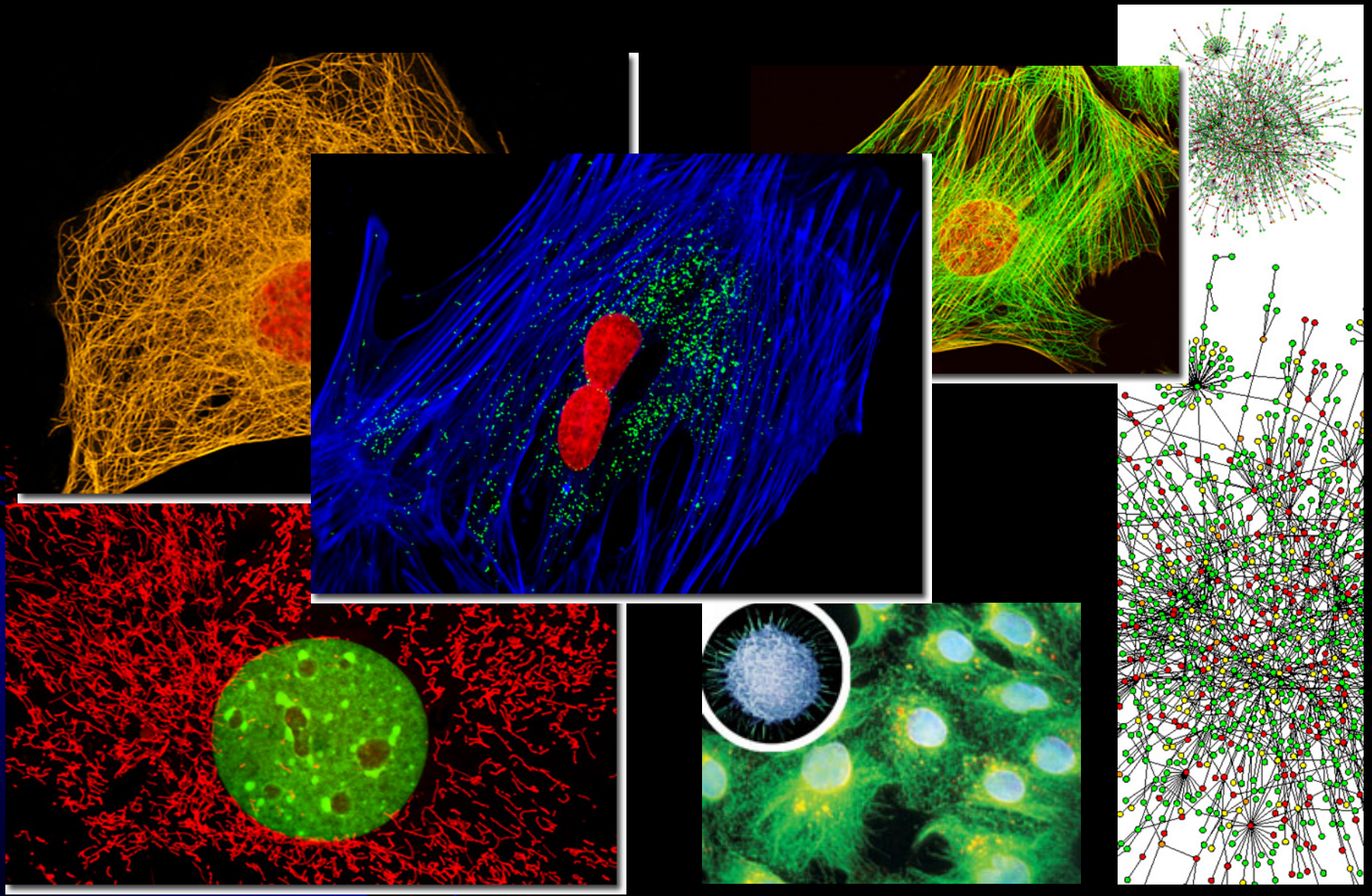
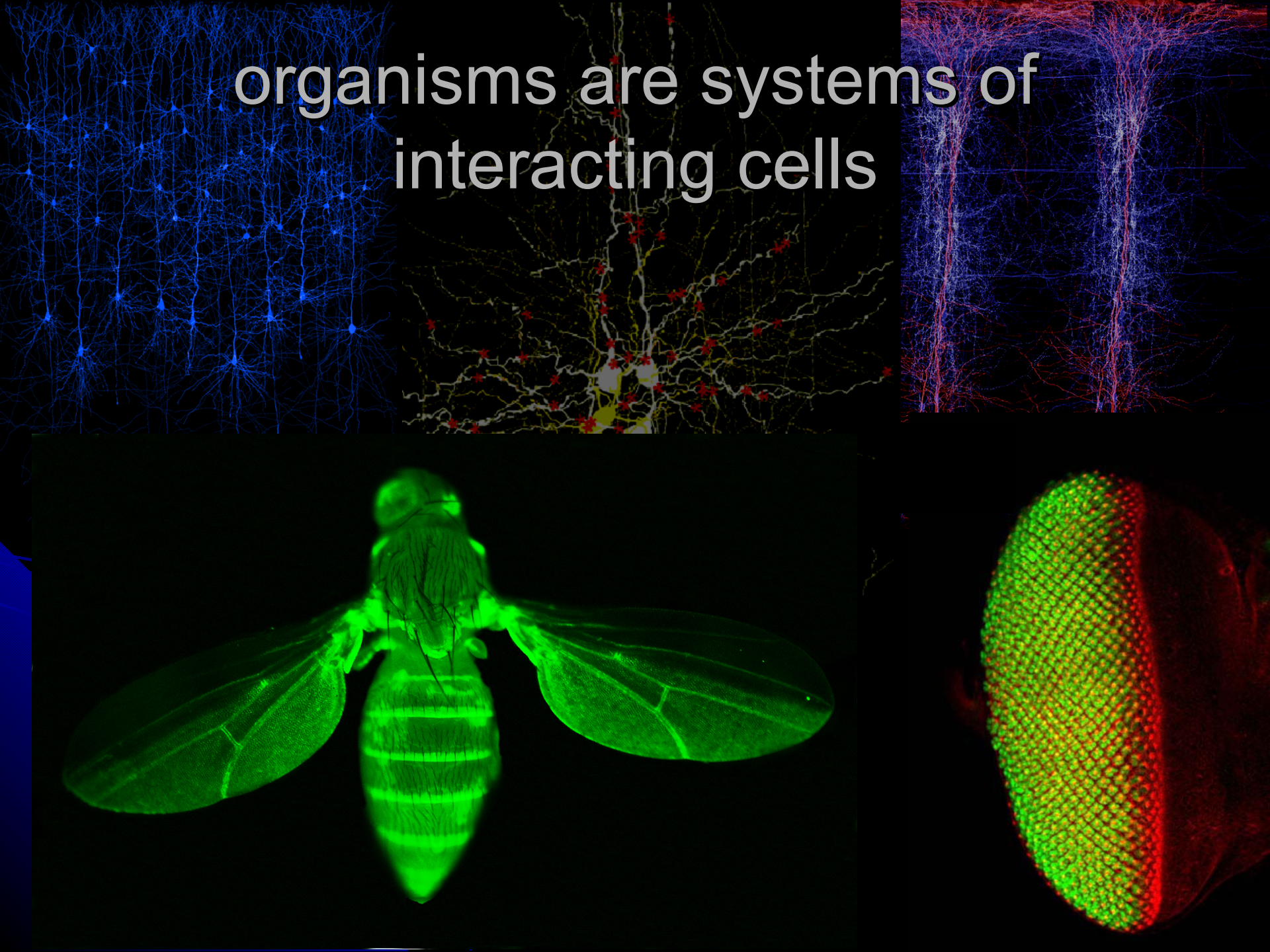
Modeling and Inference of Transcriptional Regulatory Networks

ilya shmulevich

cells are systems of interacting molecules



organisms are systems of interacting cells

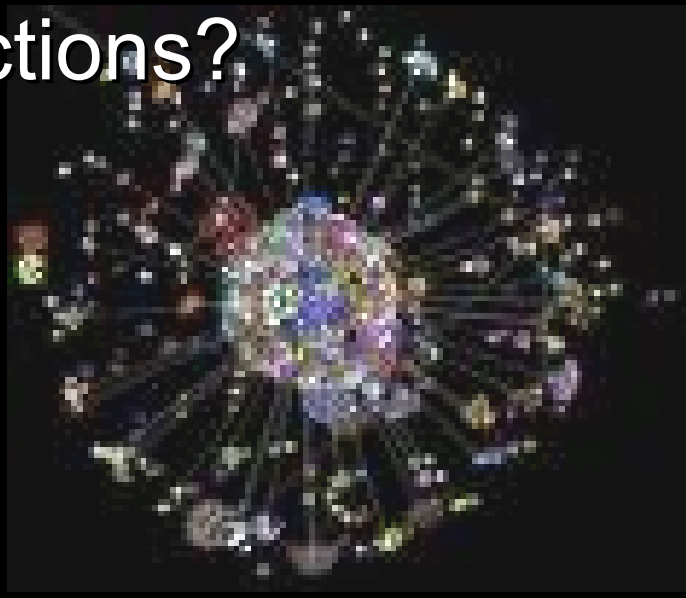


societies are systems of interacting organisms

living systems

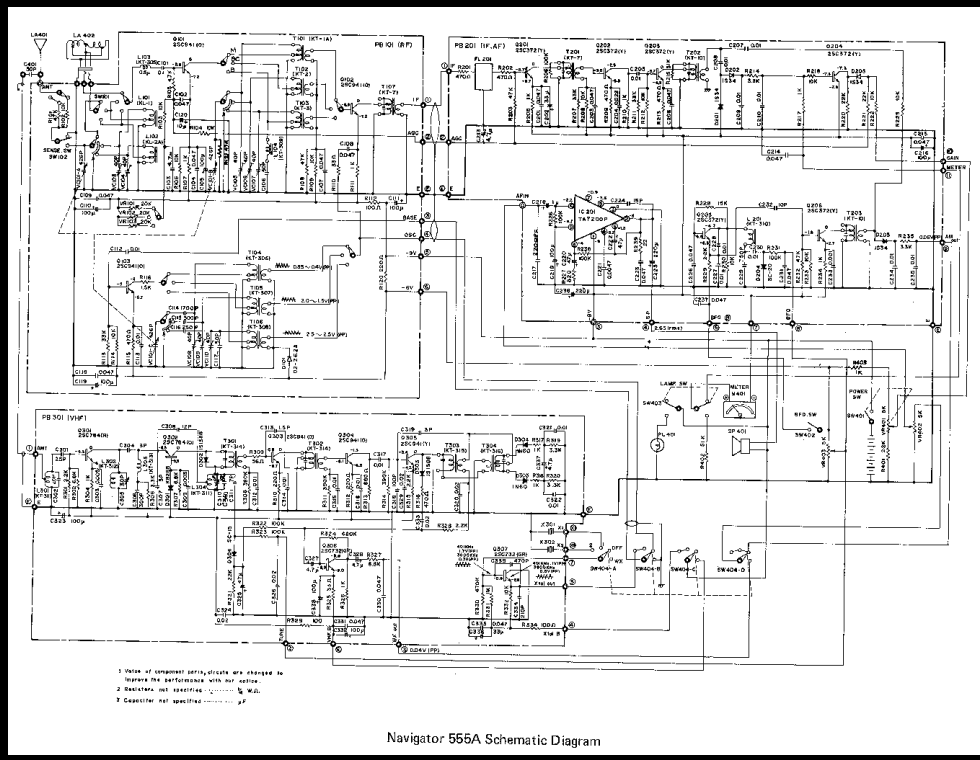
self-organized complex dynamical systems of interacting parts

how can we understand the emergent macroscopic properties of the system from its parts and their interactions?



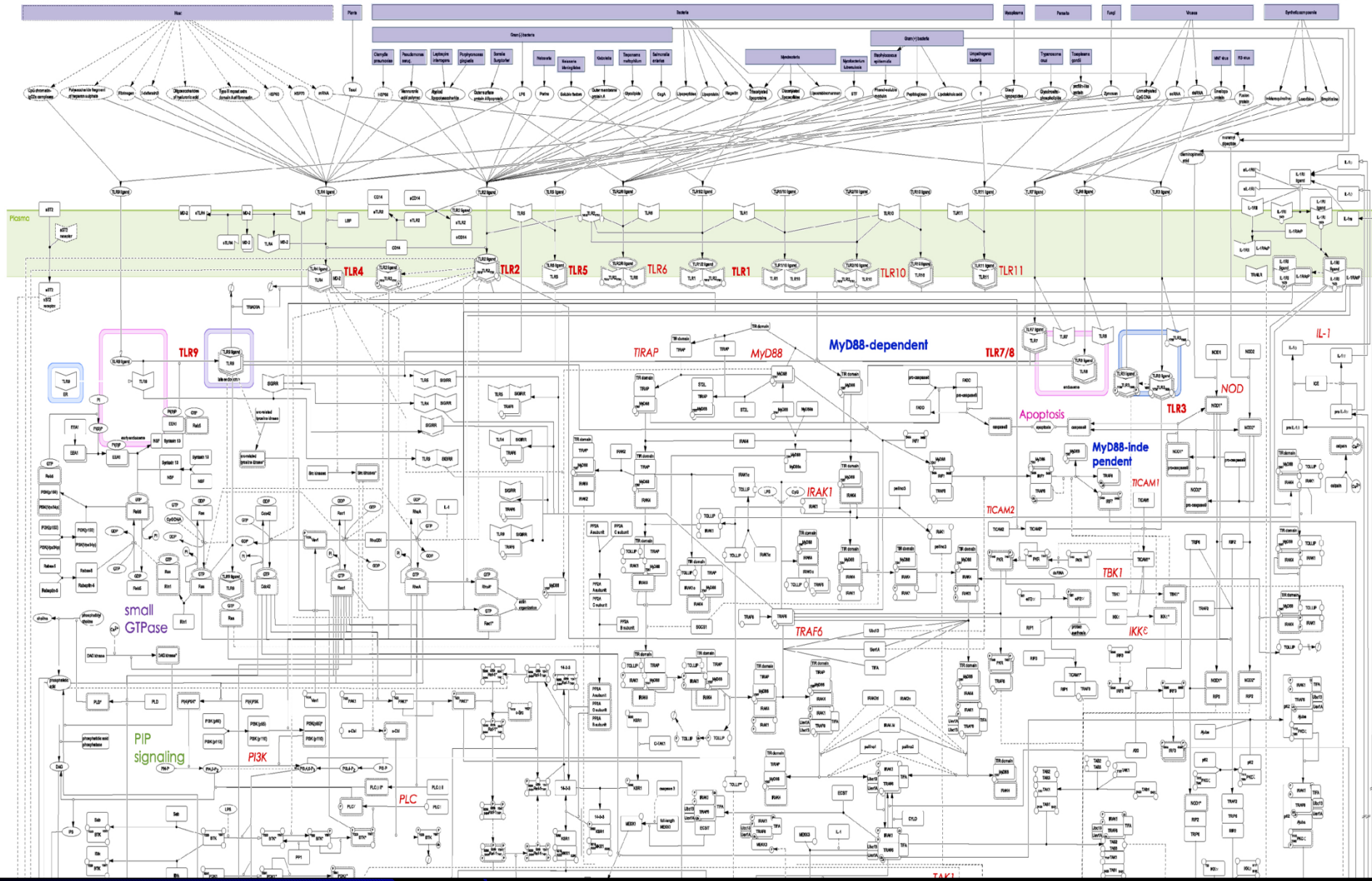
genetic networks

- Complex regulatory networks among genes and their products control cell behaviors such as:
  - cell cycle
  - apoptosis
  - cell differentiation
  - communication between cells in tissues
- A paramount problem is to **understand the dynamical interactions** among these genes, transcription factors, and signaling cascades, which govern the integrated behavior of the cell.



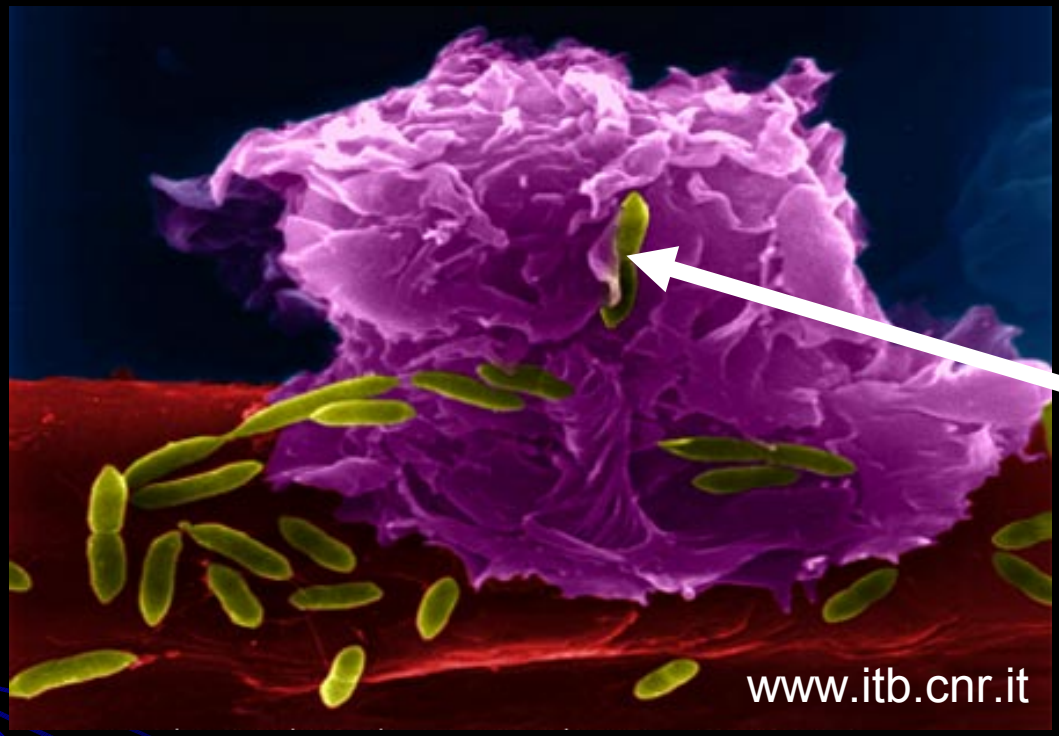
Analogy: circuit diagram

Map of the TLR signaling pathway in the macrophage



Mol Syst Biol. 2:2006.0015

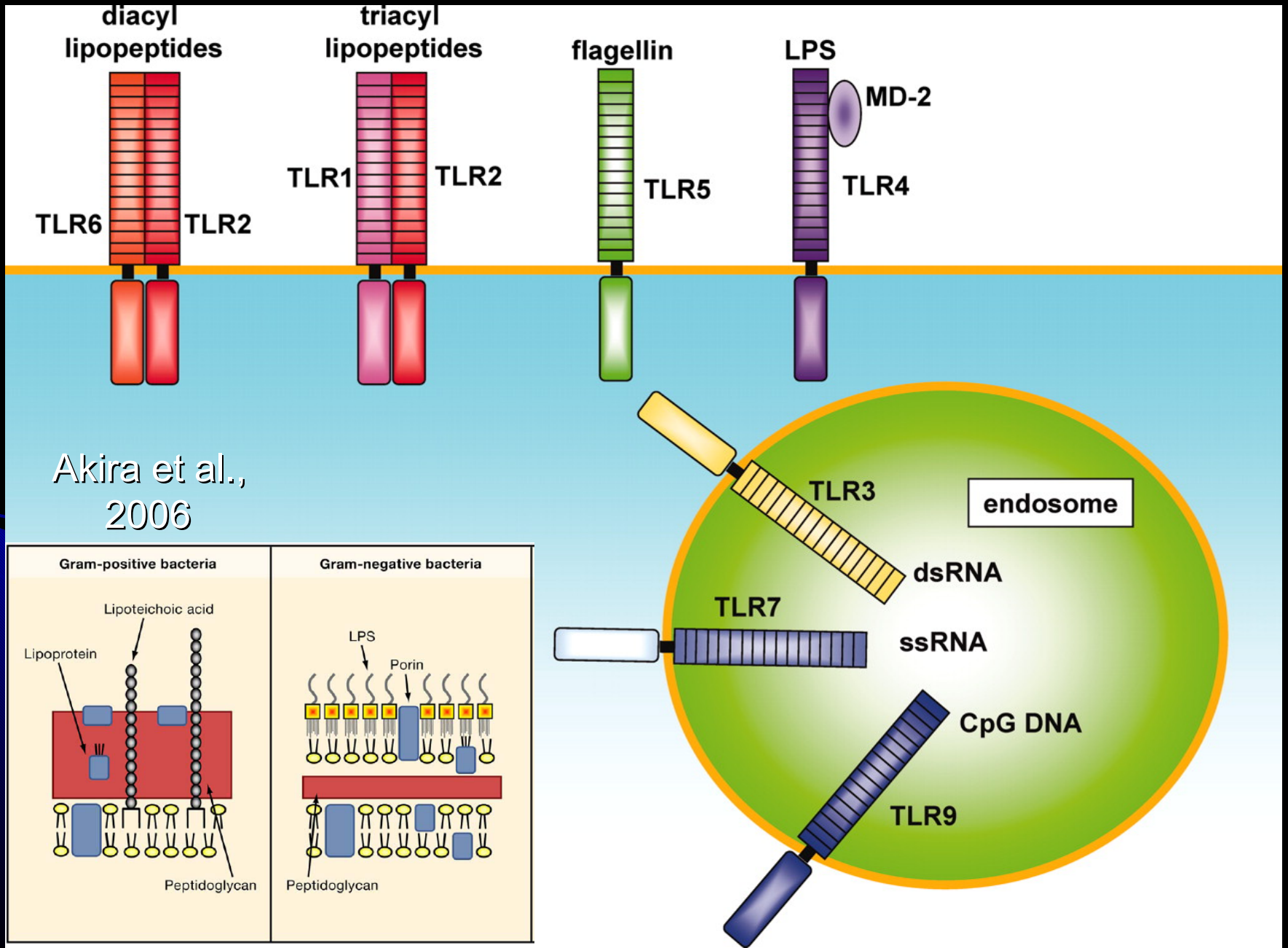
Macrophages are key immune cells



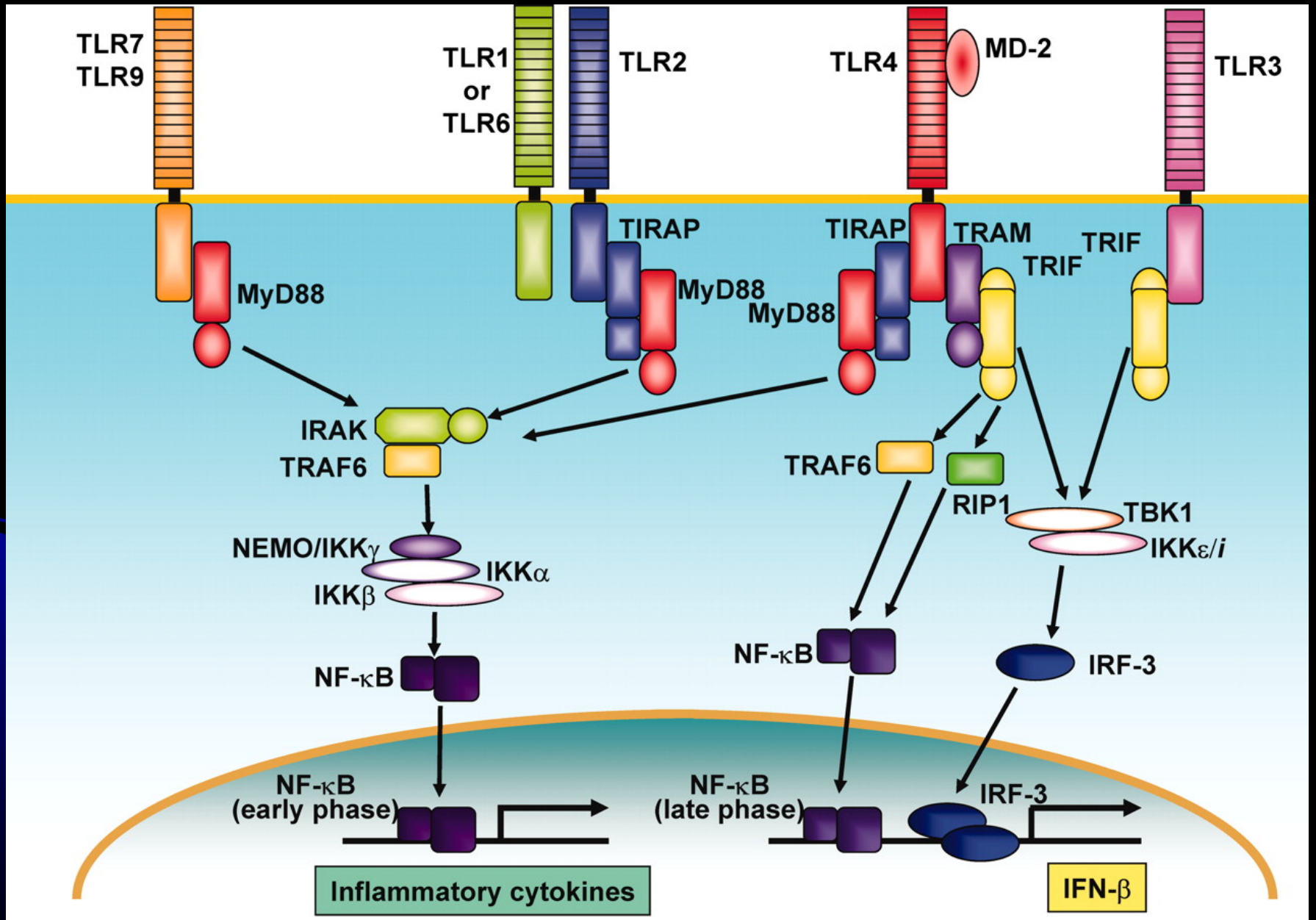
bacterium
being engulfed

- Phagocytosis
- Antigen presentation
- Secretion of proinflammatory cytokines
- Wound healing

TLRs and their associated PAMPs



TLR signaling pathway

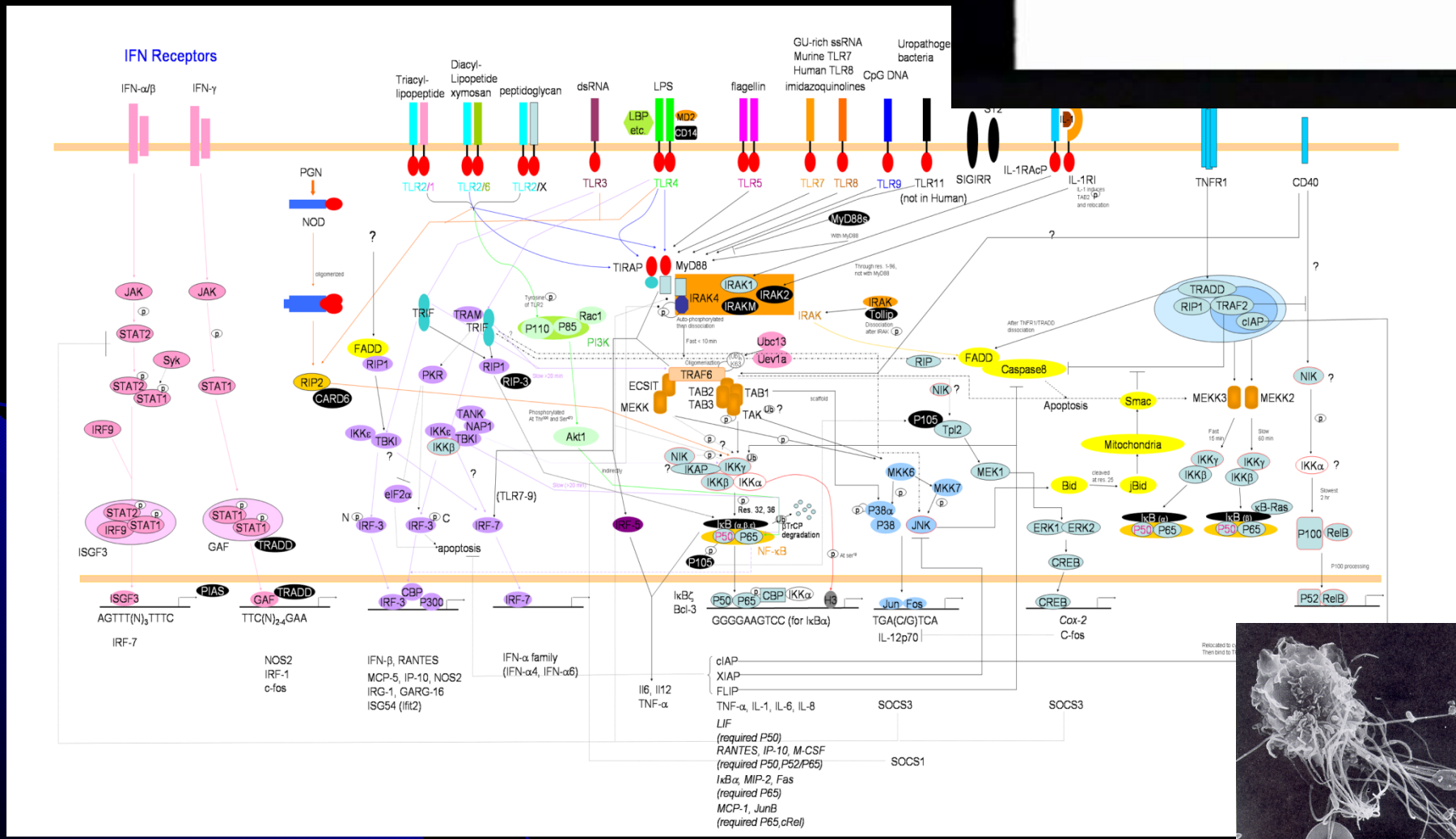


Map of the TLR signaling pathway in the macrophage

A few perspectives on

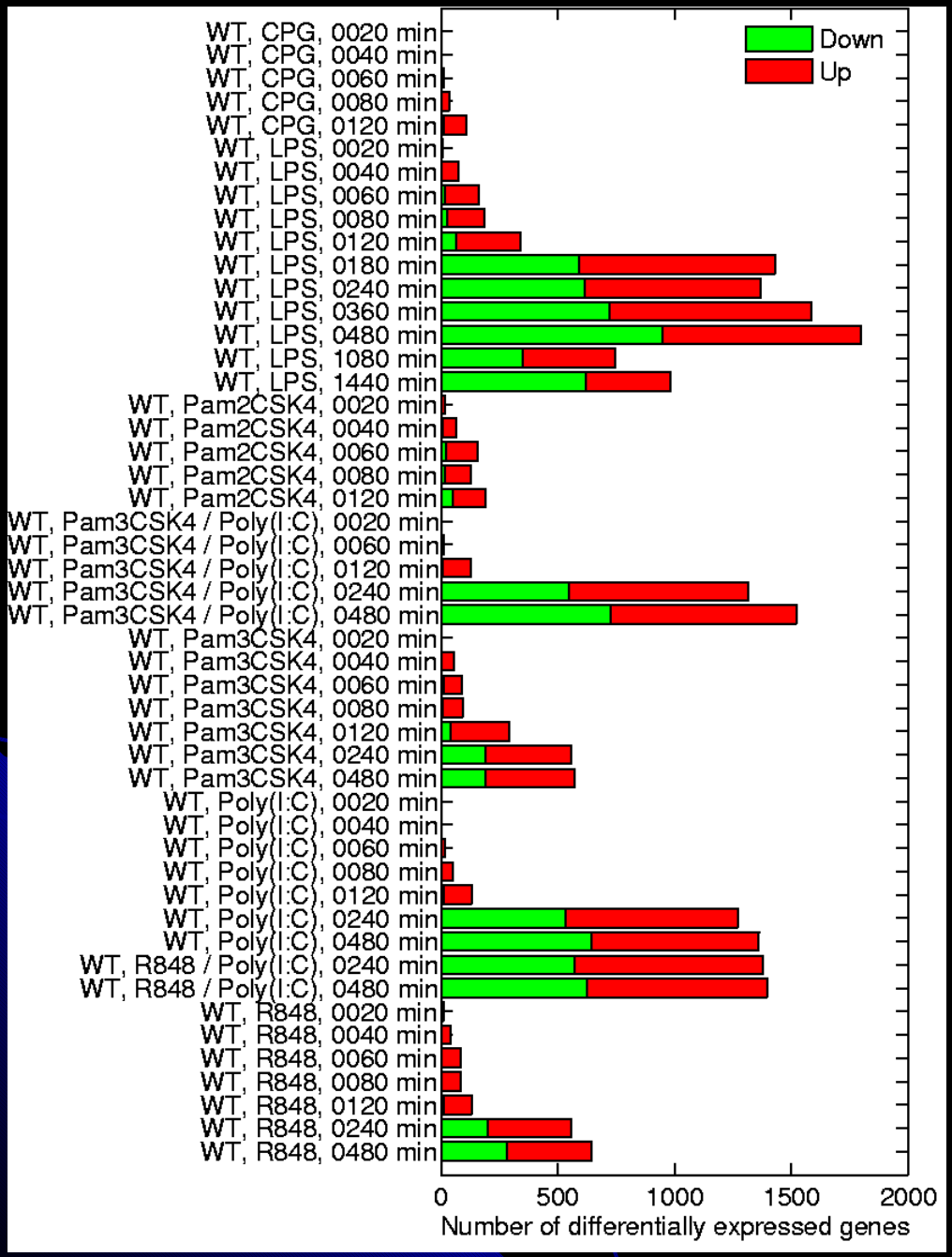
CELL MOTILITY

Division of Experimental Medicine
Brigham & Women's Hospital



The ingredients

- 130 microarray experiments (253 arrays)
  - Seven mouse strains (WT, $Ahr^{(-/-)}$, $Atf3^{(-/-)}$, $Crem^{(-/-)}$, $Cebpd^{(-/-)}$, $Myd88^{(-/-)}$, $Ticam1^{(Lps2/Lps2)}$)
  - Combinations of six stimuli (LPS, Pam_3CSK_4, Pam_2CSK_4, poly I:C, CpG, R848, T091317)
  - Time courses out to 8 hours (24 for LPS)
- Mouse genome promoters (UCSC)
- TRANSFAC Professional 10.3
- Curated list of ~1800 human TFs

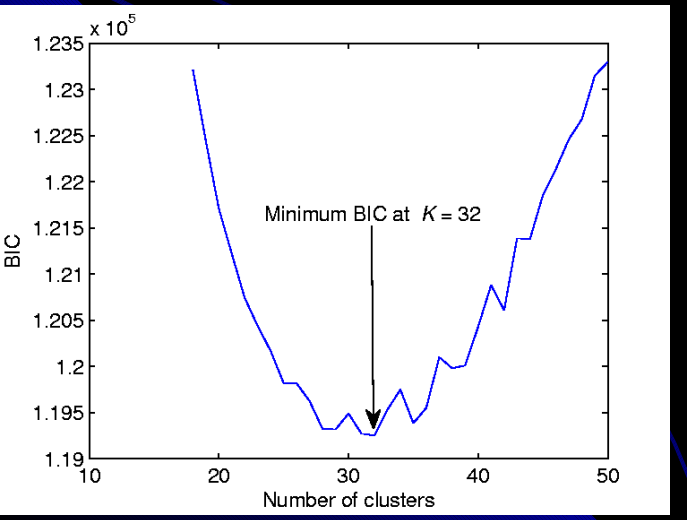


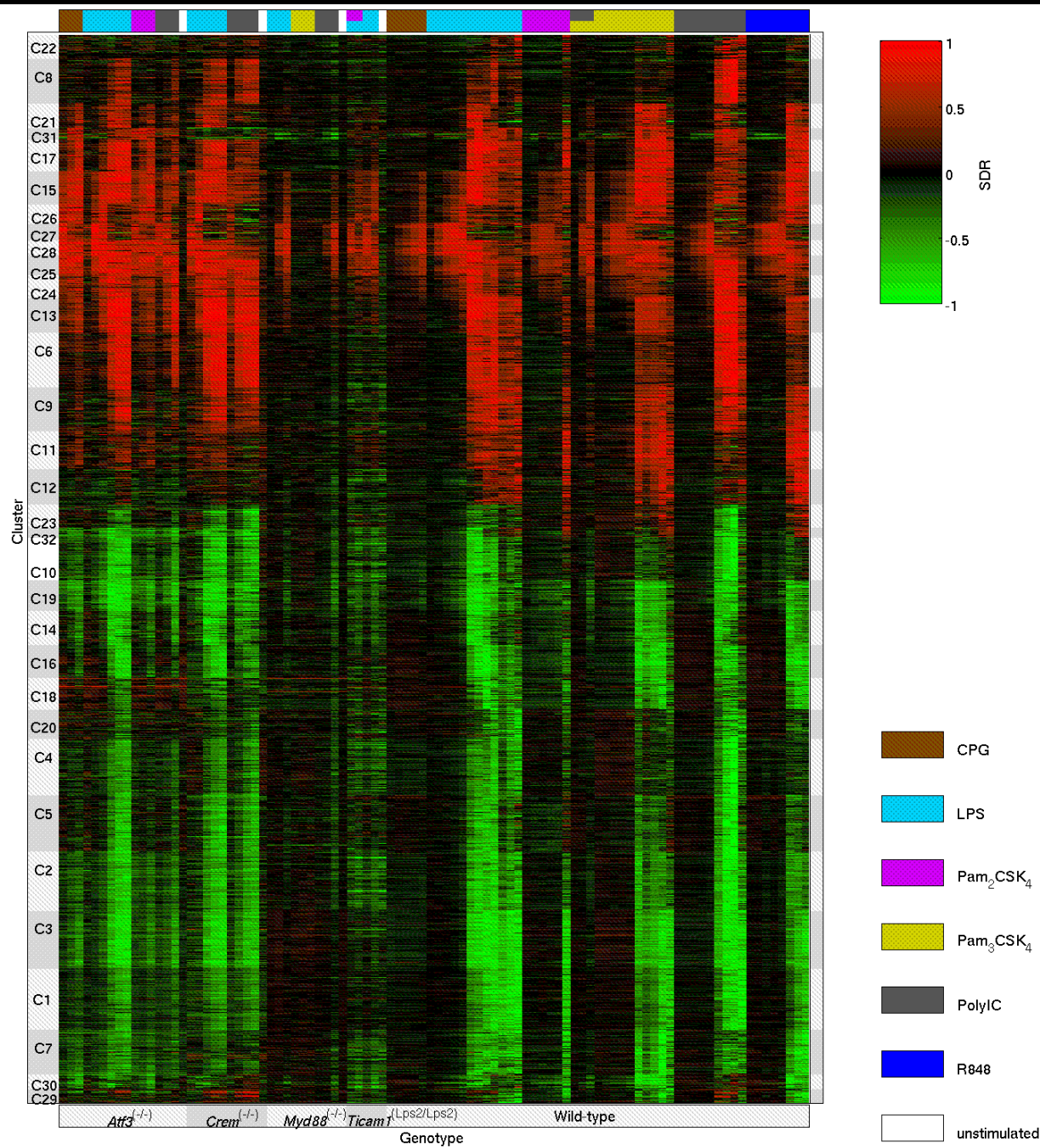
Number of differentially expressed genes vs. elapsed time (by stimulus)

Total number: 2,562

S. Ramsey, PLoS Comp Bio, 4,3, 2008.

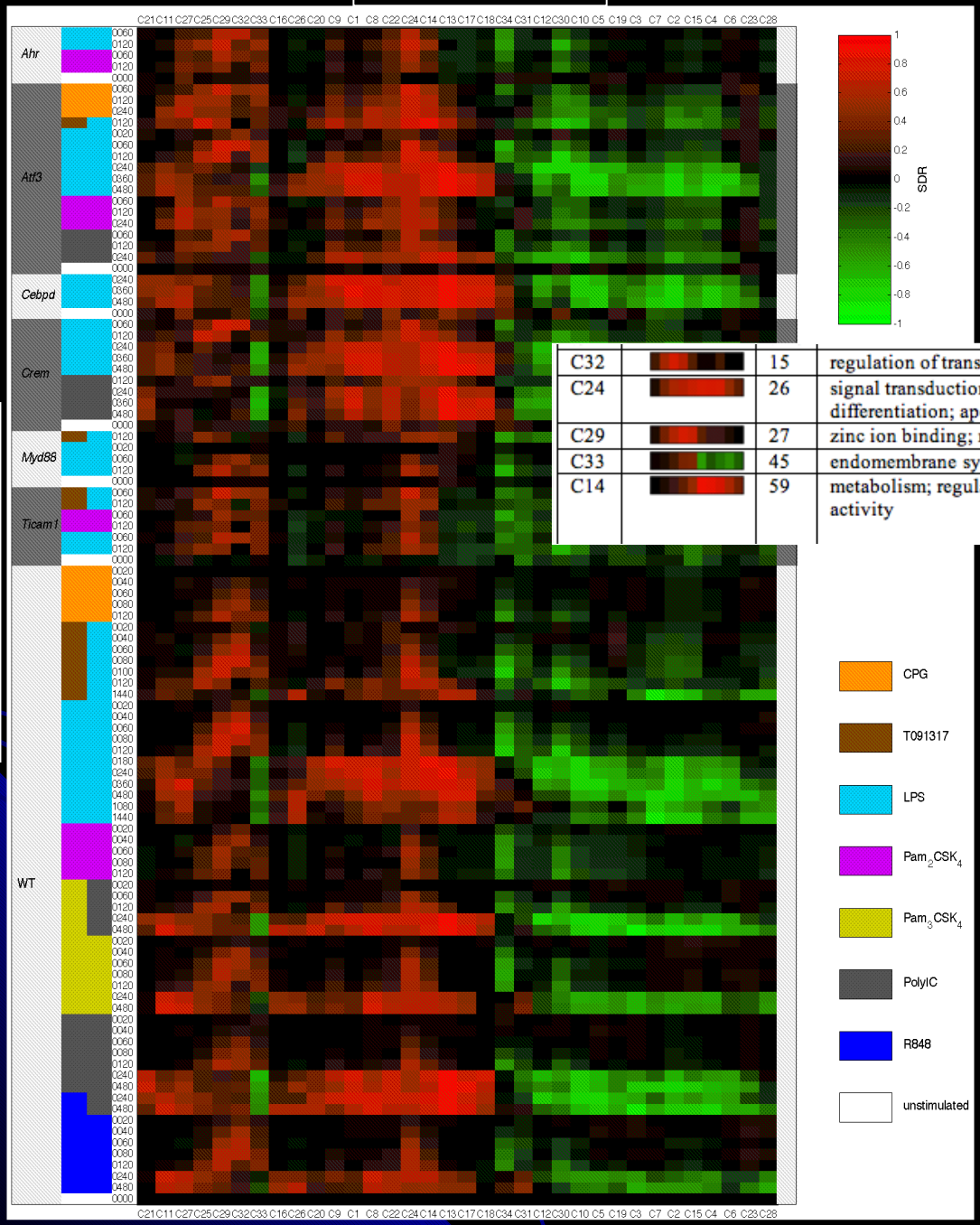
clustering of gene expression profiles





34 Clusters

130 Experiments



C32		15	regulation of transcription; response to stress	Btg2, Ewsr1, Fos, Hipk1, Tgif
C24		26	signal transduction; regulation of transcription; cell differentiation; apoptosis; cytokine activity	Atf3, Ccrn4l, Etv3, Irf1, Junb, Klf6, Nfkbiz, Prdm1
C29		27	zinc ion binding; regulation of transcription	Egr1, Egr2, Egr3, Maff
C33		45	endomembrane system	Id2, Phf19
C14		59	metabolism; regulation of transcription; cytokine activity	Arid5a, Bcl3, Hivep1, Hivep2, Klf7, Lass6, Nfil3, Nfkb1, Nfkb2, Nfkbie, Rel, Zfp263

CPG

T091317

LPS

Pam_2CSK_4

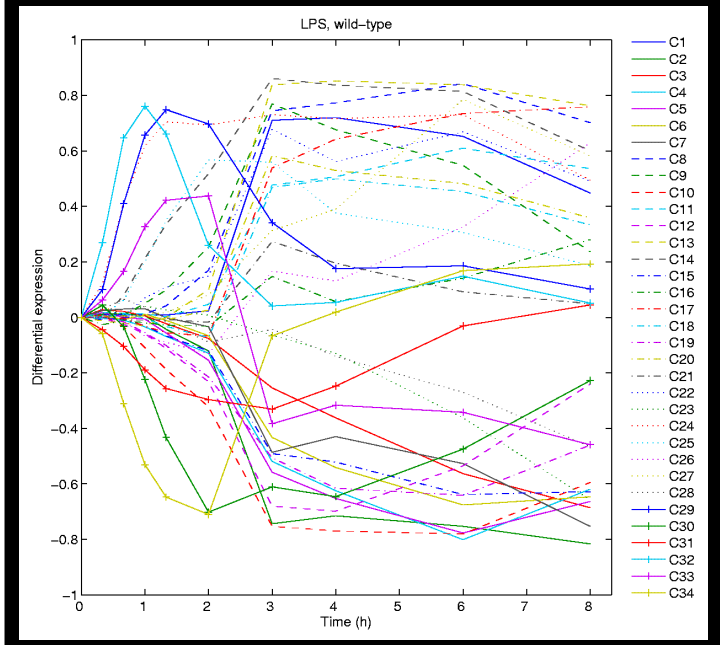
Pam_3CSK_4

PolyIC

R848

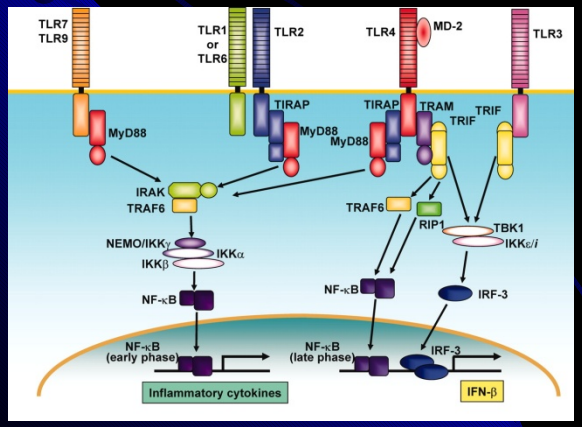
unstimulated

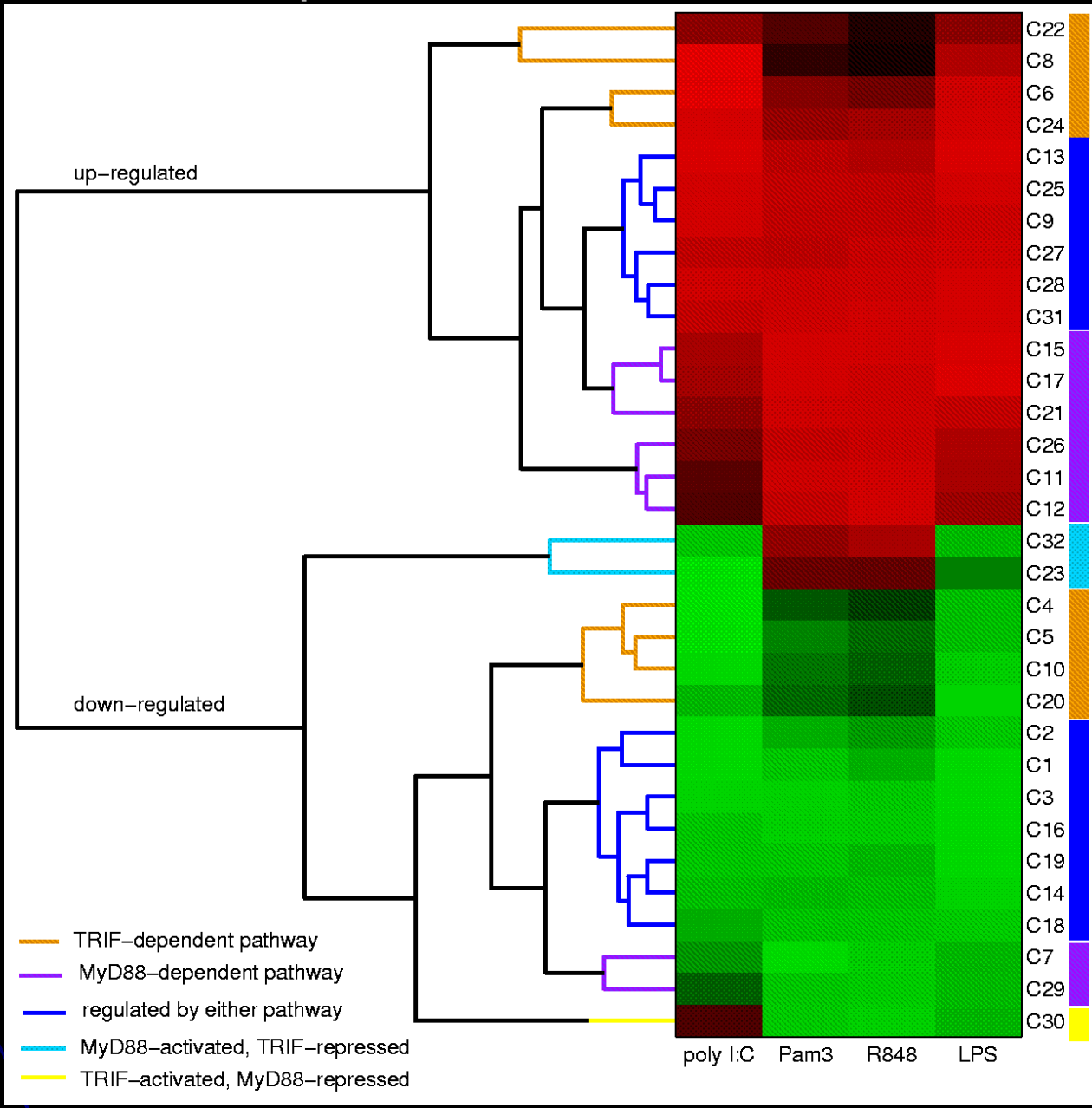
Cluster summarized expression profiles



S. Ramsey, PLoS Comp Bio, 4,3,, 2008.

Stimulus-specific cluster responses

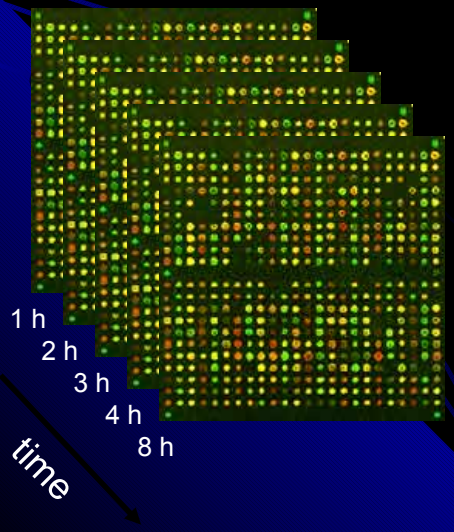




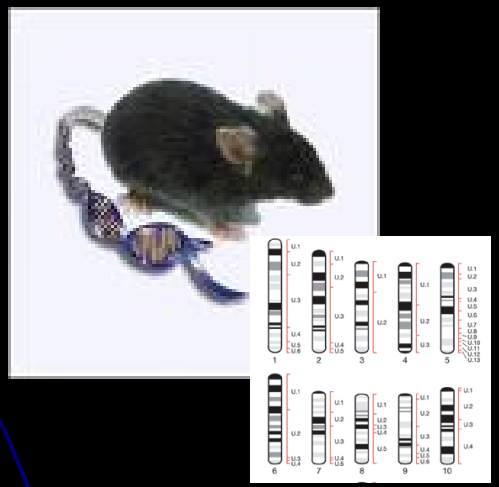
S. Ramsey, PLoS Comp Bio, 4,3, 2008.

In conjunction with transcription factor binding site prediction, we can use the *timing* of expression to identify induced transcription factors that are associated with downstream groups of genes that they regulate.

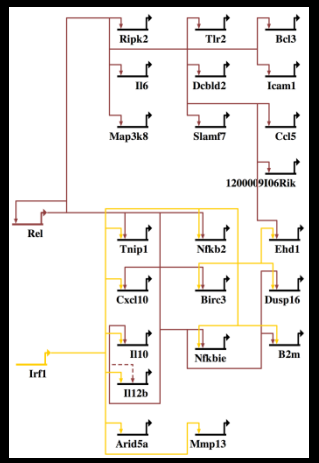
Expression *dynamics*



Genomic sequence data



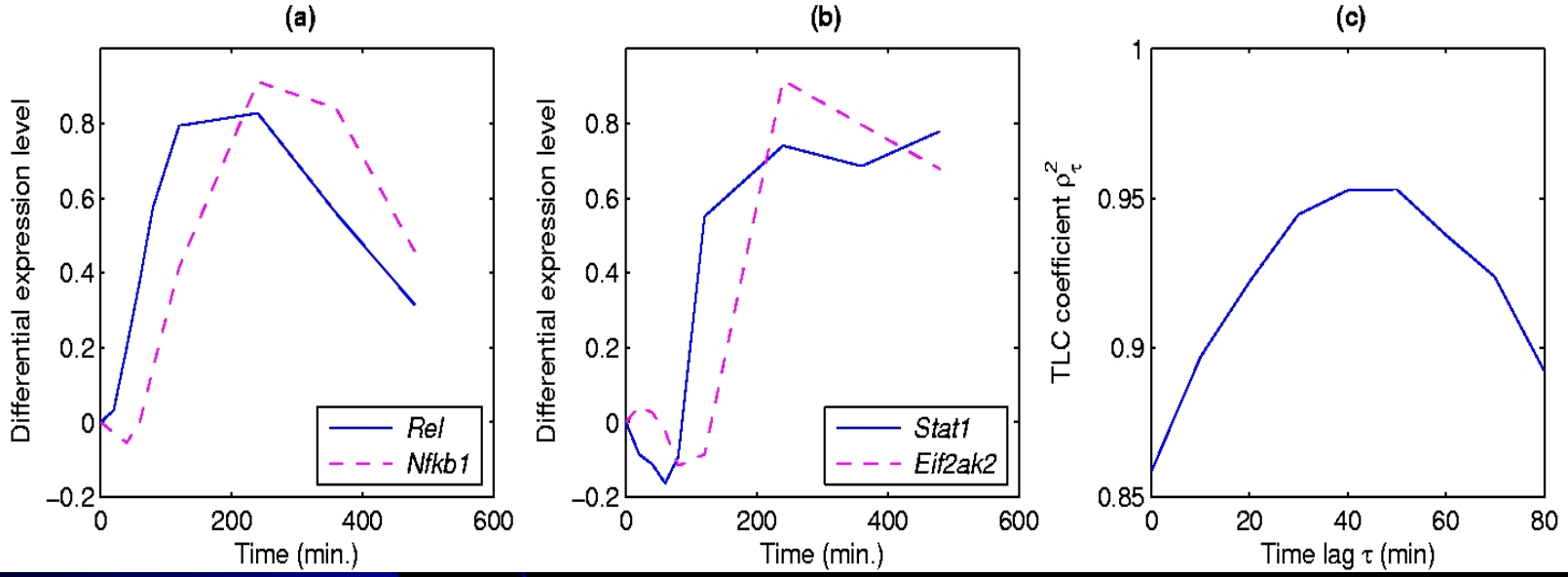
Transcriptional network



S. Ramsey

Components of the gene-gene transcriptional time delay

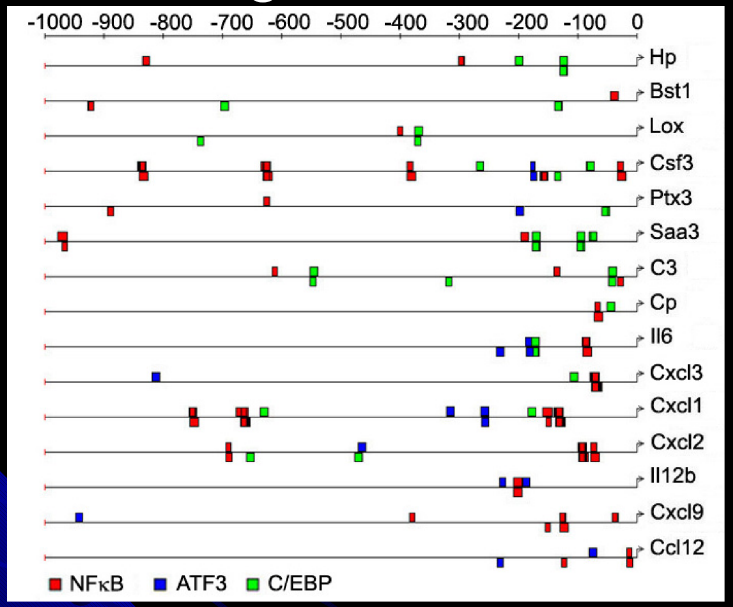
- mRNA half-life of TF gene
- Translation and folding of TF protein
- Diffusion of TF back into nucleus
- Turnover rate (half-life) for TF protein
- Transcriptional elongation of target gene



S. Ramsey, PLoS Comp Bio, 4,3, 2008.

Scan for TF binding motif matches

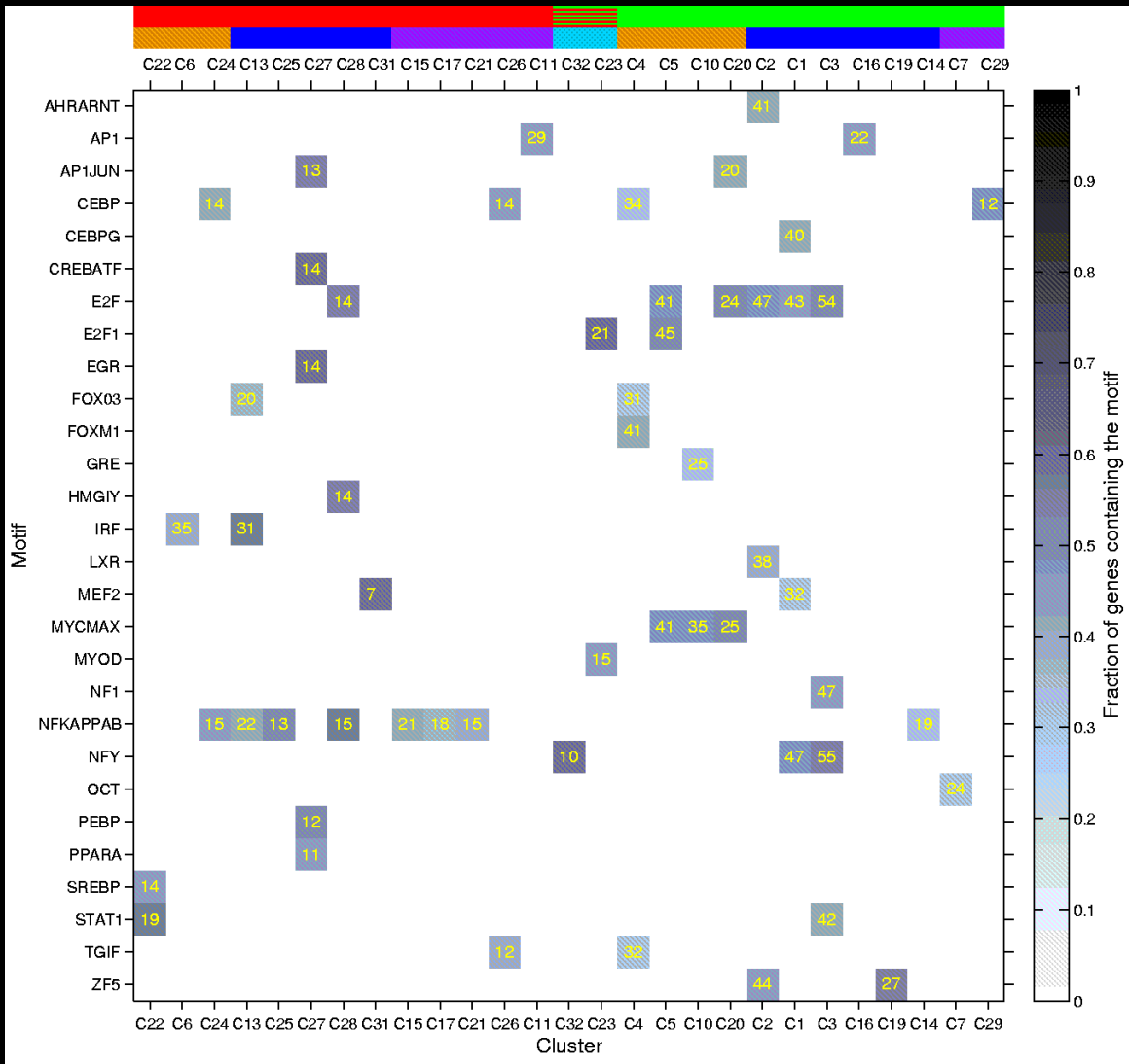
Scanning:



TF recognition site motif:

S. Ramsey, PLoS Comp Bio, 4,3, 2008.

Motif enrichment matrix:



Probabilistic Framework for Transcription Factor Binding Site Prediction

www.probtf.org

otif model $\theta^{(\pi_i)}$ at location a_i

round model

Data Fusion

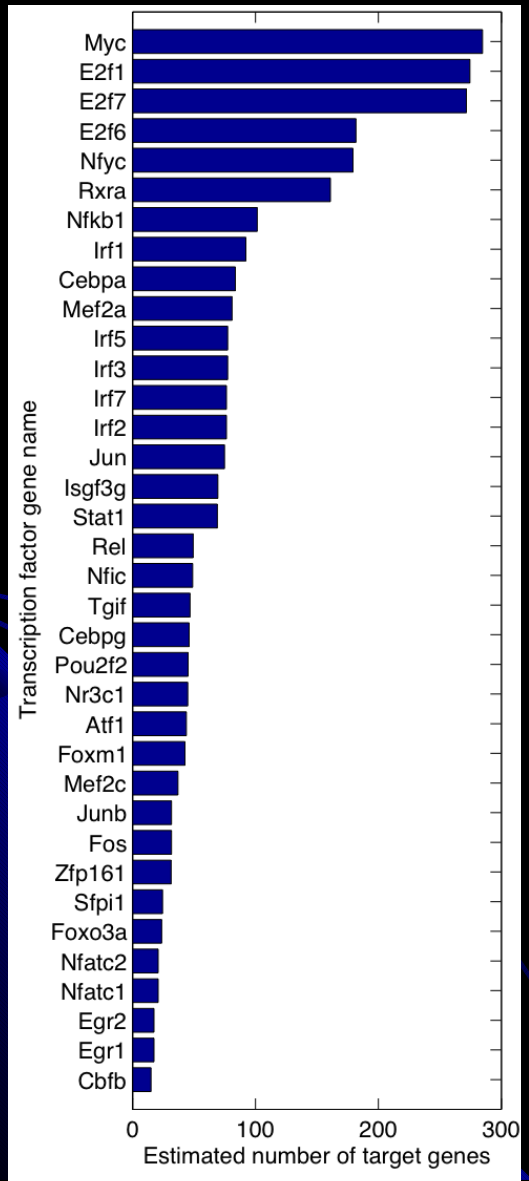
$$|A, \pi, \Theta, \phi) = P(S|A, \pi, \Theta, \phi)P(\mathcal{D}|A, \pi)$$

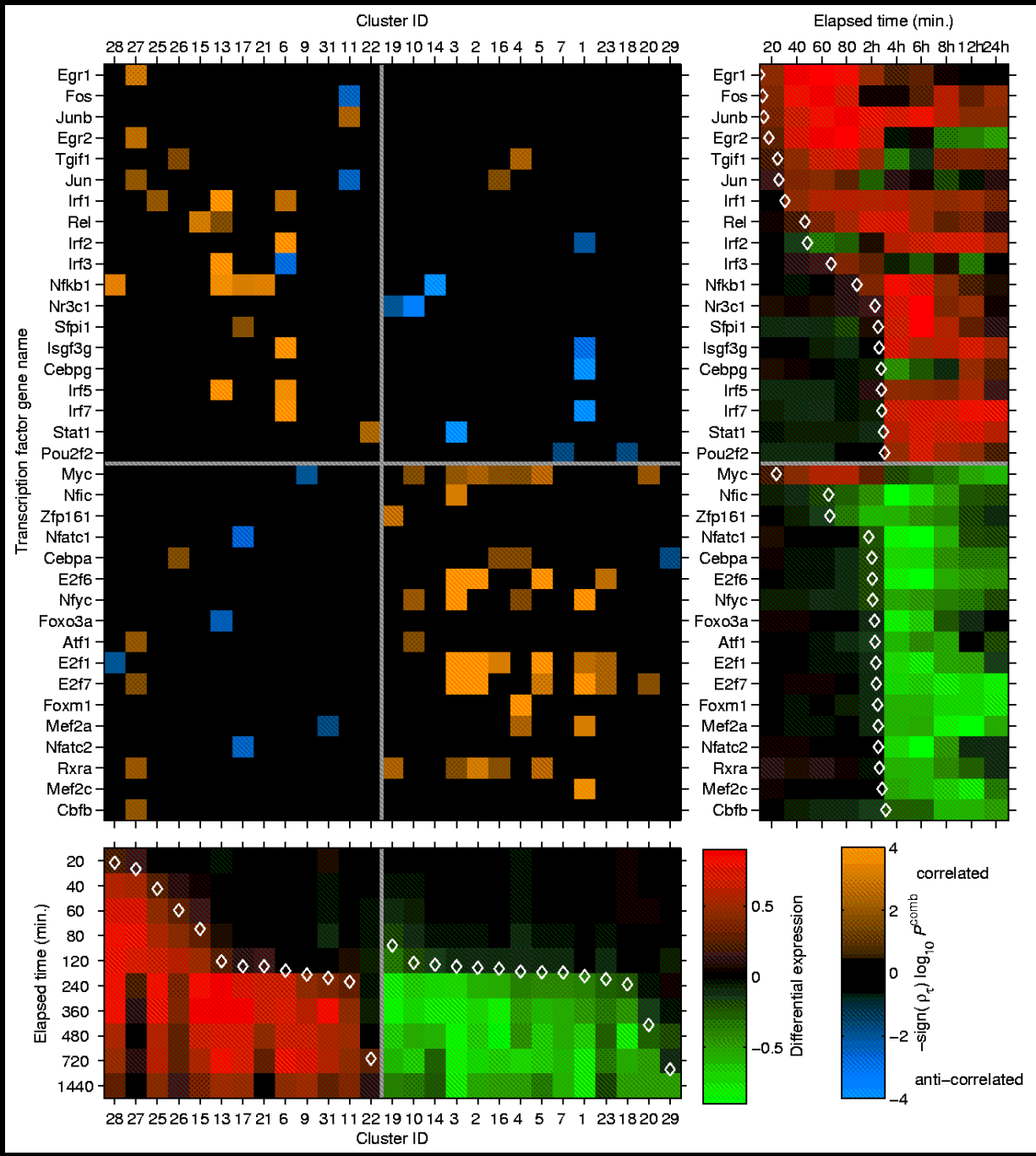
$$|S, \mathcal{D}) = \frac{P(S, \mathcal{D}|A, \pi)P(A, \pi)}{P(S, \mathcal{D})}$$

$$= \frac{P(S|A, \pi)P(\mathcal{D}|A, \pi)P(A, \pi)}{P(S, \mathcal{D})}$$

Lähdesmäki *et al* PLoS ONE, 3:3, e1820, 2008.

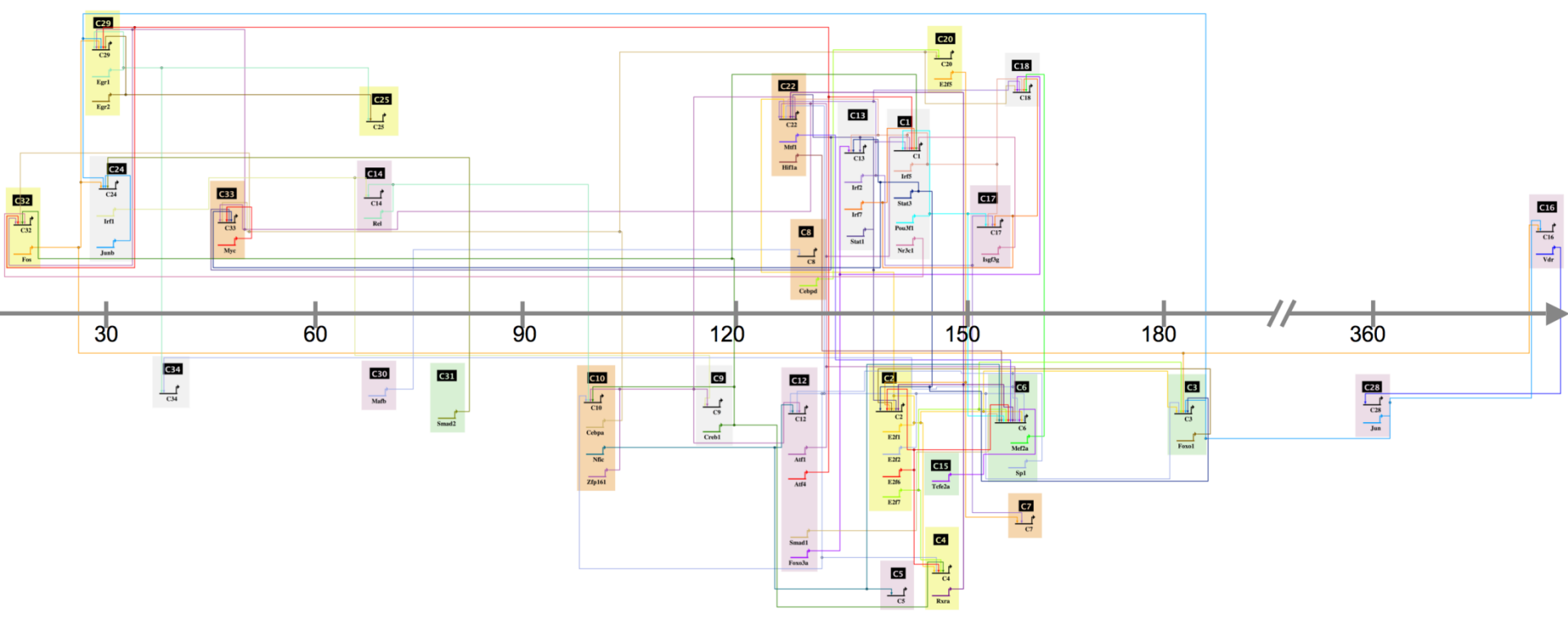
Map network





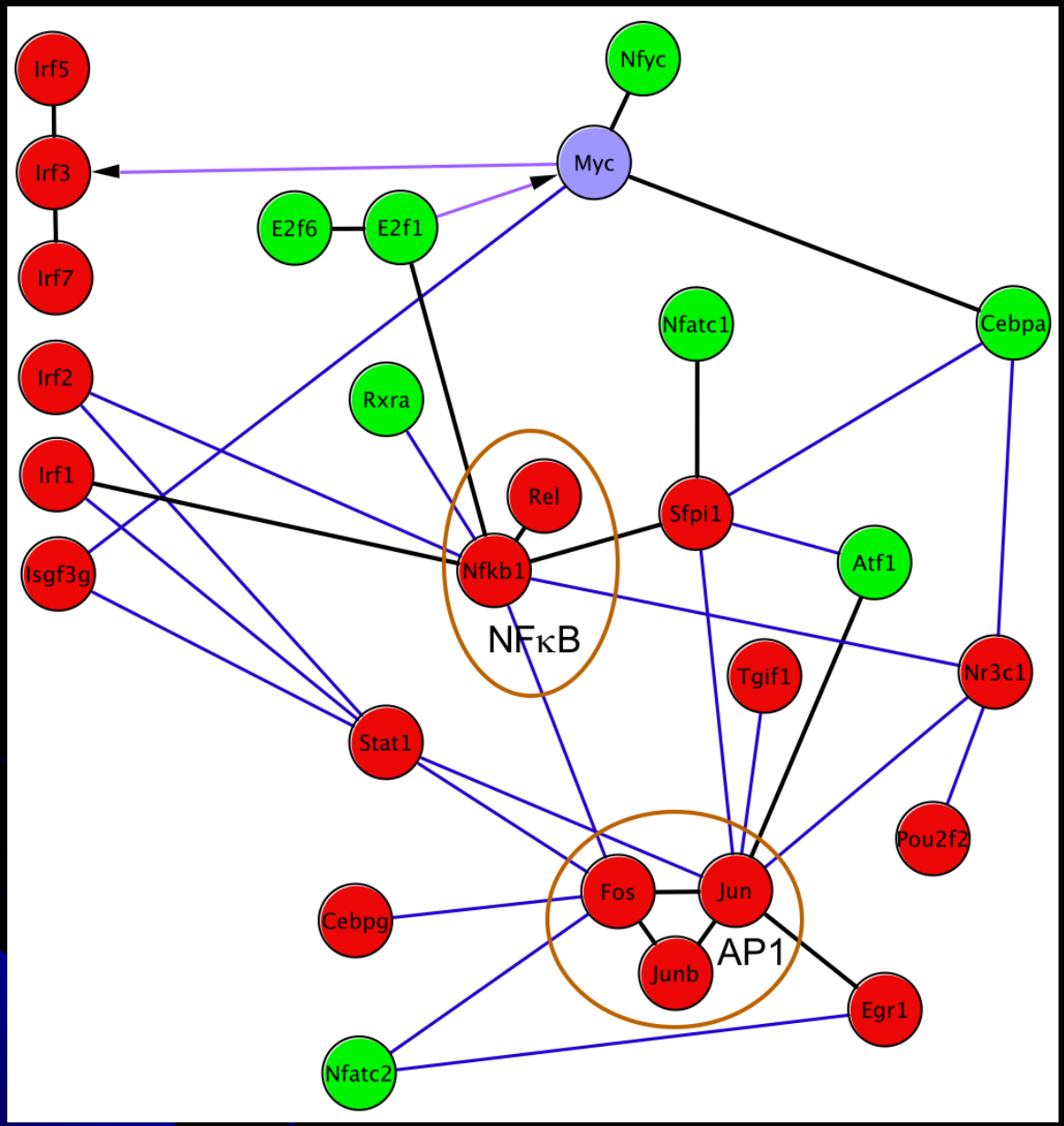
S. Ramsey, PLoS Comp Bio, 4,3, 2008.

TF-to-cluster association network



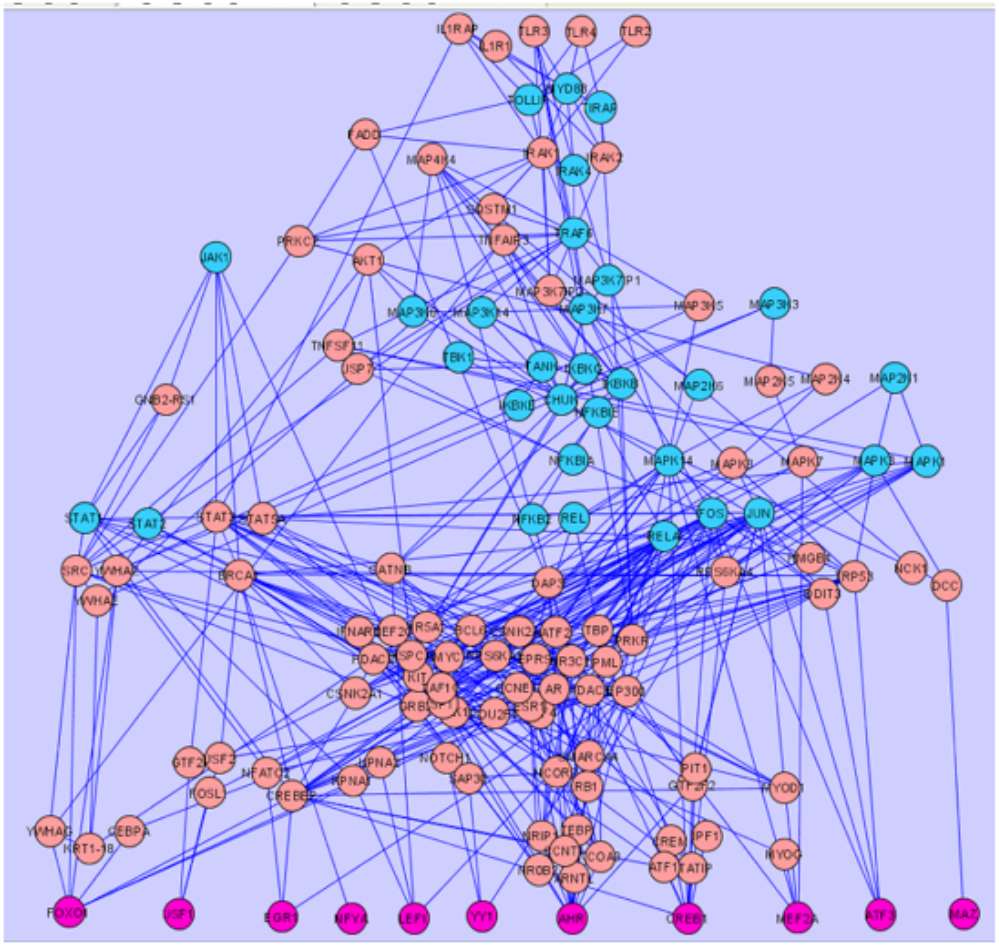
S. Ramsey, PLoS Comp Bio, 4,3, 2008.

TF interactions



S. Ramsey, PLoS Comp Bio, 4,3, 2008.

TLR signaling pathway and downstream predicted transcription factors: using protein-protein interactions



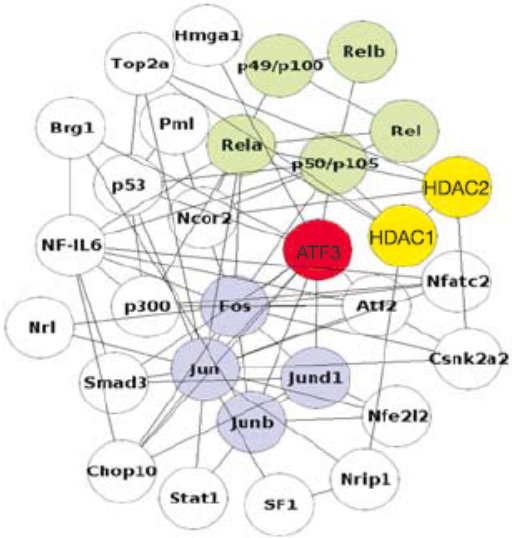
Known TLR Signaling pathway

(Known TFs)

Link to Upstream prot.

Link to Known TFs

Predicted TFs



ATF3 (red) is predicted to interact with a number of TFs, including members of the AP1 and NF-kB TF complexes.

B. Li, V. Thorsson

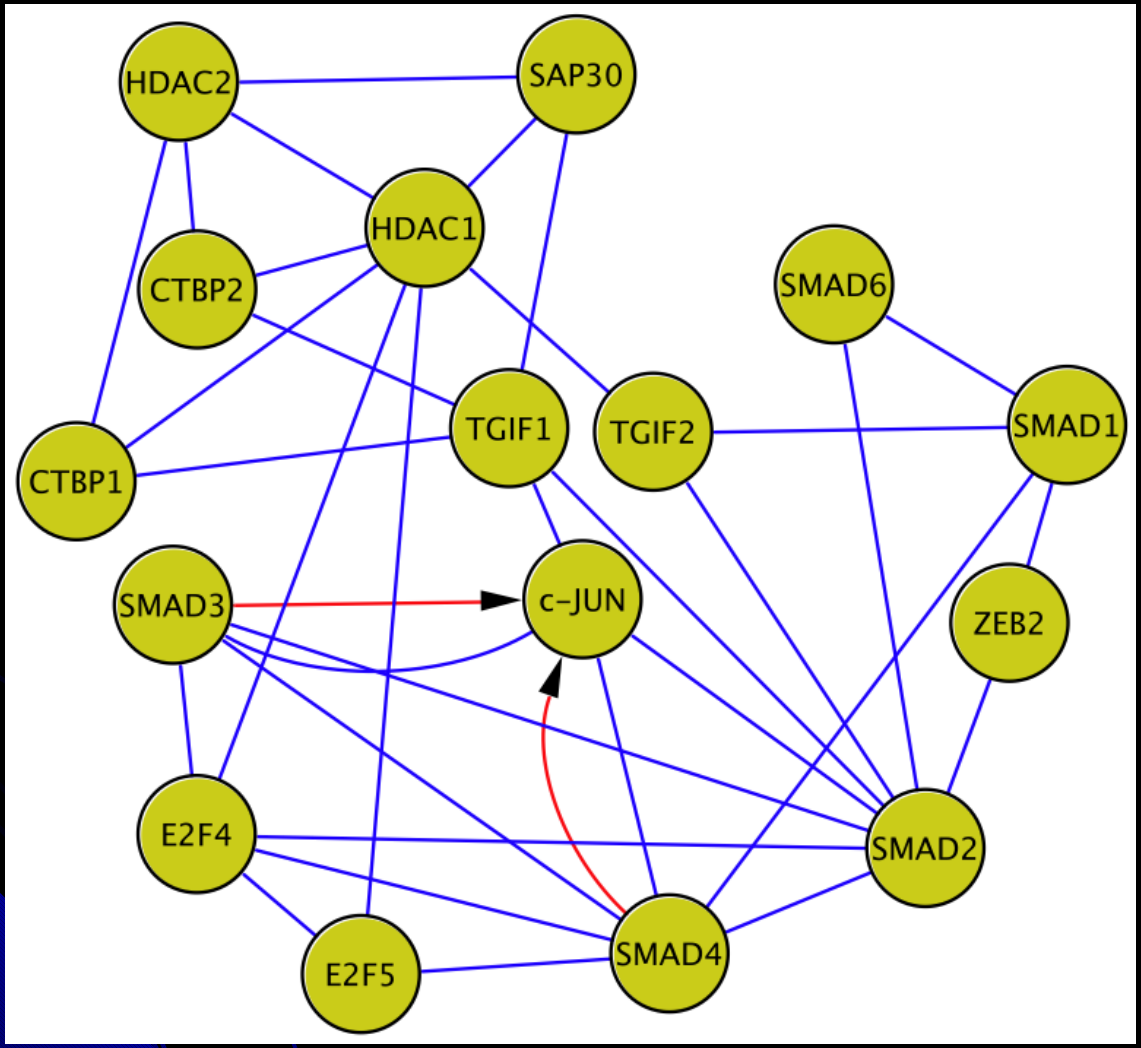
ChIP-on-chip validation

Table 2. Validation of transcription factor-to-cluster associations using ChIP-on-chip

TF	Matrix	Stim.	Clust	Time Points	In Clust	On Chip	Bound	*P*-Value
NFκB/p50	NFKB_Q6	LPS	C13	1 h, 2 h	64	23	18	1.1×10^{-3}
NFκB/p50	NFKB_Q6	LPS	C17	1 h, 2 h	58	20	11	2.5×10^{-1}
NFκB/p50	NFKAPPAB_01	LPS	C28	1 h, 2 h	28	21	20	1.1×10^{-6}
IRF1	IRF_Q6_01	LPS	C13	1 h, 2 h, 4 h	64	23	18	2.3×10^{-3}
IRF1	IRF_Q6_01	LPS	C25	1 h, 2 h, 4 h	37	22	18	8.8×10^{-4}

S. Ramsey, PLoS Comp Bio, 4,3, 2008.

TGIF enhanceosome

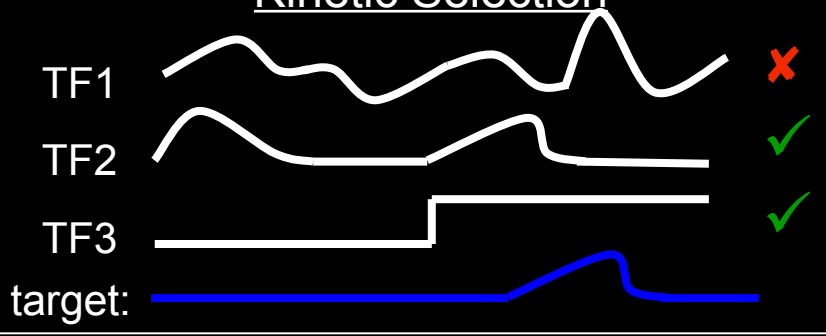


S. Ramsey, PLoS Comp Bio, 4,3, 2008.

Constrain Possible Regulators

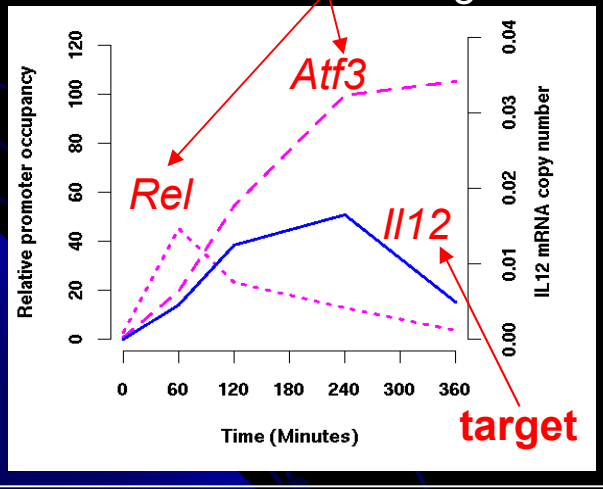
- Gene expression (timing, etc.)
- Promoter sequence analysis
- Immunoprecipitation data

Kinetic Selection

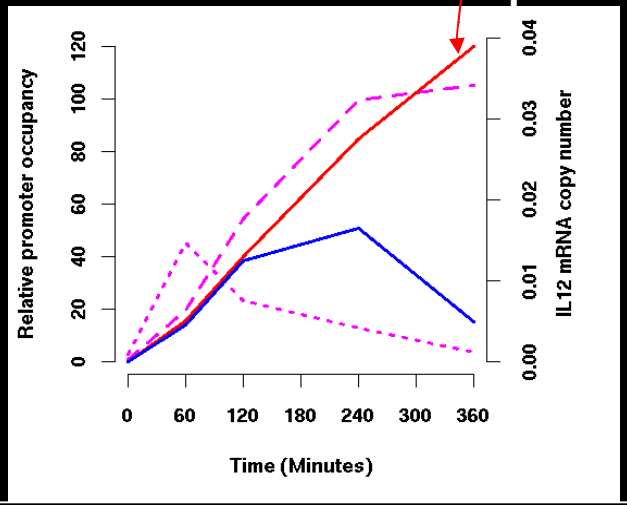


Parameter Fitting

one coefficient for each regulator:



Prediction



$$\tau \frac{d(IL12)}{dt} = -IL12 + g(\beta$$

(Gilchrist *et al.* Nature. 2006; 441(7090):173.)

V. Thorsson

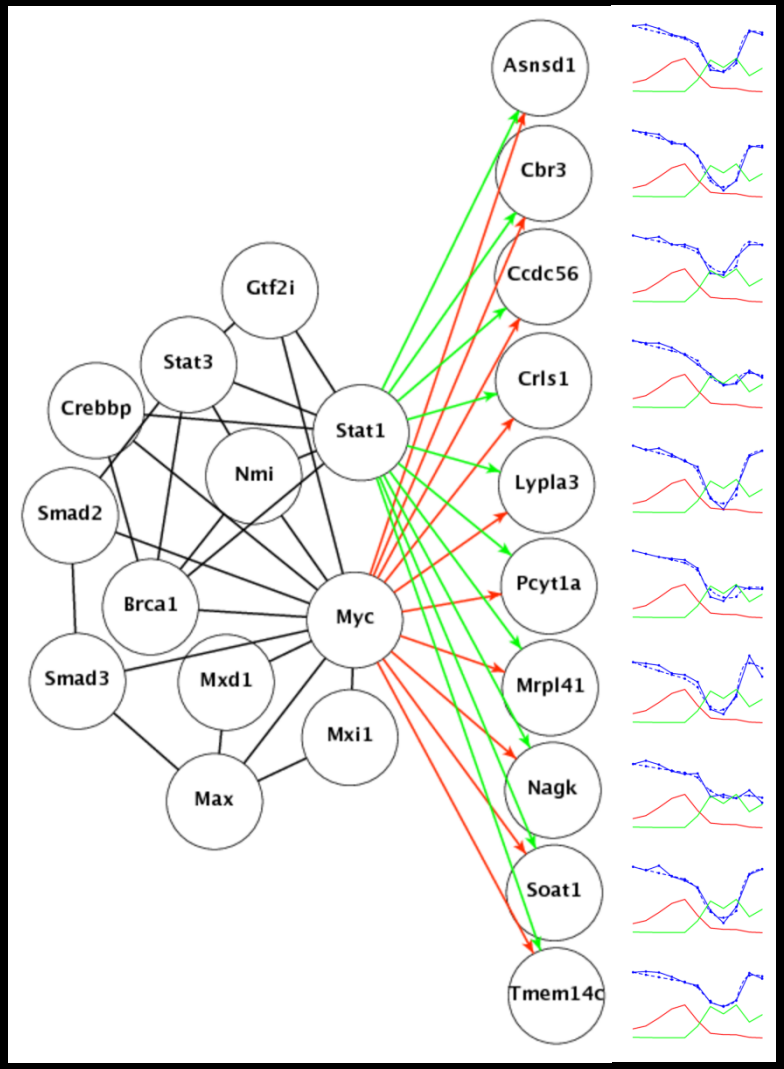
Network Predictions

Macrophage

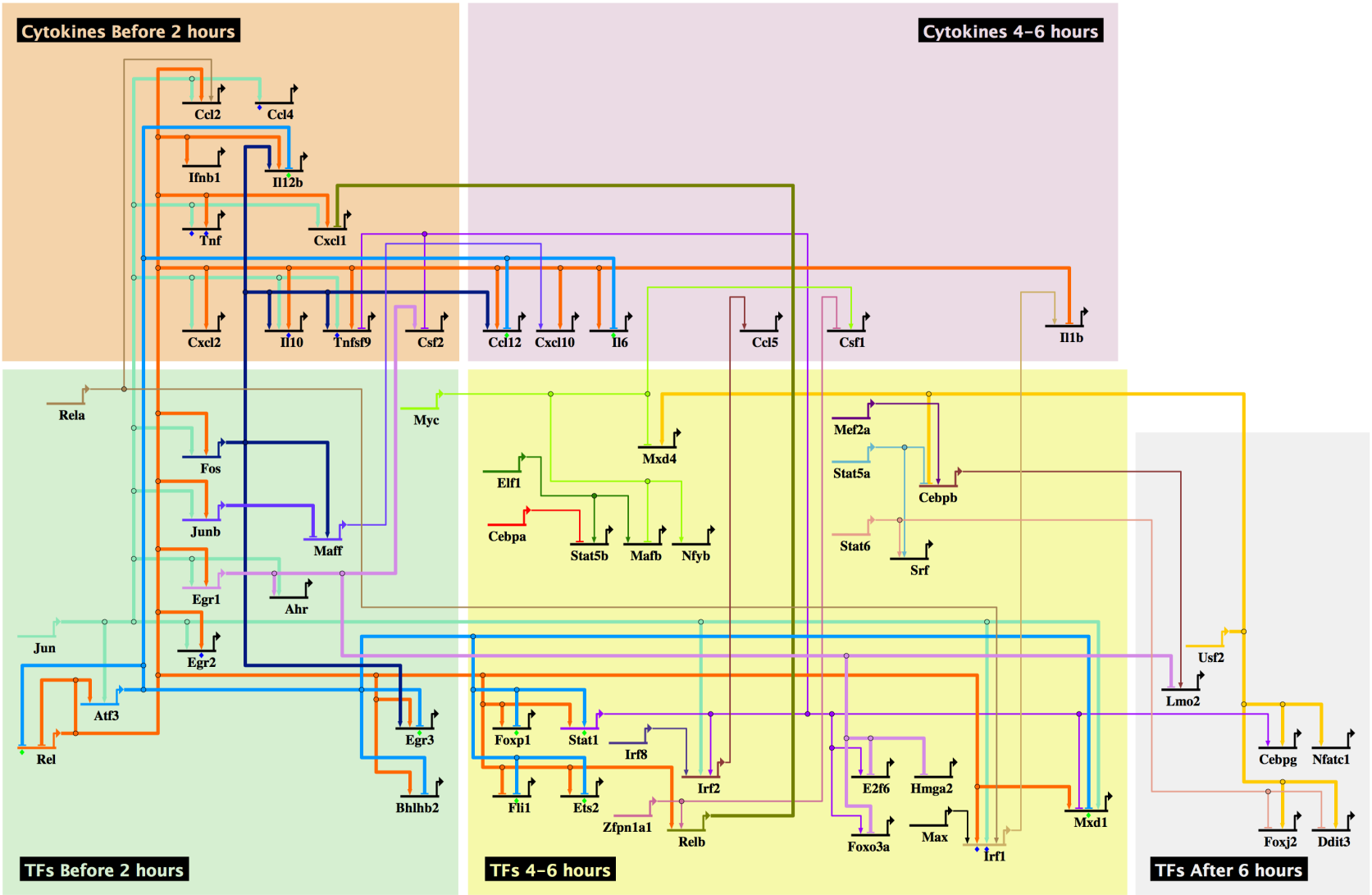
Predicted network contains co-regulated gene groups with shared:

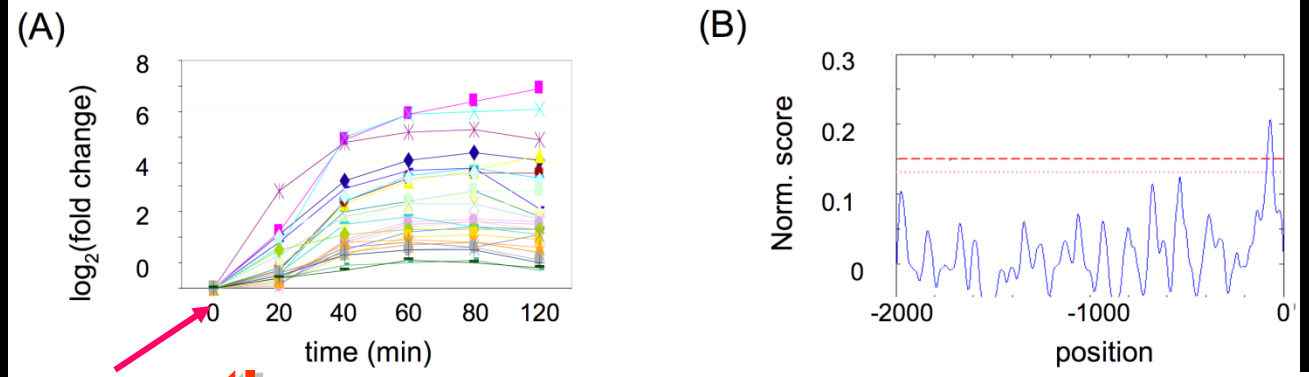
- Binding sites predictions, for single motifs or pairs of motifs
- Kinetics
- Predicted activators and repressors
- Predicted co-factors
- Function (GO categories)

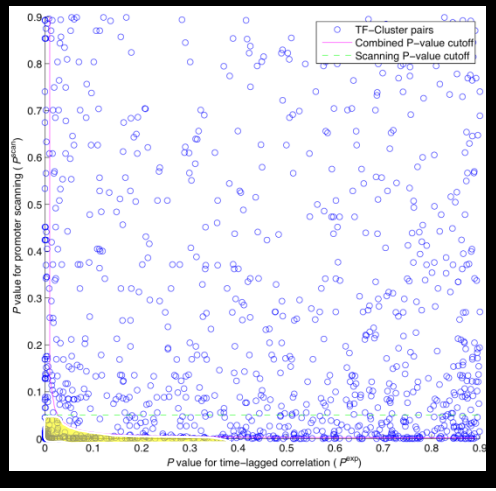
Predictions can be tested with directed binding and/or functional assays



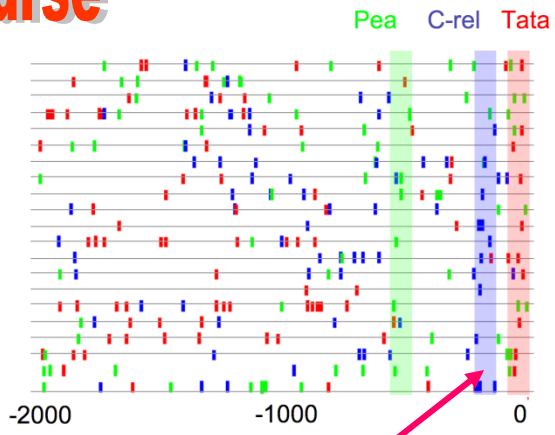
Predicted Regulation of Cytokines and Transcription Factors



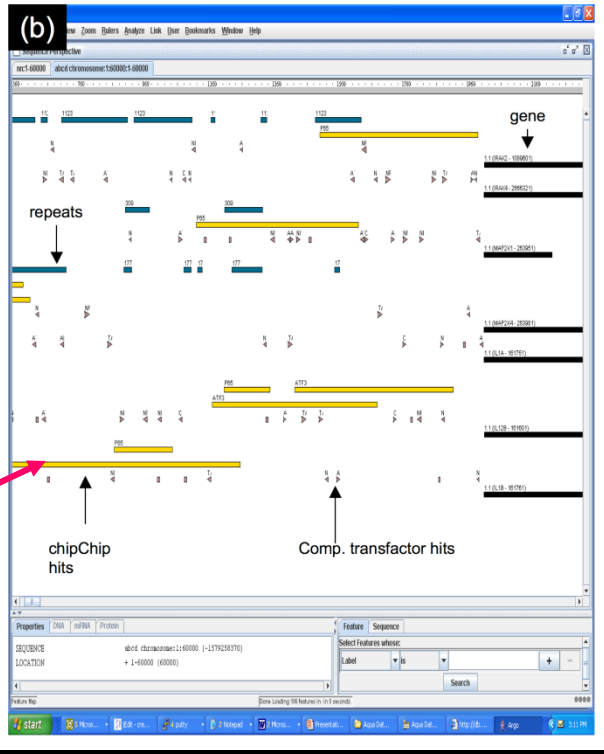




Microarray time-course data



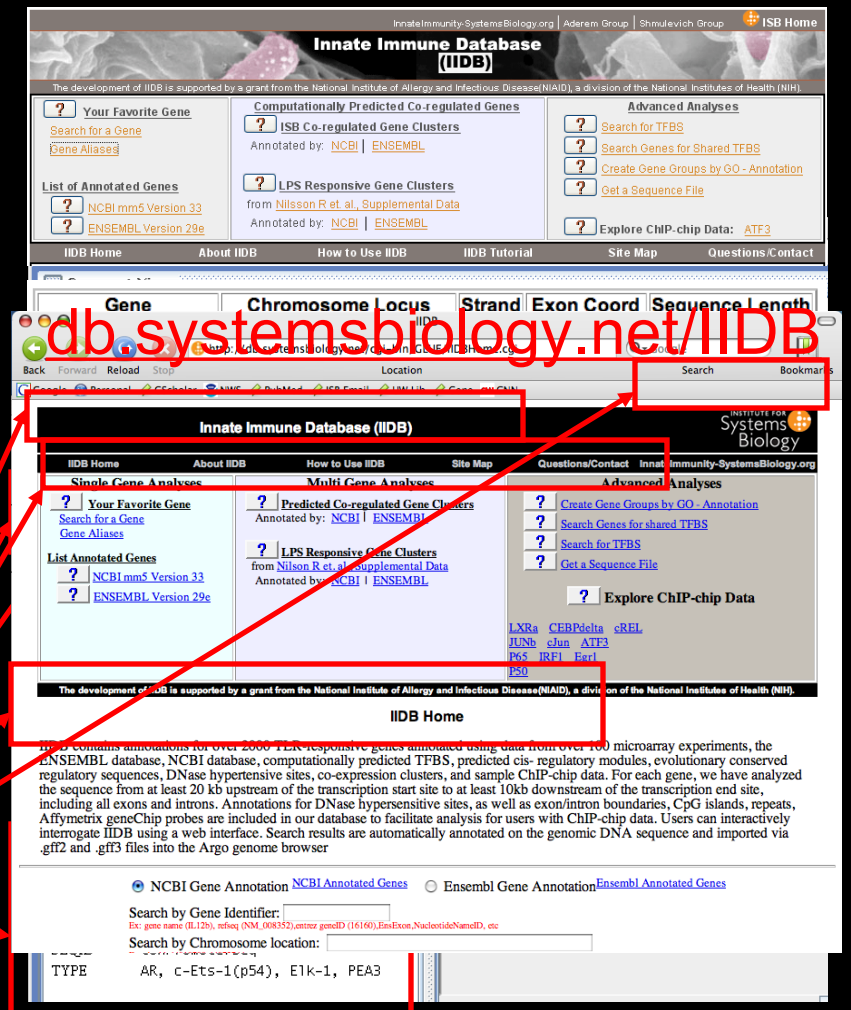
Combined density of predicted TF binding sites



ChIP-chip data

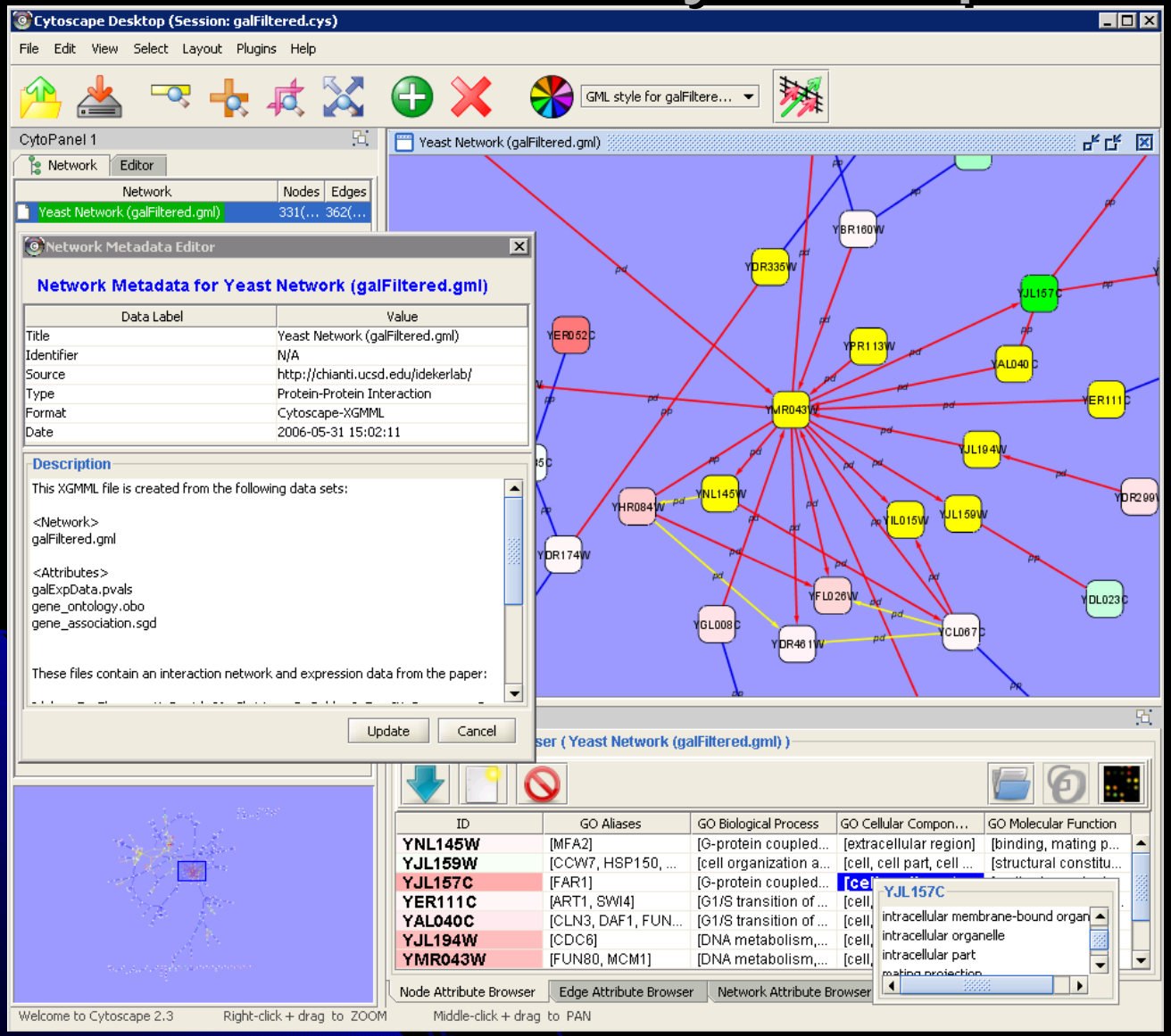
Innate Immune Database (IIDB)

- Genomic annotations and *cis*-regulatory element predictions for immune-related genes.
- Web-based software tool for querying and visualization.
- Display expression time-course data (and clusters)
- Graphical visualization of genomic annotations combining many different data types



Korb *et al*. *BMC Immunology*, 9:7, 2008.

Cytoscape



Visually Integrate gene expression, protein state, protein interactions, and protein class (ontology)

Analysis plug-in modules

Implemented in Java

(networks, attributes, network metadata, etc.)

http://www.cytoscape.org/

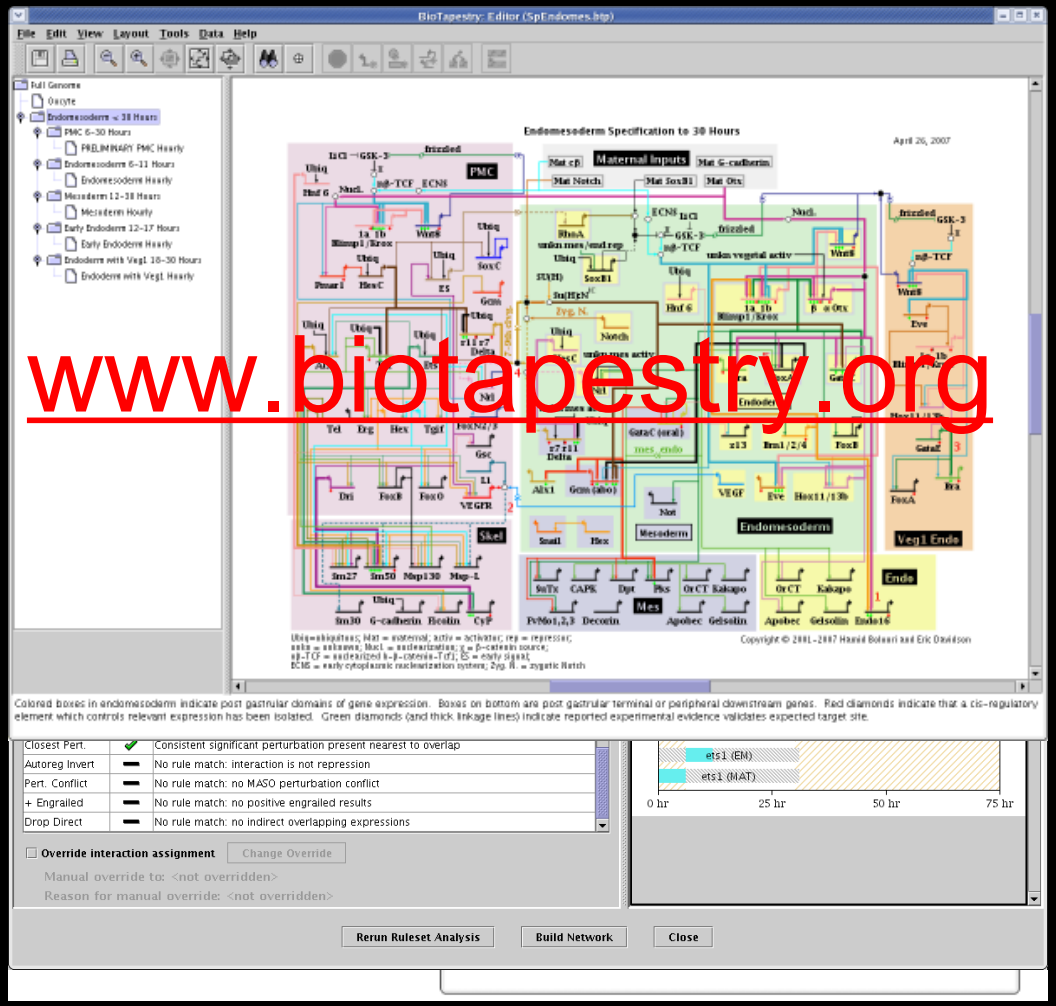
BioTapestry

Graphical application for building & visualising gene regulatory networks

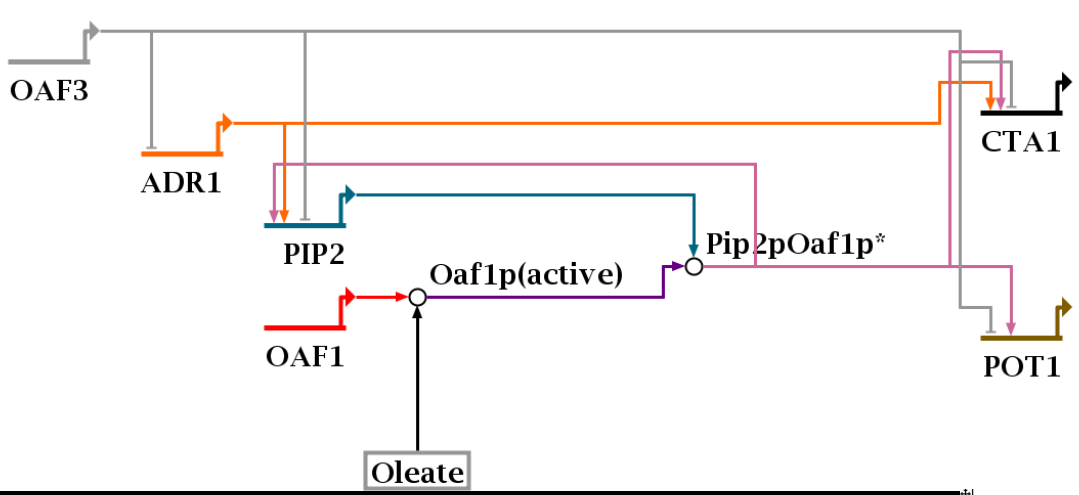
Hierarchical network model for spatially and temporally complex network activation programs

View network activity over time, based on time-course expression

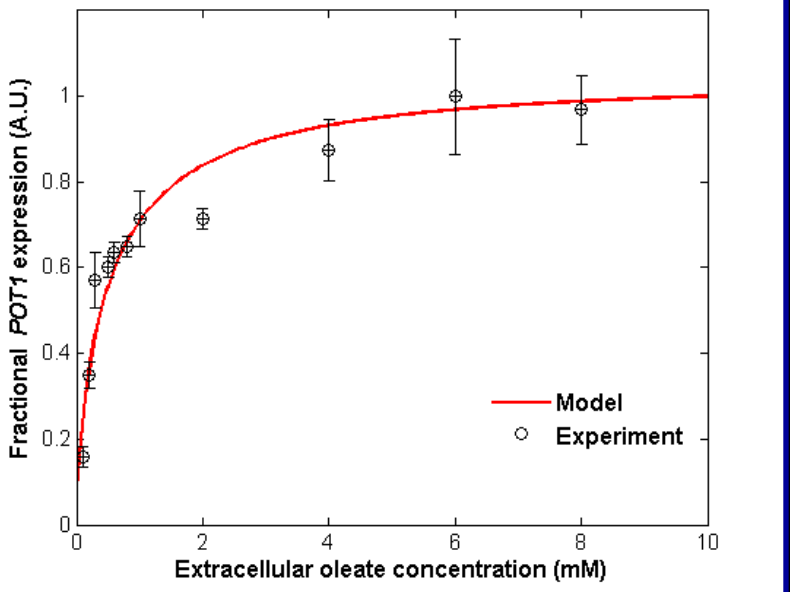
- Build networks from high-throughput data using worksheet feature (under development)



W. Longabaugh



modeling the oleate-response transcriptional network



Steady-state dose-response

$\pi_o(t) = \frac{Fp_o(t)O_{ic}}{K_{M,o} + O_{ic}}$	Activation of Oaf1p by intracellular oleate (π_o denotes activated Oaf1p)
$h(t) = \left(\frac{Q}{2K_{D,h}}\right)\left(1 + \frac{Q}{K_{D,h}}(\pi_o + p_p) - \sqrt{\left(1 + \frac{Q}{K_{D,h}}(\pi_o + p_p)\right)^2 - 4\left(\frac{Q}{K_{D,h}}\right)\pi_o p_p}\right)$	Heterdimerization of activated Oaf1p with Pip2p ($h(t)$ represents the concentration of Oaf1p-Pip2p heterodimer)
$S = \frac{O_{ic}}{K_{M,s} + O_{ic}}$	Activation of transcriptional response of *ADR1*, *OAF1*, and *OAF3* by oleate.
$\frac{dr_\gamma(t)}{dt} = k_{i,r,\gamma} f_{r,\gamma}(t - \tau_{lr}) - k_{d,r,\gamma} r_\gamma(t), \quad \forall \gamma \in \{a,c,o,y,p,f\}$	Gene expression
$\frac{dp_\gamma(t)}{dt} = k_{i,p,\gamma} r_\gamma(t - \tau_{lp}) - k_{d,p,\gamma} p_\gamma(t), \quad \forall \gamma \in \{a,c,o,y,p,f\}$	Protein expression
$f_{r,a} = \left(\frac{1}{r_{x,a}}\right)\left[r_{g,a} + S(r_{o,a} - r_{g,a})\right]$	Fractional activity of *ADR1* gene
$f_{r,c}(t) = \left(\frac{1}{r_{x,c}}\right)\left[r_{g,c} + \left\{w_p\left(\frac{h(t)}{K_{M,h} + h(t)}\right) + w_a S\left(\frac{p_a(t)}{K_{M,a} + p_a(t)}\right)\right\}(r_{o,c} - r_{g,c})\right]$	Fractional activity of *CTA1* gene
$f_{r,o} = \left(\frac{1}{r_{x,o}}\right)\left[r_{g,o} + S(r_{o,o} - r_{g,o})\right]$	Fractional activity of *OAF1* gene
$f_{r,y} = \left(\frac{1}{r_{x,y}}\right)\left[r_{g,y} + S(r_{o,y} - r_{g,y})\right]$	Fractional activity of *OAF3* gene
$f_{r,p}(t) = \left(\frac{1}{r_{x,p}}\right)\left[r_{g,p} + \left\{w_p\left(\frac{h(t)}{K_{M,h} + h(t)}\right) + w_a S\left(\frac{p_p(t)}{K_{M,a} + p_p(t)}\right)\right\}(r_{o,p} - r_{g,p})\right]$	Fractional activity of *PIP2* gene
$f_{r,f}(t) = \left(\frac{1}{r_{x,f}}\right)\left[r_{g,f} + \left(\frac{h(t)}{K_{M,h} + h(t)}\right)(r_{o,p} - r_{g,p})\right]$	Fractional activity of *POT1* gene

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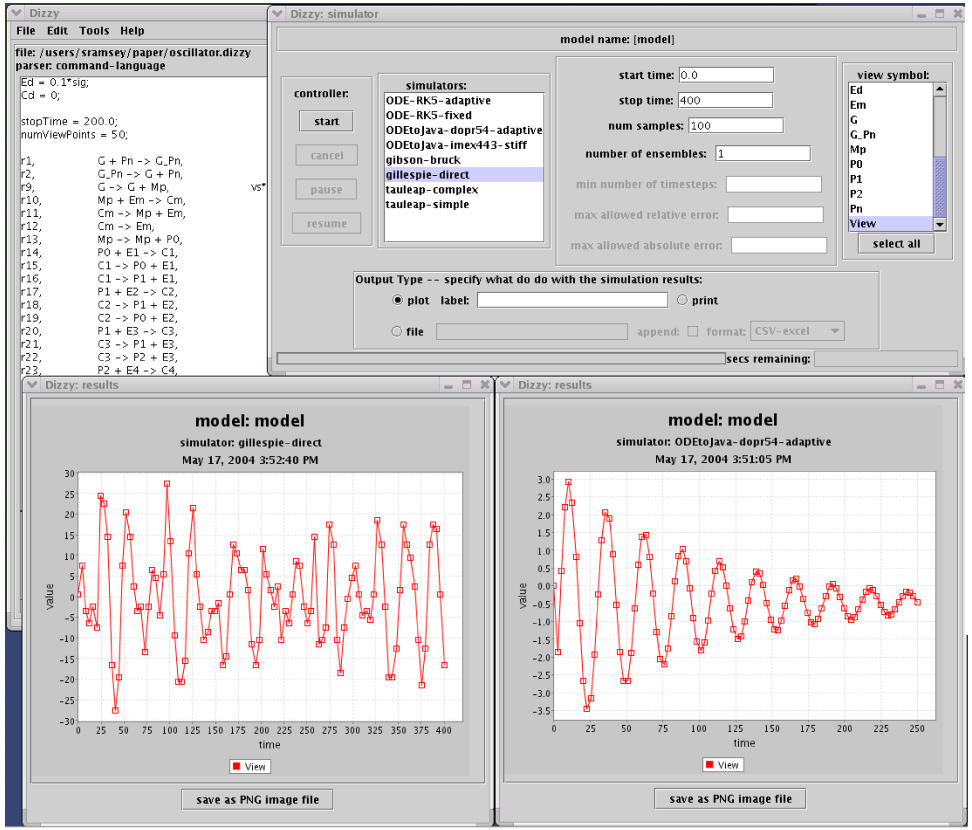
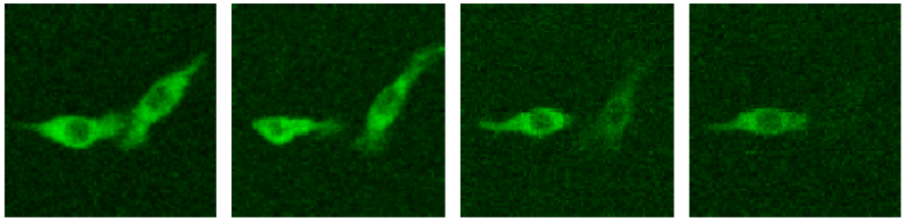
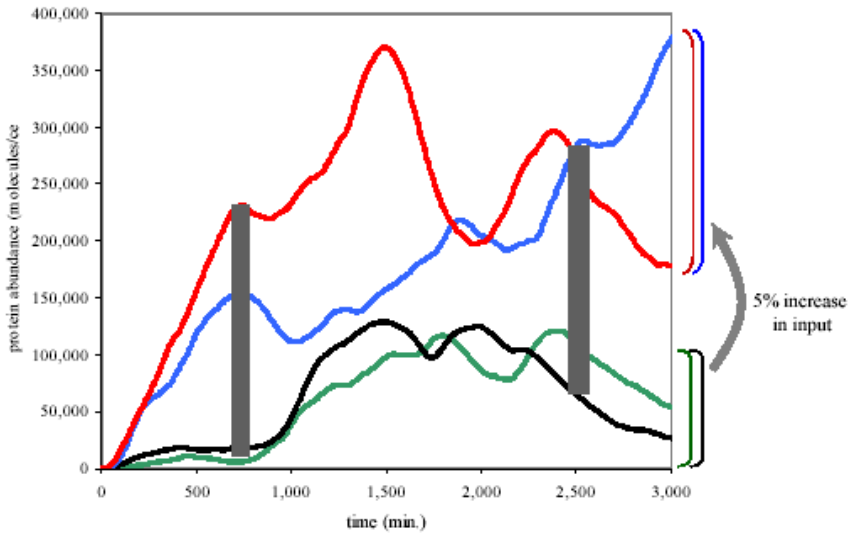
Network Simulation

Stochastic Simulation (Dizzy)



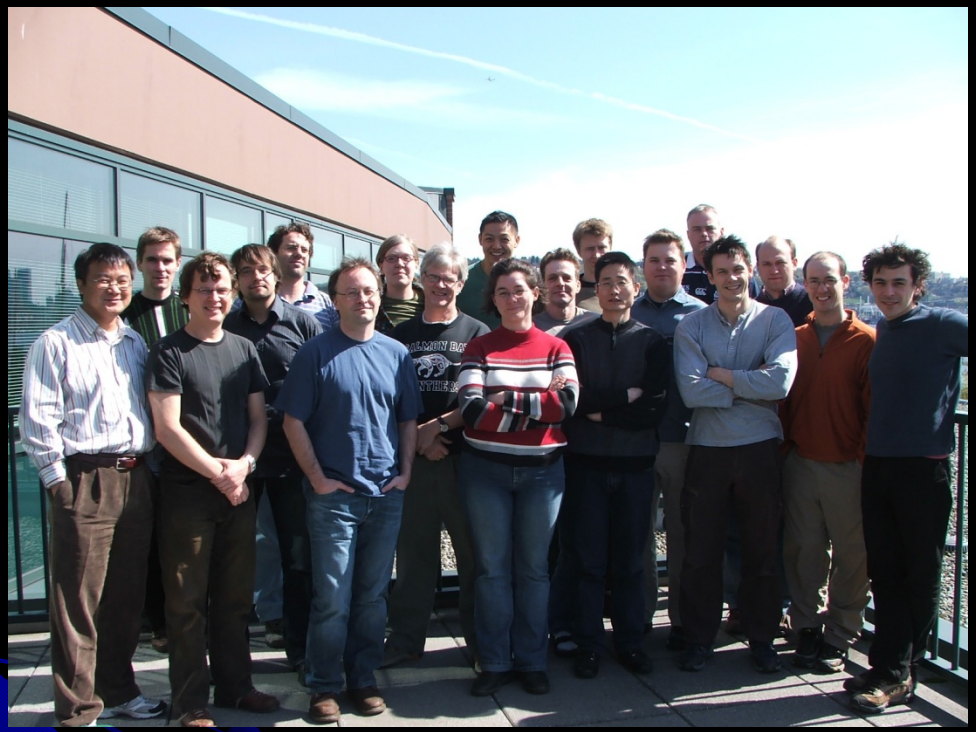




Individual macrophage cells show different levels of IkBa:GFP after stimulation by dsRNA (TLR3 ligand)

S. Ramsey

Acknowledgments



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