# SYMBOLIC SYSTEMS BIOLOGY
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# USING FORMAL METHODS TOOLS TO MODEL BIOLOGICAL PROCESSES

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**SRI International**
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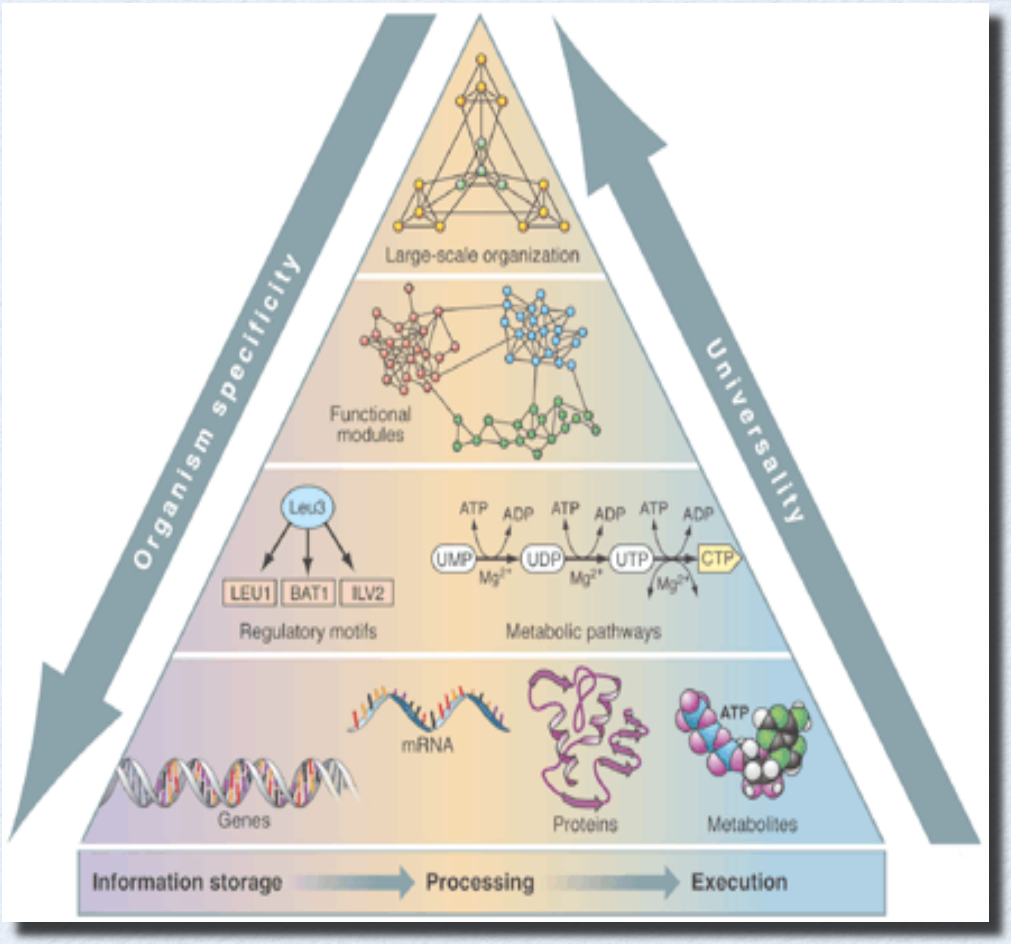
# PLAN

- Symbolic systems biology
- SSB @ SRI
  - BioCyc
  - Hybrid SAL
  - Neuron systems
  - Pathway Logic

# SYMBOLIC SYSTEMS BIOLOGY



# BIOLOGICAL SYSTEMS

- Biological processes are complex
  - signaling, regulation, defense, ....
- Huge dynamics timescales: microseconds to years
- Spatial scales over 12 orders of magnitude
  - single protein to cell to organ to organism ...
- Oceans of experimental biological data generated
- Important intuitions captured in mental models that biologists build of biological processes
- How to build in silico models from all this?

# SYMBOLIC SYSTEMS BIOLOGY

- Symbolic -- represented in a logical framework
- Systems -- how things interact and work together, integration of multiple parts, viewpoints and levels of abstraction
- Goals:
  - Develop formal models that are as close as possible to domain expert's mental models
  - Compute with, analyze and reason about complex networks
  - New insights into / understanding of biological mechanisms

# EXECUTABLE FORMAL MODELS

- Describe system states and rules for change in a formal system
- From an initial state, derive a transition graph
  - nodes -- reachable states
  - edges -- rules connecting states
- Path in transition graph ~ computation/derivation
- Many kinds of analysis available

# SYMBOLIC ANALYSIS I

- Static Analysis
  - how are elements organized -- sort hierarchy
  - control flow / dependencies
  - detection of incompleteness
- Forward simulation from a given state (prototyping)
  - run model using a specific strategy
  - fast, first exploration of a model

# SYMBOLIC ANALYSIS II

- Forward search from a given state
  - breadth first search of transition graph
  - find ALL possible outcomes
  - find only outcomes satisfying a given property
- Backward search from a given state S
  - run a model backwards from S
  - find initial states leading to S
  - find transitions that contribute to reaching S

# SYMBOLIC ANALYSIS III

- Model checking
  - determines if all pathways from a given state satisfy a given property, if not a counter example is returned
  - example property:
    - molecule X is never produced before Y
  - counter example:
    - pathway in which Y is produced after X

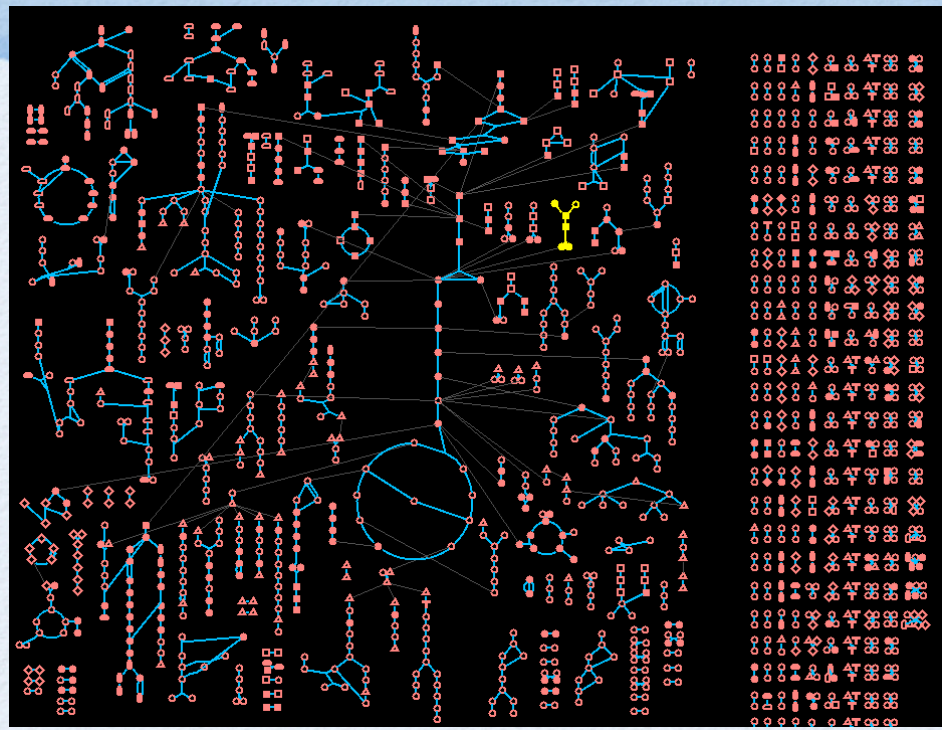
# Symbolic Analysis IV

- Constraint solving
  - Find values for a set of variables satisfying given constraints.
  - MaxSat deals with conflicts
    - weight constraints
    - find solutions that maximize the weight of satisfied constraints
  - Finding possible steady state flows of information or chemicals through a system can be formulated as a constraint problem.

# BIOCYC

http://biocyc.org/

# WHAT IS BIOCYC?



- A collection of Pathway/Genome Databases (PGDBs) databases
  - MetaCyc: reference resource for metabolic pathways
  - EcoCyc: E.Coli, HumanCyc ..
- And a tool suite
  - PathoLogic: to create PGDB from an annotated genome
  - Pathway/Genome Navigator: query, visualization, and analysis of PGDBs.
  - Pathway Editor: for pathway curation

# WHAT IS A PGDB?

- A PGDB for an organism contains
  - genome and gene products
  - metabolic pathways, reactions (elements of pathways),
  - enzymes (that enable reactions), metabolites and transporter complement;
  - the genetic control network:
  - operons, transcription factors;
  - interactions between transcription factors,
  - small-molecule ligands, and DNA binding sites

# DIET PLANNING FOR E.COLI

- Find all potential minimal nutrient sets for E. Coli
  - Start with the metabolic network, and a list of candidate nutrients
  - Define growth conditions as a set of products
  - Represent this information as a constraint system where the variables are reaction fluxes. (Cycles are tricky)
  - Managing complexity
    - Use disjunction to avoid non-linearity
    - Eliminate impossible/useless compounds
- Transform into finding prime implicants of monotone boolean function
- Solve using BDDs and Yices solver

# GOTCHA'S

- There a LOTS of solutions
  - Define a notion of equivalence on compounds and look at only canonical/reduced solutions -- hundreds rather than thousands
  - Side effect -- insights into role of equivalnce classes
- Unexpected solutions: Why does the bug grow on CO2?
- This is a great way to debug a reaction network!
- We are getting close to a fixed point.

# HYBRID SAL

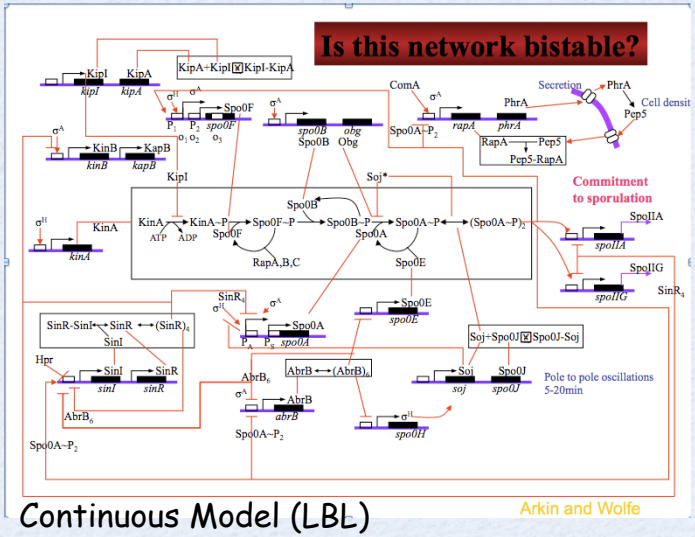
# HYBRID SYSTEMS

- Hybrid systems combine discrete and continuous variables

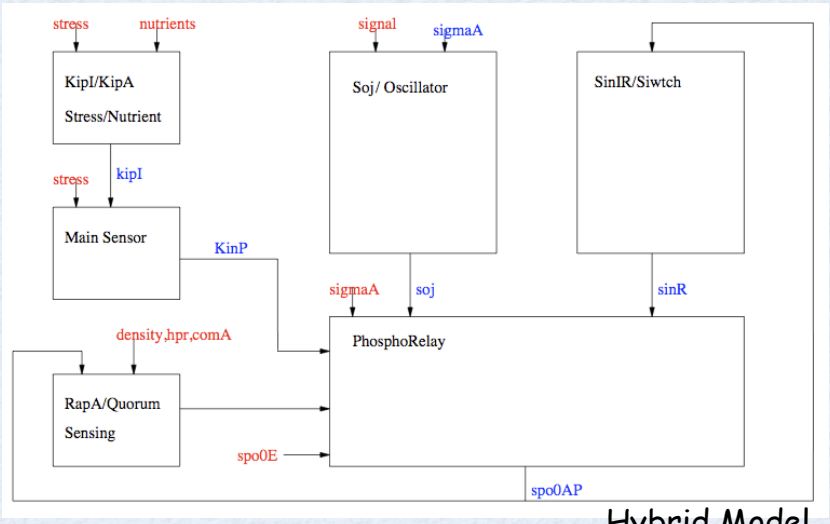
- Hybrid SAL -- hybrid systems modeling language and tools
  - sound simplification and abstraction techniques
  - unknowns represented as parameters with logical constraints
  - model checking for finite state abstractions

# B. SUBTILIS SPORULATION

- Initiated when environment not conducive to growth
- Regulated by transcriptional network
- Hybrid model developed to study bistability



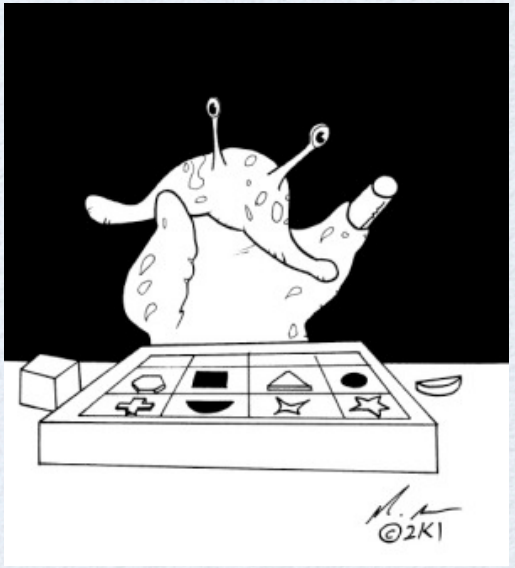

Continuous Model (LBL)




Hybrid Model

- effect of promoters and inhibitors -- discrete transitions
- unknown rate constants --- constrained parameters
- determined to be bistable for range of parameters
- bistability properties sensitive to discrete logic
- needed to refine transcriptional logic for Spo0A

# Neuron Systems: Aplysia CPG Model




- Aplysia is a sea slug often used as model to study neuron systems.
- Its neurons are large
- There are relatively few types
- It exhibits simple but interesting behaviors (biting, learning, memory) that can be correlated to neuron modules / programs

# Modeling Approaches

1. Traditional: Hodgkin Huxeley model
   - differential equations describing different ion channels
2. Simple neuron model proposed by Iz a vich
   - based on a dynamical systems view.
   - 4 parameters -- many spiking patterns
3. Automata model -- further abstraction to capture key features of the 4 parameter systems.

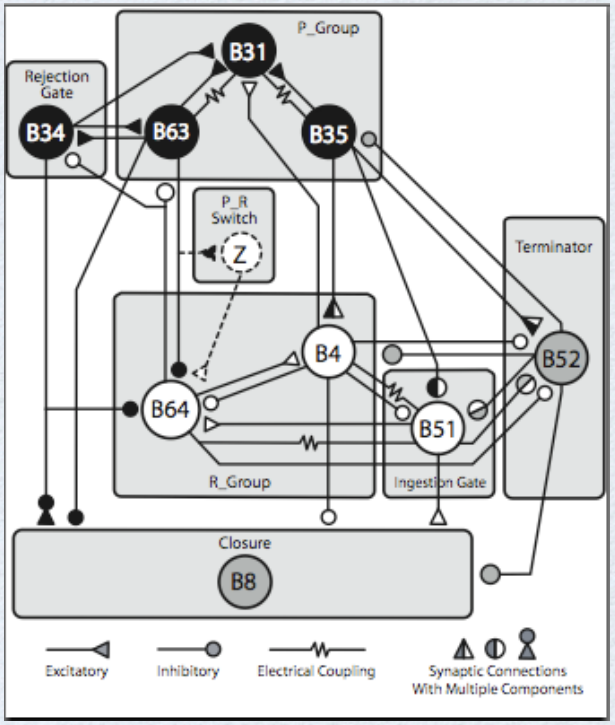
- 1-2 are analyzed by simulation
- 3 we can analyze by model-checking!

# SAL Model of Aplysia CPG

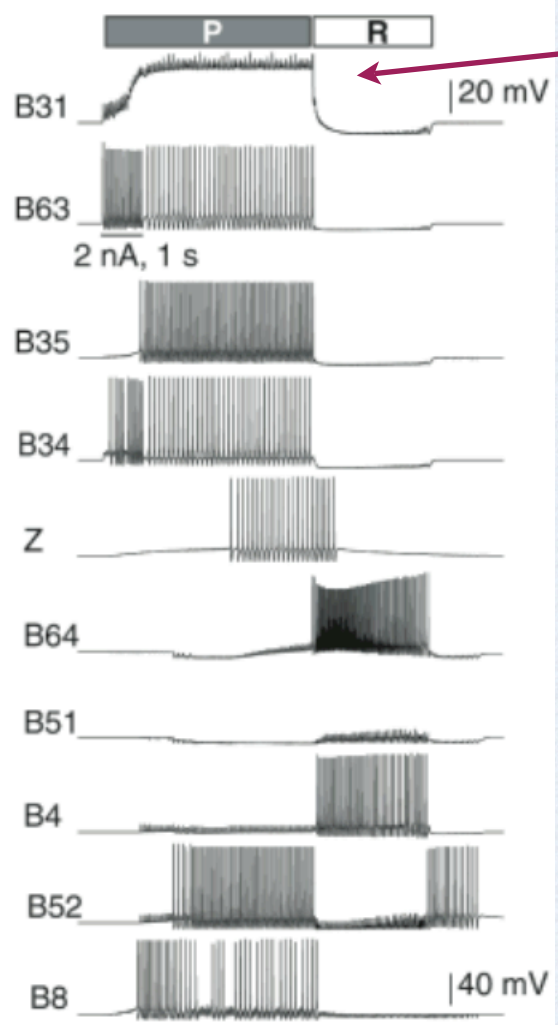
aplysia: MODULE = aplysia_wiring || aplysia_neurons || observer

```
generic[n: NEURONS]: MODULE = BEGIN
  INPUT i: SIGS
  OUTPUT o: BOOLEAN, level: LEVELS
  LOCAL sens: [1 .. 2], pir: BOOLEAN
  INITIALIZATION
     pir = FALSE; level = 0
  DEFINITION
     sens = IF (n = Z) THEN 1 ELSE 2 ENDIF;
     o = (level = N)
  TRANSITION
     [
     FIRE: level = N AND GNEURONS(n) AND NOT(pir) -->
             level' = N - 2*sens
     []
     IPII: level < N AND i' = pos -->
             level' = IF (level > N - sens) THEN N ELSE level + sens ENDIF
     []
     INII: (NOT(GNEURONS(n)) OR level < N) AND i' = neg -->
             level' = IF (level > 1) THEN level - 2 ELSE 0 ENDIF
     []
     SPIR: n = B52 AND level = 0 AND i = neg AND i' = neg -->
             pir' = TRUE; level' = N
     []
     ELSE -->
     ]
END;
```

```
aplysia_neurons: MODULE =
   (WITH INPUT ins: ARRAY NEURONS OF SIGS
    WITH OUTPUT outs: ARRAY NEURONS OF BOOLEAN
    WITH OUTPUT levels: ARRAY NEURONS OF LEVELS
      (|| (n: NEURONS): (RENAME o to outs[n], i to ins[n],
                                level to levels[n] IN generic[n])));
```




Biologists cartoon model of CPG: neurons that control biting response




Spiking patterns observed during protraction (P) and retraction (R) phases

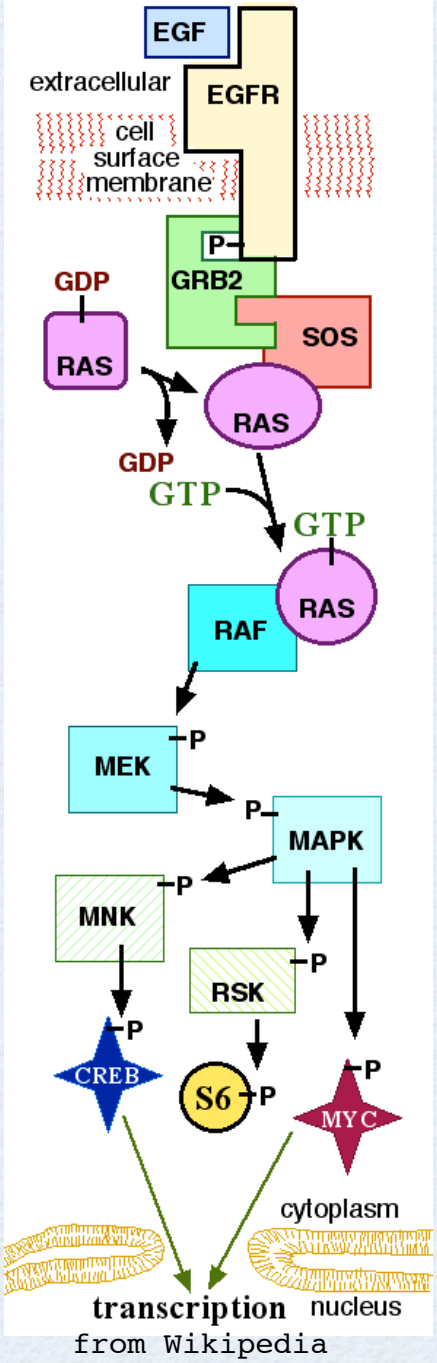
- Key features are represented as LTL formulae and model checked
  - Example P1: B31 (eventually) reaches a plateau and stays there all through the protraction phase and until the start of retraction.
  - aplysia |= F(levels[B31]=N AND U(levels[B31] >= N-1, phase=retraction));
- Model checking of similar properties was use to tune and validate the SAL model.

# Pathway Logic (PL) Representation of Signaling

http://pl.csl.sri.com/

# SIGNALING PATHWAYS

- Signaling pathways involve the modification and/or assembly of proteins and other molecules within cellular compartments into complexes that coordinate and regulate the flow of information.
- Signaling pathways are distributed in networks having stimulatory (positive) and inhibitory (negative) feedback loops, and other concurrent interactions to ensure that signals are propagated and interpreted appropriately in a particular cell or tissue.
- Signaling networks are robust and adaptive, in part because of combinatorial complex formation (several building blocks for forming the same type of complex), redundant pathways, and feedback loops.




# Egf stimulation of the Mitogen Activated Protein Kinase (MAPK) pathway.

Egf → EgfR → Grb2 → Sos1 → Ras → Raf1 → Mek → Erk

- Egf (EGF) binds to the Egf receptor (EgfR) and stimulates its protein tyrosine kinase activity to cause autophosphorylation, thus activating EgfR.
- The adaptor protein Grb2 (GRB2) and the guanine nucleotide exchange factor Sos1 (SOS) are recruited to the membrane, binding to EgfR.
- The EgfR complex activates a Ras family GTPase
- Activated Ras activates Raf1, a member of the RAF serine/threonine protein kinase family.
- Raf1 activates the protein kinase Mek (MEK), which then activates Erk (MAPK)
- ...

# REWRITING LOGIC

- Rewriting Logic is a logical formalism that is based on two simple ideas
  - states of a system are represented as elements of an algebraic data type
  - the behavior of a system is given by local transitions between states described by rewrite rules
- It is a logic for executable specification and analysis of software systems, that may be concurrent, distributed, or even mobile.
- It is also a (meta) logic for specifying and reasoning about formal systems, including itself (reflection!)

# ABOUT PATHWAY LOGIC

Pathway Logic (PL) is an approach to modeling biological processes as executable formal specifications (in Maude)

The resulting models can be queried

- using formal methods tools: given an initial state
  - execute --- find some pathway
  - search --- find all reachable states satisfying a given property
  - model-check --- find a pathway satisfying a temporal formula
- using reflection
  - find all rules that use / produce X (for example, activated Rac)
  - find rules down stream of a given rule or component
  - translate to alternative formalism and export

# PATHWAY LOGIC ORGANIZATION

A Pathway Logic (PL) system has four parts

- Theops --- sorts and operations
- Components --- specific proteins, chemicals ...
- Rules --- signal transduction reactions
- Dishes --- candidate initial states

Knowledge base: Theops + Components + Rules

Equational part: Theops + Components

A PL cell signaling model is generated from

- a knowledge base
- a dish

# RULE 1: RECEPTOR BINDING

If a dish contains an EgfR ligand (?ErbB1L:ErbB1L) outside a cell with EgfR in the cell membrane then the ligand binds to exterior part of the receptor and the receptor is activated.

```
rl[1.EgfR.act]:
 ?ErbB1L:ErbB1L  [CellType:CellType | ct {CLm | clm EgfR}]
  =>
 [CellType:CellType | ct {CLm | clm ([EgfR - act] : ?ErbB1L:ErbB1L)} ] .
```

Rule 1 applies to rasDish

```
 PD(Egf [Cell | {CLm | EgfR PIP2}{CLi | [Hras - GDP] Src}
                {CLc | Gab1 Grb2 Pi3k Plcg Sos1}])
```

with the match

```
  ?ErbB1L:ErbB1L := Egf
  clm := PIP2
  ct := {CLi | [Hras - GDP]  Src} {CLc | Gab1 Grb2 Pi3k Plcg Sos1}
```

giving rasDish1

```
  PD([Cell |
     {CLm | ([EgfR - act] : Egf)  PIP2}
     {CLi | [Hras - GDP]  Src}
     {CLc | Gab1 Grb2 Pi3k Plcg Sos1}]) .
```

# HOW DO WE INFER RULES?

Rules are inferred from evidence, called datums, curated from the literature.

Consider the rule converting Rala-GDP to Rala-GTP in response to Egf stimulation

```
rl[1064.Rala.irt.Egf]:
{EgfRC | EgfR -act : Egf Pi3k RalGds clm }
{CLi | cli [Rala - GDP]}
=>
{EgfRC | {EgfR - act} : Egf Pi3k RalGds [Rala - GTP] clm }
{CLi | cli } .
```

Evidence for this rule includes:

```
DID#05387:   Rala[Ab] GTP[BD-PD] is increased irt Egf (tnr)
cells: Cos7 in BMLS
inhibited by: Wortmannnin [chem]  --- Pi3k Inhibitor
inhibited by: LY294002 [chem]          ""
source: 15034142-Fig-5c

DID#12876:  xRala[xAb]IP GTP/GDP[32Pi-TLC] is increased irt Egf
cells: Cos1-xRalGds in BMS
times: 0 1+ 2++ 3++ 4+ 5 min
reqs: xRalGds [omission]
inhibited by: xRalGds-C203S "membrane-binding-mutant" [substitution]
comment: cells were pretreated with Vanadate 30 min before Egf treatment
source: 9416833-Fig-2
```

# THE PATHWAY LOGIC ASSISTANT (PLA)

# The Pathway Logic Assistant (PLA)

- Provides a means to interact with a PL model
- Manages multiple representations
  - Maude module (logical representation)
  - PetriNet (process representation for efficient query)
  - Graph (for interactive visualization)
- Exports Representations to other tools
  - Lola (and SAL model checkers)
  - Dot -- graph layout
  - JLambda (interactive visualization, Java side)

# rasNet

Hras activated

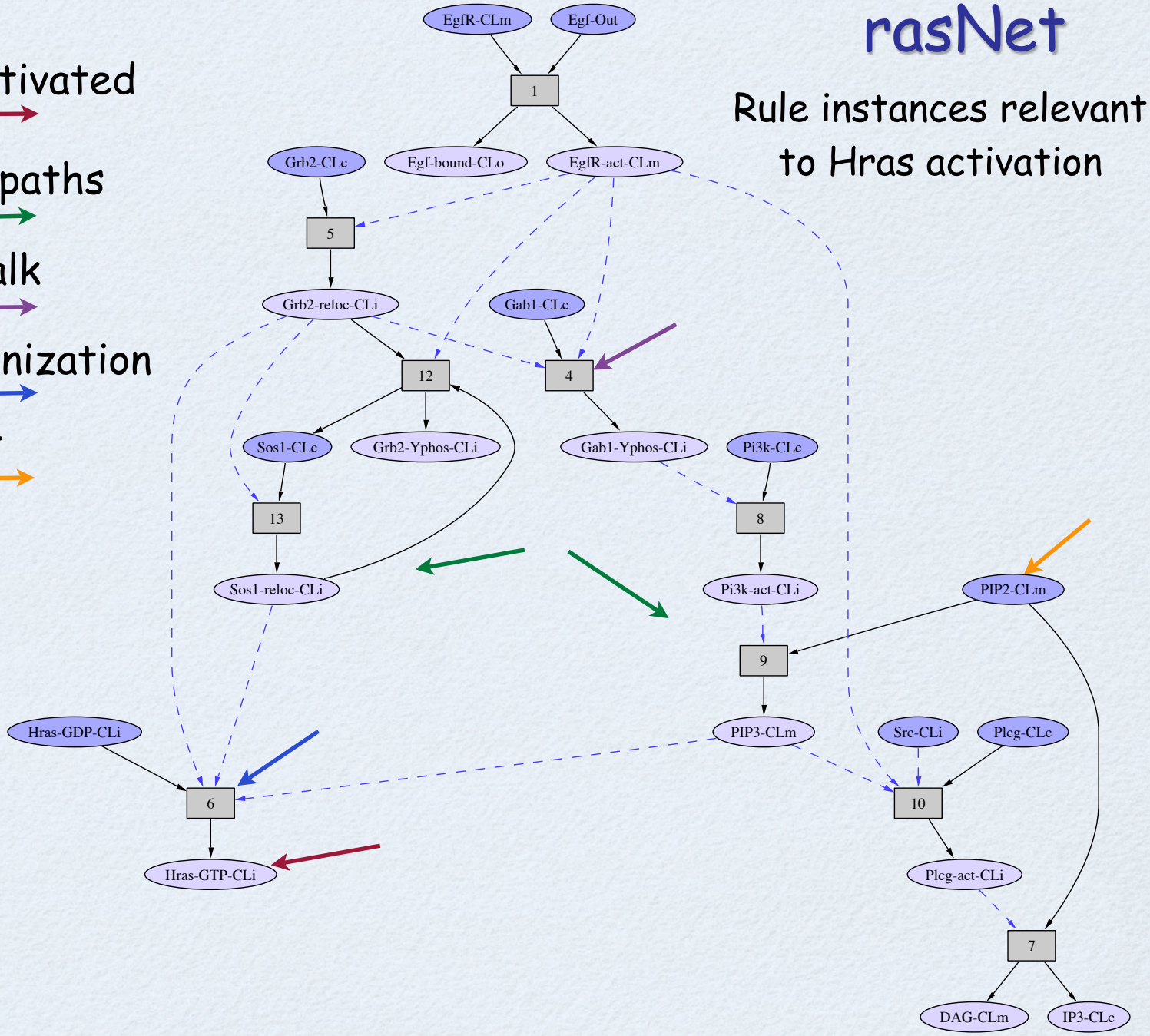
Parallel paths

Cross talk

Synchronization

Conflict

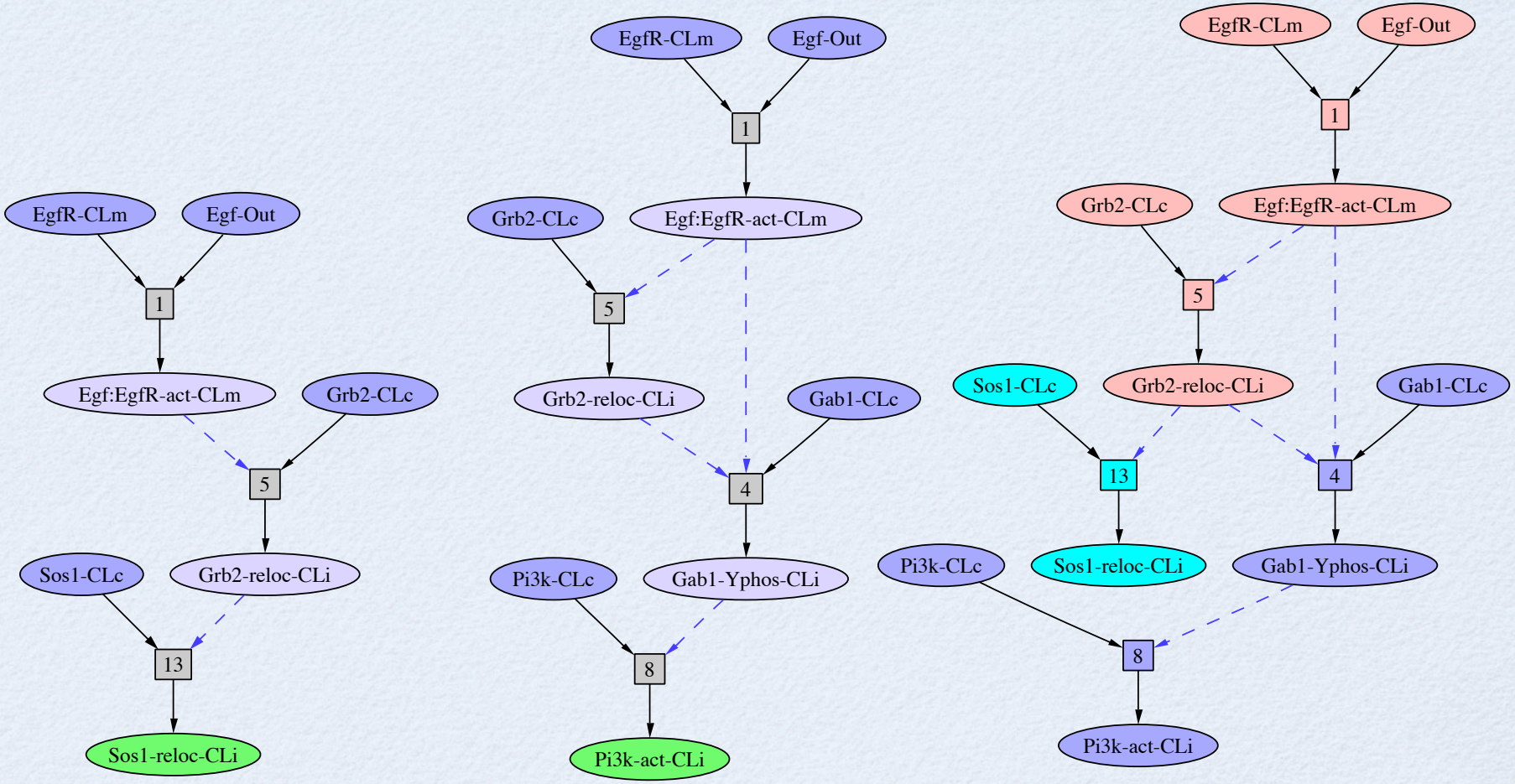
Rule instances relevant to Hras activation



# A SIMPLE QUERY LANGUAGE

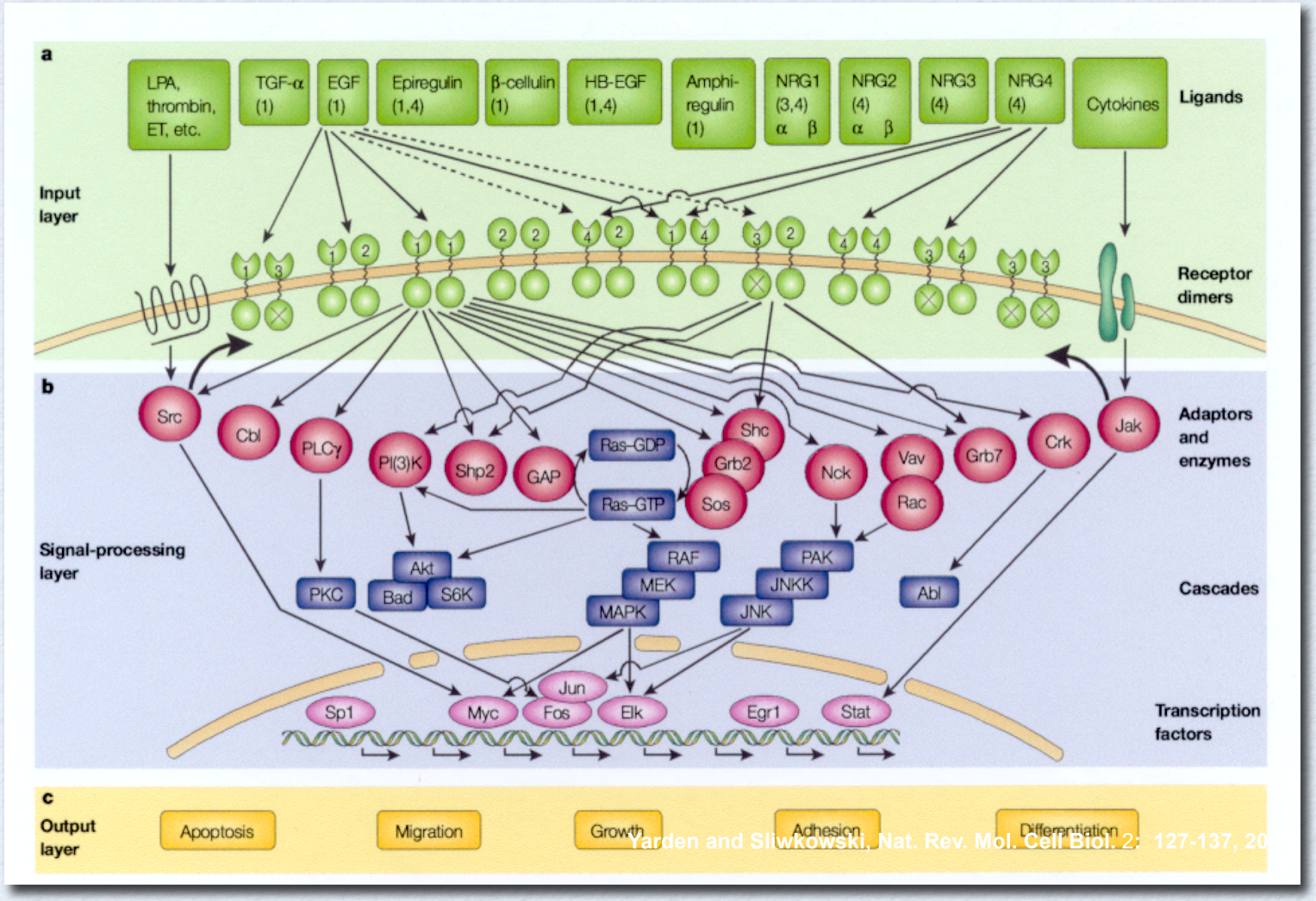
- Given a Petri net with transitions P and initial marking O (for occurrences) there are two types of query
  - subnet
  - findPath - a computation / unfolding
- For each type there are three parameters
  - G: a goal set---occurrences required to be present at the end of a path
  - A: an avoid set---occurrences that must not appear in any transition fired
  - H: as list of identifiers of transitions that must not be fired
- subnet returns a subnet containing all (minimal) such pathways (using backward and forward collection)
- findPath returns a pathway (transition list) generating a computation satisfying the requiremments (using model checking on the negation).

# PATHWAYS IN RAS NET



# FULL MODEL OF EGF STIMULATION

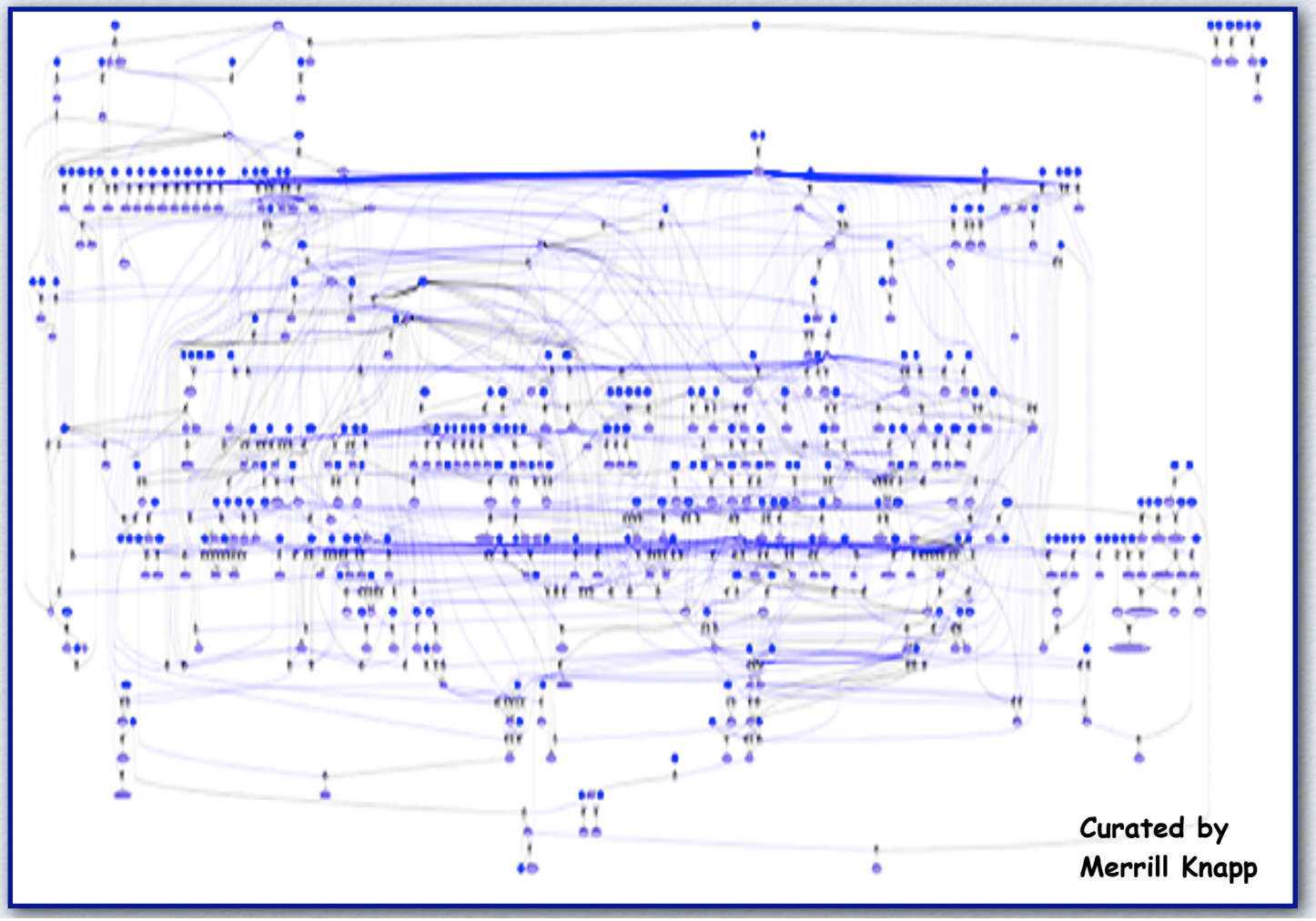
# THE ERBB NETWORK (CARTOON FORM)




Yarden and Sliwkowski, Nat. Rev. Mol. Cell Biol. 2: 127-137, 20

# PL EGF MODEL

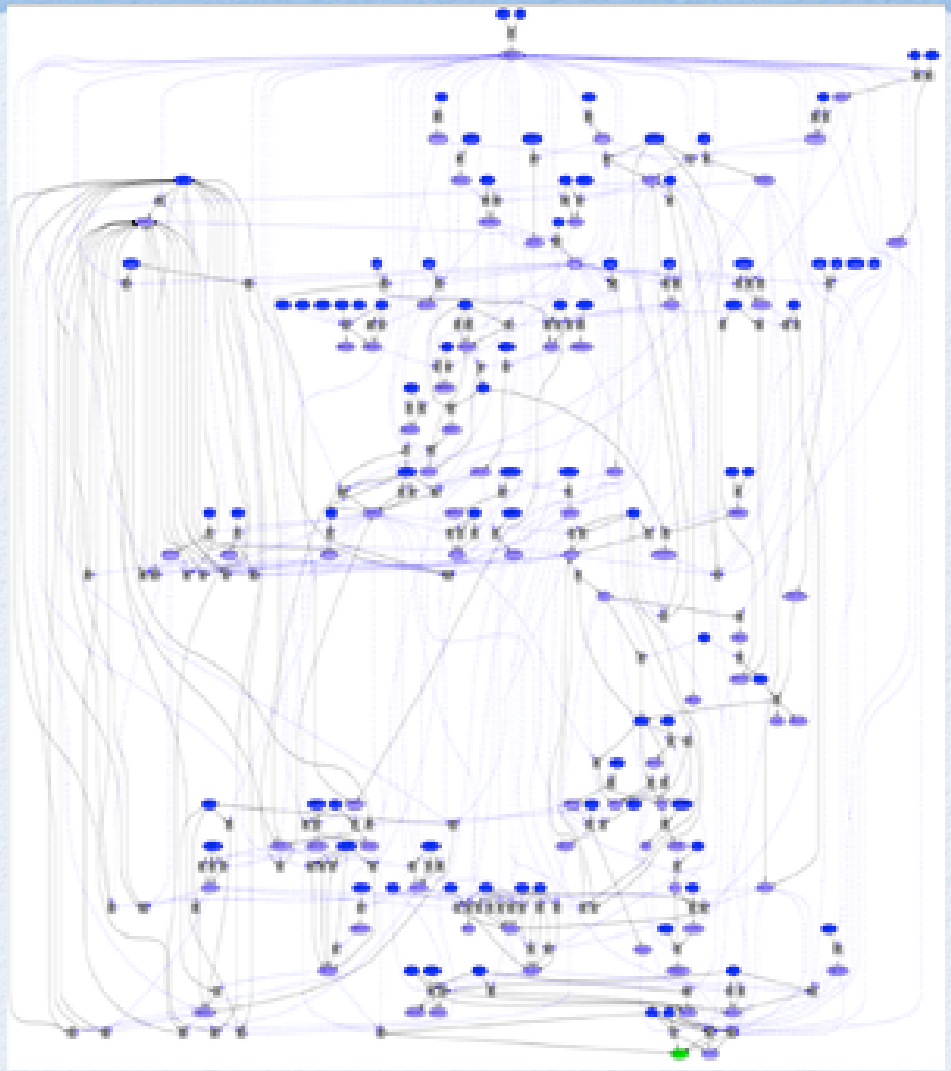
Events that could occur in response to Egf



# SUBNET RELEVANT TO ERK ACTIVATION

Subnet containing all pathways leading to activation of Erk.

Obtained by backwards followed by forwards collection

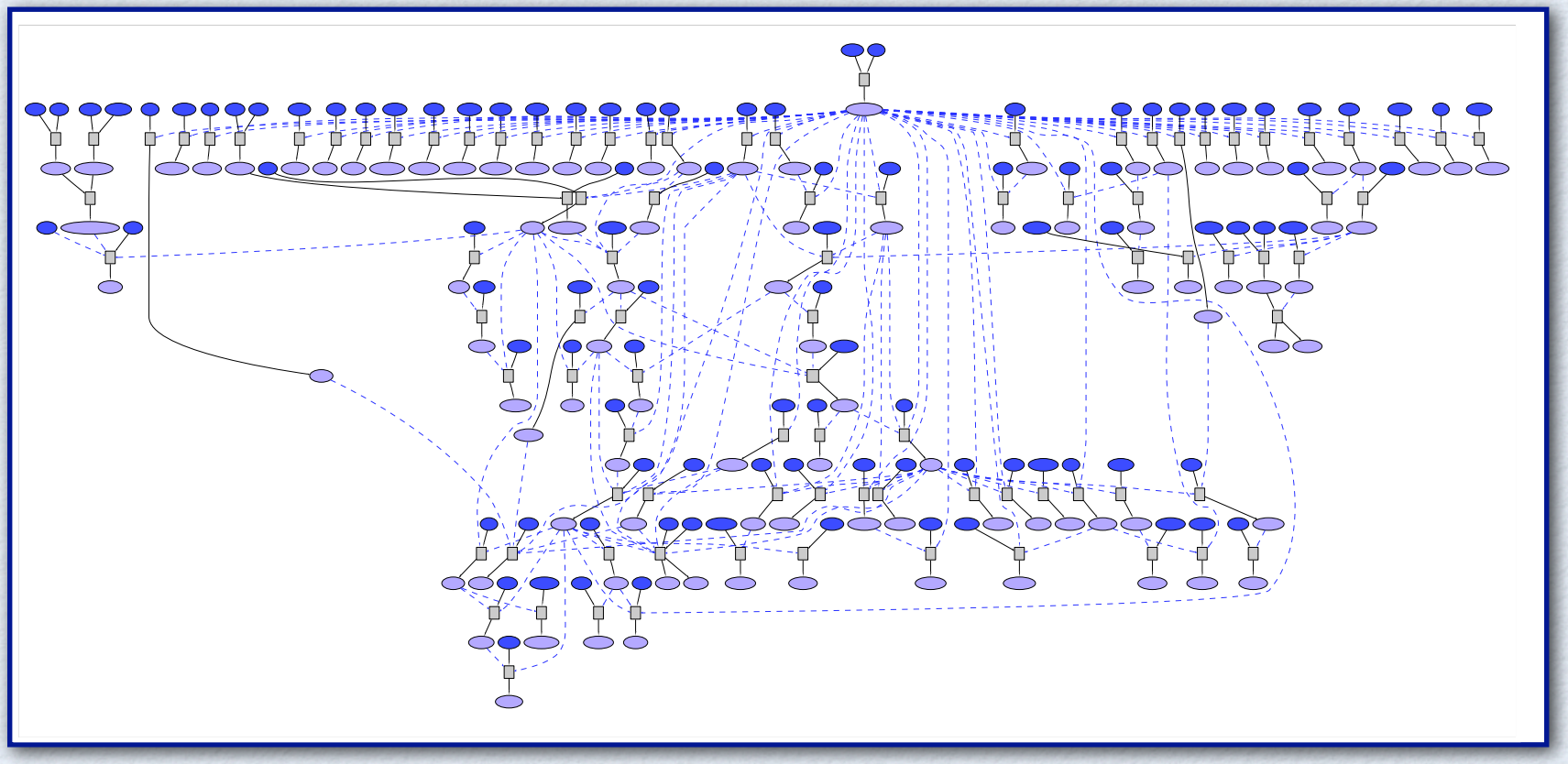


# CONSTRAINING THE EGF MAP

The idea is to go from all possible pathways to a plausible set, given the context.

- a list of 85 protein state changes demonstrated experimentally to occur in response to a short stimulus with Egf were set as goals and a set of concurrent paths were produced by PLA. This subnet ensures that the paths used to reach chosen goals are mutually compatible.
- (reachability of all of the goals is also a test of the model)
- Egf Rules, with requirements specific to Egf signaling, were given preference over Common Rules

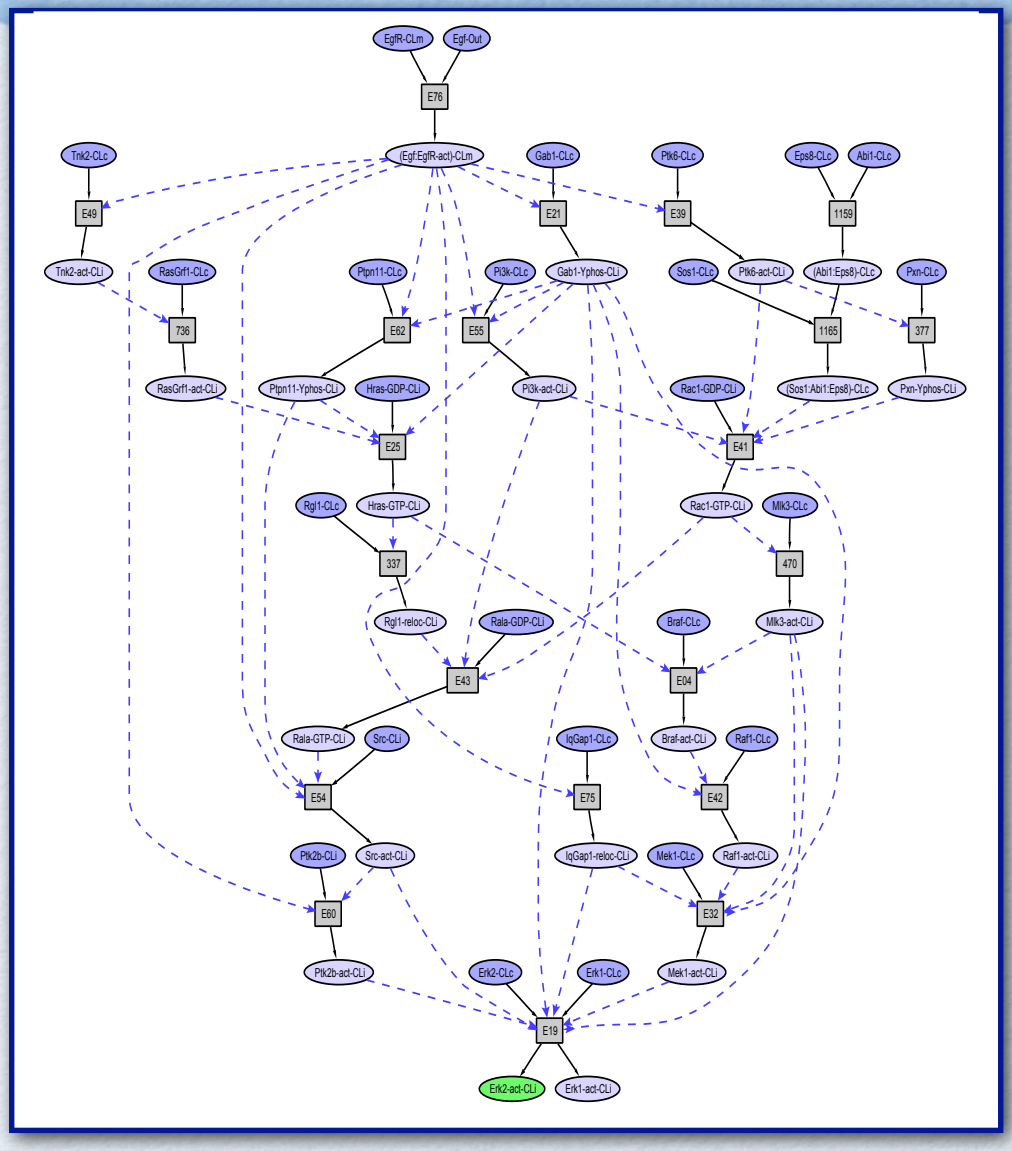
# THE CONSTRAINED EGF MAP



# ACTIVATION OF ERK IRT EGF

The path leading to activation of Erk1/2 in the constrained Egf network.

This path exists in the context of all the other experimental observations,



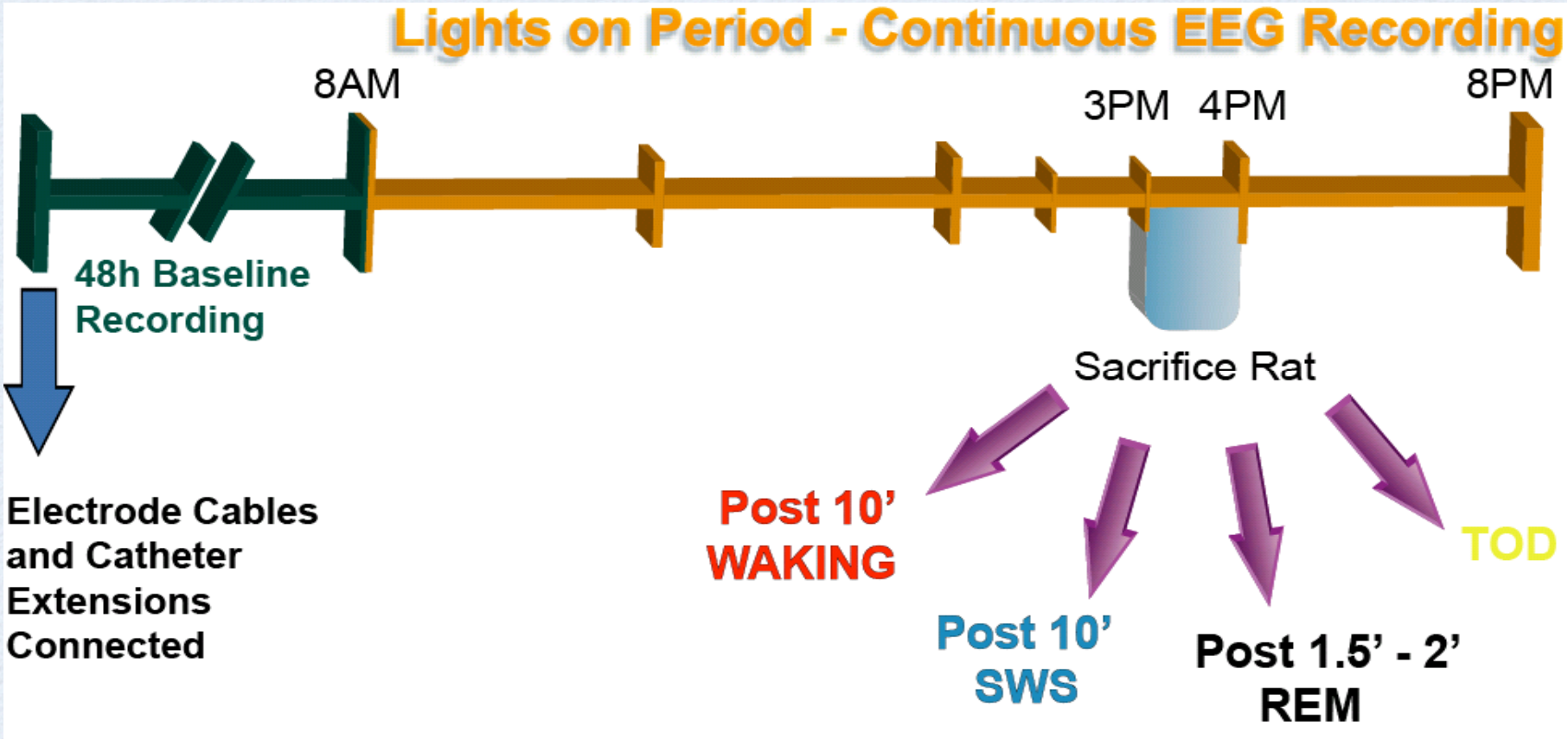
# SLEEP

(with MaryAnn Greco and Merrill Knapp)

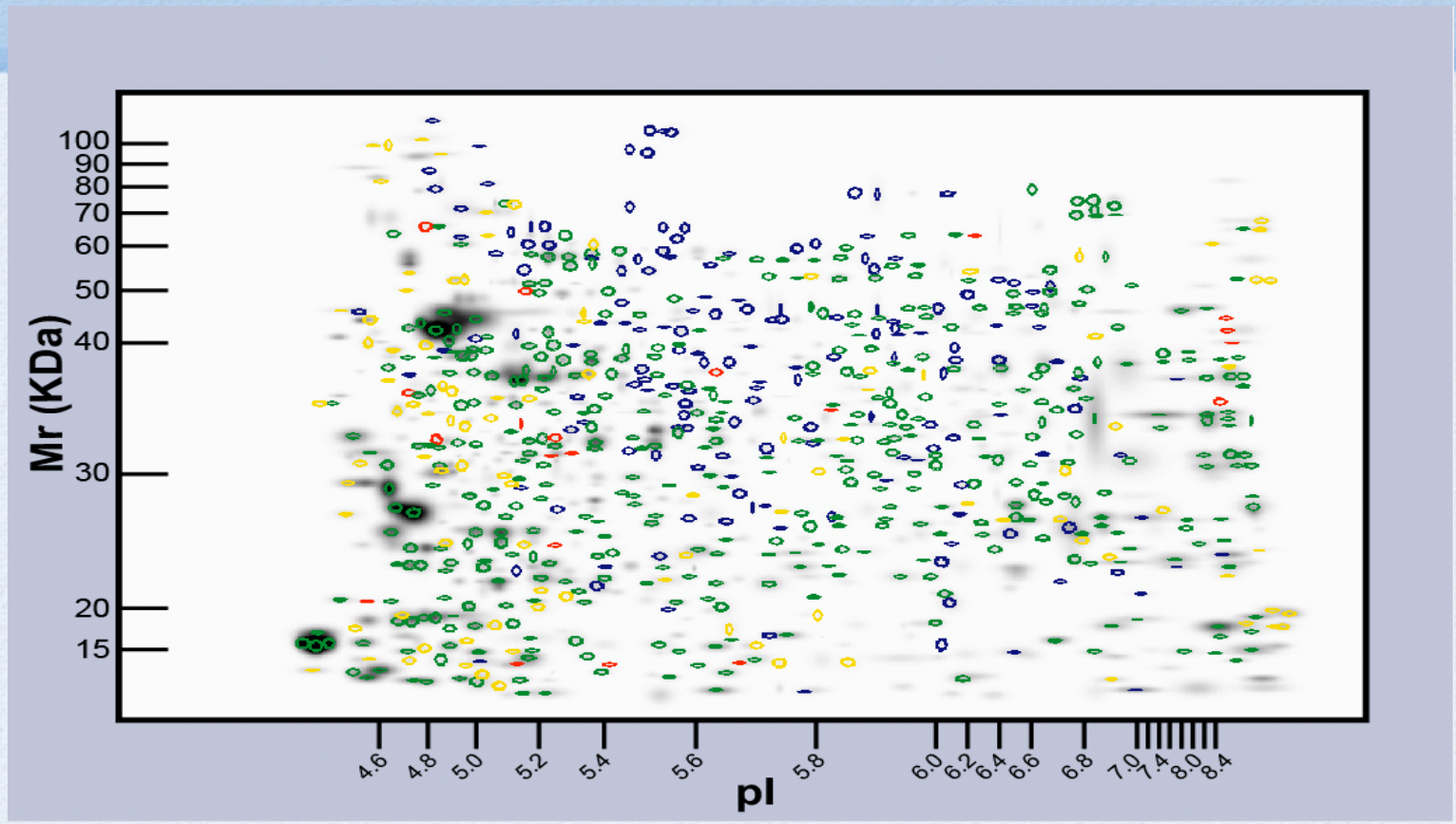
# THE QUESTION

- What is the function of sleep?
- What are your cells doing when you sleep? vs awake?
- Rat model -- proteomics from different organs at different sleep states

# NATURAL SLEEP PARADIGM



# 2D MASTER GEL

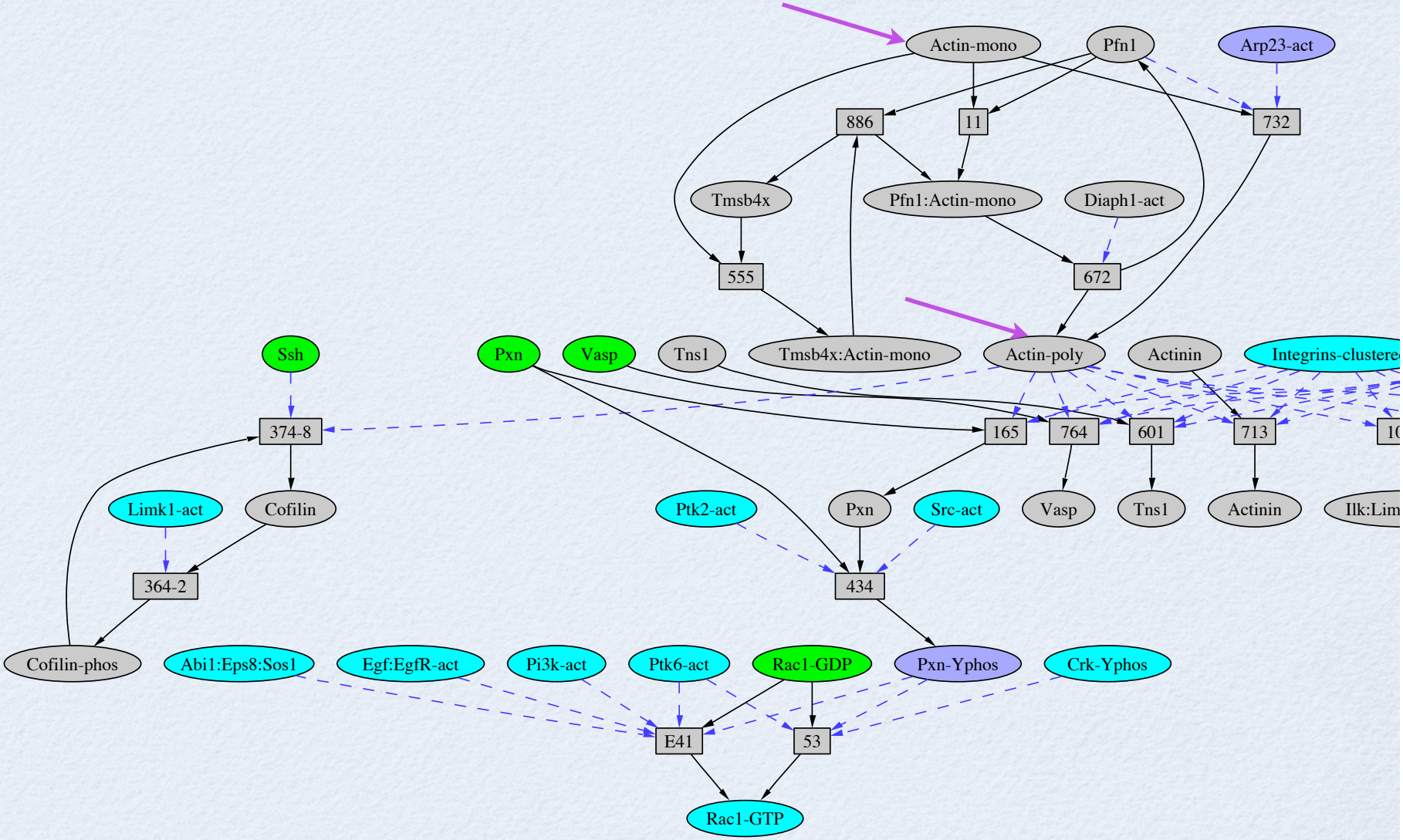



Proteins unique to different states were identified

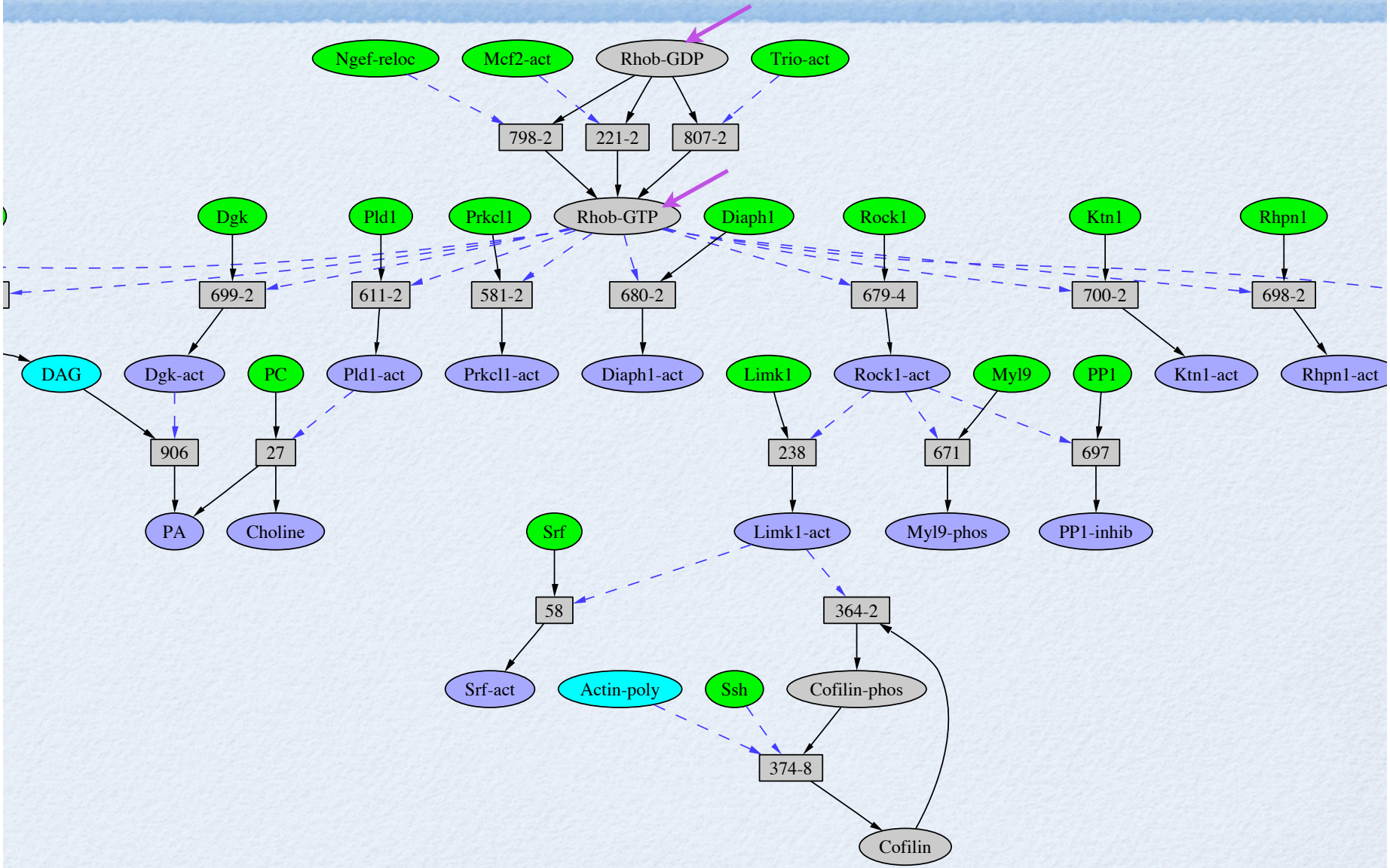
Those modeled in PL included Actin and Rhob

Use the PLA explorer to find signaling connections

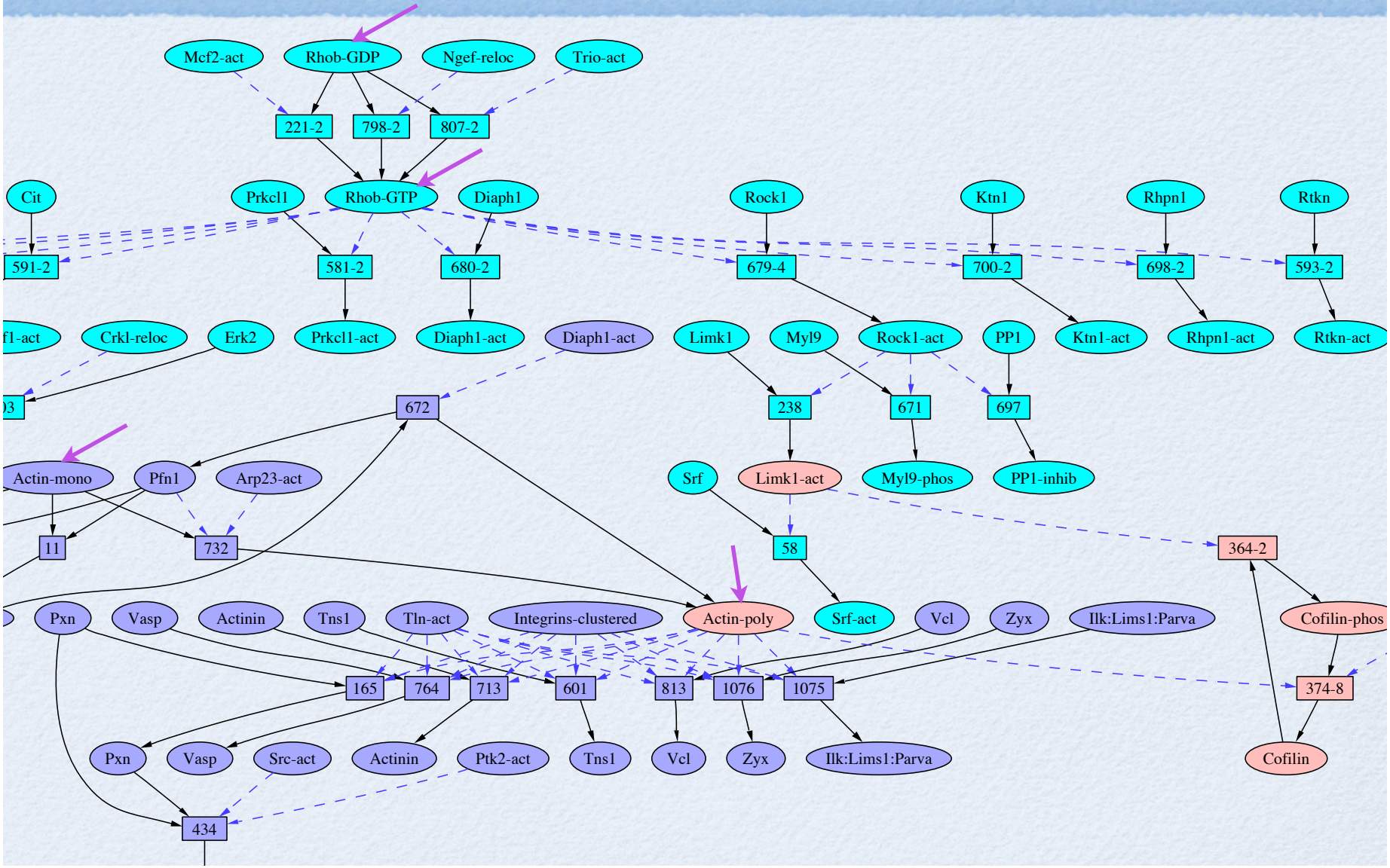
# EXPLORING PL KB FROM ACTIN



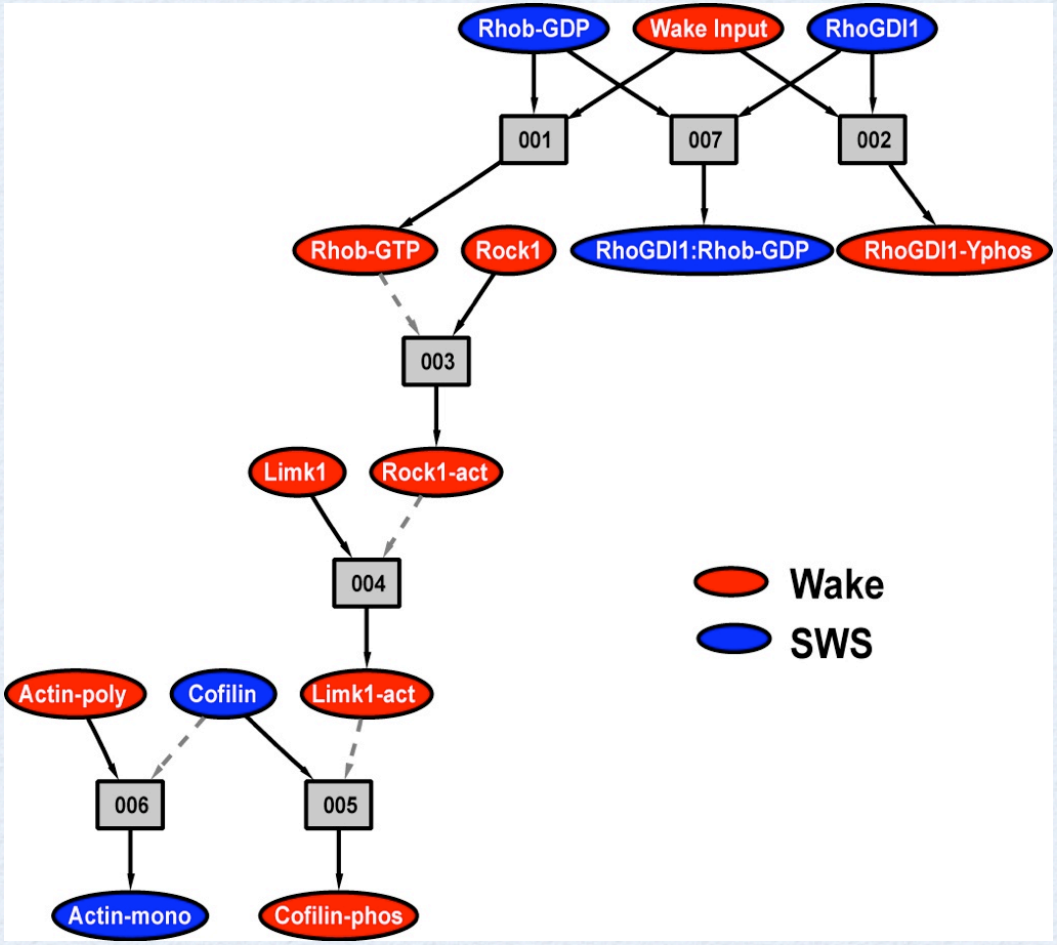
# EXPLORING PL KB FROM RHOB



# COMPARING THE EXPLORE NETS



# A HYPOTHETICAL MODEL PATHWAY RELATING STATE AND SYNAPTIC PLASTICITY




Wake state:
unknown signal(s)
=> phosphorylation of Rock1
=> activation of Limk1
=> phosphorylation of cofilin
=> increase in polymerized actin
(Phosphorylated cofilin is unable to depolymerize actin)

SWS:
RhoDG11 binds Rhob-GDP
(is not phosphorylated)
=> Rock1, Limk1, and cofillin would not be phosphorylated and
=> actin depolymerization
=> decrease in synaptic weight

**TODO -- test the hypothesis**

# Choosing a formalism

# REQUIREMENTS

- What do you want to represent?
- What questions do you want to ask?
- What material/data is available, what must be estimated/ hypothesized?
- How are you going to validate?
- What are you familiar with?
- What formalism features:
  - Language primitives, semantic models, tools
  - model analysis/validation vs system analysis

# What to Model?

- Species: Individual, concentration, population
- Structure: compartments, binding, location
- Process:
  - non-determinism -- computation trees
  - stochastic / probablistic -- MC
  - deterministic -- ODE
- Quantitative: kinetics, dynamics,
- Qualitative: interactions, causal relations ...
- Effects of perturbation
  - Knockout / KnockIn / Mutations
  - Stimulus / Stress

# What questions?

- Examples:
  - All the ways to phosphorylate Erk?
  - How fast can signal get to nucleus?
  - Is Pi3k required for activation of Raf?
- Categories:
  - Reachability
  - Information/material flow
  - Dynamical features
    - steady states, stable states, oscillations
    - competition/race conditions/interference
  - Kinetic profiles

# Some points in design space

- Data source:
  - KEGG/SBML library -> Model -> Analysis
  - Data Curation -> Datums -> Rules -> Model -> Analysis
- Representation of components/species
  - blackbox --- constant declaration
  - explicit attributes of state --- Algebraic specification
  - as behaviors --- process calculus

# More points in design space

- Properties/questions -- tools
  - qualitative -- search/model checking/constraint solving
  - stochastic -- simulation/ statistical/stochastic mc
  - continuous/kinetic -- ODE solvers ...
- View/perspective
  - physical agent point of view -- interaction centric
    - rules emerge from process descriptions
  - state/event -- centric
    - states are static structures,
    - rules specify behavior / interactions directly

# FUTURE CHALLENGES

- Integration of signaling and metabolic networks
- Modeling action of transcription factors
- Modeling domain-domain interactions
- Adding semiquantitative information
- Algorithms to discover meaningful subnets
- Integrating models

# SRI SSB TEAM AND COLLABORATORS

- PL Founders:
  - Patrick Lincoln
  - Keith Laderoute
- PL Core team
  - Carolyn Talcott
  - Steven Eker
  - Merrill Knapp
  - Andy Poggio
  - Malabika Sarker
  - Ashish Tiwari
  - Frederic Vigneault
- BioCyc
  - Peter Karp
  - Markus Krumenaker
  - Alex Shearer
- SRI MP Biologists
  - Anna Lisa d'Andrea
  - MaryAnn Greco
- SRI SV (CADRE)
  - Krishna Kodukula
  - Amit Galande
  - Rajeev Vaidyanathan
- LBNL -- Cancer Biology
  - Joe Gray
  - Paul Spellman
  - Laura Heiser
- Walter Reed
  - Marti Jett
- U Texas Houston/San Antonio
  - Doug Baxter
  - Riccardo Mozzachiodi

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