UNIVERSITY OF TWENTE.

Formal Methods & Tools.

Scalable Multi-core Model Checking: Technology & Applications of Brute Force
Part IV: Biology

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30, 31 October 2014

VTSA 2014, Luxembourg

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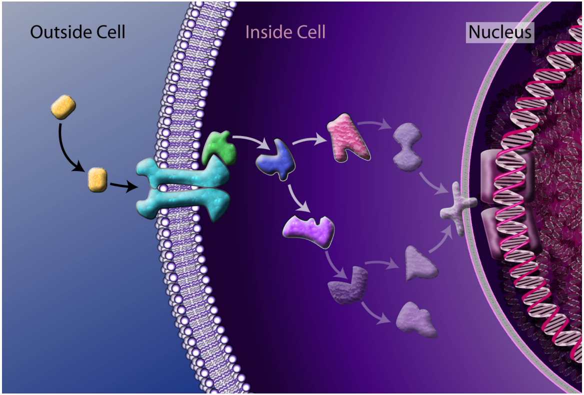
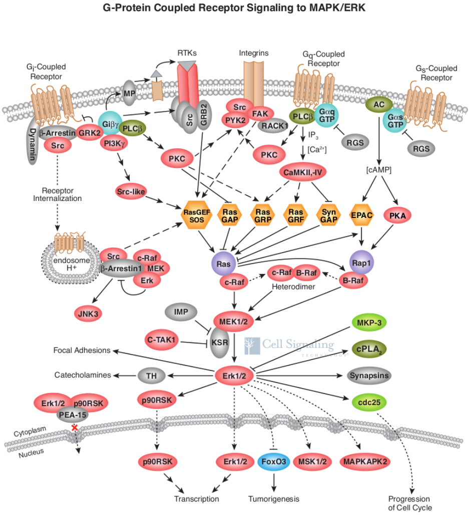
Signaling Pathway in Cell Biology



Kinase pathways: spreading the Phosphor token

- Biochemical equilibrium reactions:
  - $E + S + ATP \rightleftharpoons ES + ATP \rightarrow ES^P + ADP \rightleftharpoons E + S^P + ADP$
- Simplify to one interaction (here activiation): $E \longrightarrow S$

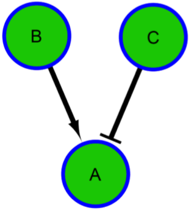
Signaling Network in Cell Biology



Complex network dynamics

- Node interactions:
    - activation
    - inhibition
- Crosstalk and Feedback
- Ultimate questions:
    - understand & control
    - key to finding a cure of “system” diseases
    - cancer, diabetes, arthritis

How to model signaling networks?



Mathematical models (ODE) [Gillespie '77]

$$\frac{dA}{dt} = k_1 \cdot B - k_2 \cdot C - k_3 \cdot A$$

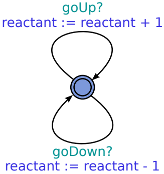
- A, B, C are molecule concentrations
- k_1, k_2, k_3 are kinetic parameters
- Precise, strong tools (simulation, stability)
- Difficult, too many parameters are unknown

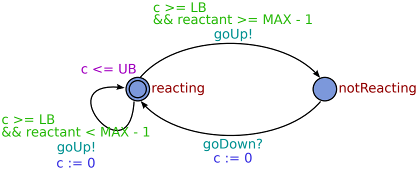
Boolean networks [Kauffman'69]

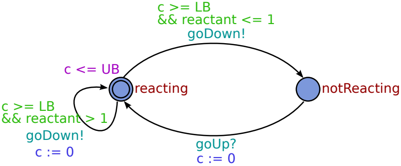
$$B \wedge \neg C \implies A$$

- Easy to handle, biologically relevant
- No timing, no concentrations at all
- So how to execute this?

Simplified version of Timed Automata


goUp?
reactant := reactant + 1
goDown?
reactant := reactant - 1


c >= LB
&& reactant >= MAX - 1
goUp!
c <= UB
reactant
notReacting
c >= LB
&& reactant < MAX - 1
goUp!
c := 0
goDown?
c := 0


c >= LB
&& reactant <= 1
goDown!
c <= UB
reacting
notReacting
c >= LB
&& reactant > 1
goDown!
c := 0
goUp?
c := 0

Basic modeling ideas

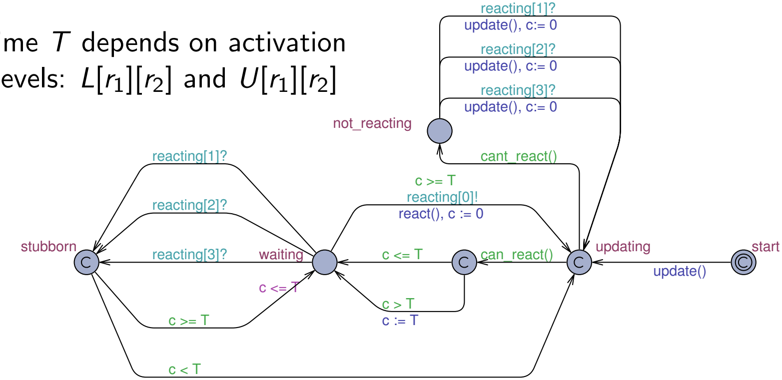
- Discretized activity levels
- Clocks constrained by upperbound and lowerbound
- Activation/Deactivation is communicated over channels

Using Networks of Timed Automata

Modeling Assumptions

- Every reactant is modeled by a Timed Automaton
- It maintains a discrete activation level: $\left\lfloor \frac{active}{active+inactive} \right\rfloor$
- Clocks trigger when the activation level goes up or down
- Activation/Inhibition: broadcast communication between automata

time T depends on activation levels: $L[r_1][r_2]$ and $U[r_1][r_2]$



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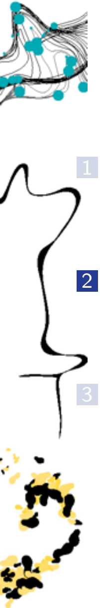
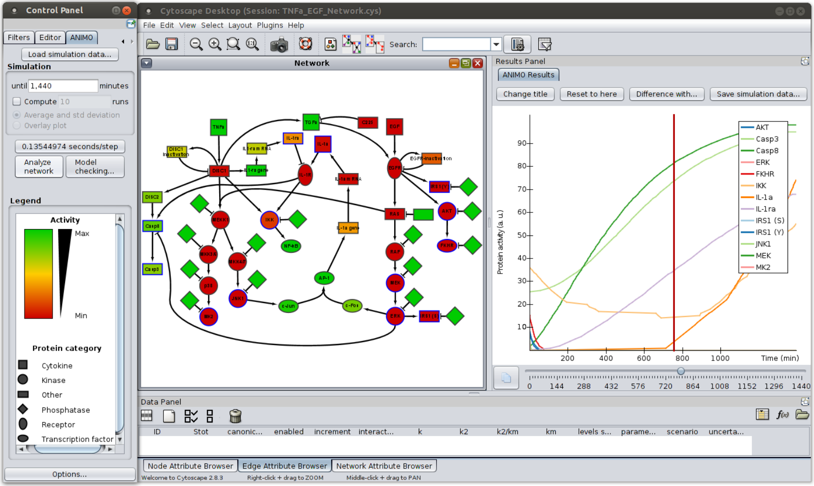


ANIMO: Analysis of Networks by Interactive Modeling

SCHIVO, SCHOLMA, KARPERIEN, LANGERAK, vdPOL, POST, URQUIDI, VET, WANDERS,
(FMT, HMI, BIOENG) [BIBE'12] [GENE'13] [J-BHI'14]



until 1,440 minutes

☐ Compute 10 runs

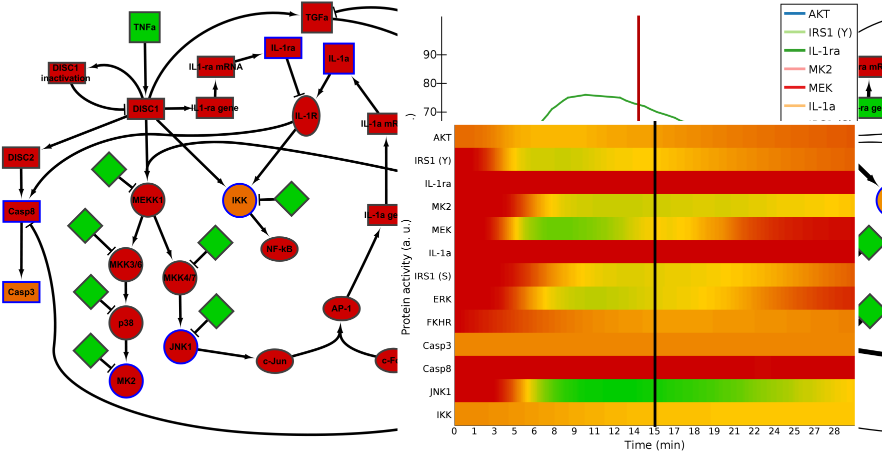
○ Average and std deviation

○ Overlay plot

0.13544974 seconds/step

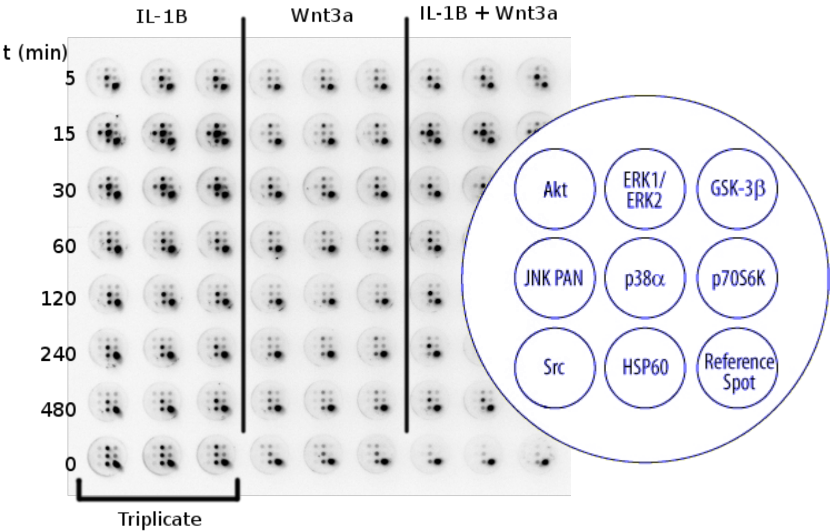
ANIMO is a Cytoscape plugin, running UPPAAL in the background

ANIMO workflow



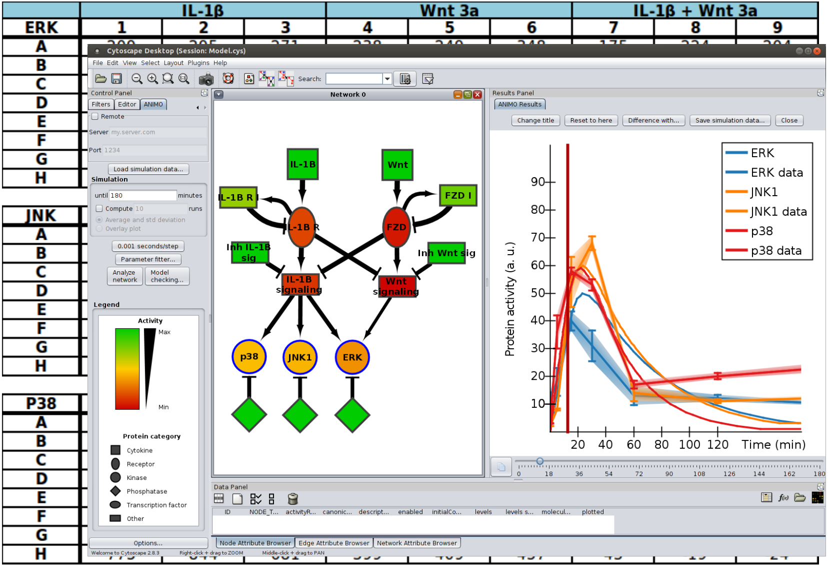
Draw topology, initial conditions, and investigate the behaviour
Node colors/edges show activation level; view as graphs, heatmap.

Model Validation by Wet-Lab Experiments



Phosphorylation of proteins in human chondrocytes:
Time series under three experimental conditions.

Validation by Wet-Lab Experiments



☐ Remote

Server my.server.com

Port 1234

Simulation

until 180 minutes

☐ Compute 10 runs

● Average and std deviation

○ Overlay plot

ID NODE_T... activityR... canonic... descript... enabled initialCo... levels levels s... molecul... plotted

Welcome to Cytoscape 2.8.3 Right-click + drag to ZOOM Middle-click + drag to PAN

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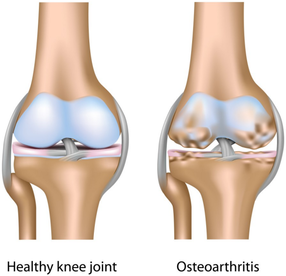
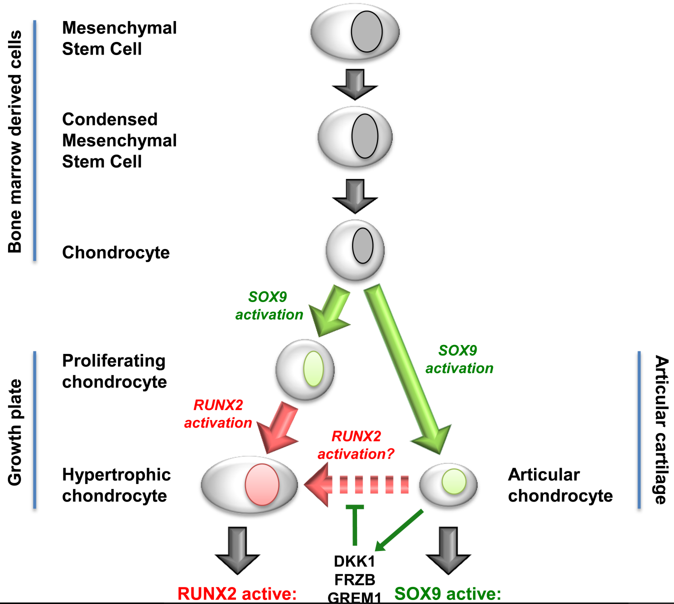
Osteoarthritis

Osteoarthritis

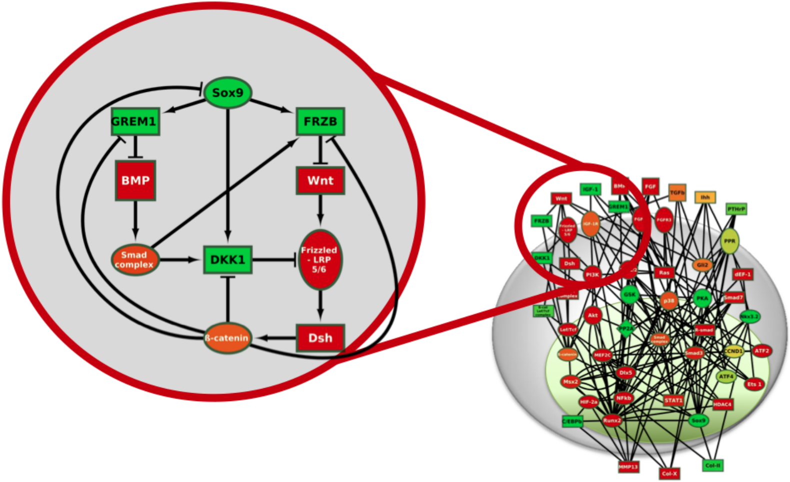
- Mesenchymal stem cells can differentiate to
  - either Osteoblasts (bone)
  - or Chondrocytes (cartilage)
- Osteoarthritis: articular cartilage dries, wears out, forms bone
- Pain in “bone-to-bone” joints
- 60% of the population (> 65 years) will show symptoms
- Characterized by transcription factors: SOX9 or RUNX2



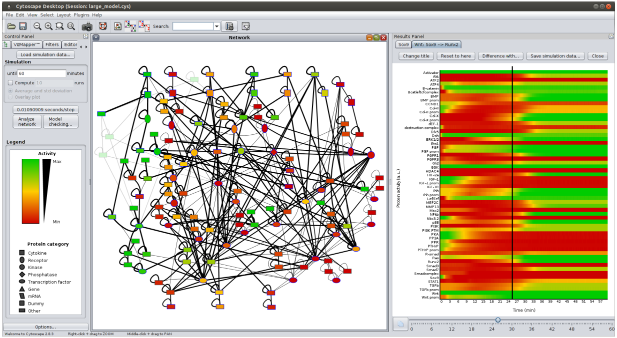
Development of Chondrocyte (cell fate)



ECHO: the Executable Chondrocyte



Put ECHO in ANIMO



Size

- 7 inputs
- 123 nodes
- 354 links
- Sox9, Runx2 as output

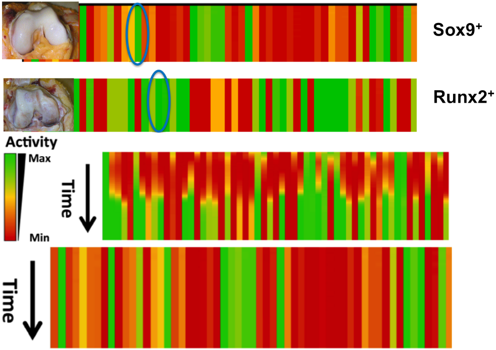
Starting point: Boolean Network
Kerkhofs et al (U Leuven), PLoS One 7(4), 2012

Validation by simulating results from literature

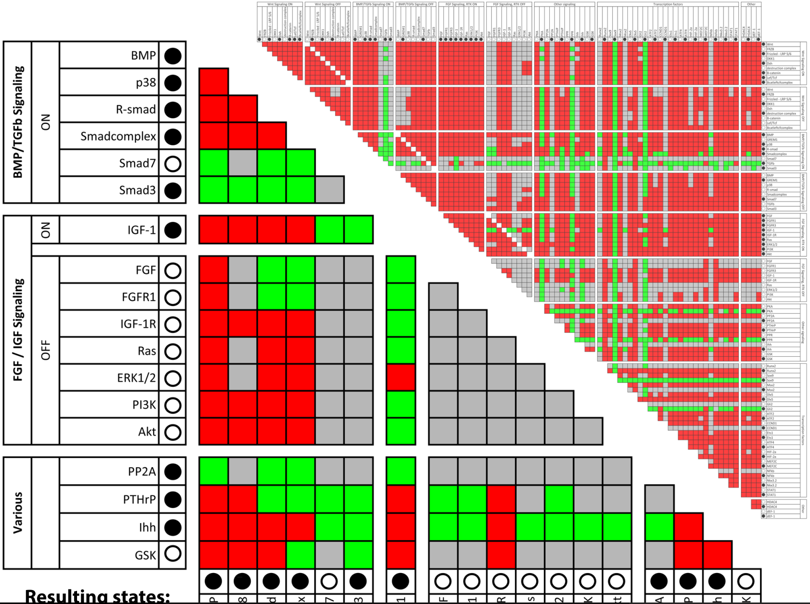
Basic validation by simulation

- Exhaustive / Monte Carlo simulation with 3^7 input conditions
- Knock-out or overexpress individual nodes in the network

- There are only two stable states (SOX9 and RUNX2)
- WNT protein pushes SOX9 stable states to RUNX2
- DKK, FRZB and GREM stabilize healthy cartilage

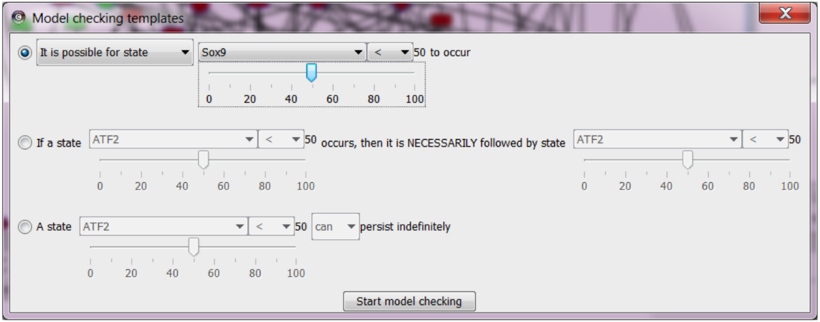


In search for new knowledge: parameter sweeps



Resulting states:

Multi-core Model Checking for Biological Applications?



Model checking templates

● It is possible for state Sox9 < 50 to occur

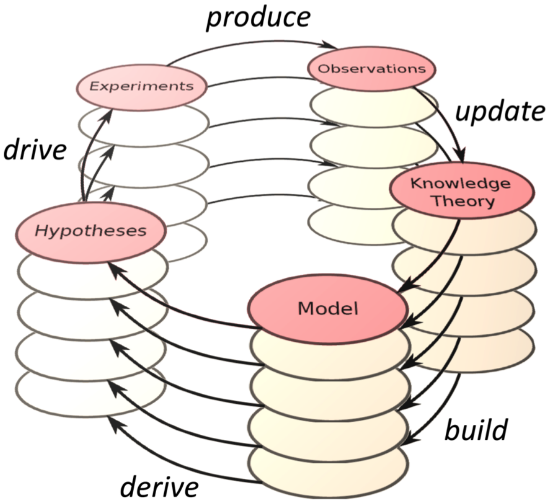
○ If a state ATF2 < 50 occurs, then it is NECESSARILY followed by state ATF2 < 50

○ A state ATF2 < 50 can persist indefinitely

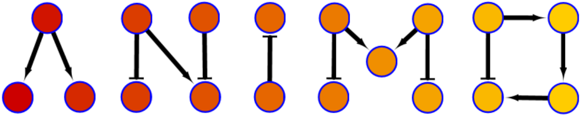
Key questions (biological relevant answers)

- Which input combination/series causes a switch RUNX2 → SOX9
- Which interactions should be inhibited to prevent SOX9 → RUNX2

The Empirical Research Cycle/Spiral



Literature on ANIMO



Signaling Networks in Biology

- http://fmt.cs.utwente.nl/tools/animo/
- Stefano Schivo, Jetse Scholma, B. Wanders, R. Urquidi, P. van der Vet, M. Karperien, R. Langerak, J. van de Pol, J.N. Post, (BIBE'12, J-BHI'14)
Modelling biological pathway dynamics with Timed Automata
- Jetse Scholma, Stefano Schivo, R. Urquidi, J. van de Pol, M. Karperien, J. Post, ... GENE 533 (2013)
Biological networks 101: computational modeling for molecular biologists
- Stefano Schivo, Jetse Scholma, Marcel Karperien, Janine N. Post, Jaco van de Pol, Rom Langerak, ... (SynCoP 2014)
Setting Parameters for Biological Models With ANIMO