Study of signalling pathways using constrained-based modelling techniques

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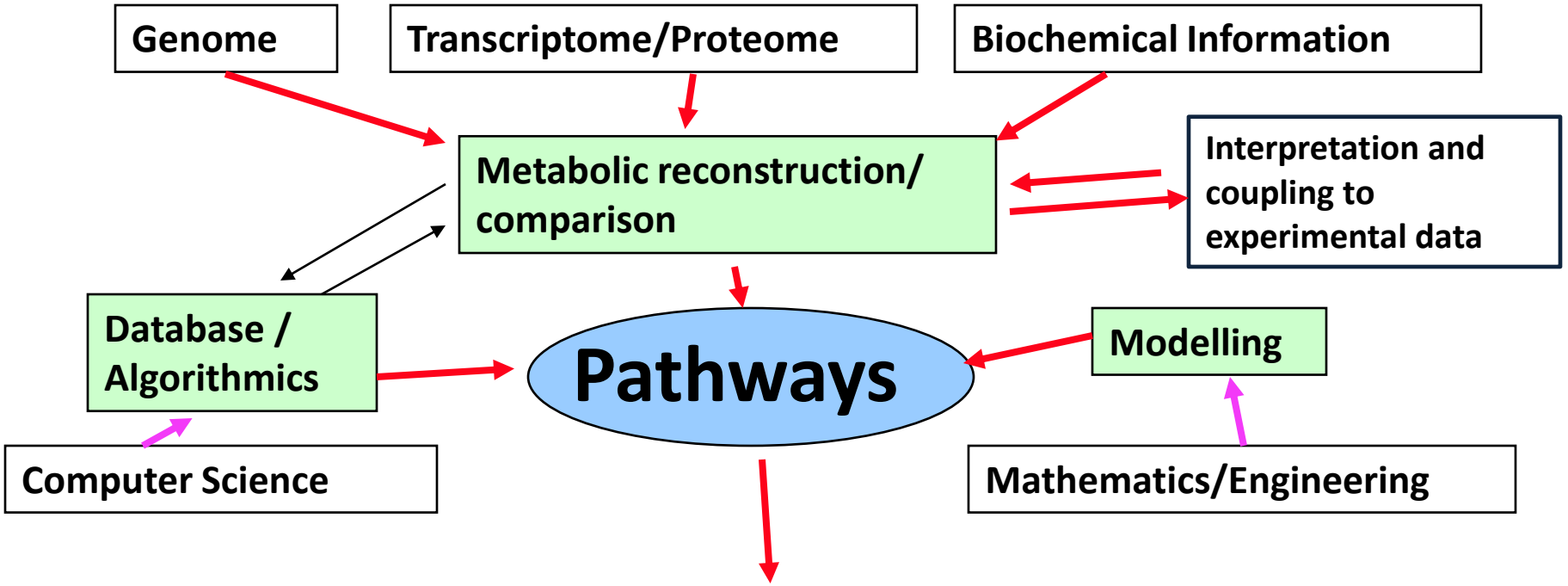
ICM 2010 Satellite Meeting, HICC, Hyderabad

A “get-together”

- Remarkable advances to explain the components of cellular pathways and gene networks
- They provide a snapshot of the complete genetic activity of a cell



- Better understanding of cellular physiology
- Identification of missing links or new pathways and routes
- Identification of target molecules
- **Understanding the unicellular metabolism and signalling events**

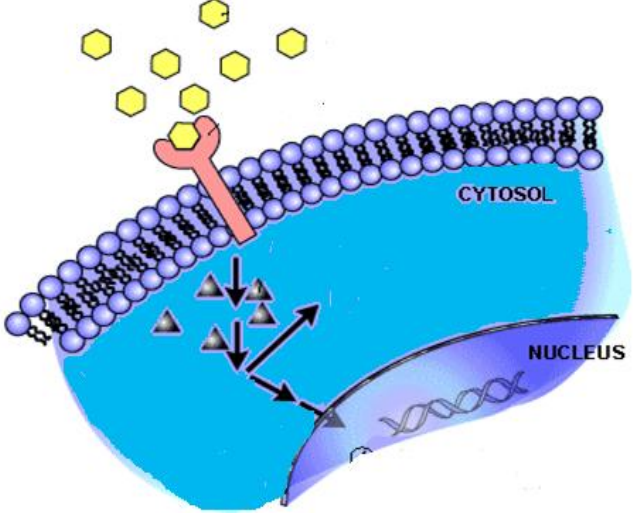
Objective

To study T-cell Receptor and MAPK signalling pathways and hence identify important molecules that are involved in Immune and Cancer cells

Our Work

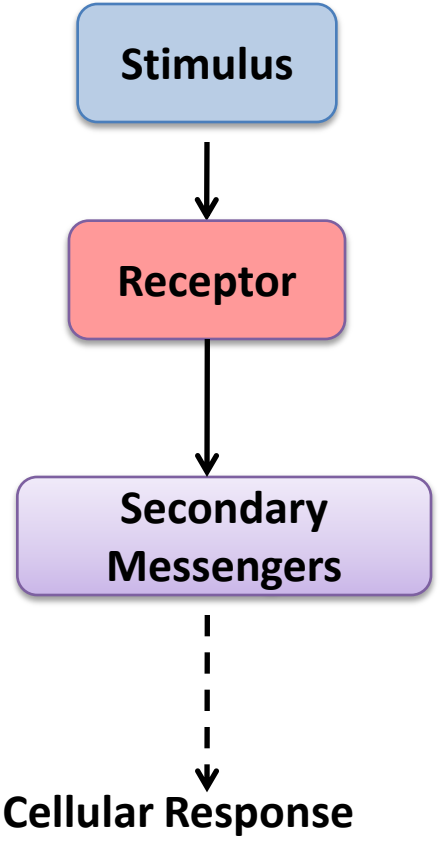
- Reconstruction of T-cell Receptor and MAPK Signalling Pathways
- Mathematical Modeling of the reconstructed pathways using Boolean formalism
- Application of Constrained-based technique
  - Logical Steady State Analysis
- Identification of optimal intervention points (targets) and optimal / shortest / alternative paths
- Connecting to other pathways related to immune and cancers

Signalling pathways



- The cascade of processes by which an extra-cellular signal (typically *hormone, neurotransmitter, antigen*) interacts with a receptor at the cell surface, causing a change in the level of a secondary messenger (for example, calcium or cyclic AMP)

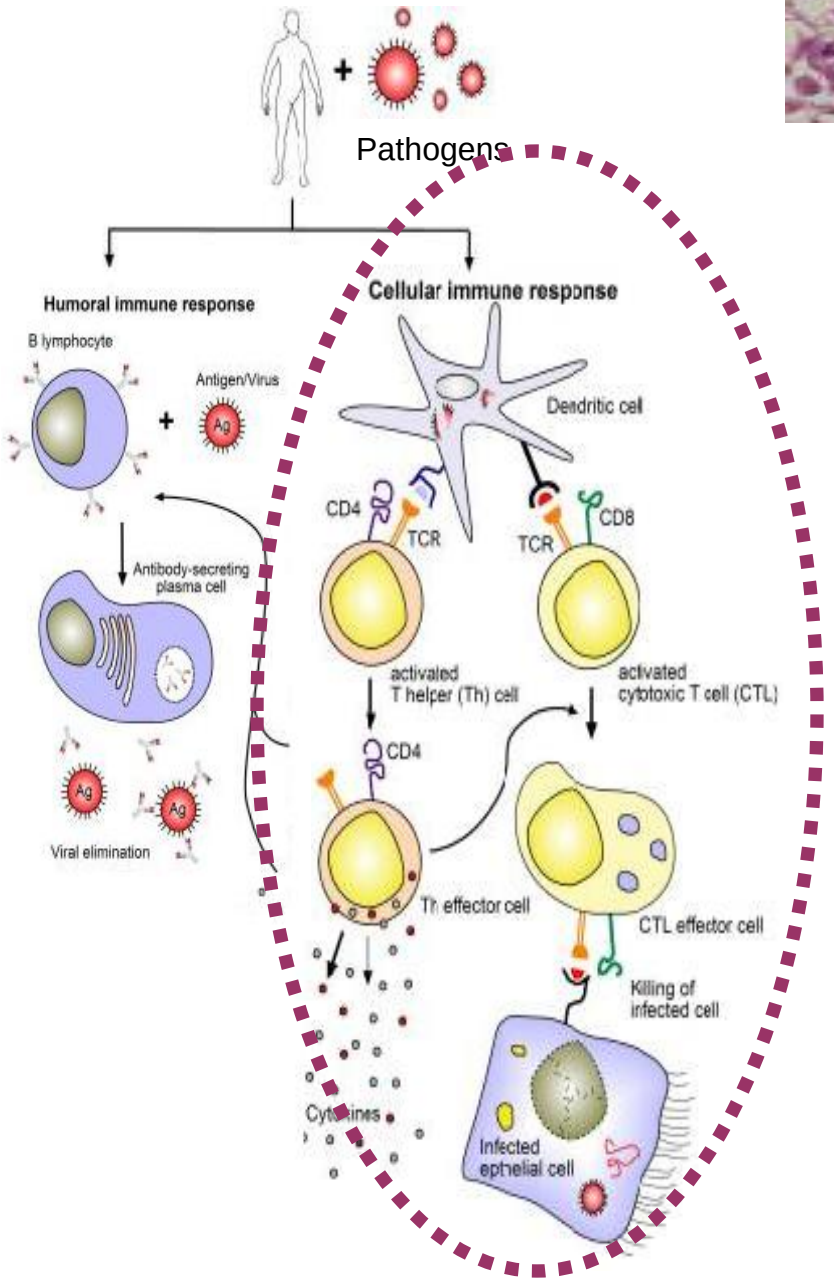
- Effects a change in the cell's functioning (for example, *Cellular proliferation and differentiation*, *cytokine production*, *apoptosis*, *expression of genes*).

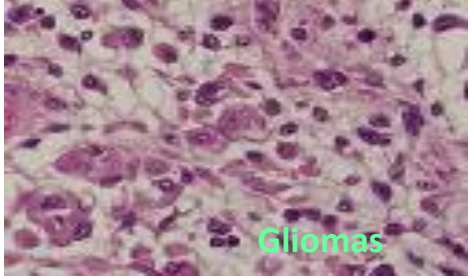


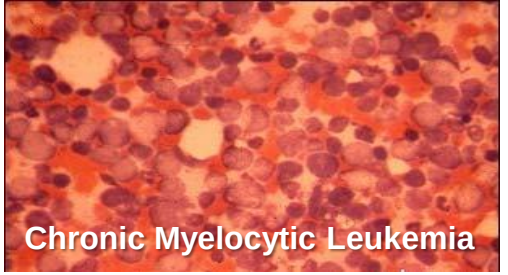
Why model Signal Networks?

- **To understand the biochemical processes involved in the cell**
- **Identify difference of mechanism between species**
- **Identification and Regulation of Key Species**
- **Predict the effects of drugs on Signal Transduction**
  - e.g. what protein should be disrupted to prevent T-cell Hyper-proliferation
- **Many disease processes involve changes in the normal pathways**
  - **e.g. change in signalling pattern in cancer cells**

Immune System and the Problem of Cancer







- **Cancer: one of the greatest killers in world**
  - 15 percent of all human deaths - 7.6 million deaths in 2007
- **The mechanisms of establishment and destruction of the disease is still not clear and there is a need to address not only preventive measures but also more successful treatment strategies**
- **Mathematical understanding of the problem captures some essential characteristics of cancer cell kinetics**

Efforts along these lines are now being investigated through *Tumor-Immune* interaction or *Immunotherapy*

The question is how much we can activate the immune system (specifically CTLs) or increase the production of cytokines to control the tumour progression?

Identification and Modelling of Pathways in Immune (T-cells) and Cancer Cells

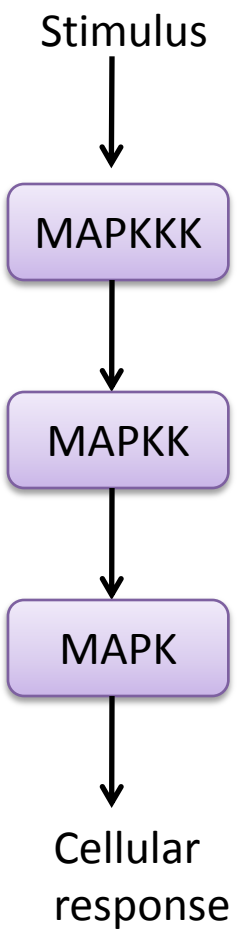
Major Pathways

- **MAPK Pathway**
- TGF-Beta Pathway
- PI3-Kinase Pathway
- Calcium Pathway
- Signal Transduction by Growth Factor Receptors
- Cell Cycle
  - CKI-CDK-RB Pathway
  - ARF-MDM2-p53 Pathway
- mTOR Pathway
- VEGF Pathway
- WNT Pathway
- **T Cell Receptor Pathway**
- Apoptosis

Mitogen Activated Protein Kinase pathway (MAPK)

- Mitogens - Chemical substances that triggers mitosis
- Ser/Thr specific protein kinases
- Occurs in almost all kinds of cells
- Responses like Proliferation, differentiation
- Specific Mutations cause uncontrolled proliferation → Cancer

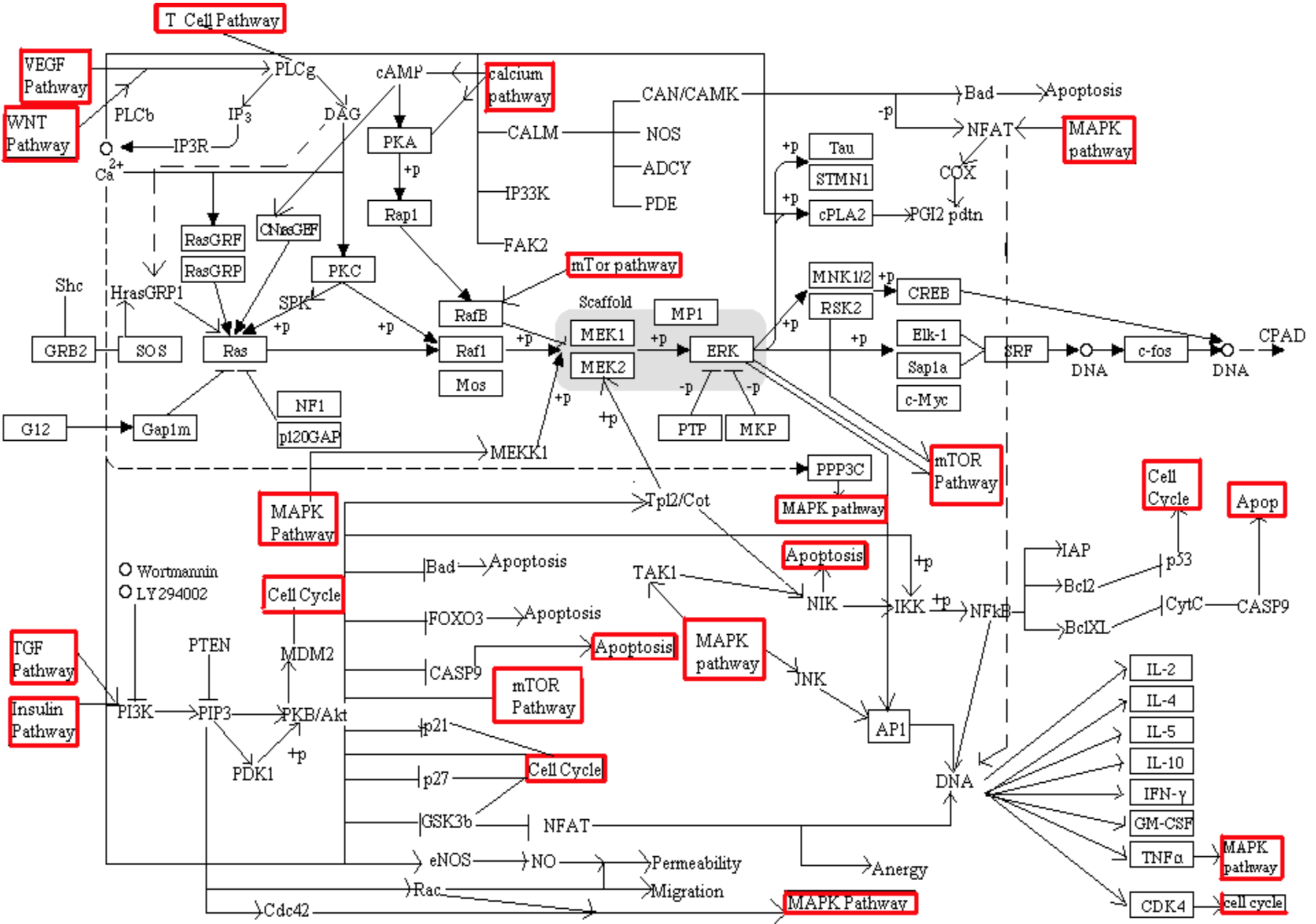
✓ **Studying this pathway can help understanding the progression of the disease**



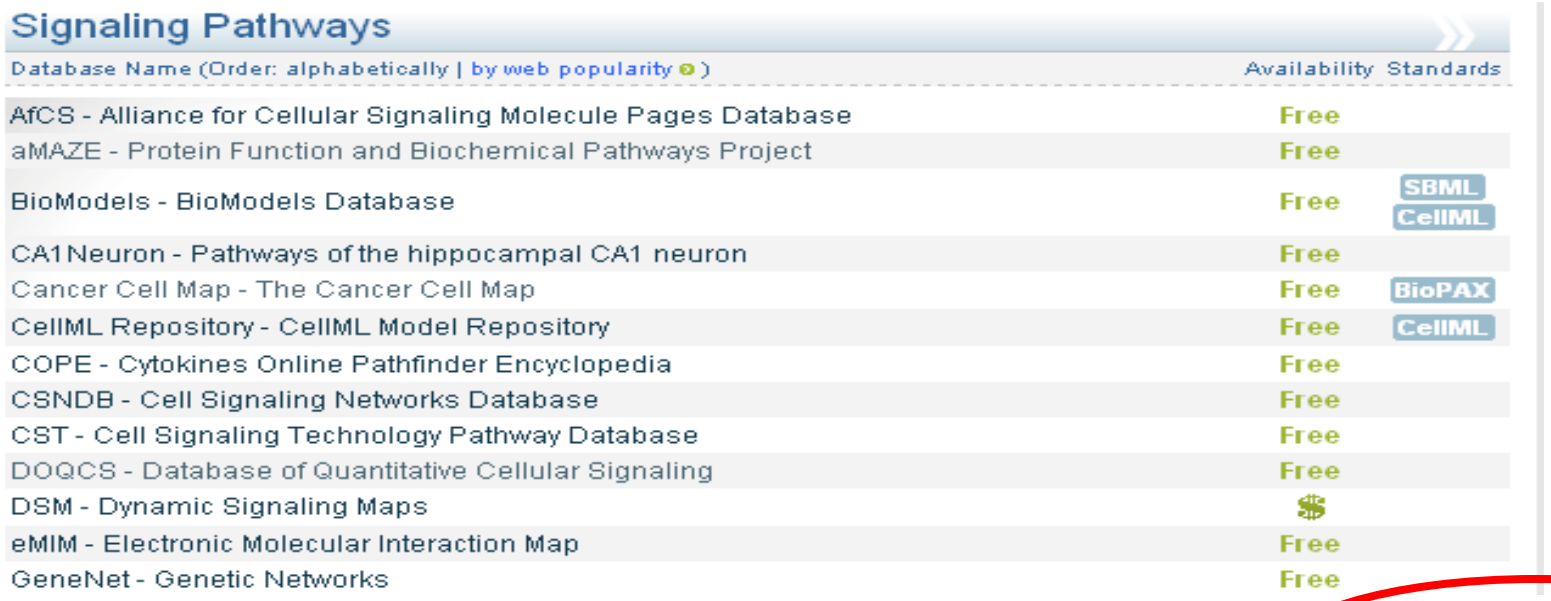
T-cell Receptor Pathway

- Major Signal transduction pathways in T-cells
- Involved in cytokine production, activation of apoptosis and other pathways
- Identification of T11TS/SLFA3 induce pathway

Basic Network



Signalling Pathway Databases



Signaling Pathways

Database Name (Order: alphabetically | by web popularity)	Availability	Standards
AfCS - Alliance for Cellular Signaling Molecule Pages Database	Free	
aMAZE - Protein Function and Biochemical Pathways Project	Free	
BioModels - BioModels Database	Free	SBML CellML
CA1 Neuron - Pathways of the hippocampal CA1 neuron	Free	
Cancer Cell Map - The Cancer Cell Map	Free	BioPAX
CellML Repository - CellML Model Repository	Free	CellML
COPE - Cytokines Online Pathfinder Encyclopedia	Free	
CSNDB - Cell Signaling Networks Database	Free	
CST - Cell Signaling Technology Pathway Database	Free	
DOQCS - Database of Quantitative Cellular Signaling	Free	
DSM - Dynamic Signaling Maps	$	
eMIM - Electronic Molecular Interaction Map	Free	
GeneNet - Genetic Networks	Free	

RECONSTRUCTION

Problems

- Single molecule activated by multiple molecules
- Difficulty in targeting molecules produced inside the cell
- Multiple pathways connected through multiple molecules
- Single molecule activates multiple pathways
- **To get a comprehensive picture of the entire pathway**

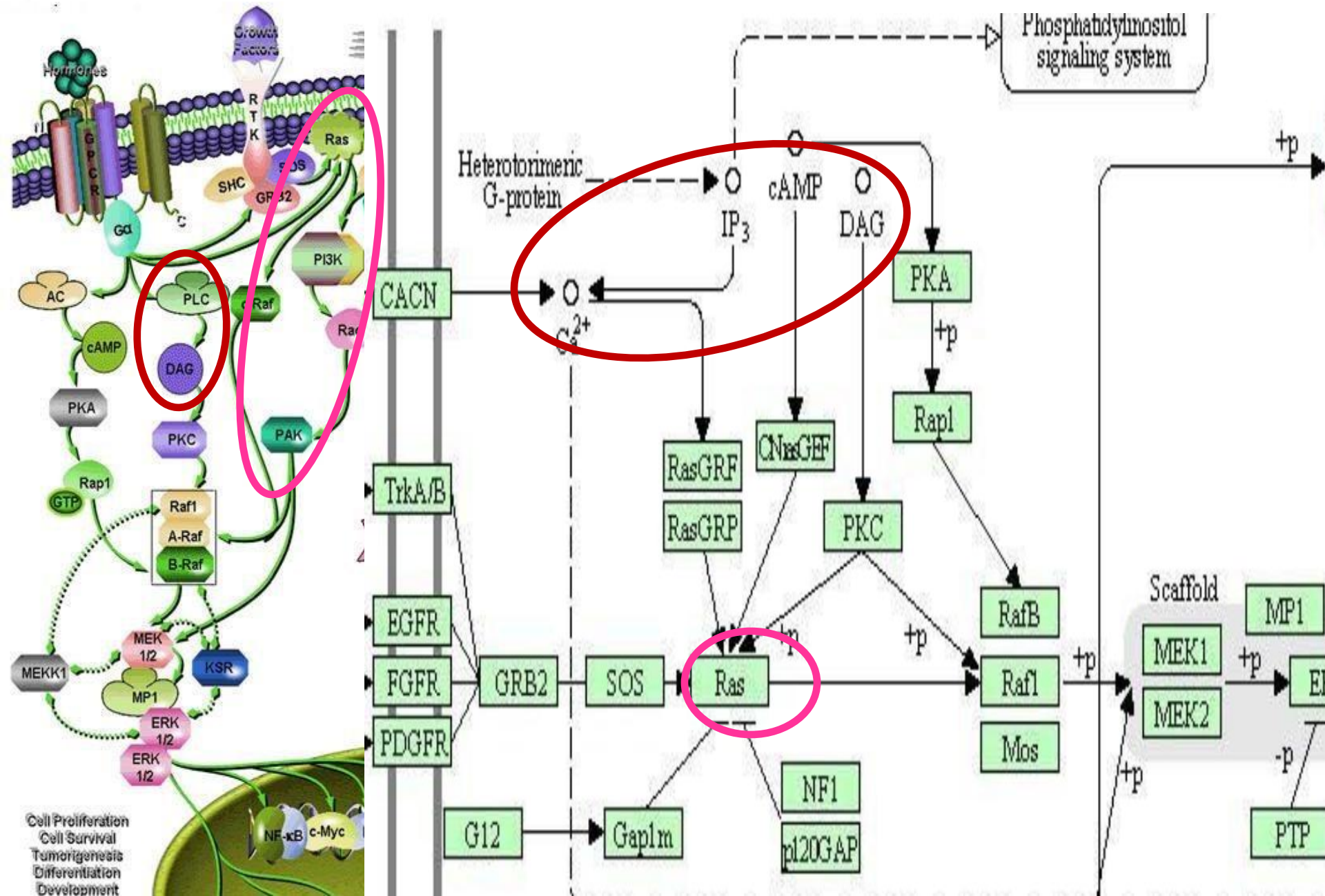
Why do we need reconstruction?

- Numerous signaling databases present online
- Inconsistent & Incomplete data available on different databases.

Database	Molecules in MAPK signaling pathway	Interactions in MAPK signaling pathway
KEGG	**179**	**110**
Protein Lounge	**120**	**75**
Cell Signaling	**155**(90+35+30)	**120**(60+25+35)
Panther	**36**	**44**
BioModels	**34**(26+8)	**30**(20+10)
NPID	**80**(45+35)	**95**(55+40)

We studied > 15 databases for T-cell Receptor and MAPK signaling pathways and cross-checked with > 100 published papers

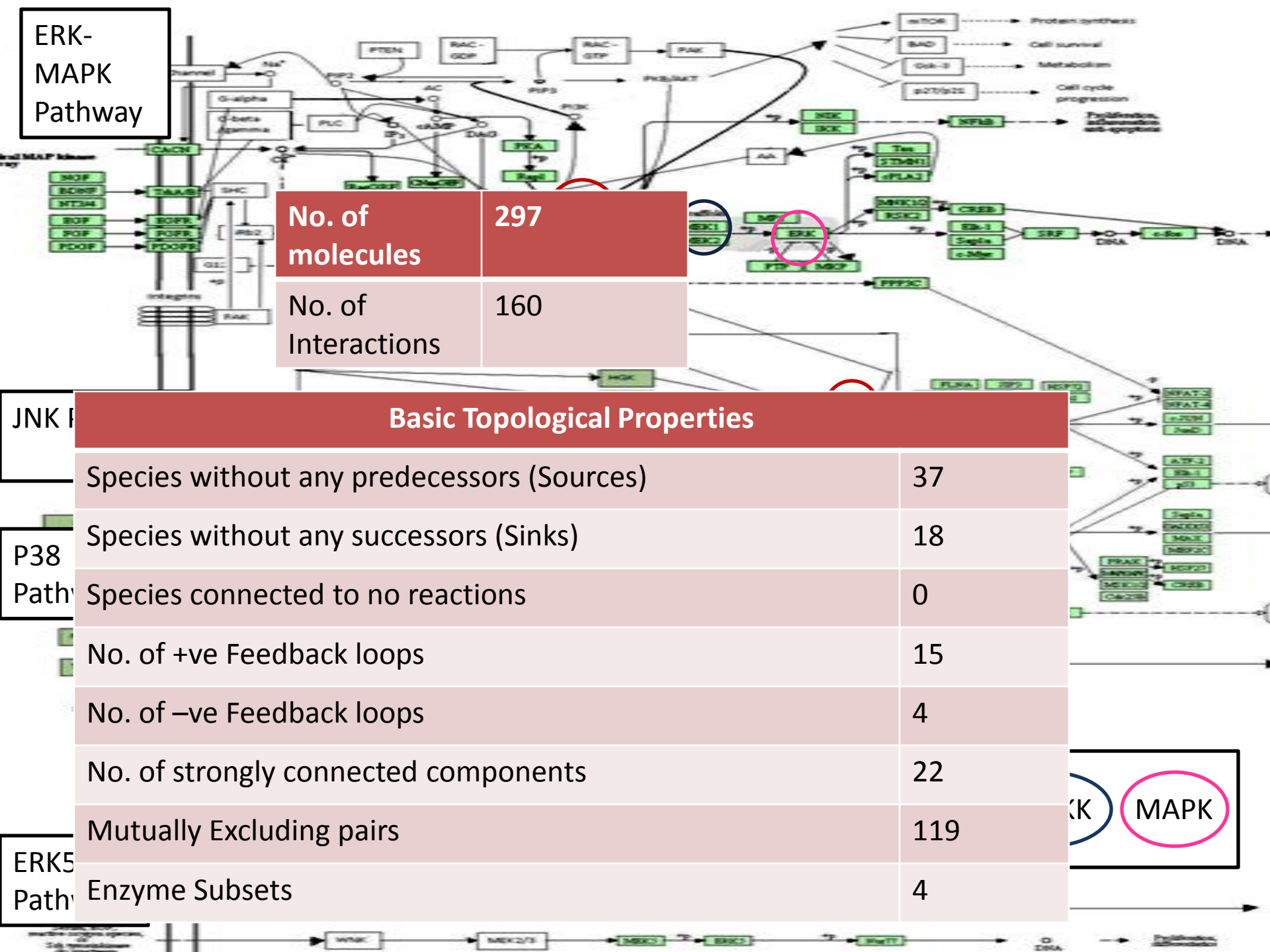
Protein Lounge vs KEGG

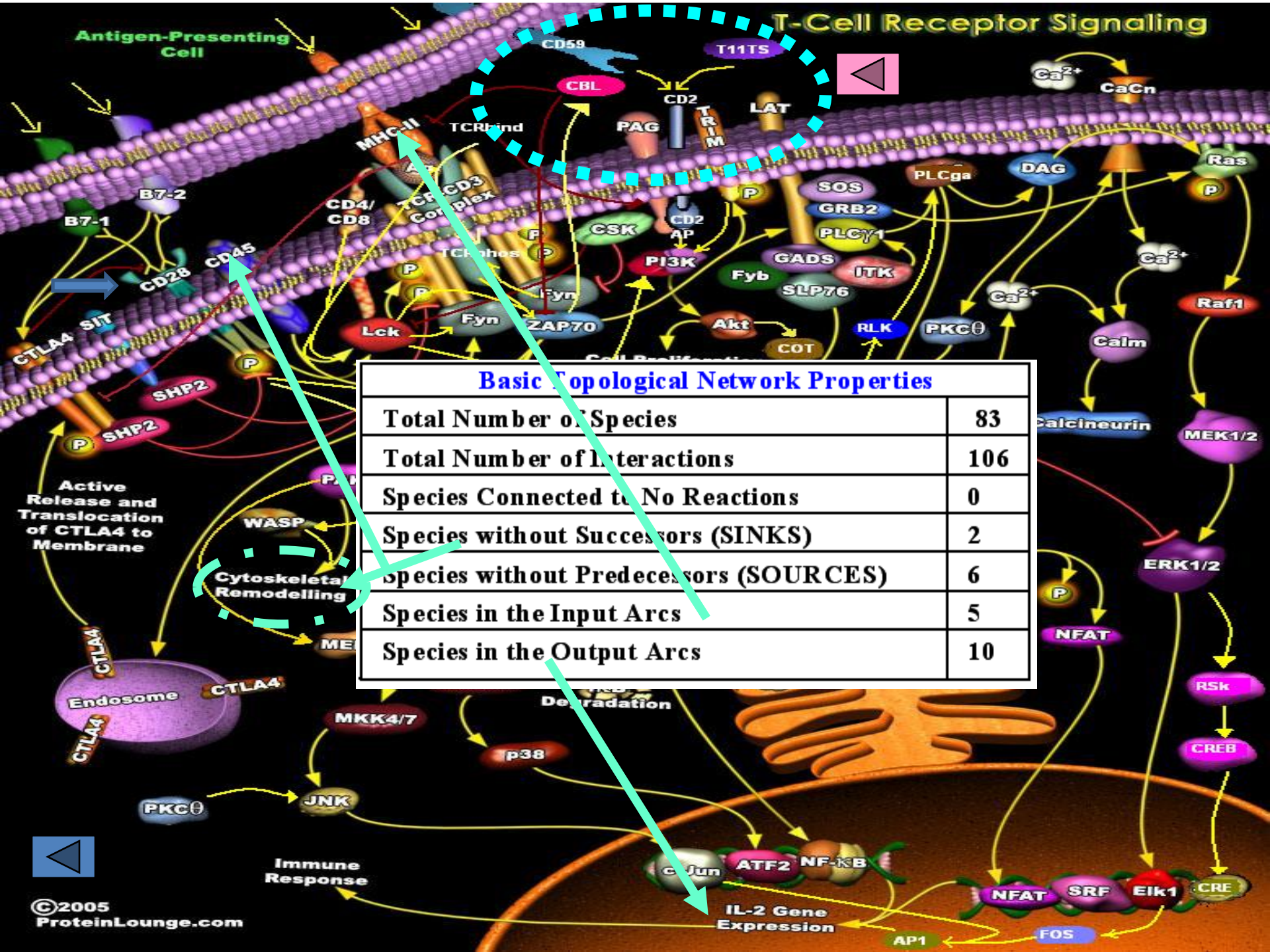


ERK-MAPK Pathway

No. of molecules	297
No. of Interactions	160

Basic Topological Properties	
Species without any predecessors (Sources)	37
Species without any successors (Sinks)	18
Species connected to no reactions	0
No. of +ve Feedback loops	15
No. of –ve Feedback loops	4
No. of strongly connected components	22
Mutually Excluding pairs	119
Enzyme Subsets	4





Basic Topological Network Properties	
Total Number of Species	83
Total Number of Interactions	106
Species Connected to No Reactions	0
Species without Successors (SINKS)	2
Species without Predecessors (SOURCES)	6
Species in the Input Arcs	5
Species in the Output Arcs	10

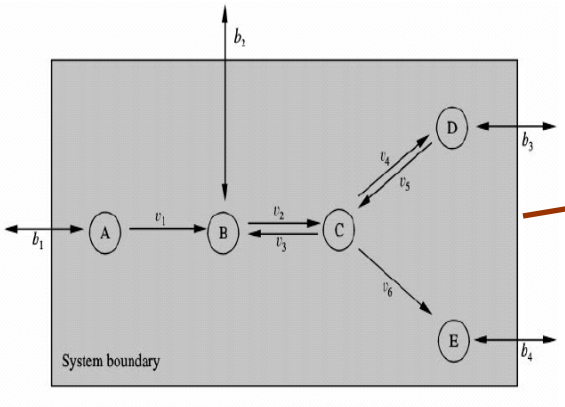
Recent Methodologies

Optimization techniques are being applied to pathways to understand the control architecture and functional properties

---- e.g. Flux Balance Analysis (FBA), and similar techniques (LSSA) are also used to study the signalling pathways

Flux Balance Analysis: Creating an *in silico* model in order to describe an organisms metabolism in steady state

The entire process from a bird's eye



$$\begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 1 & -1 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 1 & -1 & -1 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & -1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

Internal flux constraints

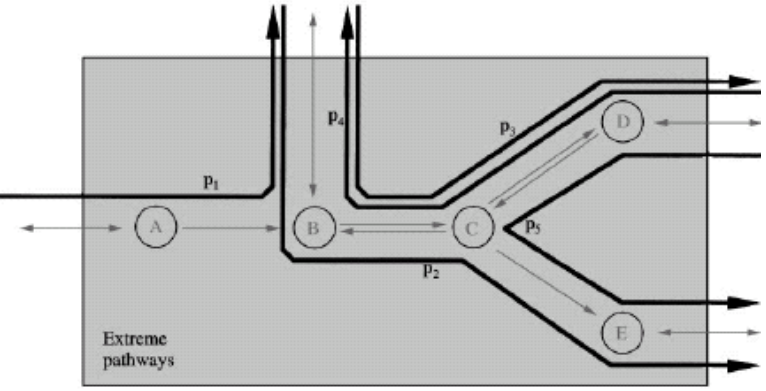
$$v_j \geq 0, \quad j = 1,\ldots,6$$

Exchange flux constraints

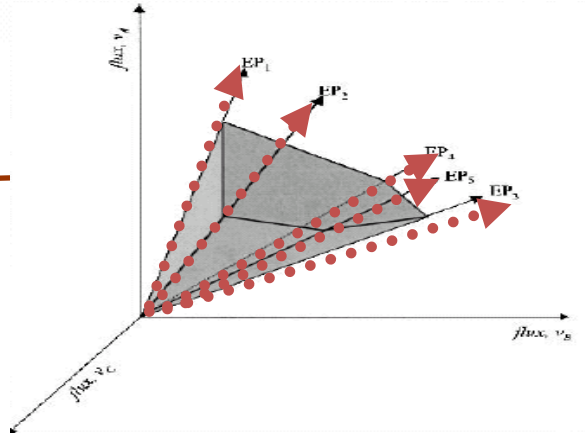
$$-\infty \leq b_j \leq +\infty, \quad j = 1,\ldots,4$$

Compute steady state flux Convex

+ constraints



Identify Extreme pathways (using algorithm presented in Schilling 2000)



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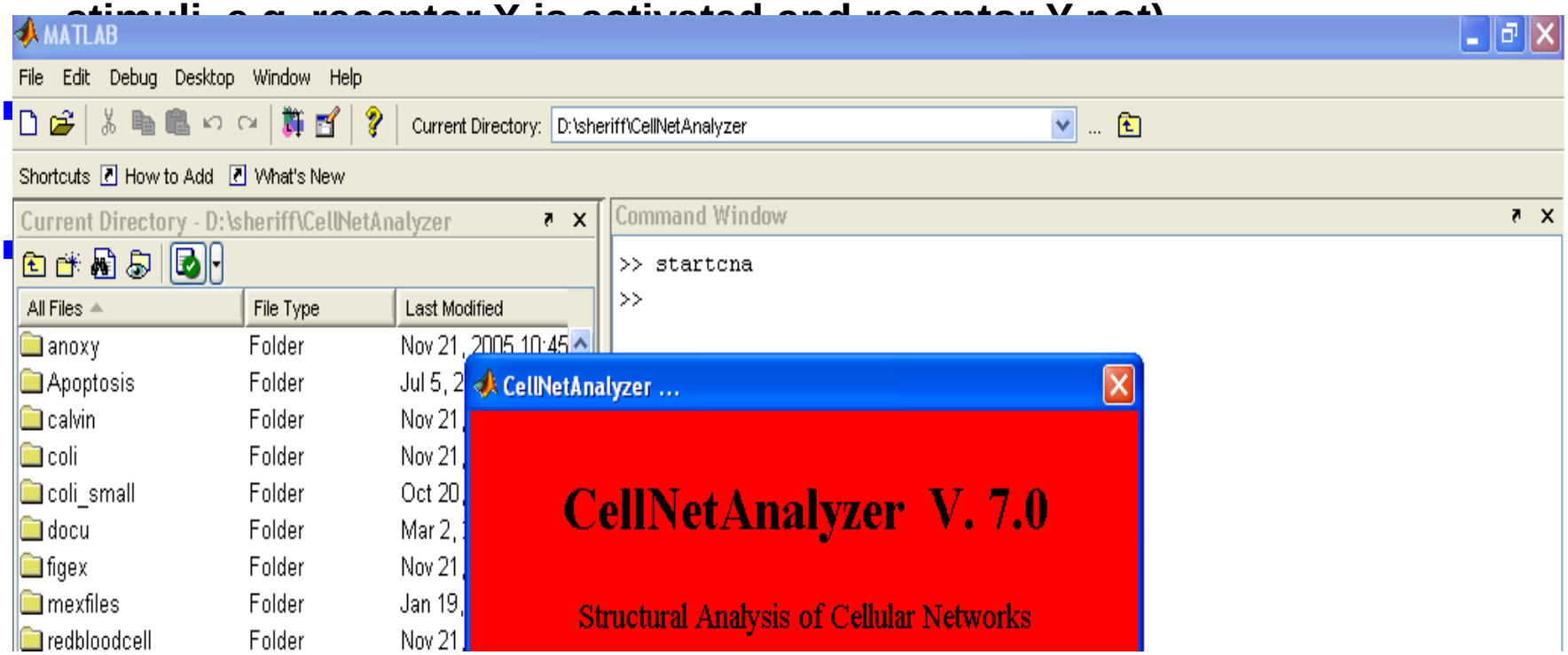
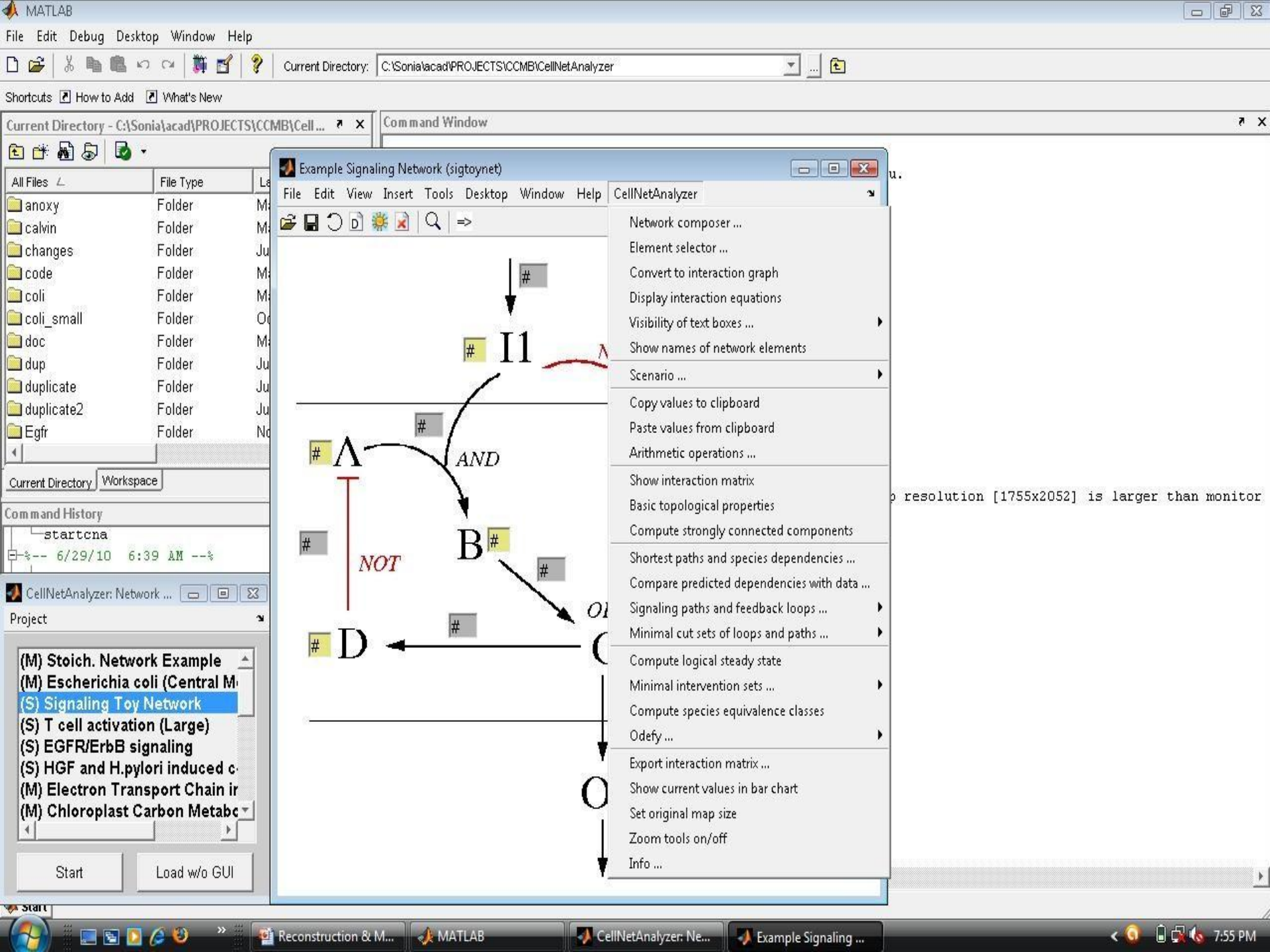
Logical Steady State Analysis (LSSA)

- **Boolean or logical networks have been extensively used for modelling signaling or gene regulatory networks, including feedback circuits.**
- **LSSA allows to form a user-defined scenario (consisting of a set of input stimuli, e.g. receptor X is activated and receptor Y not)**



Klamt et al., BMC Bioinformatics (2006)


MATLAB
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Shortcuts How to Add What's New
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doc Folder
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(S) Signaling Toy Network
(S) T cell activation (Large)
(S) EGFR/ErbB signaling
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(M) Electron Transport Chain ir
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File Edit View Insert Tools Desktop Window Help CellNetAnalyzer
I1
A
AND
B
NOT
D
Network composer ...
Element selector ...
Convert to interaction graph
Display interaction equations
Visibility of text boxes ...
Show names of network elements
Scenario ...
Copy values to clipboard
Paste values from clipboard
Arithmetic operations ...
Show interaction matrix
Basic topological properties
Compute strongly connected components
Shortest paths and species dependencies ...
Compare predicted dependencies with data ...
Signaling paths and feedback loops ...
Minimal cut sets of loops and paths ...
Compute logical steady state
Minimal intervention sets ...
Compute species equivalence classes
Odefy ...
Export interaction matrix ...
Show current values in bar chart
Set original map size
Zoom tools on/off
Info ...
Reconstruction & M...
MATLAB
CellNetAnalyzer: Ne...
Example Signaling ...
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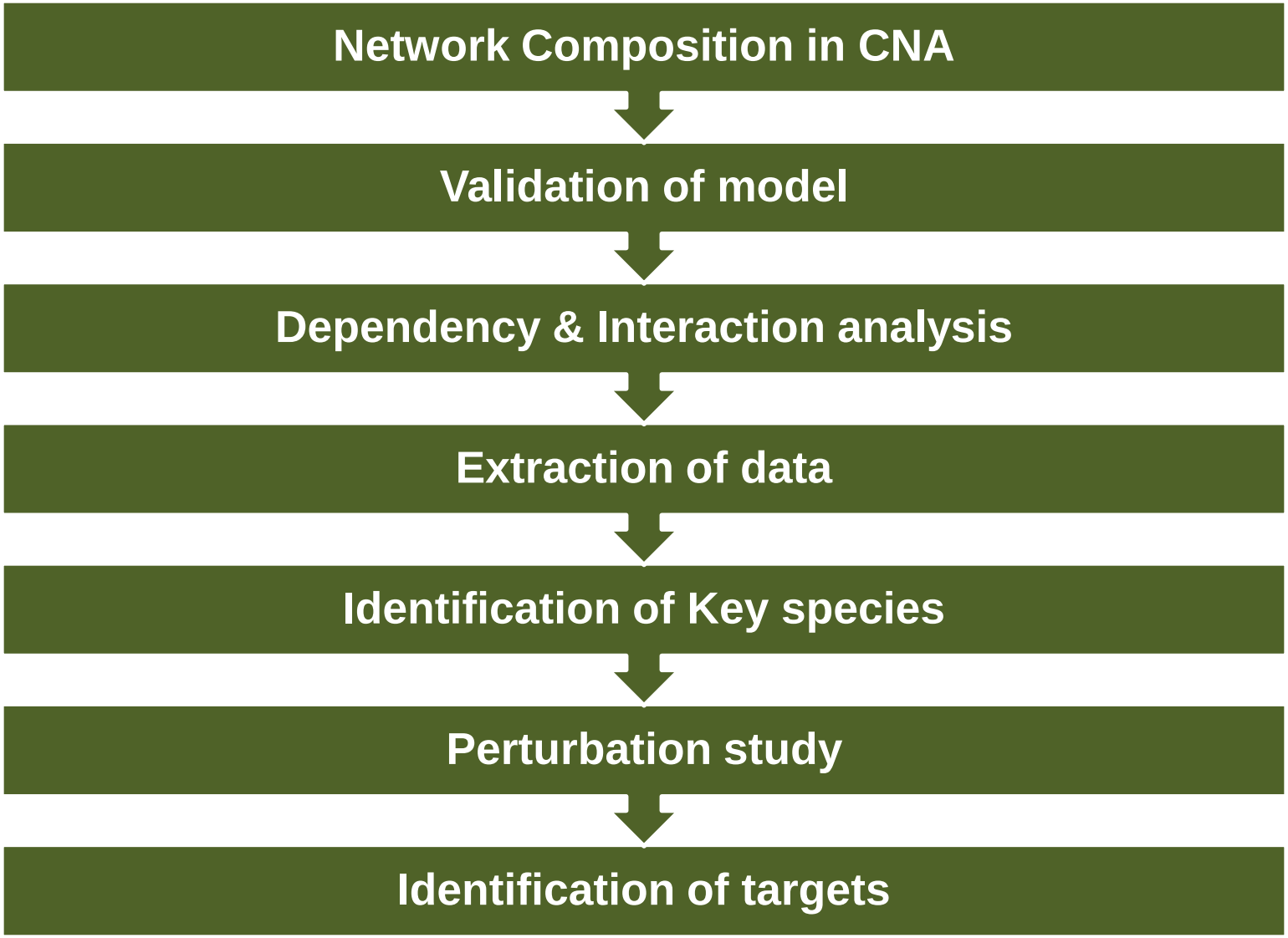
Procedures followed



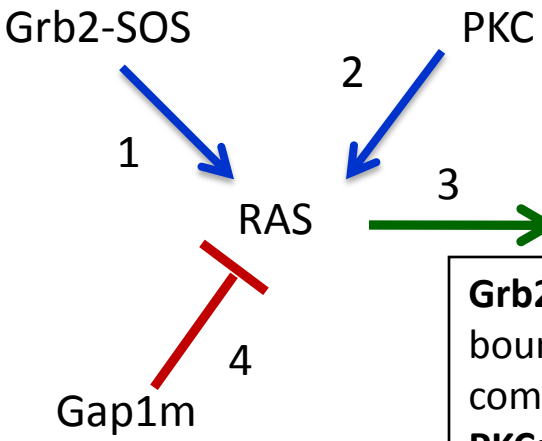
Logical Equations & Interaction Graphs

Grb2-SOS	PKC	OR ✓	AND
0	0	0	0
0	1	1	0
1	0	1	0
1	1	1	1

Activators = Grb2-SOS + PKC

Activators	!Gap1m	OR	AND ✓
0	0 → 1	1	0
0	1 → 0	0	0
1	0 → 1	1	1
1	1 → 0	1	0

RAS = Activators.!Gap1m



Grb2-SOS: Growth factor receptor-bound protein – Son of Sevenless (GEF) complex
PKC: Protein Kinase C
Gap1m: RAS GTP-ase activating protein (GTP hydrolysis)
Ras: GTP-ases

PI3K: Phosphoinositide 3 kinase

! = NOT (or Inhibition)

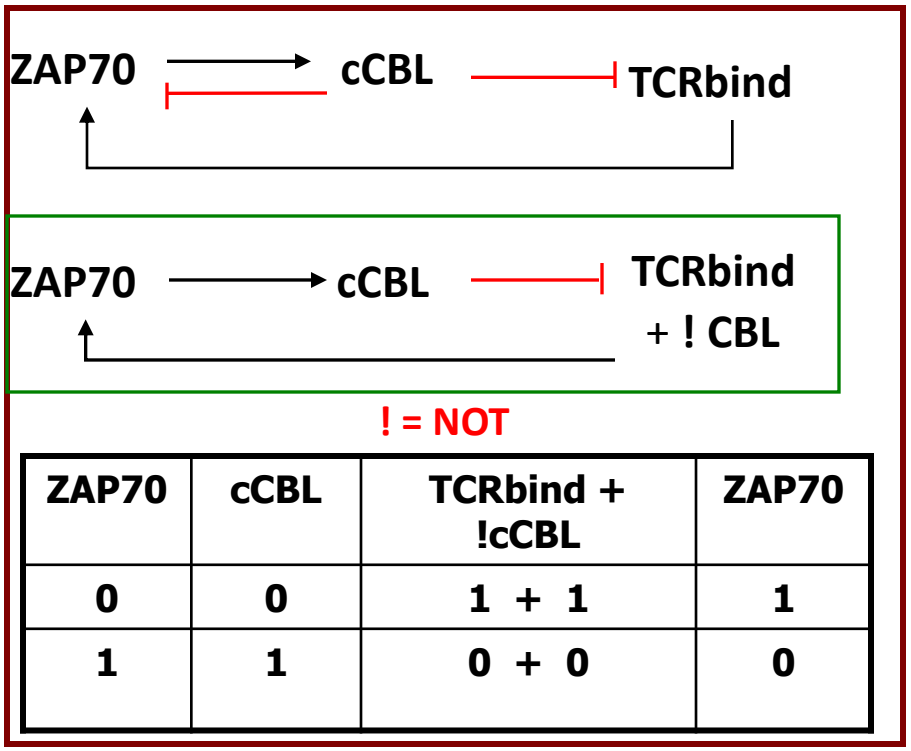
Interaction Graph

1(+)	2(+)	3(-)	4(+)	
-1	0	0	0	Grb2-SOS
0	-1	0	0	PKC
0	0	1	0	PI3K
0	0	0	-1	Gap1m
1	1	-1	1	RAS

Interaction Hyper-graph

(1+2)&!4	3	
-1	0	Grb2-SOS
-1	0	PKC
0	1	PI3K
-1	0	Gap1m
1	-1	RAS

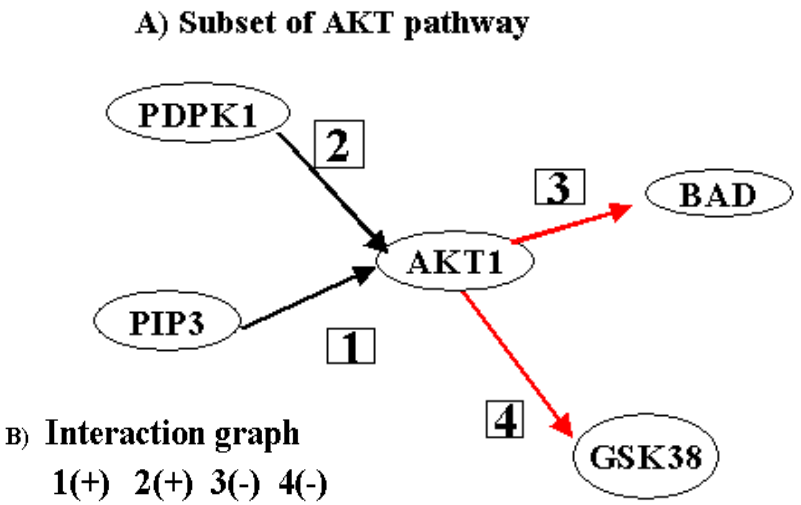
Logical equations and Interaction graphs



! = NOT

ZAP70	cCBL	TCRbind + !cCBL	ZAP70
0	0	1 + 1	1
1	1	0 + 0	0

TCRbind: T-Cell Receptor bound complex (TCR +MHCpeptide), ZAP70: Zeta-Chain (TCR) Associated Protein Kinase of 70 kDa, cCBL: cbl oncogene, E3 ubiquitin ligase



B) Interaction graph

	1(+)	2(+)	3(-)	4(-)	
	-1	0	0	0	PIP3
	0	-1	0	0	PDPk1
	1	1	-1	-1	AKT1
	0	0	1	0	BAD
	0	0	0	1	GSK38

C) Interaction hyper-graph

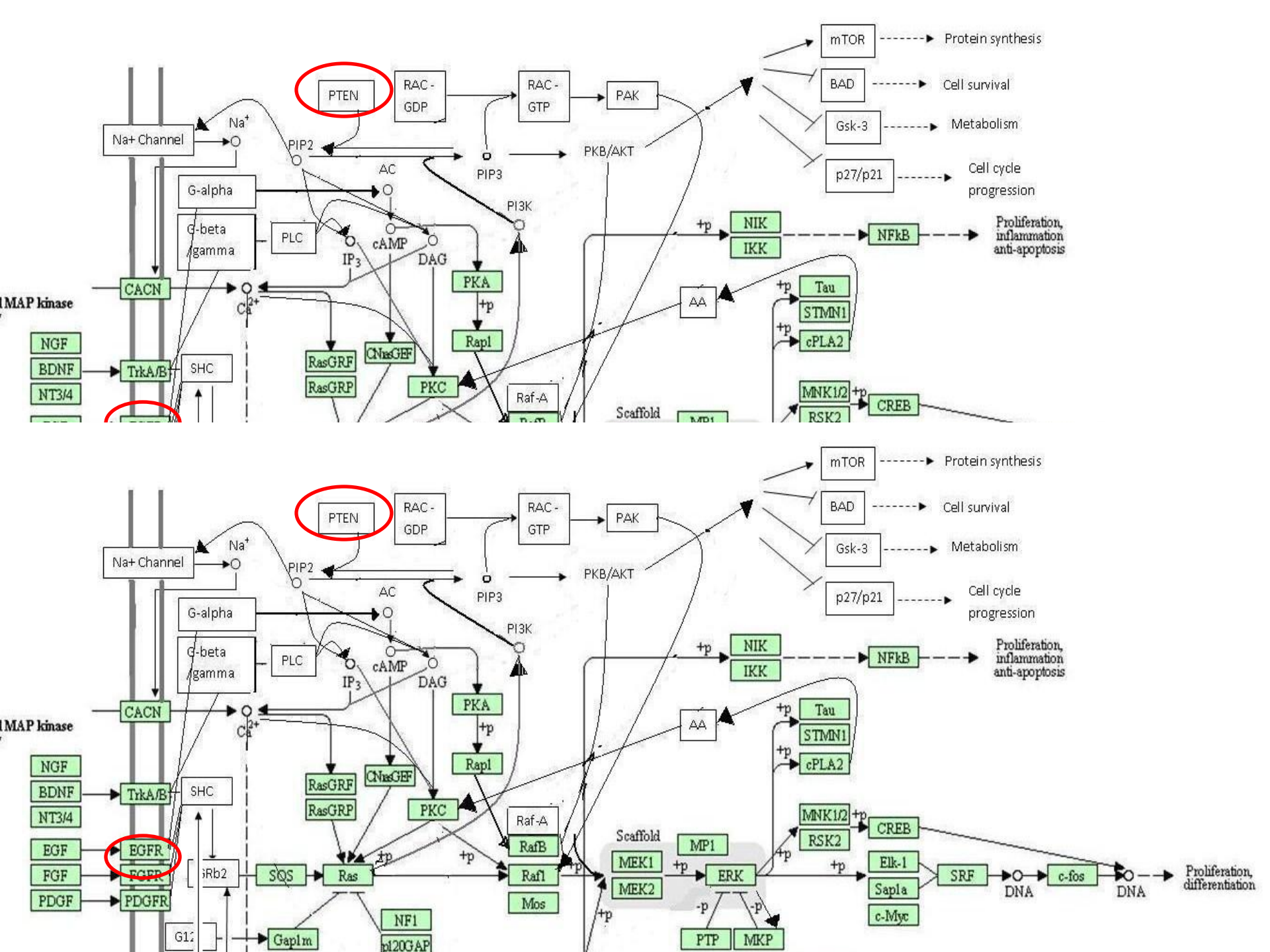
	1&2	3	4	
	-1	0	0	PIP3
	-1	0	0	PDPk1
	1	-1	-1	AKT1
	0	1	0	BAD
	0	0	1	GSK38

PDPK1: 3-phosphoinositide dependent protein kinase-1, PIP3: Phosphatidylinositol (3,4,5)-trisphosphate, Akt1: PKB protein kinase B. BAD: Bcl-2-associated death promoter (BAD) protien, GSK3B: Glycogen synthase kinase 3 beta

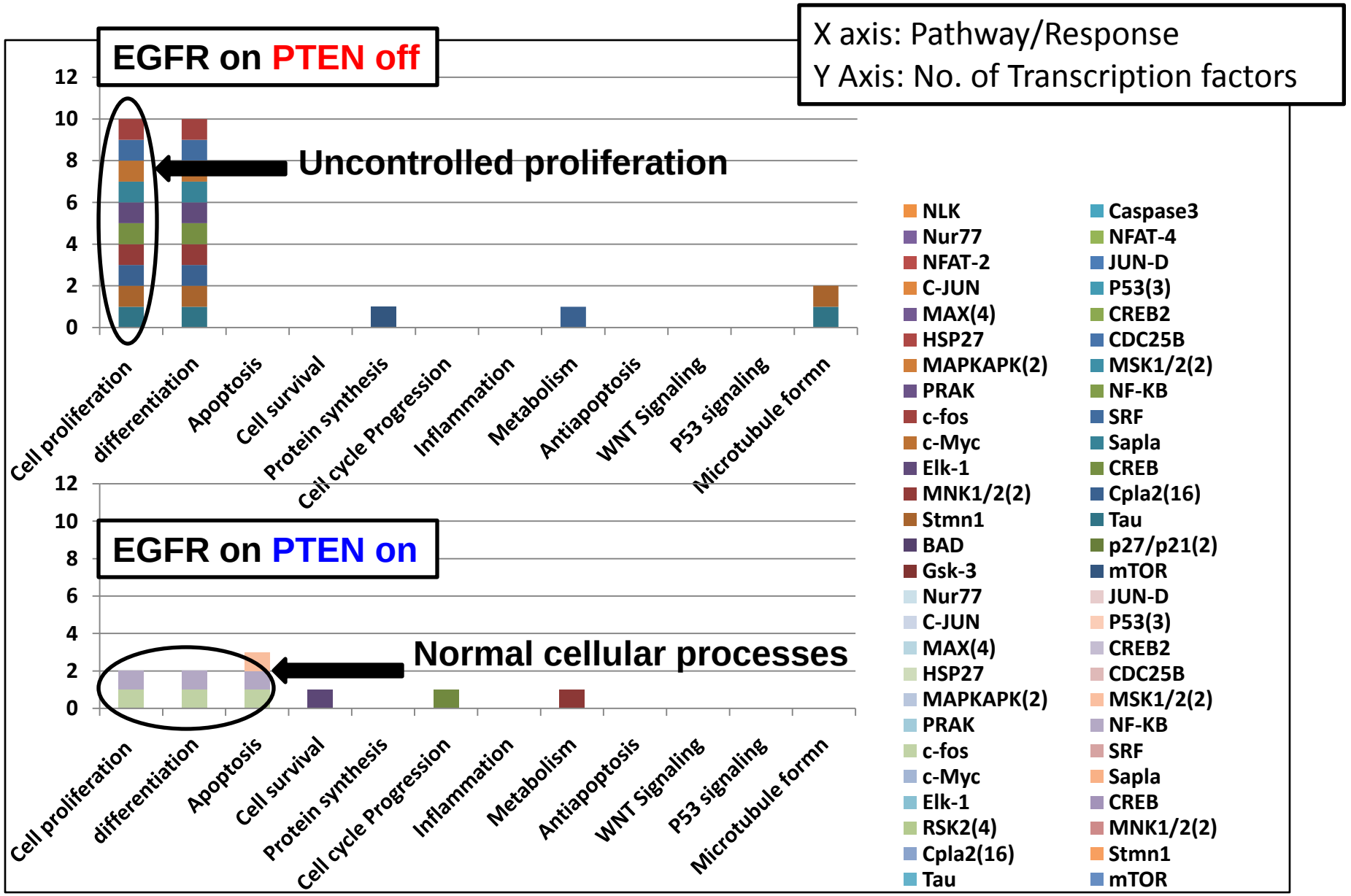
The signed graph can be stored by an $m \times q$ incidence matrix B in which the columns correspond to the q arcs (interactions) and the rows to the m nodes (species).

For the k-th arc $\{i, j, s\}$ a (-1) is stored in the k-th column of B for the tail vertex (i) and (+1) for the head vertex (j) of arc k.

$B_{i,k} = -1$ and $B_{j,k} = 1$ and $B_{l,k} = 0$ ($l \neq i, j$). For storing the signs, a q-vector s is introduced whose k-th element is (+1) if arc k is positive and (-1) if k is negative.

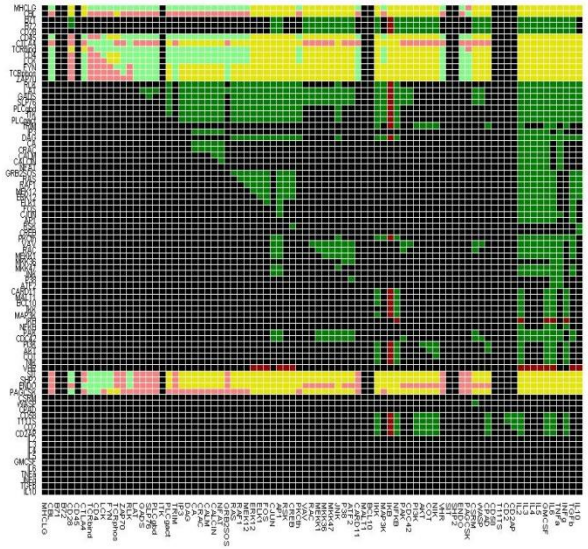

PTEN
RAC - GDP
RAC - GTP
PAK
mTOR
Protein synthesis
BAD
Cell survival
Gsk-3
Metabolism
p27/p21
Cell cycle progression
Na+ Channel
Na+
PIP2
PIP3
PKB/AKT
AC
G-alpha
G-beta /gamma
PLC
PI3K
IP3
cAMP
DAG
+p
NIK
IKK
NFkB
Proliferation, inflammation anti-apoptosis
CACN
Ca2+
PKA
AA
Tau
STMN1
cPLA2
NGF
BDNF
NT3/4
TrkA/B
SHC
RasGRF
RasGRP
CNrasGEF
Rap1
PKC
Raf-A
MNK1/2
CREB
RSK2
Scaffold
MAP kinase
EGF
FGF
PDGF
EGFR
FGFR
PDGFR
iRb2
SOS
Ras
RafB
Raf1
Mos
MP1
MEK1
MEK2
ERK
PTP
MKP
Elk-1
Sap1a
c-Myc
SRF
c-fos
DNA
Proliferation, differentiation
G12
Gap1m
NF1
p120GAP

Effect on Transcription factors varying PTEN

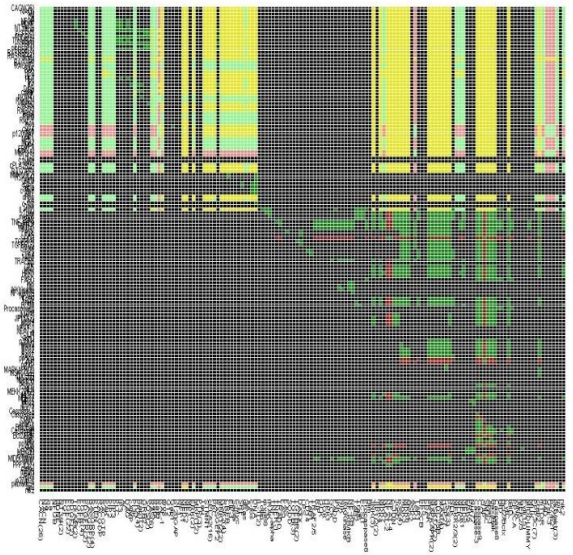


Logical Interpretation and Analysis

T-cell Receptor Pathway

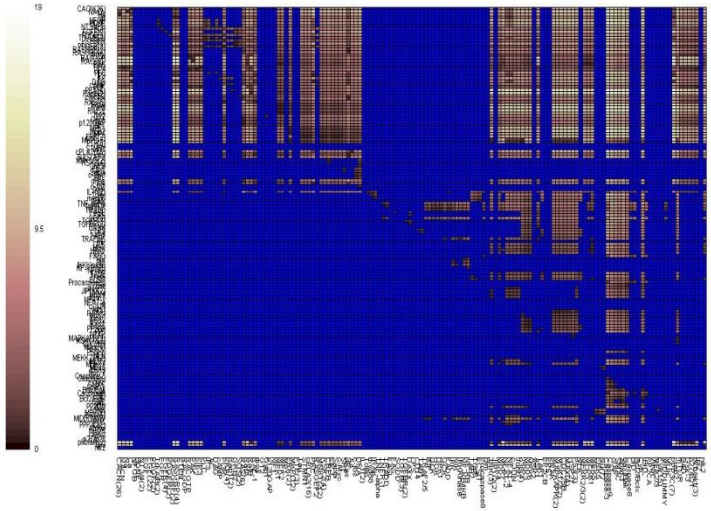


MAPK Pathway

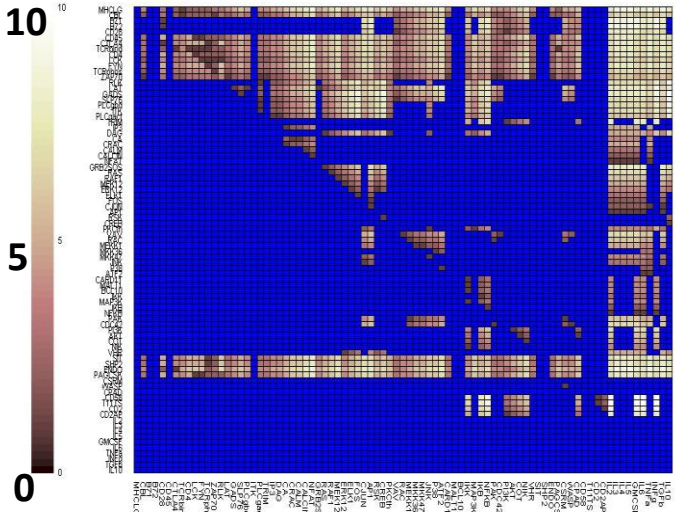


Dependency Matrix

- No influence of i on j
- i has activating and inhibiting effect on j
- i is a pure inhibitor of j
- i is a pure activator of j
- i is an independent inhibitor of j
- i is an independent activator of j



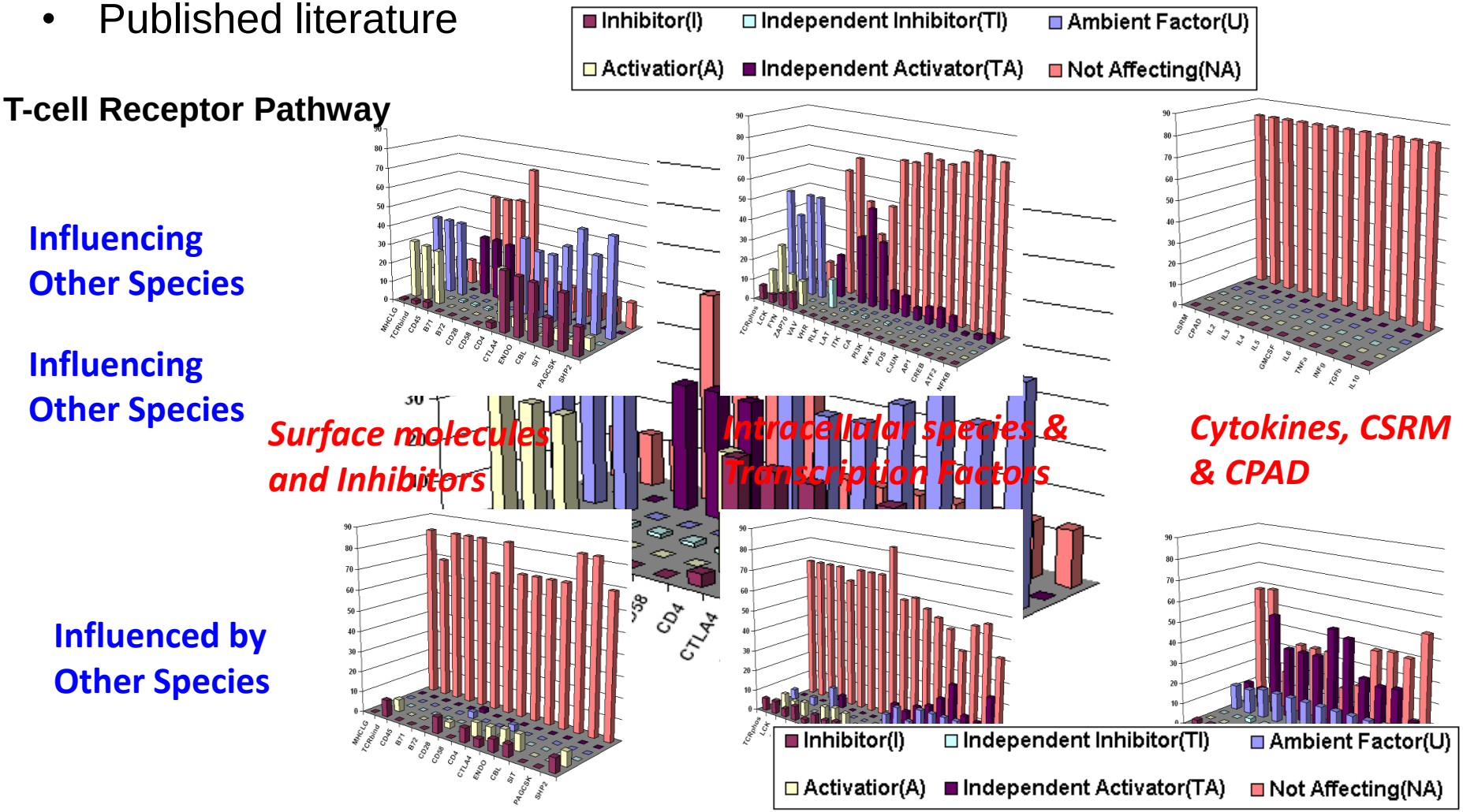
Shortest Path

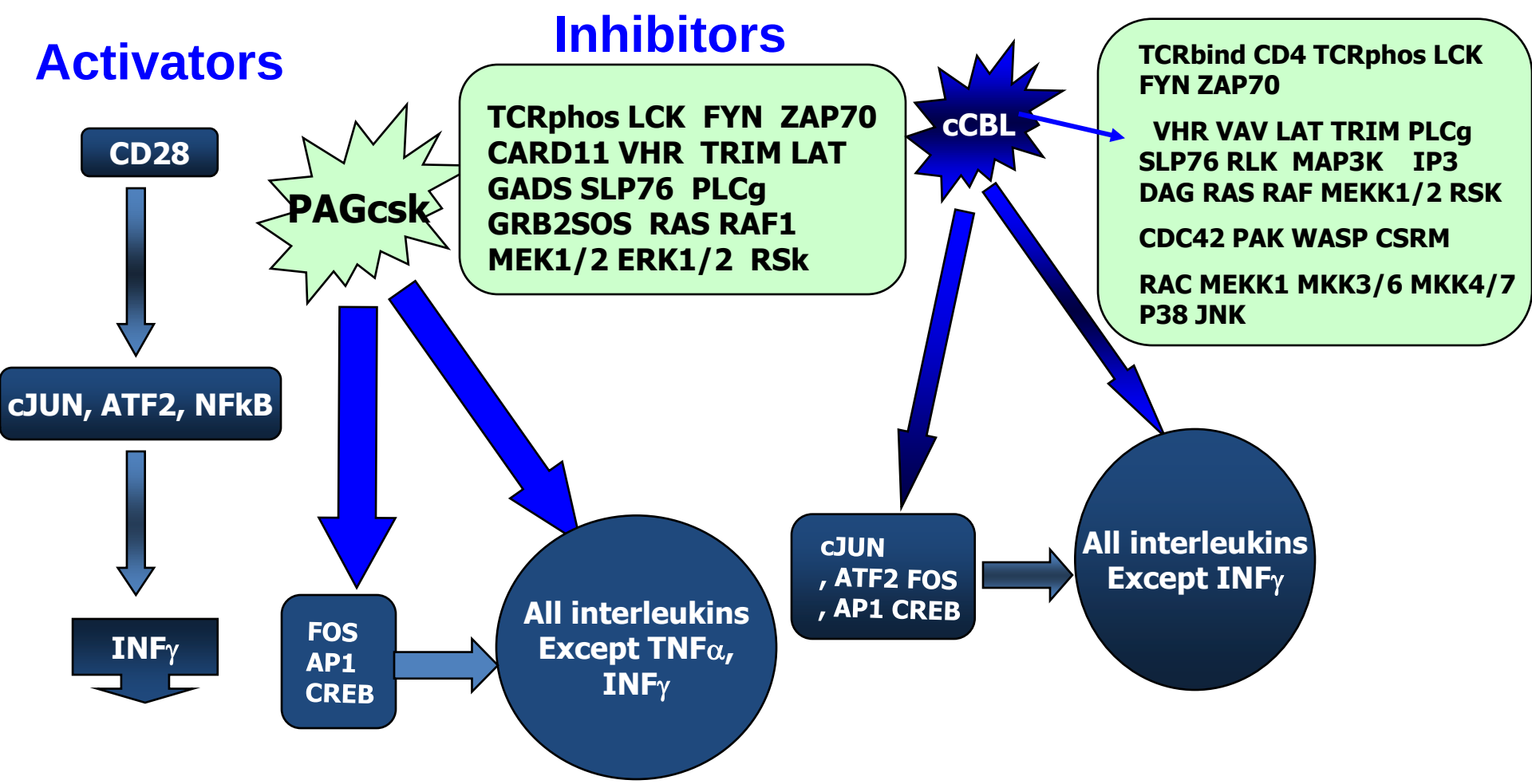


X Axis: Species
Y Axis: Species

Identification of Key Species

- Interactions with more number of molecules
- Influencing low number but crucial molecules
- Transcription factors leading to important pathways/cellular responses
- Published literature

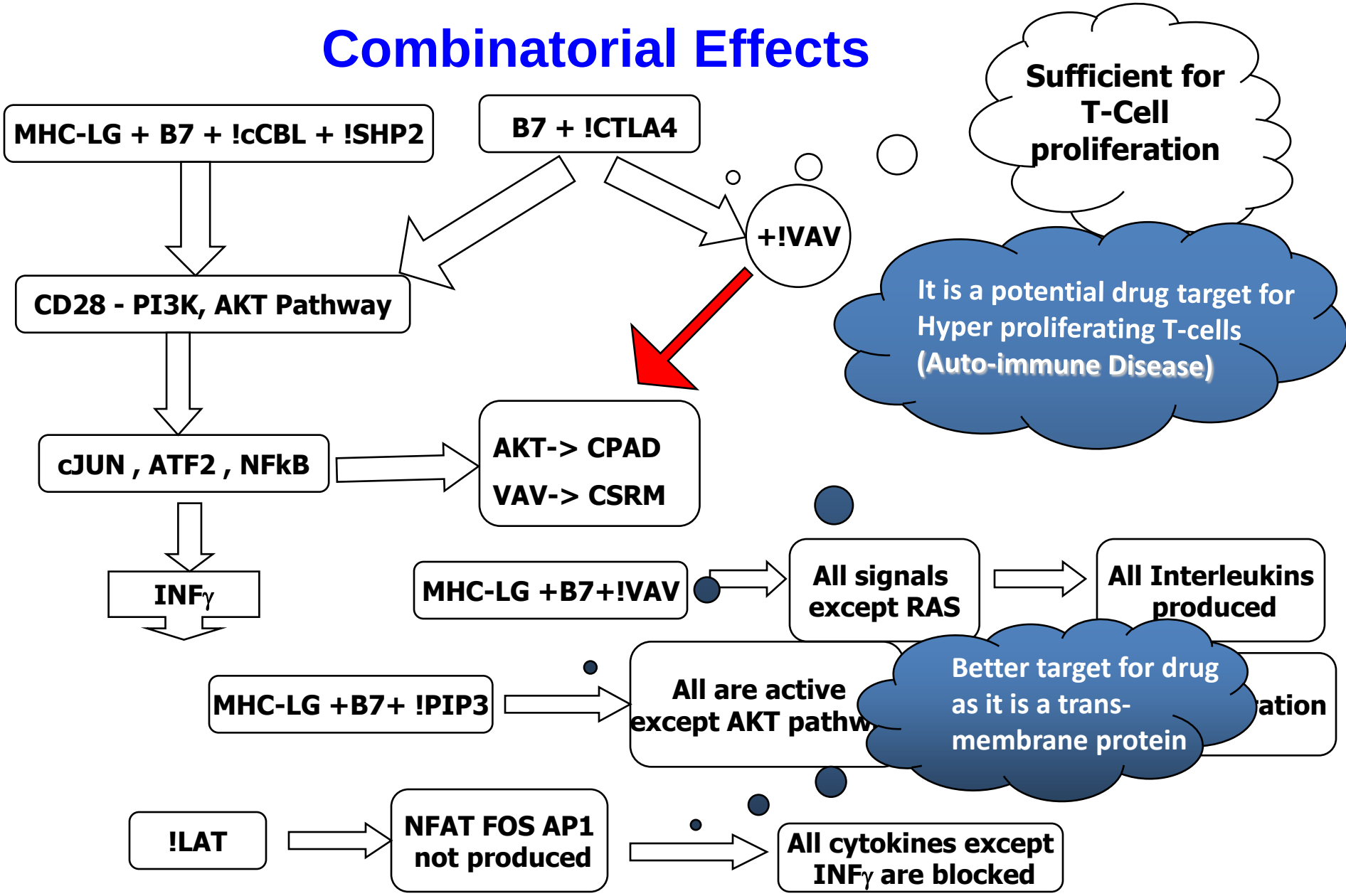




CD28:Cluster of Determinants 28, ATF2: Activating Transcription Factor-2, TCRphos:T-Cell Receptor phosphorylated, FYN: Src family tyrosine kinase, LCK: leukocyte-specific protein tyrosine kinase, FOS: Cellular Oncogene Fos, CREB: Cyclic AMP Reponse element(CRE) binding protein, TRIM: T-Cell Receptor Interacting Molecule etc.

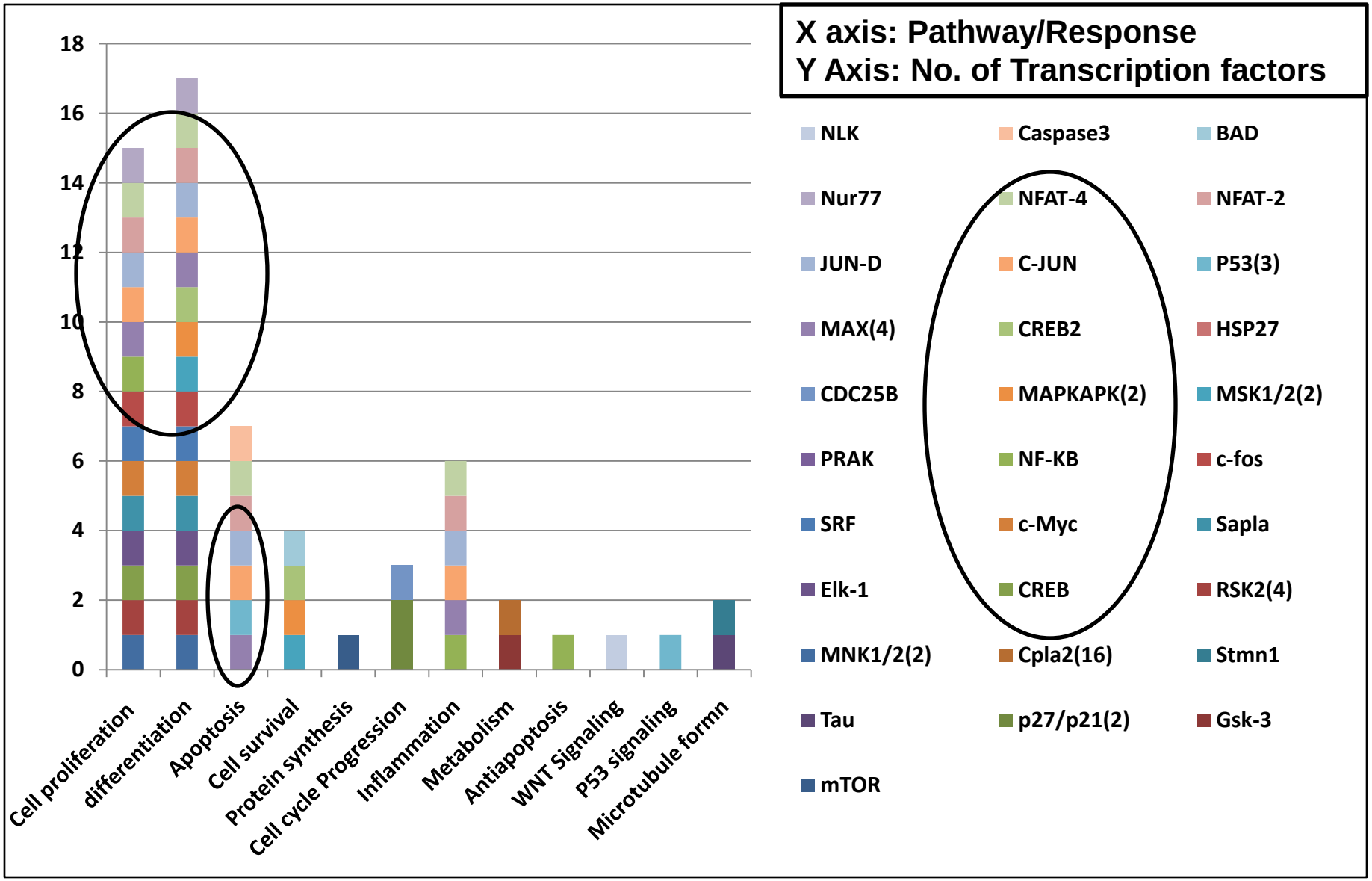
VAV: uanine nucleotide exchange factors for Rho family GTPase, RAC: GTP-binding proteins members of the Rho family, LAT: Linker for Activation of T-Cells, PAK: p21/CDC42/Rac1-Activated Kinase-1, DAG: Diacylglycerol, IP3: Inositol Triphosphate

Combinatorial Effects



PI3K: Phosphoinositide-3-Kinase-Class-3, CTLA4: cytotoxic T-lymphocyte-associated protein 4, SHP2: SH2-containing Protein tyrosine Phosphatase-2

Transcription factors leading to significant effects on Functional Response and other pathways



Conclusion

- **This study is important to identify important molecules, related sub-pathways for further experimental study and Identifies possible alternative pathways and optimal intervention points (targets)**
- **Signalling networks for T-Cell Receptor pathway that gets activated by various external molecules (B7-1,B7-2,MHC-peptide,CD58, etc) on binding with their respective receptors (CD28,CTLA4,TCR,CD2) are analysed using LSSA.**
- **TNFα produced by T-Cell binds to the death receptors in the glioma cells and induce apoptosis in them. FAS ligand (activated by IL2) also binds to the death receptor and induce apoptosis.**
- **Study of MAPK pathways also reveal few key molecules, IL-1, B-Raf, which can act as better target for controlling cancer.**
- **LAT could be an effective drug target for the auto-immune disease where hyper proliferation of T-Cell occurs. LAT is a trans-membrane protein, which could be easily targeted from the exterior of the cell and could be used as immuno – suppressant.**
- **Our study helps to identify the target species in the entire T-Cell receptor and MAPK pathway, and could be helpful for potential drug targeting.**
- **The entire approach constitutes a primary study of *system level understanding* of the immune and cancer cells.**

Emerging area to study the entire system

Acknowledgement

INSA-IAS-NASI Summer Students

M. Rahuman Sheriff
Naveen Joshi
Sonia Chothani

Dr. Somdatta Sinha (Group Leader)

T_C Cell vs. Cancer cell

Thank You