

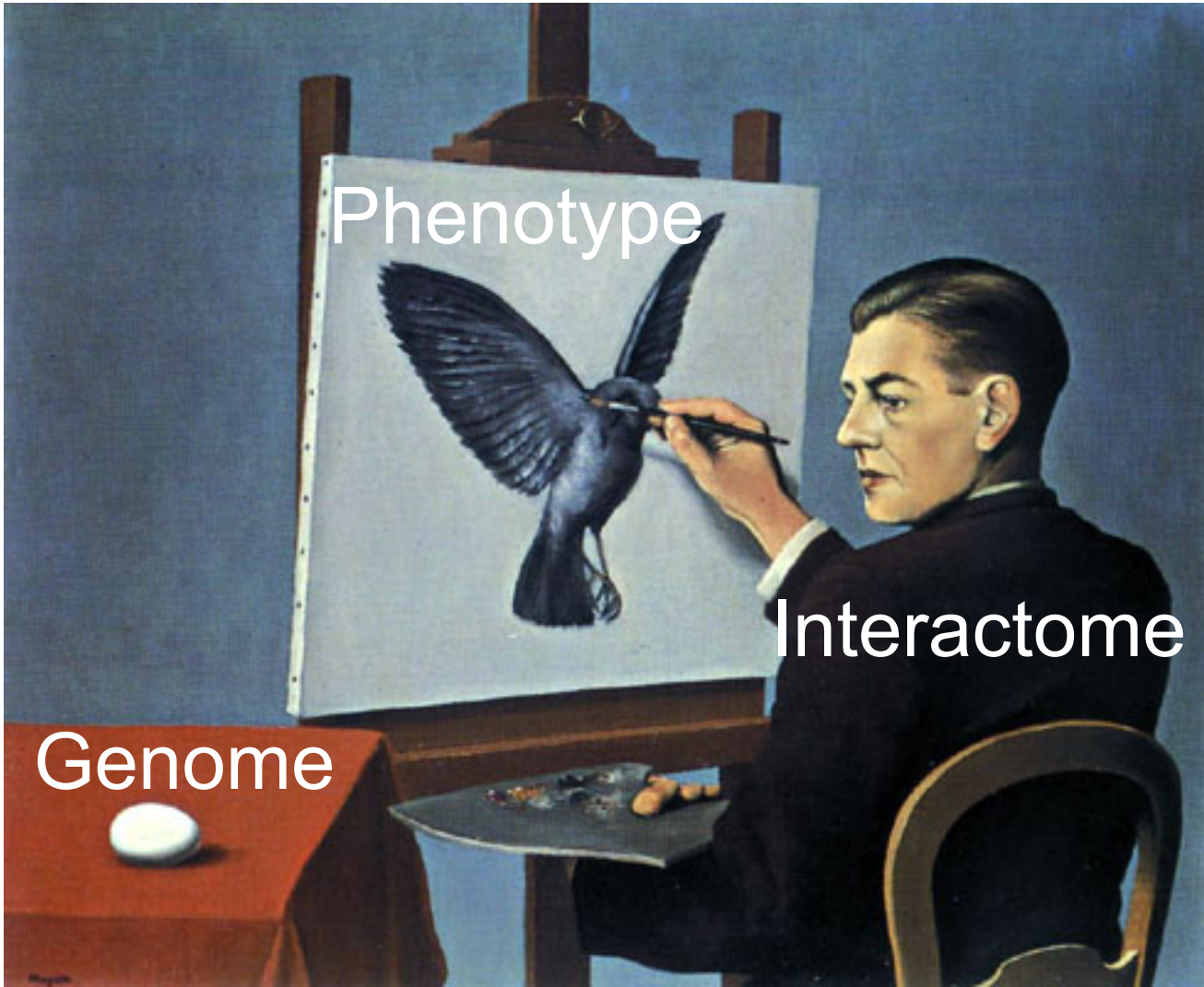


Protein – protein interactions

*Interactome networks drive
molecular organisation of the cell*

Dr. Jean-Claude Twizere

Jean-claude.twizere@ulg.ac.be



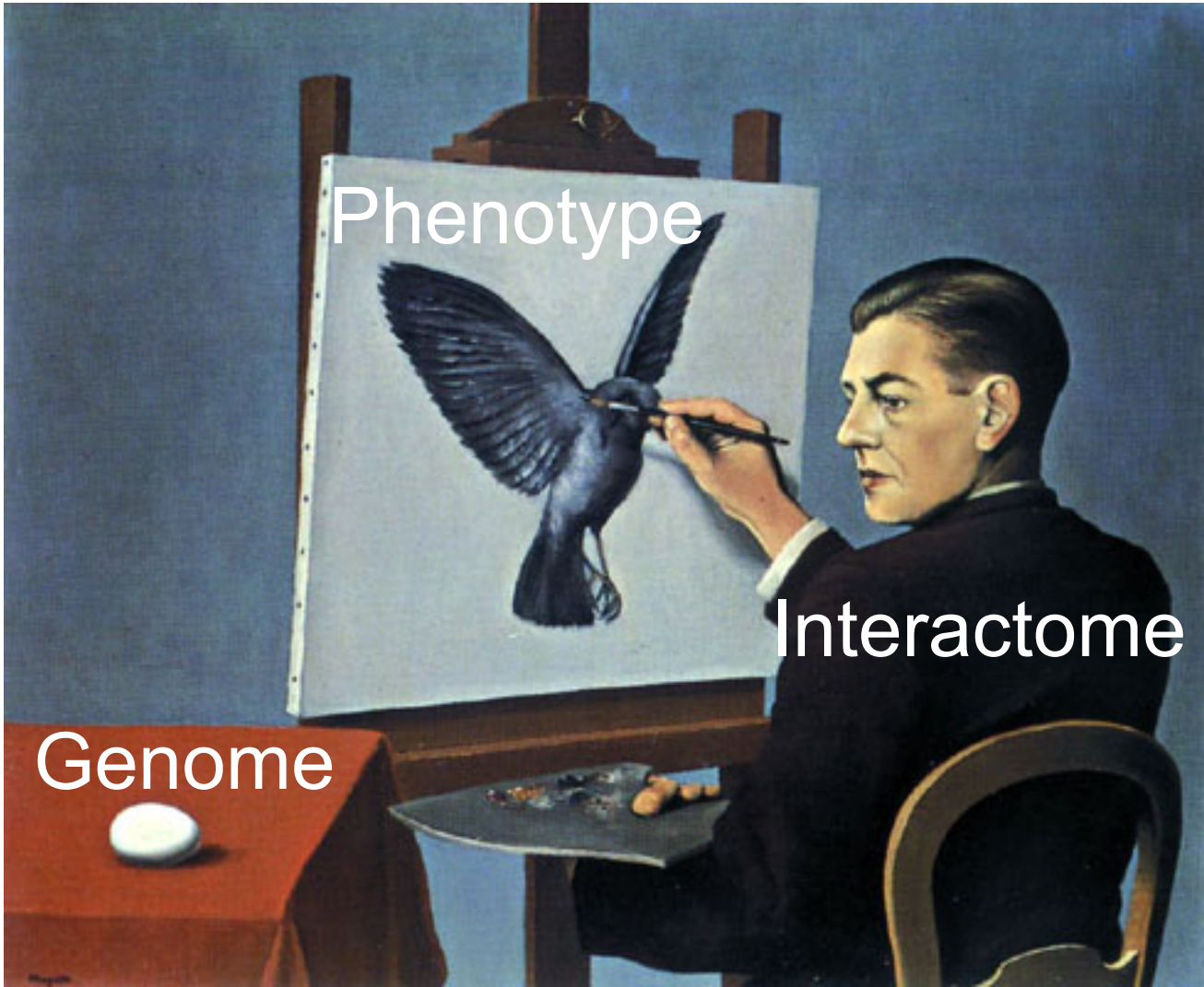
Phenotype

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 interactions
- Interaction networks essential to understand biology, disease and/or drug action

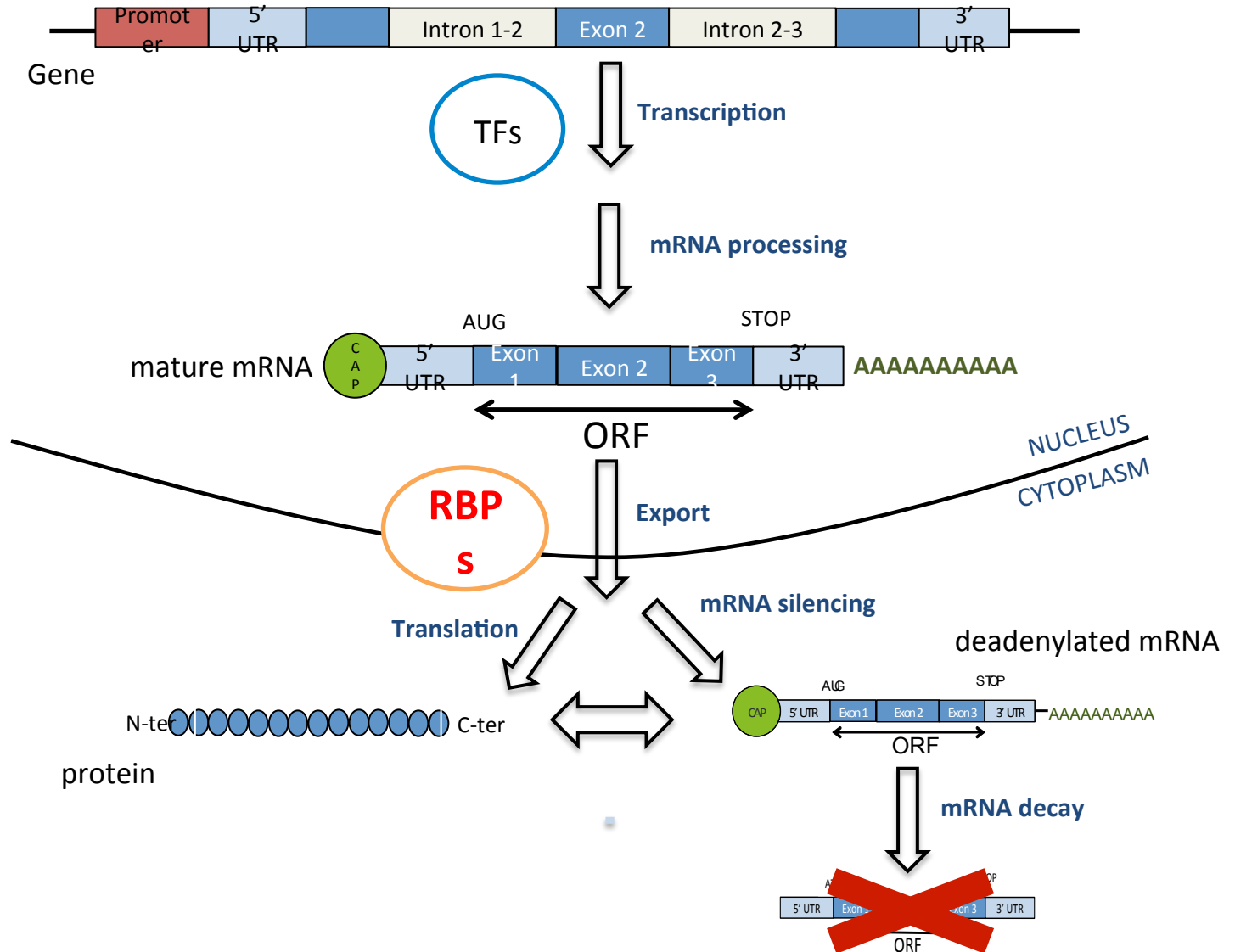


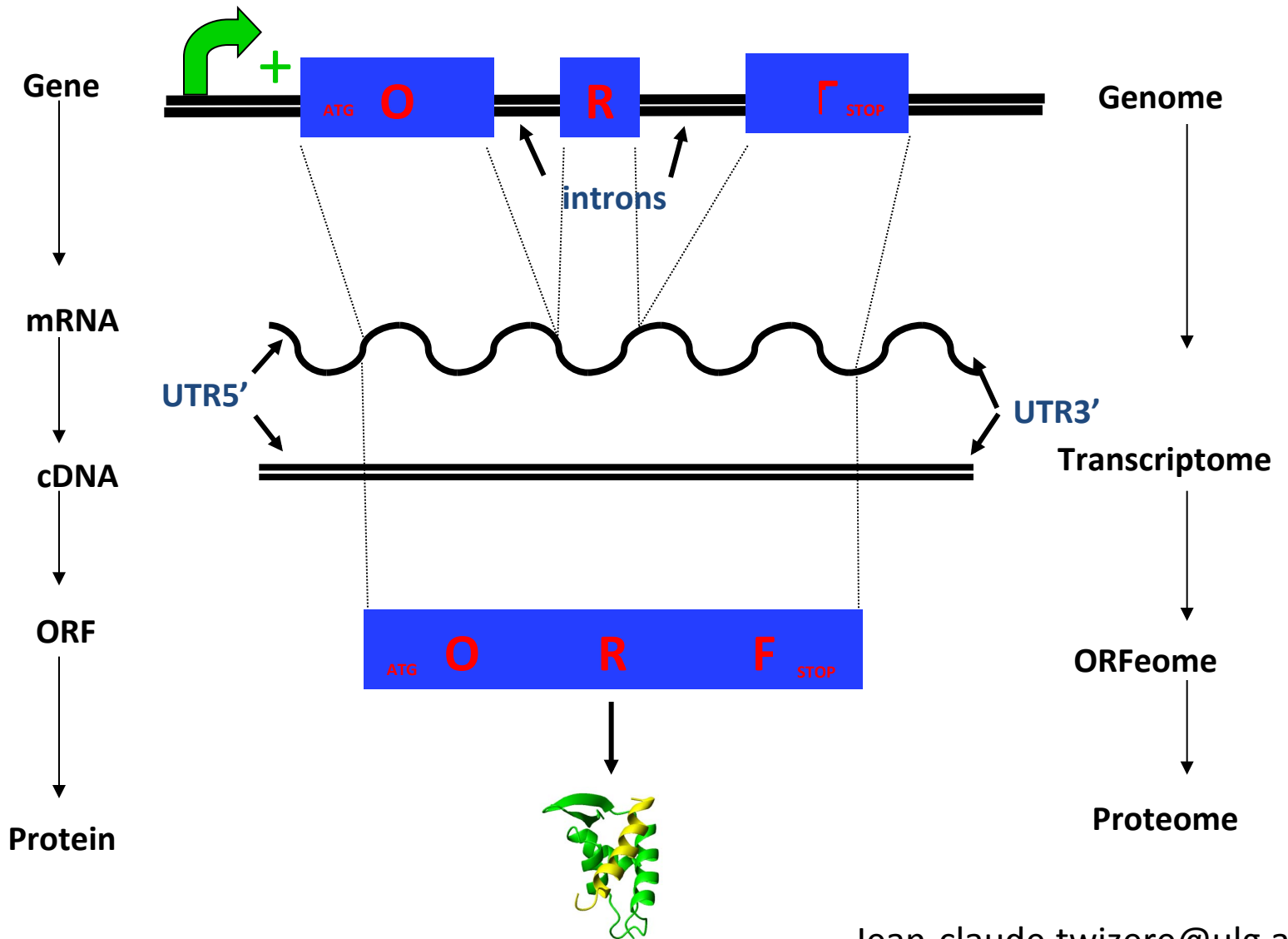
Phenotype

Interactome

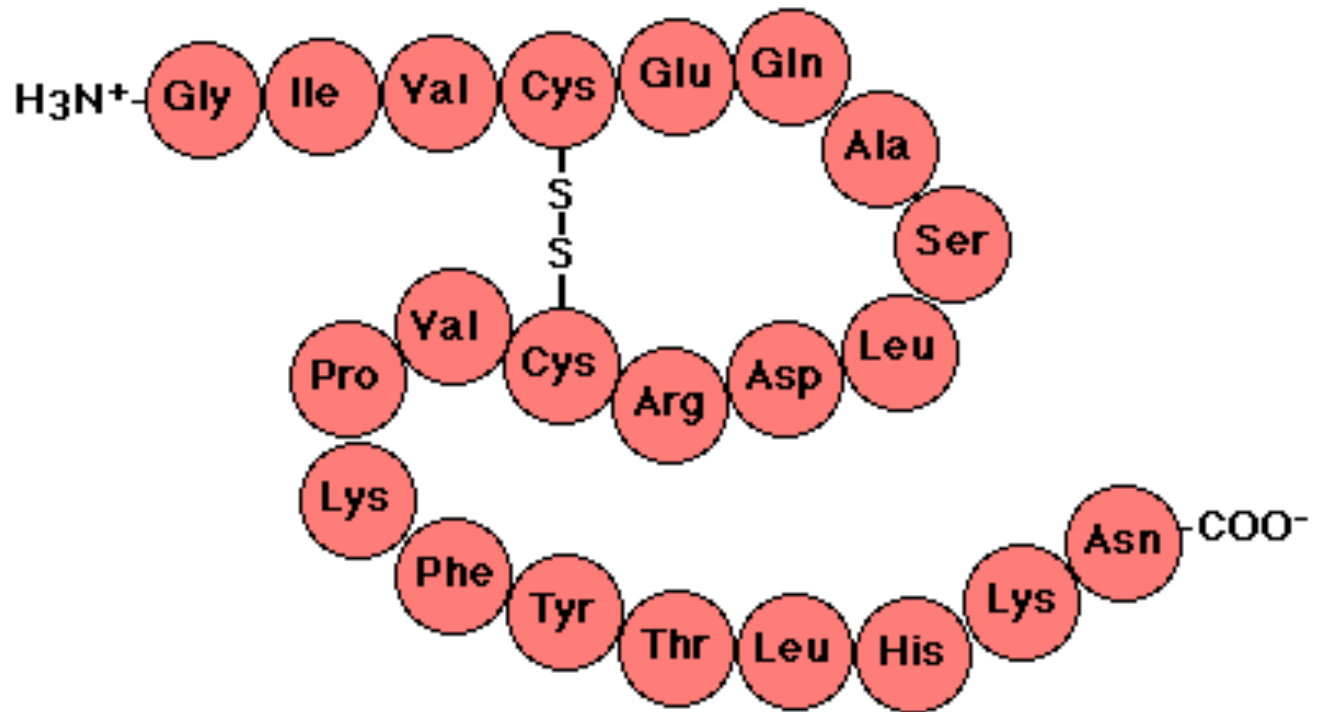
Genome

Gene expression regulation



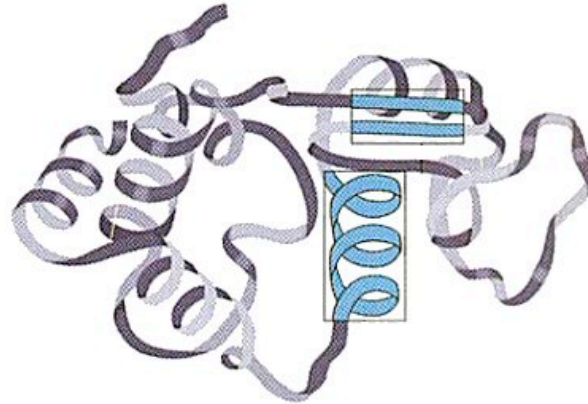


Primary structure

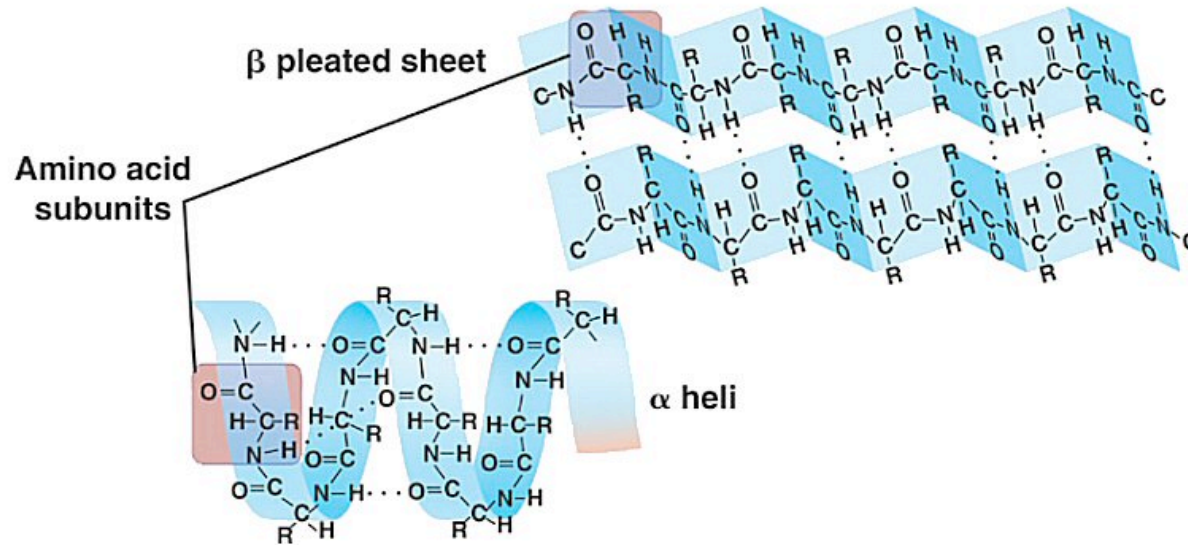


Secondary and tertiary structure

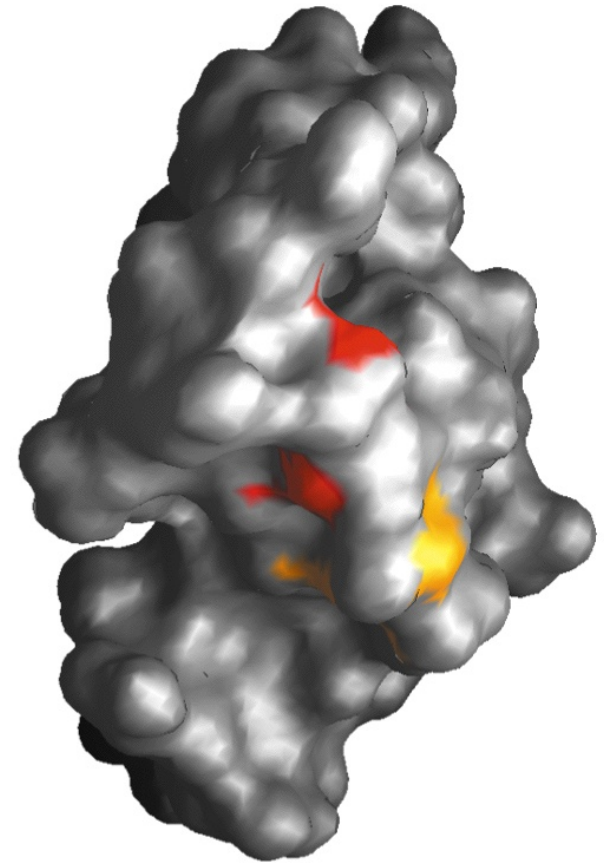
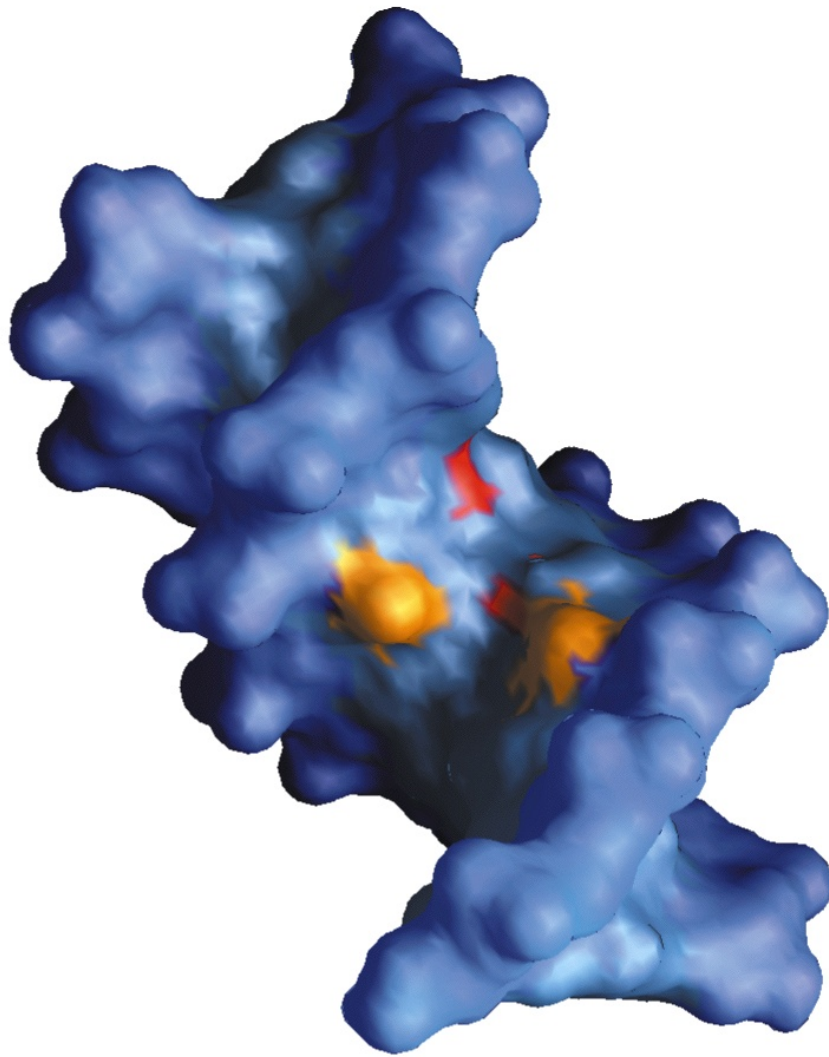
III.



II.

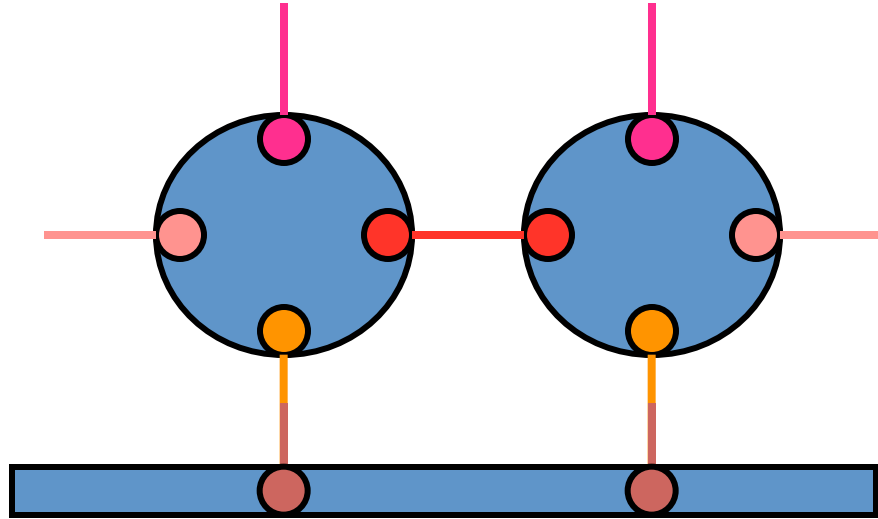


Quaternary structure



(d)

Protein Interactions

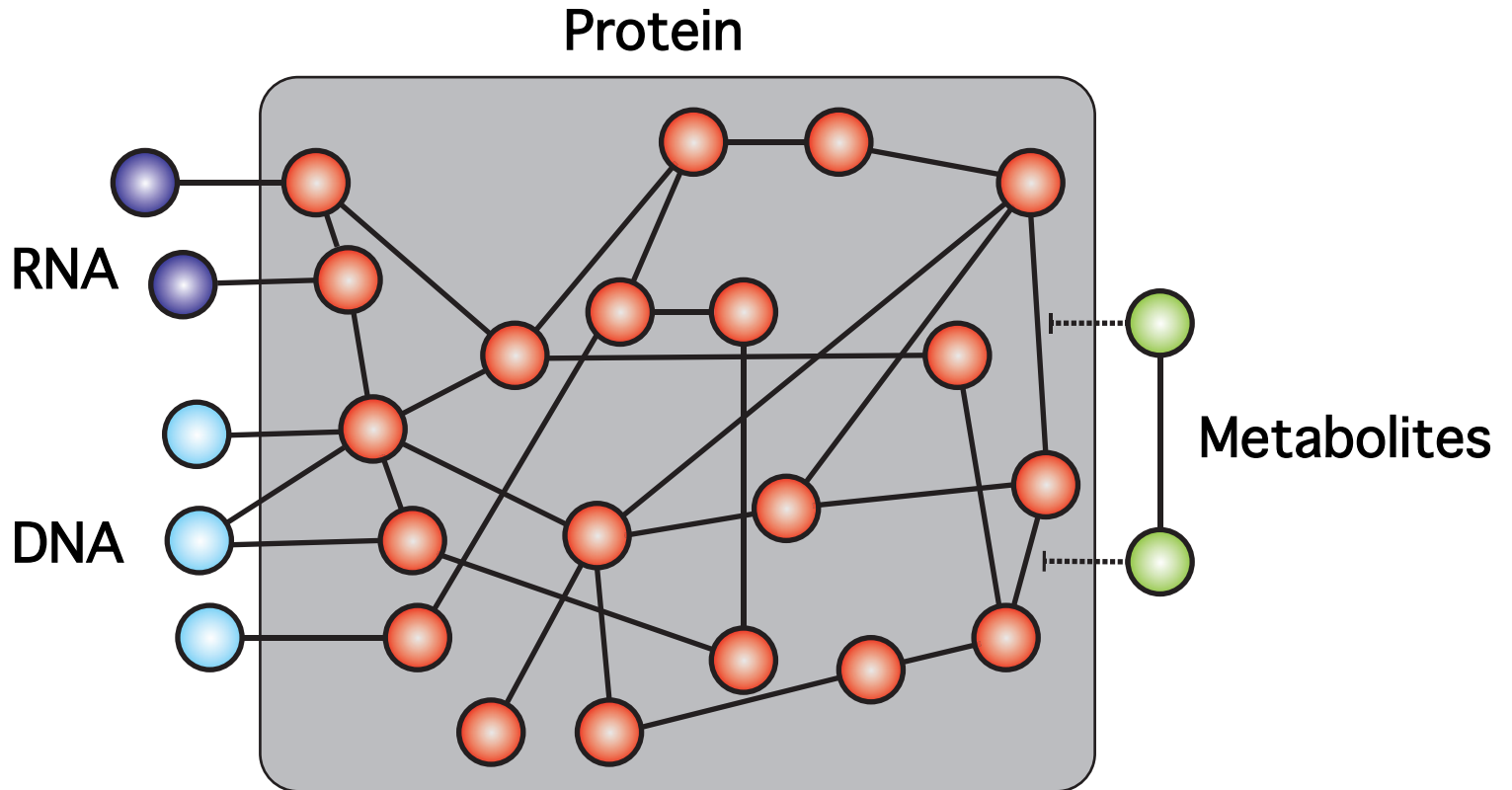


Homodimerization and DNA/protein interaction

Protein-protein interactions

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

The protein interactome network



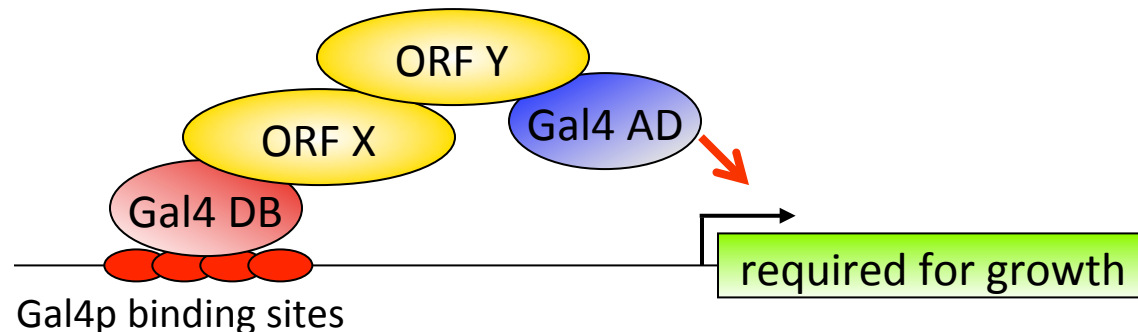
Nodes: Proteins, DNA, RNA or Metabolites

Edges: Bio-physical interactions

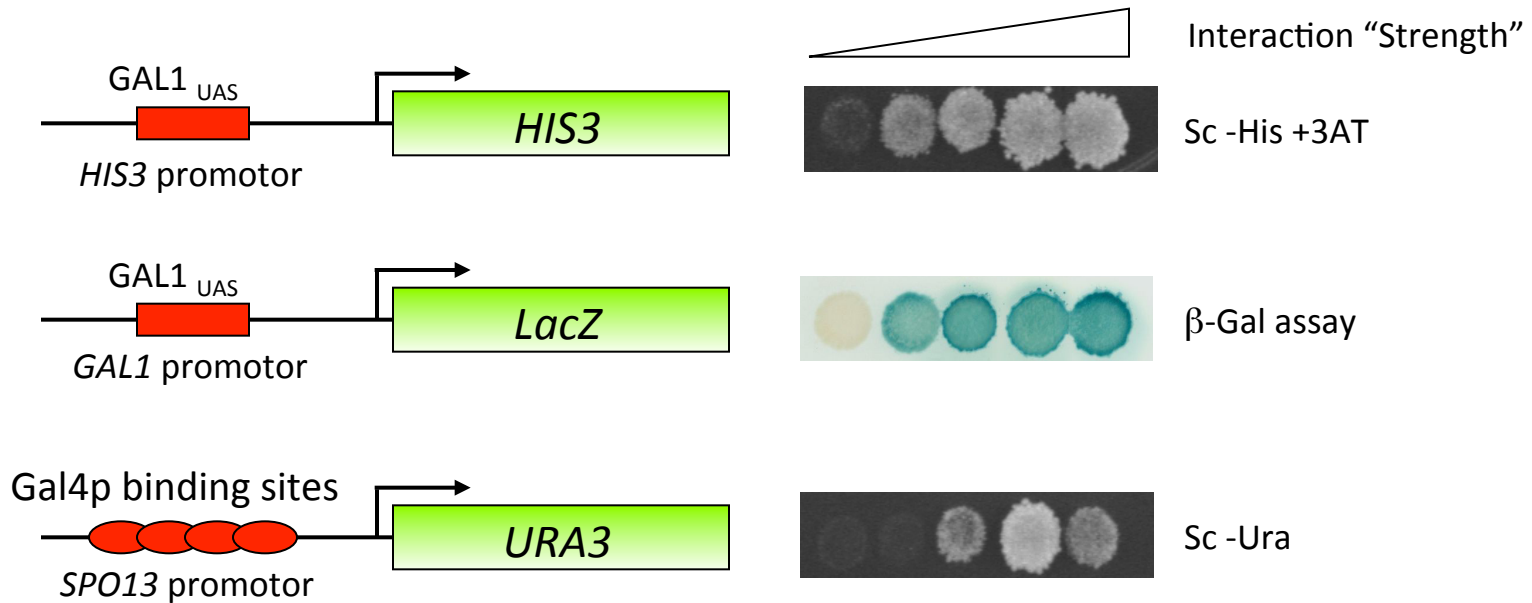
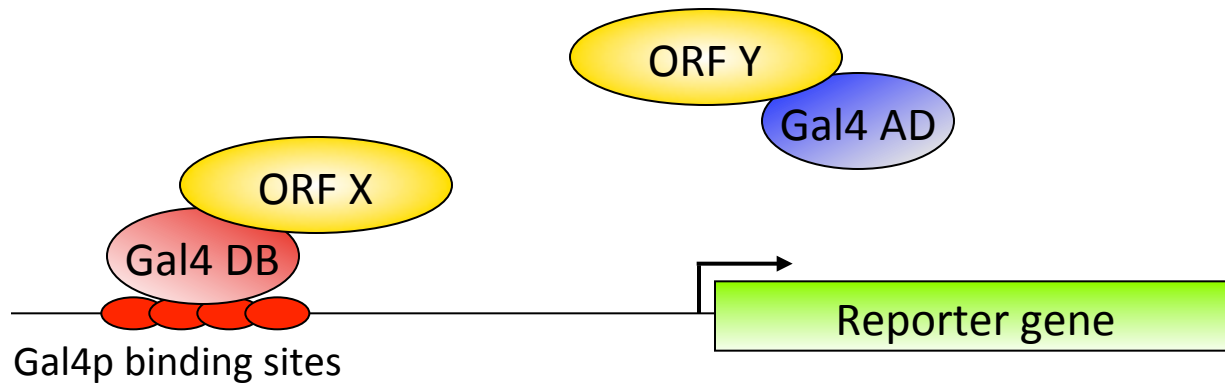
Discovering interactions: Yeast two-hybrid

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



Yeast two-hybrid

Reagents (retroviruses side)

genes + fragments

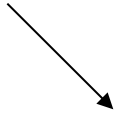


Gateway cloning

DB, AD expression vectors

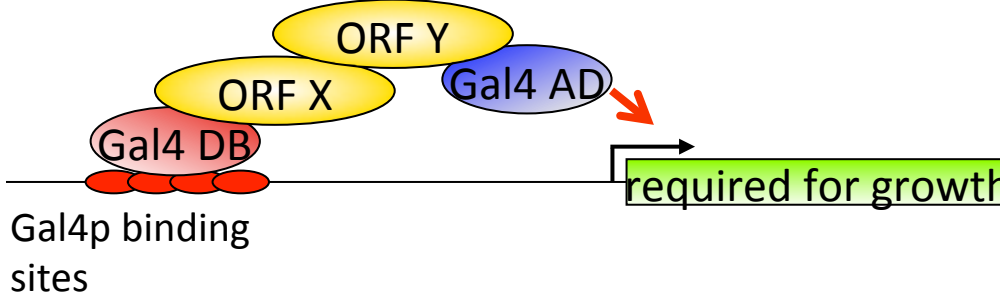
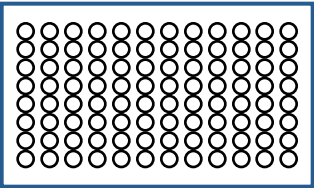
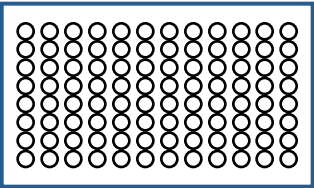


Yeast transformation

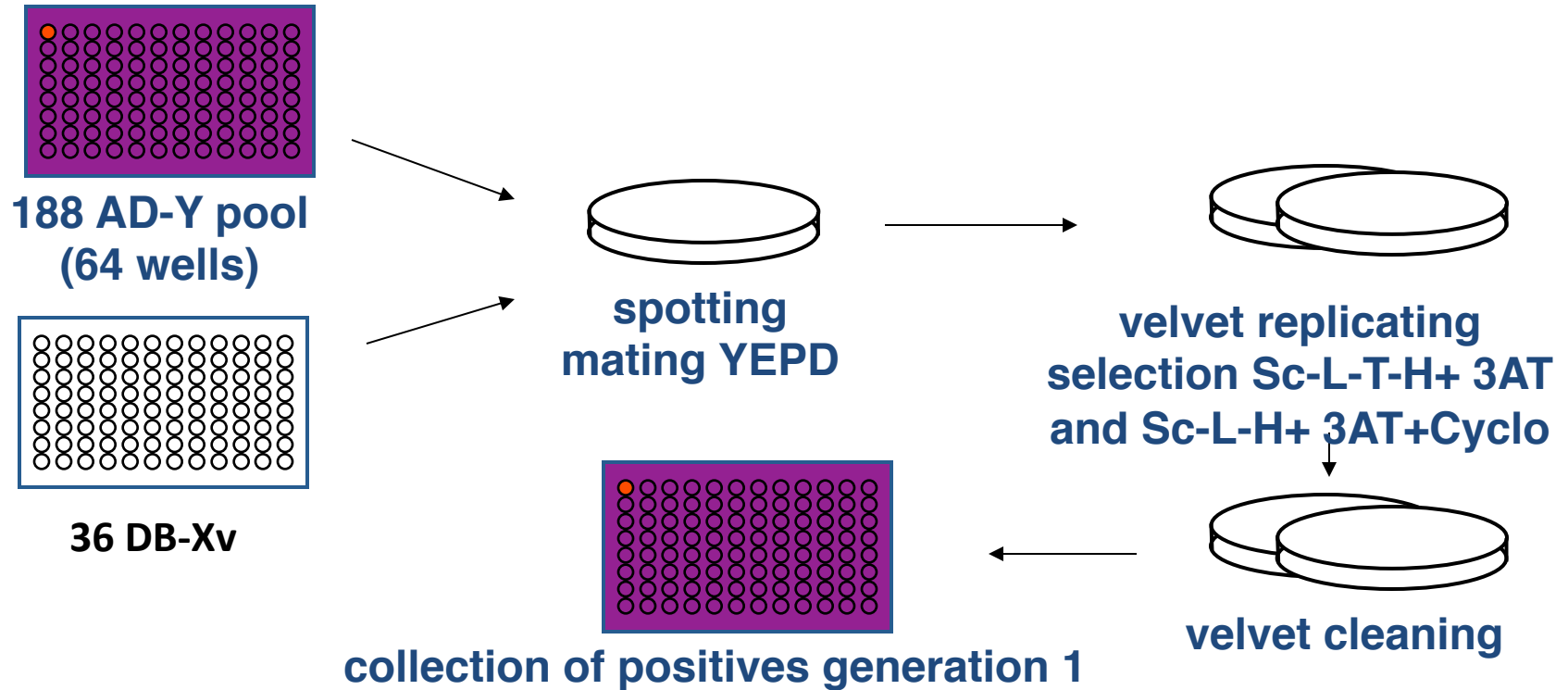


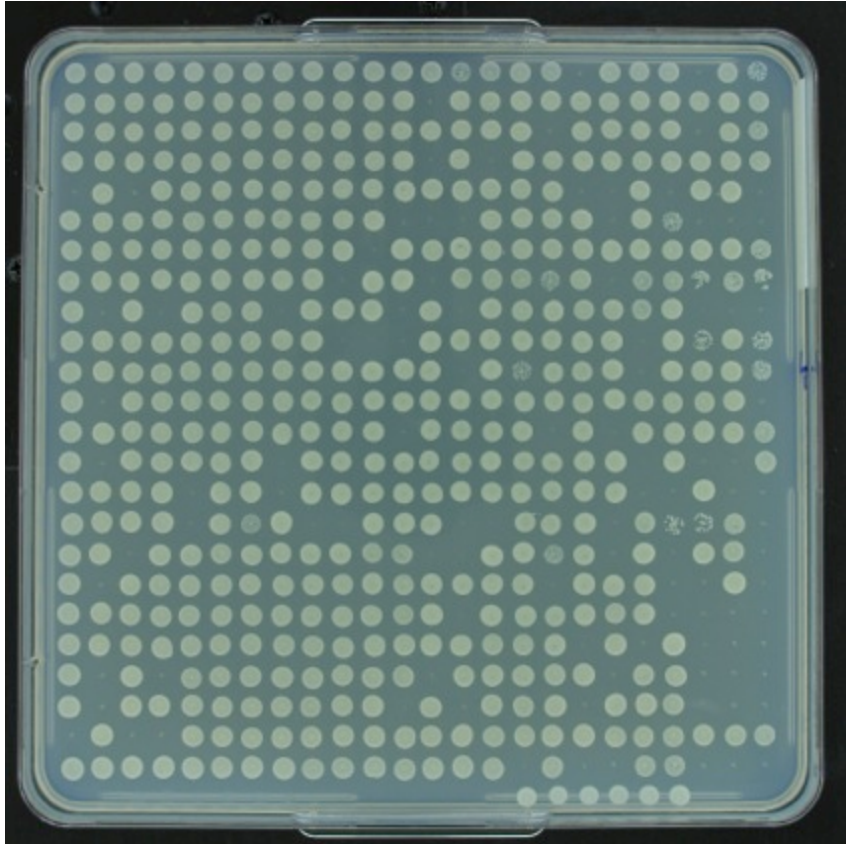
Mat a (Y8900 or Mav103)

Mat a (Y8800 or Mav203)

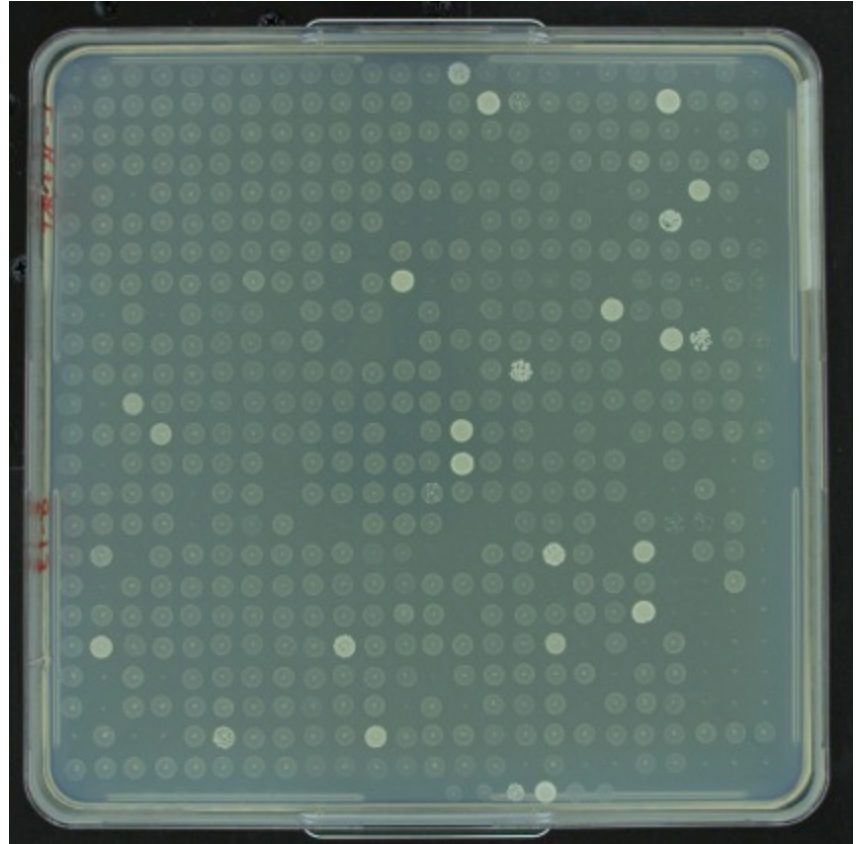


High-throughput Y2H mating



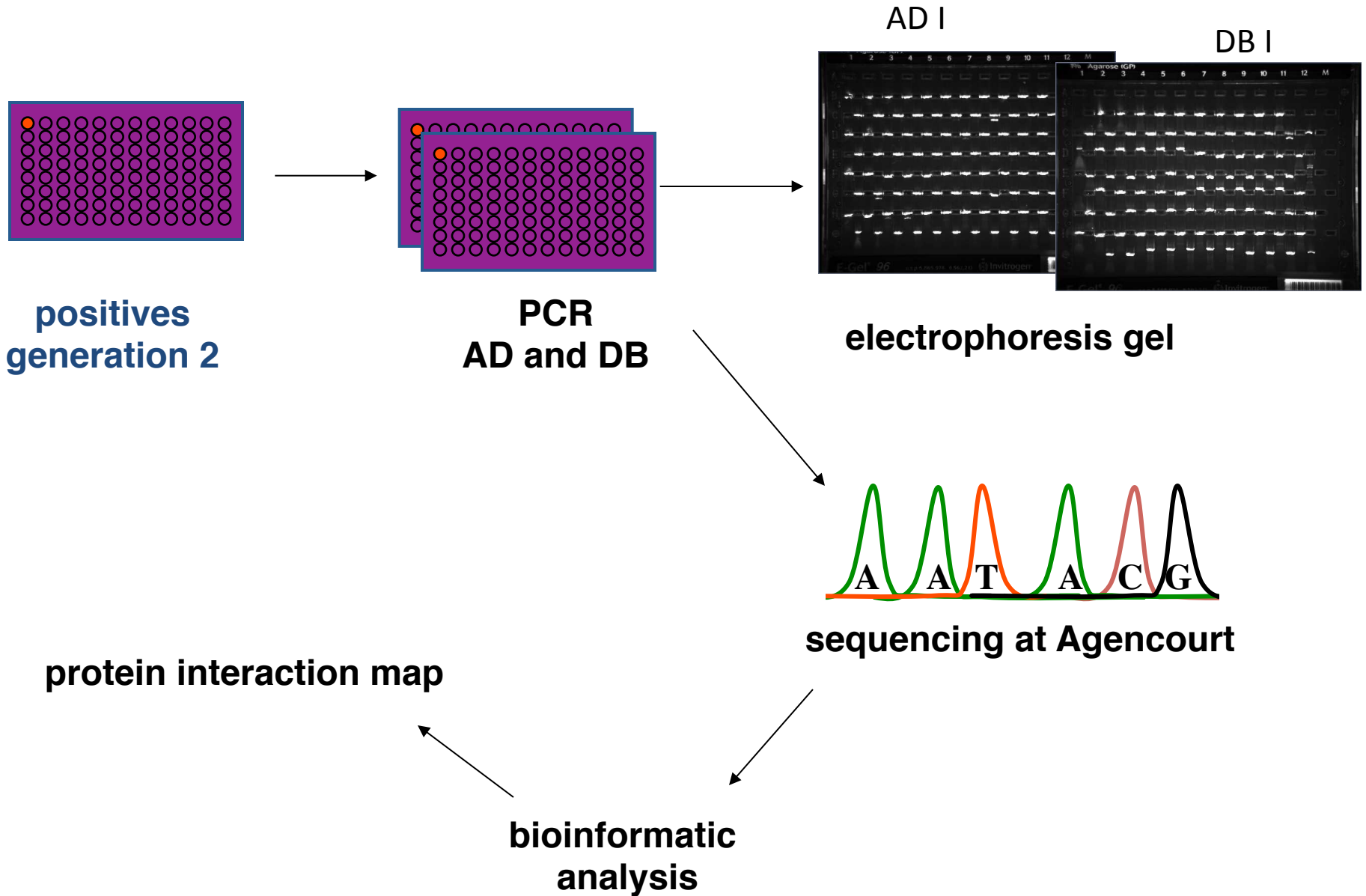


SC-LT

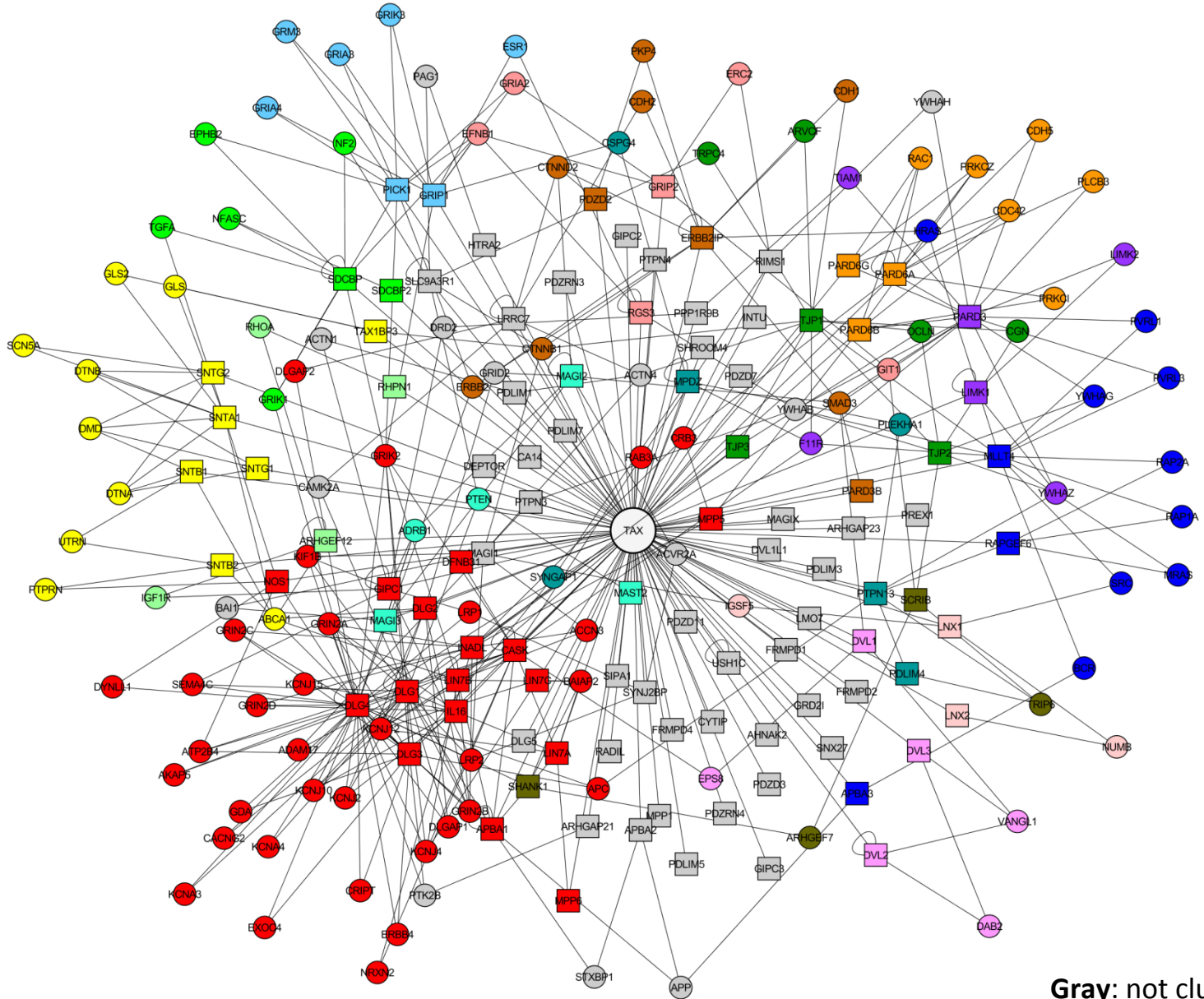


SC-LTH +1 mM 3AT

Sequencing



TAX / PDZ PROTEINS INTERACTOME

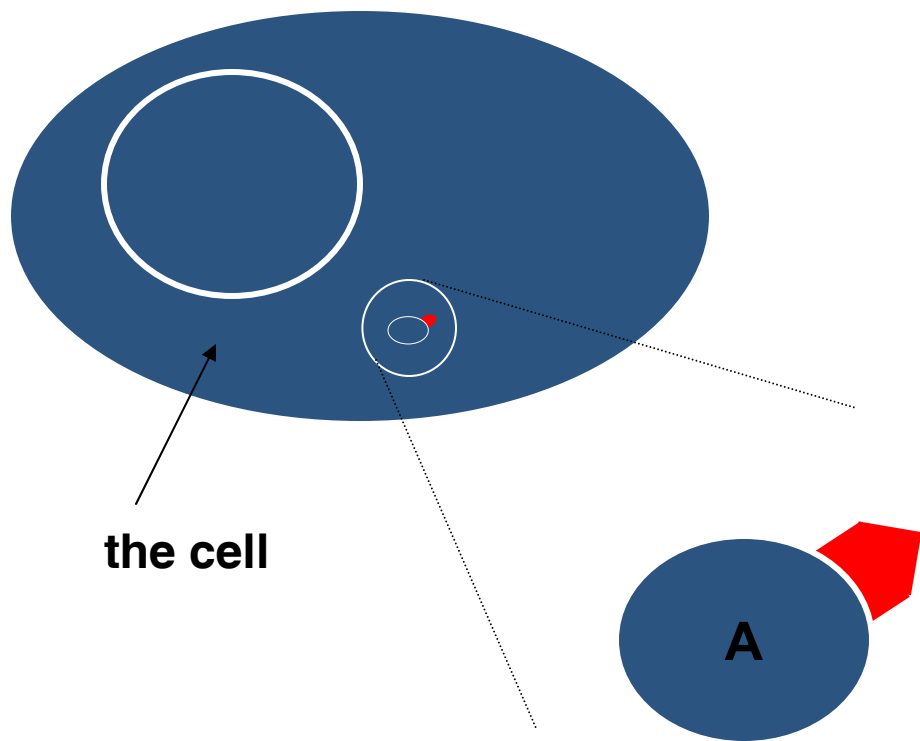


Gray: not clustered
Other color: clustered

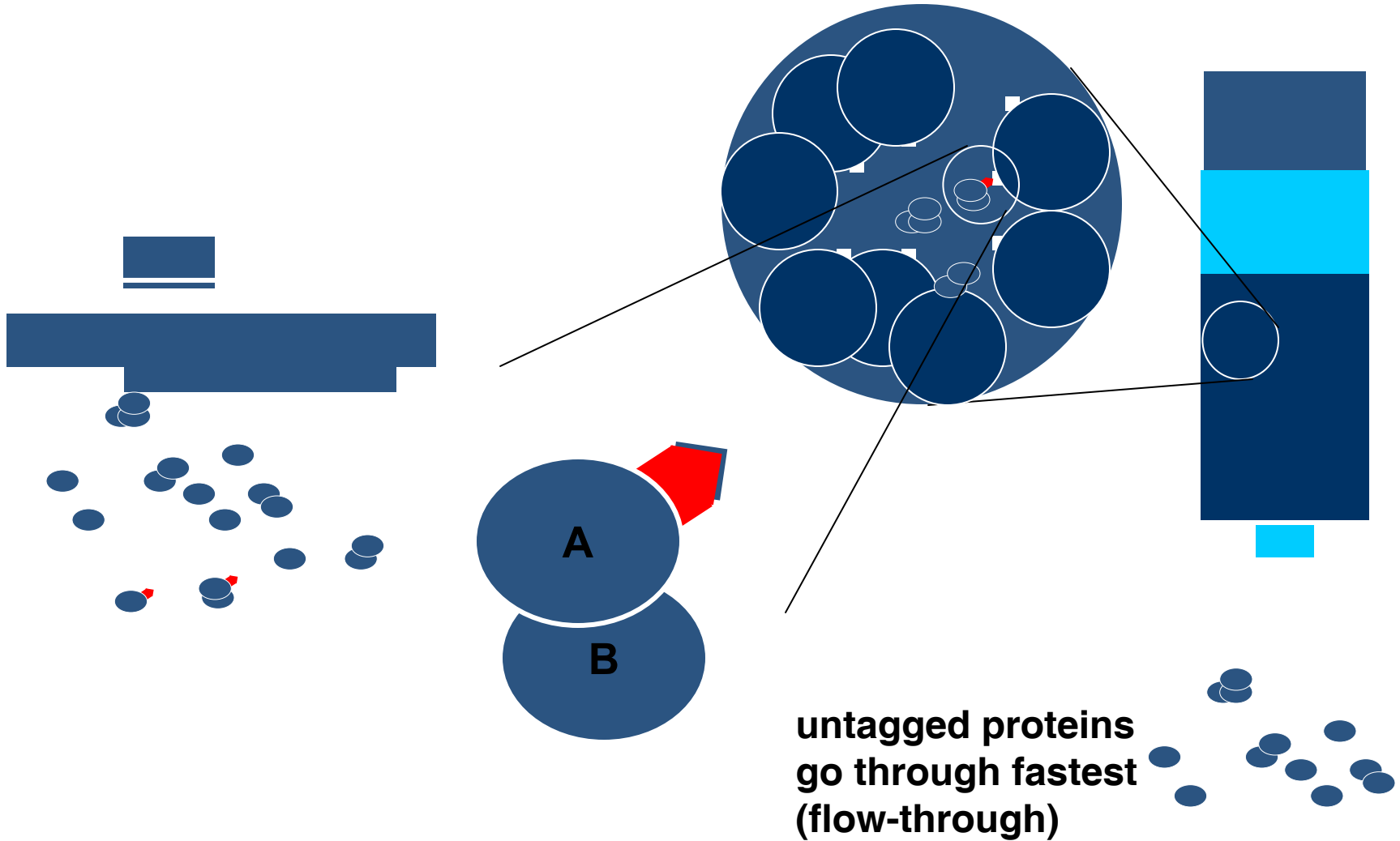
Affinity purification/mass spectrometry

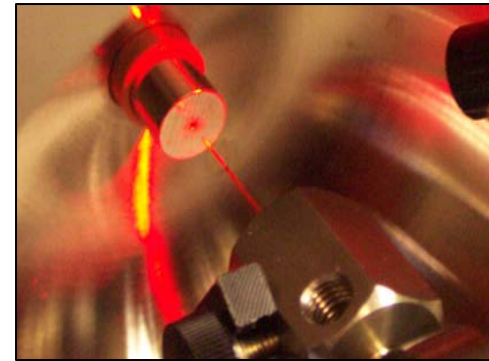
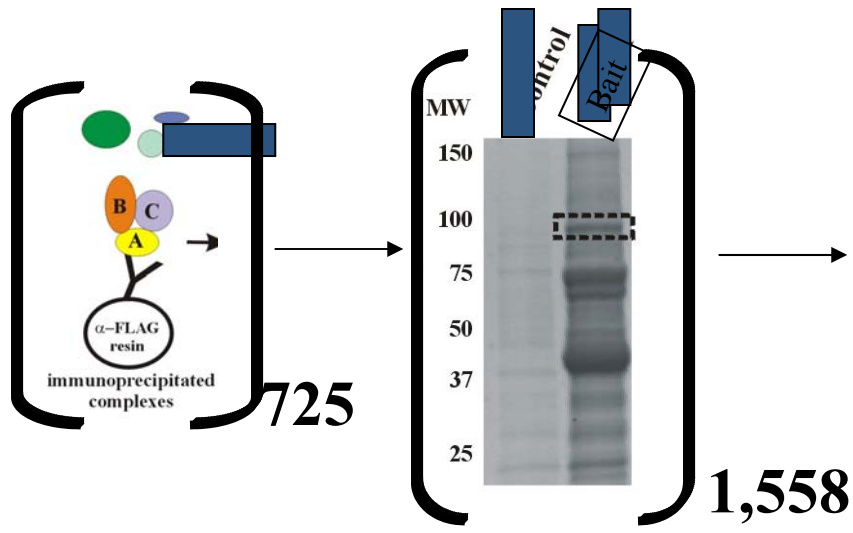


Affinity purification/mass spectrometry

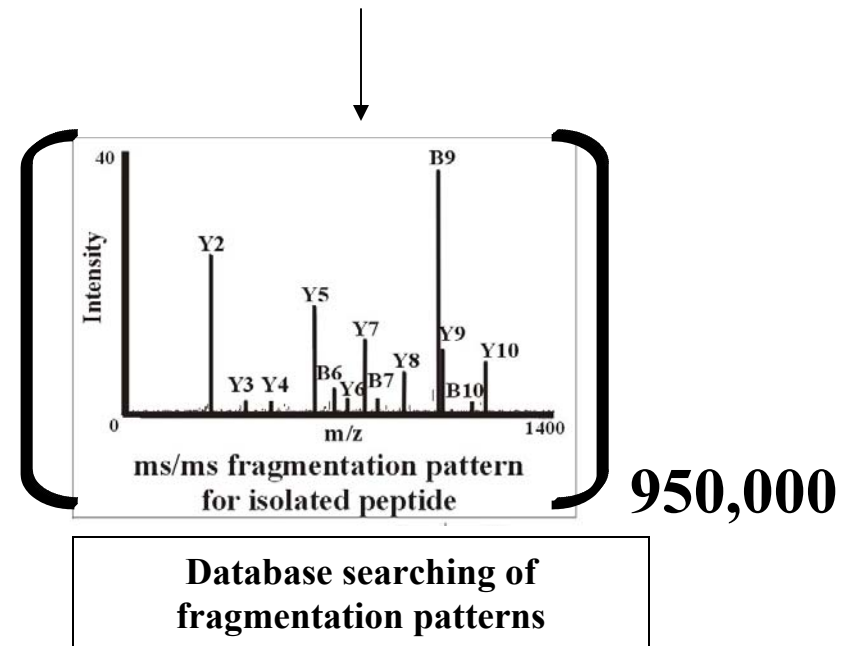
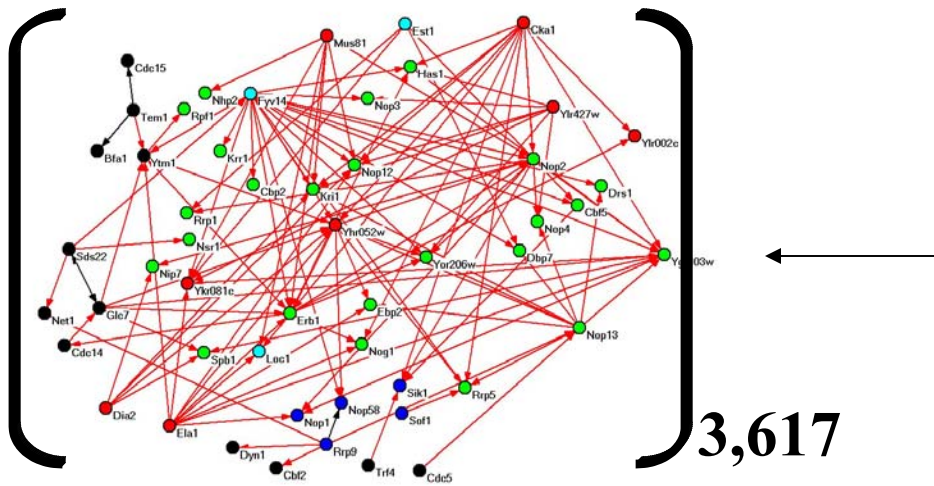


Affinity purification/mass spectrometry

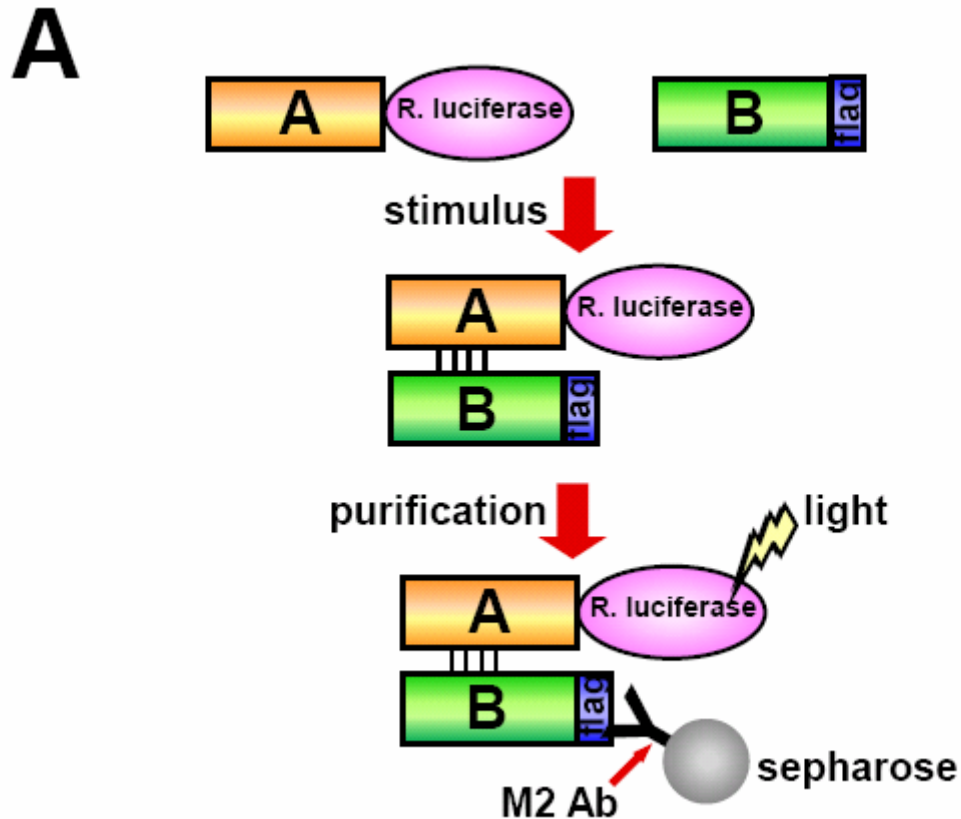




HTP-MS/MS
Network



LUminescence-based Mammalian IntERactome mapping (LUMIER)

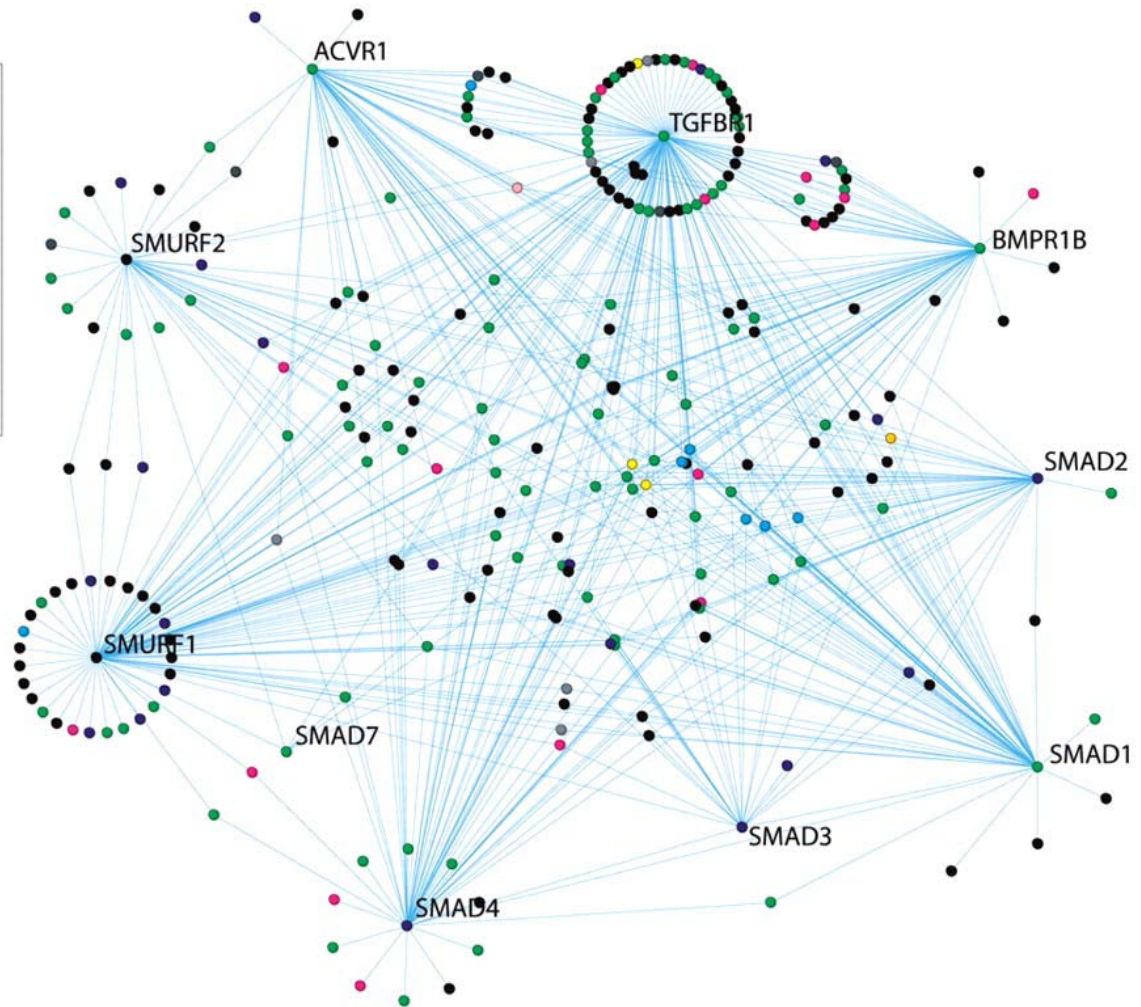
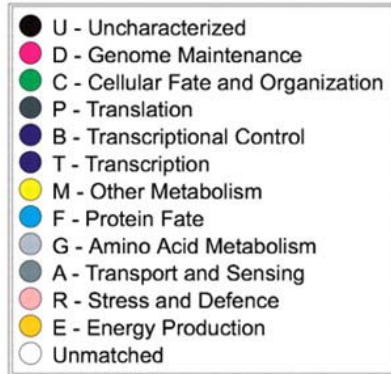


Barrios-Rodiles M, et al.

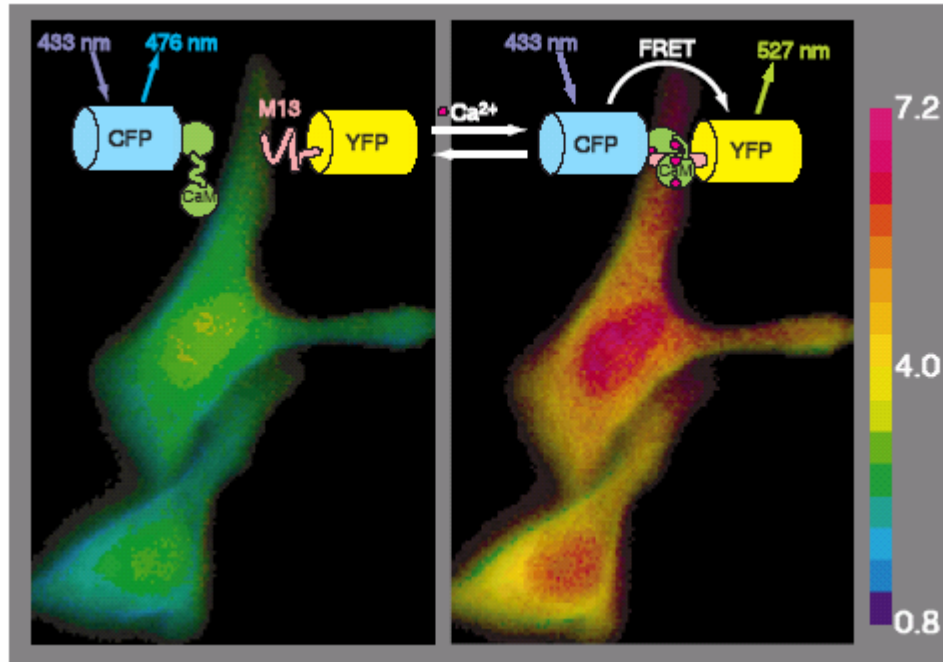
High-throughput mapping of a dynamic signaling network in mammalian cells.
Science. 2005 Mar 11;307(5715):1621-5.

High-throughput screening in 293 cells using the Lumier approach

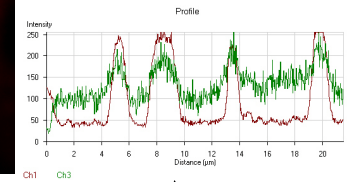
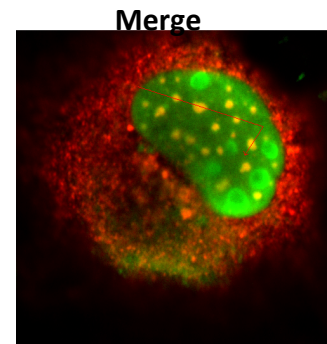
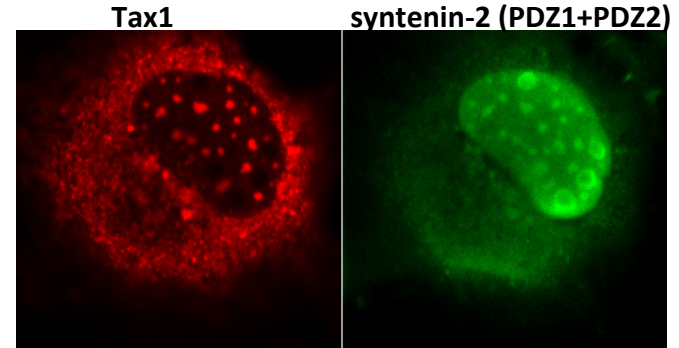
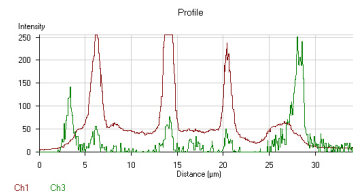
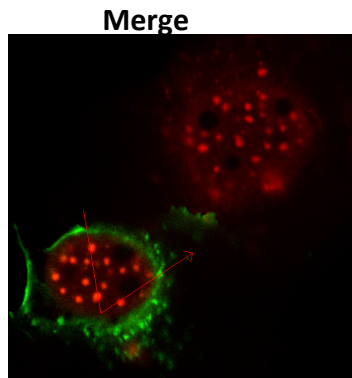
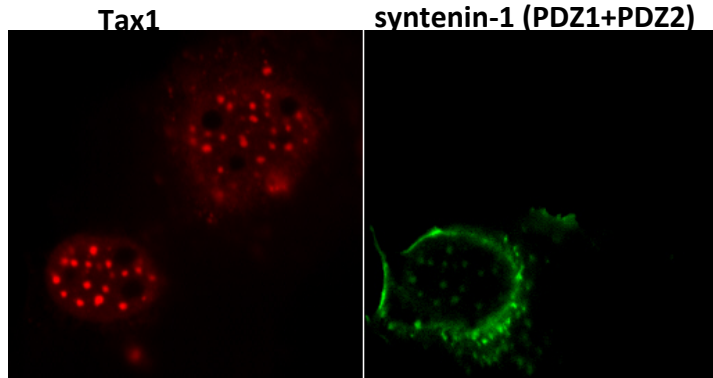
A



Monitoring assembly: FRET



Monitoring interactions: co-localization

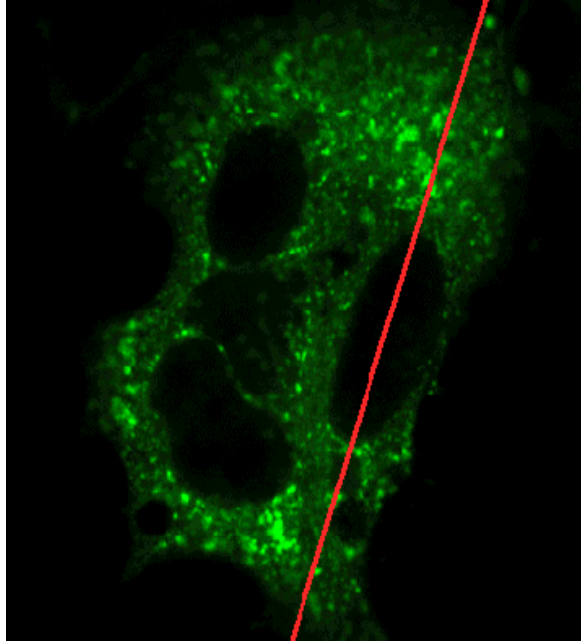


colocalization between syntenin-2 (PDZ1+PDZ2) and Tax1

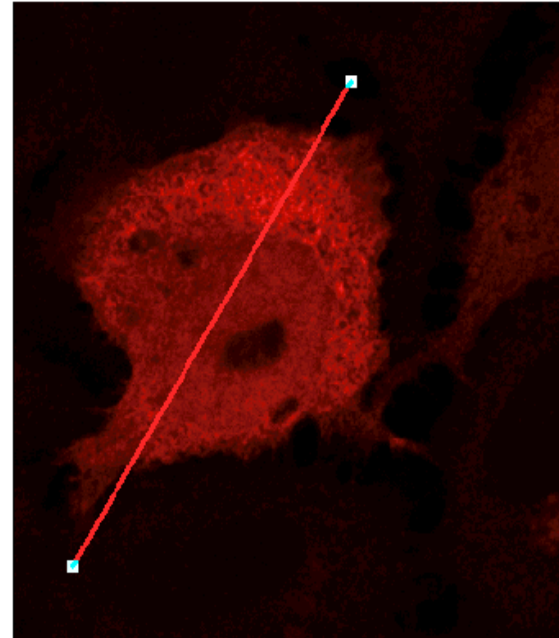
No colocalization between syntenin-1 (PDZ1+PDZ2) and Tax1

Monitoring interactions: localization change

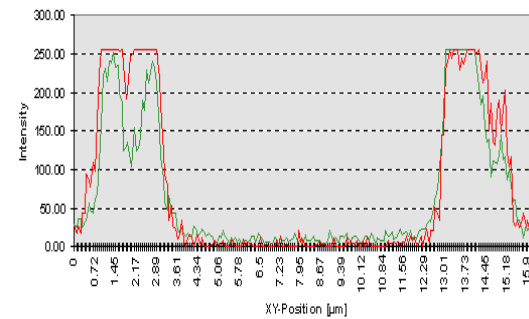
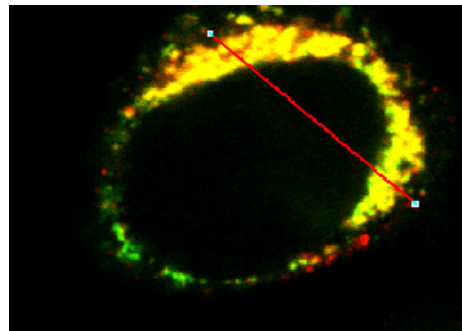
GFPTTP



Tax

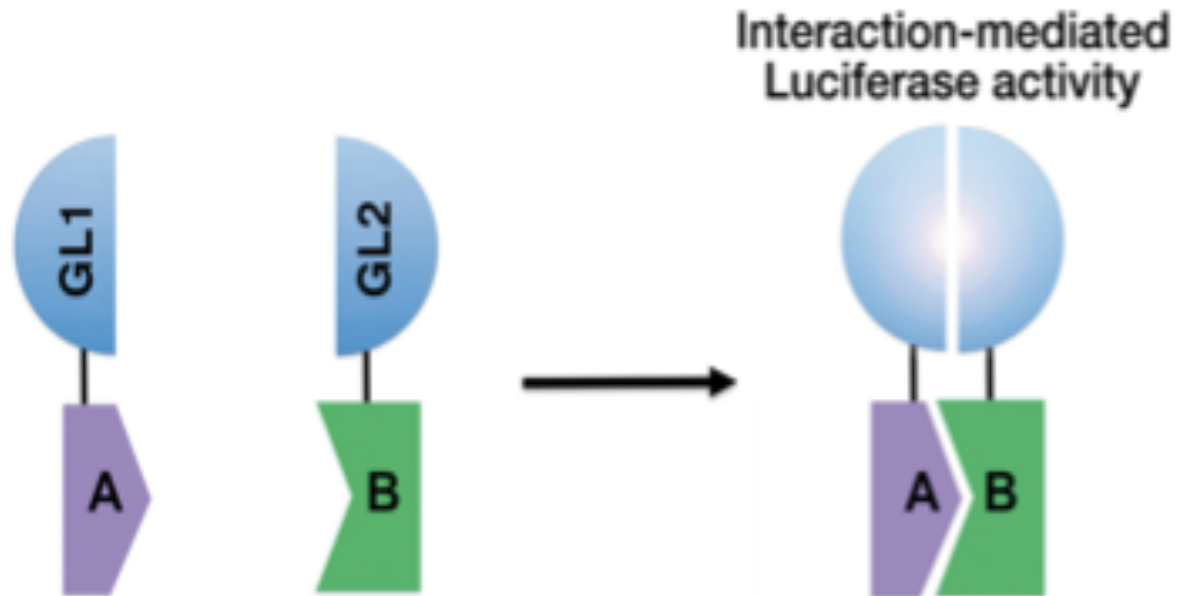


Tax1
et TTP

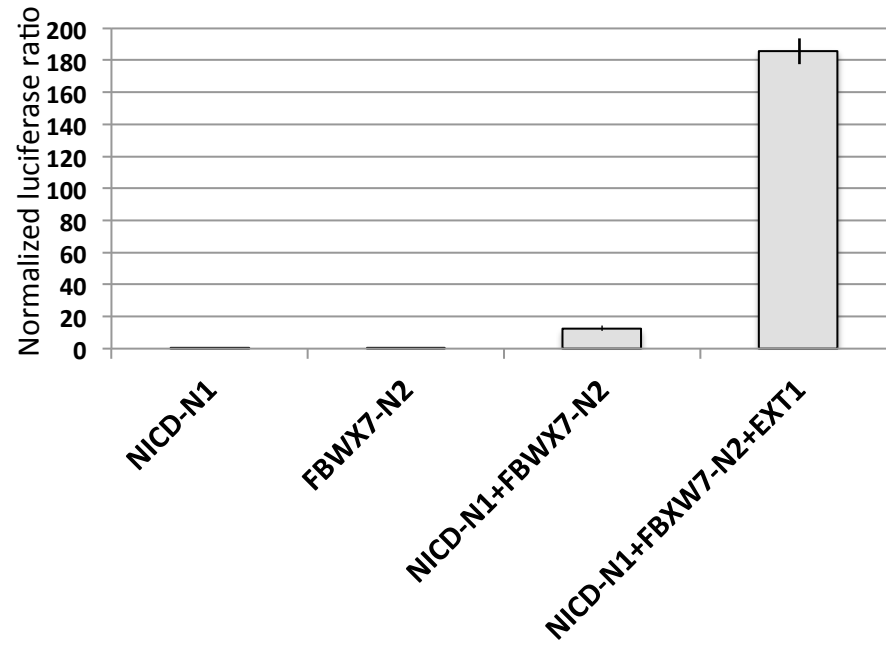


Monitoring interactions: protein complementation

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

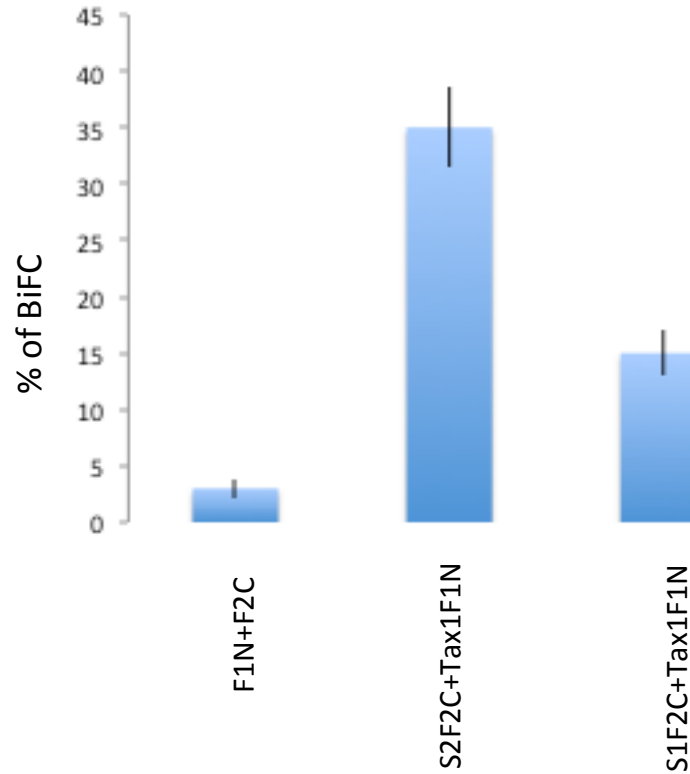
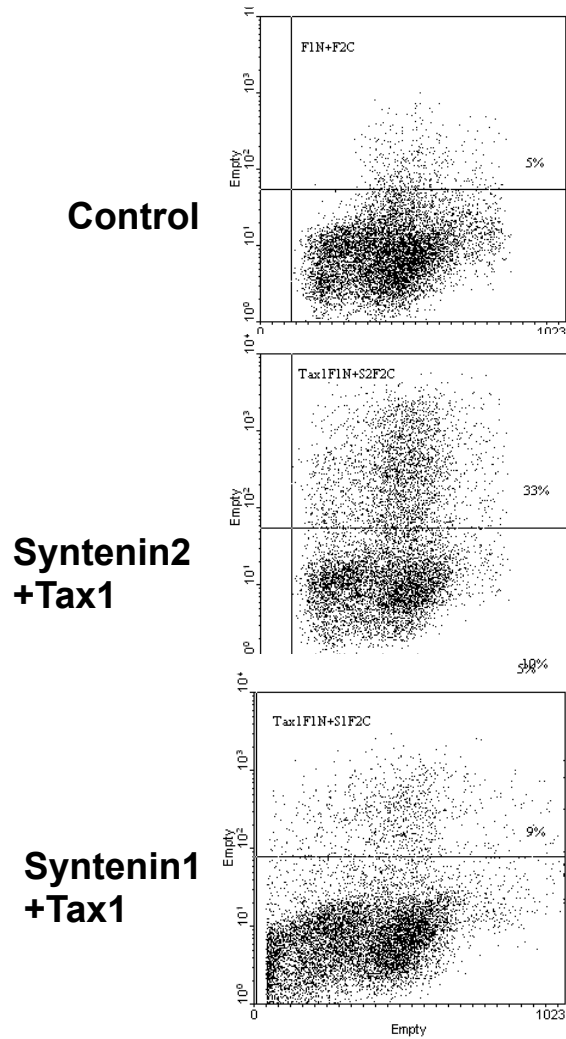


Protein complementation assay



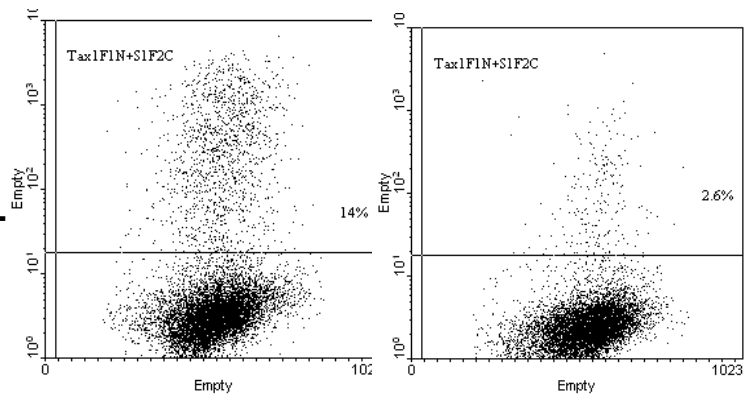
Monitoring interactions: protein complementation

Bi-molecular fluorescence complementation

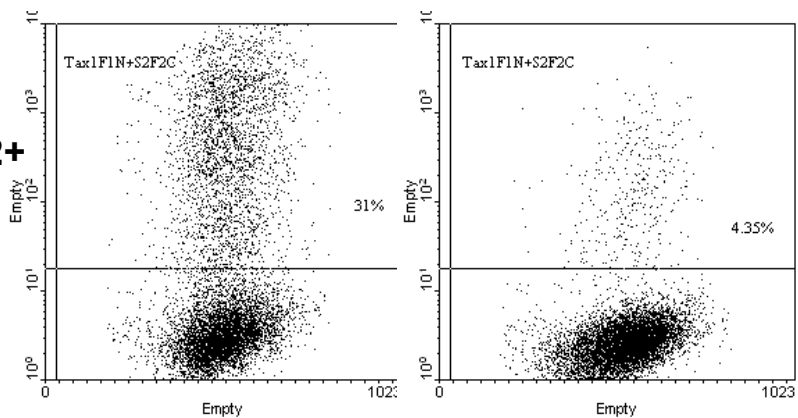


FJ9 inhibits Tax/syntenin interaction

**Syntenin1+
Tax1**



**Syntenin2+
Tax1**



% of BiFC



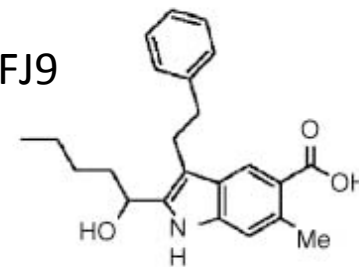
S2+Tax1

S2+Tax1+100uMFJ9

S1+Tax1

S1+Tax1+100uMFJ9

FJ9

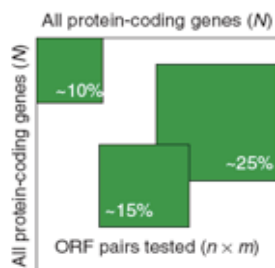


Protein-protein interactions

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

Empirical framework

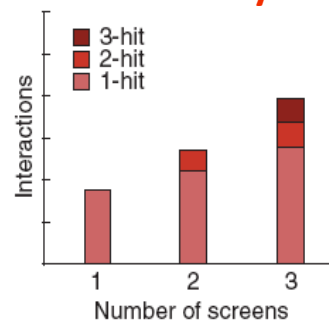
Completeness



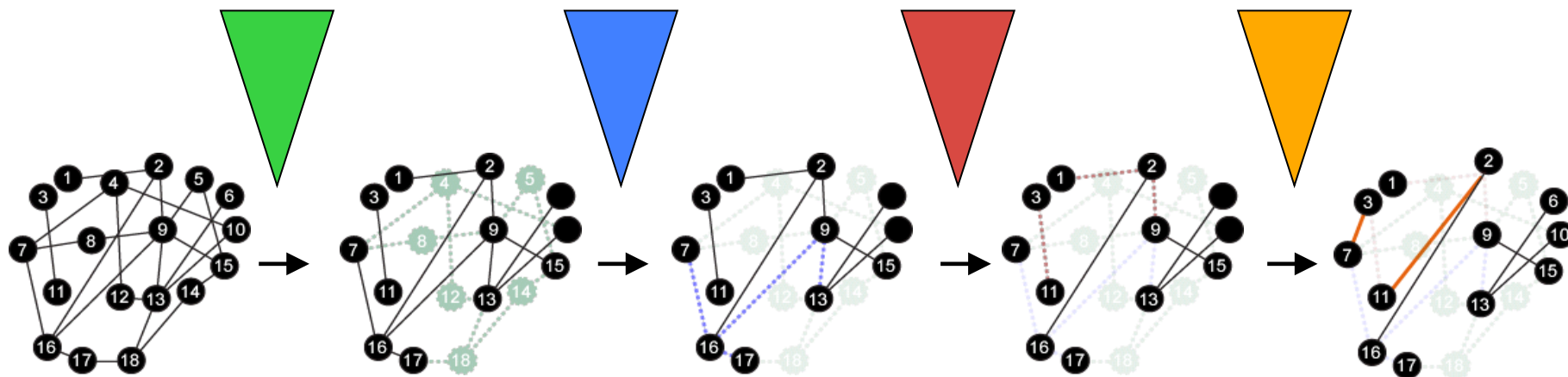
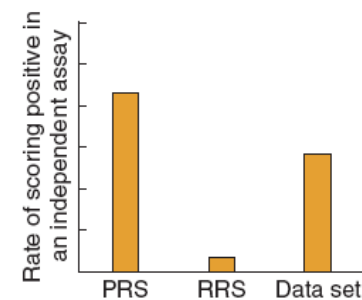
Assay sensitivity



Sampling sensitivity



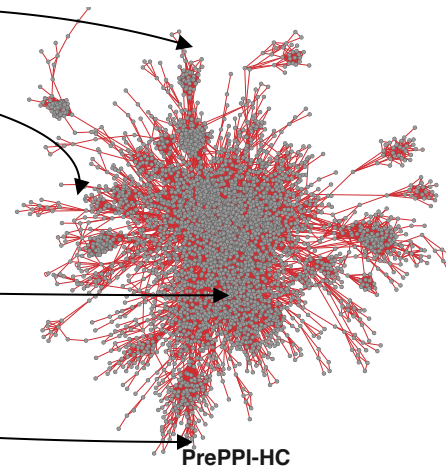
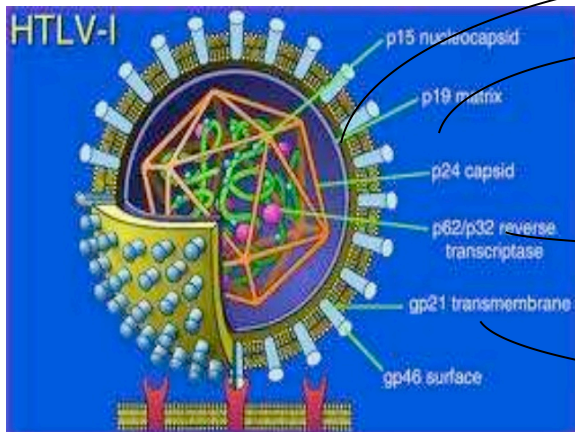
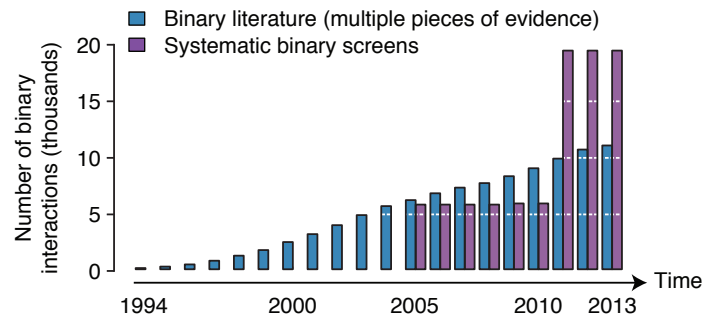
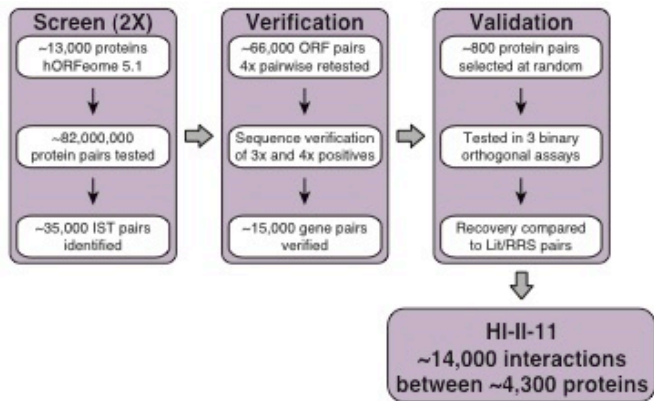
Precision



True network

Mapped network

Interactomes mapping applications



Host – Pathogens interactome

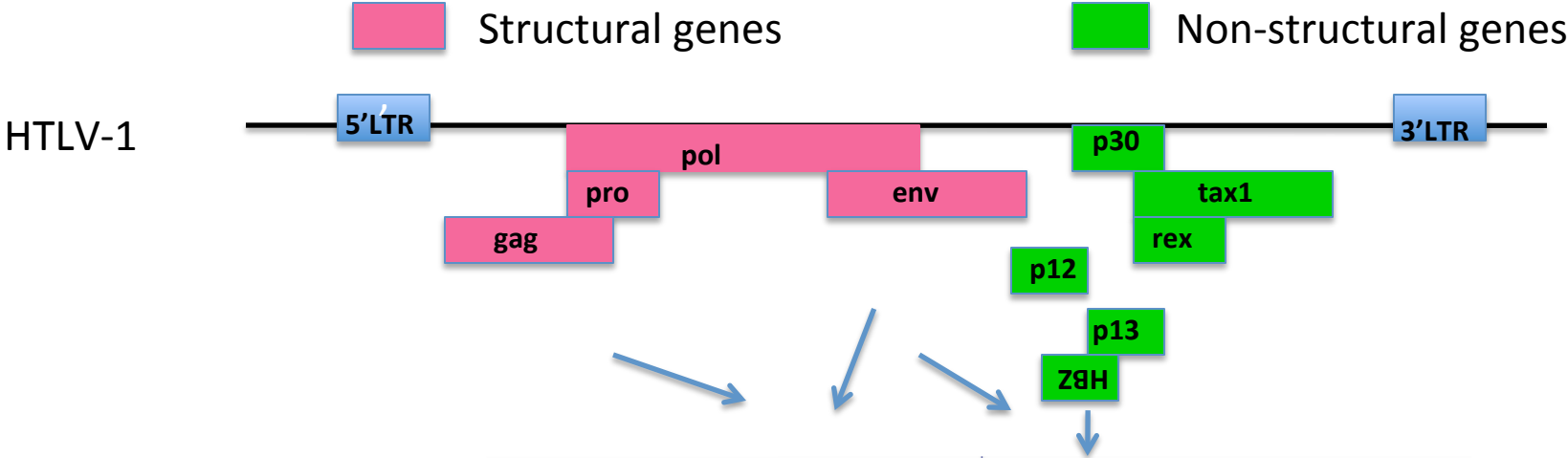
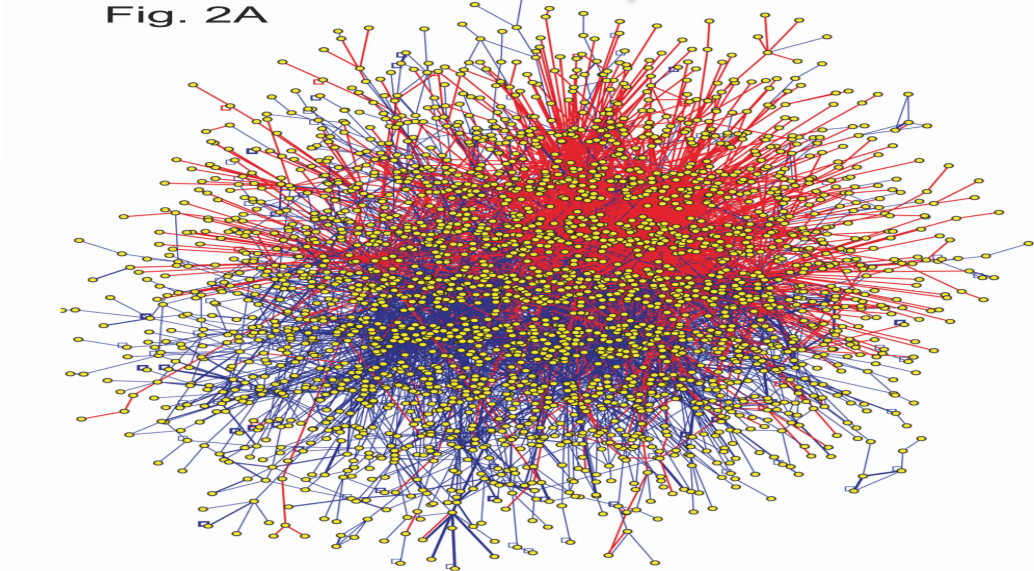
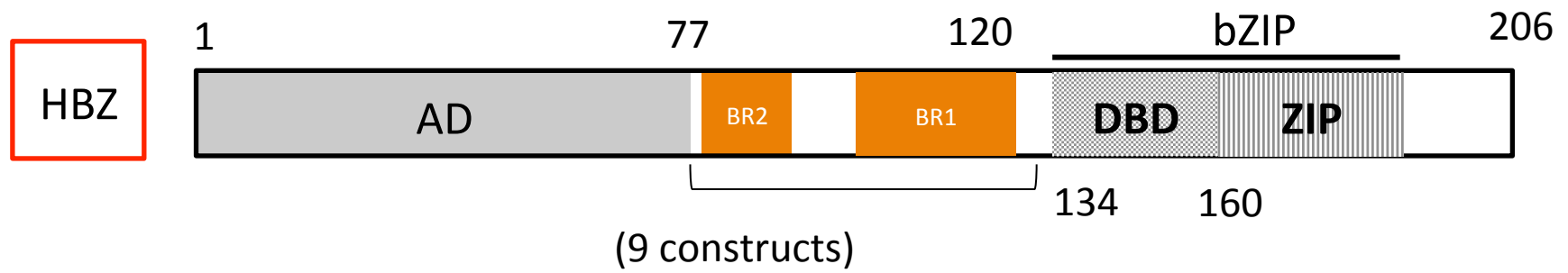
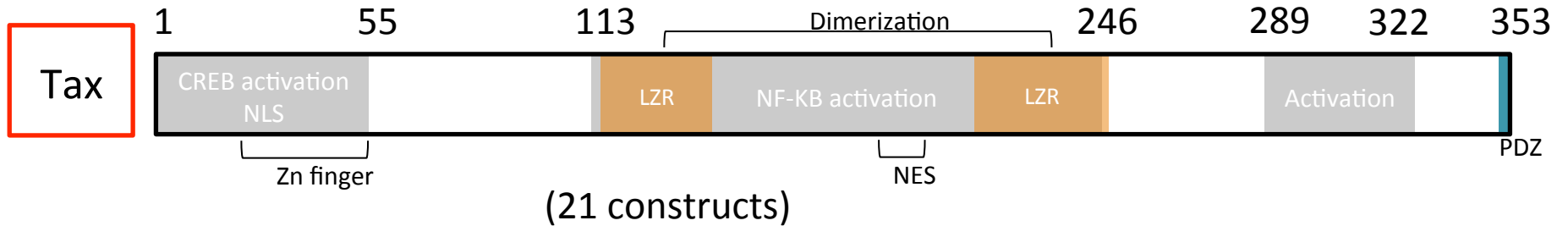


Fig. 2A



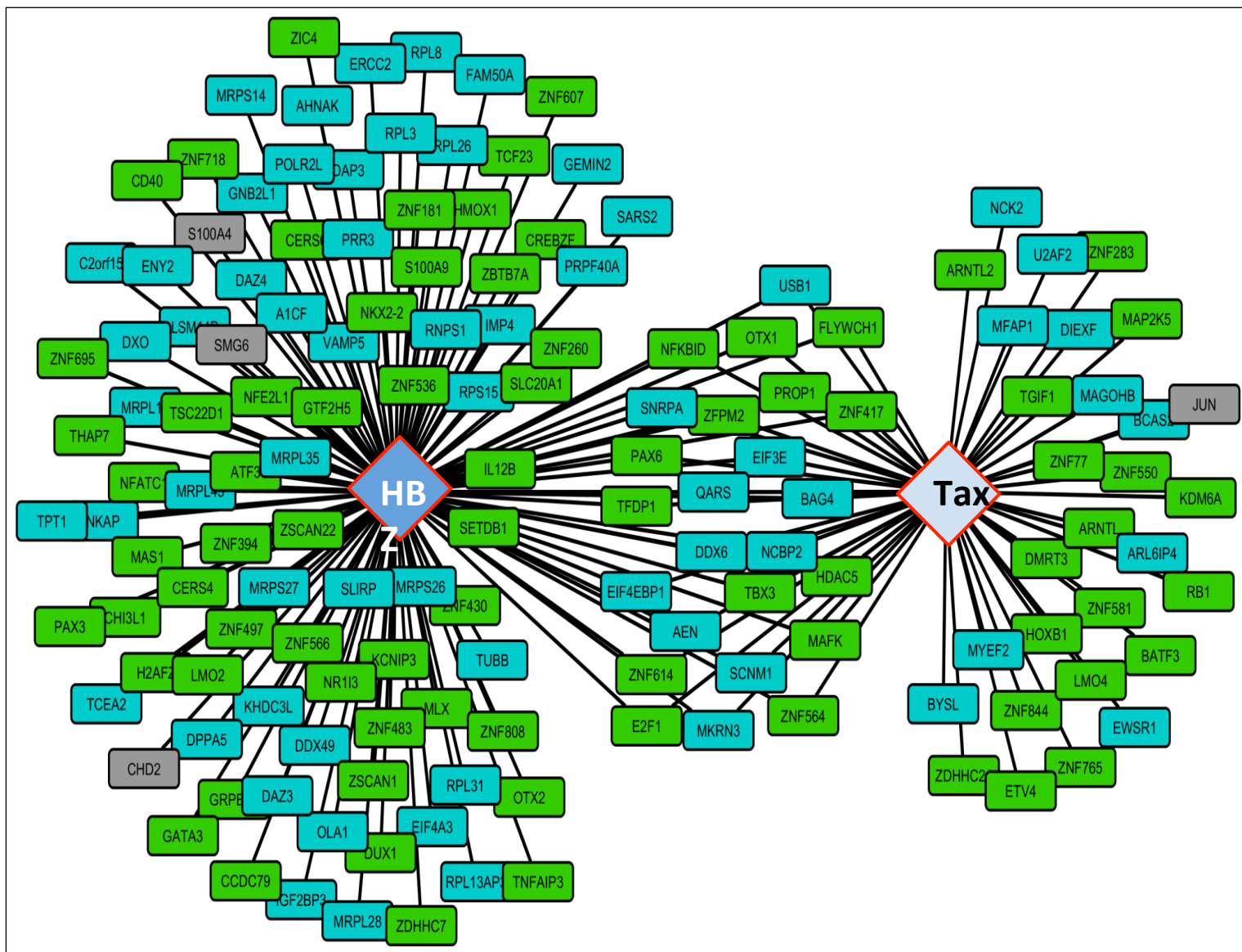
Preliminary results

Cloning of Tax and HBZ constructs

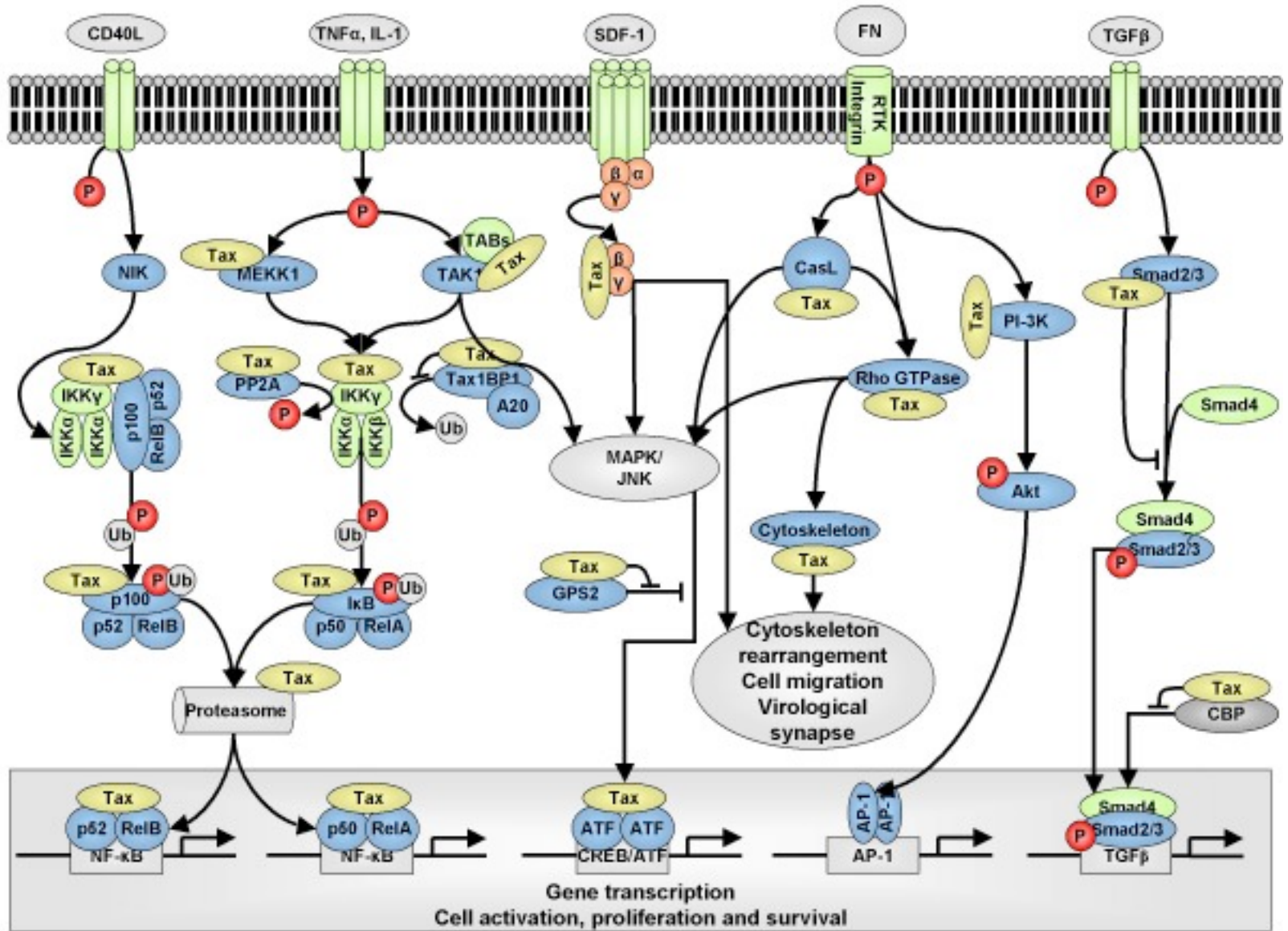


Comprehensive mapping of Tax/HBZ interactome with Transcriptional and Post-transcriptional regulators

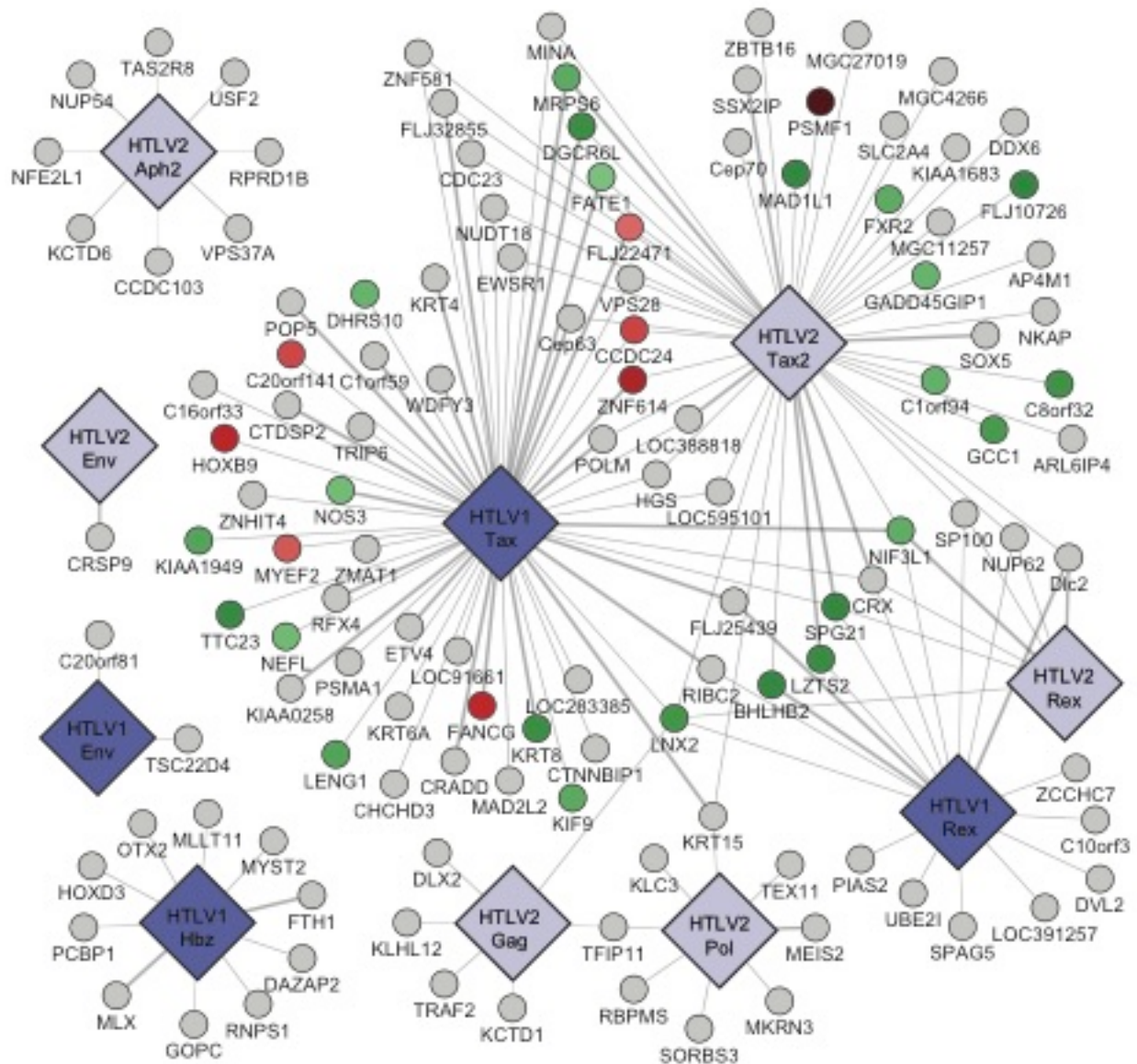
- RBPs
- TFs
- RBPs/TFs



History

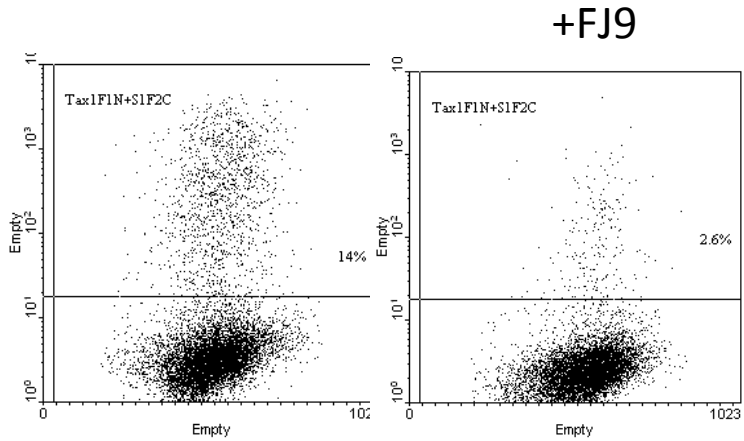


A host – pathogen interactome for HTLV1/2

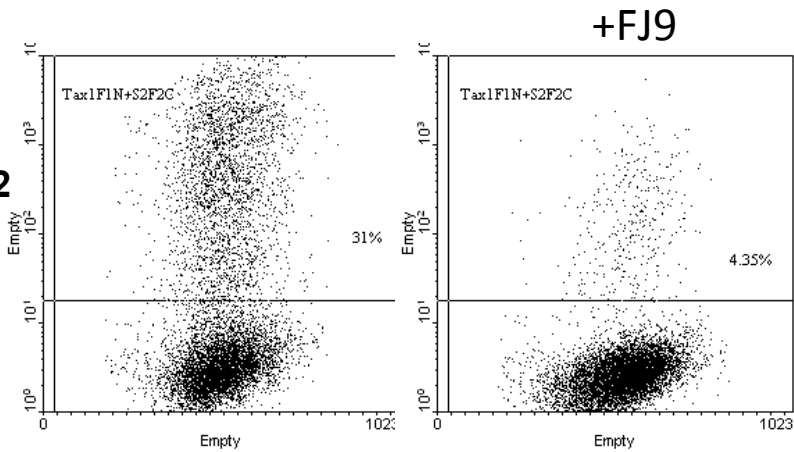


Inhibition of protein – protein interactions by small molecules

**Syntenin1
+Tax1**



**Syntenin2
+Tax1**



% of BiFC

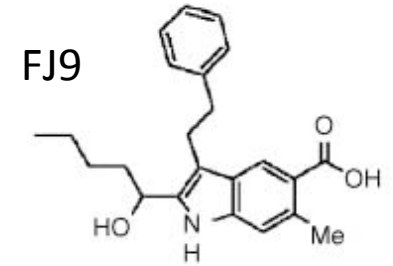


S2+Tax1

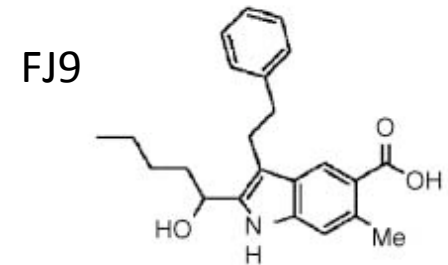
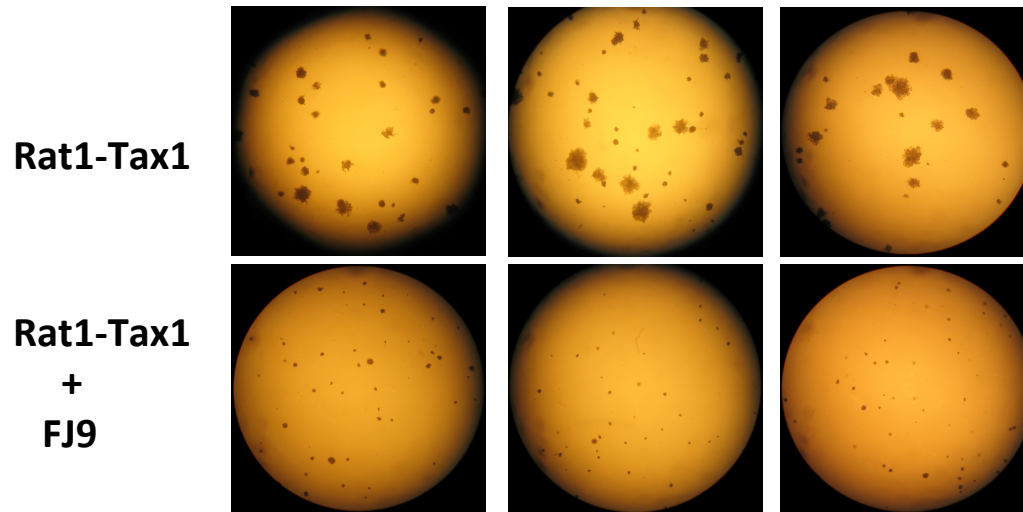
S2+Tax1+100uMFJ9

S1+Tax1

S1+Tax1+100uMFJ9



Inhibition of protein – protein and cellular transformation

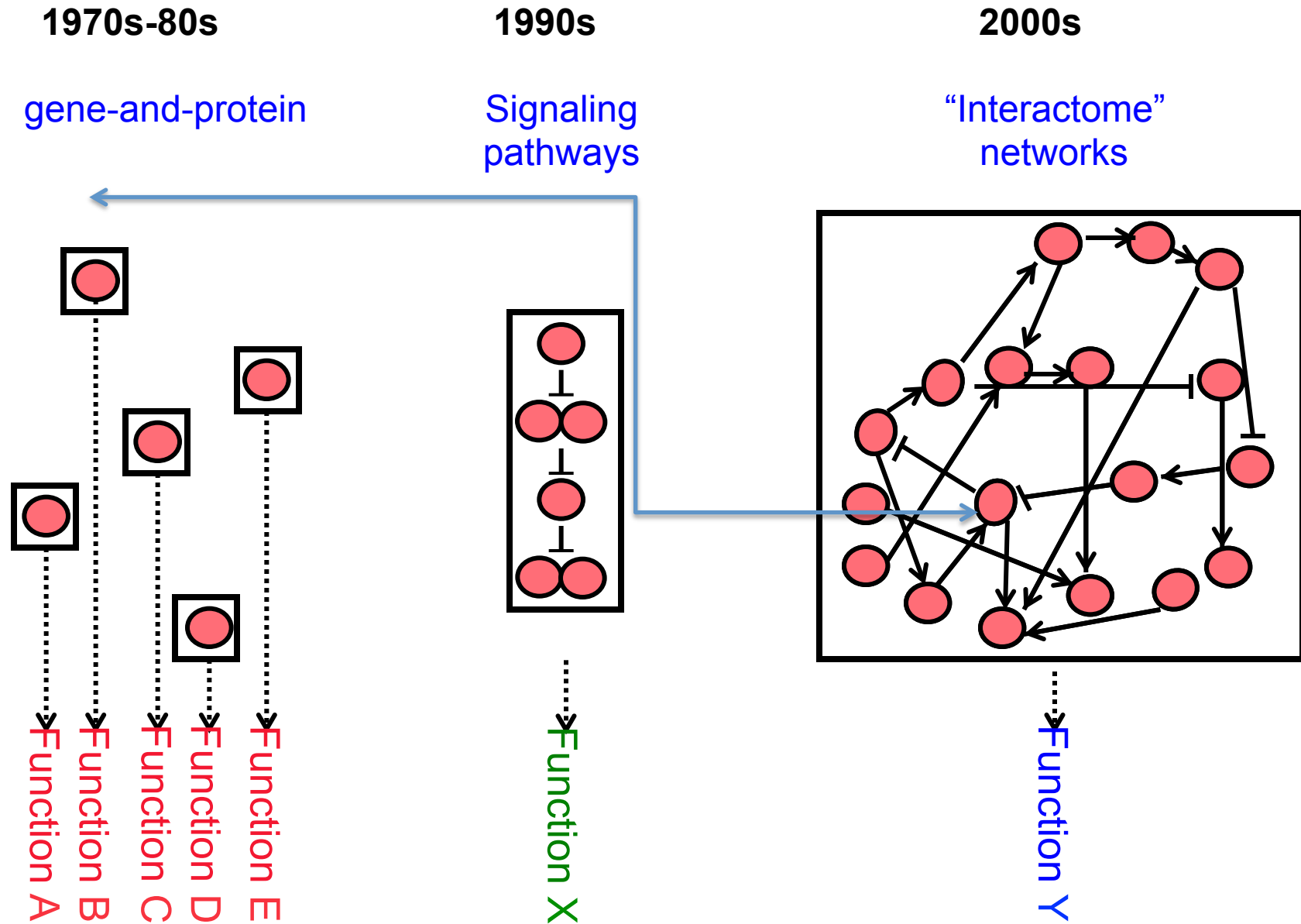


Disruption of Tax/ PDZ interaction inhibited Tax transformation as measured by a decrease in size and number of Tax-induced Rat-1 foci.

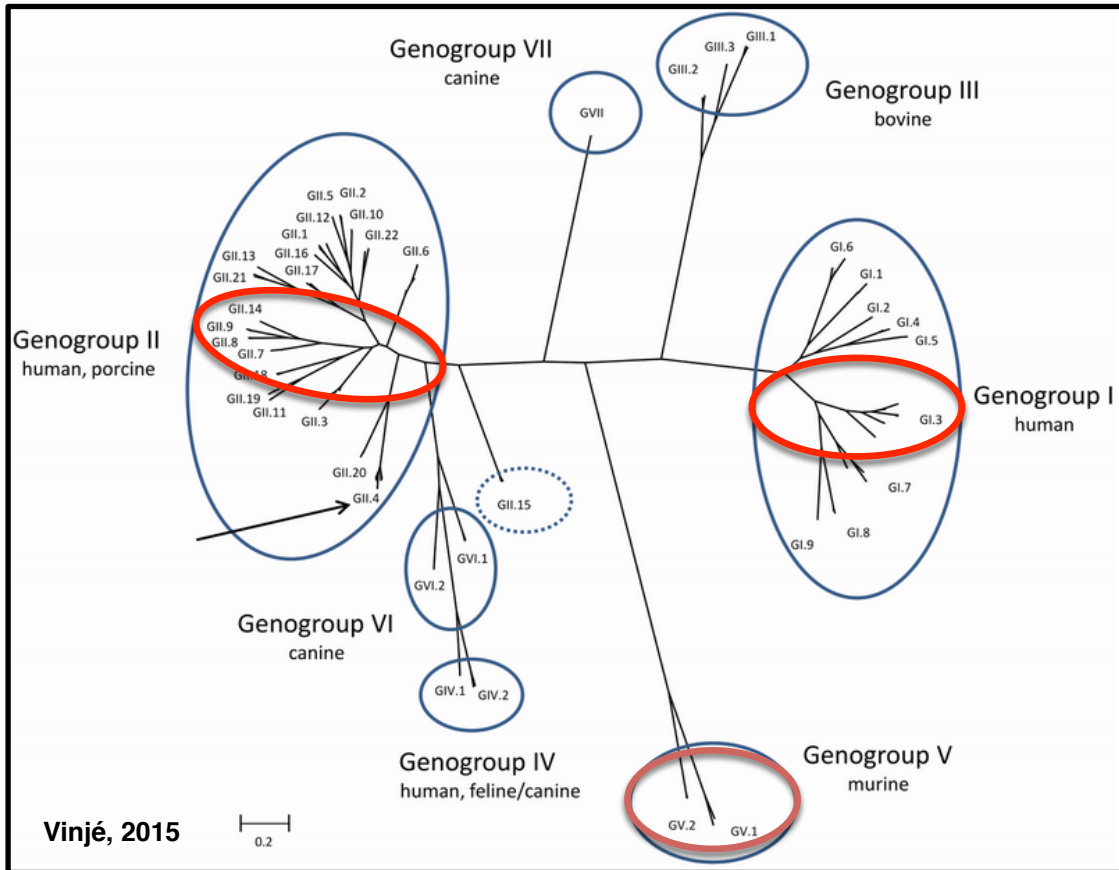


PDZ proteins involved in Tax1 transformation activity

Models for overall functional organization of the cell



Noroviruses Interactome



GI.1 (ORF2+ORF3)

GI.2

GI.3

GI.4

GI.5

GI.6

GI.7

GI.8

GI.9

GII.1

GII.2

GII.3

GII.4

GII.5

GII.6

GII.7

GII.8

GII.9

GII.10

GII.12

GII.13

GII.14

GII.15

GII.16

GII.17

GII.20

GII.21

GII.22

GII.4 1974

GII.4 1987

GII.4 1997

GII.4 2002

GII.4 2002a

GII.4 2004

GII.4 2005

GII.4 2006 (complete)

GII.4 2009

GII.4 2012 (ORF2+ORF3)

MNV1

CW1 (complete; P-domain)

CR6 (complete)

CW3 (complete)

Marco Grodzki, PhD

Mapping an interactome network

All proteins

All
proteins

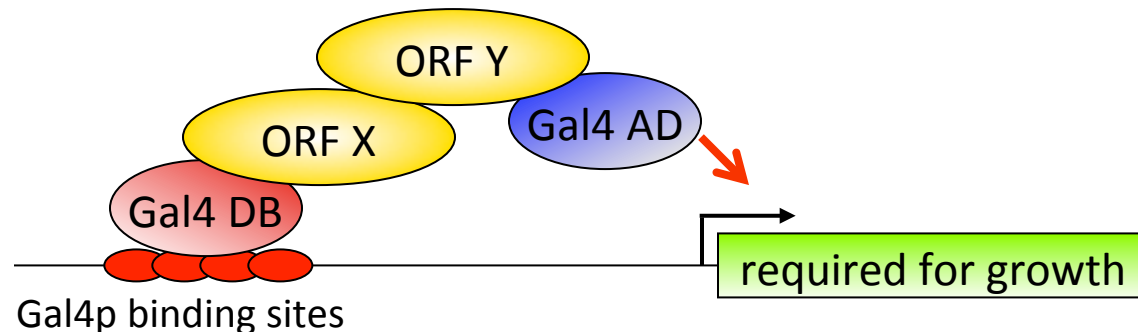
Test

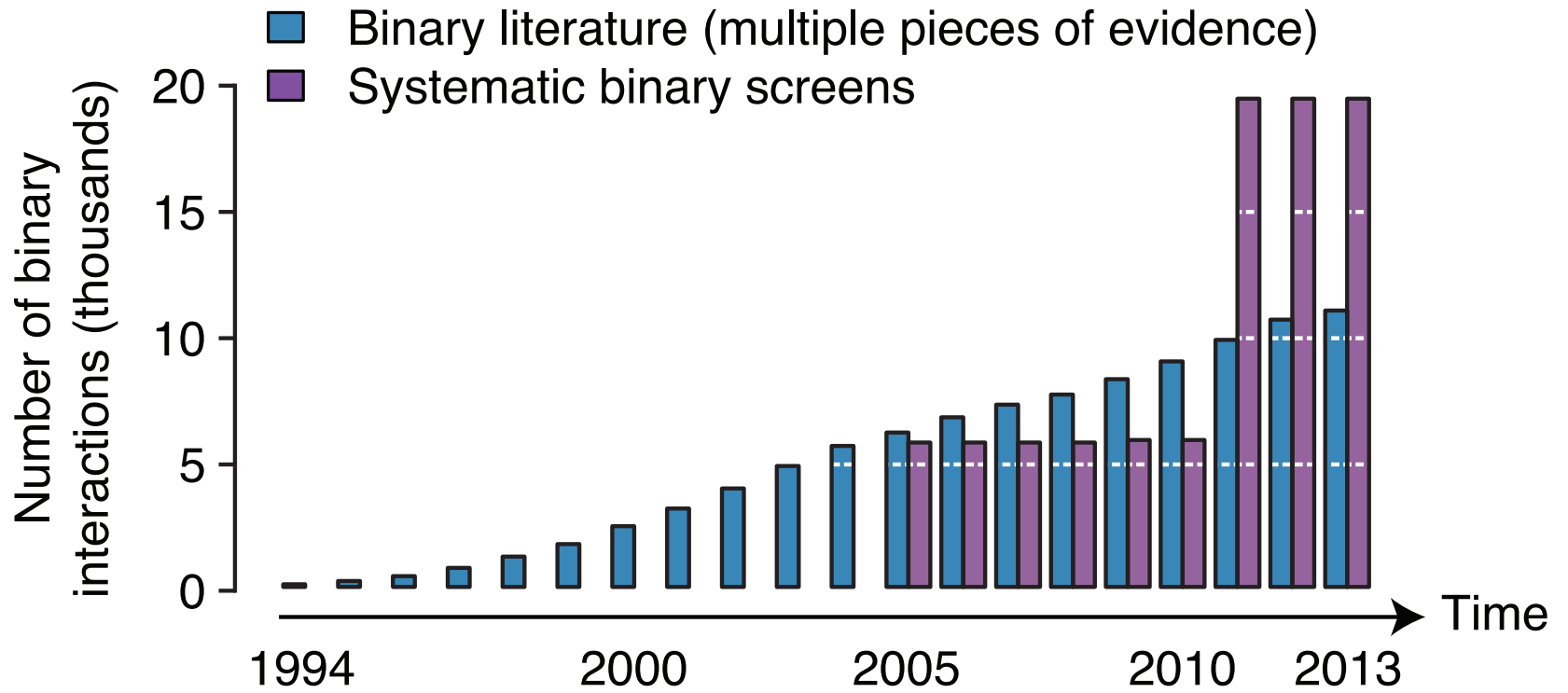
all pairwise combinations

for **possible** physical interactions

Yeast two-hybrid

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



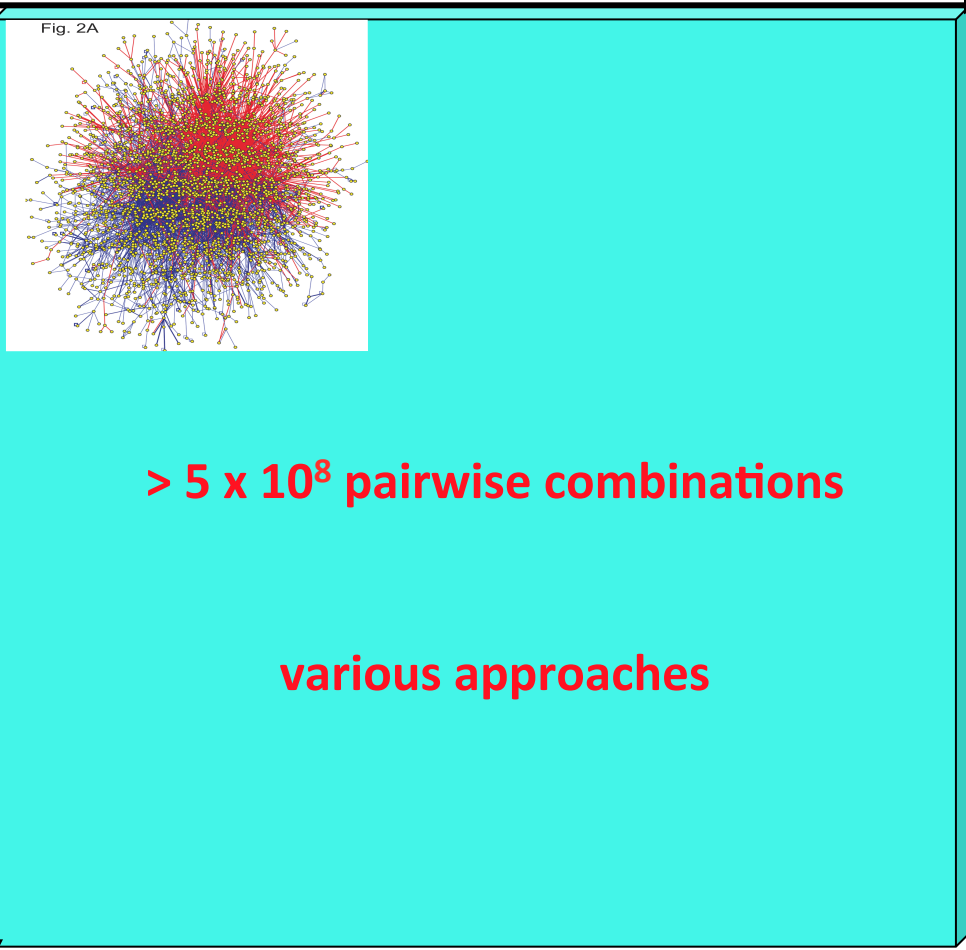


Human interactome

Rual et al.; Nature 437, 1173-1178

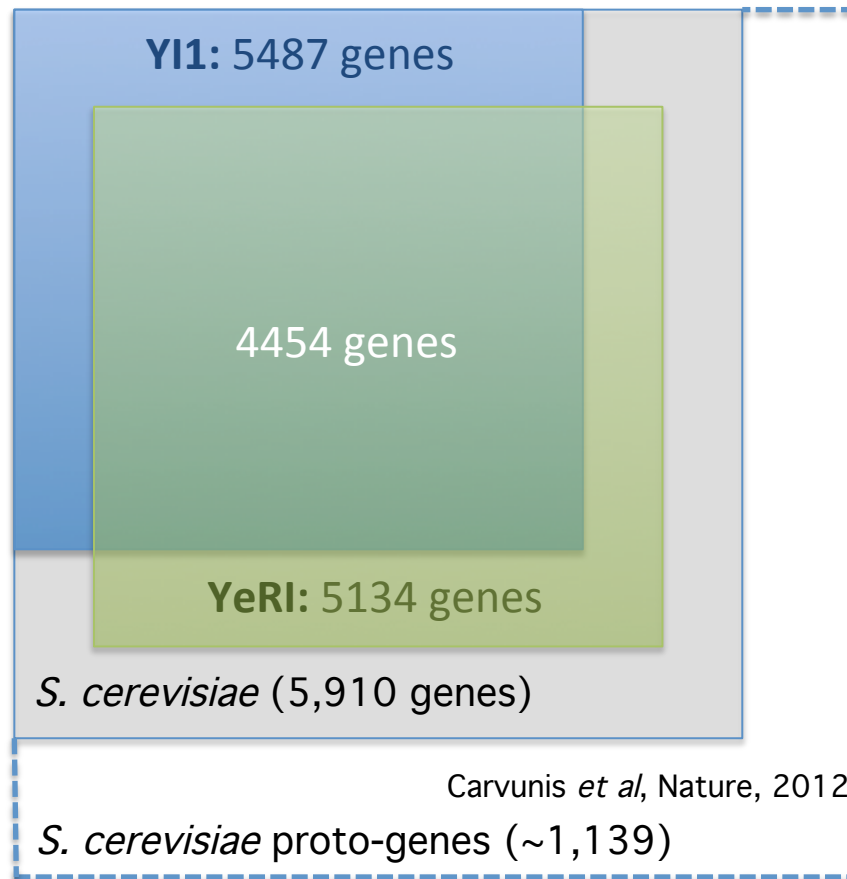
Stelzl et al.; Cell 122 (6), 957-68

>22,000 proteins



>22,000 proteins

Towards completeness of the yeast interactome

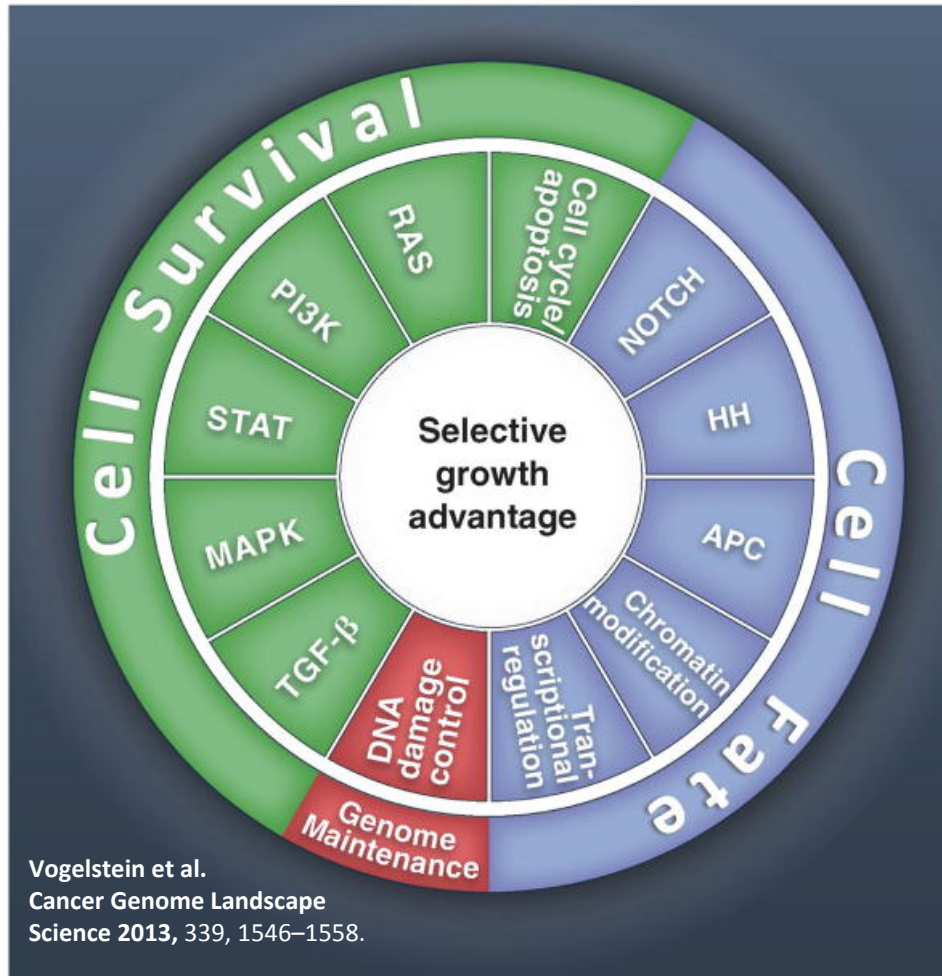


Genomic mutations landscape in cancer

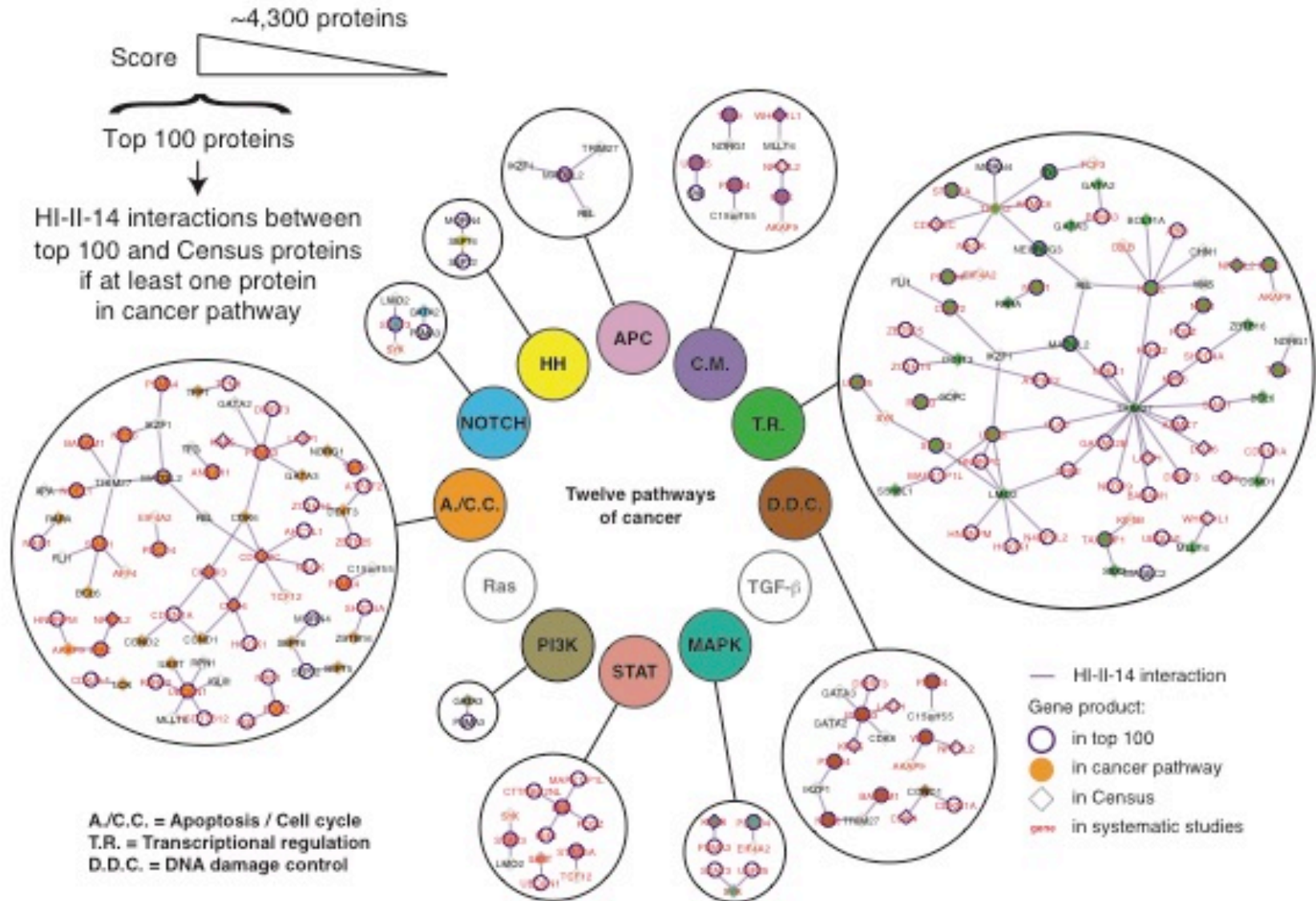
Cancer Pathways

~ 500 cancer census genes

~140 cancer driver genes



Guilt by association partners of known cancer genes

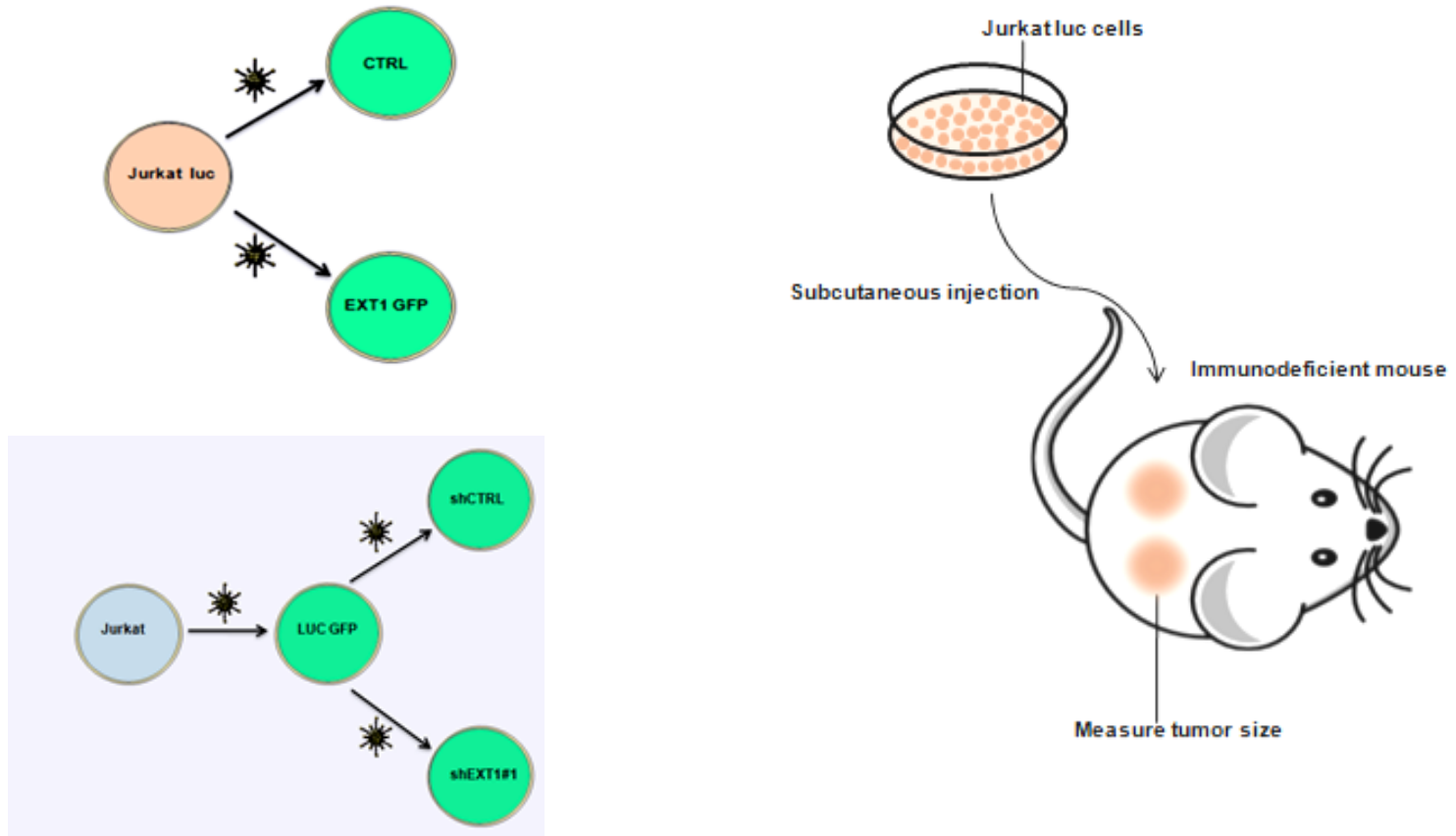


1. The role of EXT1 in T-ALL

Silencing/over-expression of EXT1 in a T-ALL in *in vivo* model

Tumor xenograft experiment

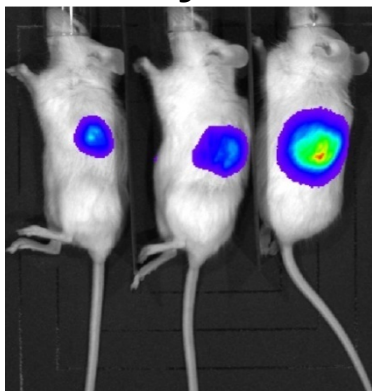
Based on bioluminescence imaging (BLI) with luciferase reporter



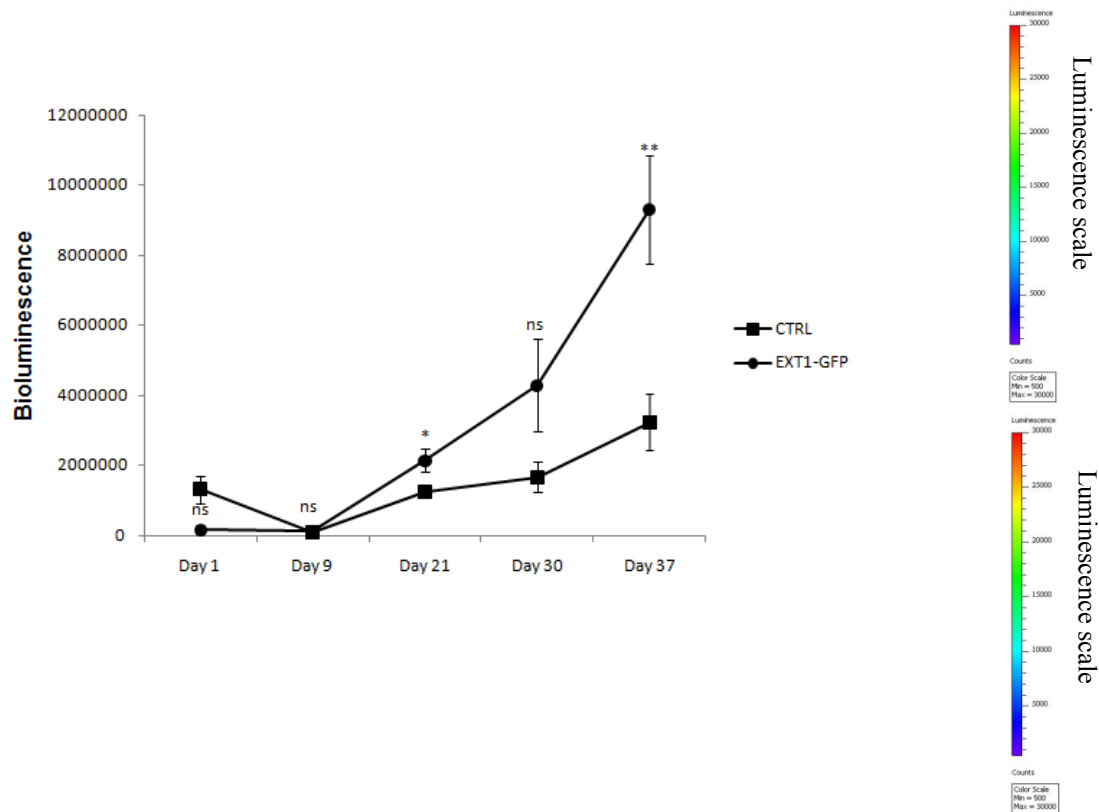
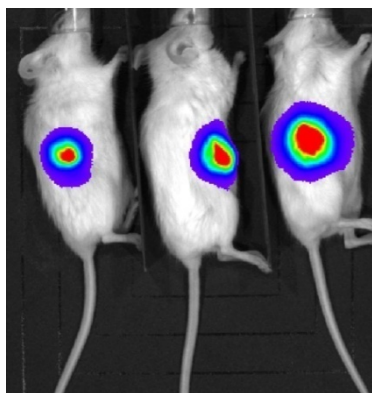
The role of EXT1 in T-ALL

Day 37

Jurkat CTRL



Jurkat EXT1



Injection of Jurkat over-expressing EXT1 in NOD-SCID mice resulted in a significant increase of the leukemic burden

Applications of interactome mapping

- Organisms Interactome mapping
- Novel disease-related genes
- Host-Pathogens interactomes