

# Identification of cancer-specific signaling dynamics and gene regulatory network

RTK Consortium  
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RIKEN GSC  
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# What is critical to cell determination?

Changes in protein-protein interaction of signaling pathways?

= well addressed in the past studies

Dynamics?

= transient vs. sustained activation

What about gene expression patterns?

= under investigation

Linkage between dynamics-gene regulatory network-cell fate

= totally unknown

# Importance of dynamics study of RTK

1. Deregulation of signal transduction pathway is involved in many diseases. - 50% of cancer
2. < 20% of human genome encodes signal transduction-related proteins.

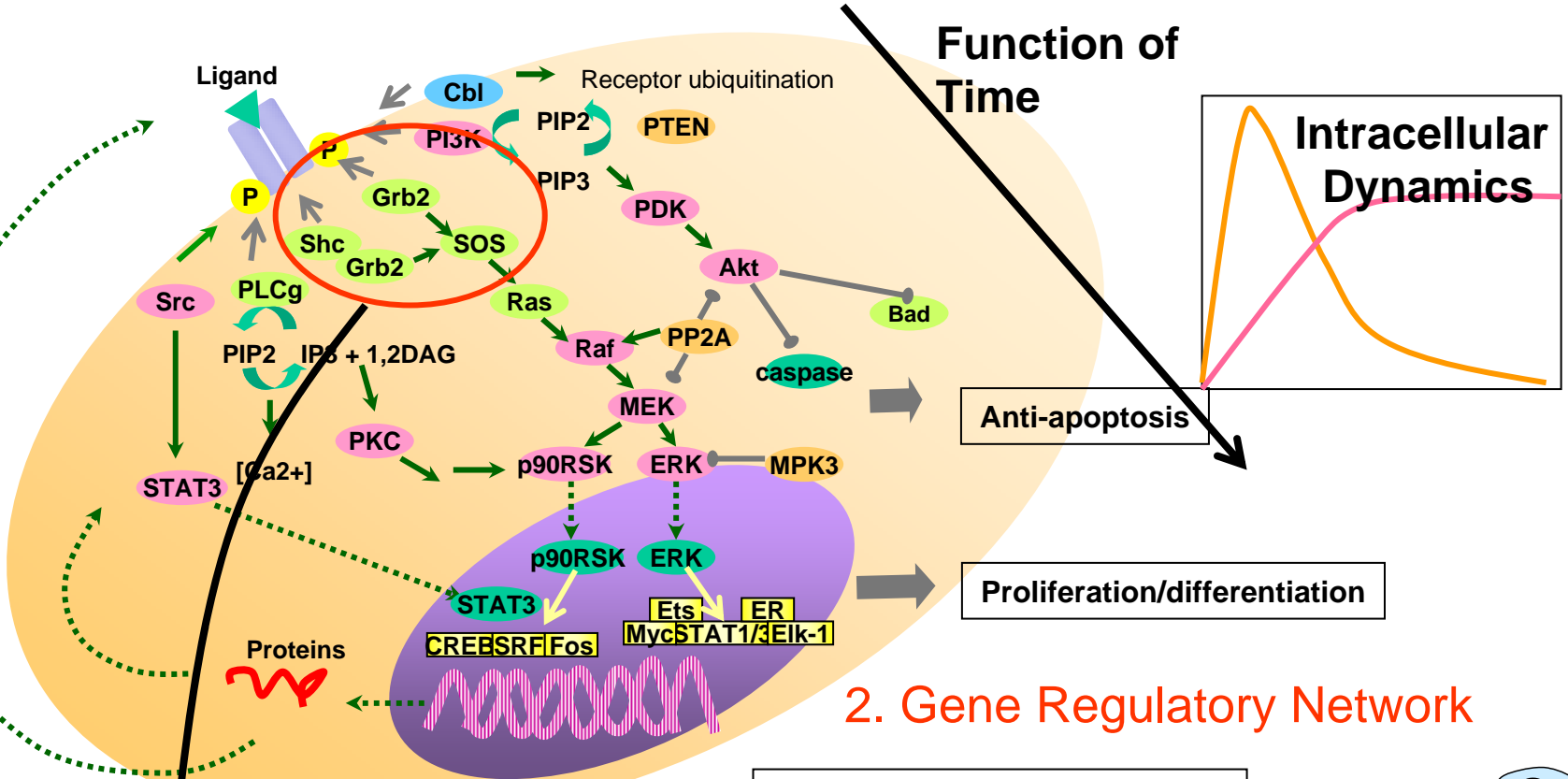
However.....

Signal transduction is transient.

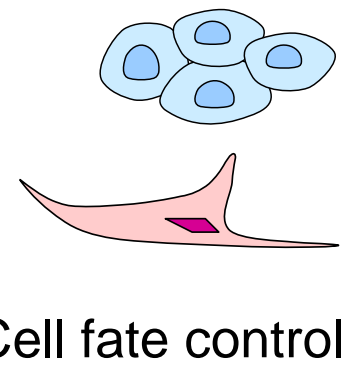
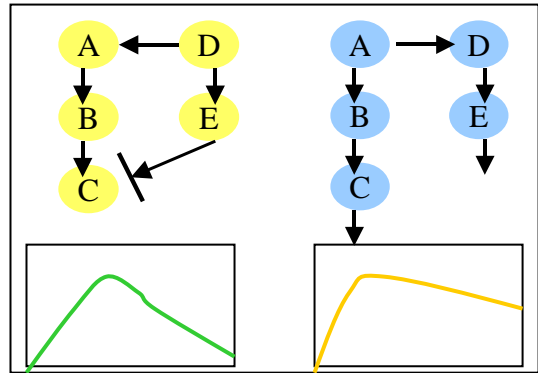
Protein modification, protein-protein interaction, kinase activation, gene expression.

Dynamics, not static, study is essential.

# 1. Kinetic Modeling of ErbB Signaling Network



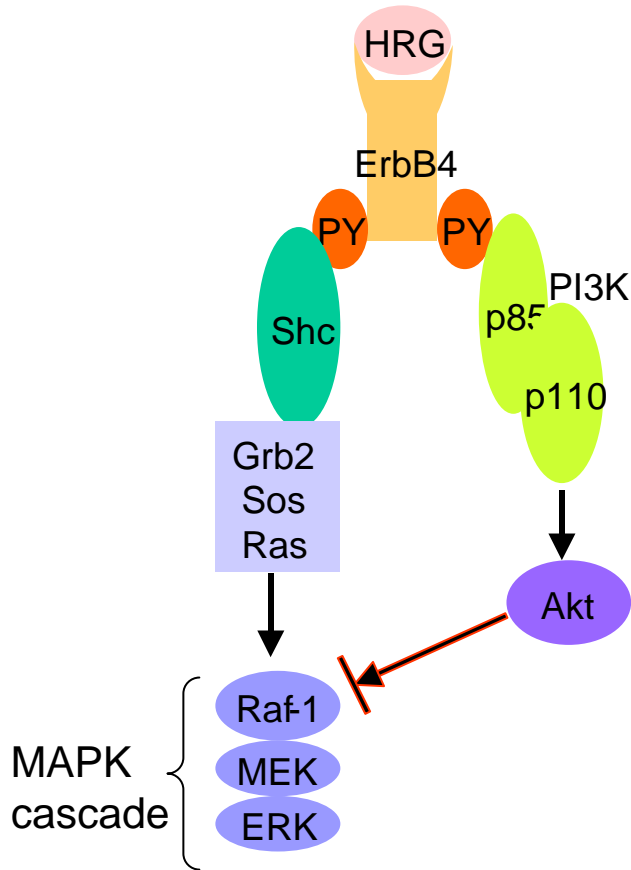
# 2. Gene Regulatory Network



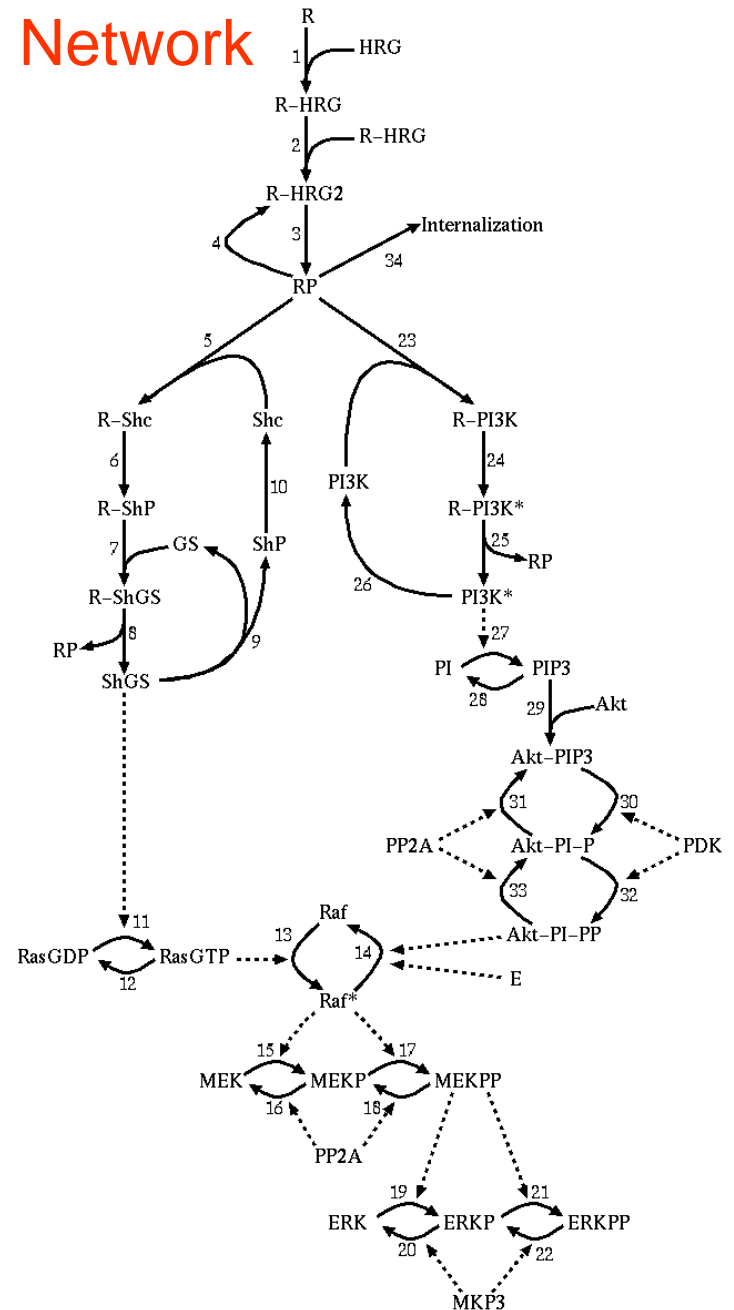
# 3. Application: Molecular Simulation of PPI

# 1. Kinetic Modeling of ErbB Signaling Network

## ErbB4 Model

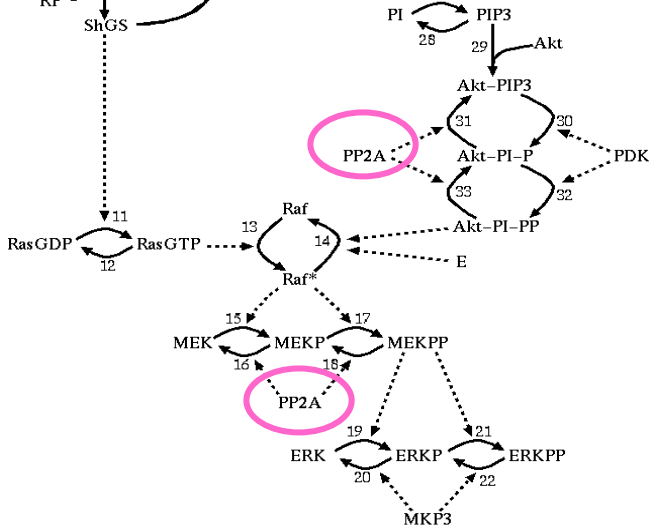


modeling



Experimentally confirmed:  
Heregulin induced-signal transduction pathway  
in ErbB4 expressing CHO cell.

# Effect of PP2A



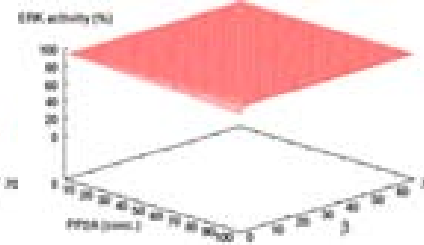
$$K_m = \alpha \times K_{MEK / \text{or Akt}} \text{ (Catalytic activity of PP2A)}$$

$\alpha$  (multiplicative factor)

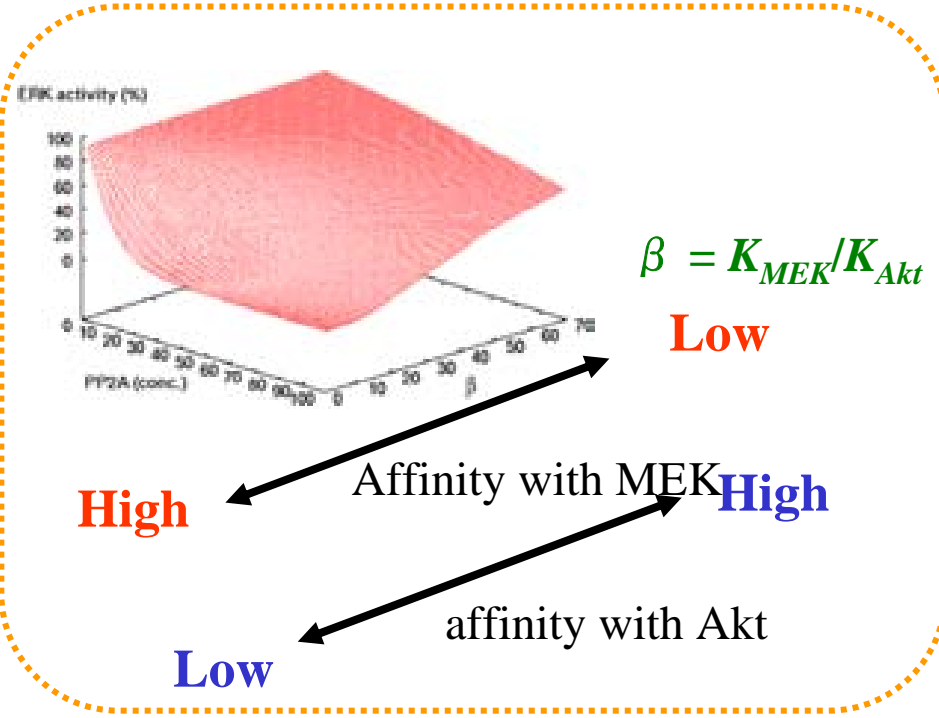
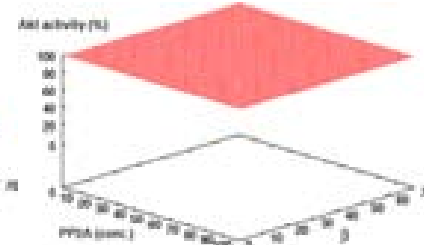
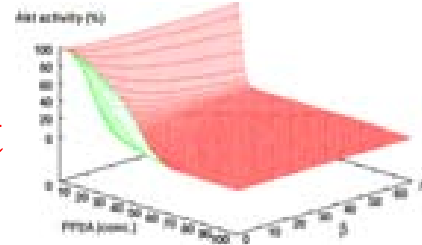
1

$10^4$

ERK



Akt



Low PP2A conc.  
Large  $K_m$

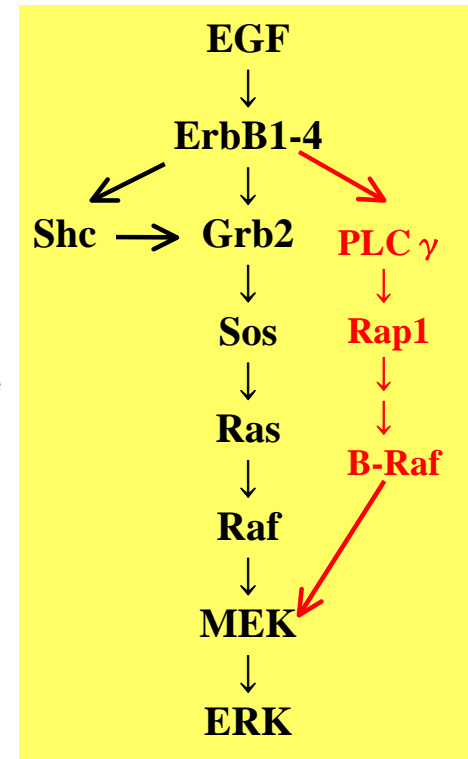


No Raf-Akt cross-talk

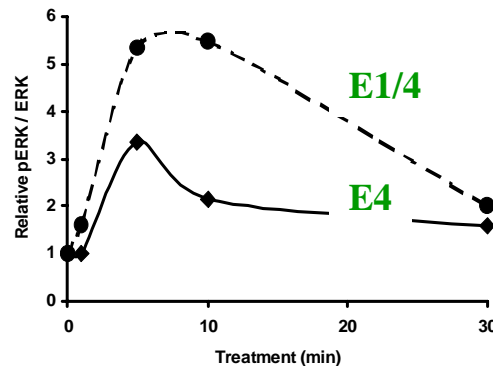
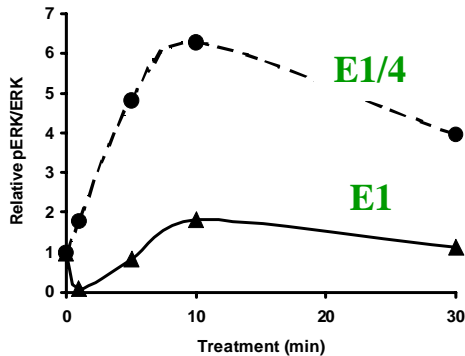
# Leading a hypothesis from simulation: PP2A effect

Ex. Specific B-Raf activation in ErbB1-4 cells

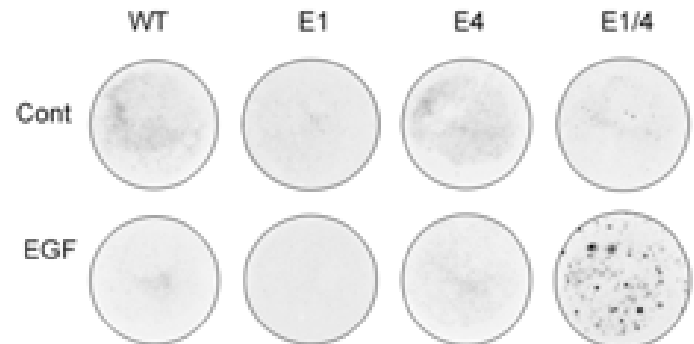
Cell:	E1				E4				E1/4			
EGF	-	+	-	+	-	+	-	+	-	+	-	+
OA	-	-	+	+	-	-	+	+	-	-	+	+



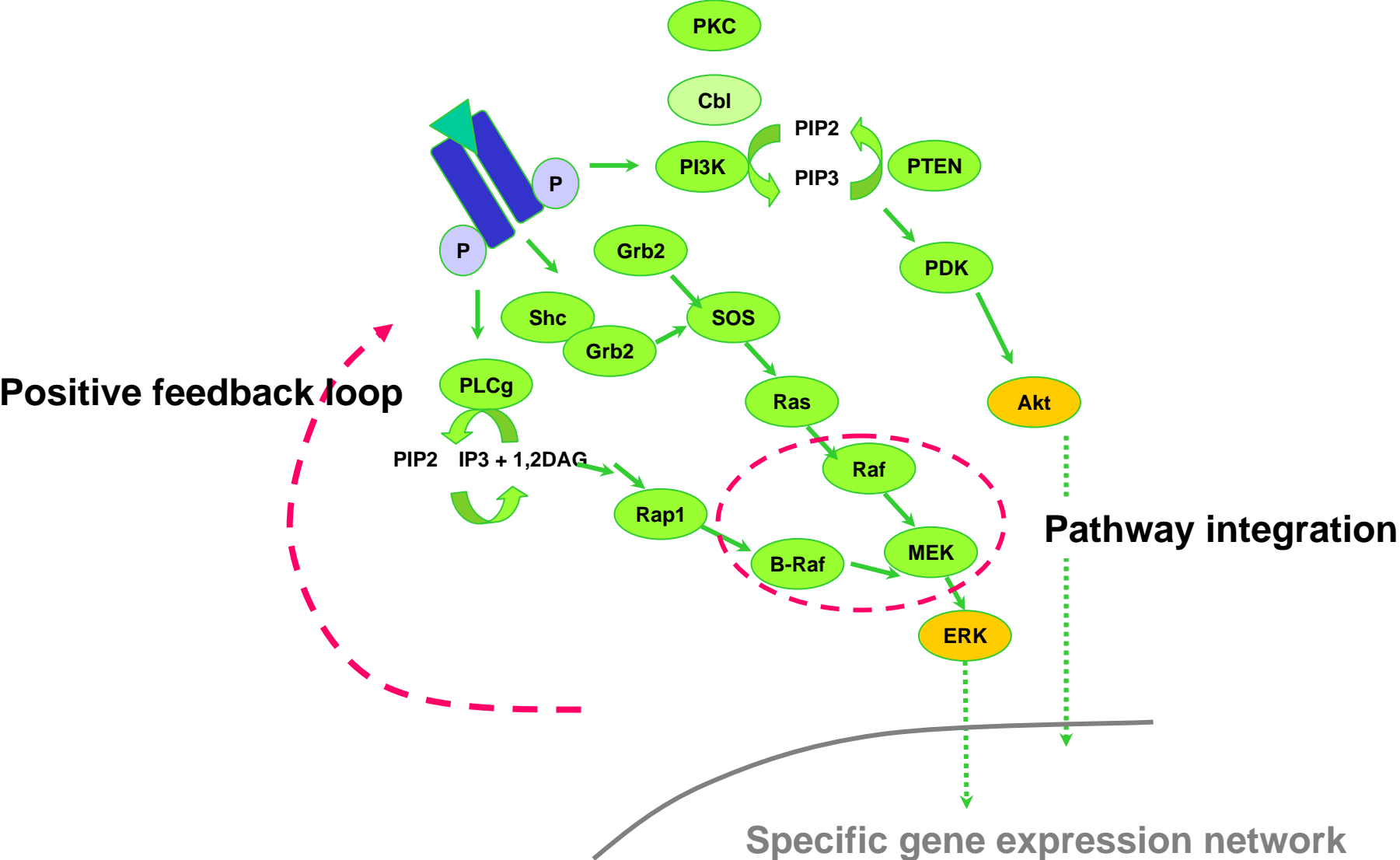
Higher ERK activity in E1/4 cells



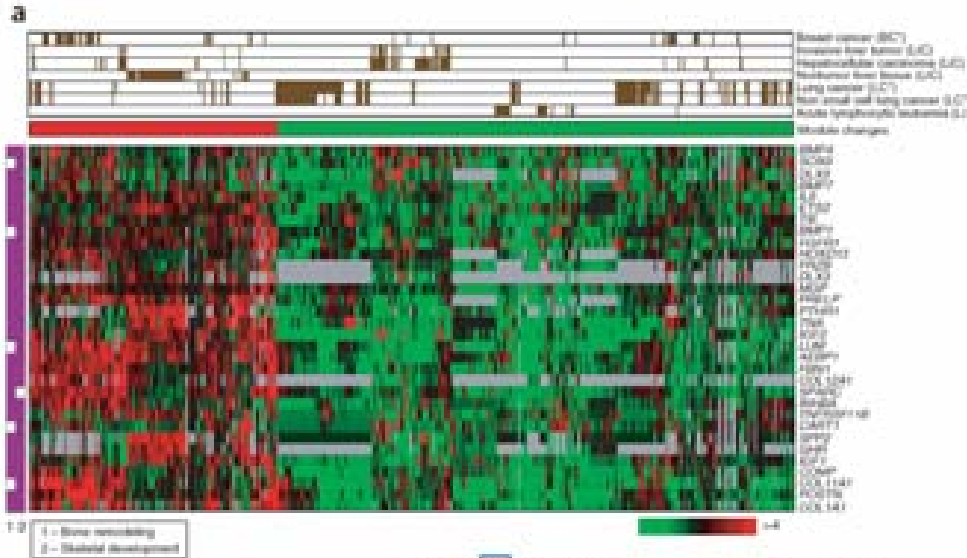
E1/4 cell specific transformation



# Possible signal amplification mechanism in cellular transformation



# Cell-, Condition-specific expression modules



14,145 genes

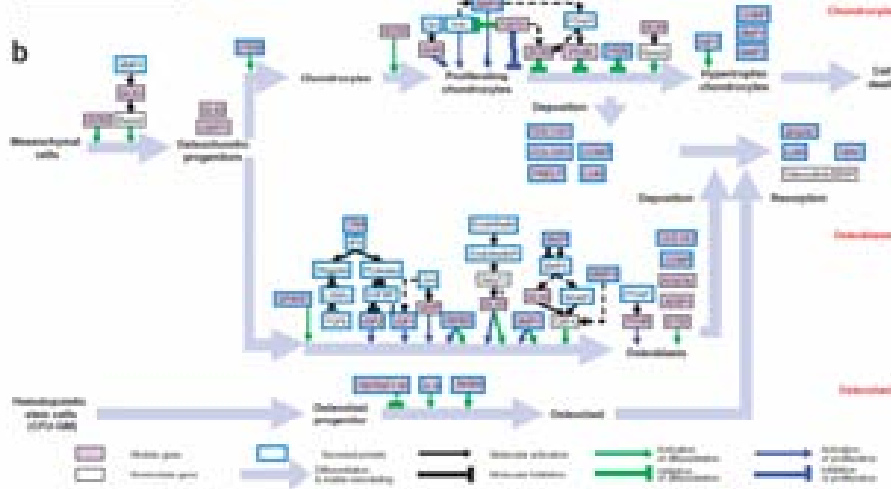
1,975 arrays

- different experimental sets!

22 tumor types



Hierarchical clustering  
 Probability scores  
 Validation analysis  
 Clinical annotations



Cancer specific modules  
 Condition specific modules

However...

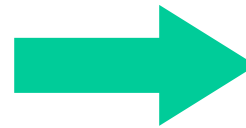
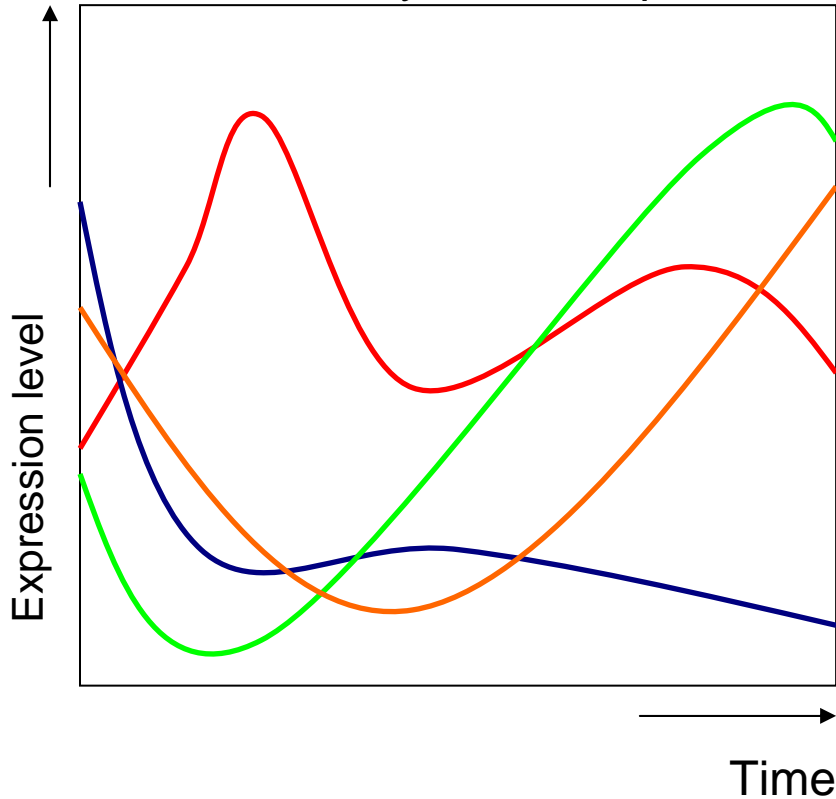
RTK regulates gene expression rapidly.

Dynamic **time-series data** is essential

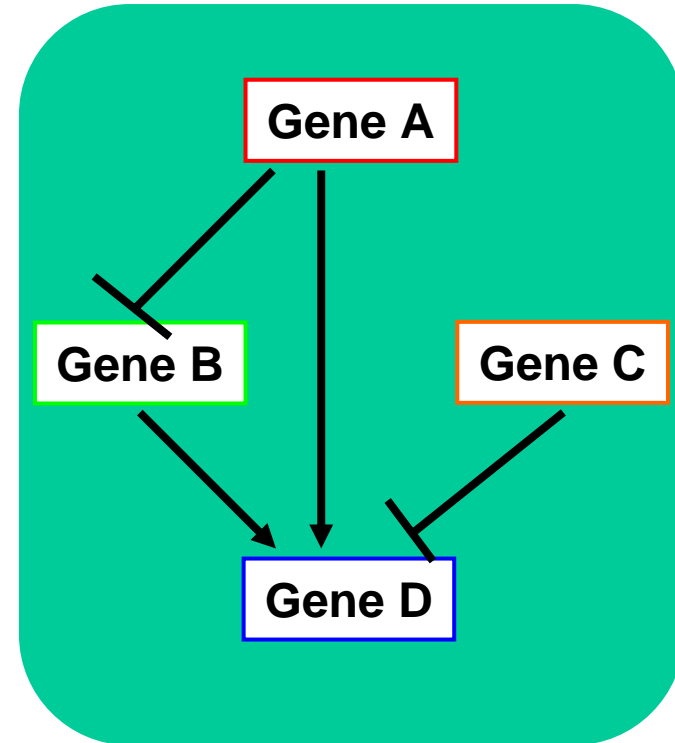
A module map showing conditional activity of expression modules in cancer.

## 2. Gene Regulatory Network

Time course data of mRNA expression  
(Microarray, GeneChip, RT-PCR)

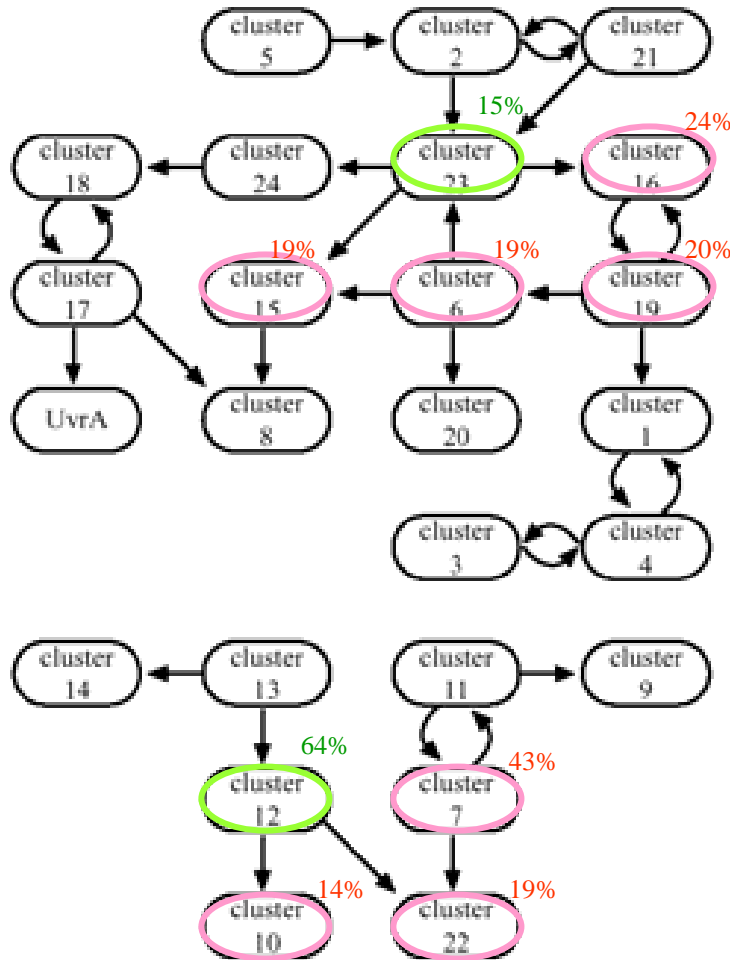


Genetic network  
S-System  
Bayesian network etc



# Prediction of Gene regulatory network

## - A S-system example



612 putative ORF



→ 24 clusters + disrupted gene

14 time points data



Prediction of the regulatory relationship between 25 clusters

Prediction of gene function

-  Cluster containing large # of **Energy metabolism** related genes
-  Cluster containing large # of **unknown function** genes

# Project target

## Identification of cancer cell-specific signaling pathway

- signaling architecture (crosstalk, positive/negative feedbacks)
- dynamics (duration, amplitude, timing)

Relationship and specificity



1. Development of modeling/analytical methods

## Identification of cancer cell-specific gene regulatory network

- Regulatory network modules
- Metagenes



2. Application

Clinical diagnosis  
Pharmaceutical development

Backup from RIKEN

Structure elucidation  
Direct protein interaction  
-Dr. Yokoyama  
Screening (in silico)  
-Dr. Taiji  
Disease model  
etc

# Untangling the wires: A strategy to trace functional interactions in signaling and gene networks

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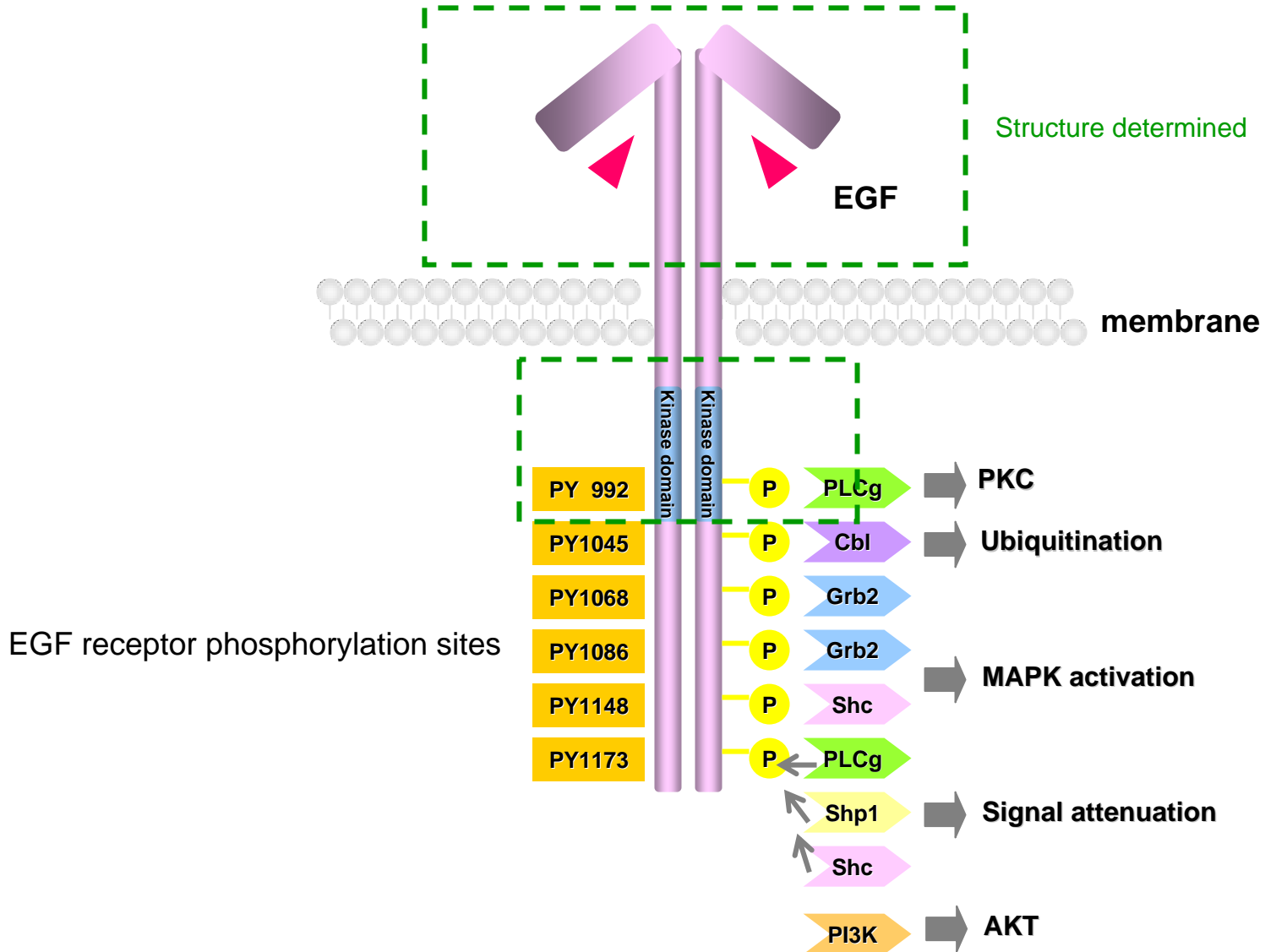
Communicated by Rudolf E. Kalman, Swiss Federal Institute of Technology, Gainesville, FL, July 25, 2002 (received for review March 30, 2002)

Emerging technologies have enabled the acquisition of large genomics and proteomics data sets. However, current methodologies for analysis do not permit interpretation of the data in ways that unravel cellular networking. We propose a quantitative method for determining functional interactions in cellular signaling and gene networks. It can be used to explore cell systems at a mechanistic level or applied within a “modular” framework, which dramatically decreases the number of variables to be assayed. This method is based on a mathematical derivation that demonstrates how the topology and strength of network connections can be retrieved from experimentally measured network responses to successive perturbations of all modules. Importantly, our analysis can reveal functional interactions even when the components of the system are not all known. Under these circumstances, some connections retrieved by the analysis will not be direct but correspond to the interaction routes through unidentified elements. The method is tested and illustrated by using computer-generated responses of a modeled mitogen-activated protein kinase cascade and gene network.

**A**dvances in high-throughput genomics and proteomics analysis facilitate the monitoring of the expression levels of large gene

The daunting challenge of understanding the coordinated behavior of numerous molecular interactions can be facilitated by analyzing them within a “modular” framework (12, 13). A complex cellular network can be divided conceptually into reaction groups referred to as functional units or modules. Each module consists of several signaling or gene interactions and performs one or more identifiable tasks. For instance, each of the three tiers of the mitogen-activated protein kinase (MAPK) cascade can be considered as a functional module that involves unphosphorylated, mono-phosphorylated, and bisphosphorylated forms of a protein kinase and the reactions converting these forms. Modules need not be rigid, and entire MAPK cascades can serve as functional modules in a signaling network that involves growth factor and stress-activated pathways. For gene networks, modules can involve mRNAs of a particular gene or gene cluster with regulatory interaction loops running through metabolic and signaling pathways (14). Modules can be interconnected in multiple ways, many of which may be unknown, even when the network components are identified in genetic and biochemical studies. Fig. 1 illustrates such potential interactions for a three-module cascade and dynamic connections as well as possible unknown components for a gene-

### 3. Molecular Simulation of protein-protein interaction

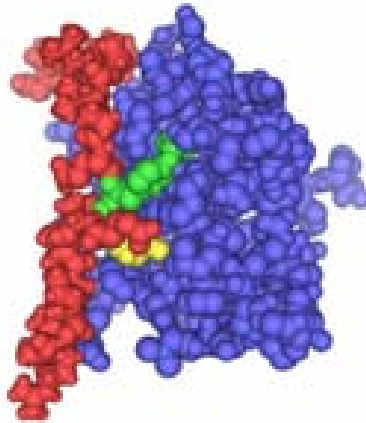


# MD simulation

- prediction of binding kinetics

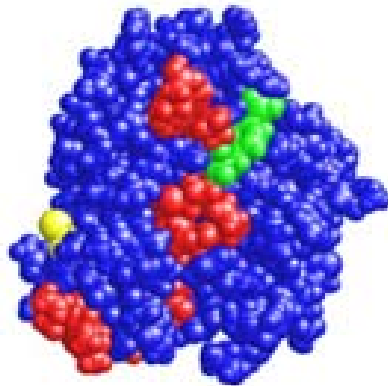
ErbB peptides

-Grb2 SH2



ErbB peptides

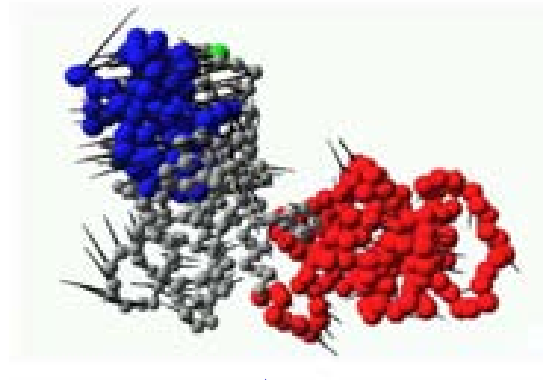
-PI3K p85  
SH2



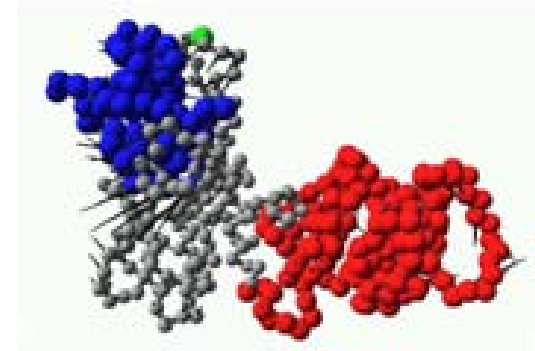
- prediction of domain motion

Shc

Unphosphorylated



pY317 phosphorylated



# Experiment in RIKEN: Cancer cell line (example; NCI60)

## 1. Gene expression analysis

- Affymetrix human array (ORF, tiling array?)
- transcription and translation ?

## 2. Intracellular signal transduction analysis

- western blot / multiplex method

### (1) Growth hormone response

- ligand concentration variations

### (2) Time course (10 min - 72 hrs: >10 points)

### (3) Inhibitor / mutant effect

*Suggestions for gene expression analysis and modeling method!*

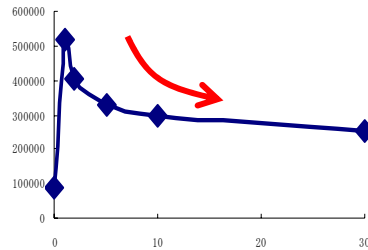
# Ligand variation in signaling dynamics and gene expression in ErbB expressing cancer cells

Differentiation

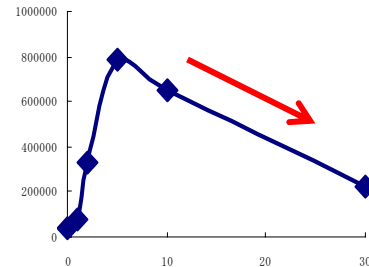
EGF



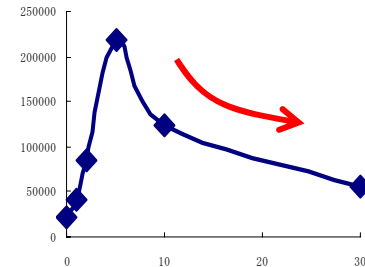
Phospho-receptor



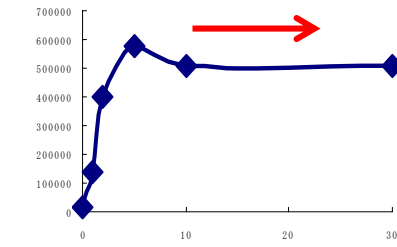
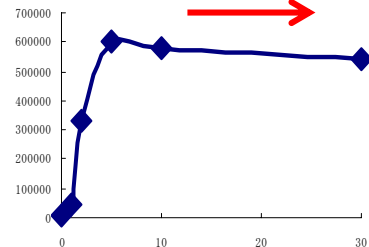
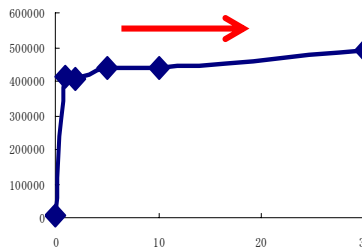
Phospho-ERK



Phospho-Akt



HRG



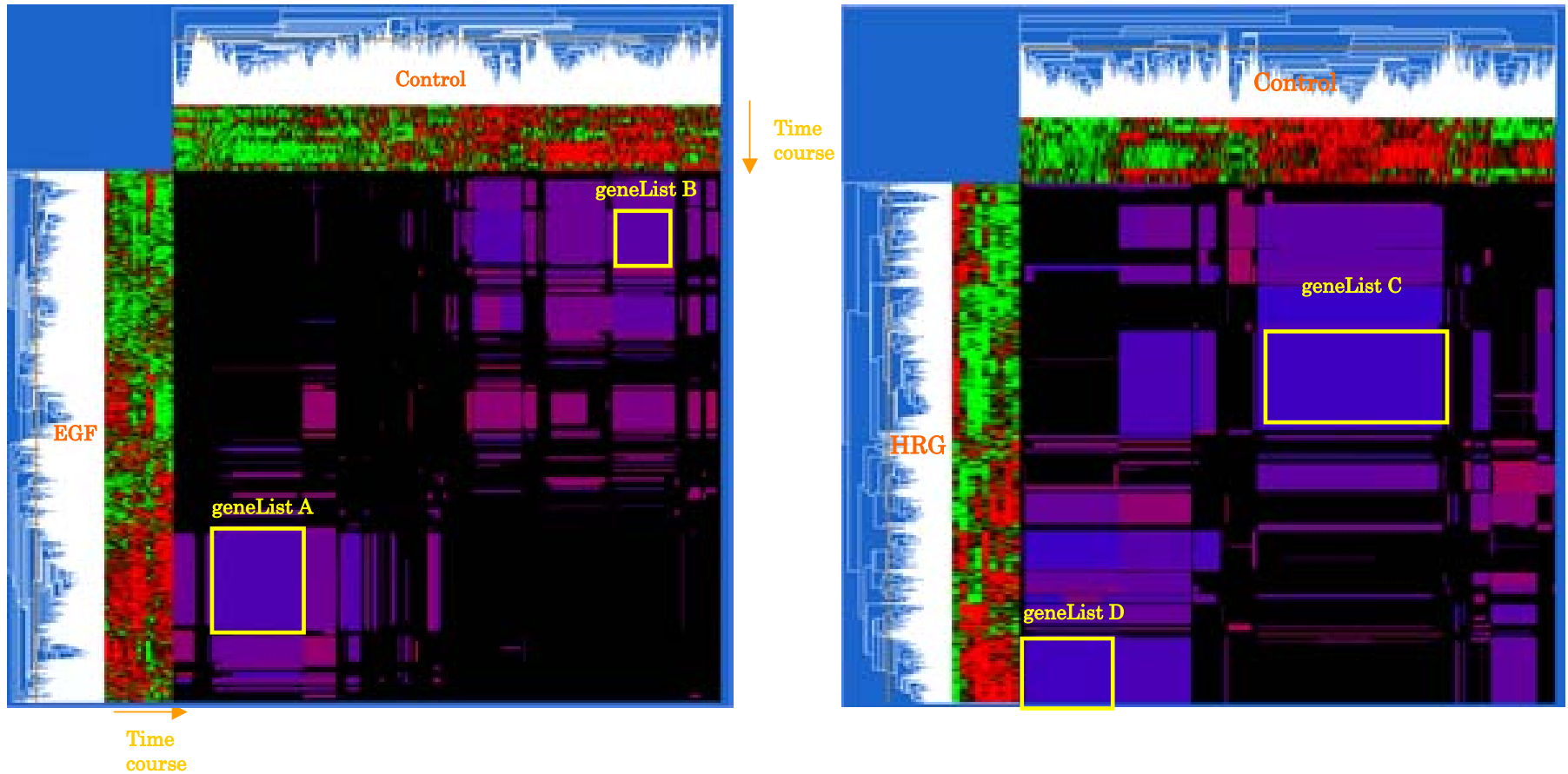
Control



Change in kinetics: induced by different PPI or pathway architectures?  
Signal patterns: on/off switch for specific gene expression?

# Ligand specific gene expression: an example of hierarchical analysis

5 min – 24 hrs time points after ligand administration

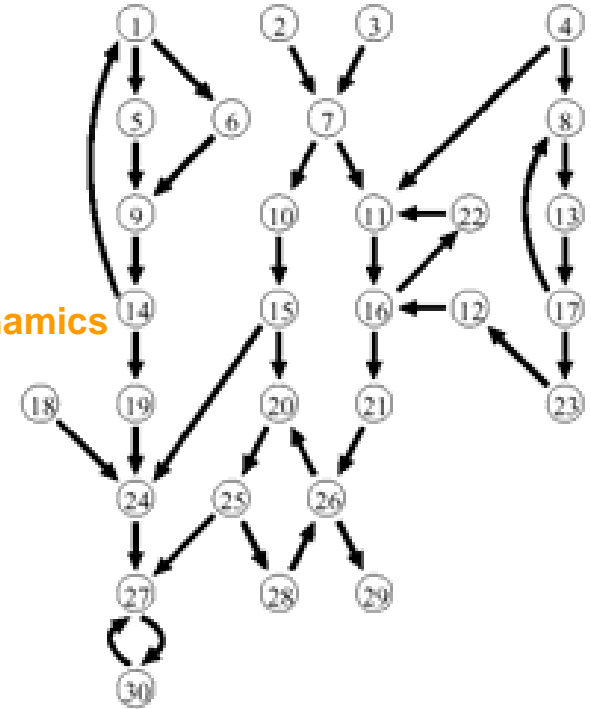
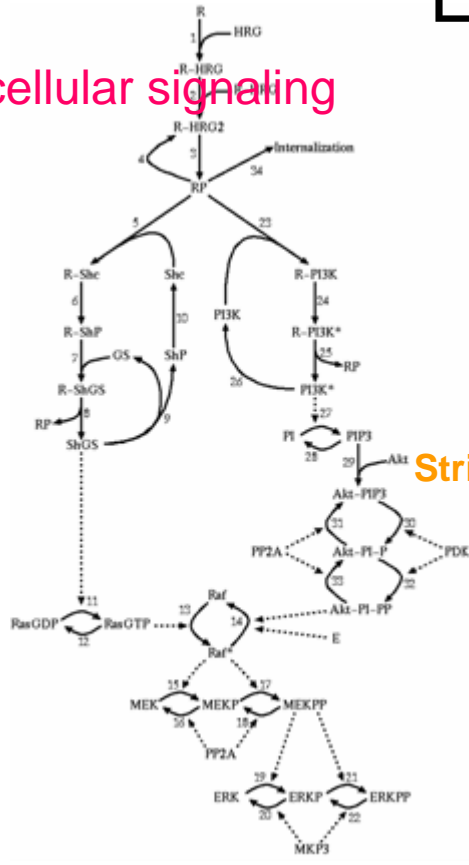


Common or distinctive patterns / network ?

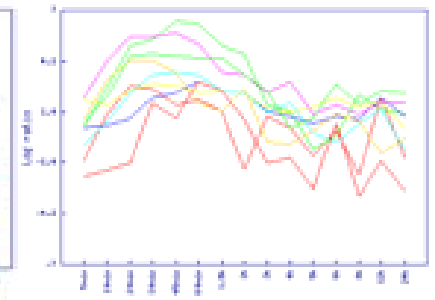
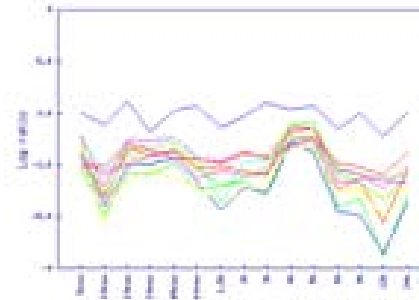
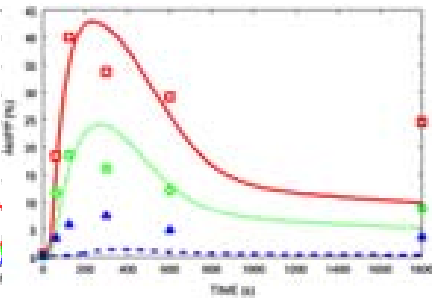
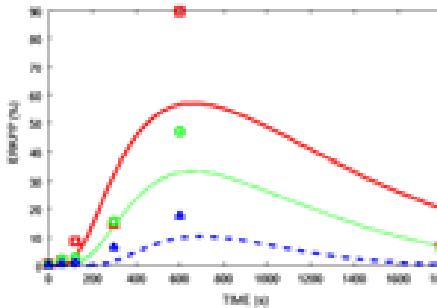
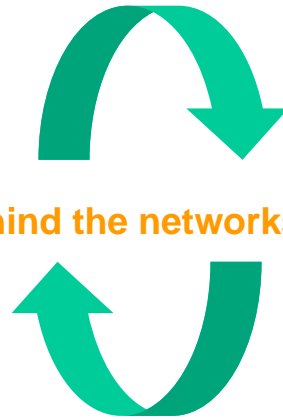
# Linking two networks

Intracellular signaling

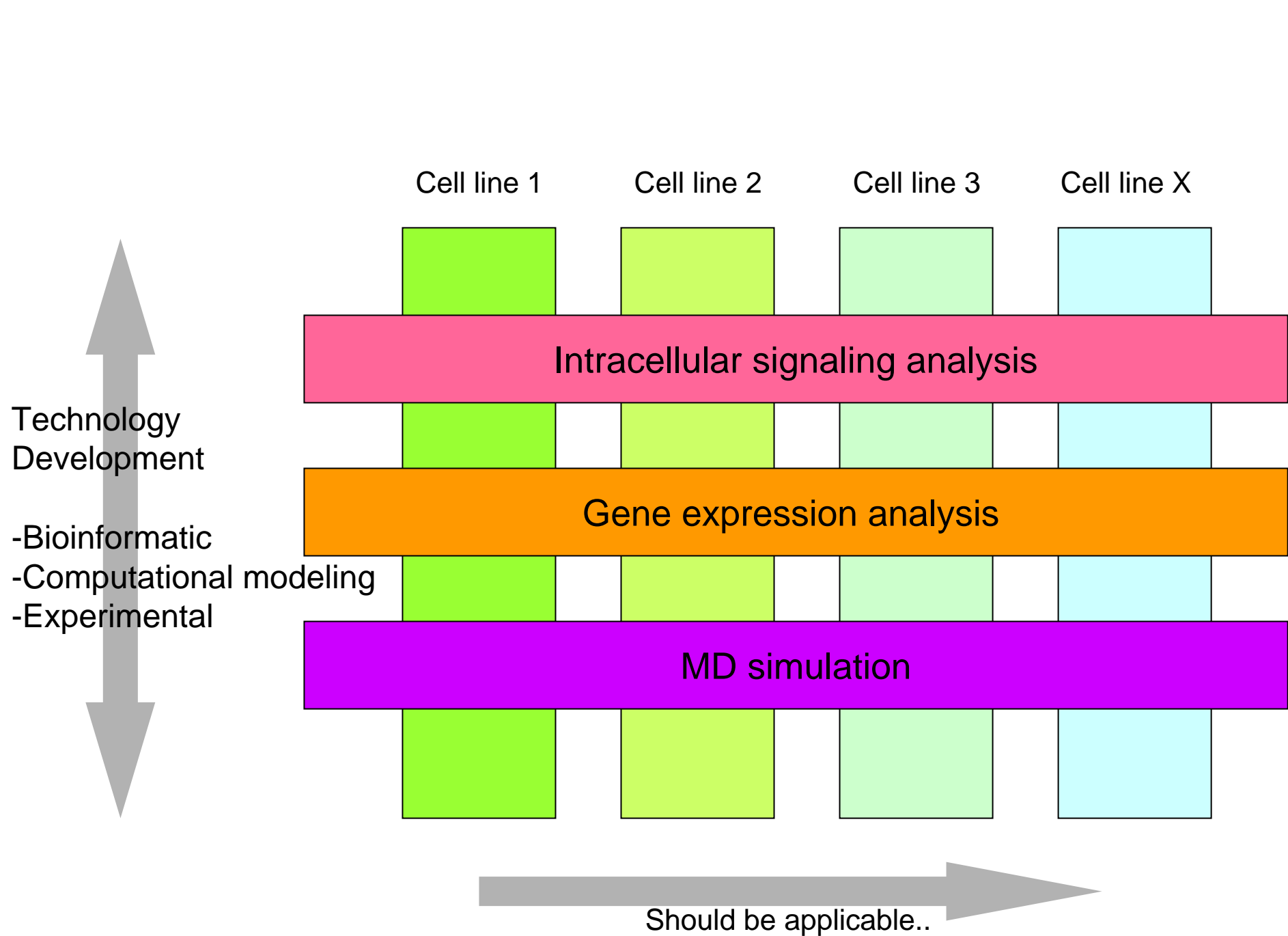
Gene regulatory network



Strings behind the networks / dynamics



two dynamics



We would like to address...

Cancer-cell specific....

- Intracellular signaling dynamics
- Signal amplification mechanism - common architectures?
- Key regulators
- Specific gene expression pattern, network & dynamics

We would like to develop methodologies...

- Gene expression analysis - bioinformatics
- Molecular dynamics simulation for PPI analysis
- HTP PPI, phosphorylation detection methods etc