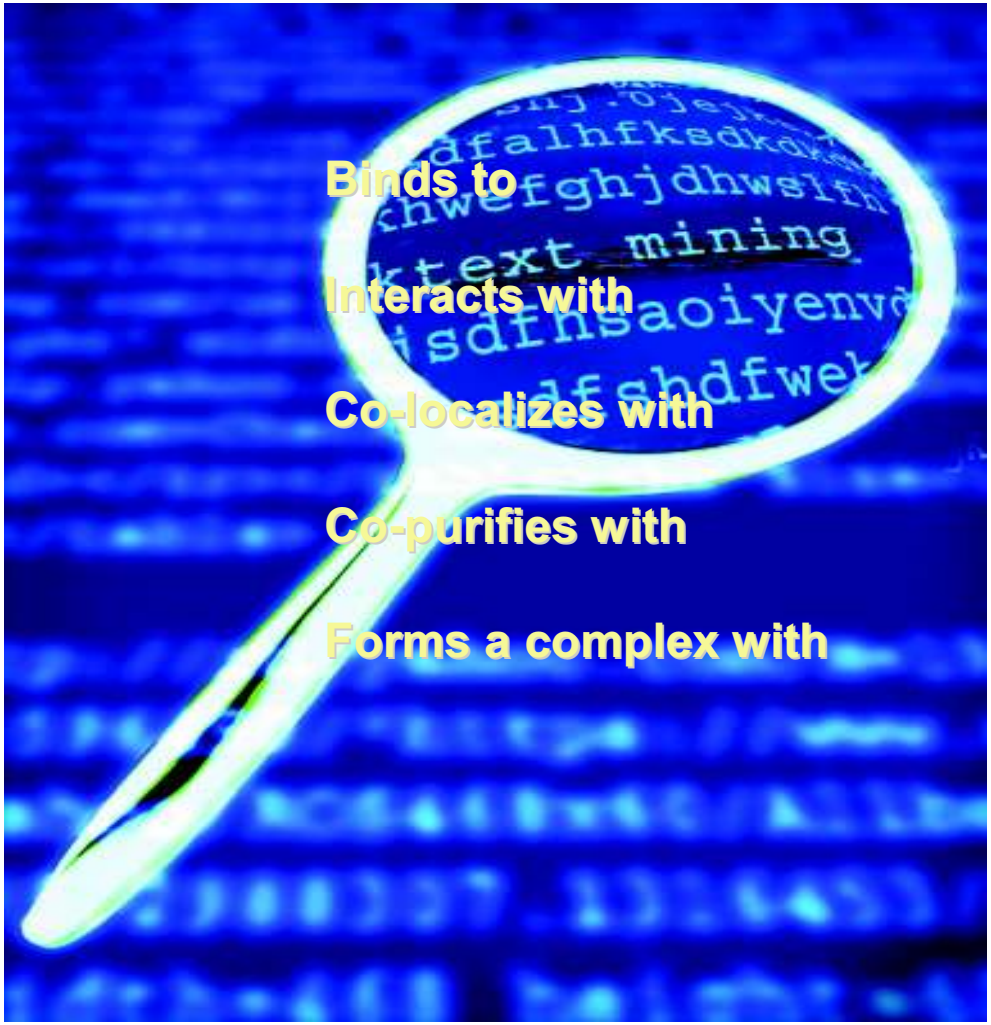
A Prototype of the HumanVirus Interact ome Resource (HVIR)

“ Tax1



Protein B ”

Alex Pothen
M. Zubair
Kurt Maly

Chris Osgood
John Semmes

Eastern Virginia Medical School
Established 1973

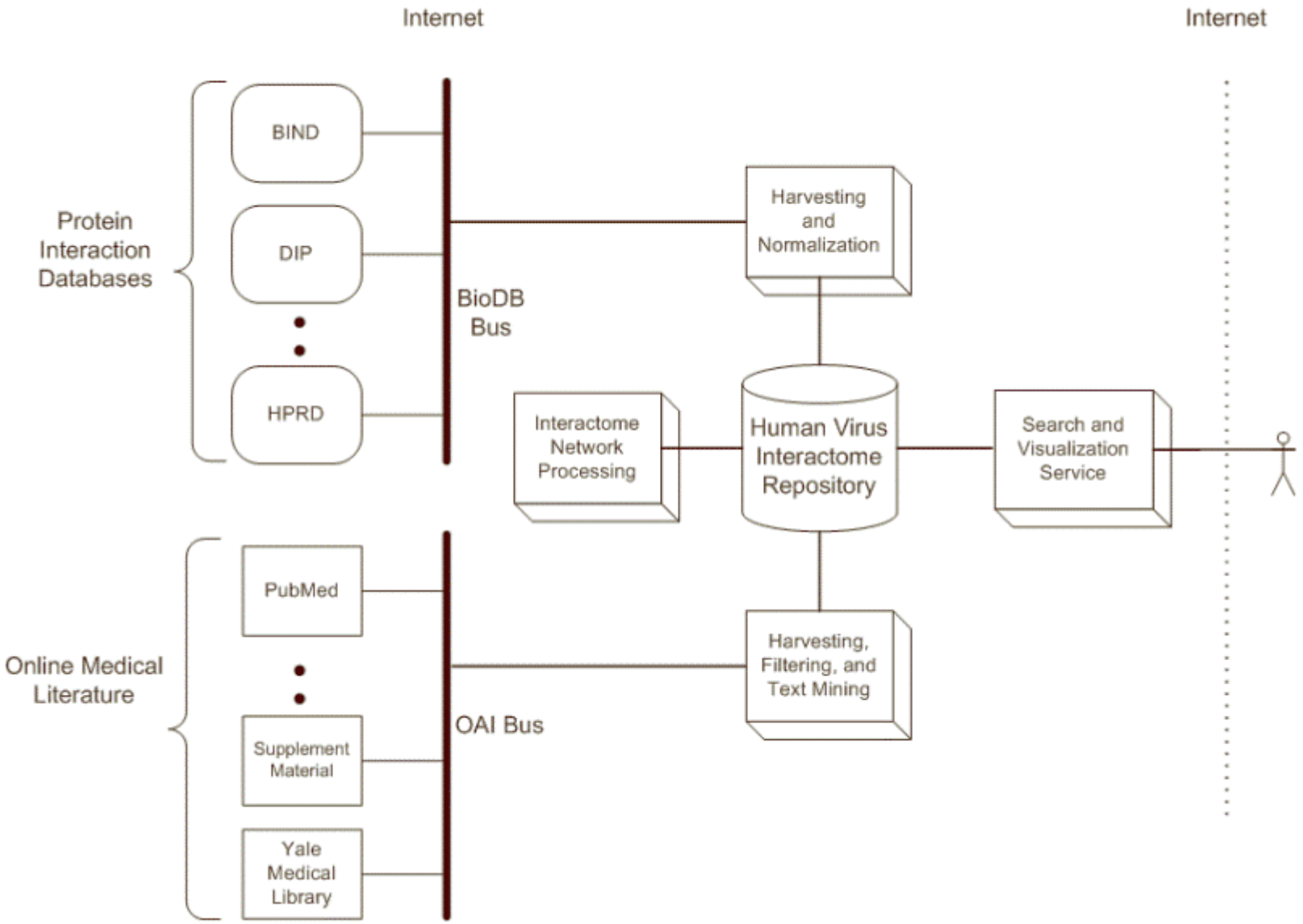
ODU

Viruses in the News

- HIV, SARS, Avian Flu, Human flu pandemics
- A virus conjectured to be cause of mammalian extinctions in the Pleistocene
- Viral proteins interacting with human proteins are responsible for infection and transmission; targets for therapies
- Currently no automated tools to mine published viral-human protein interactions

Coping with Growing Proteomic Information

- Recent advances in protein science
  - High throughput experimental methodologies: Yeast 2-hybrid system, Tagged affinity purification, etc.
- On-line literature and protein interactions databases growing rapidly (>16 Million abstracts in PubMed)
- Need for automated tools to aggregate data, process it, and present it visually in biologically meaningful ways
- Need standards for representing data, and tools that support interoperable databases

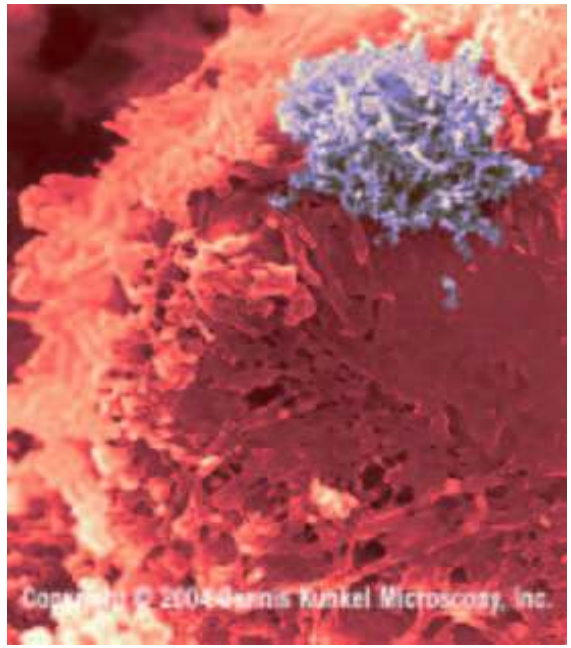

Internet
Internet
BIND
DIP
HPRD
Protein Interaction Databases
BioDB Bus
Harvesting and Normalization
Interactome Network Processing
Human Virus Interactome Repository
Search and Visualization Service
PubMed
Supplement Material
Yale Medical Library
Online Medical Literature
OAI Bus
Harvesting, Filtering, and Text Mining

The HVIR Framework

- **Viral-human protein interactions mined from the literature, e.g., PubMed**
- **Human interactome from curated databases, e.g., Human Protein Reference Database (HPRD)**
- **Integrate the data into a repository, HVIR**
  - Standards for representing protein interactions
  - Unique IDs from International Protein Index
  - Semi-automated curation
  - Regularly harvest new data from literature, databases
  - Build tools to be interoperable

The HVIR Framework

- **Organize interactions network in biologically meaningful ways**
- **Visualize the network for interactive exploration**
- **Make biological inferences, guide further expts.**
- **Initially create this tool for the Human T-cell Lukemia virus (HTLV-1), its protein, Tax**



HVIR

PROTEIN-PROTEIN INTERACTION SEARCH

Please enter protein names/IDs with any delimiter:

Protein ID: REQUIRED (ex. ACTG)

aldh2

SUBMIT

Gene Name

Number of entries: 1

Protein : (This entry)

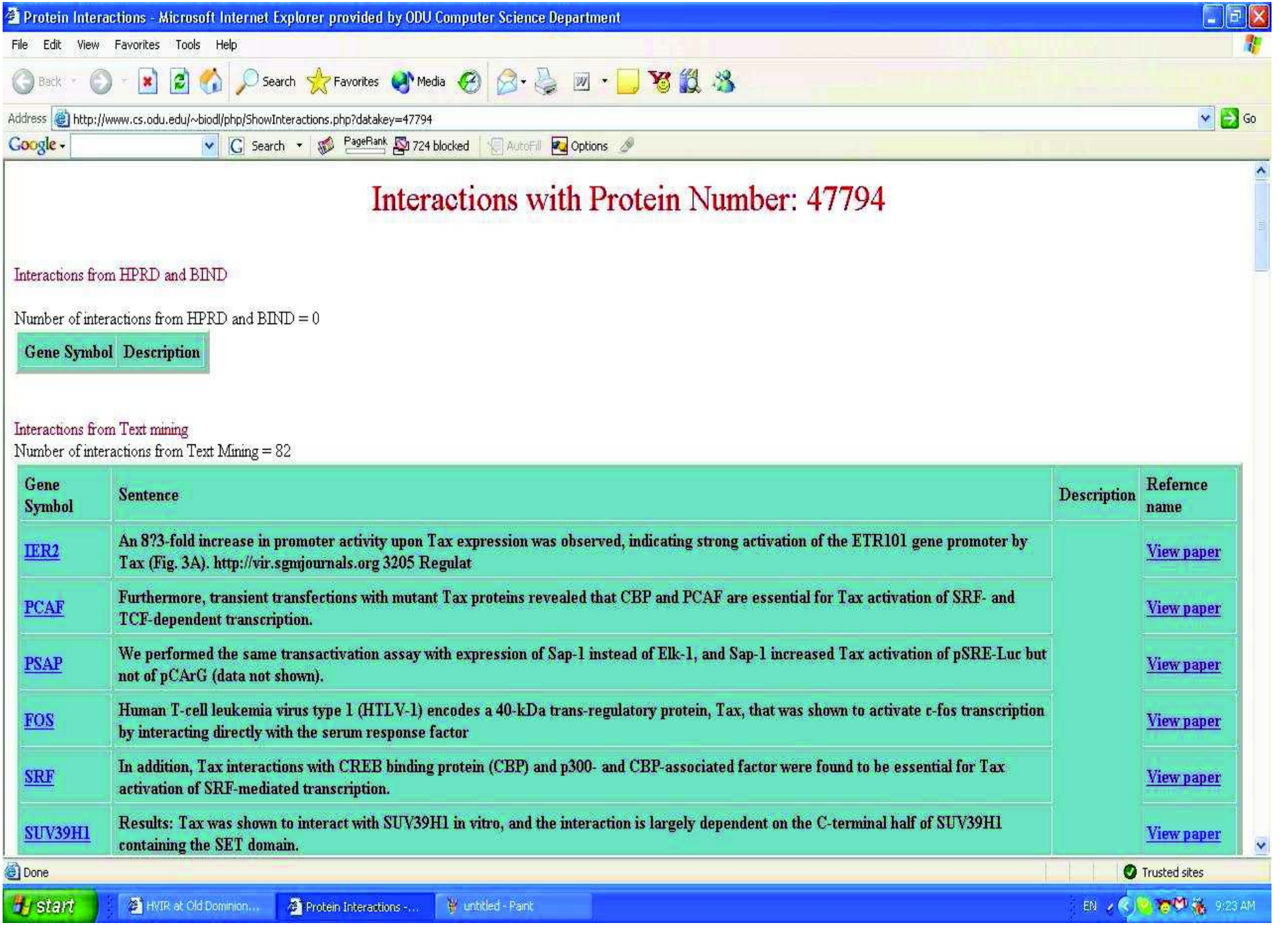
PID: 3, GeneSymbol: ALDH2, ProteinType: 1 View Graph

Gene Alternate Name

Number of entries: 1

Protein : (This entry)

PID: 3, GeneSymbol: ALDH2, ProteinType: 8


Protein Interactions - Microsoft Internet Explorer provided by ODU Computer Science Department
File Edit View Favorites Tools Help
Back Search Favorites Media
Address http://www.cs.odu.edu/~biodl/php/ShowInteractions.php?datakey=47794
Go
Google Search PageRank 724 blocked AutoFill Options
Interactions with Protein Number: 47794
Interactions from HPRD and BIND
Number of interactions from HPRD and BIND = 0
Gene Symbol Description
Interactions from Text mining
Number of interactions from Text Mining = 82
Gene Symbol
Sentence
Description
Refernce name
IER2
An 8?3-fold increase in promoter activity upon Tax expression was observed, indicating strong activation of the ETR101 gene promoter by Tax (Fig. 3A). http://vir.sgmjournals.org 3205 Regulat
View paper
PCAF
Furthermore, transient transfections with mutant Tax proteins revealed that CBP and PCAF are essential for Tax activation of SRF- and TCF-dependent transcription.
View paper
PSAP
We performed the same transactivation assay with expression of Sap-1 instead of Elk-1, and Sap-1 increased Tax activation of pSRE-Luc but not of pCArG (data not shown).
View paper
FOS
Human T-cell leukemia virus type 1 (HTLV-1) encodes a 40-kDa trans-regulatory protein, Tax, that was shown to activate c-fos transcription by interacting directly with the serum response factor
View paper
SRF
In addition, Tax interactions with CREB binding protein (CBP) and p300- and CBP-associated factor were found to be essential for Tax activation of SRF-mediated transcription.
View paper
SUV39H1
Results: Tax was shown to interact with SUV39H1 in vitro, and the interaction is largely dependent on the C-terminal half of SUV39H1 containing the SET domain.
View paper
Done
Trusted sites
start
HVIR at Old Dominion...
Protein Interactions -...
untitled - Paint
EN
9:23 AM

cannot activate viral gene expression from the HTLV-1 long terminal repeat (LTR) due to the inability of Tax-M47 to interact with the p300- and CBP-associated factor (PCAF) (15, 22). No activation of pSRE-Luc was seen with Tax-M47 (Fig. 4A). Tax with the G148V mutation (Tax-G148V), which is analogous to Tax-M22 (T130S and L131A), can activate the CREB and SRF transcriptional pathways but cannot activate NF-κB due to a defect in associating with IKKγ (2, 8, 9, 22, 23). Tax-G148V retained almost 50% of wild-type Tax activity on pSRE-Luc (Fig. 4A). Both Tax-H3S and Tax-K88A are incapable of activating the HTLV-1 promoter, but they differ in their functional defects: Tax-H3S is unable to interact with the bZIP domain of CREB (1), whereas Tax-K88A is unable to bind CBP (10). Tax-H3S activated the SRE enhancer by almost 70% in Jurkat cells compared to the level of enhancement by wild-type Tax, whereas Tax-K88A was unable to activate pSRE-Luc (5% of the wild-type level of Tax) (Fig. 4A). To make certain that the defect in SRE activation was not due to a defect in TCF binding, we performed a GST pull-down assay with Tax-M47 and Tax-K88A. Compared to wild-type Tax (Fig. 4B, lane 2), Tax-M47 (Fig. 4B, lane 3) and Tax-K88A (Fig. 4B, lane 4) were not impaired in their ability to bind Elk-1. However, the inability of Tax-M47 and Tax-K88A to interact with PCAF and CBP, respectively, was detrimental to Tax transactivation of pSRE-Luc. None of the proteins bound the GST-only matrix (Fig. 4B, lanes 6 to 8), and the input proteins (Fig. 4C, lanes 1 to 3) are shown for comparison of the amounts of protein retained on GST–Elk-1 beads. The data show that both the CBP and PCAF binding domains of Tax are required for Tax activation of transcription from the SRE enhancer and that these Tax motifs are not important for Elk-1 binding in vitro.

J Virol

We have presented the results of functional experiments showing that Tax activation of SRF-dependent transcription is dependent on TCF binding to the SRE. Furthermore, transient transfections with mutant Tax proteins revealed that CBP and PCAF are essential for Tax activation of SRF- and TCF-dependent transcription. We also demonstrated that Tax interacts directly with TCFs in vitro, which suggests that Tax activation of the SRE requires contacts with TCFs as well as SRF. We are currently analyzing the composition and assembly of complexes formed on the SRE in the presence of Tax using nuclear extracts and recombinant proteins.

The relative importance of various cellular transcription factor pathways to HTLV-1-mediated T-cell transformation has been examined using the mutant proteins Tax-M47 and -M22 (20, 22). The former is defective for activation of the CREB and SRF pathways, while the latter is unable to activate NF-κB. In the past, the effects of these mutations have been interpreted in the context of a CREB–versus–NF-κB

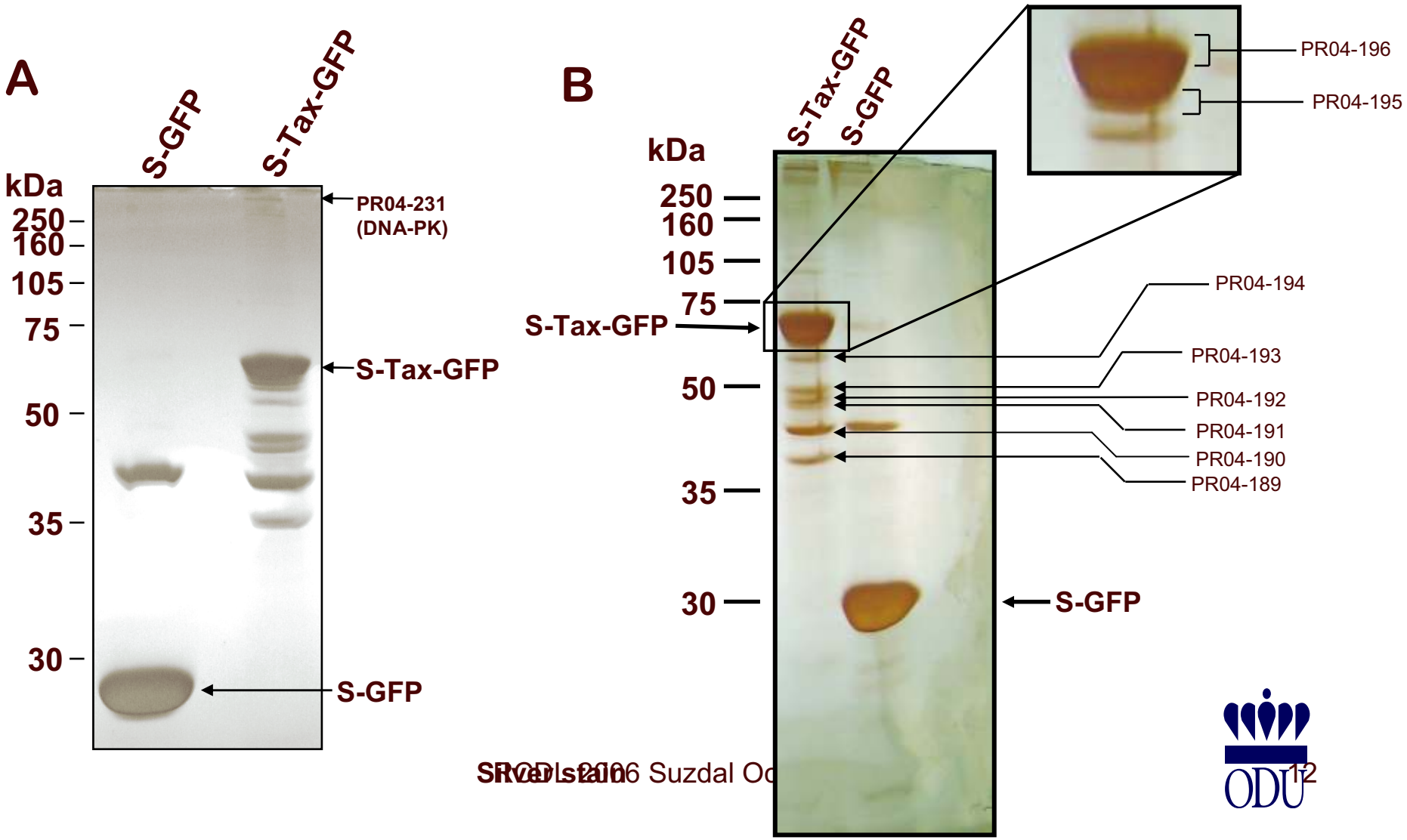
Four Objectives of HVIR

- Tools for creating and sharing protein interaction data
- Tools for processing and organizing interaction networks
- Tools for validating interactions
- Tools for evaluating effectiveness and scalability of the tools above

Four Objectives of HVIR

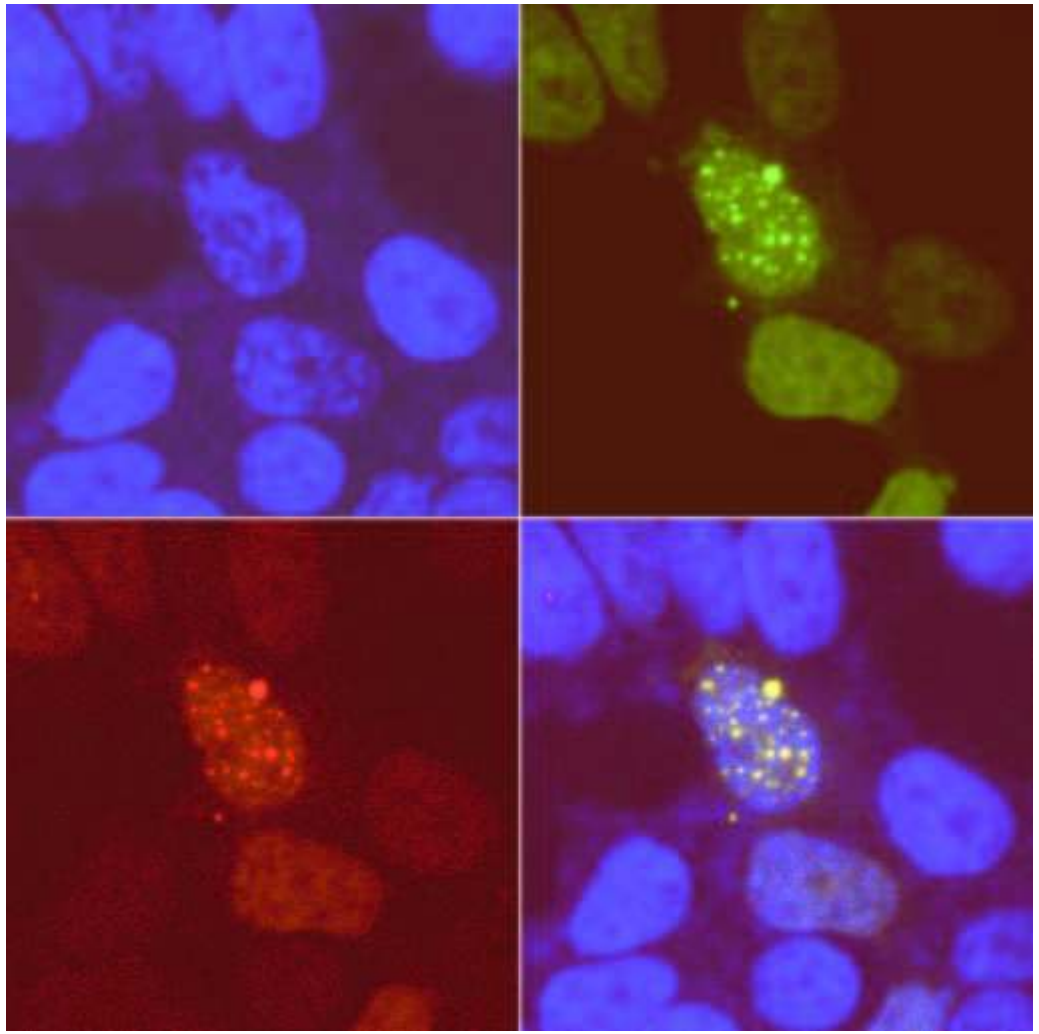
- Creating and sharing interactions: literature mining, standards for representing interactions data, protocols for harvesting data from multiple databases (Open Architecture Initiative)
- Processing interactome networks: clustering using multiple criteria, visualization tools for exploring networks
- Validating content: assign confidence values using probabilistic models, curate ones with low confidence
- Evaluate effectiveness: focus groups of users evaluate how HVIR guides experimentation

Identifying proteins in Tax complex (Durkin, Semmes)



Tax co-localizes with activated DNA-PK (Durkin, Semmes)

Nuclear stain



STaxGFP

DNA-PK
p-T2609

merge

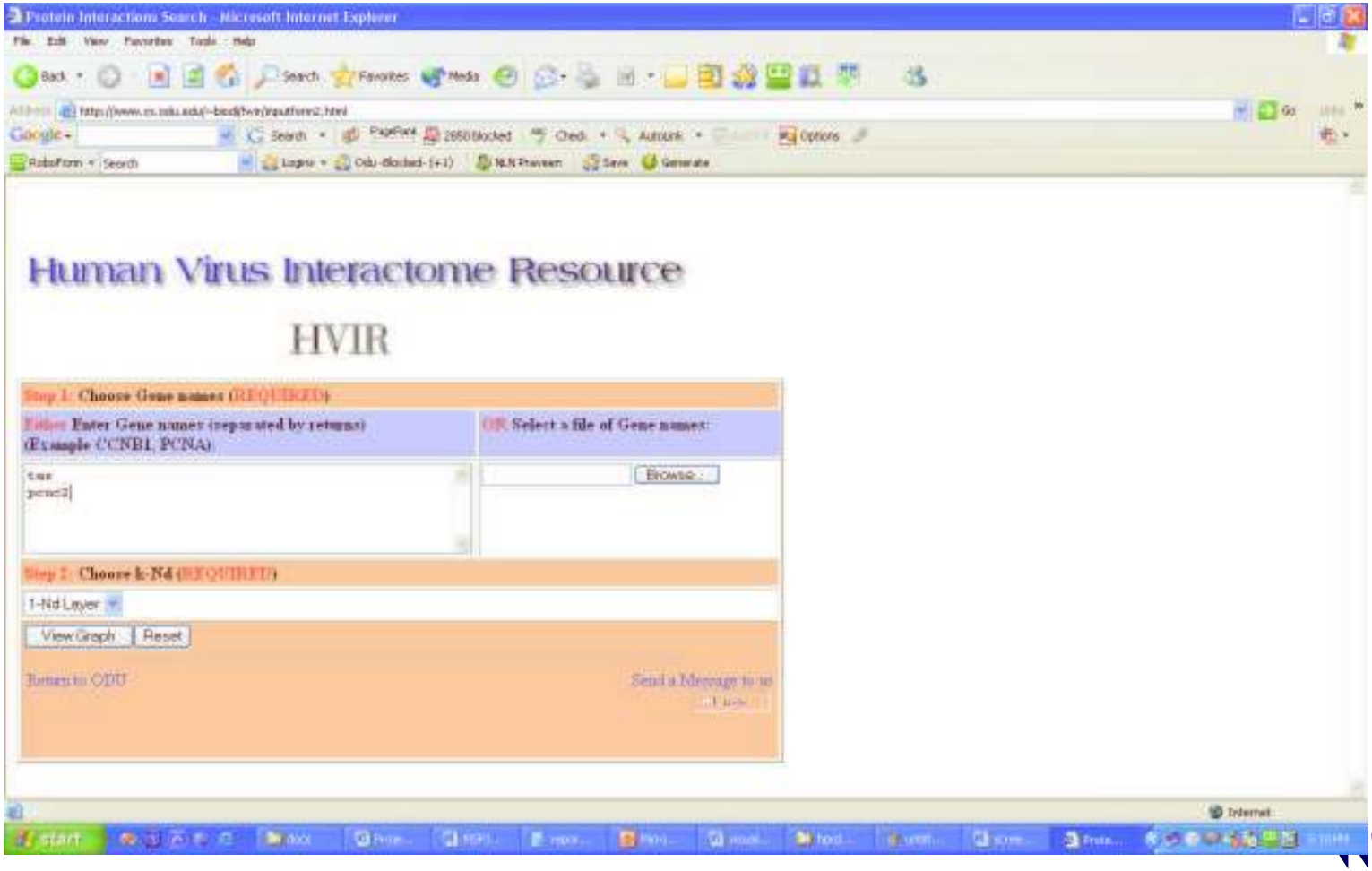
Summary of Work

- **Designed HVIR to provide virologists with data on protein interaction networks**
- **Four major sets of tools: creating interaction data, processing it, validating it, and evaluating effectiveness.**
- **Built a prototype for the HTLV-1 virus in collaboration with virologists and demonstrated it to them.**
- **The Tax interactome now known to include 82 proteins vs. 8 when we began.**
- **Seeking funding to build and extend HVIR.**

Future work

- Detailed study of the Tax interactome to generate predictions and validate utility of HVIR.
- Build HVIR and make it available for use by biologists.
- Employ a second virus, cytomegalovirus (CMV), with a larger set of proteins to study scalability (Julie Kerry, EVMS).
- Promote standards for data representation and interoperable protocols for data harvesting.

HVIR Input form



Tax interactors

a HumanVirus Interactome Resource

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Dominion Univ

Interactions with Protein Number: 47794

Interactions from HPRD and BIND

Number of interactions from HPRD and BIND = 0

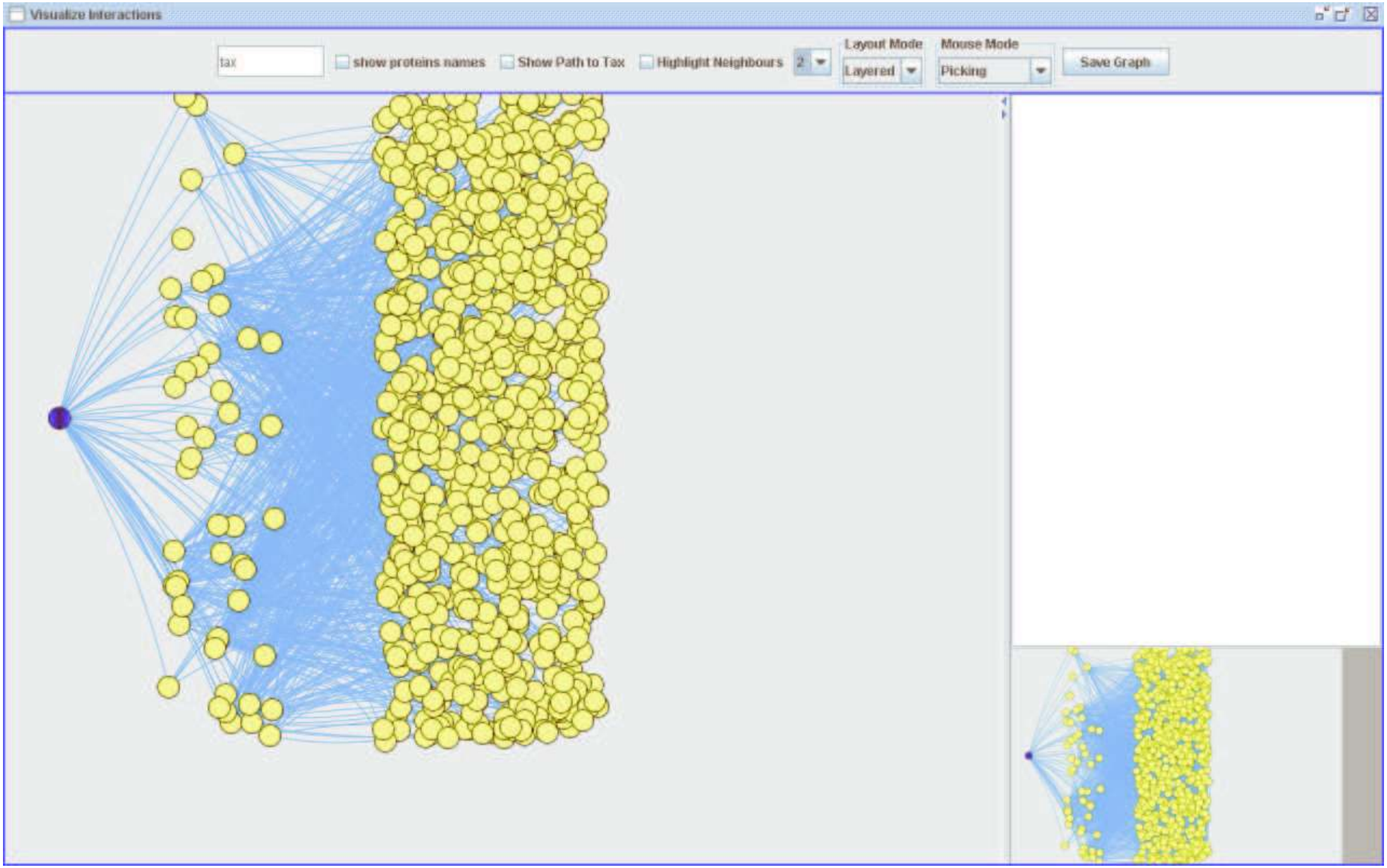
Gene Symbol	Description

Interactions from Text mining

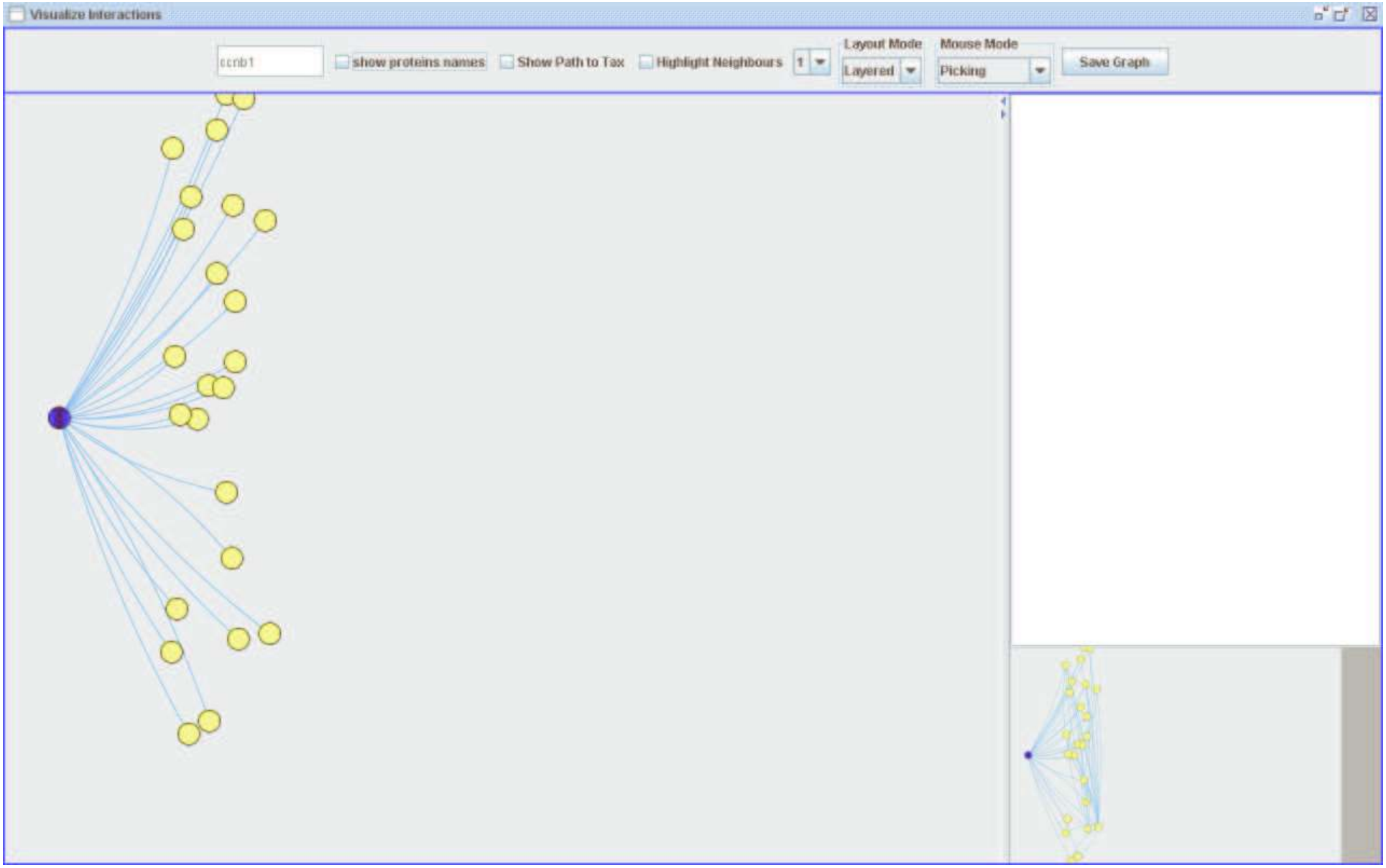
Number of interactions from Text Mining = 45

Gene Symbol	Sentence	Description	Refernce name
IER2	An 8?3-fold increase in promoter activity upon Tax expression was observed, indicating strong activation of the ETR101 gene promoter by Tax (Fig. 3A). http://vir.sgmjournals.org 3205 Regulat		View paper
PCAF	Furthermore, transient transfections with mutant Tax proteins revealed that CBP and PCAF are essential for Tax activation of SRF- and TCF-dependent transcription.		View paper
PSAP	We performed the same transactivation assay with expression of Sap-1 instead of Elk-1, and Sap-1 increased Tax activation of pSRE-Luc but not of pCArG (data not		View paper

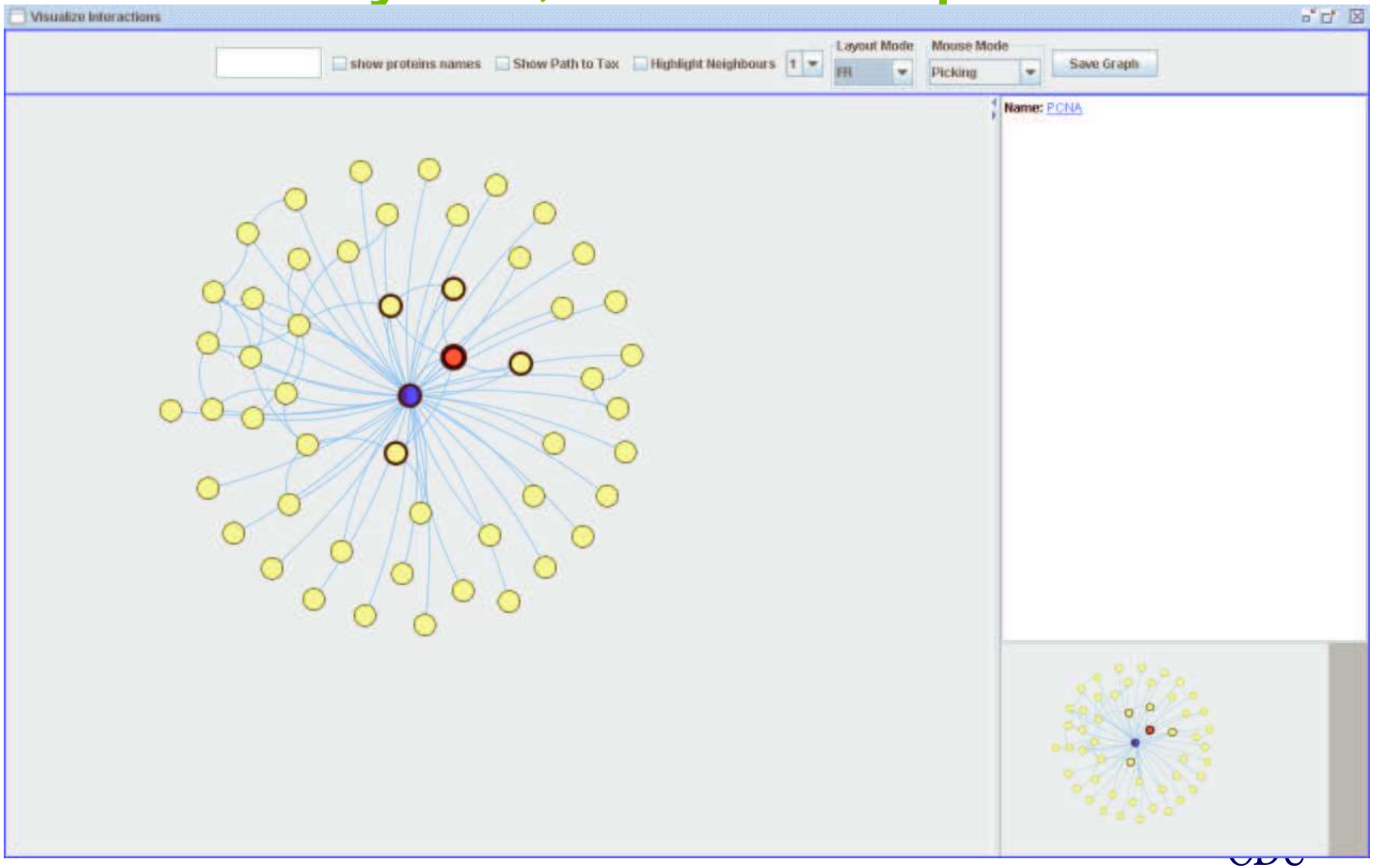
Second neighbors of Tax



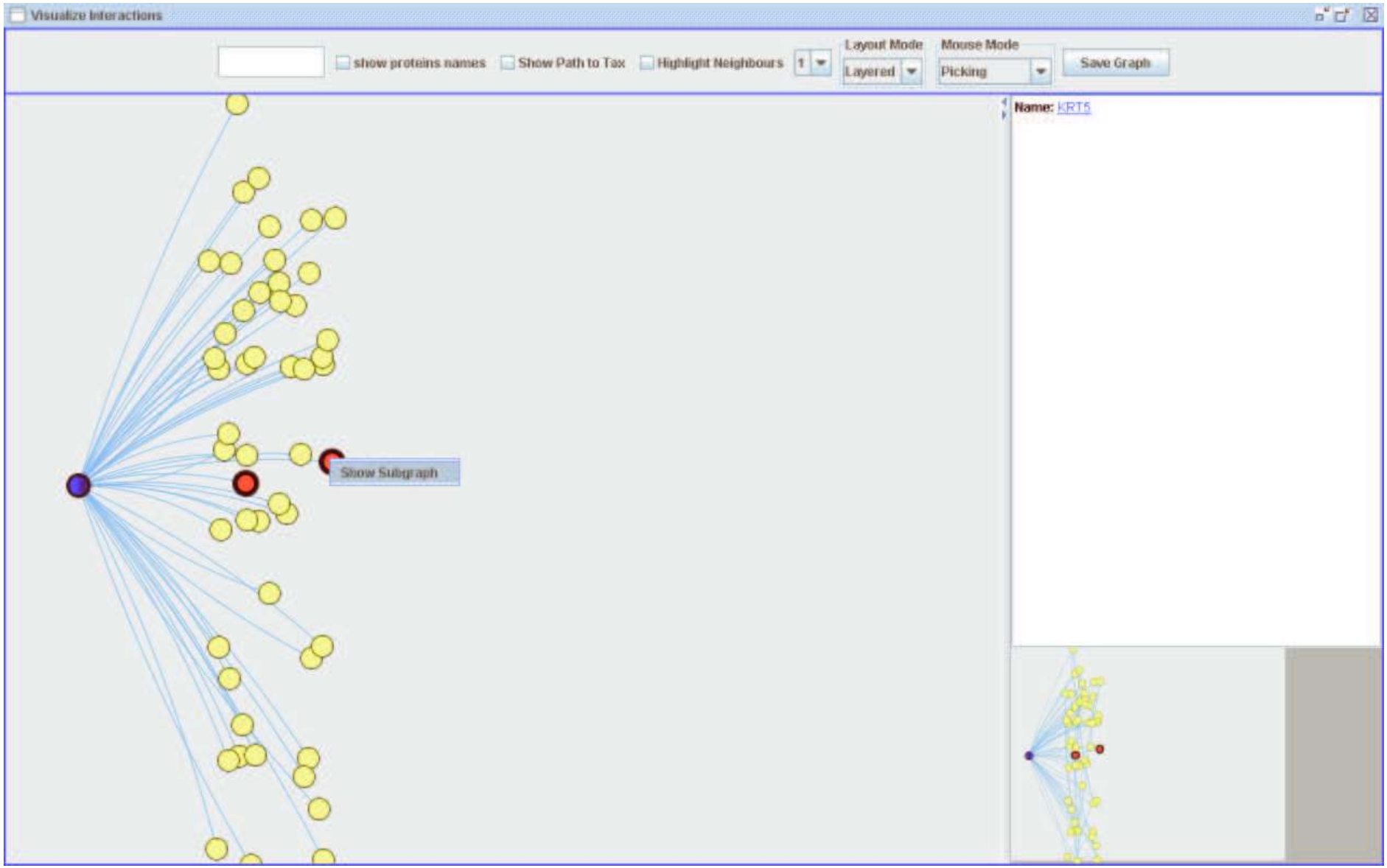
Local network of selected protein



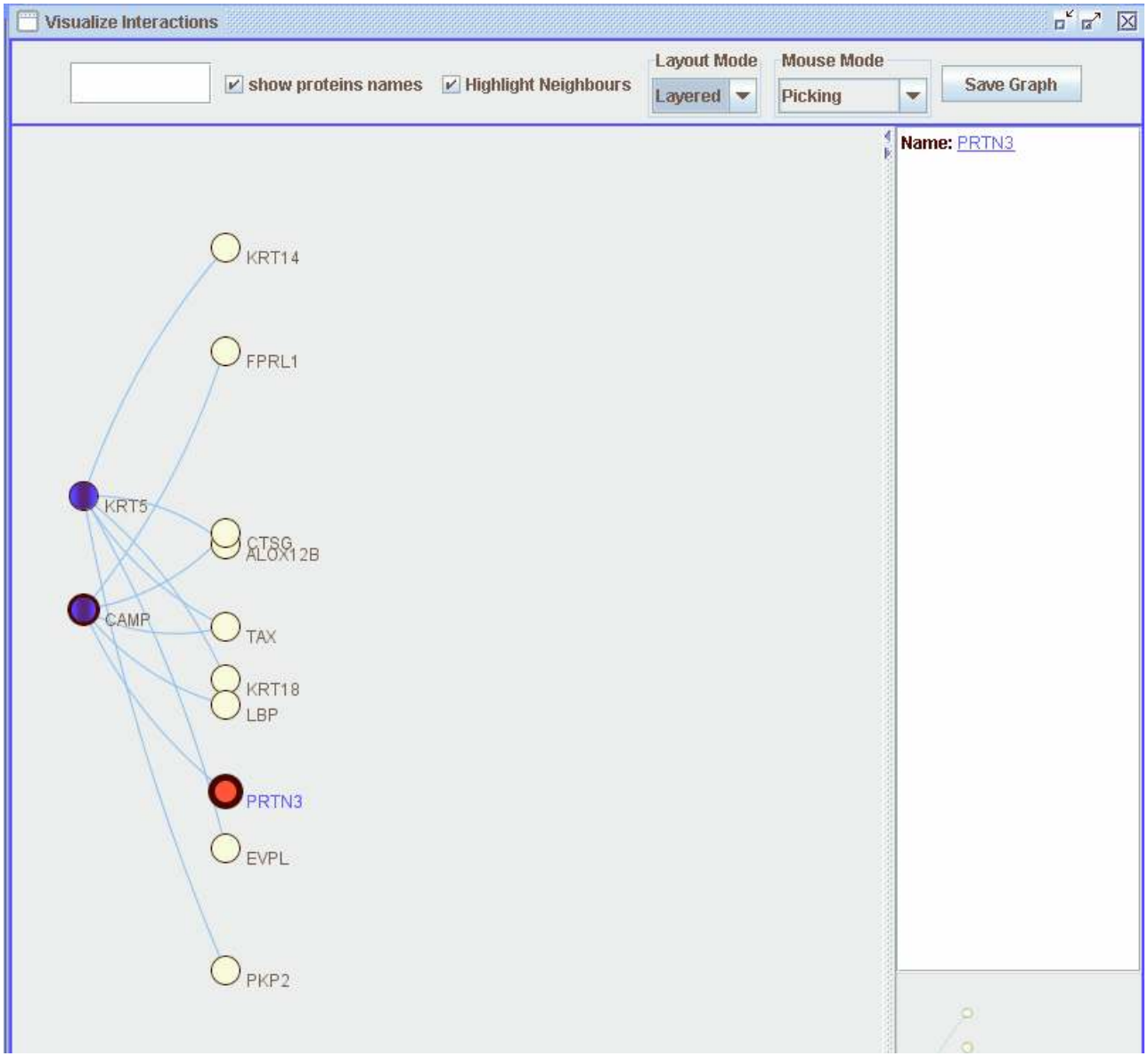
FR Layout, selected proteins



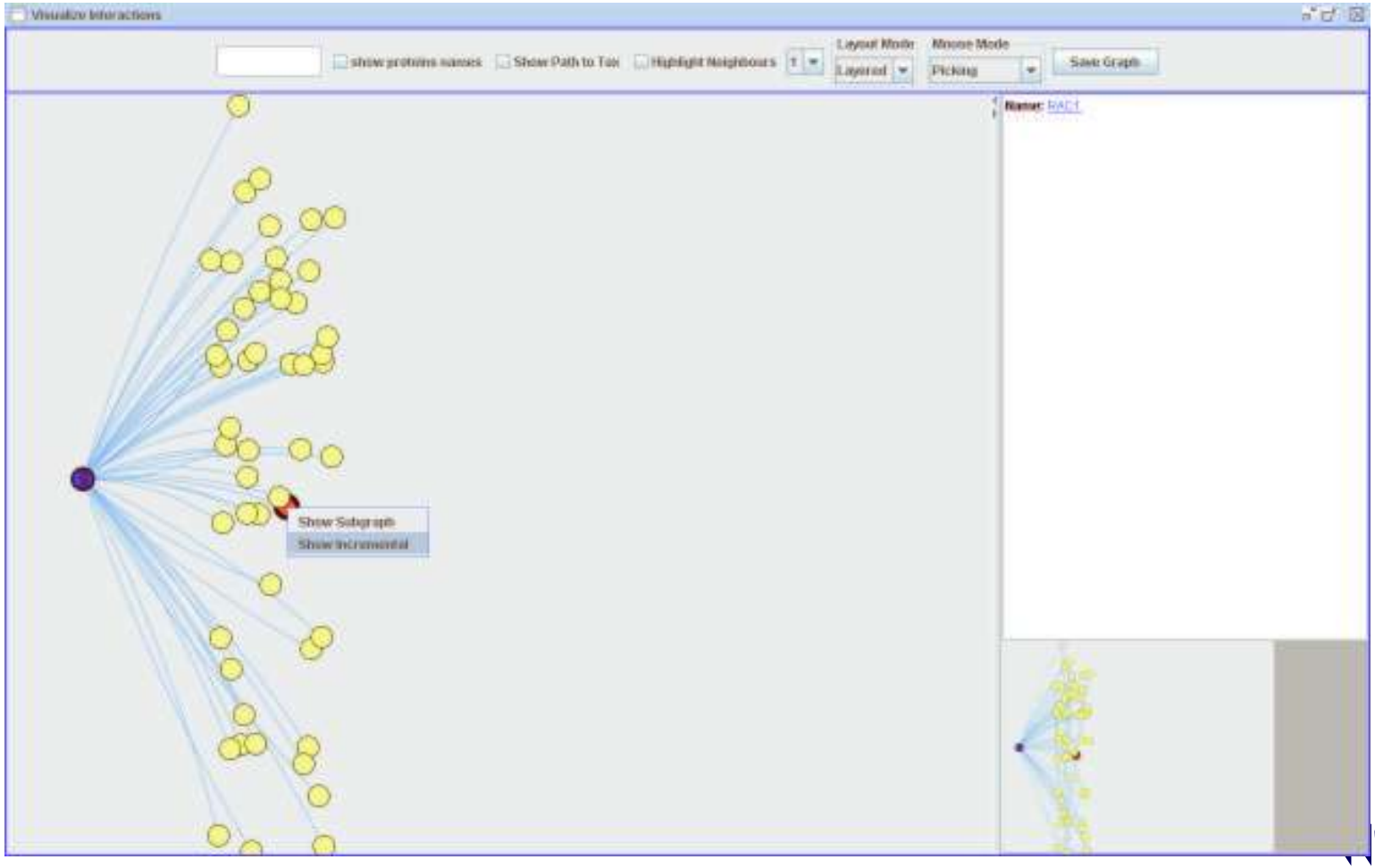
A subnetwork of selected proteins



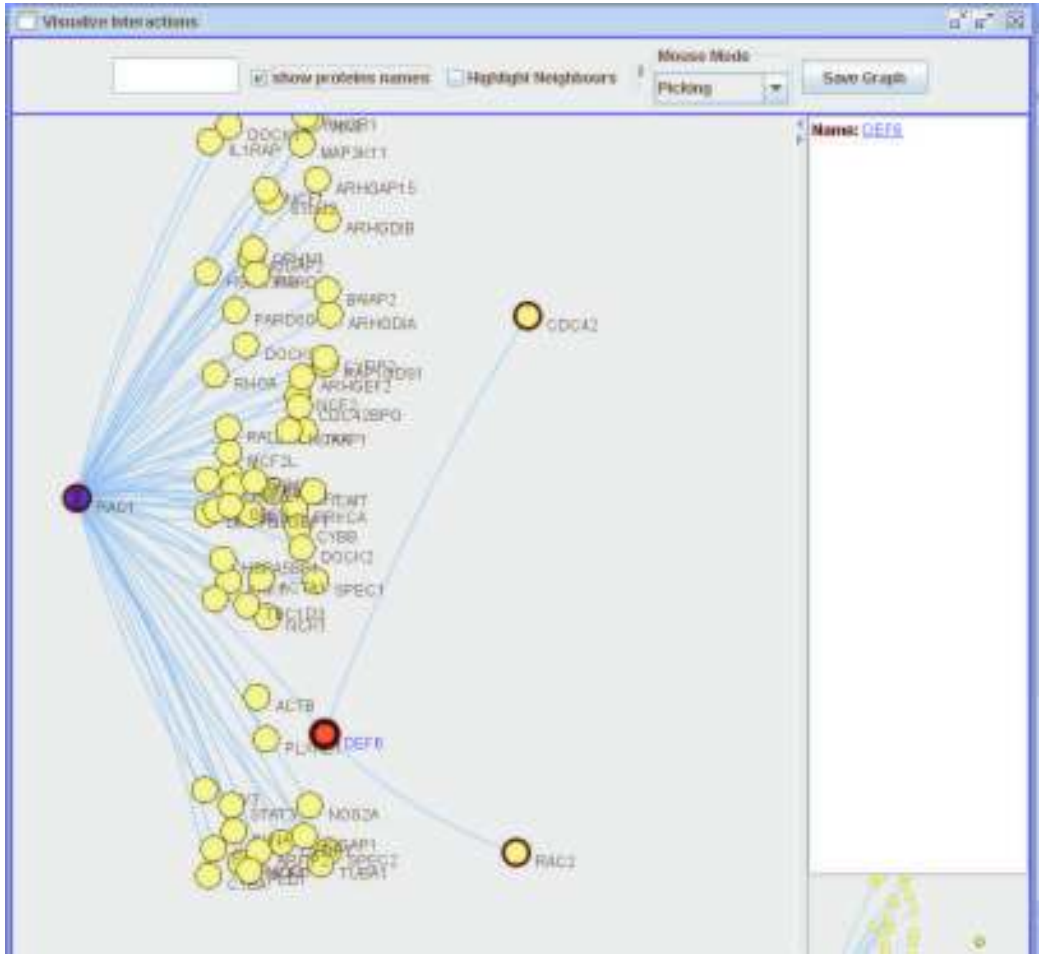
A subnetwork of sel. proteins



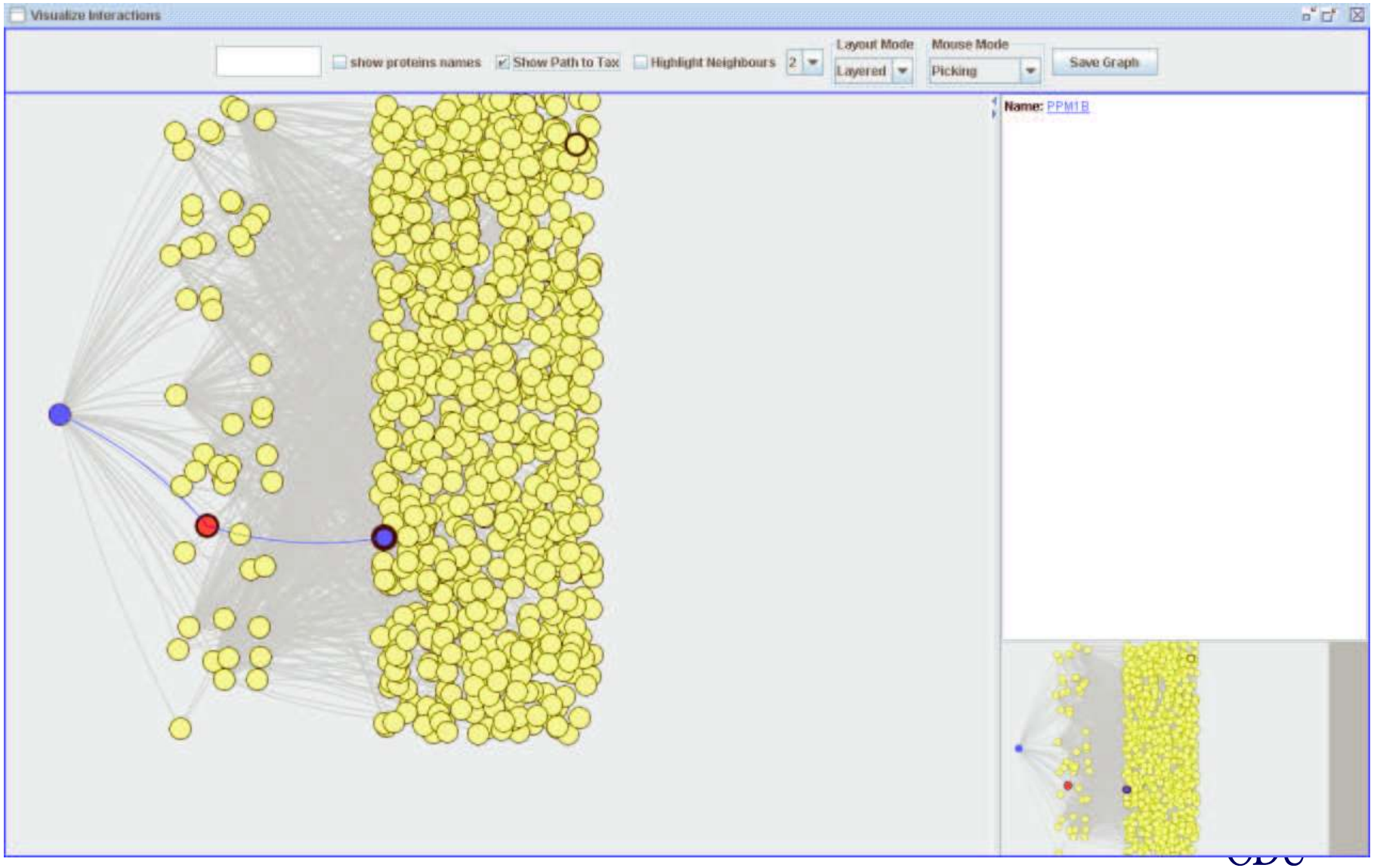
Incremental Navigation



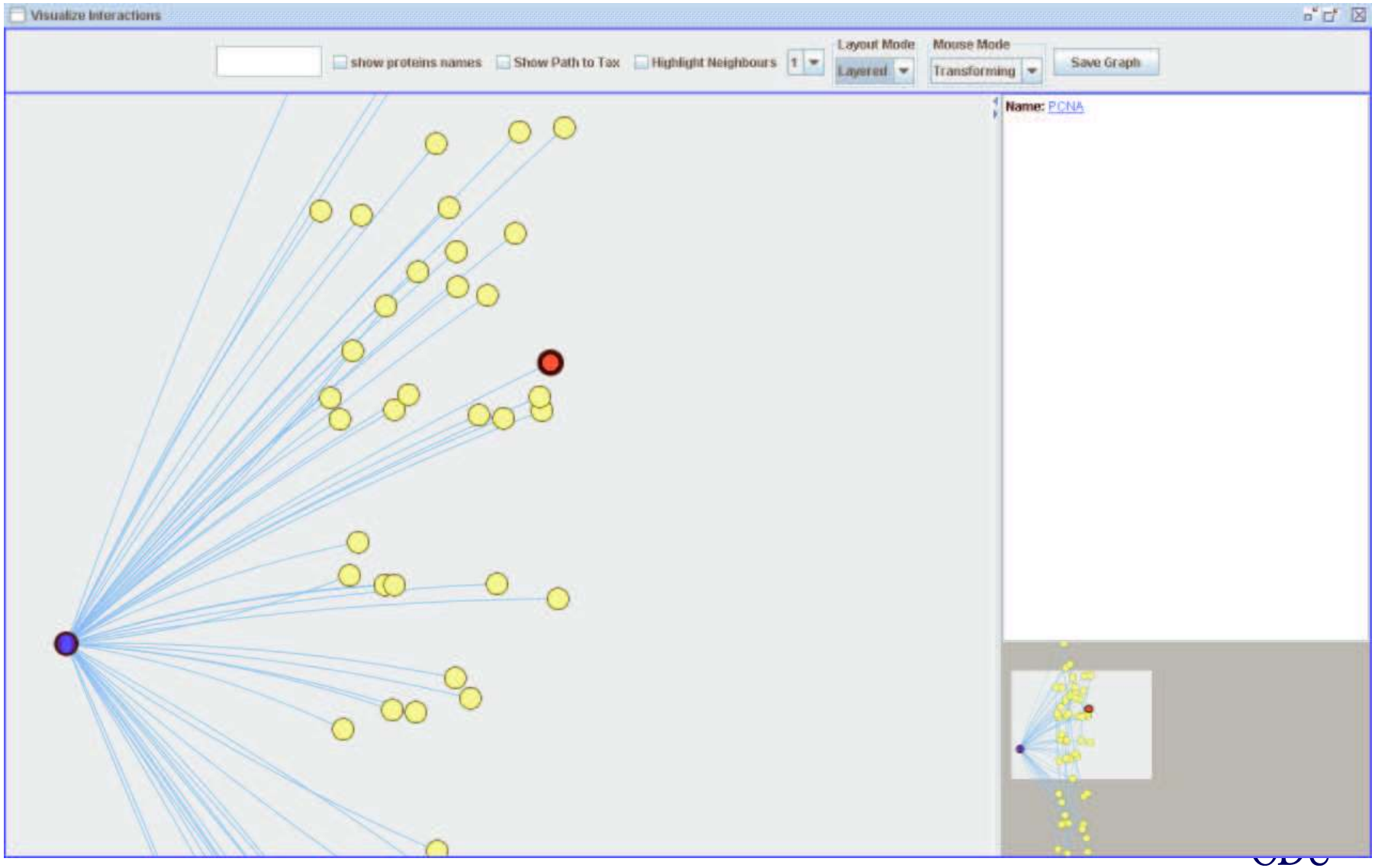
Incremental Navigation



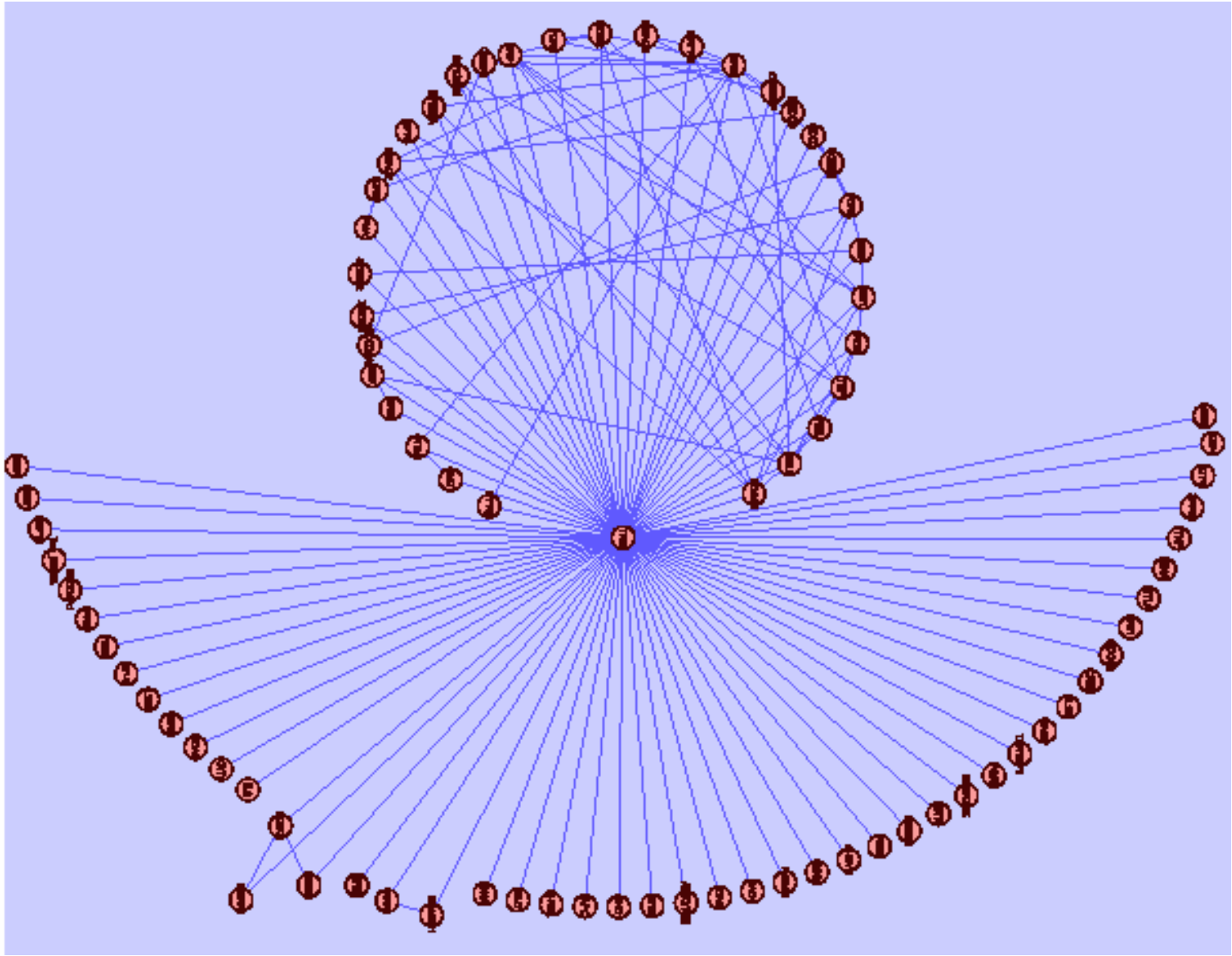
Shortest path from Tax



Zooming in on a subnetwork



Current version of Tax network



N = 82