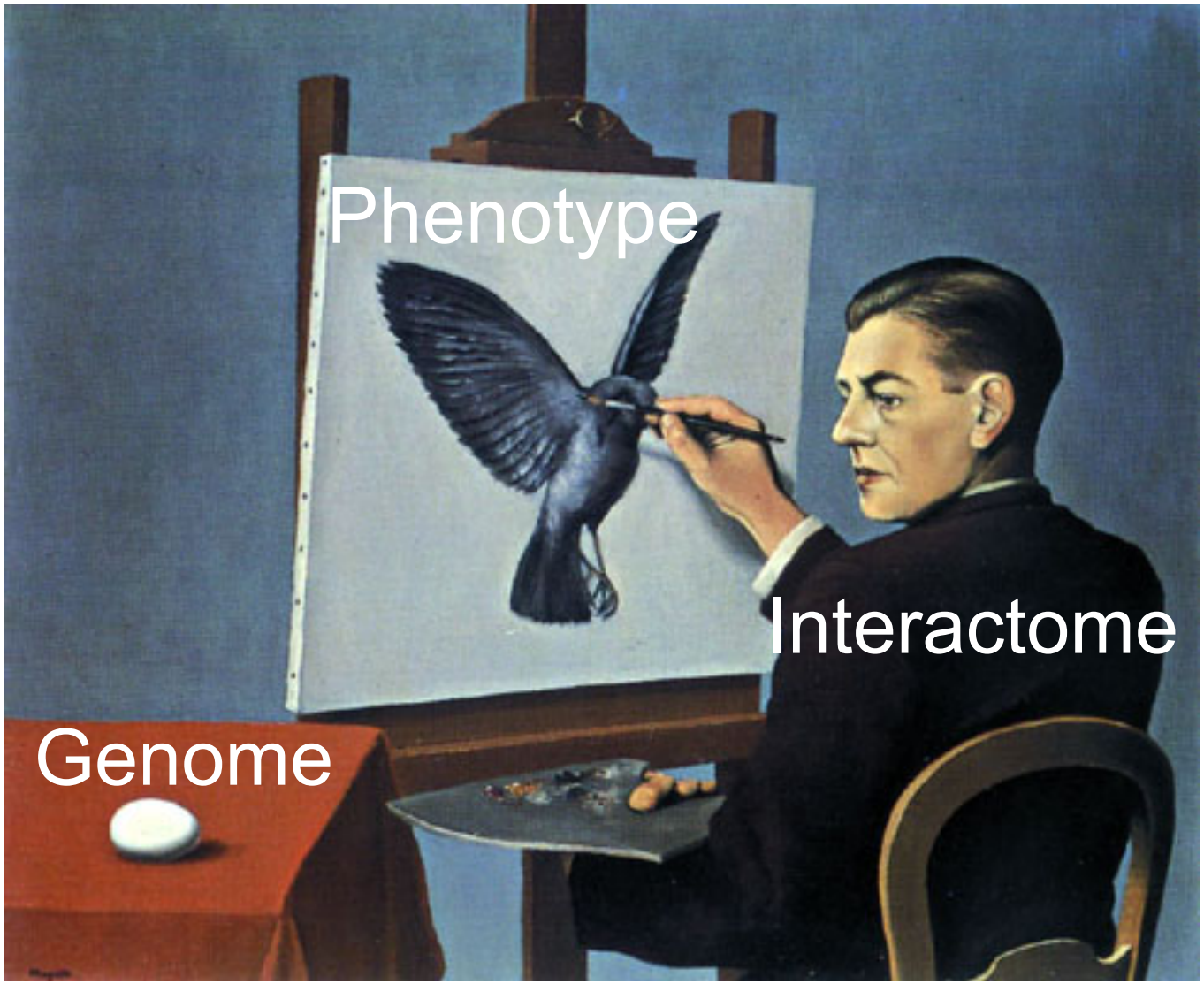
Signaling Transduction Unit (Prof. F. Dequiedt)
Protein signaling and Interactions Lab

Protein – protein interactions

Interactome networks drive molecular organisation of the cell

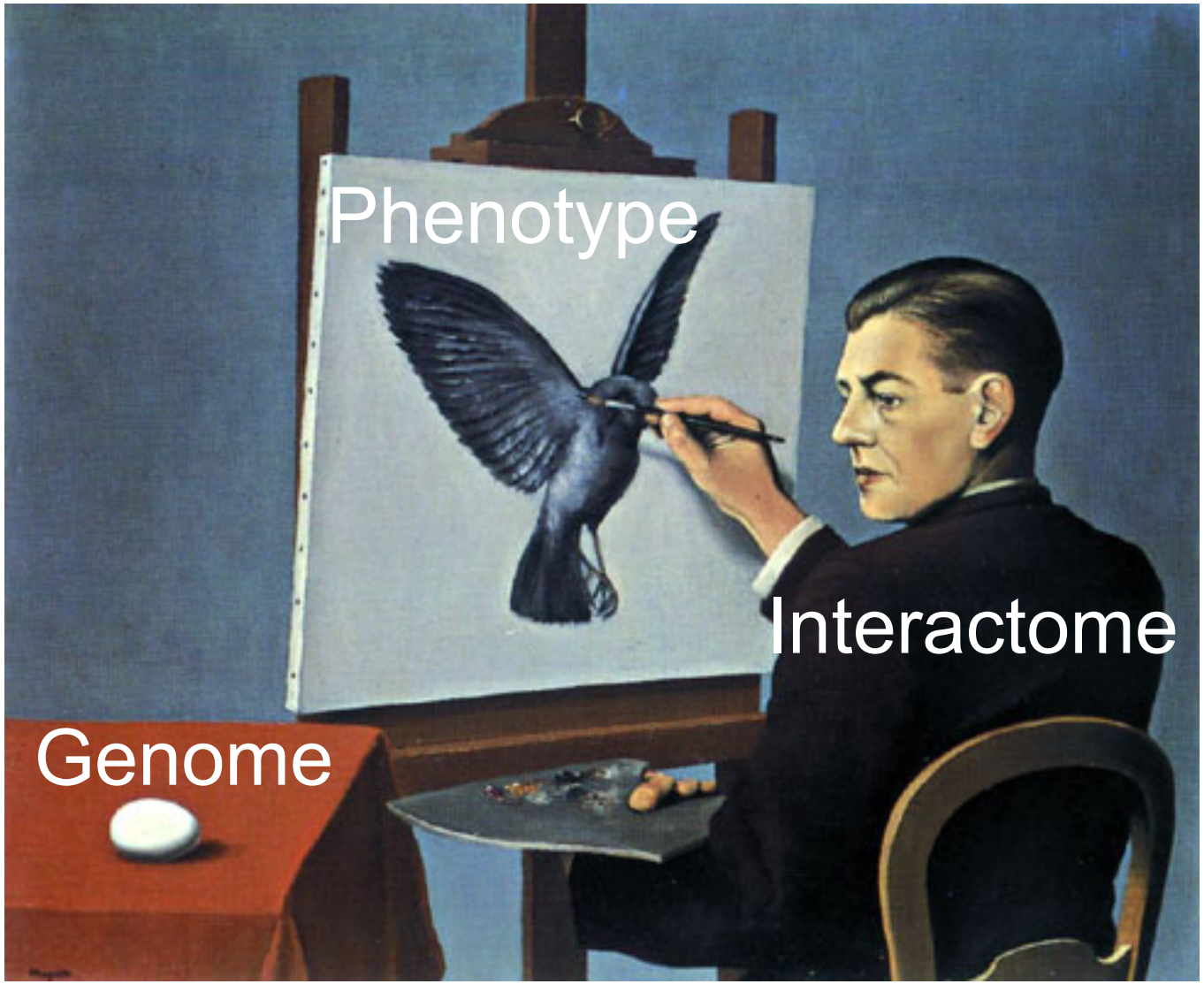
Dr. Jean-Claude Twizere

Jean-claude.twizere@ulg.ac.be


Phenotype
Interactome
Genome

Molecular organisation of the cell

- Elucidation of Genomes, proteomes, their components and interactions
- Functional organization remains largely unknown
- Cellular function is the result of coordinated intecations
- Interaction networks essential to understand biology, disease and/or drug action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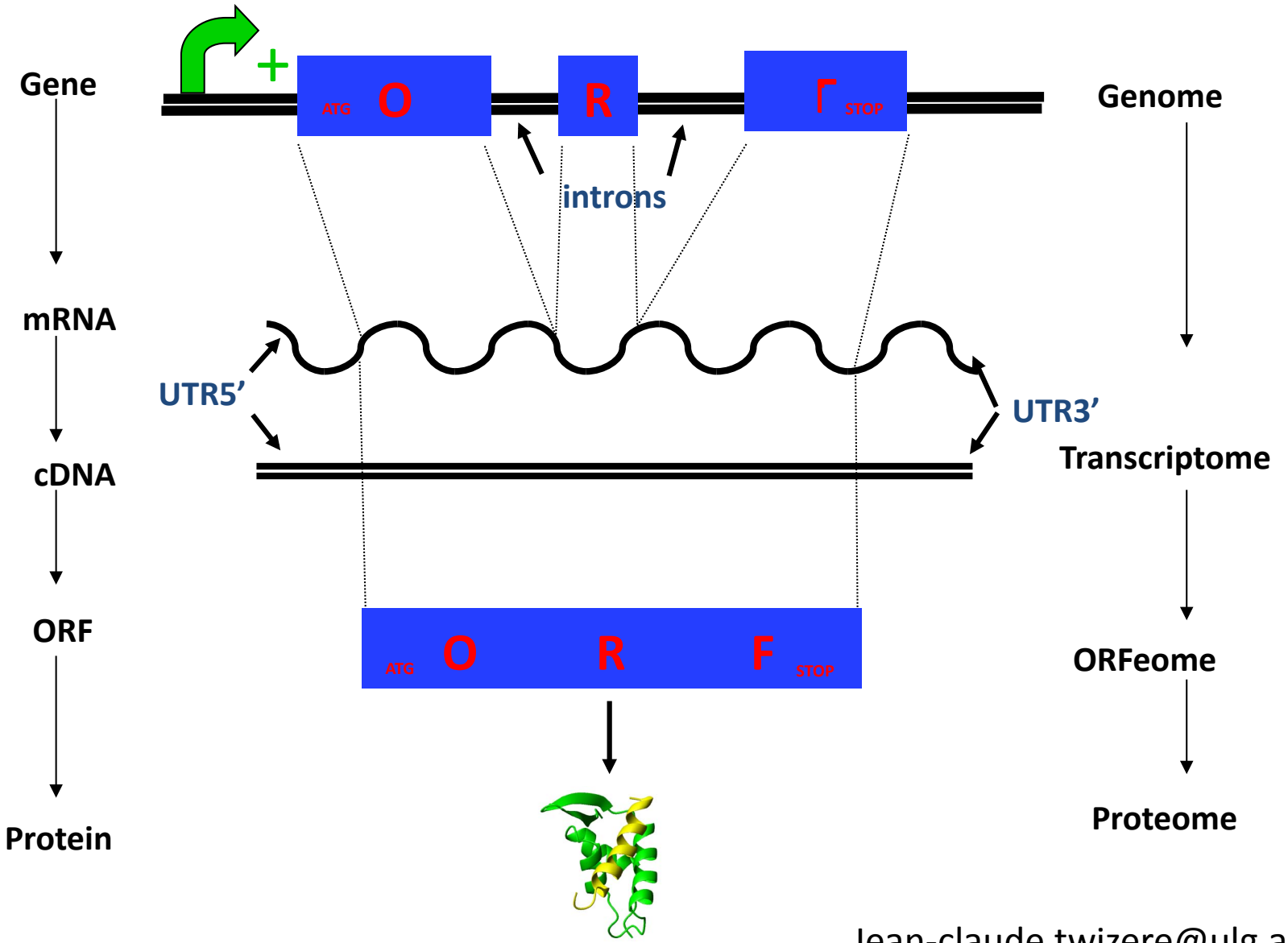

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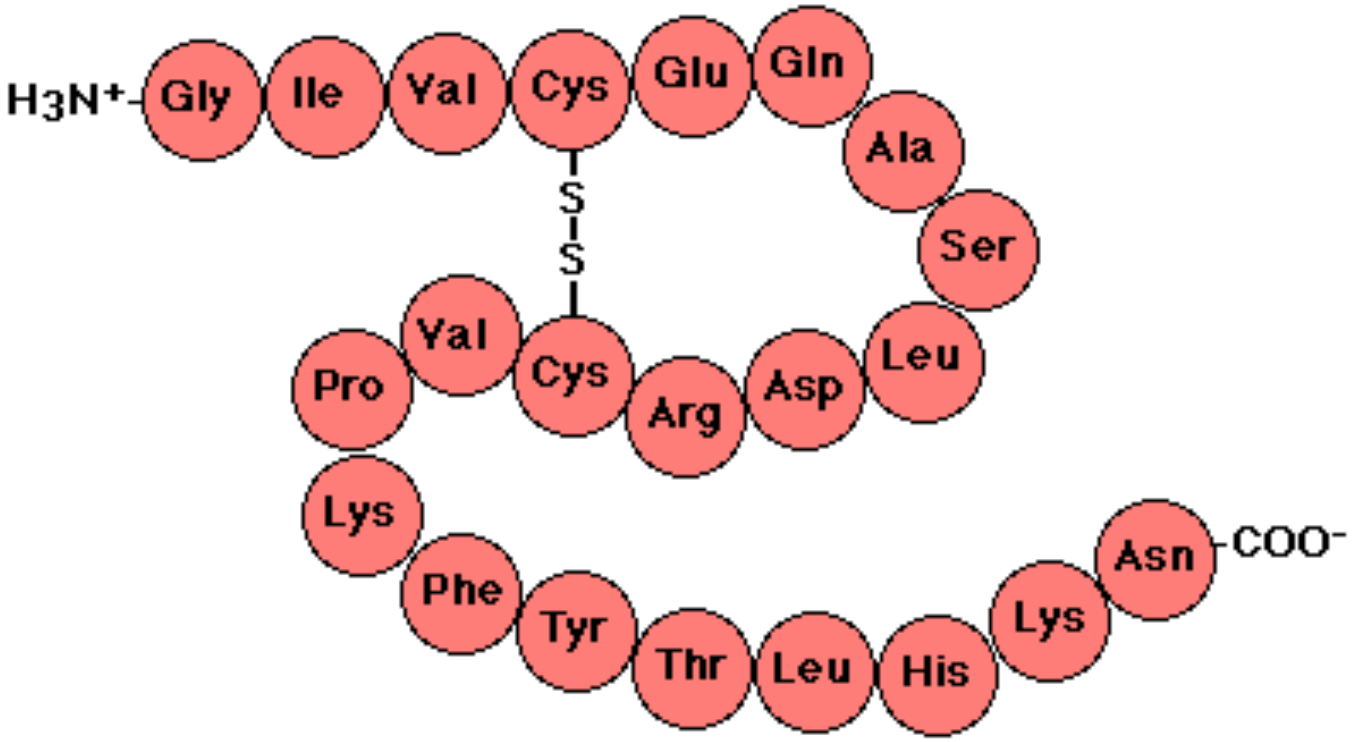
Primary structure



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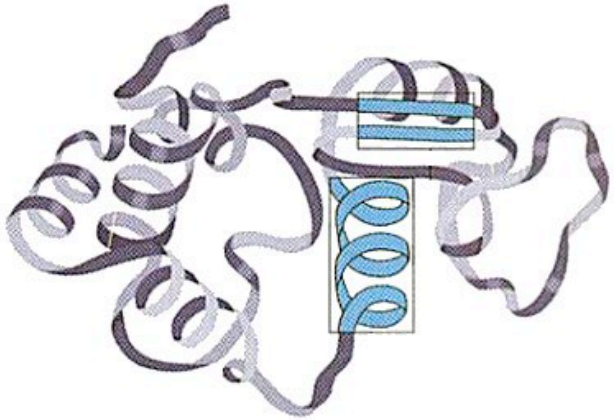
Secondary and tertiary structure

III.



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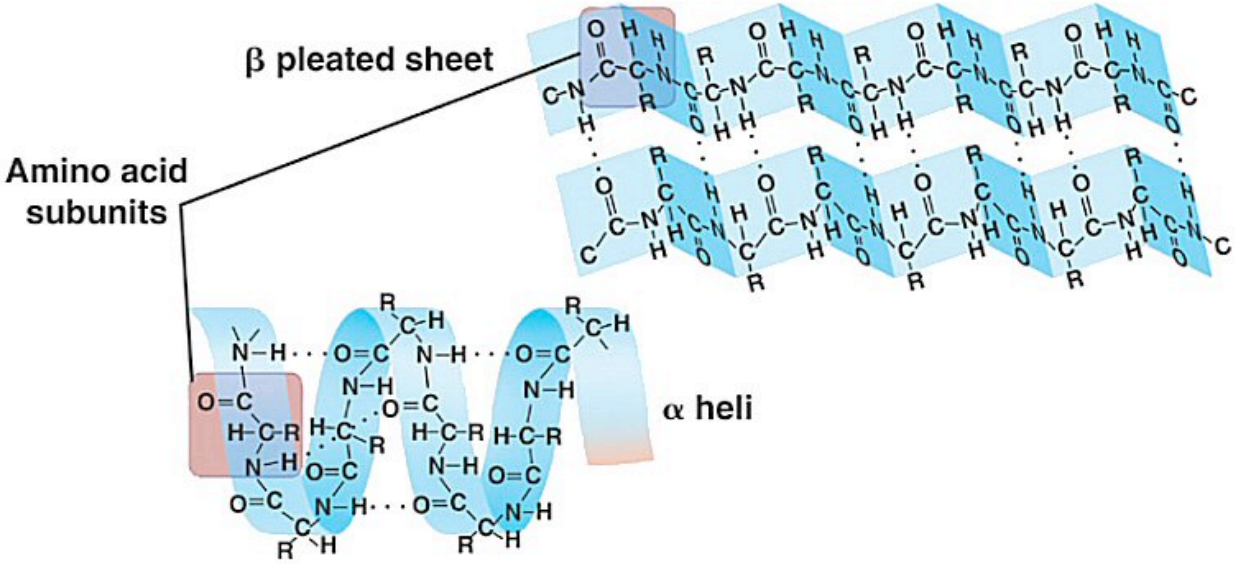
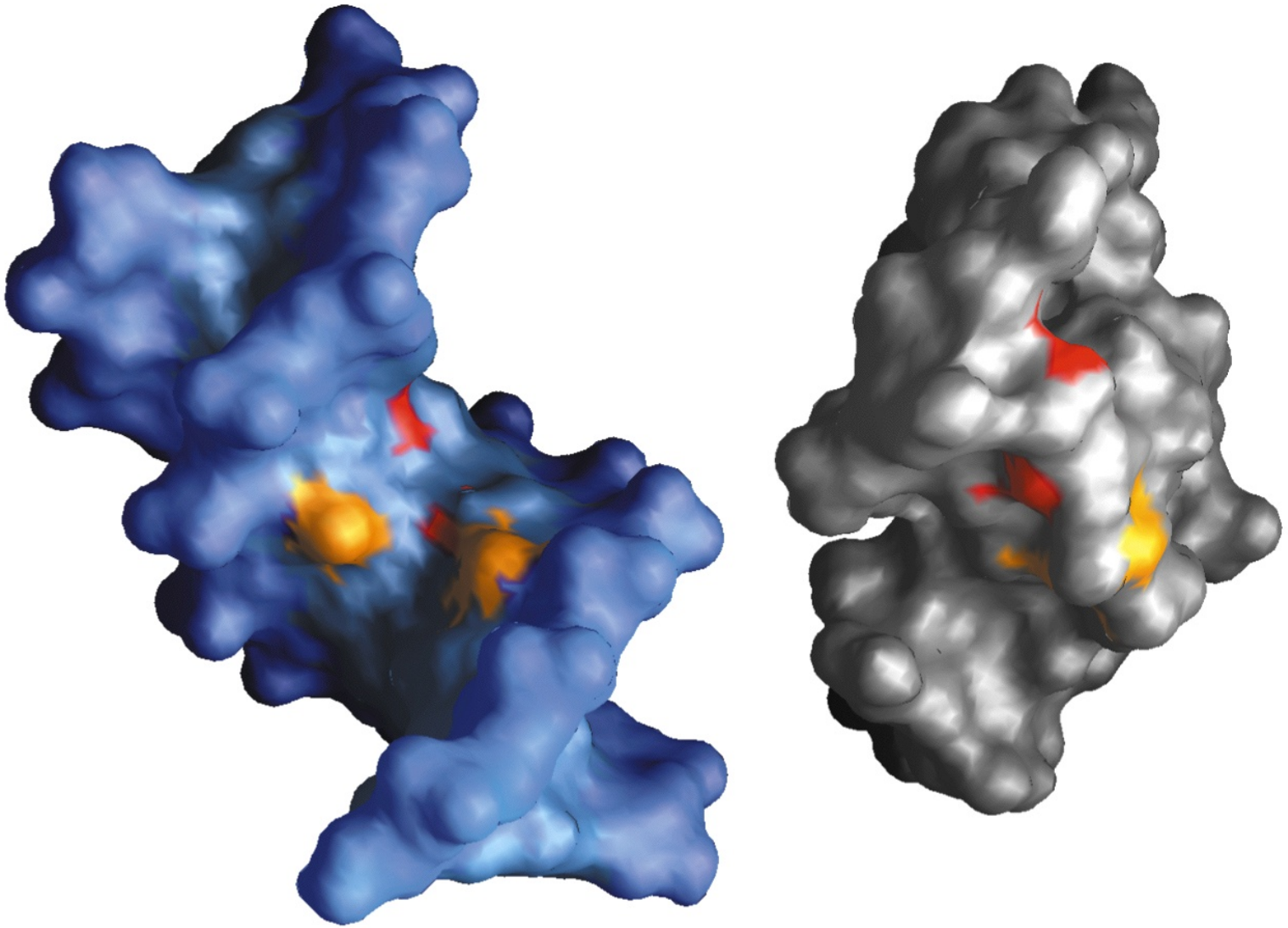
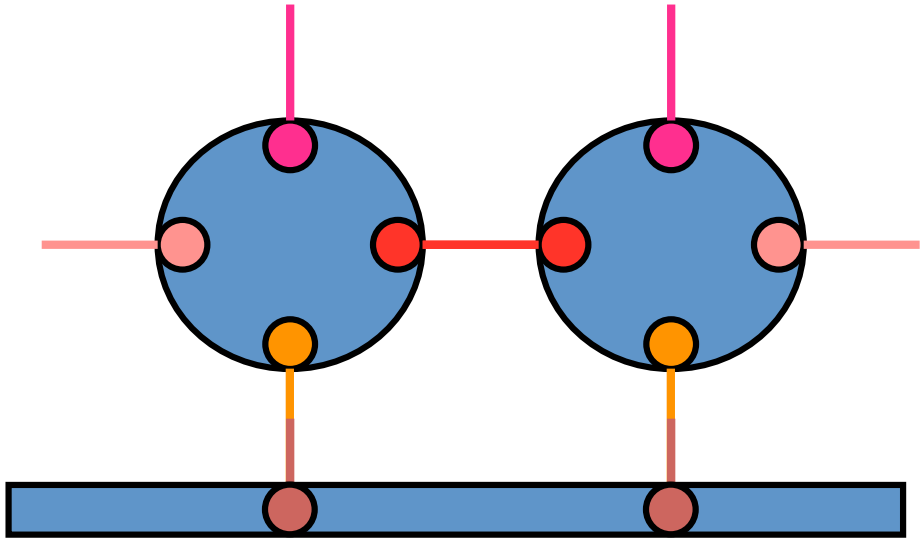


Quaternary structure



(d)

Protein Interactions

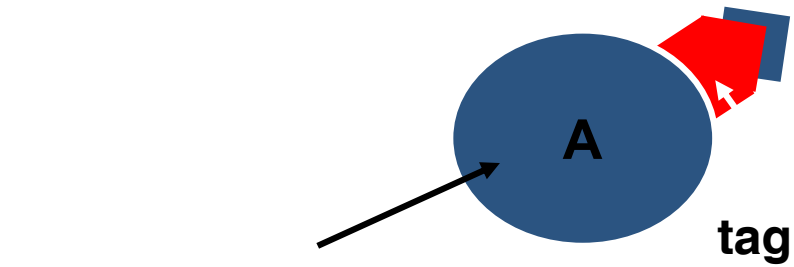


Homodimerization and DNA/protein interaction

Protein-protein interactions

- Affinity purification
- Y2H hybrid
- Energy transfer (Fluorescence = FRET, heat =ITC)
- Co-localisation (Fluorescence based)
- Protein complementation
    - Luciferase based
    - Fluorescence based

Affinity purification/mass spectrometry

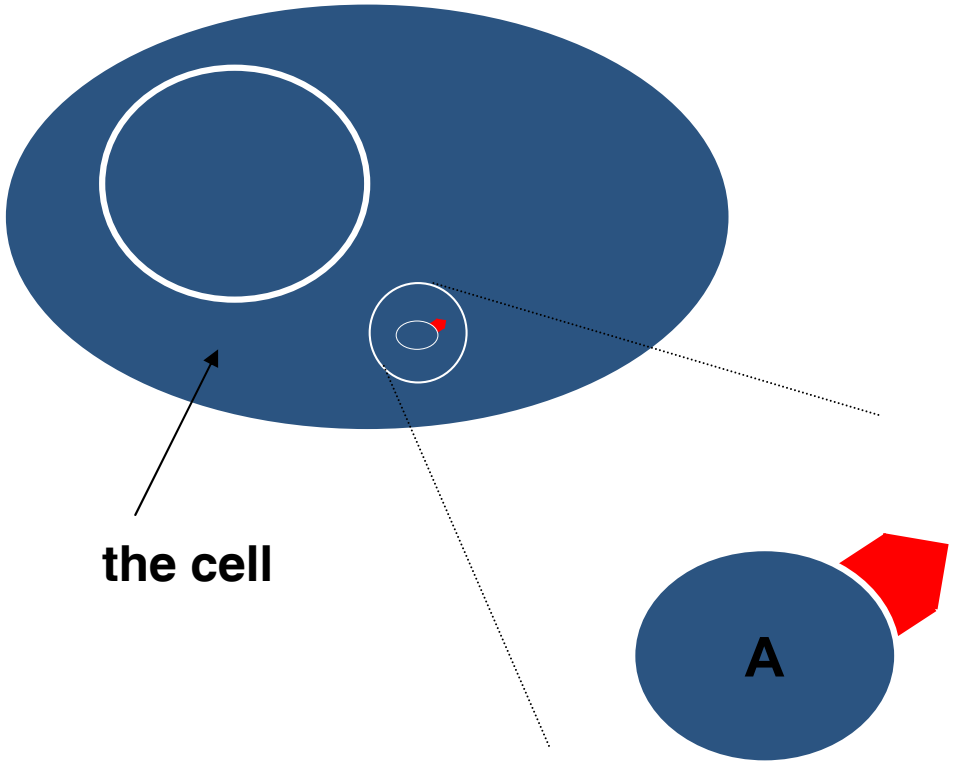


this molecule will bind the ‘tag’.

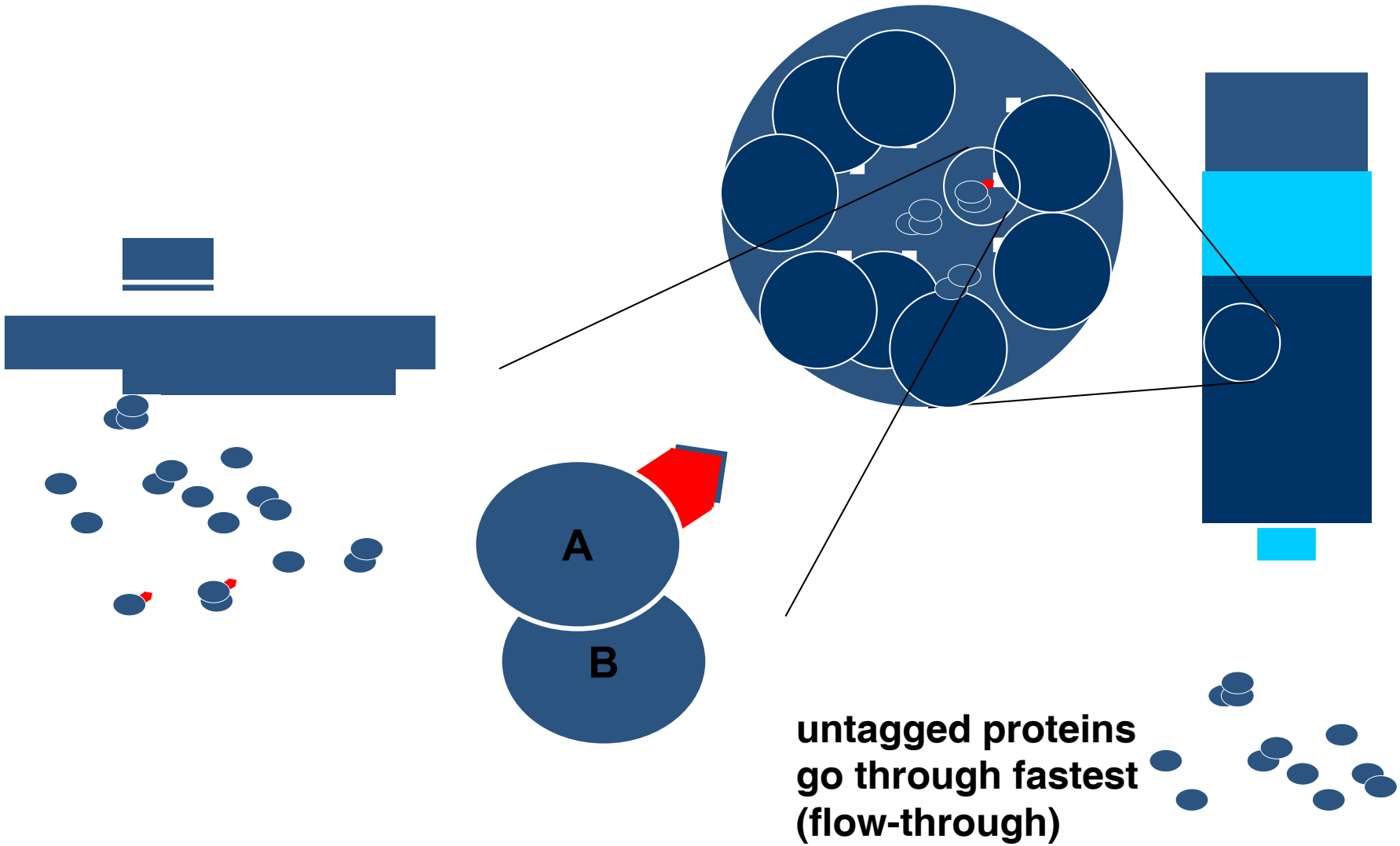
Protein of interest

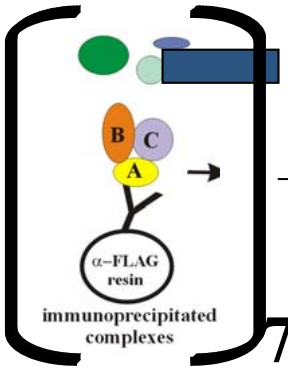
tag modification
(e.g. HA/GST/His, FLAG, MYC, TAP)

Affinity purification/mass spectrometry



Affinity purification/mass spectrometry





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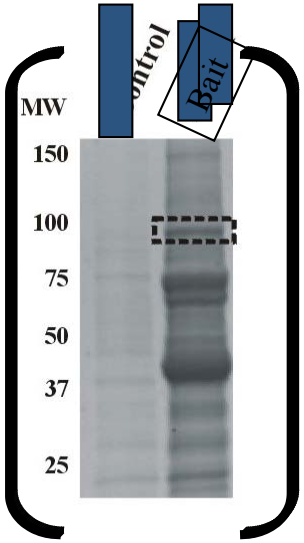

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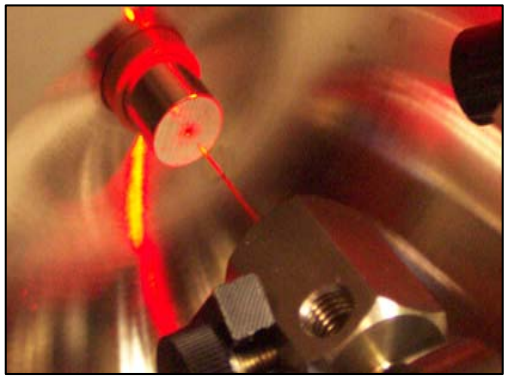
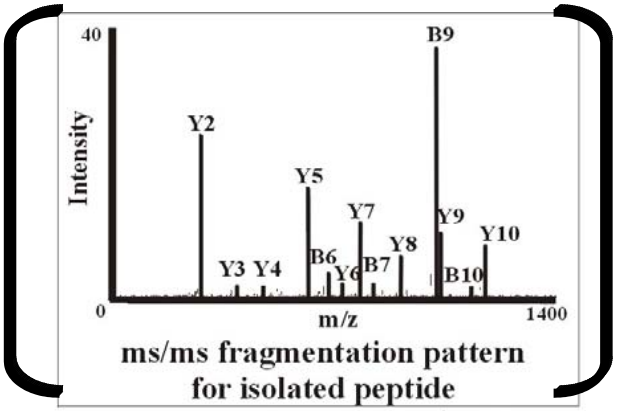
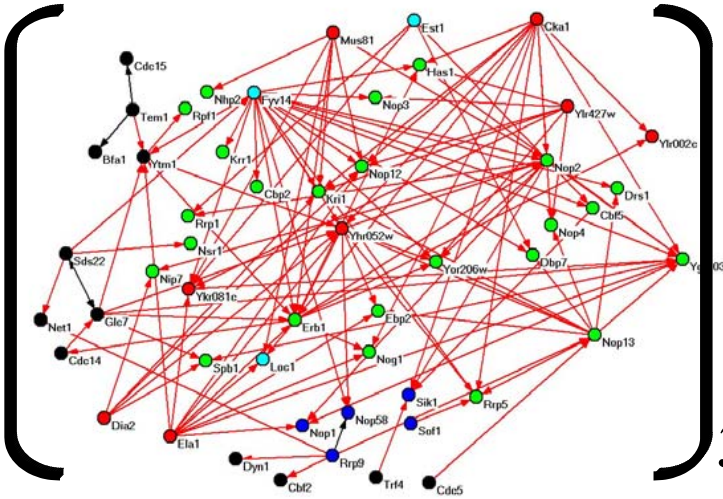


HTP-MS/MS Network



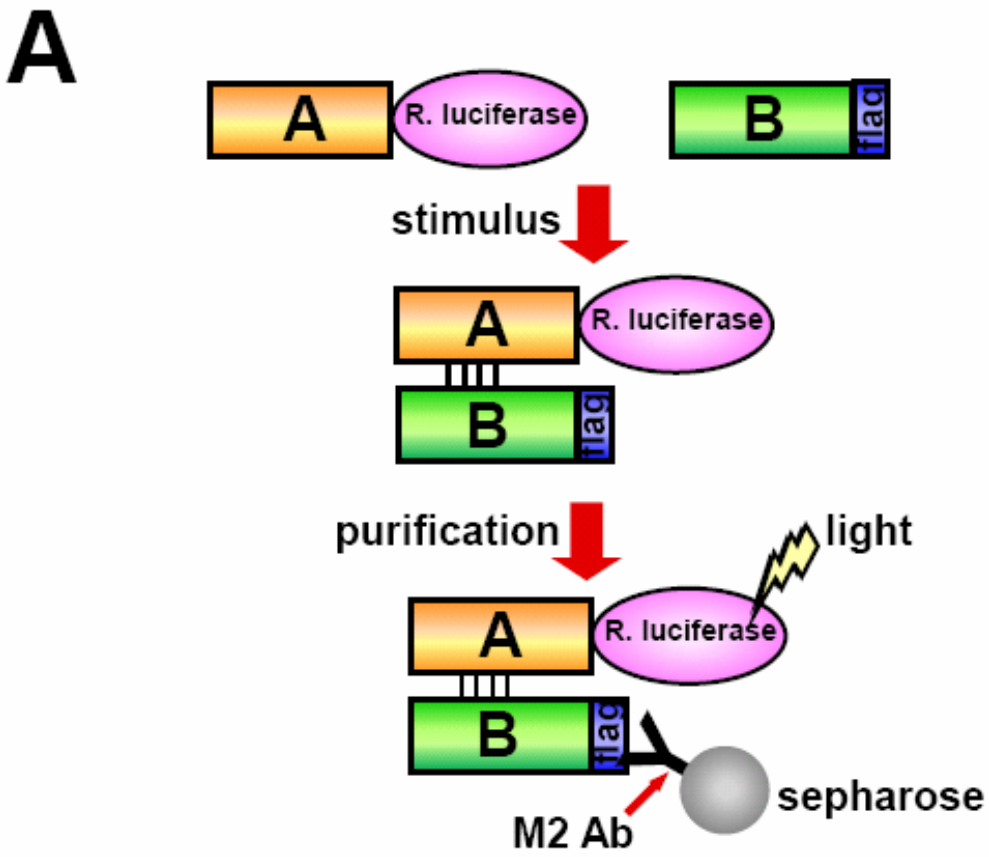
950,000

Database searching of fragmentation patterns



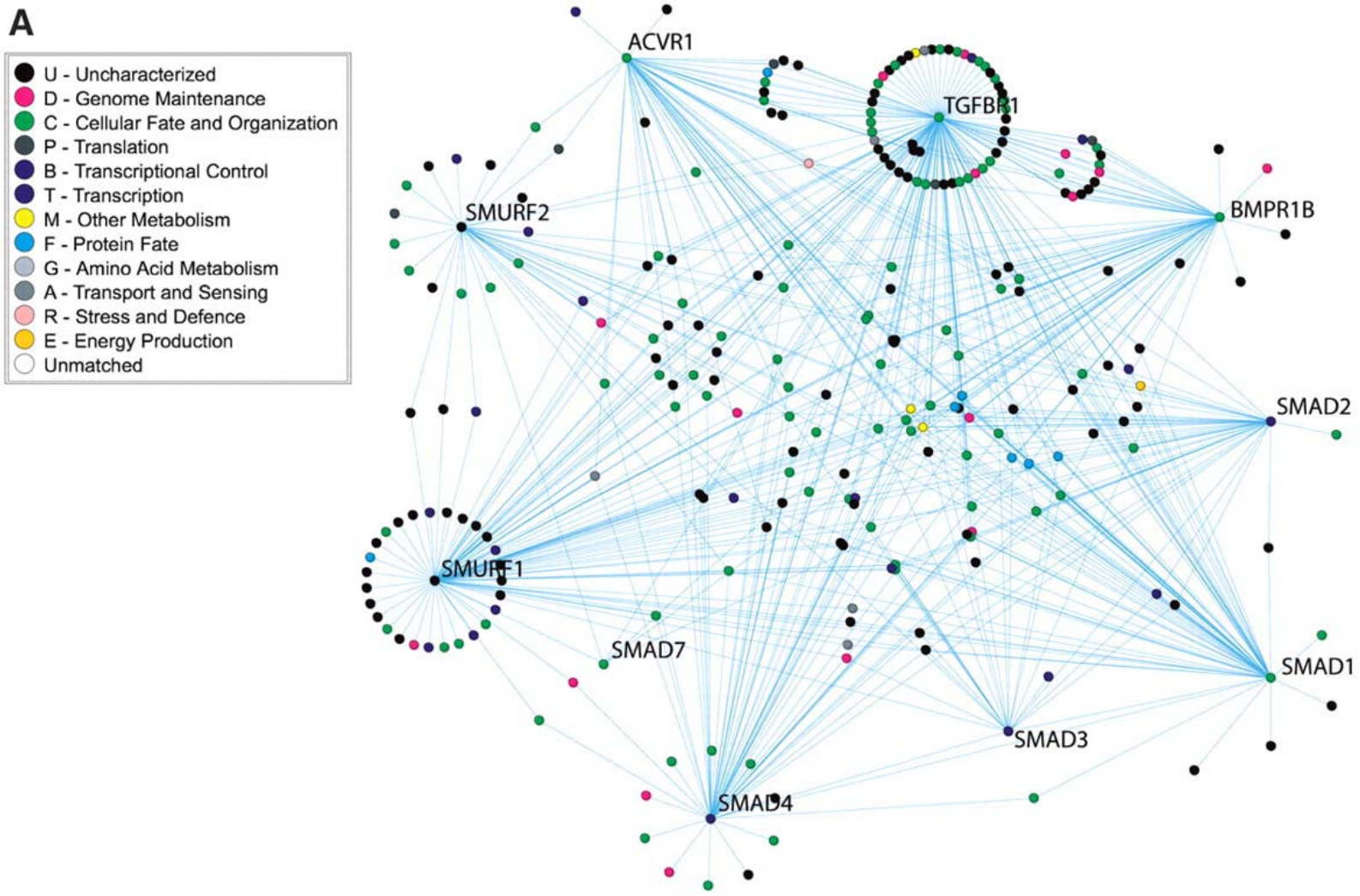
3,617

LUminescence-based Mammalian IntERactome mapping (LUMIER)



Barrios-Rodiles M, et al.
High-throughput mapping of a dynamic signaling network in mammalian cells.
Science. 2005 Mar 11;307(5715):1621-5.

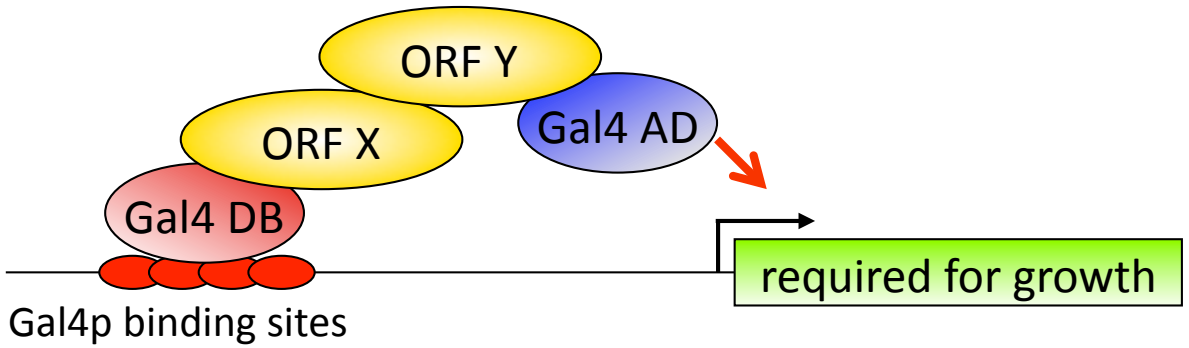
High-throughput screening in 293 cells using the Lumier approach



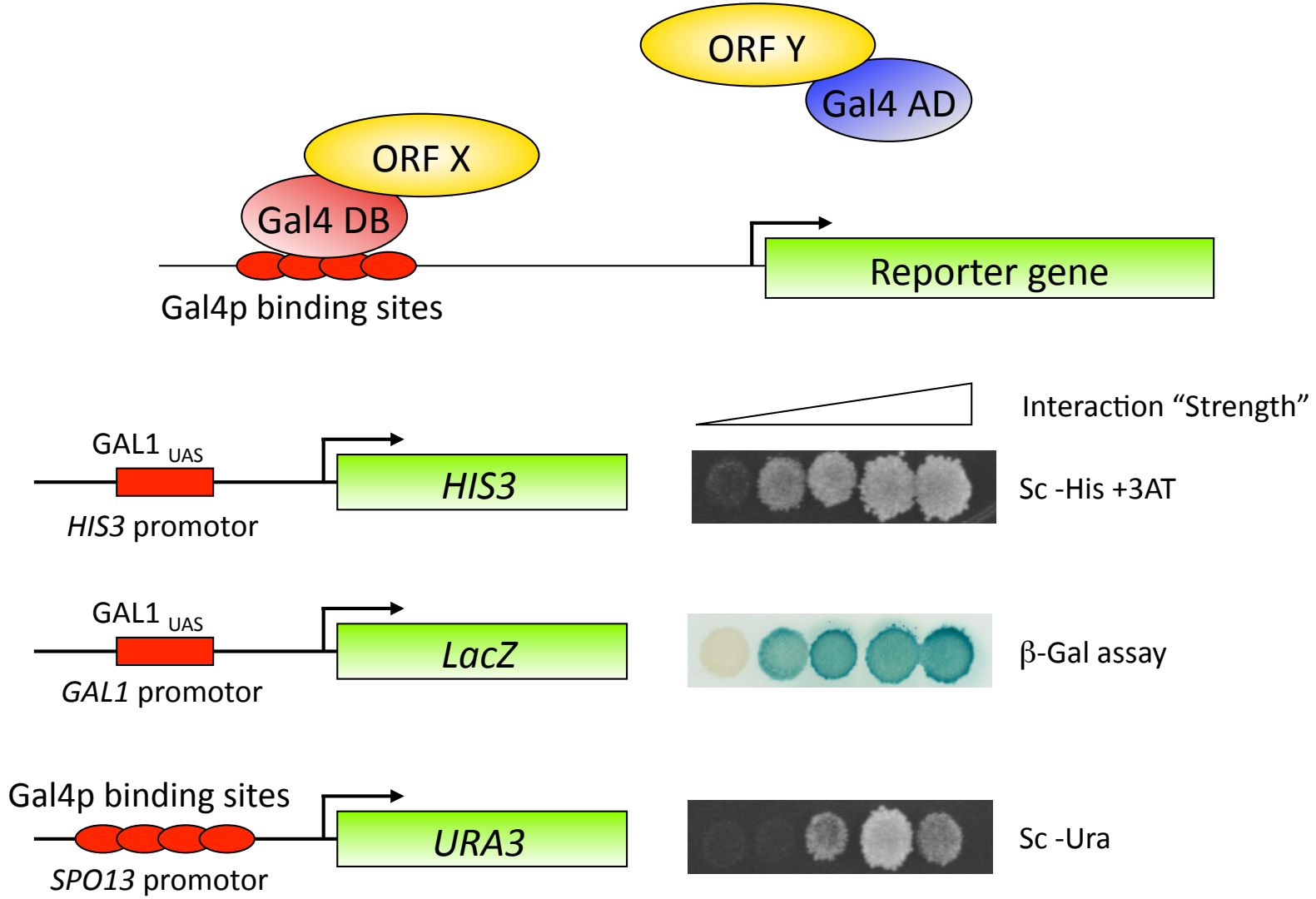
Discovering interactions: Yeast two-hybrid

Yeast two-hybrid

- Reconstitution of GAL4 transcription factor
- Fusion proteins DB-ORFX and ORFY-AD
- Reporter gene

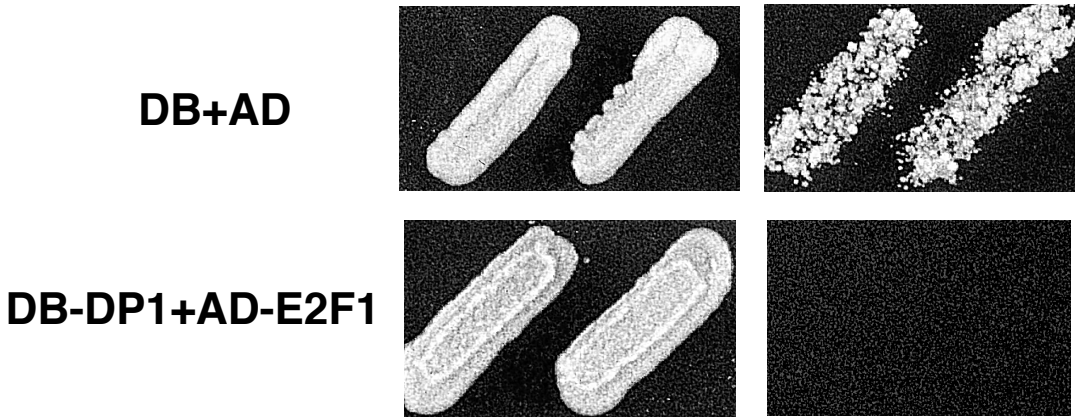


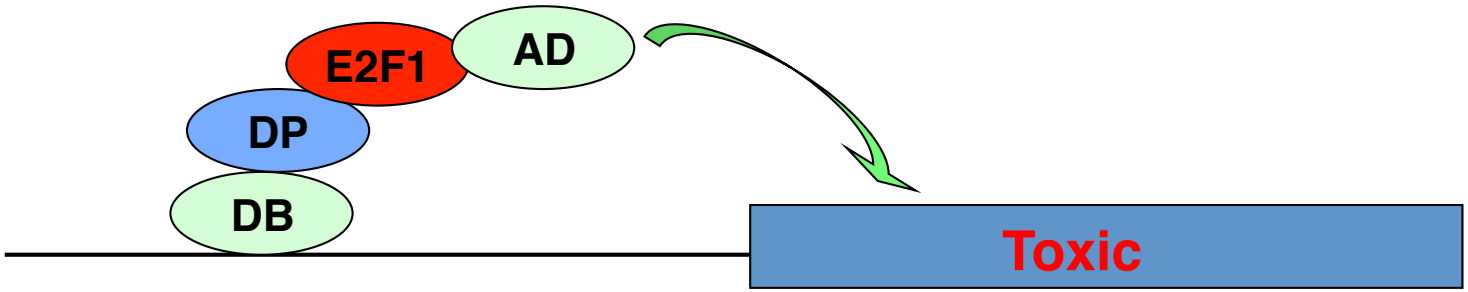
A positive selection of the protein – protein interactions



Negative selection against protein-protein interactions

Reverse two-hybrid





Yeast two-hybrid

Reagents (retroviruses side)

genes + fragments

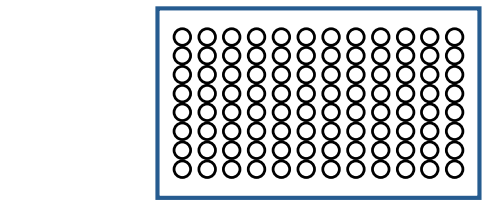
Gateway cloning

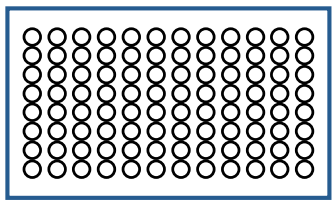
DB, AD expression vectors

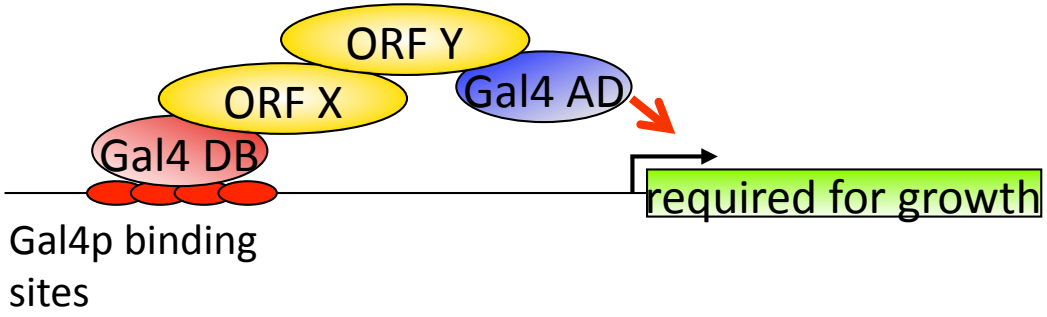
Yeast transformation

Mat a (Y8900 or Mav103)

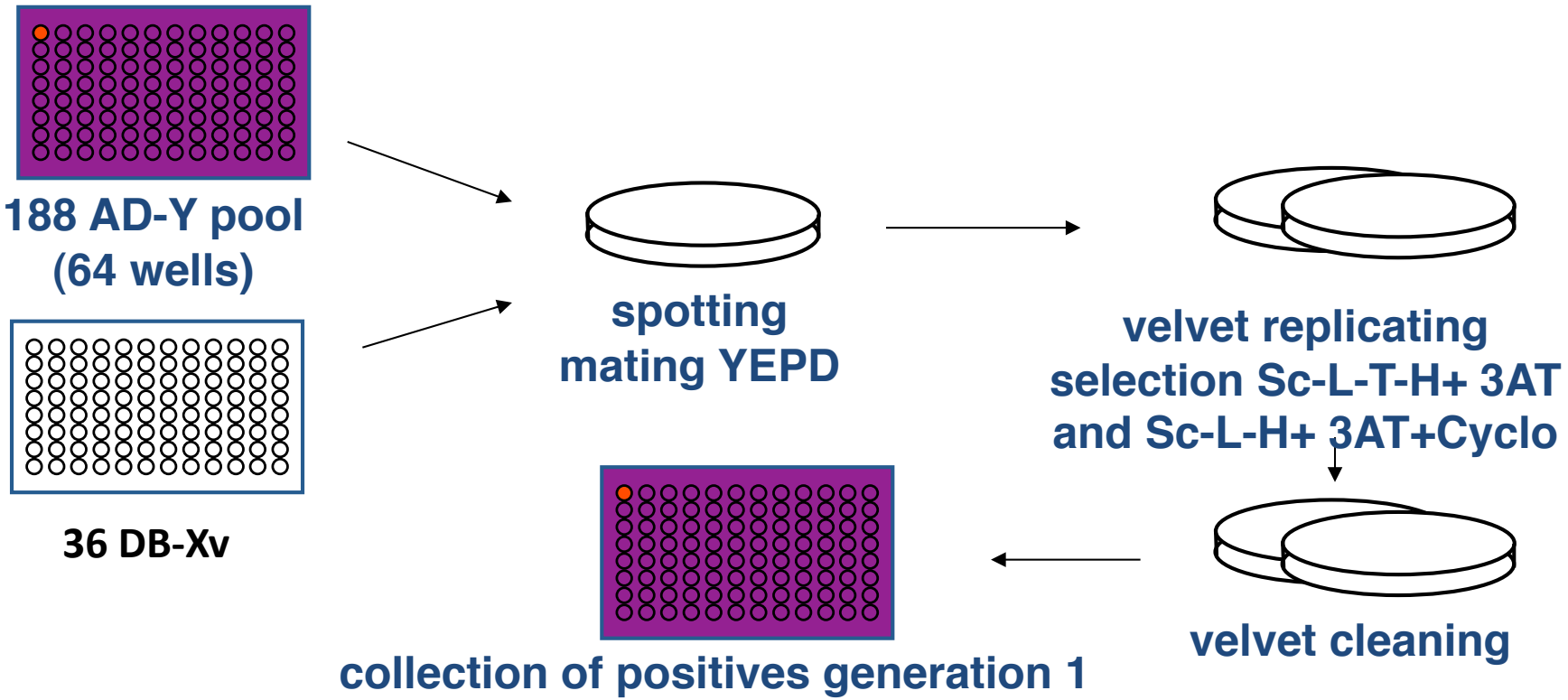
Mat a (Y8800 or Mav203)







High-throughput Y2H mating



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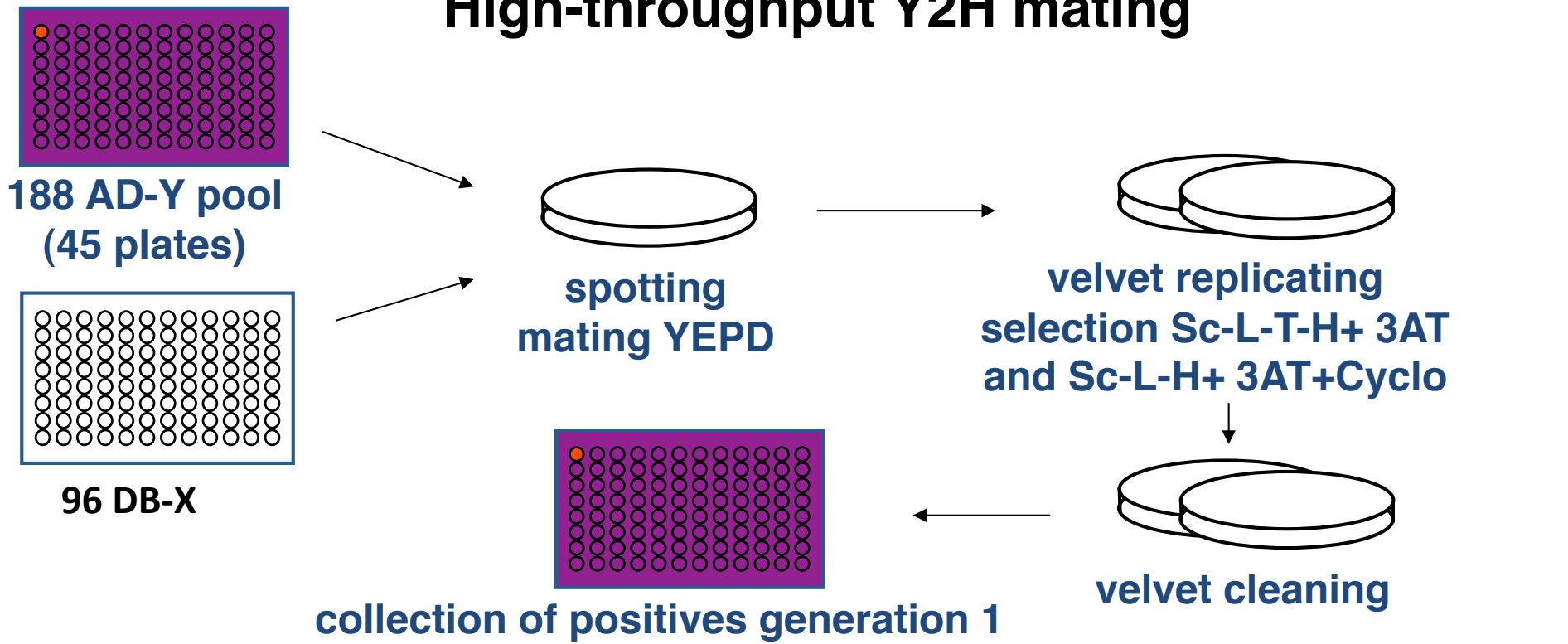
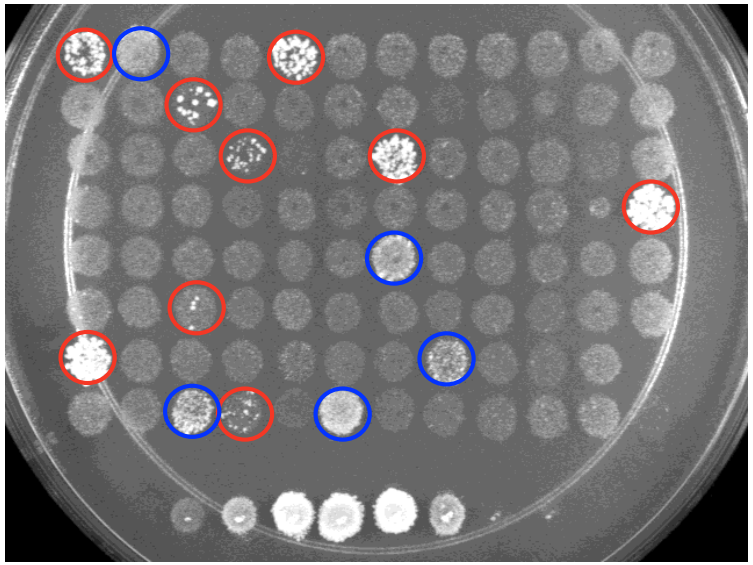
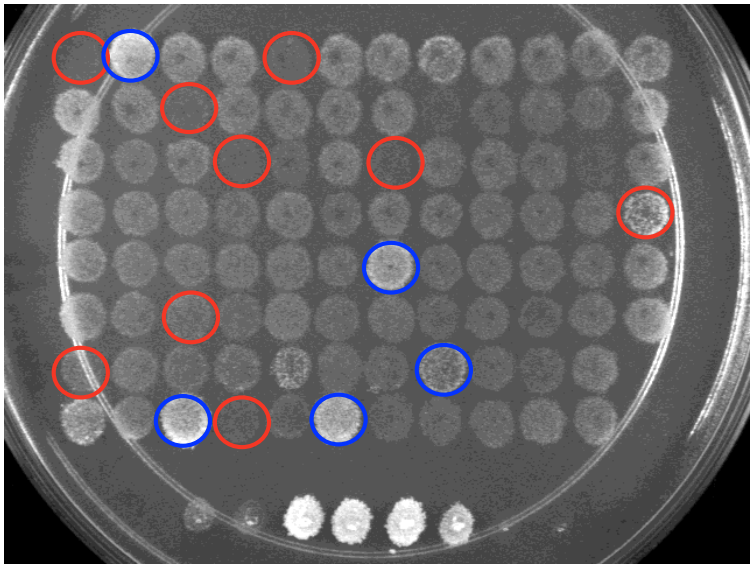




3AT-H-L-T

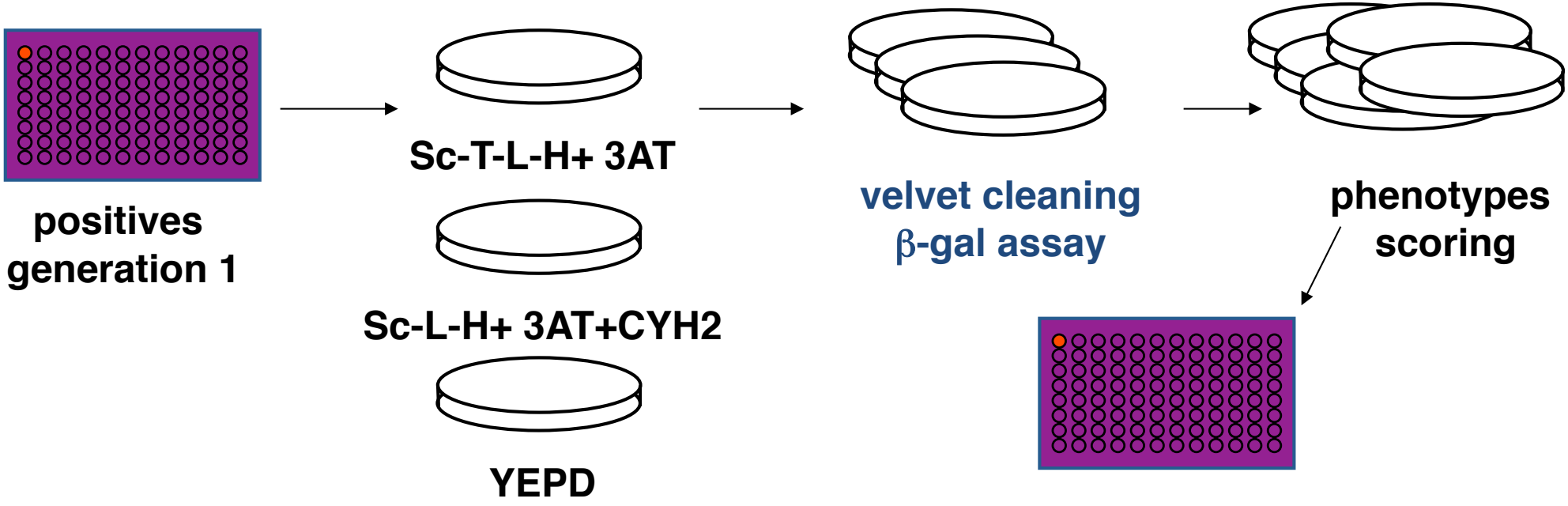


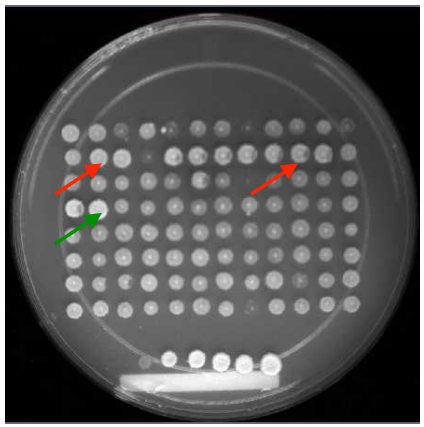
3AT-H-L+cycloheximide

interaction

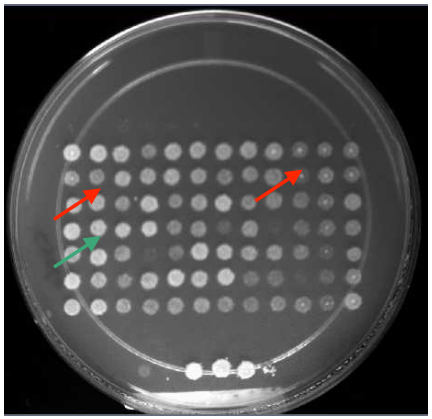
auto-activator

Y2H phenotyping

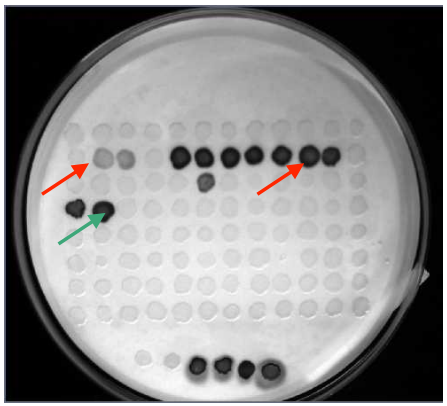




3AT-H-L-T



3AT-H-L+cycloheximide

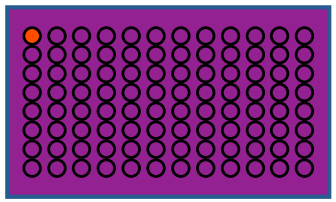


β-gal assay

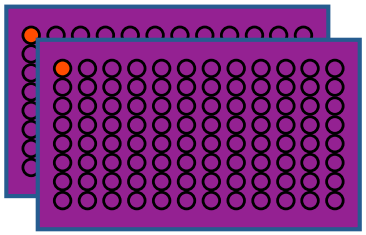
: auto-activation

: interaction

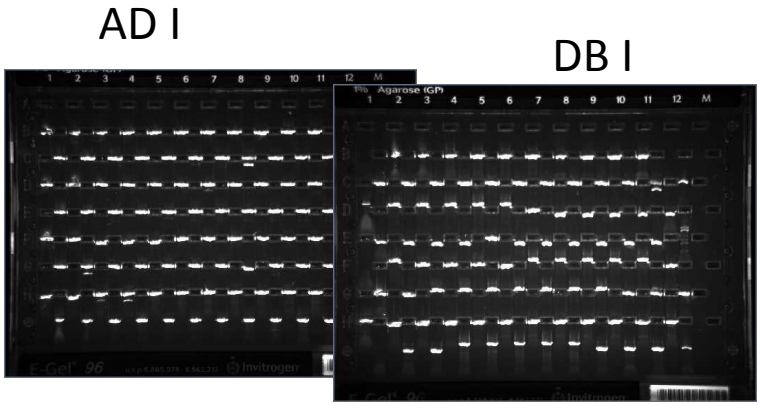
Sequencing



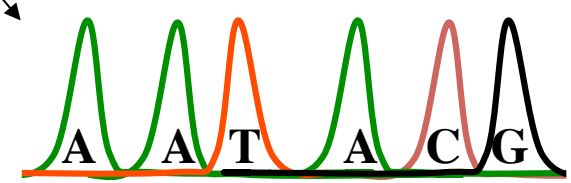
positives
generation 2



PCR
AD and DB



electrophoresis gel

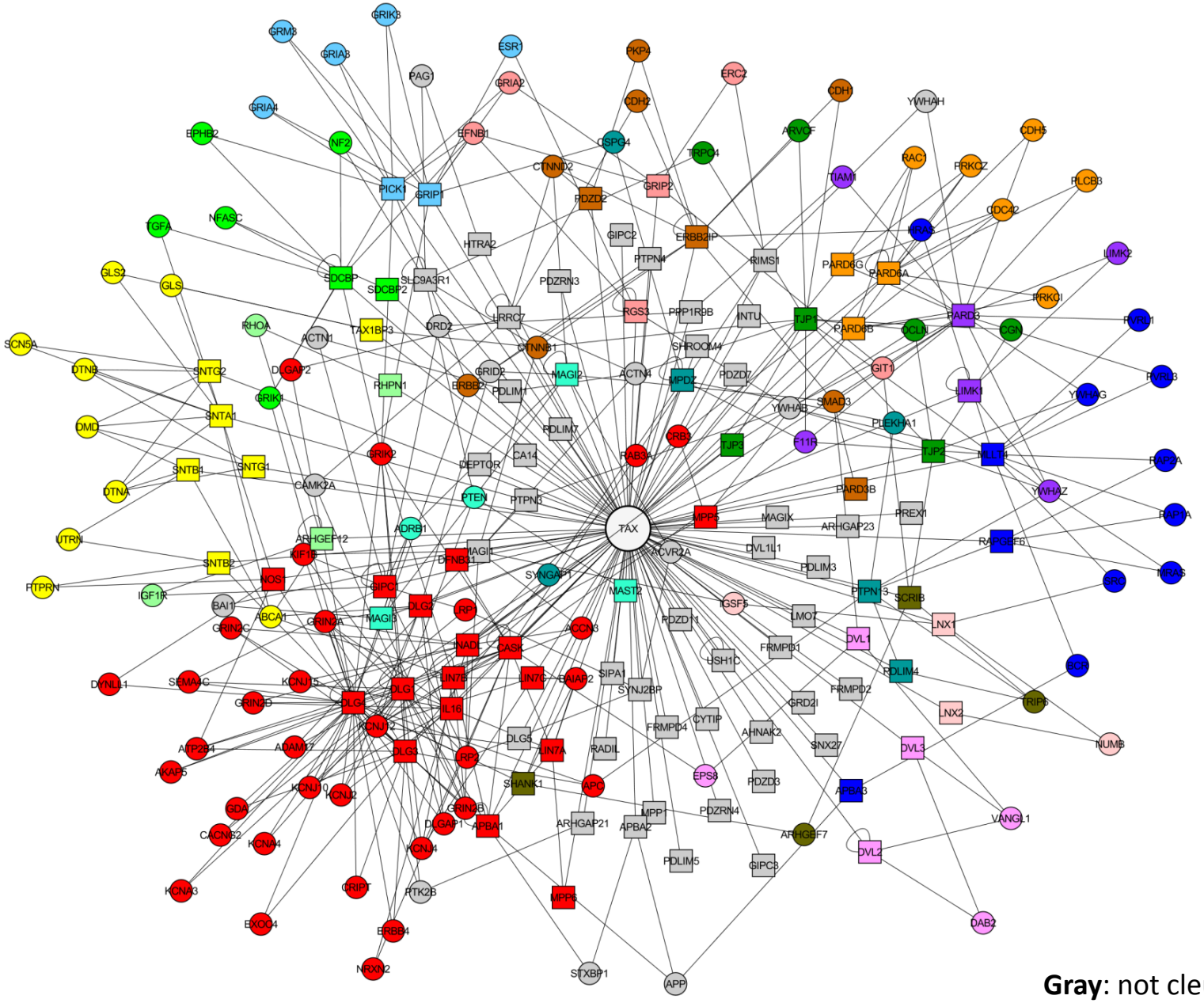


sequencing at Agencourt

protein interaction map

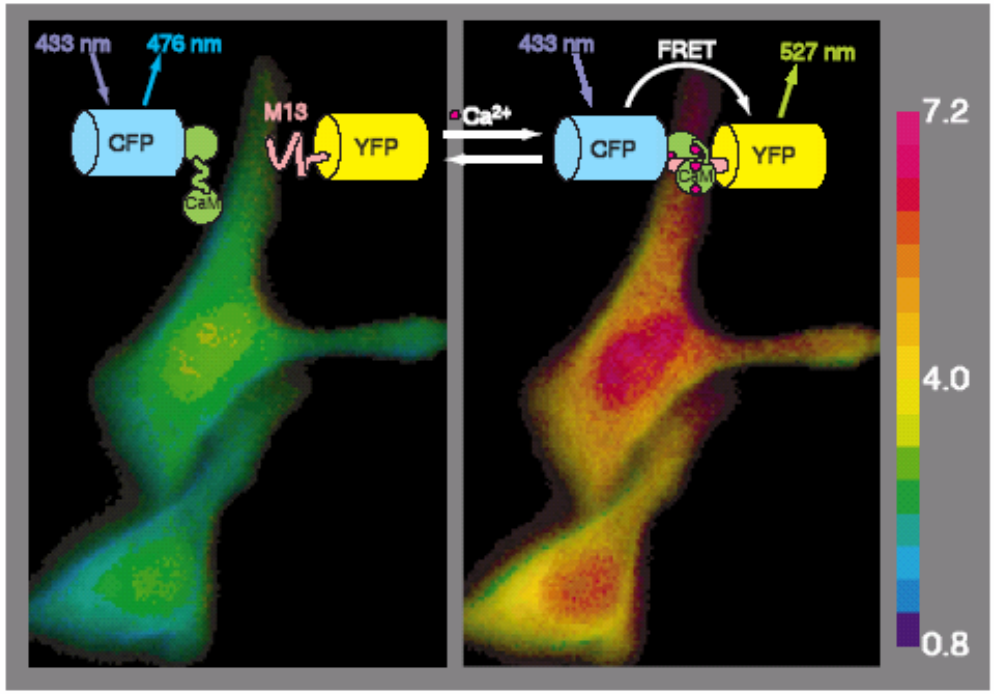
bioinformatic
analysis

TAX / PDZ PROTEINS INTERACTOME

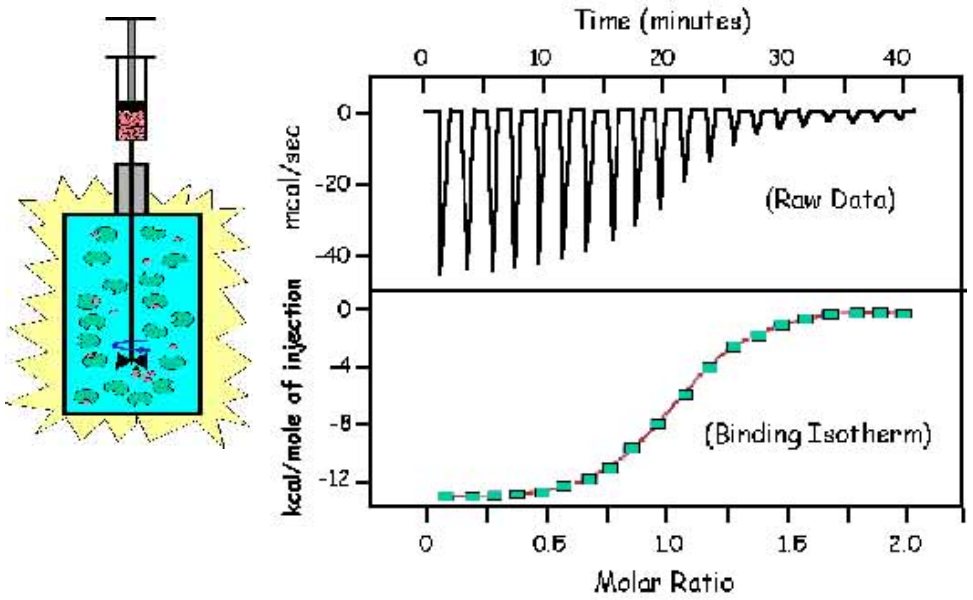


Gray: not cleustered
Other color: clustered

Monitoring assembly: FRET



Monitoring assembly: ITC



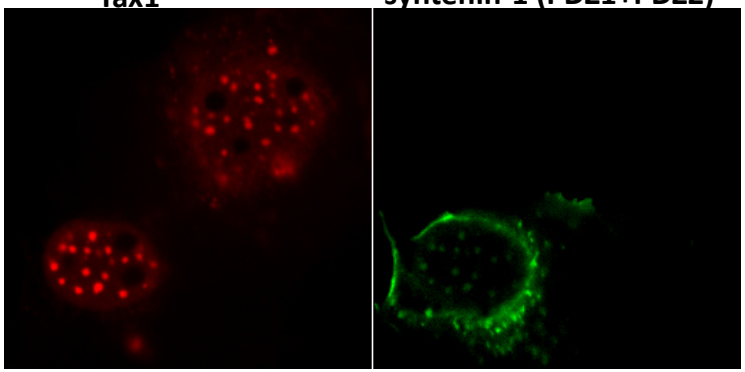
Isothermal Titration Calorimetry (ITC)
Principle of measurement:
change in heat

- one concentrated sample (protein, ligand, etc.) is injected into chamber containing the second reactant (*e.g.*, protein)
- a titration with increasing sample is performed
- heat which is evolved is detected by instrument
- measure k_d and n (stoichiometry of binding)

Monitoring interactions: co-localization

Tax1

syntenin-1 (PDZ1+PDZ2)



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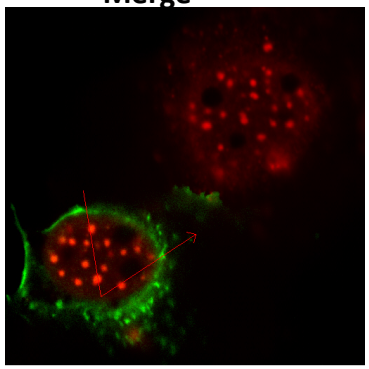
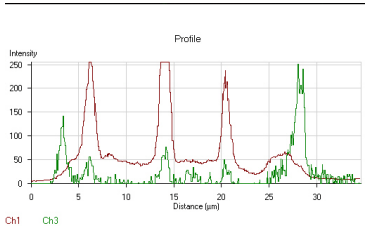
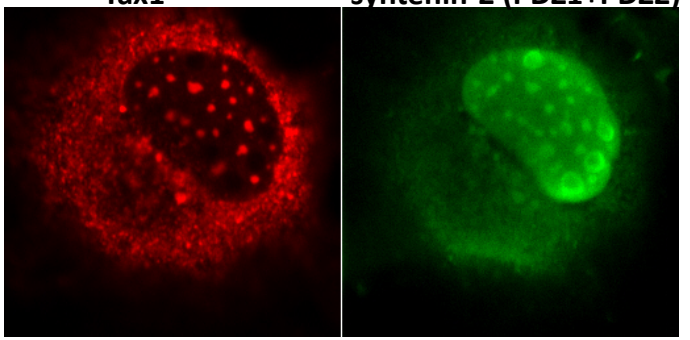




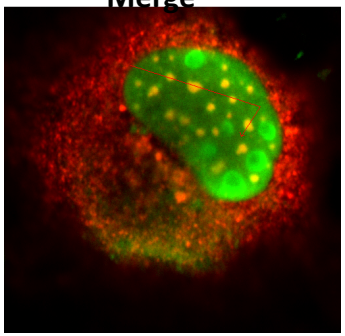
No colocalization between syntenin-1 (PDZ1+PDZ2) and Tax1

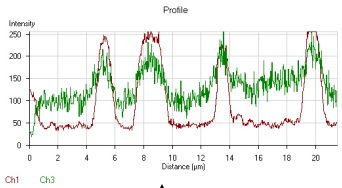
Tax1

syntenin-2 (PDZ1+PDZ2)



Merge

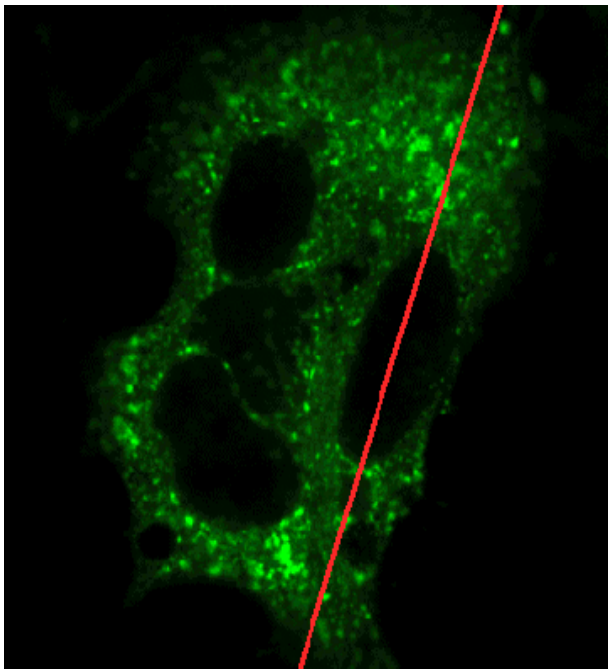




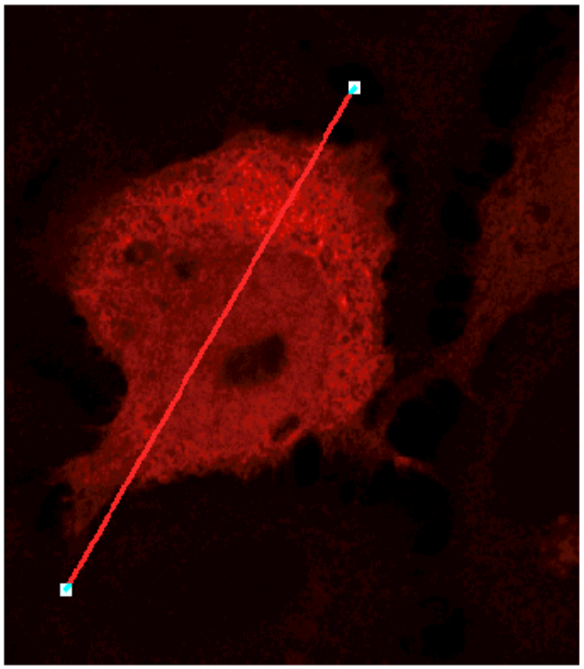
colocalization between syntenin-2 (PDZ1+PDZ2) and Tax1

Monitoring interactions: localization change

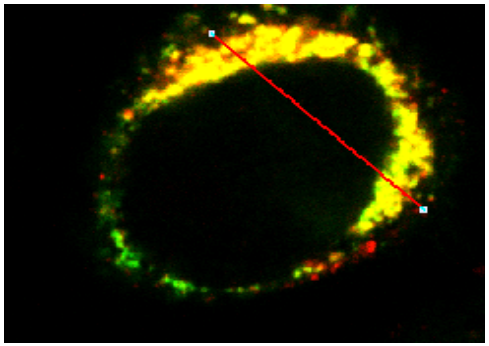
GFPTTP

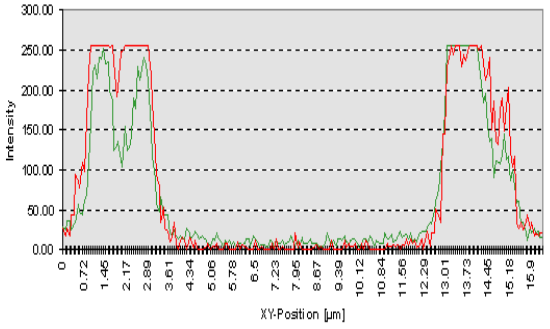


Tax



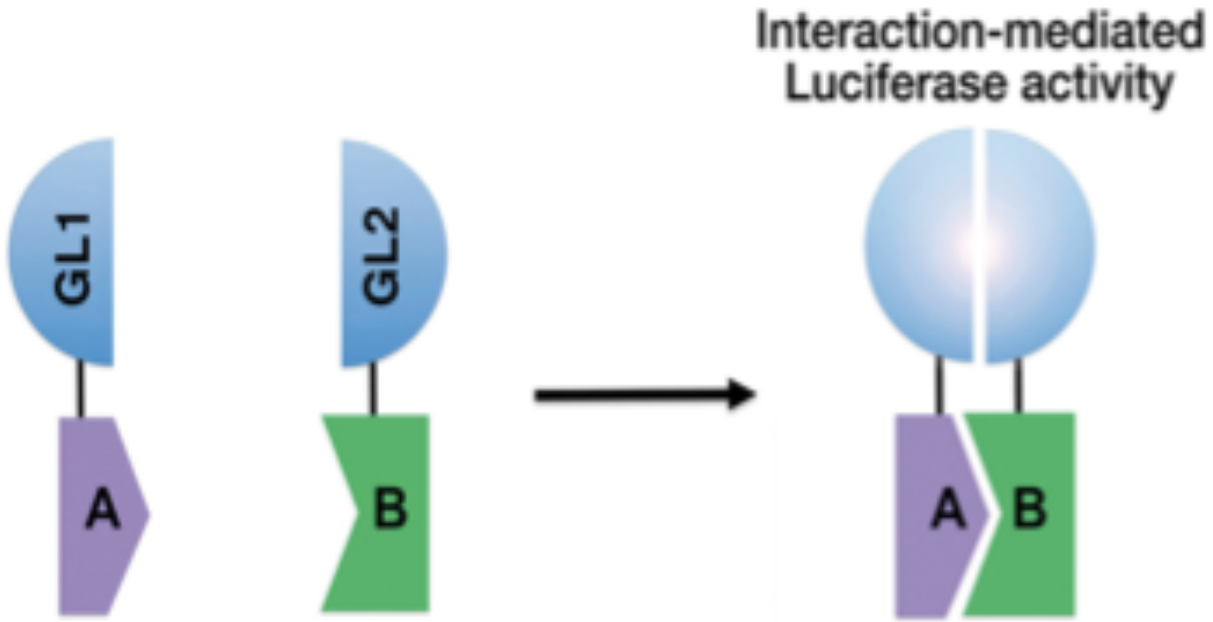
Tax1 et TTP





Monitoring interactions: protein complementation

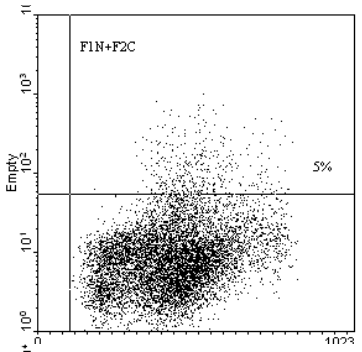
Gaussia princeps luciferase (GL)-based protein complementation assay (PCA)



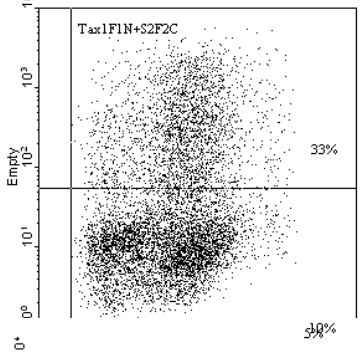
Monitoring interactions: protein complementation

Bi-molecular fluorescence complementation

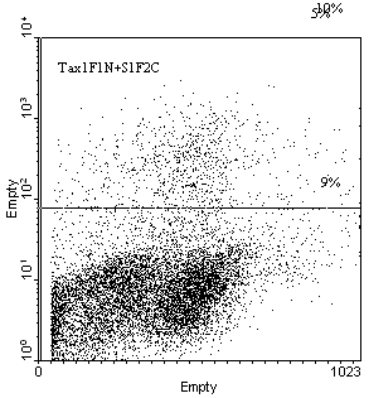
Control

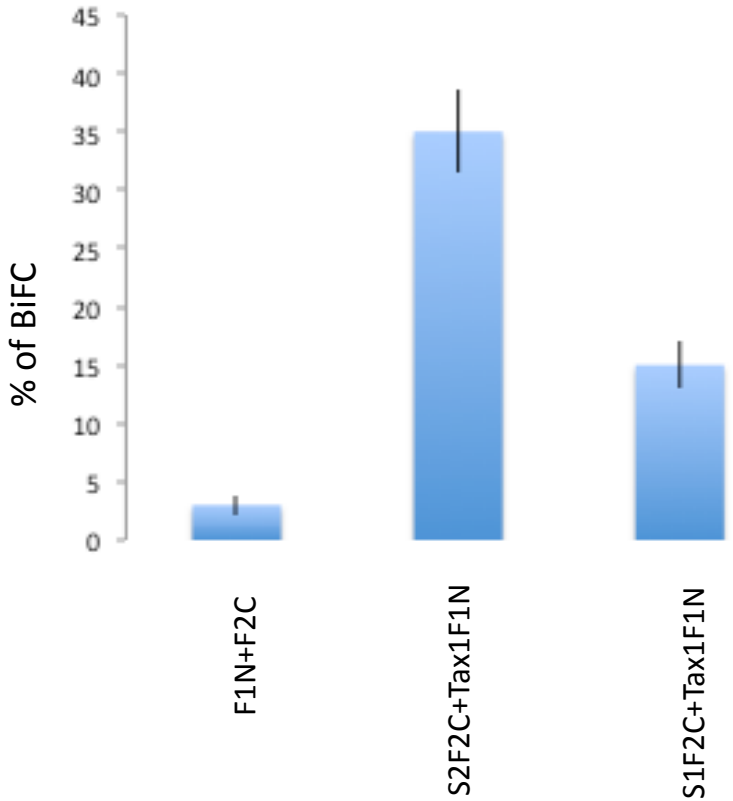


Syntenin2 +Tax1



Syntenin1 +Tax1





Protein-protein interactions

- Affinity purification
- Y2H hybrid
- Energy transfer (Fluorescence = FRET, heat =ITC)
- Co-localisation (Fluorescence based)
- Protein complementation
    - Luciferase based
    - Fluorescence based

Interactomes mapping applications

Models for overall functional organization of the cell

1970s-80s

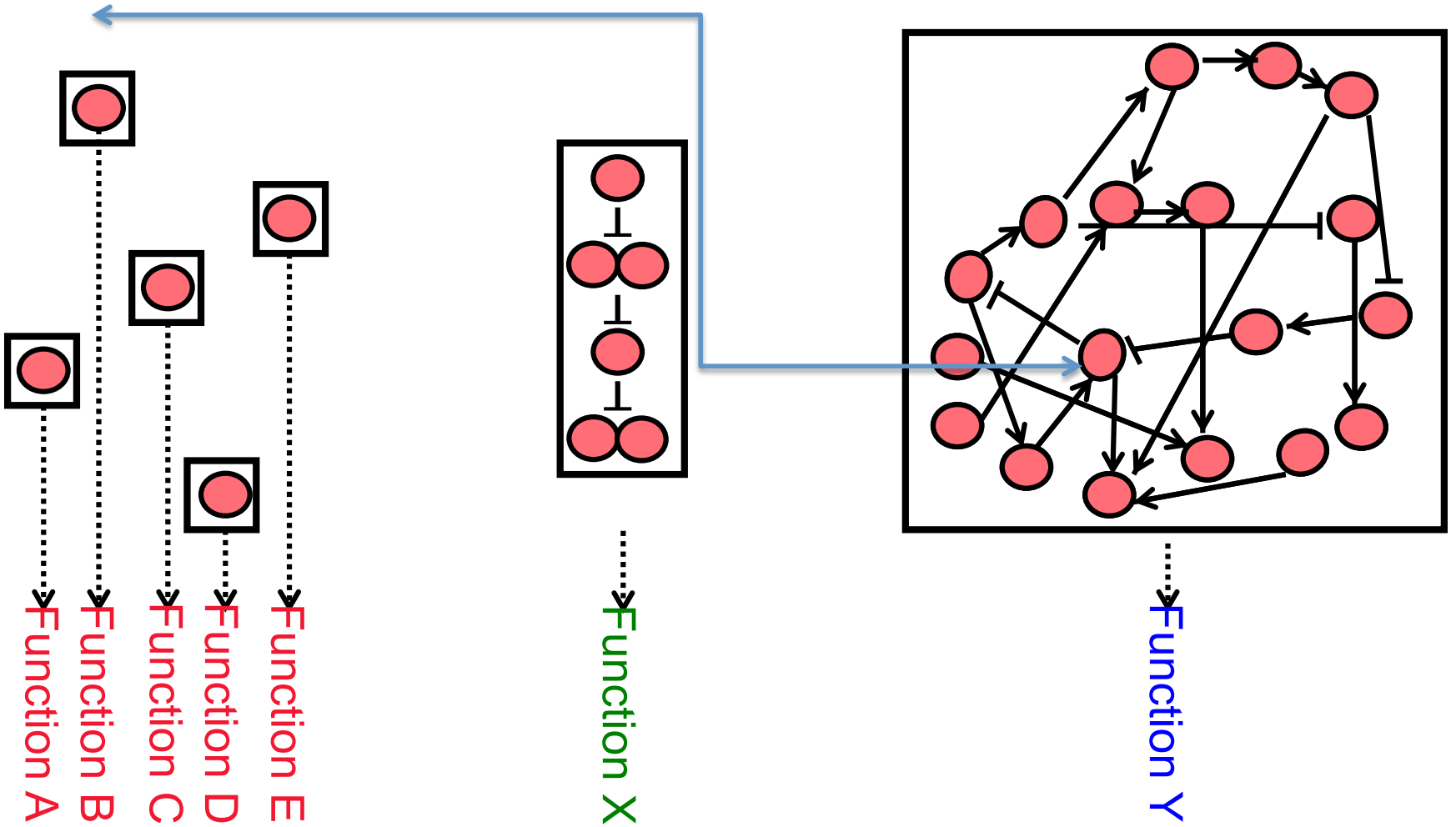
gene-and-protein

1990s

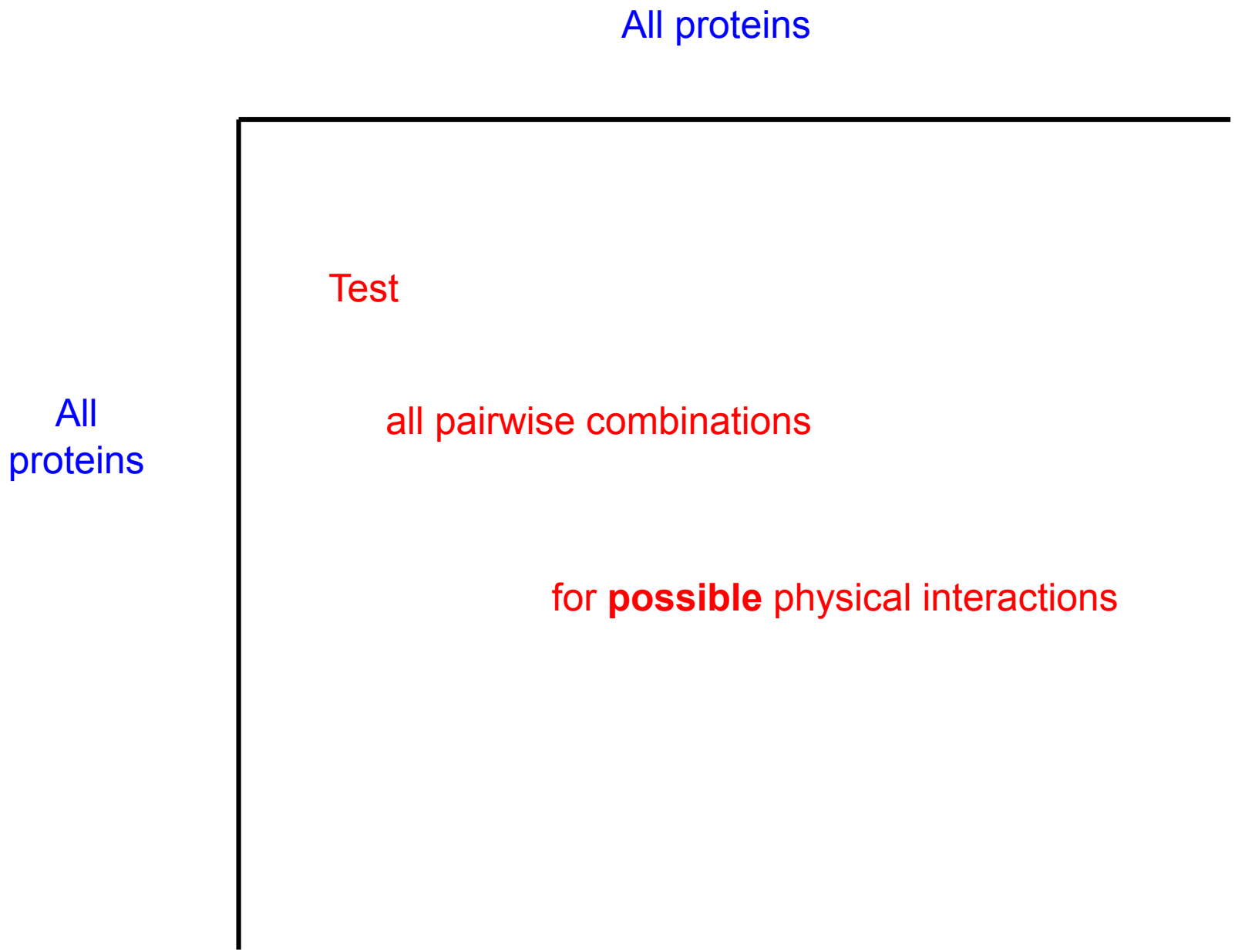
Signaling pathways

2000s

"Interactome" networks

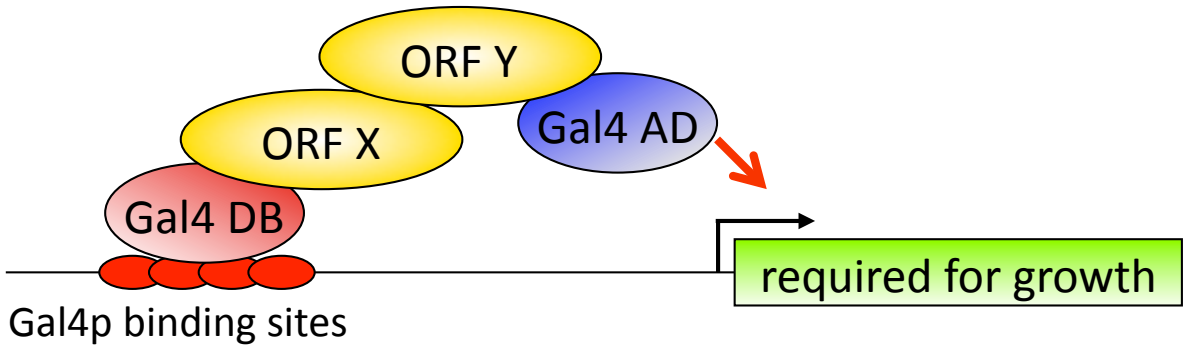


Mapping an interactome network

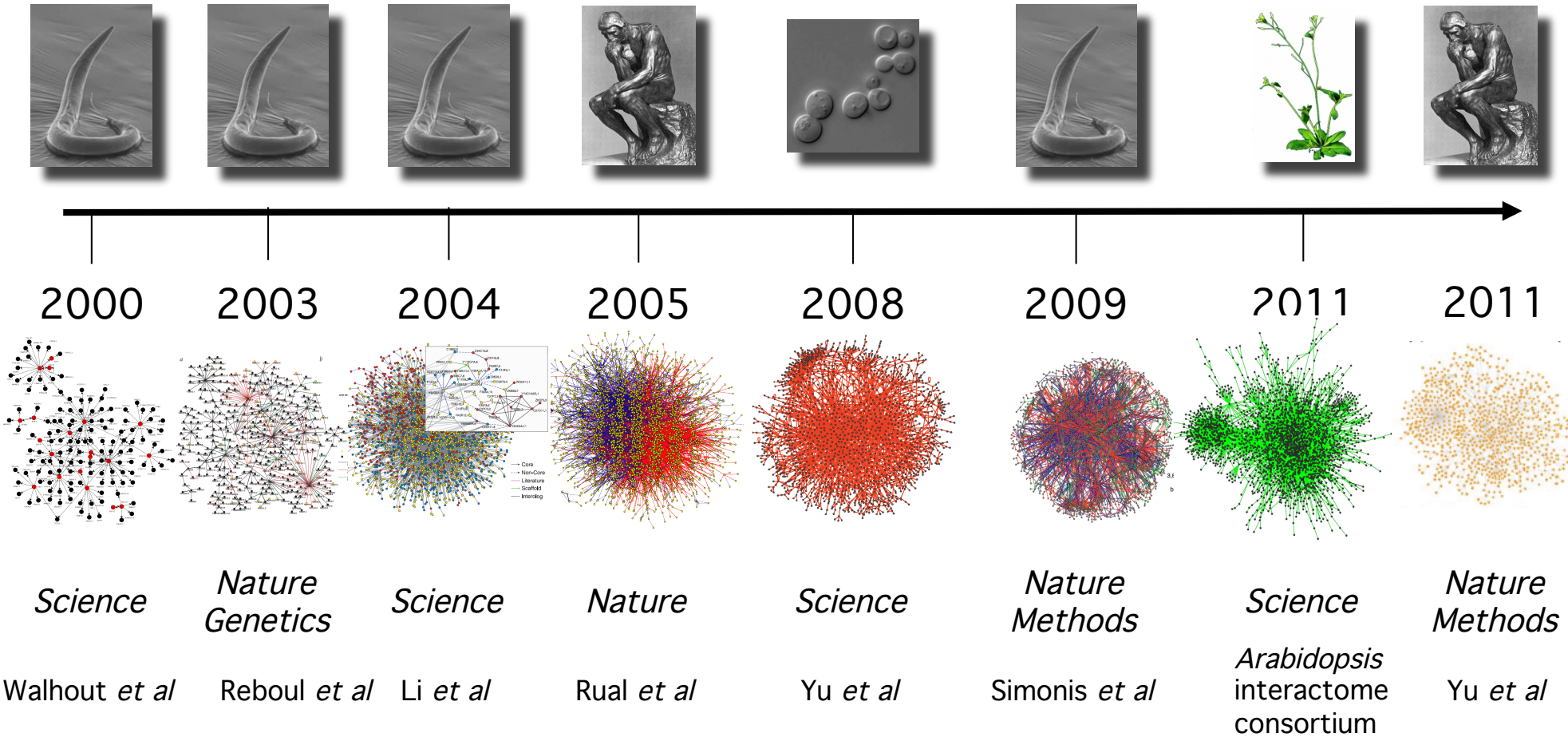


Yeast two-hybrid

- Reconstitution of GAL4 transcription factor
- Fusion proteins DB-ORFX and ORFY-AD
- Reporter gene



Whole organisms mapping

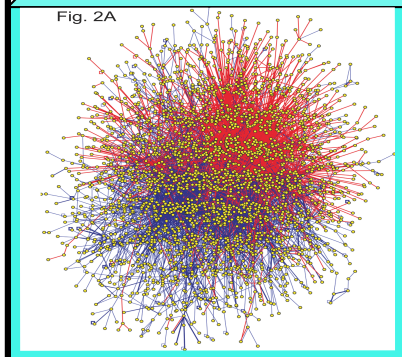


The initial focus on the multicellular model organism *Caenorhabditis elegans* has since been extended to the yeast *Saccharomyces cerevisiae*, the plant *Arabidopsis thaliana*, and human, as well as to interactions between viral and host proteins.

Human interactome

Rual et al.; Nature 437, 1173-1178
Stelzl et al.; Cell 122 (6), 957-68

>22,000 proteins

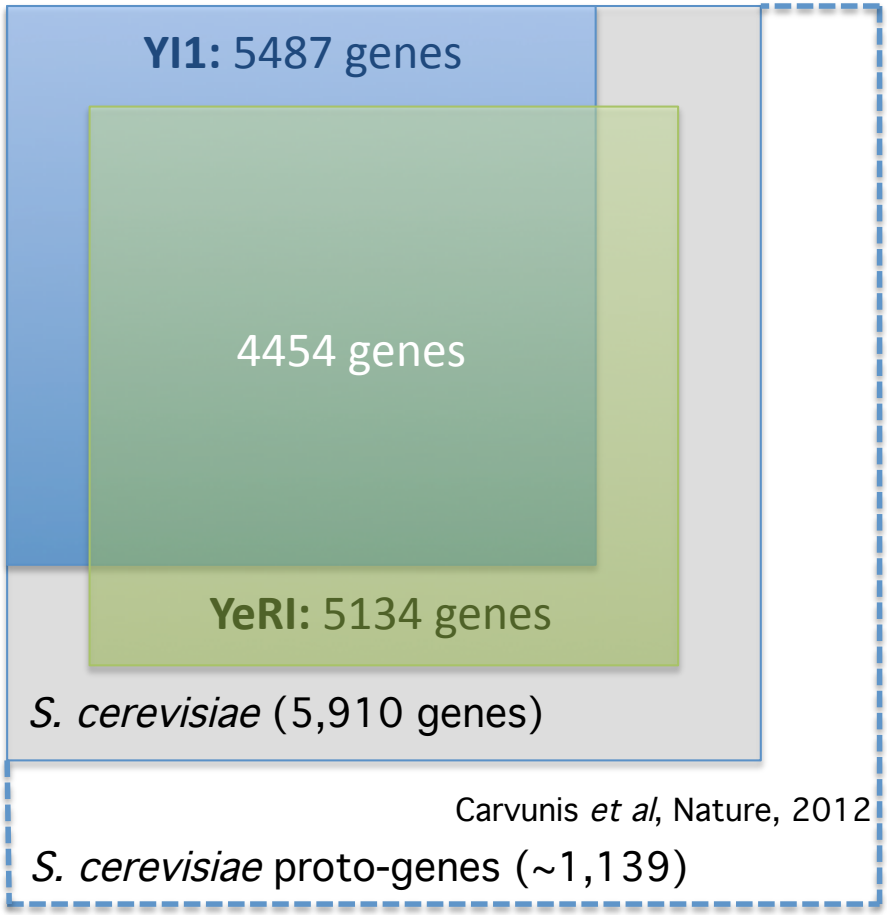


> 5 x 10^8 pairwise combinations

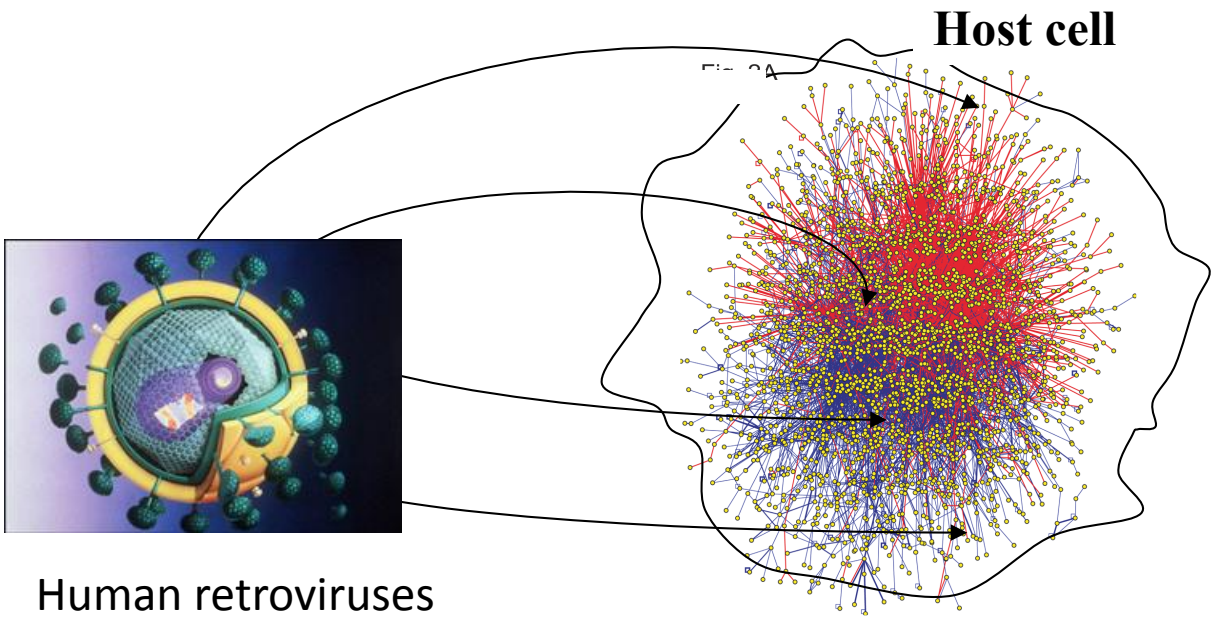
various approaches

>22,000 proteins

Towards completeness of the yeast interactome

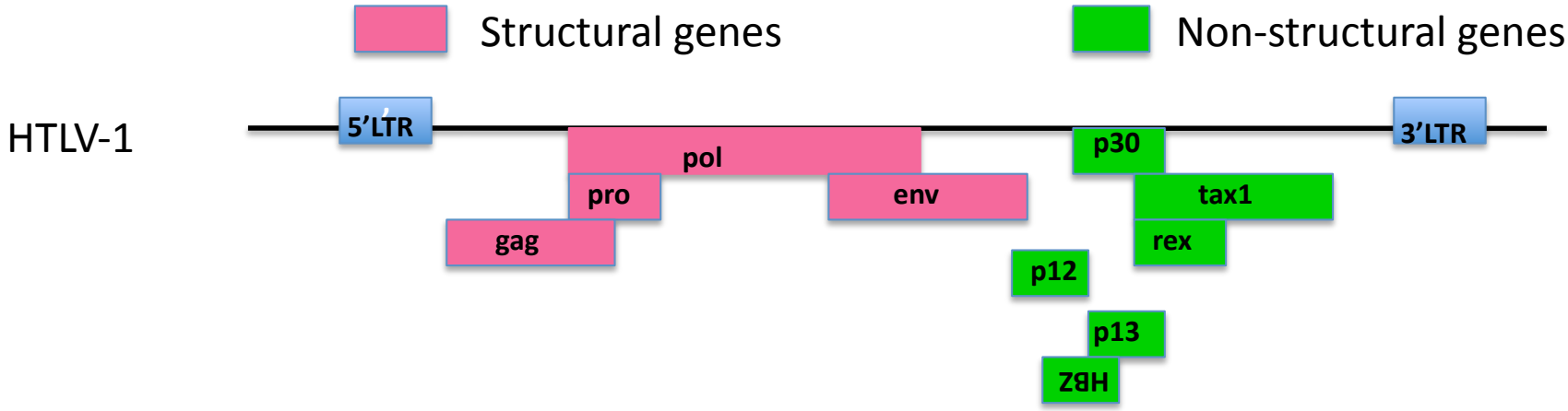


Host – Pathogens interactome

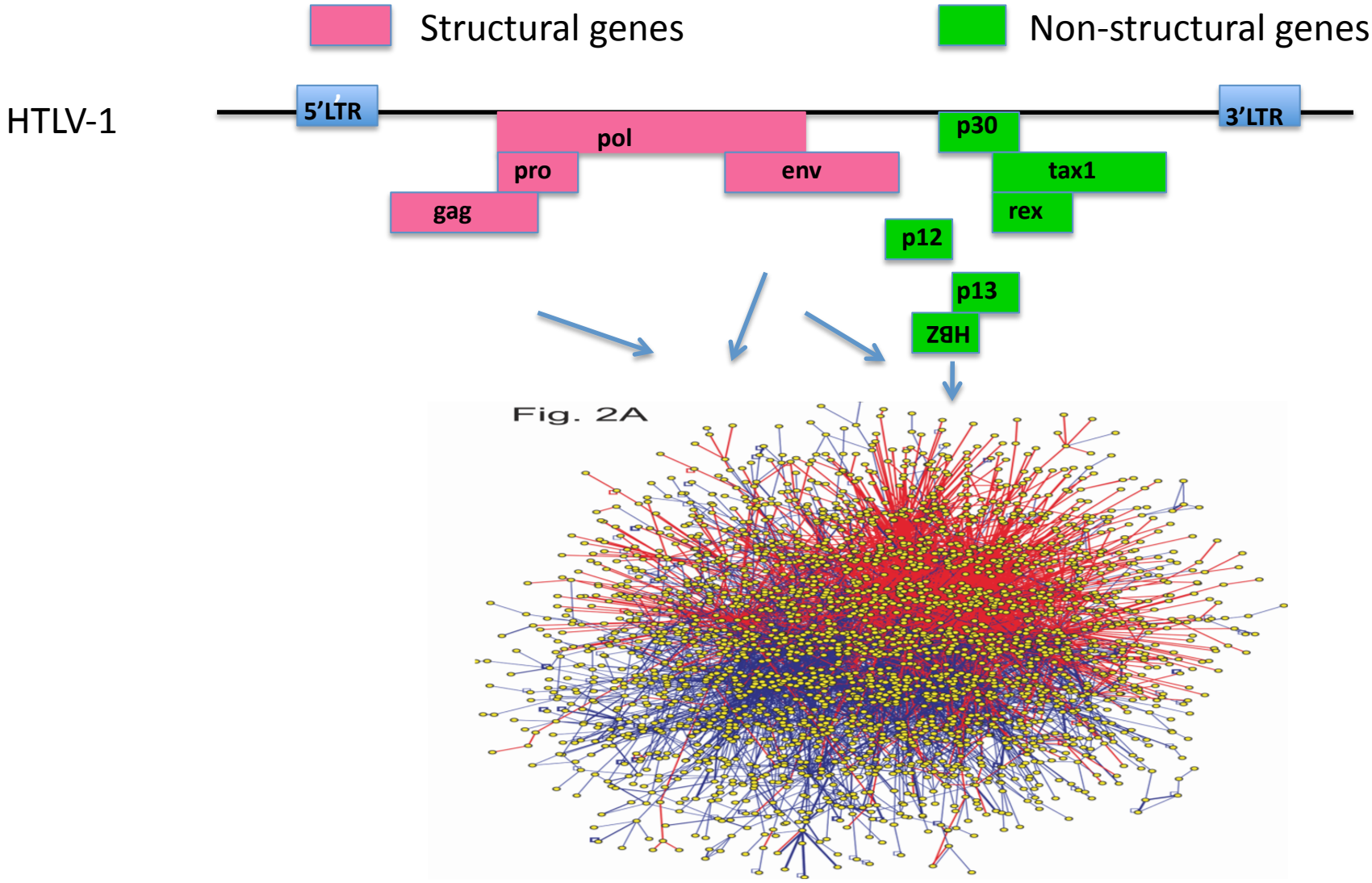


Human retroviruses

Host – Pathogens interactome



Host – Pathogens interactome



Yeast two-hybrid

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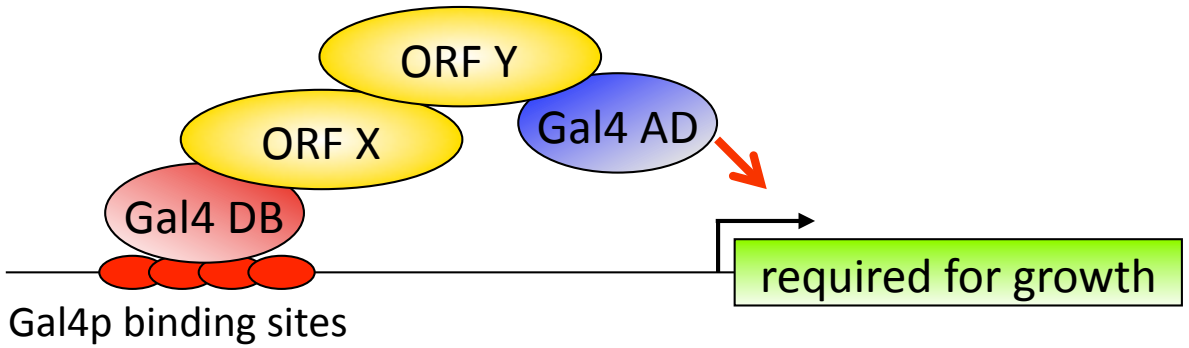
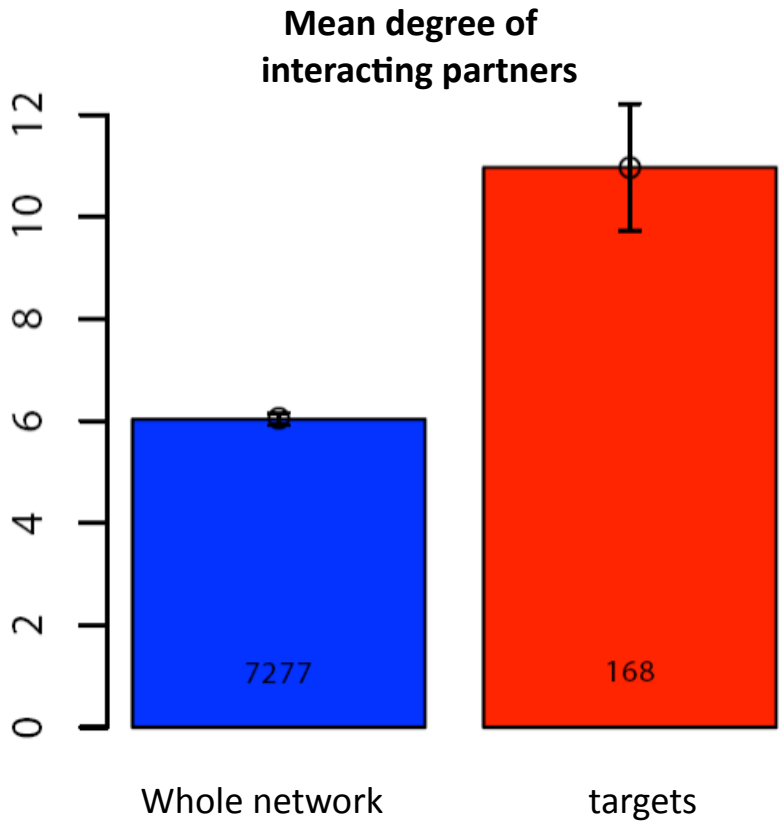
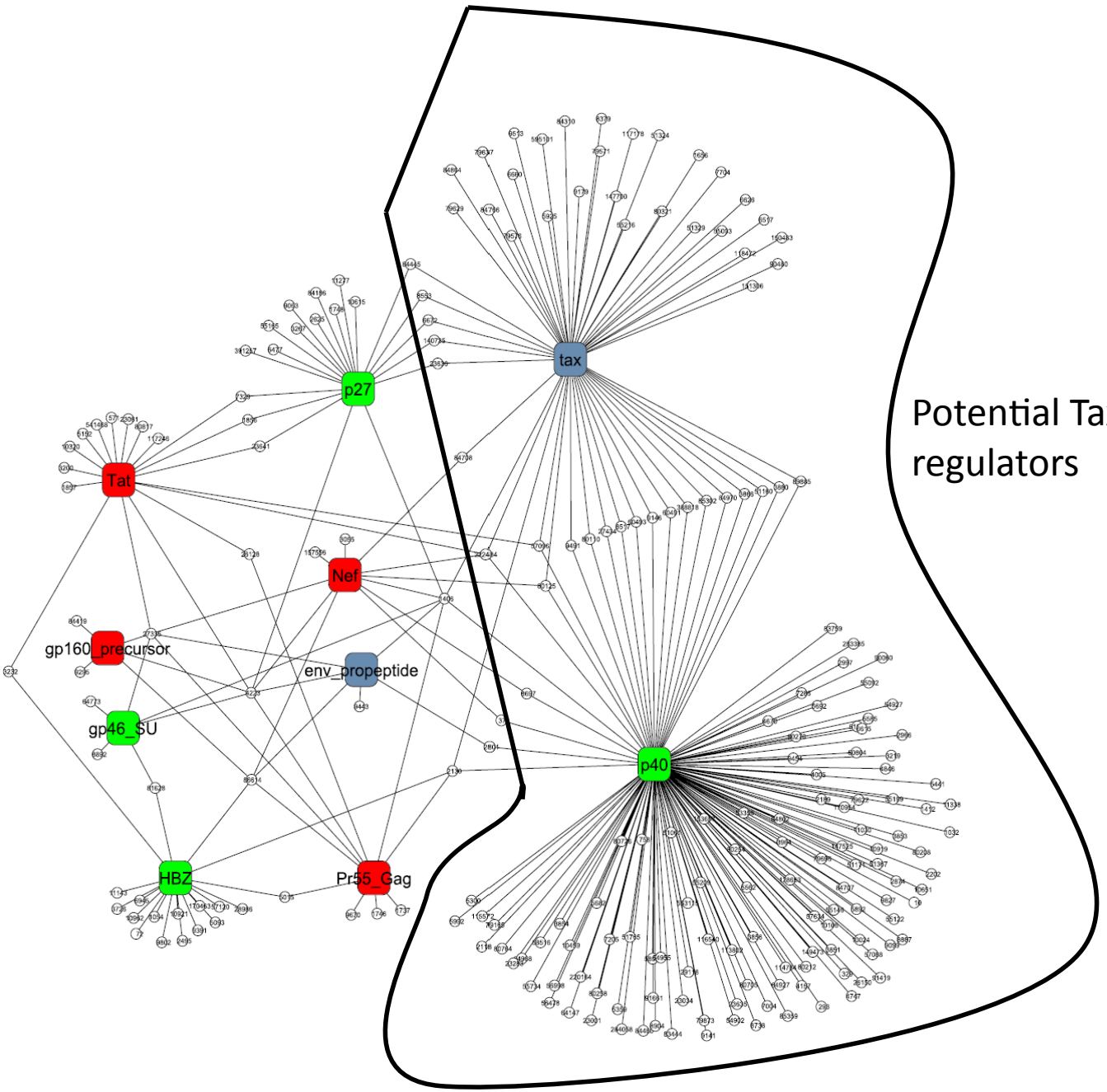


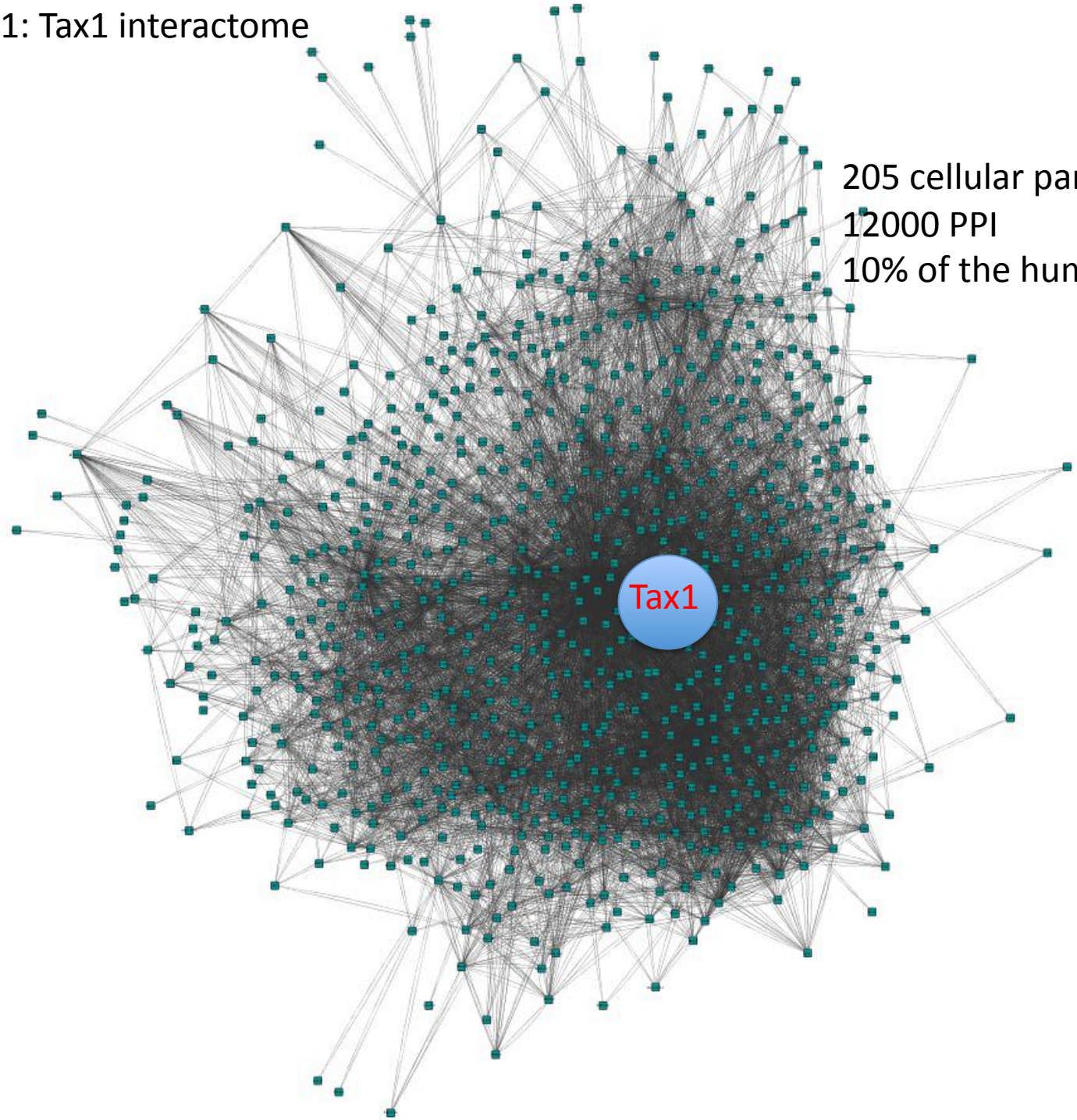


Viral proteins tend to target highly connected proteins in the human interactome

From Interactome networks to biological functions?

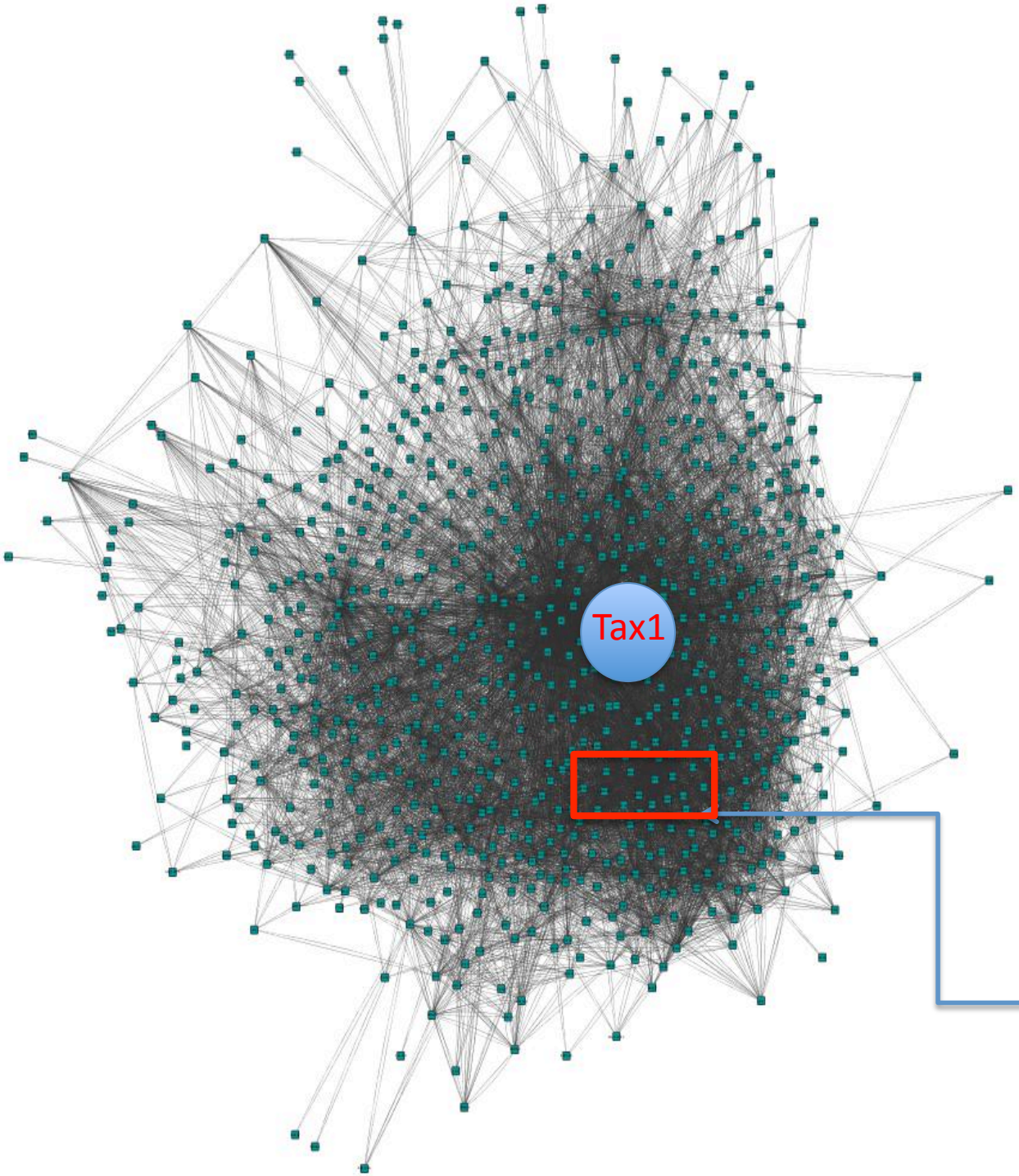


Project 1: Tax1 interactome



205 cellular partners
12000 PPI
10% of the human interactome

-Cell Cycle
-CREB/ATF
-MAPK
-NF-kB
-GPCR
-Wnt
-Notch



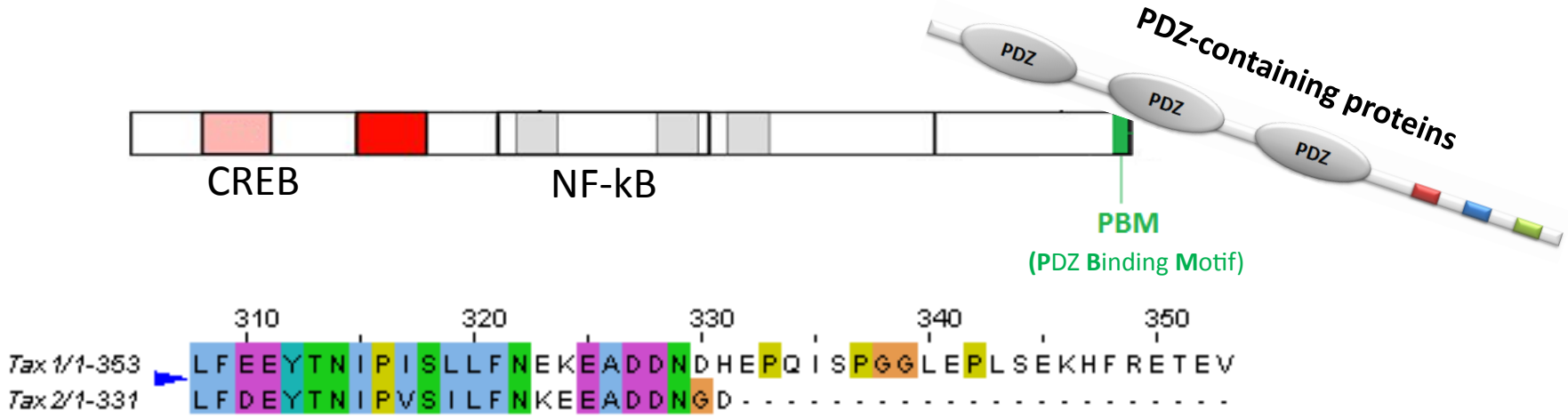
Tax1 interactome

841 cellular partners
12000 PPI
10% of the human interactome

PDZ containing proteins

Host – Pathogens interactome

Tax1



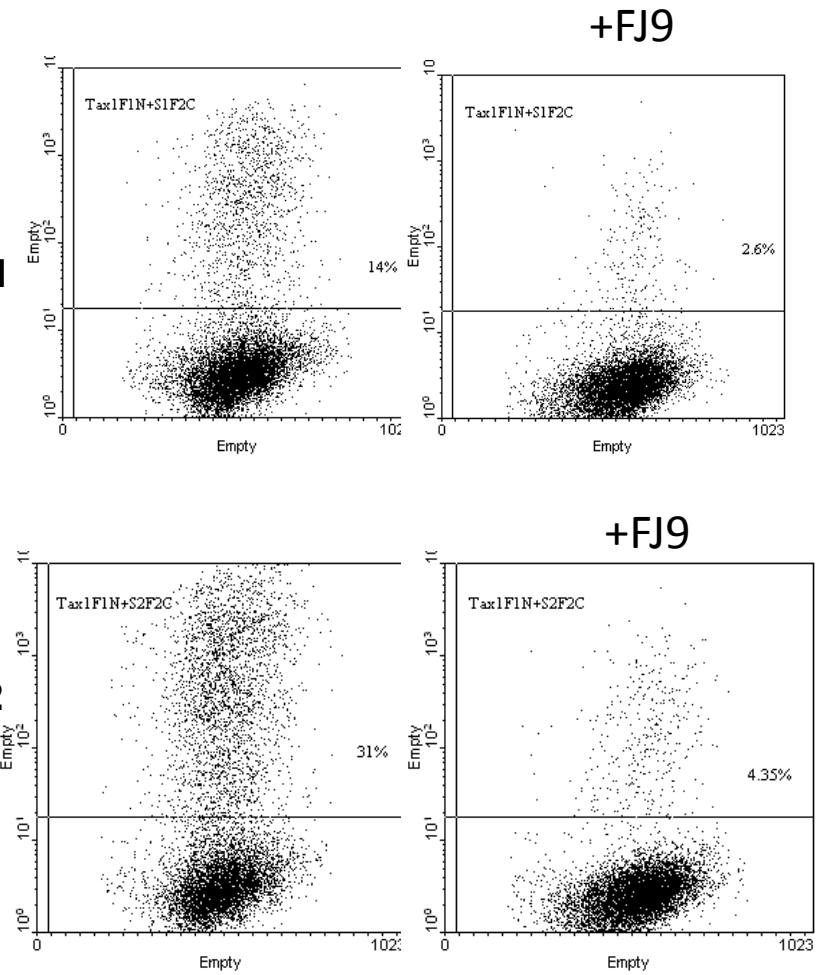
Tax2

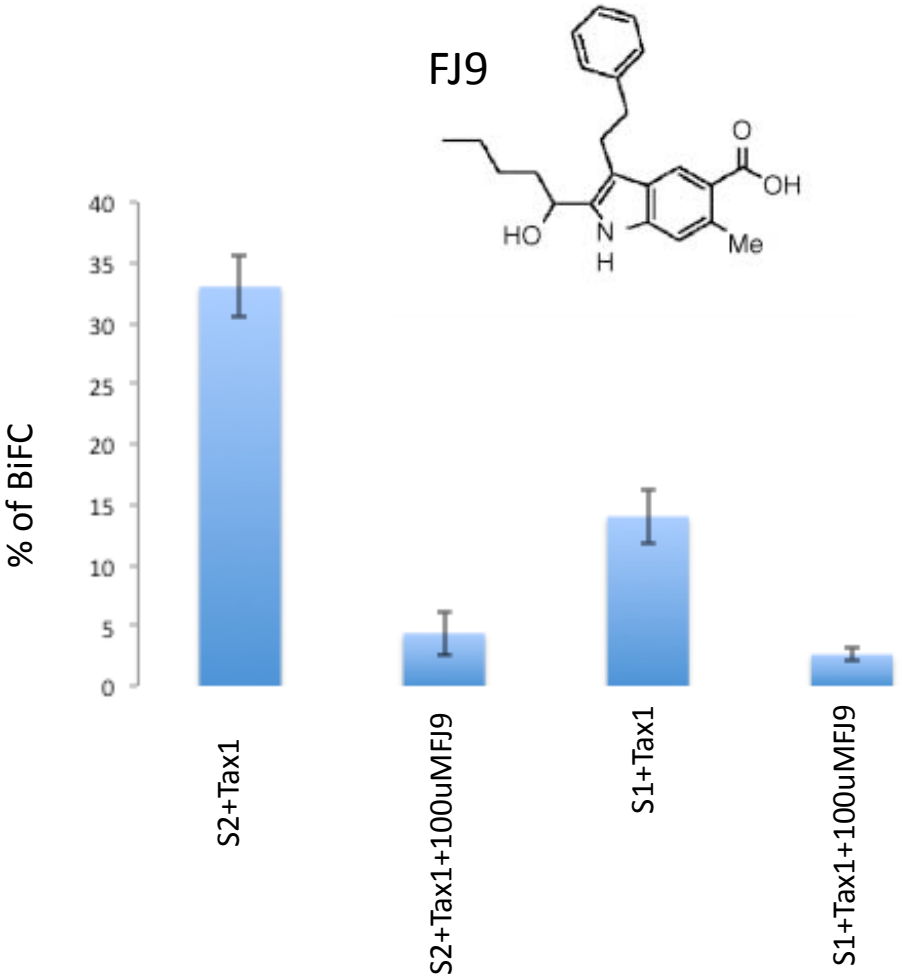
No PBM

Inhibition of protein – protein interactions by small molecules

Syntenin1 +Tax1

Syntenin2 +Tax1

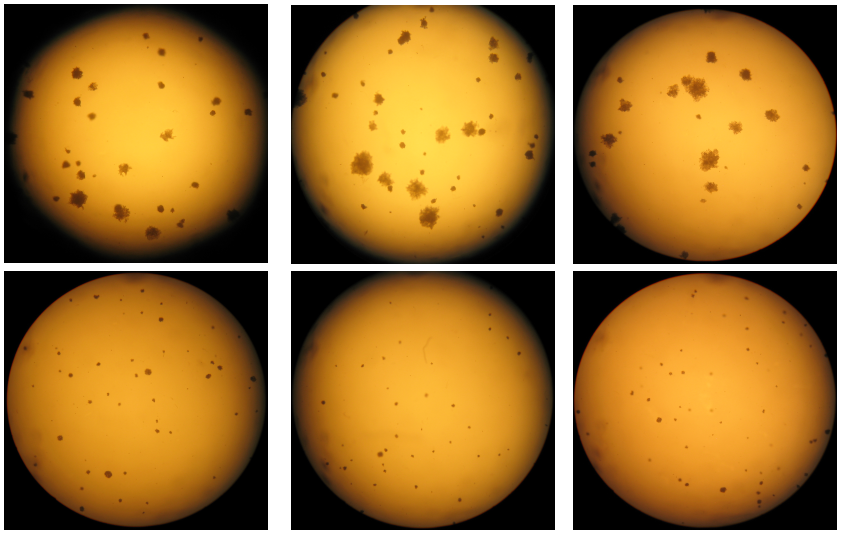




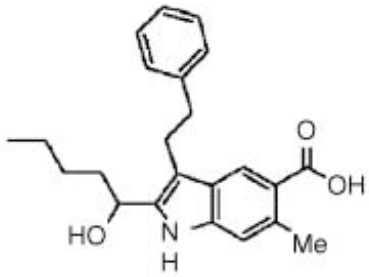
Inhibition of protein – protein and cellular transformation

Rat1-Tax1

Rat1-Tax1 + FJ9



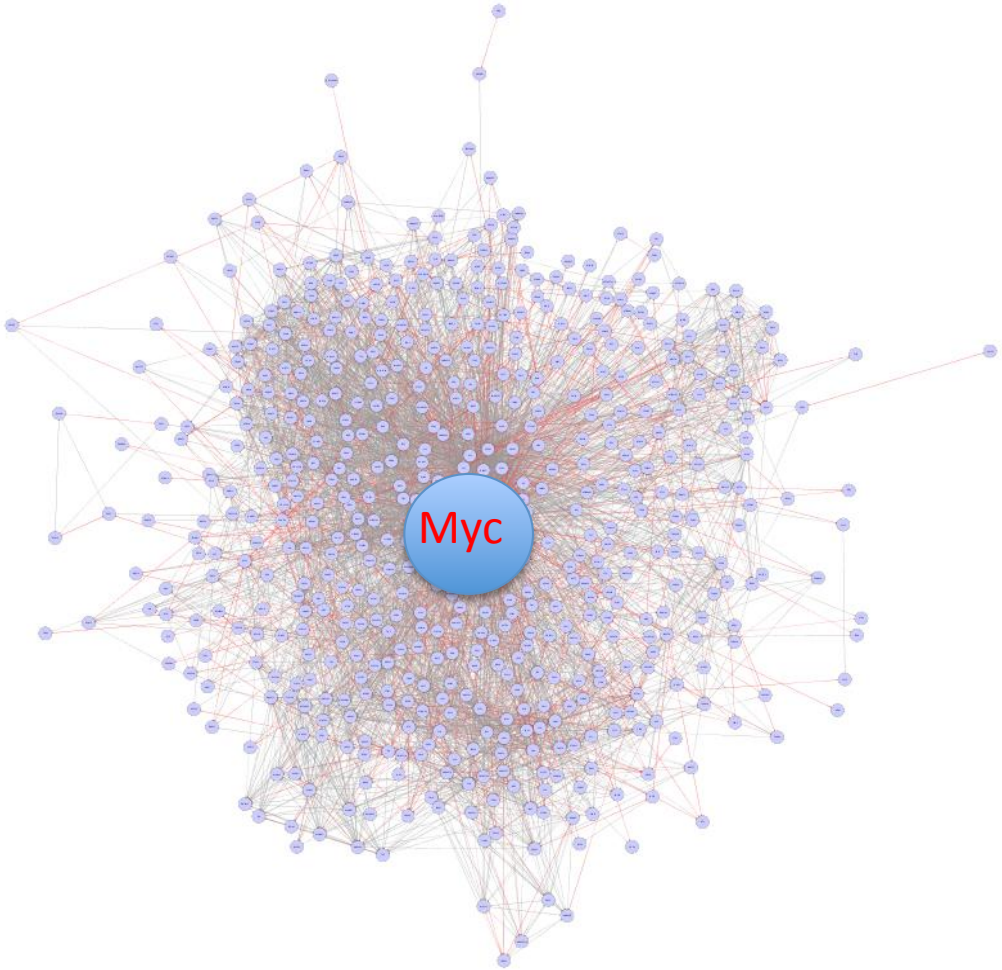
FJ9



Disruption of Tax/ PDZ interaction inhibited Tax transformation as measured by a decrease in size and number of Tax-induced Rat-1 foci.

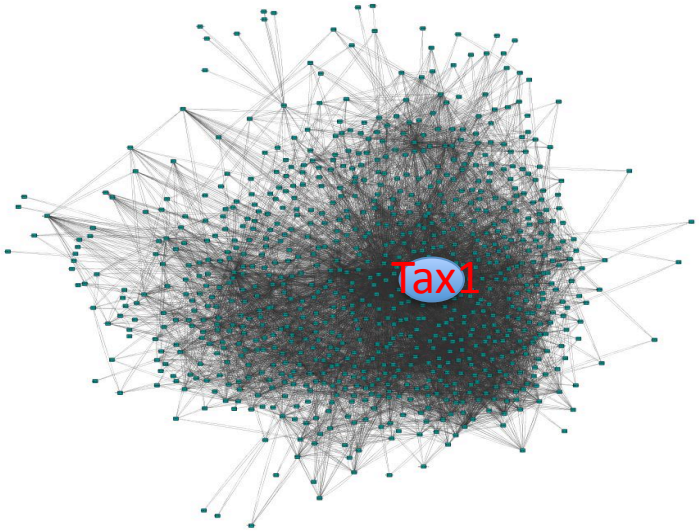
PDZ proteins involved in Tax1 transformation activity

Cellular oncogenes vs viral oncogenes

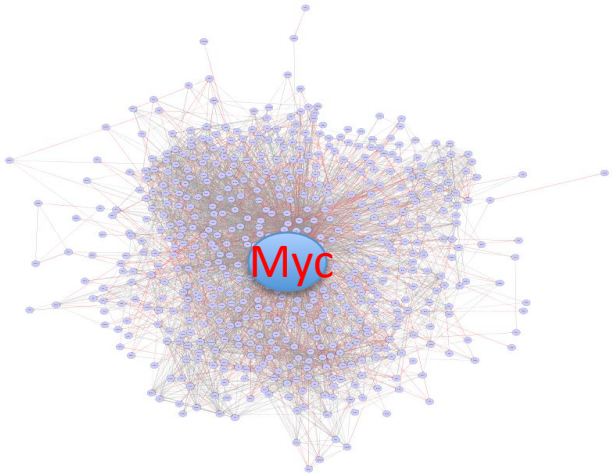


Most TEL-AML1 perturbed PPI interactions involve the Myc cellular oncogene

Viral oncogene interactome



Cellular oncogene interactome



Hypothesis:

Unrelated diseases may have a shared genetic/interactome basis?