Dynamic Topology of Biological Networks

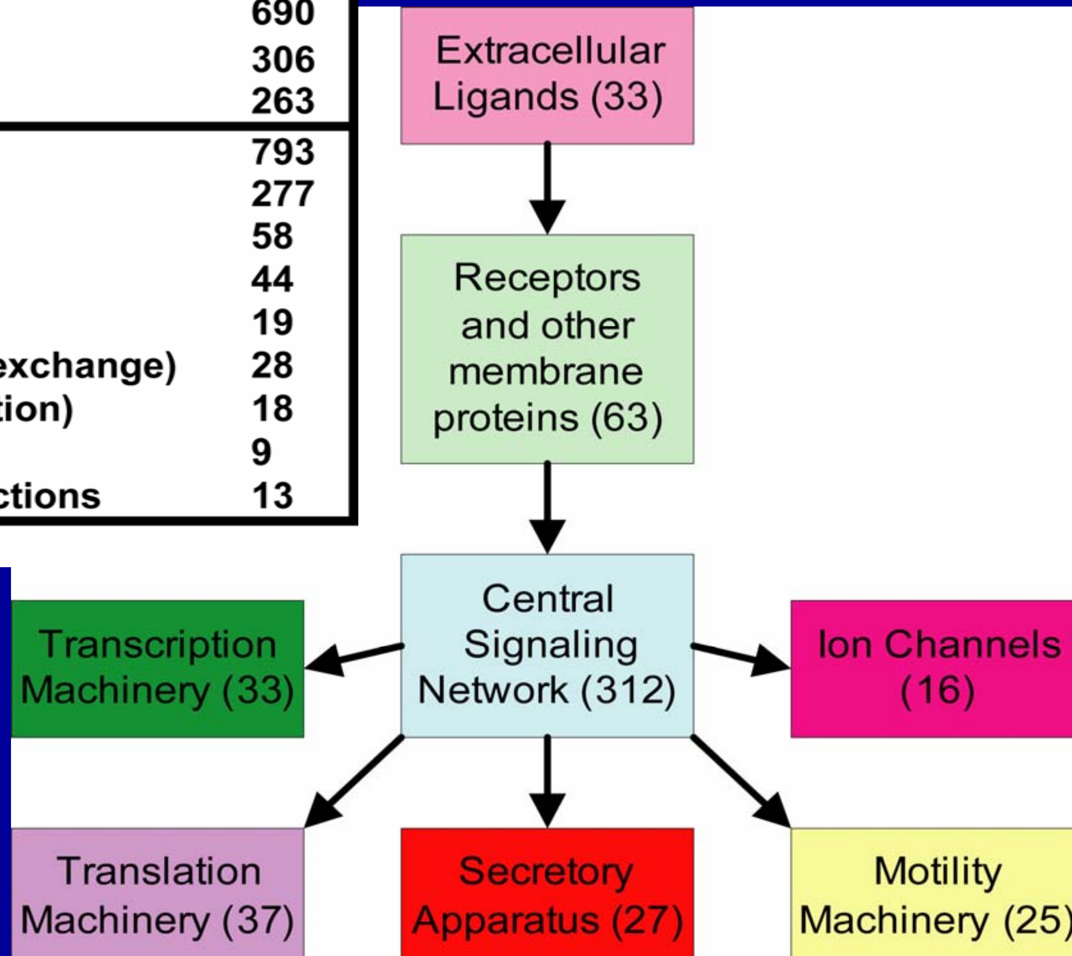
Functional Consequences

Ravi Iyengar, Ph.D.
Mount Sinai School of Medicine
December 19, 2006

MOUNT SINAI SCHOOL OF MEDICINE

The CA1 hippocampal neuron as a network of interacting components

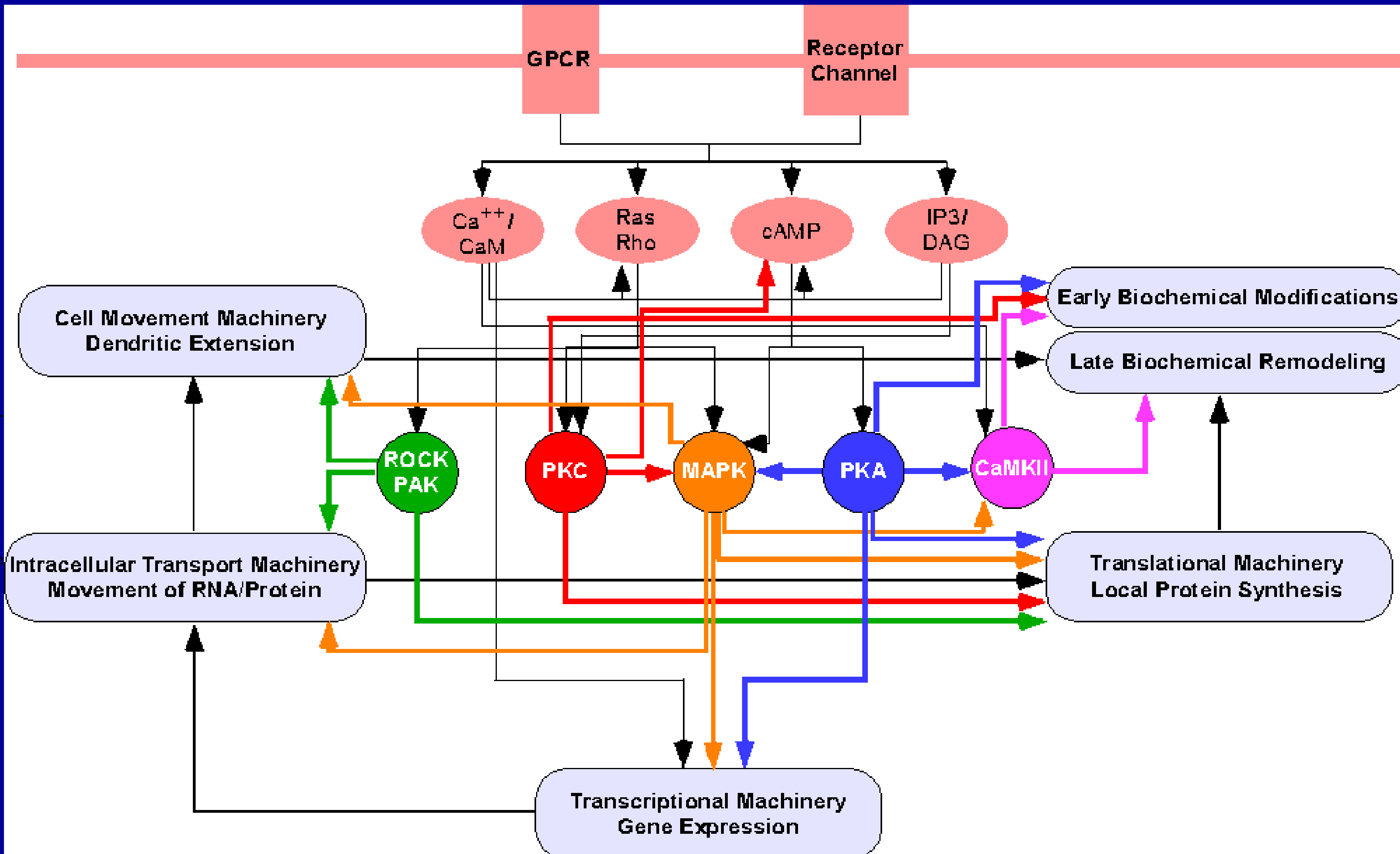
Number of nodes (components)	546
Number of links (connections)	1259
Number of references	1202
Activation links	690
Inhibitory links	306
Neutral links	263
Binding	793
Phosphorylation	277
Dephosphorylation	58
Enzymatic cleavage	44
Channels to Ions	19
GEF (small GTPase exchange)	28
GAP (GTPase activation)	18
Ubiquitination	9
Other enzymatic reactions	13



Avi Ma'ayan

Maayan et al (2005)
***Science* 309:1078**

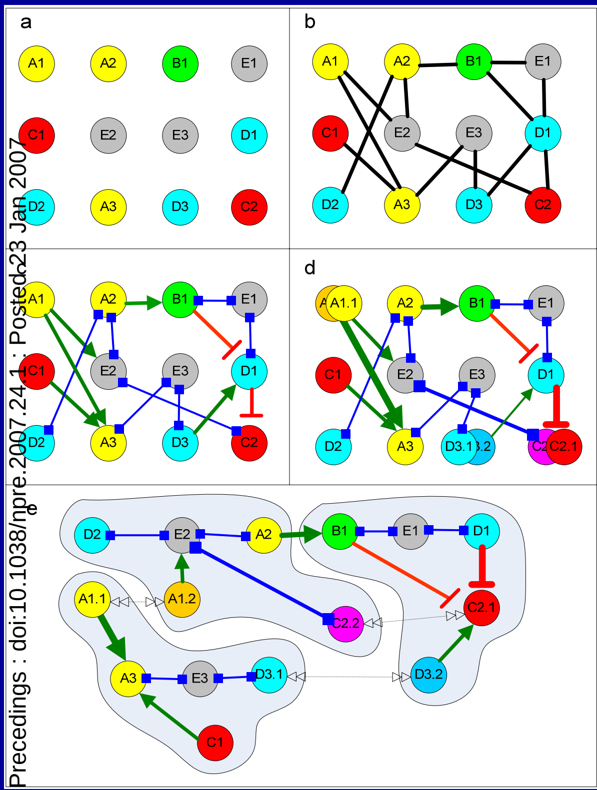
Schematic Representation of a Hippocampal Neuron

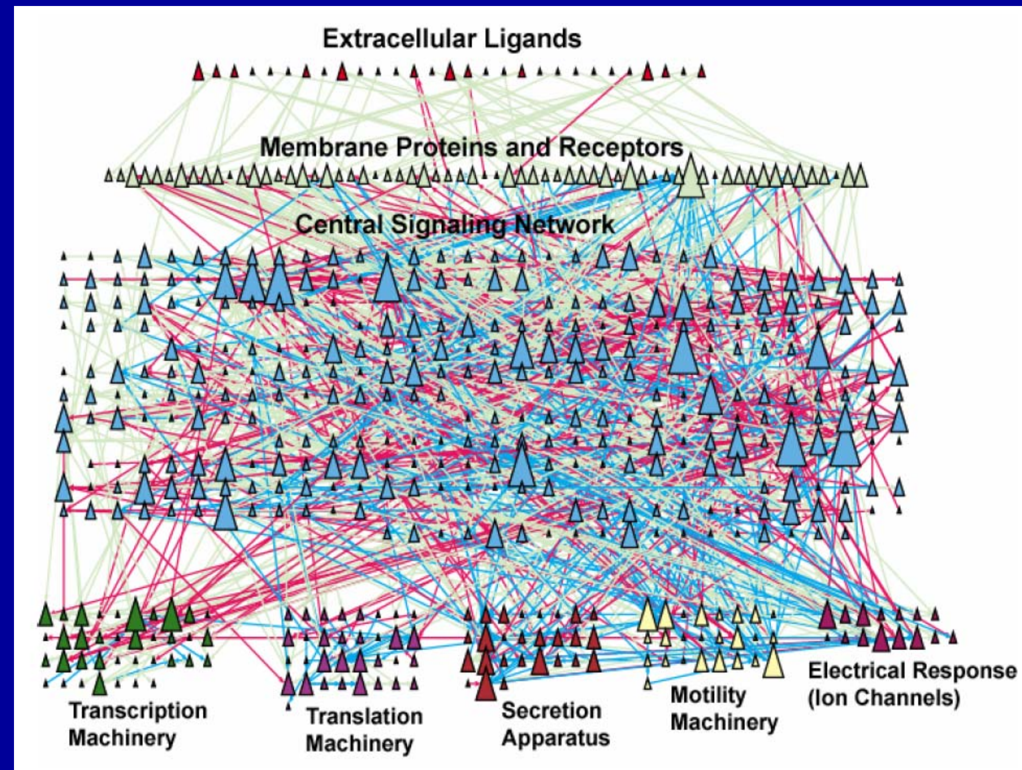


Jordan Landau Iyengar *Cell (2000) 103:193*

The Cell as a Directed Graph

Hippocampal neuron 2004 v





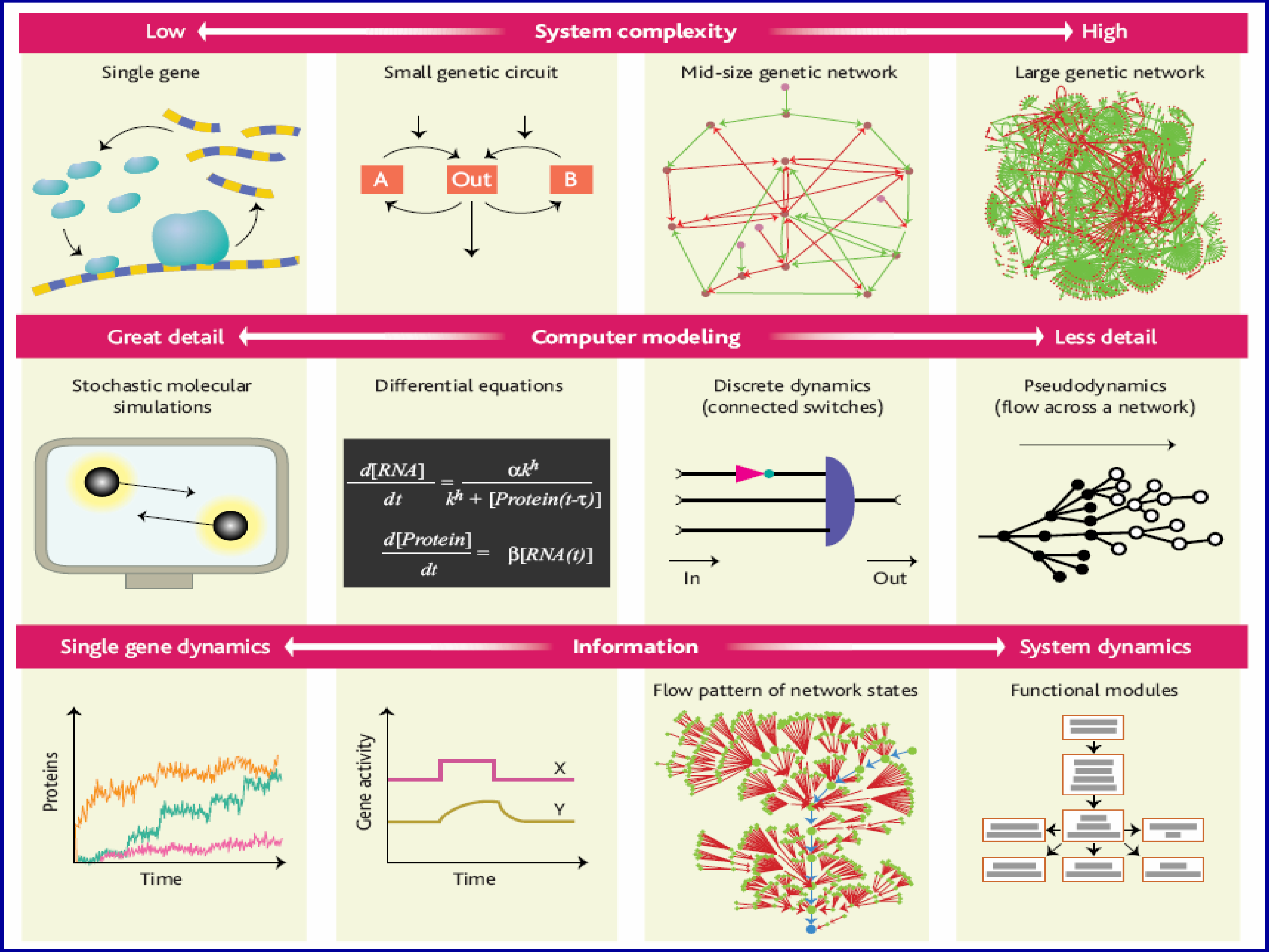
Multiple levels of representation of cellular interactions as networks

We currently use directed graphs (c) for our studies

How do we identify regulatory patterns in such complex systems?

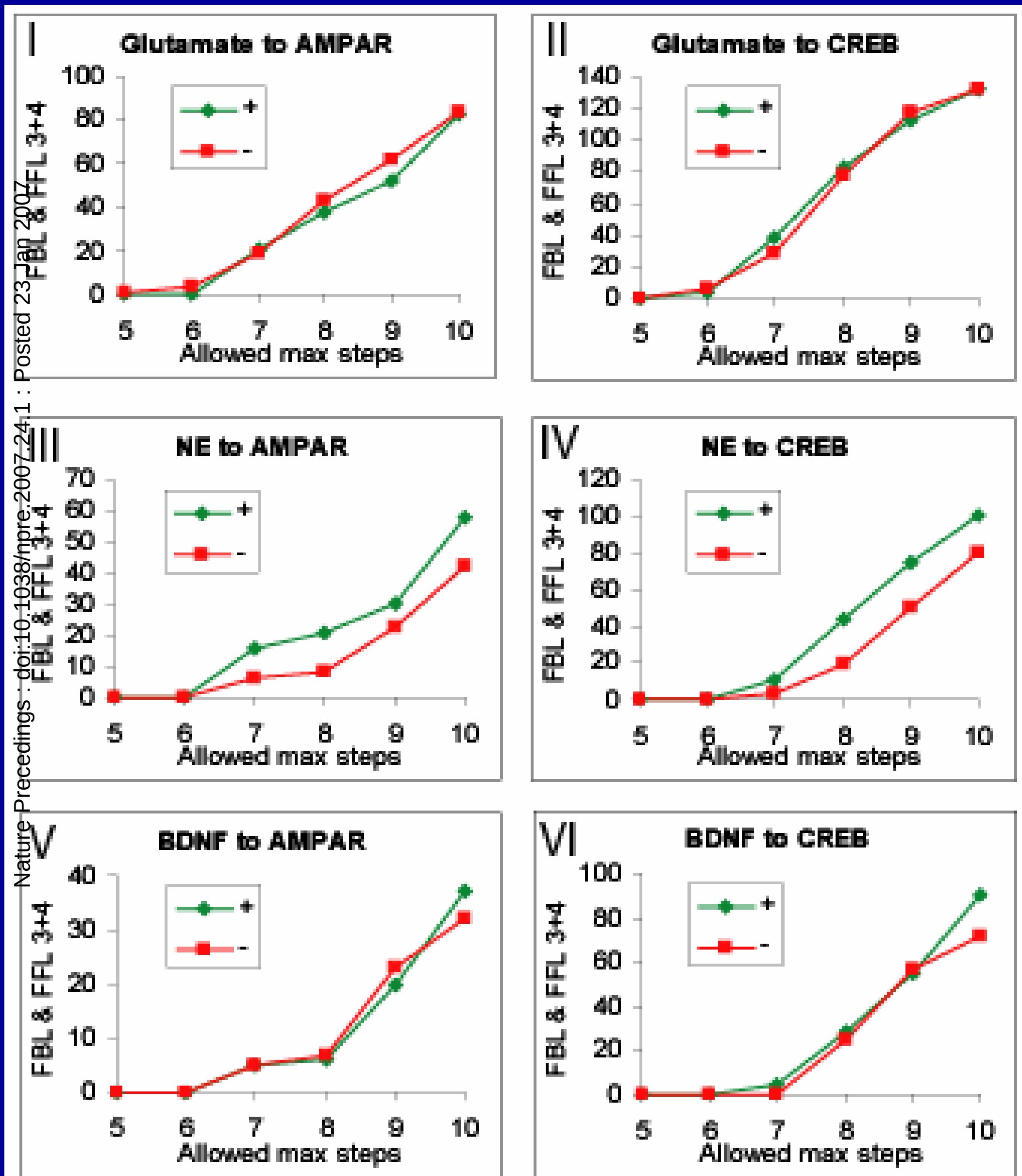
Network Sciences (graph theory)

Methods to study small to large biochemical systems range from simple to complex approaches



S. Bornholdt *Science* **310**, 5747 (2005)

Sub-networks (modules) from Ligands to CREB

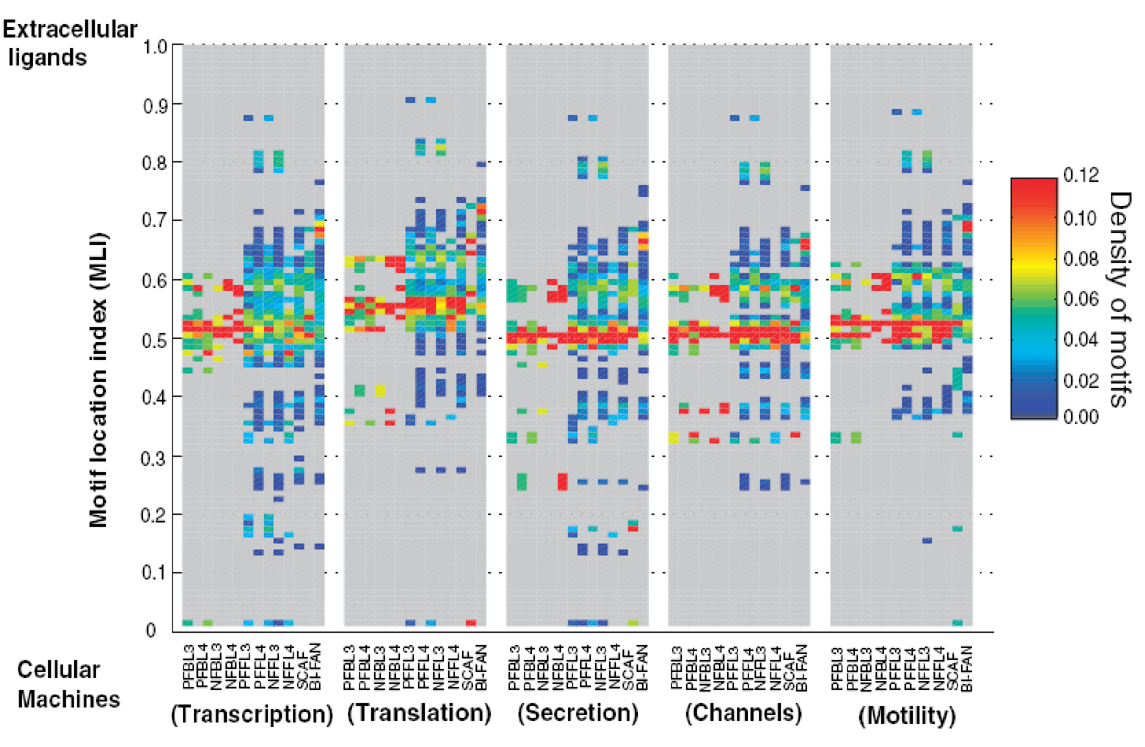


Recruitment of *motifs* within specified paths from receptor to effectors as connectivity propagates

NE, which induces plasticity (state change) in CA1 neuron induces more +ve motifs as compared to –ve motifs as signal (functional connectivity) propagates through the network

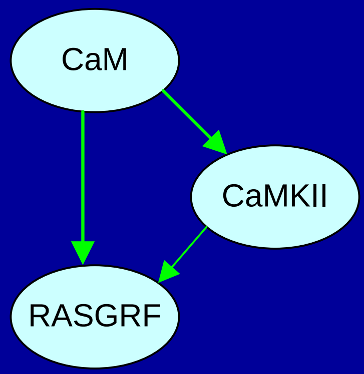
Preferential recruitments of positive motifs may trigger processes that lead to state change in cells

Maps Defining Functional Locations of Motifs Between Ligands and Cellular Machines



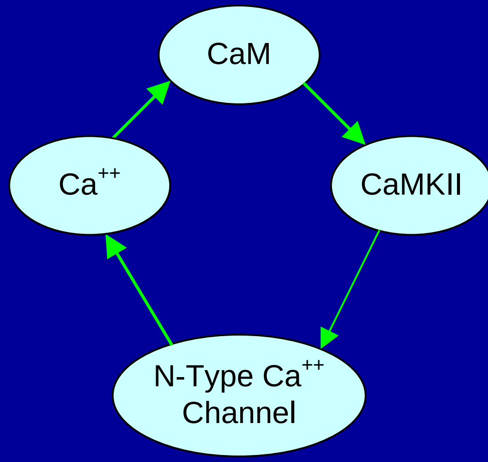
$$MLI = \frac{\sum_{i=1}^{n}\left(\frac{CPLM_i}{CPLM_i + CPLL_i}\right)}{n}$$

Properties of Network Motifs



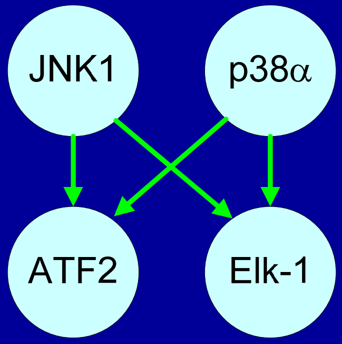
Feed-forward Motif

- Provides redundancy
- Leads to signal prolongation
- Coincidence detection



Feedback Loop

- Signal amplification
- Leads to signal prolongation
- Bistability and switching



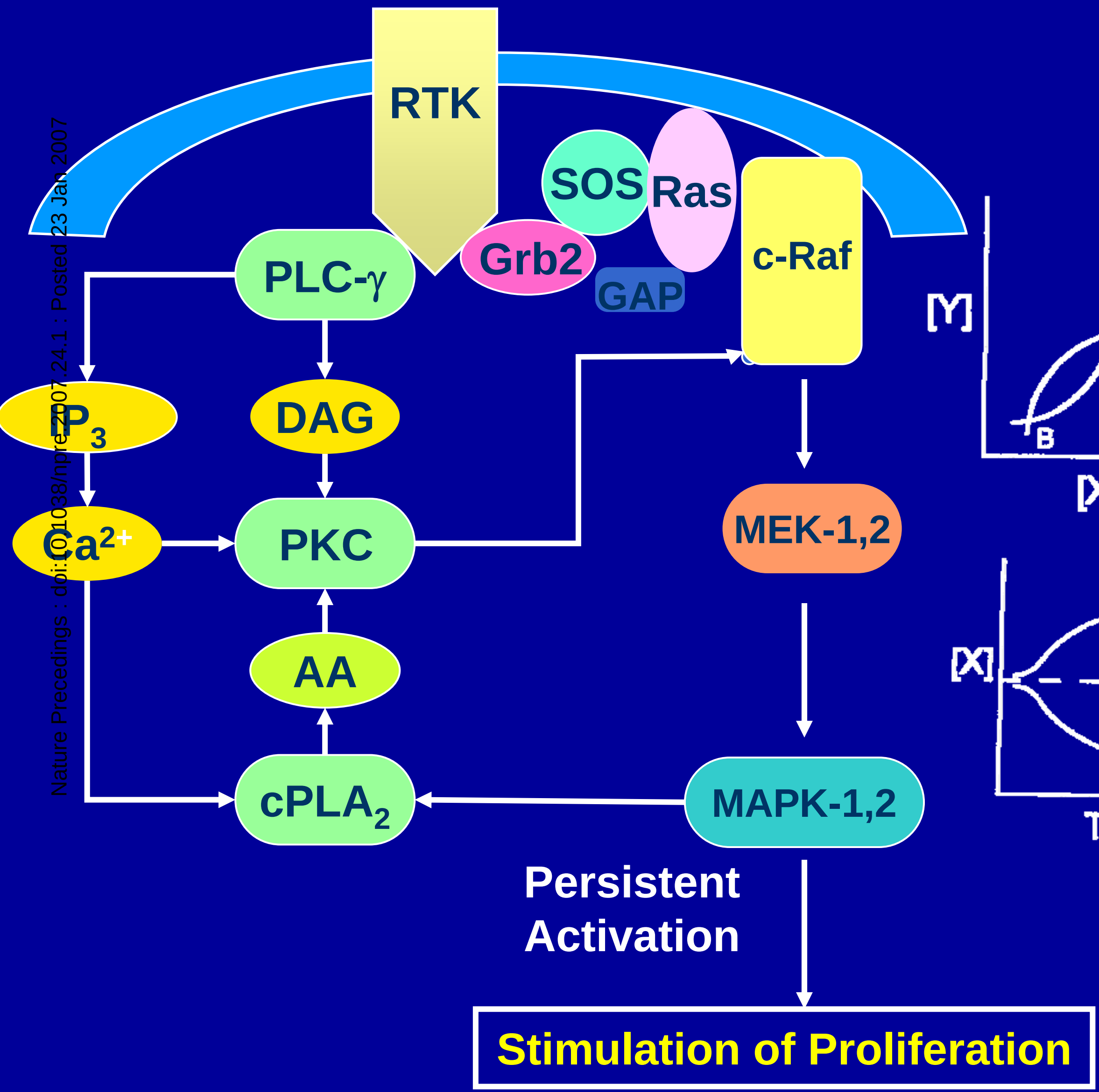
Bifan Motif

- Noise filtering
- Coincidence detection
- Signal sorting

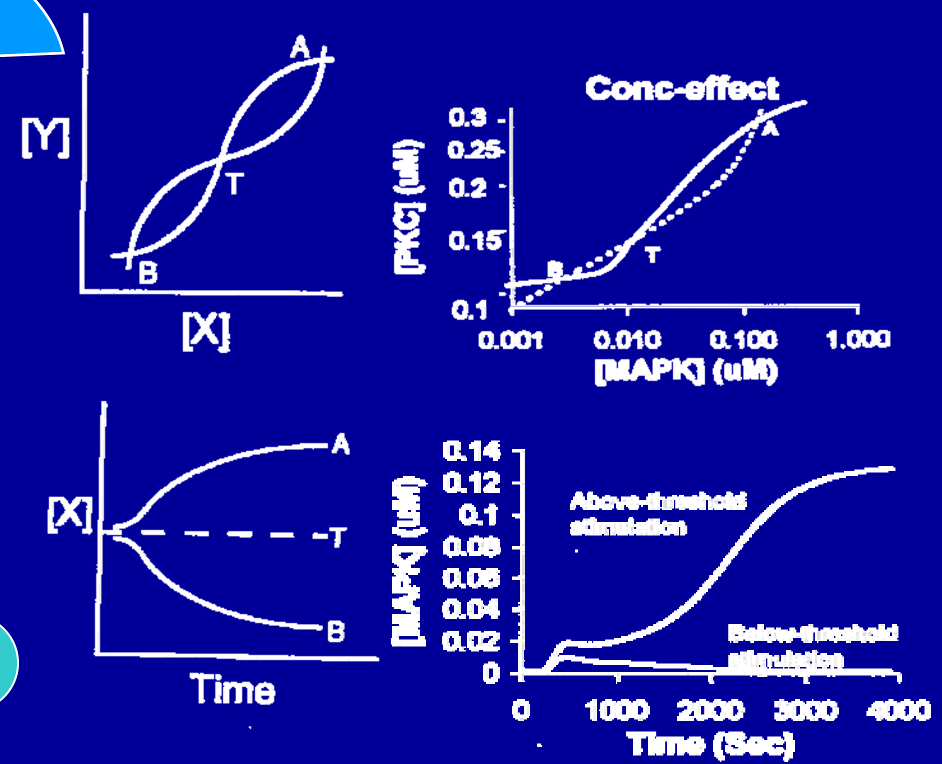
Three common motifs originally described by Uri Alon and colleagues

Milo et al. *Science*, **298**, 824 (2002)

Bistable behavior of the positive feedback loop



Ordinary differential Eq based- Models



Upi Bhalla

Science (1999) 283:381

Bifan network motifs

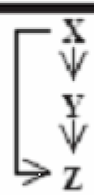
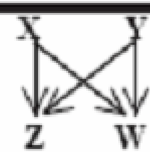
Signal processing by the p38/JNK protein kinases cross regulating the transcription factors ATF2/Elk -1

Azi Lipshatz

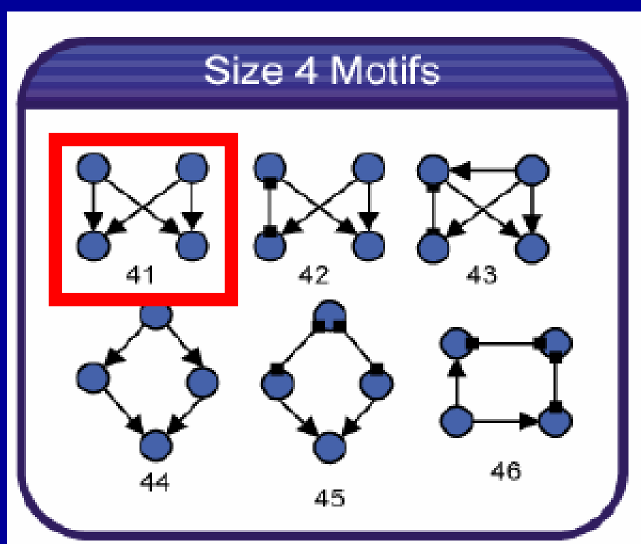
Sudarshan Purshottaman

Avi Ma’ayan

Bifans are the most abundant network motifs in biological regulatory networks

Network	Nodes	Edges	N_{real}	$N_{rand} \pm SD$	Z score	N_{real}	$N_{rand} \pm SD$	Z score	N_{real}	$N_{rand} \pm SD$	Z score
Gene regulation (transcription)					**Feed-forward loop**			**Bi-fan**			
E. coli	424	519	40	7 ± 3	10	203	47 ± 12	13			
*S. cerevisiae**	685	1,052	70	11 ± 4	14	1812	300 ± 40	41			

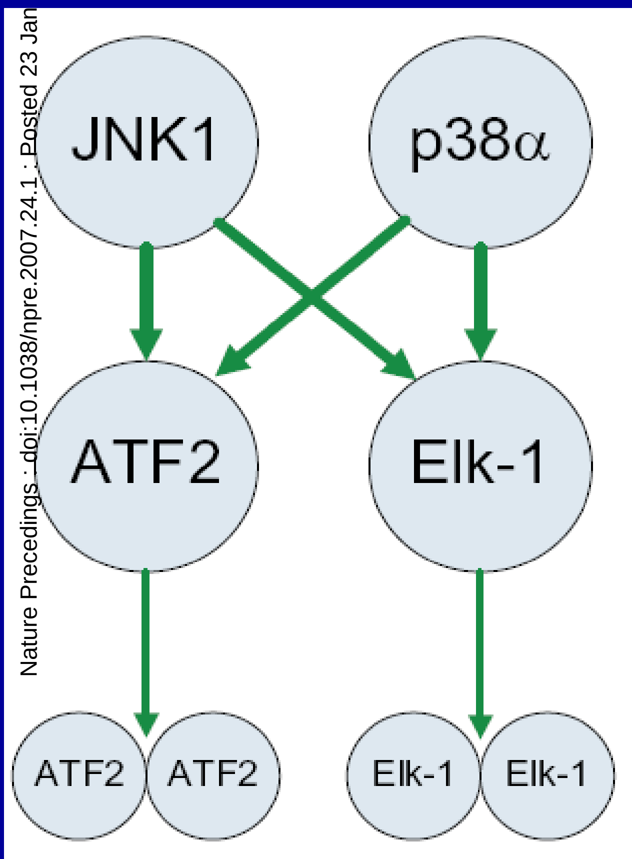
Milo et al. *Science*, **298**, 824 (2002)

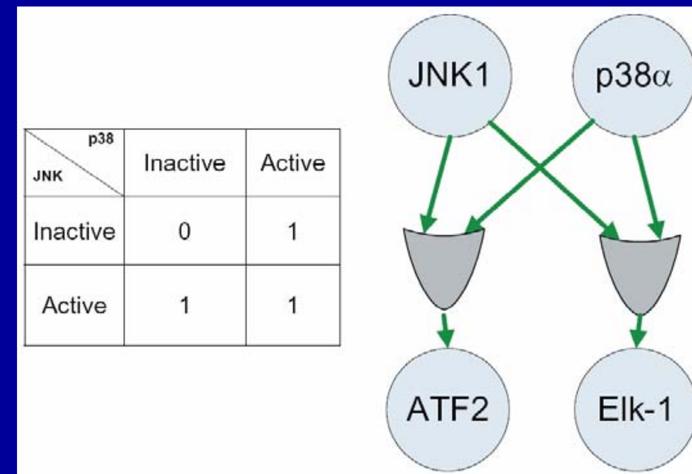


	Motifs counts		
Motif #	**CN***	**SN****	**Z-score**
31	16	4.8 ± 2.8	3.98
32	22	9.3 ± 3.3	3.84
33	14	8.1 ± 2.6	2.30
34	36	12.5 ± 3.7	6.38
35	32	12.2 ± 3.8	5.16
36	25	9.8 ± 3.7	4.12
41	1011	186.6 ± 32.6	25.31
42	[illegible]	[illegible]	[illegible]
43	26	7.2 ± 4.8	3.91
44	303	104.0 ± 15.5	12.88
45	57	17.8 ± 7.3	5.39
46	105	40.0 ± 10.9	5.97
47	49	31.5 ± 8.3	2.12

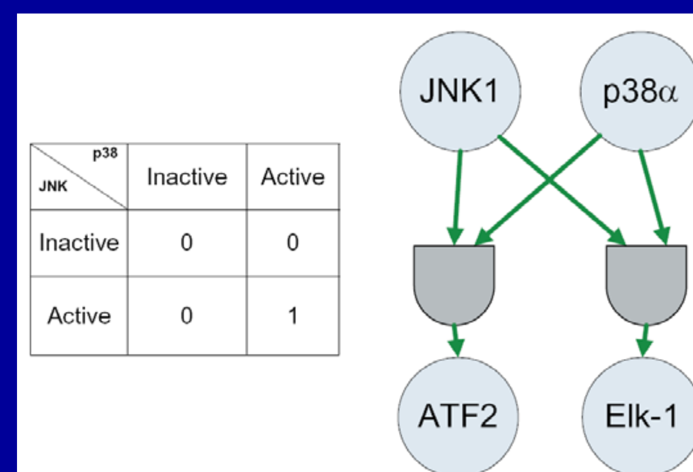
* CN- Cellular Network.
** SN- Shuffled networks.
Mean ± SD computed for 100 shuffled networks.

Two protein kinases phosphorylate two transcription factors



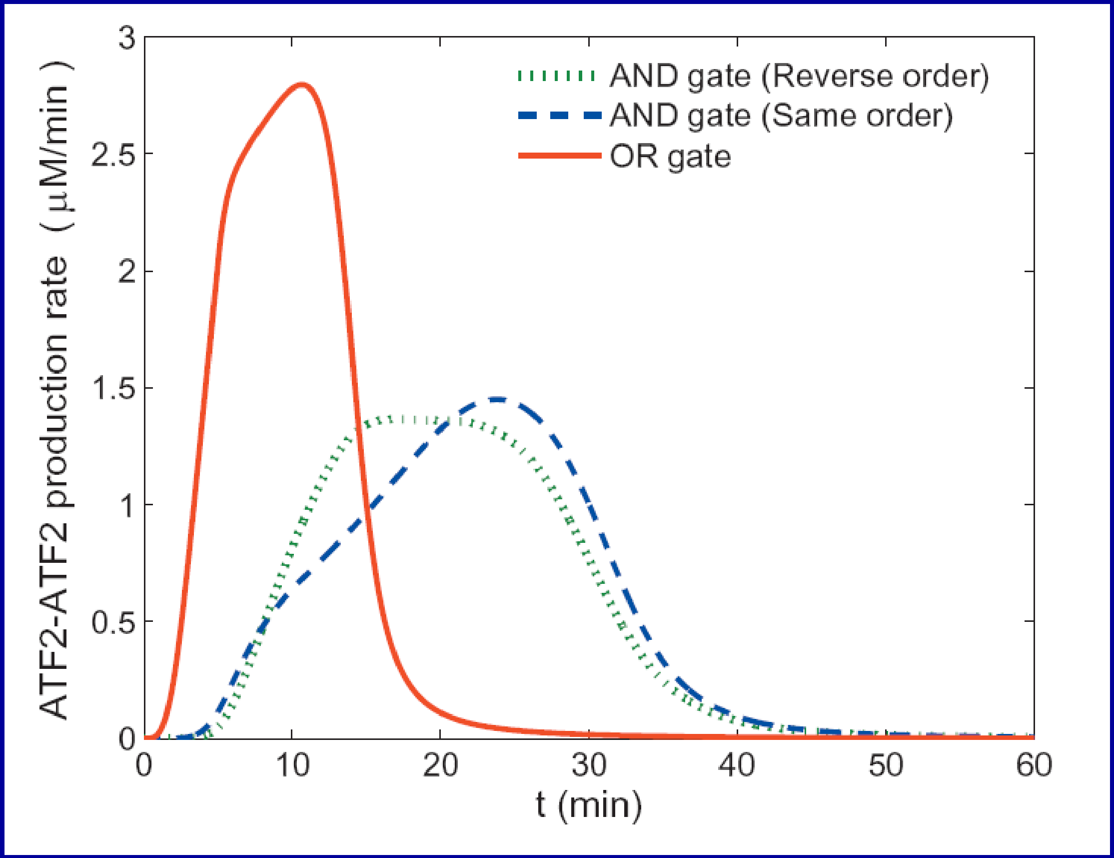


JNK \ p38	Inactive	Active
Inactive	0	1
Active	1	1



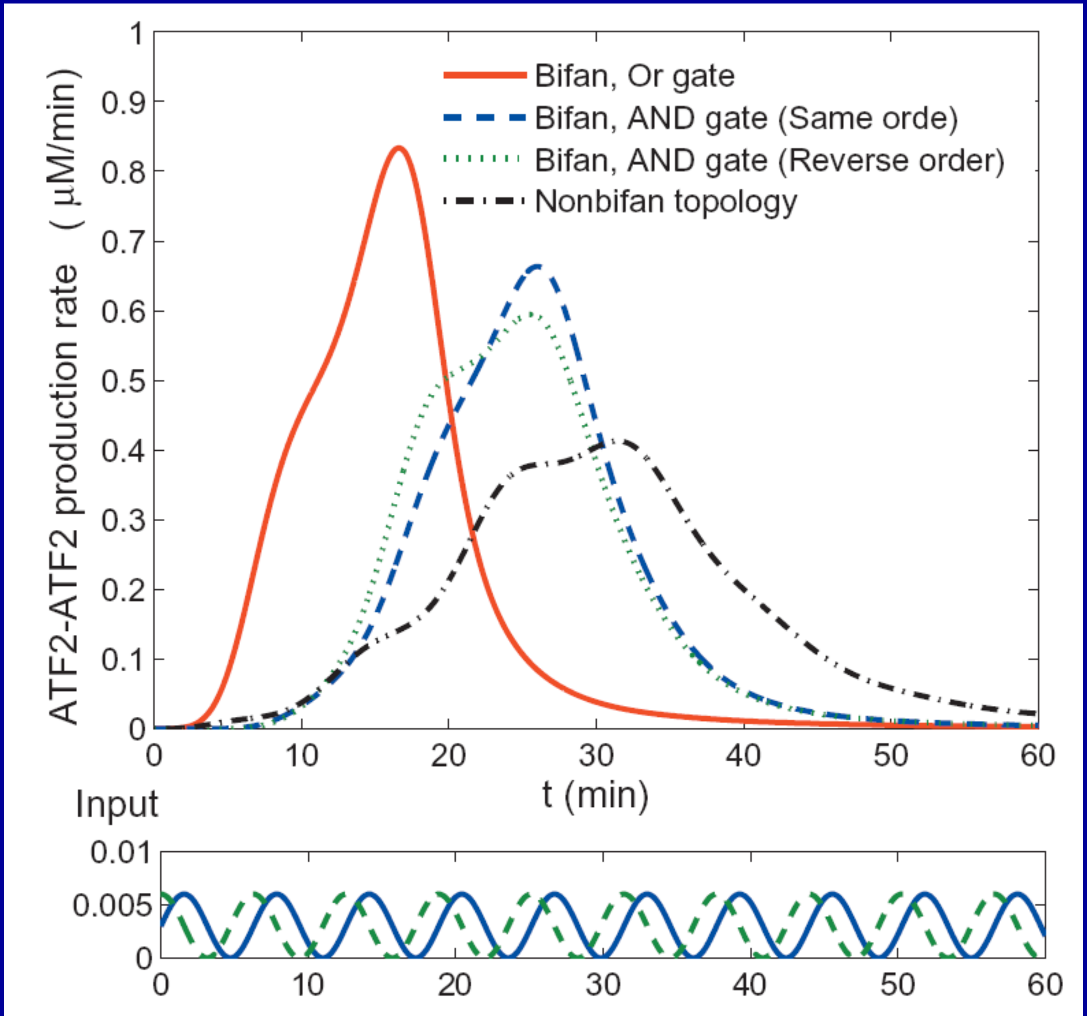
JNK \ p38	Inactive	Active
Inactive	0	0
Active	0	1

Comparing OR and AND Gating by Bifans



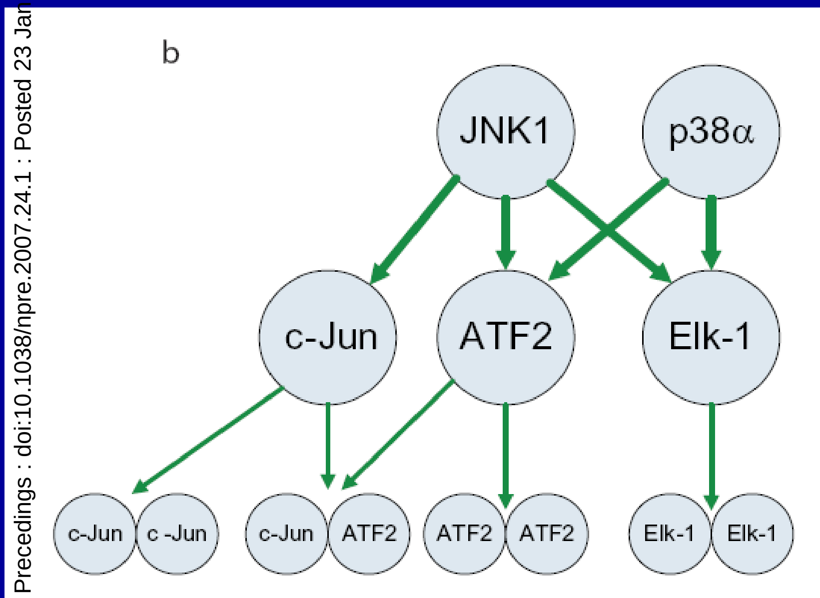
OR gates are sharp and transient
AND gates prolong signals and are shallower

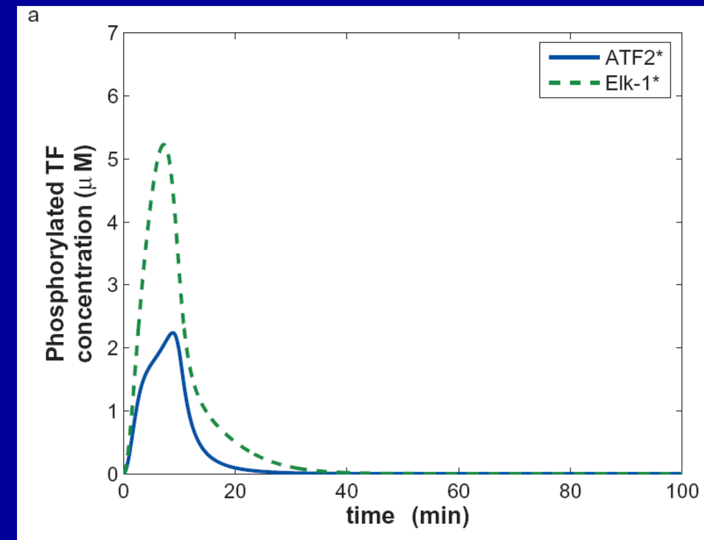
Bifans can Filter Noise



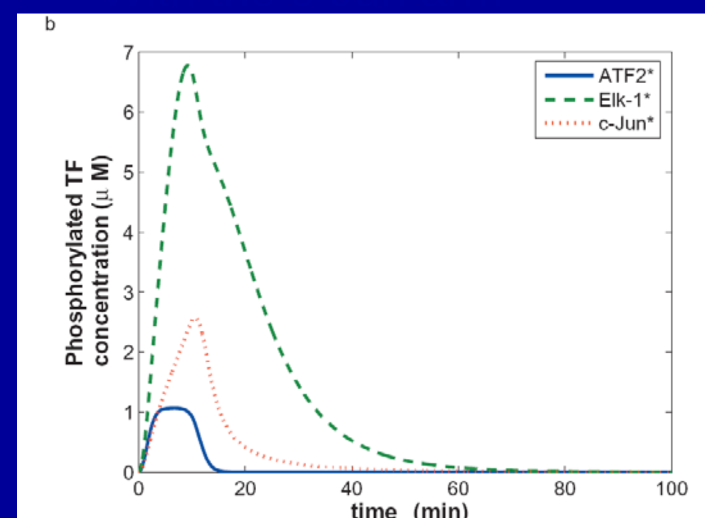
Smoother output from bifans when input signals are choppy

Extending the Bifan by Adding c-Jun

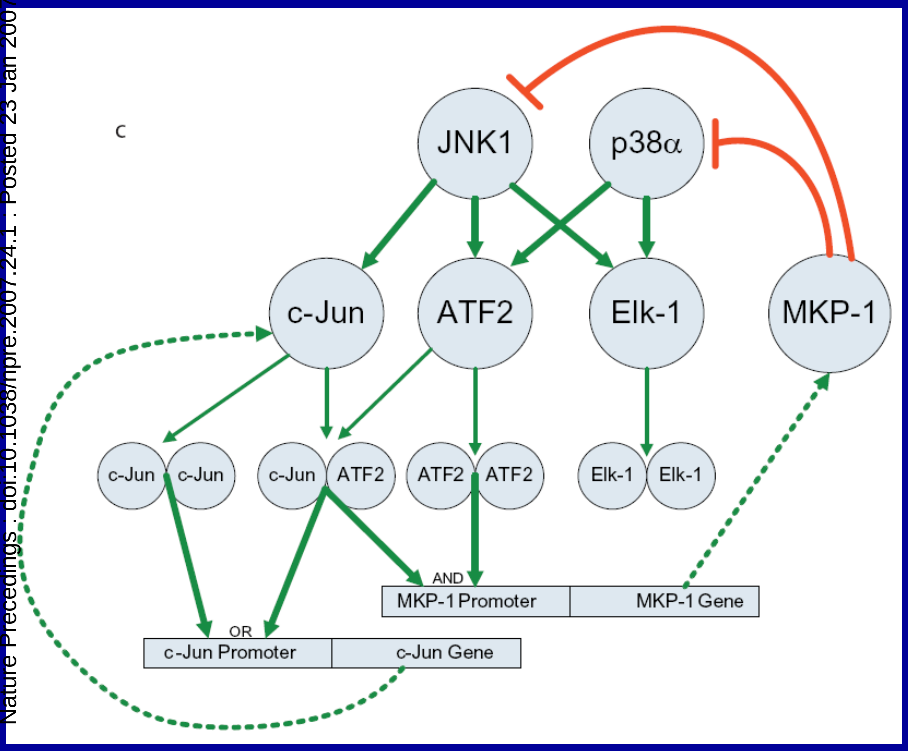


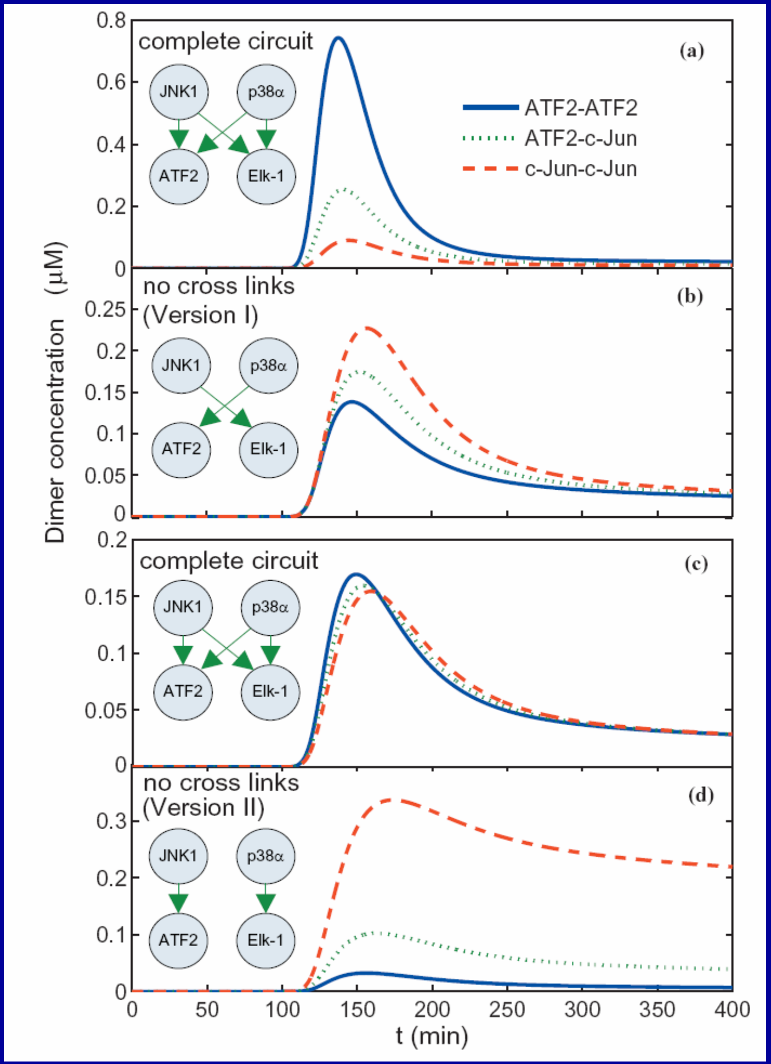


The context of the bifan affects the dynamics



Extending the circuit by adding transcriptional feedback





The bifan configuration can be used to control TFs homo and hetrodimer concentrations to tightly regulate gene expression

Conclusions

- OR gate bifans produce transient with high amplitude output

  AND gate bifans prolongs signals and produce shallower output

- Bifans can filter noise

- The context of the bifan dramatically affects the signal output

  Models of transcriptional feedback coupled with bifans show that the bifan configuration can be used to regulate homo and hetrodimer TFs concentrations

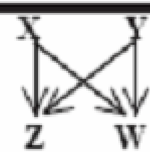
A feedforward motif and its functional significance

Modulation of Kidney Podocyte Differentiation by a Protein Tyrosine Phosphatase-SL-mediated Feedforward Motif from β-adrenergic receptors to CREB

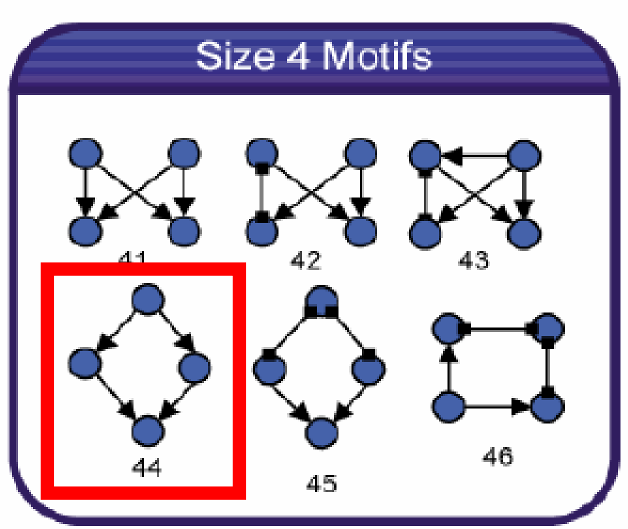
Narat John Eungdamrong

John Cijiang He

Feed Forward Motifs

Network	Nodes	Edges	N_{real}	$N_{rand} \pm SD$	Z score	N_{real}	$N_{rand} \pm SD$	Z score	N_{real}	$N_{rand} \pm SD$	Z score
Gene regulation (transcription)					**Feed-forward loop**			**Bi-fan**			
E. coli	424	519	40	7 ± 3	10	203	47 ± 12	13			
*S. cerevisiae**	685	1,052	70	11 ± 4	14	1812	300 ± 40	41			

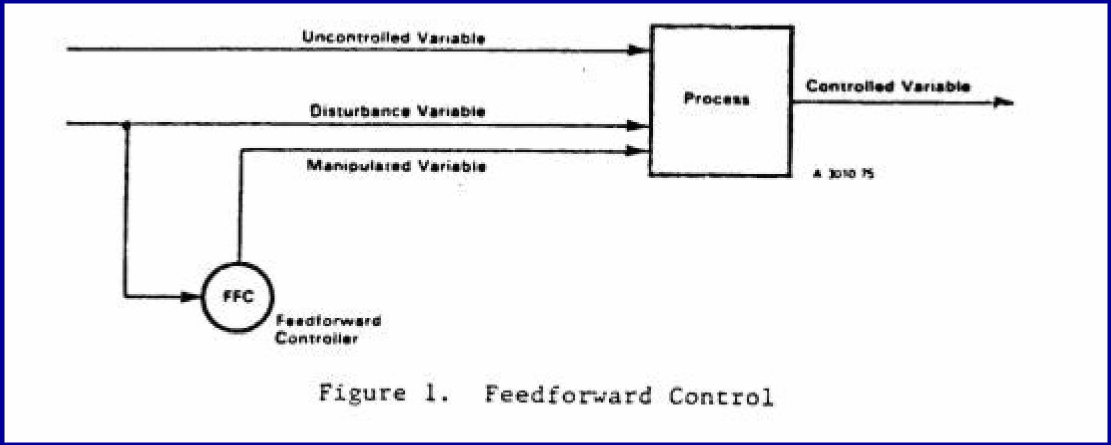
Milo et al. *Science*, **298**, 824 (2002)



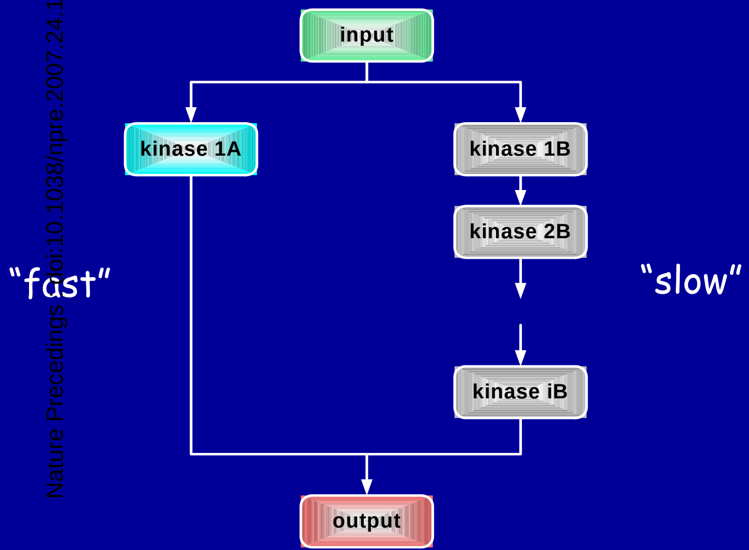
	Motifs counts		
Motif #	**CN***	**SN****	**Z-score**
31	16	4.8 ± 2.8	3.98
32	22	9.3 ± 3.3	3.84
33	14	8.1 ± 2.6	2.30
34	36	12.5 ± 3.7	6.38
35	32	12.2 ± 3.8	5.16
36	25	9.8 ± 3.7	4.12
41	1011	186.6 ± 32.6	25.31
42	108	68.2 ± 14.8	2.69
[illegible]	[illegible]	[illegible]	[illegible]
44	303	104.0 ± 15.5	12.88
45	57	17.8 ± 7.3	5.39
46	105	40.0 ± 10.9	5.97
47	49	31.5 ± 8.3	2.12

* CN- Cellular Network.
** SN- Shuffled networks.
Mean ± SD computed for 100 shuffled networks.

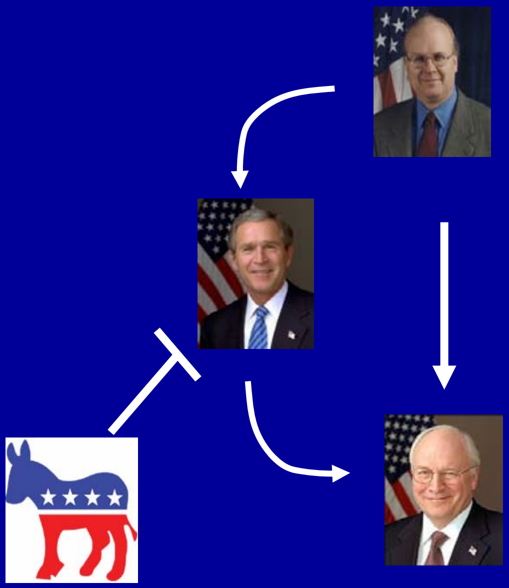
FFMs in Networks



Process Control



Signaling System

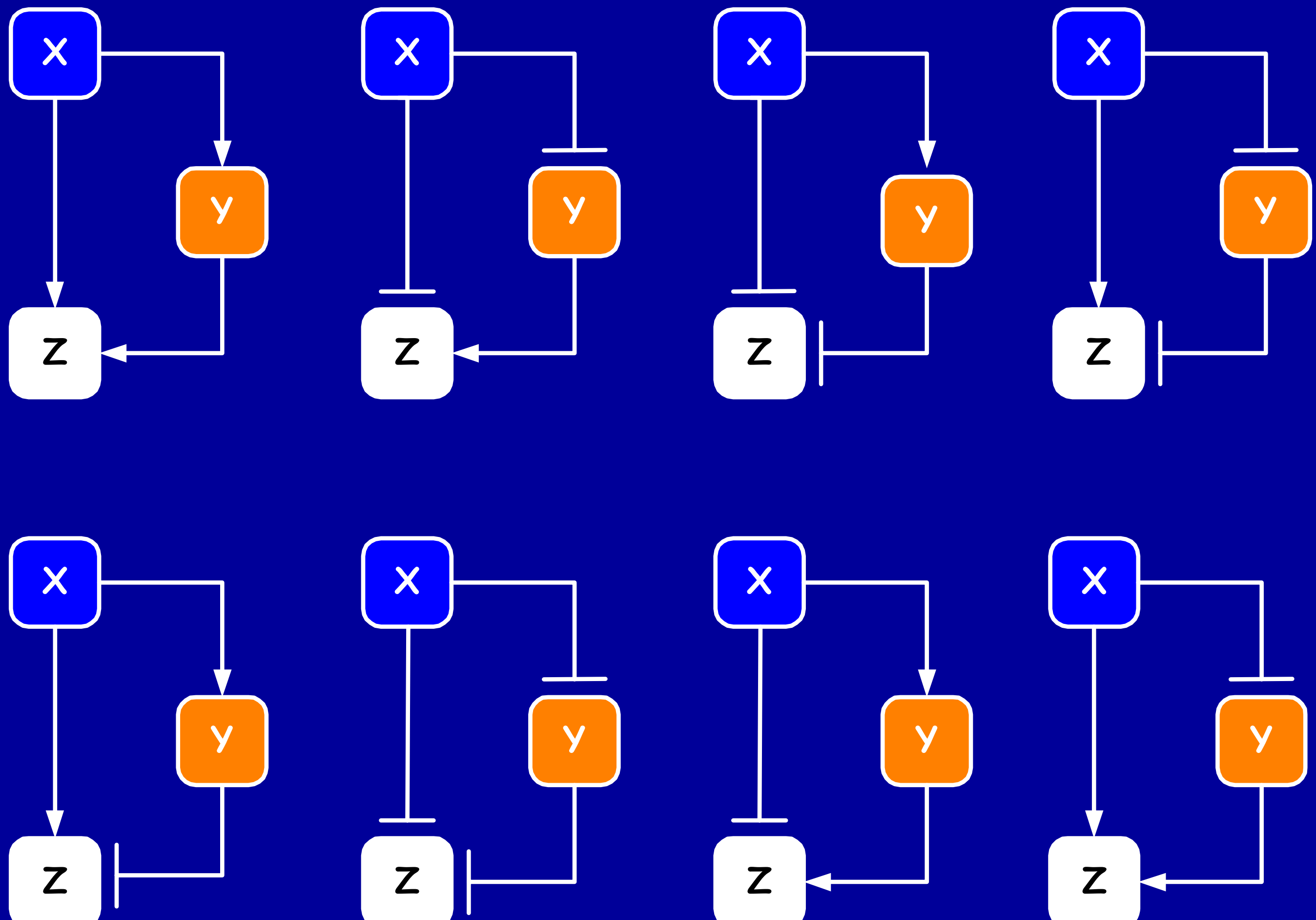


Social Network

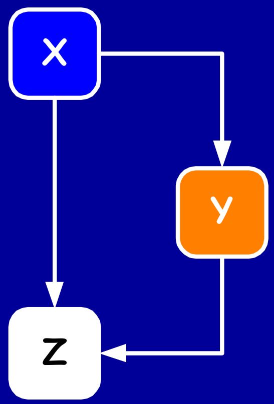
Various types of FFMs

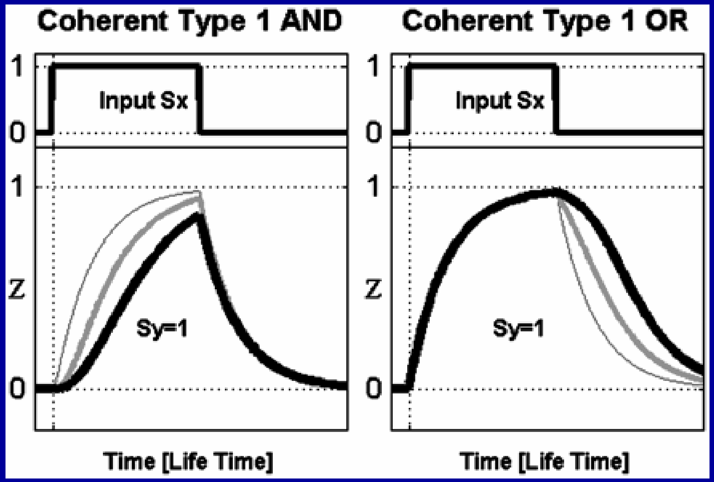
"coherent"

"incoherent"



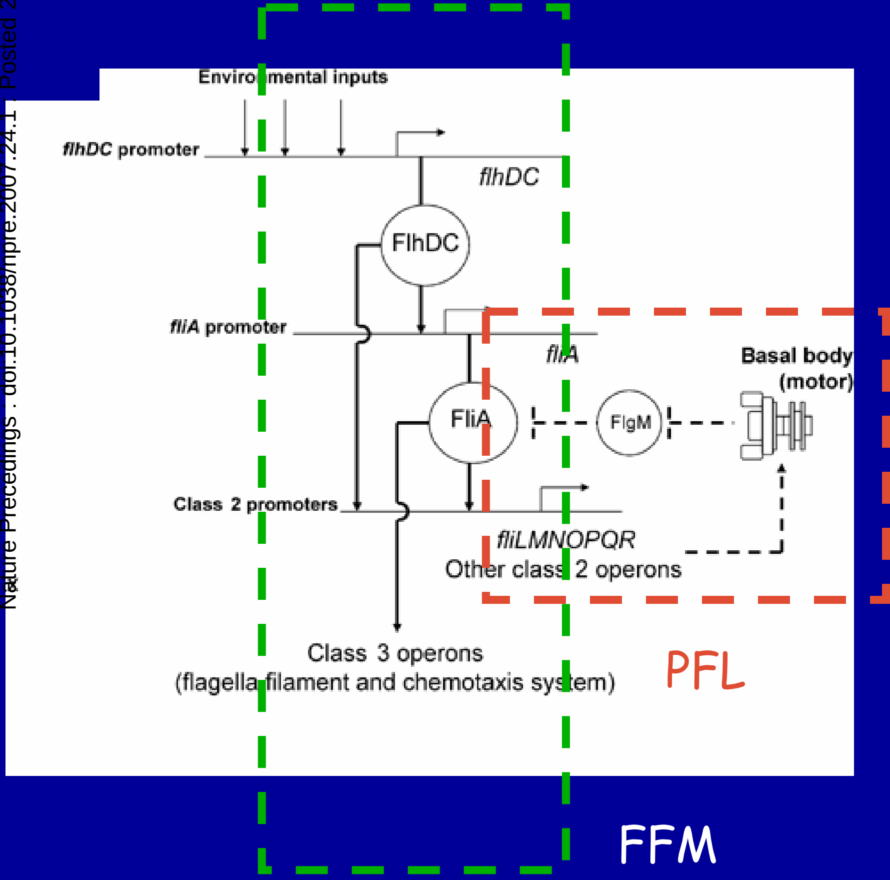
Coherent FFM can yield prolonged outputs

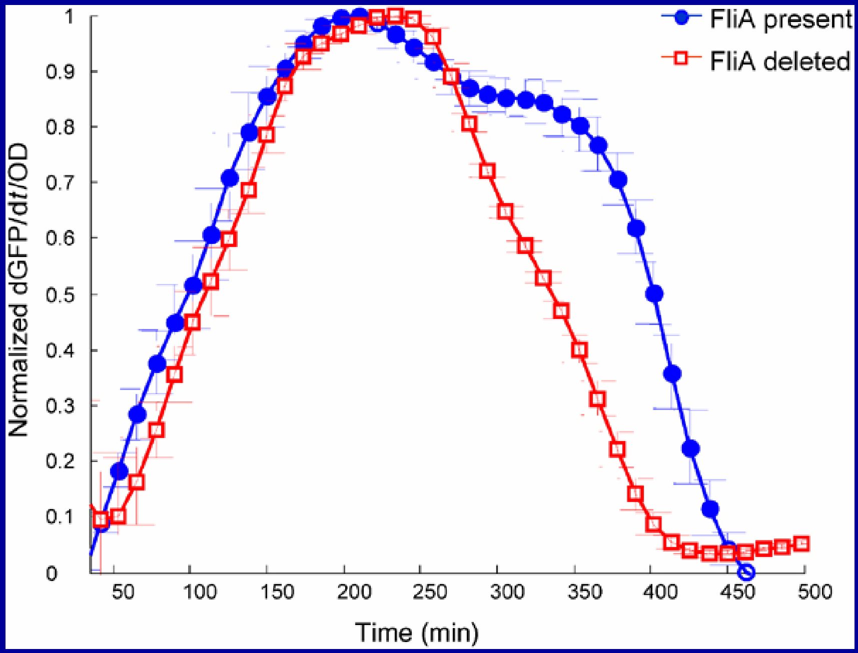




- Coherent FFM with an OR gate (e.g. Z can be activated by either X or Y) can prolong output.
- This results from the time required for Y to deactivate.
- Difference in the kinetics of the “long” and “short” pathways does not significantly alter the motif behavior.

Coherent OR FFM can contribute to prolonged signaling in transcriptional networks





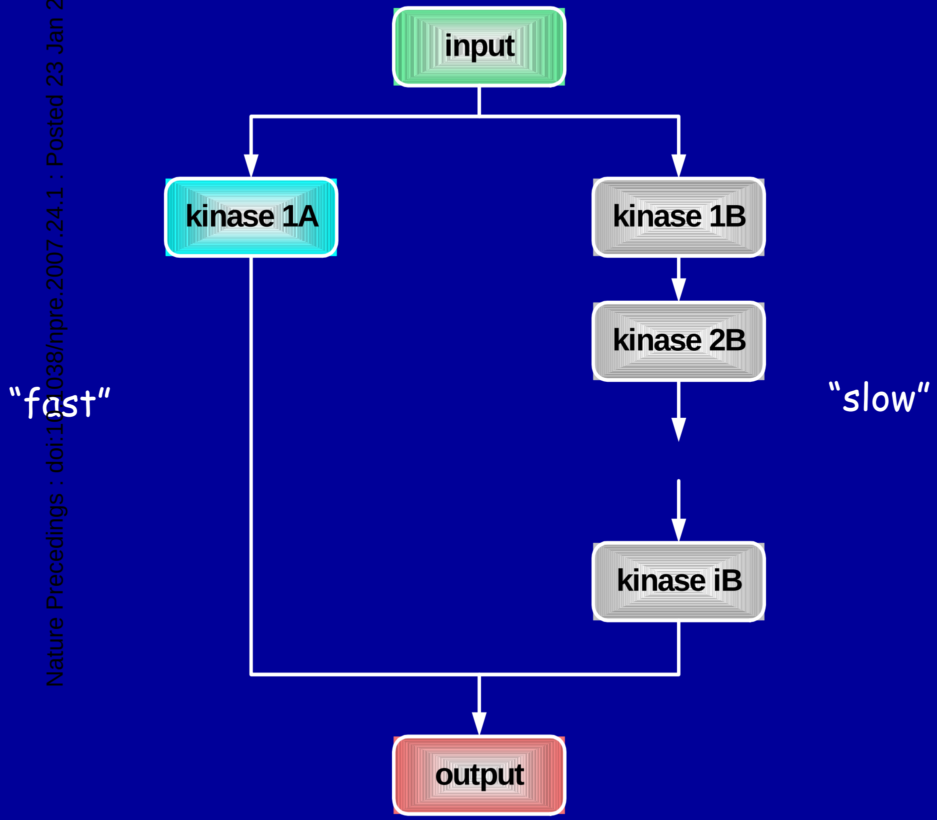
(Mol Syst Biol. 2005 Mar 29.)

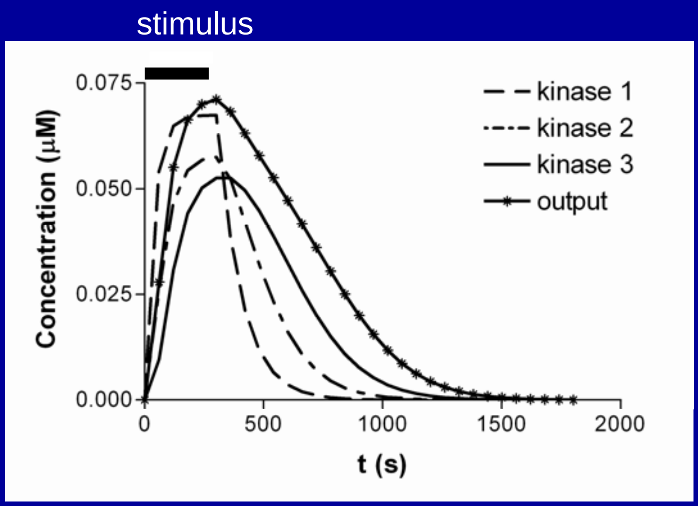
Key Questions

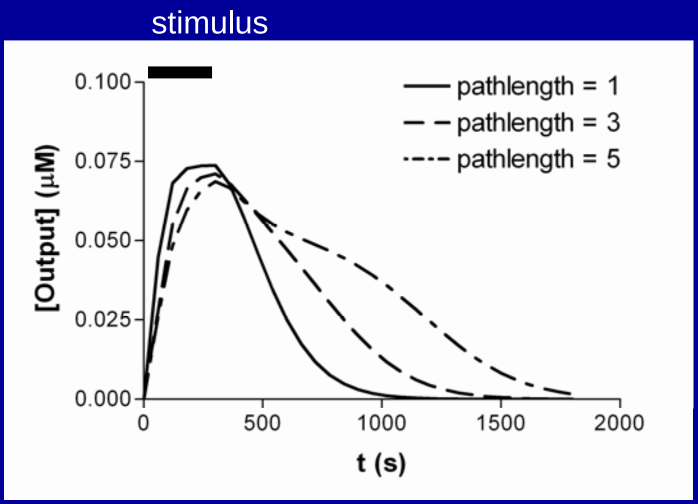
- What is the regulatory role of FFMs in signaling networks?
- Can FFM sustain signaling in absence of a PFL?
- Does modulation of FFMs result in meaningful functional changes?

What is the regulatory role of FFMs in signaling networks?

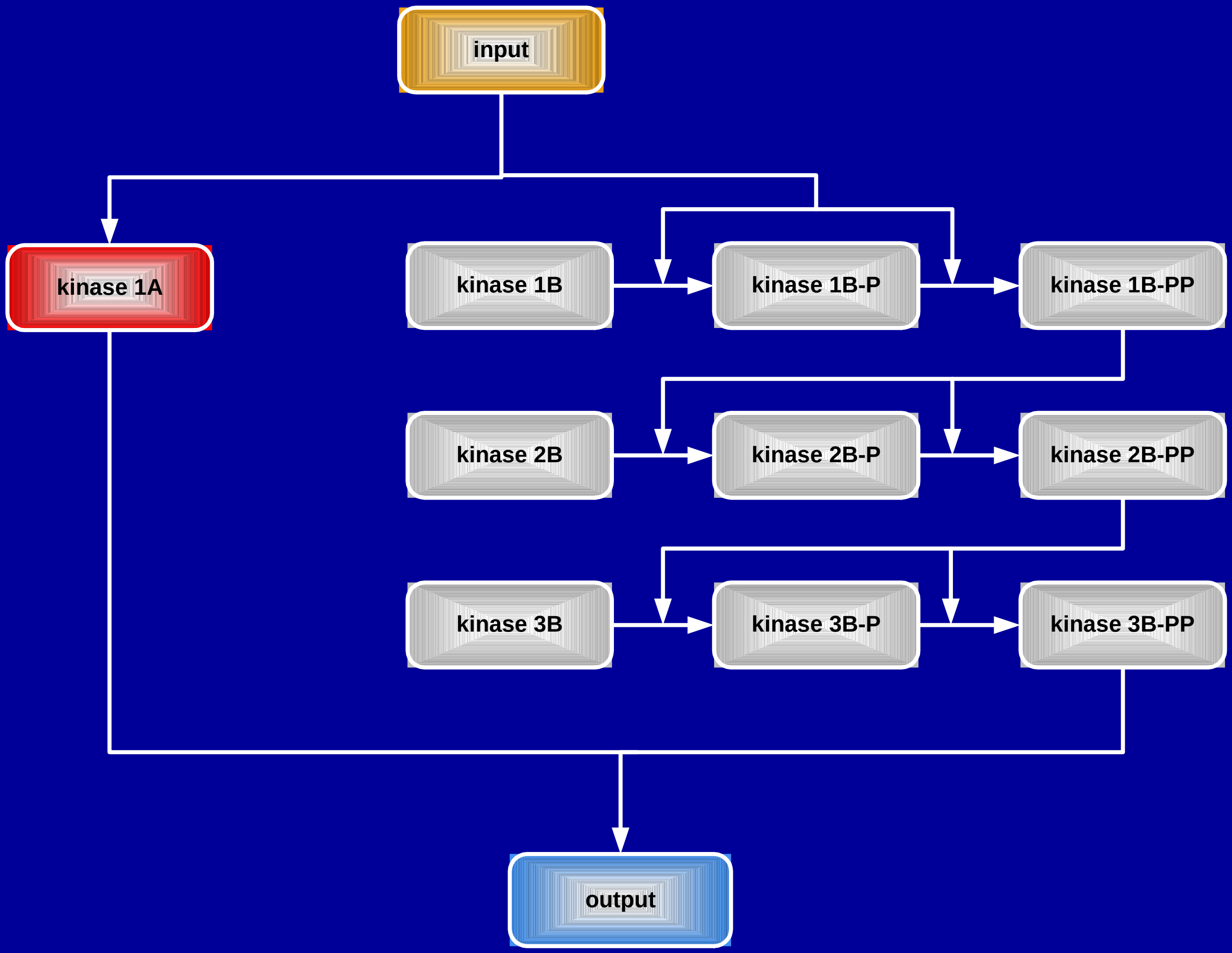
Increasing pathlength increases length of output signal





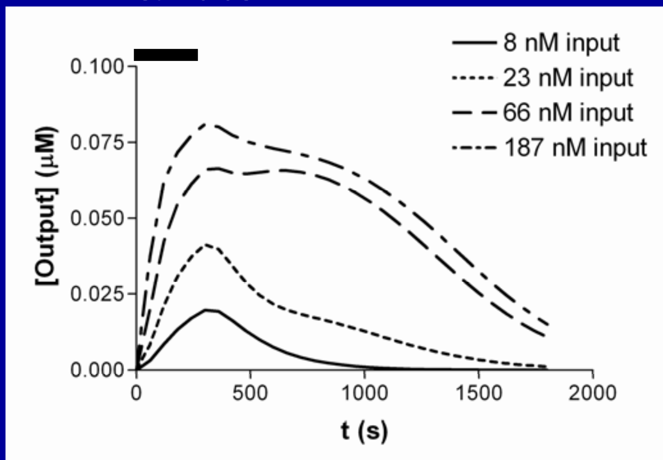


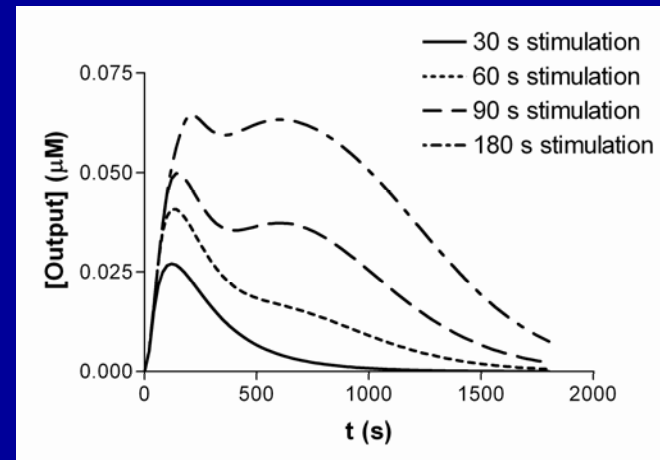
Effect of multisite phosphorylation on FFM behavior



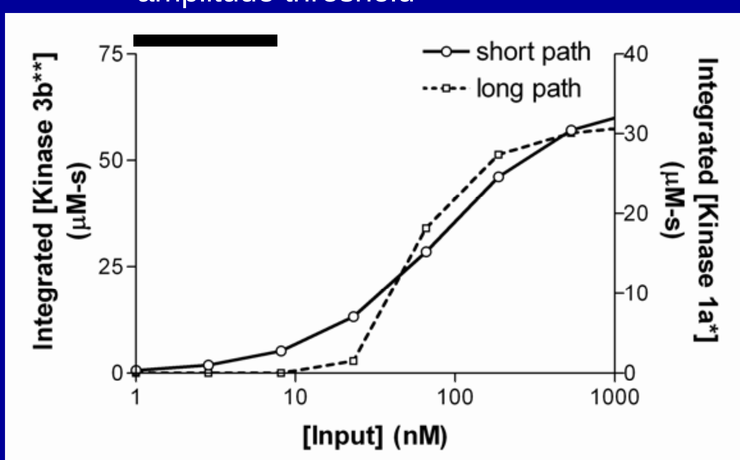
Noise Filtering by ultrasensitive FFMs

stimulus

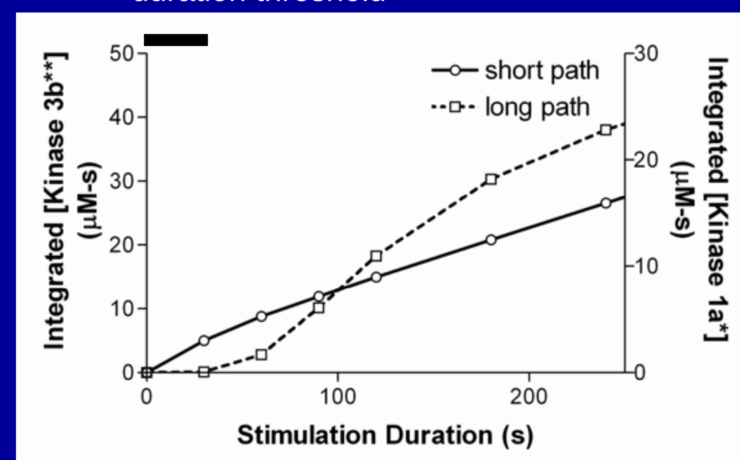




amplitude threshold



duration threshold



Conclusions

- Simple FFM can sustain signaling.
- Phosphatase activity sets the timescale for signaling.
- Motif behaviors are relatively robust to perturbation in [input] and changes in kinetic parameters.
- Increasing pathlength can prolong signal output.
- Incorporation of an ultrasensitive cascade allows detection of stimulus strength (amplitude & duration).

Can a feedforward motif prolong signal output in absence of a positive feedback loop?

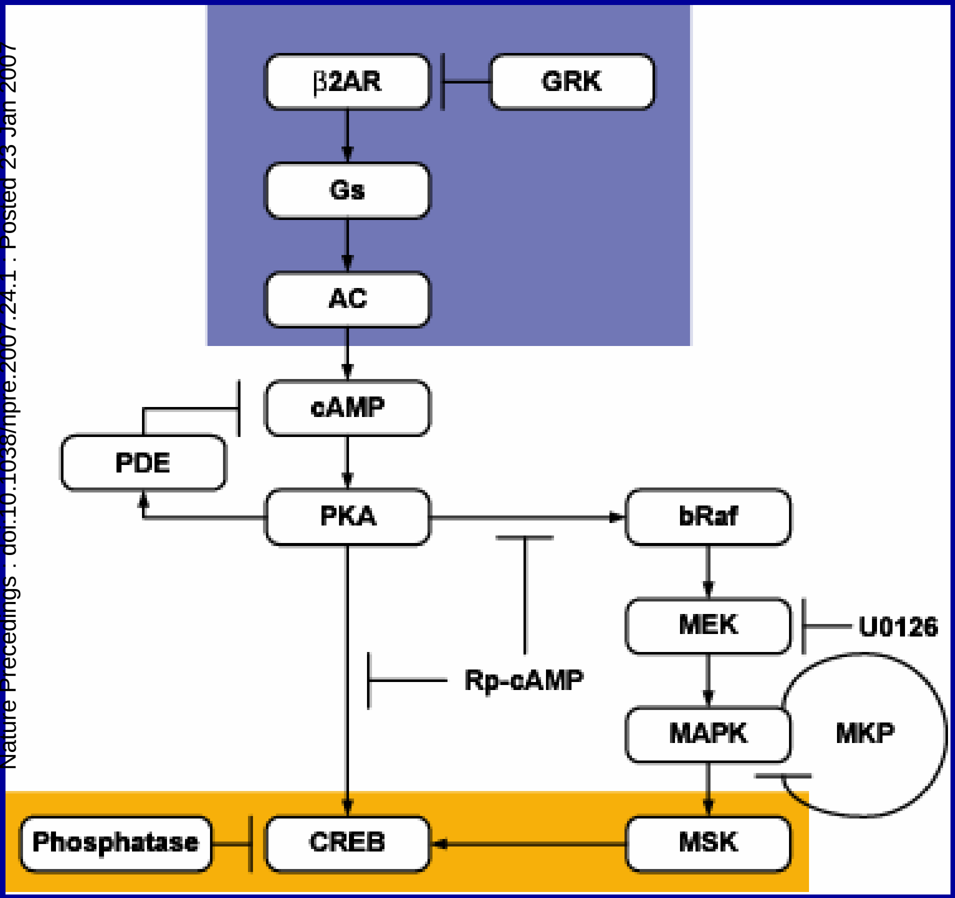
CREB activation as an experimental model for studying FFM

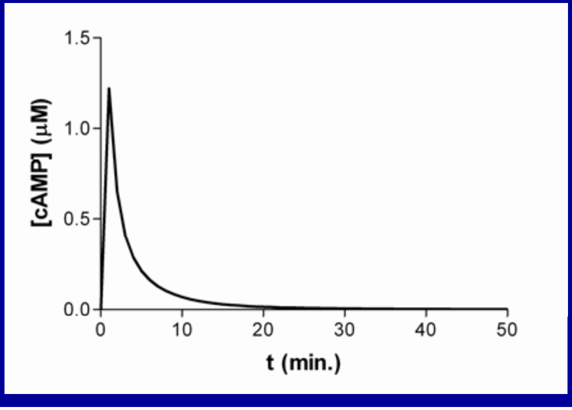
- CREB, a key regulator of gene expression, is activated by phosphorylation on Ser-133.

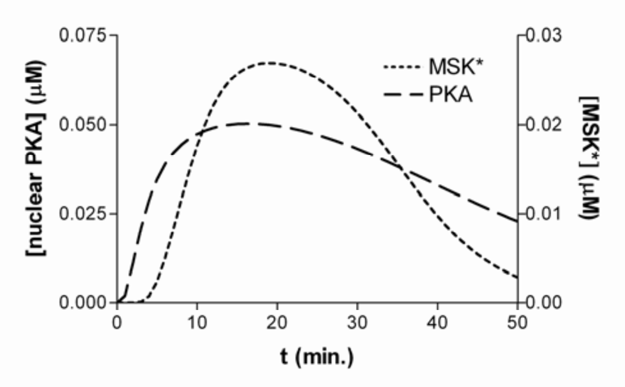
- CREB is responsive to multiple intracellular signaling pathways

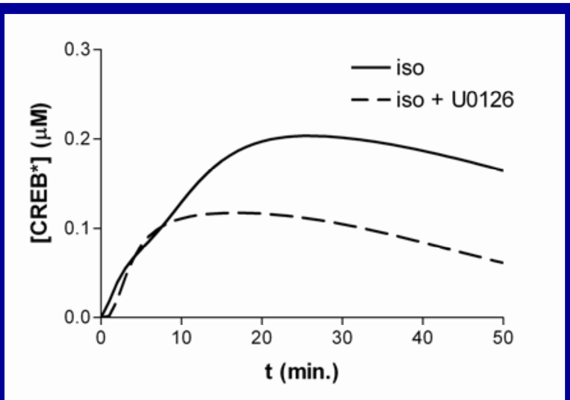
(e.g. Ser133 is phosphorylated by PKA, and MSK)

A Feedforward Motif in CREB Activation in Kidney Podocytes

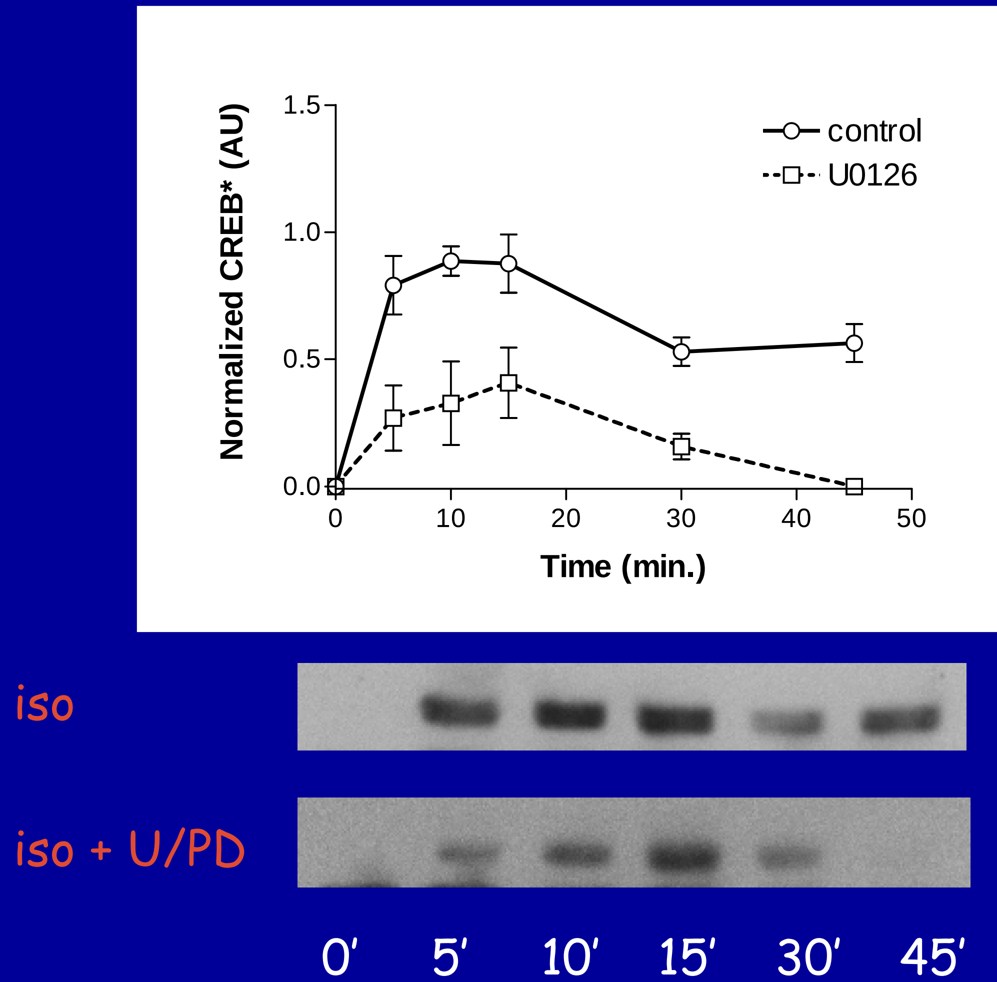




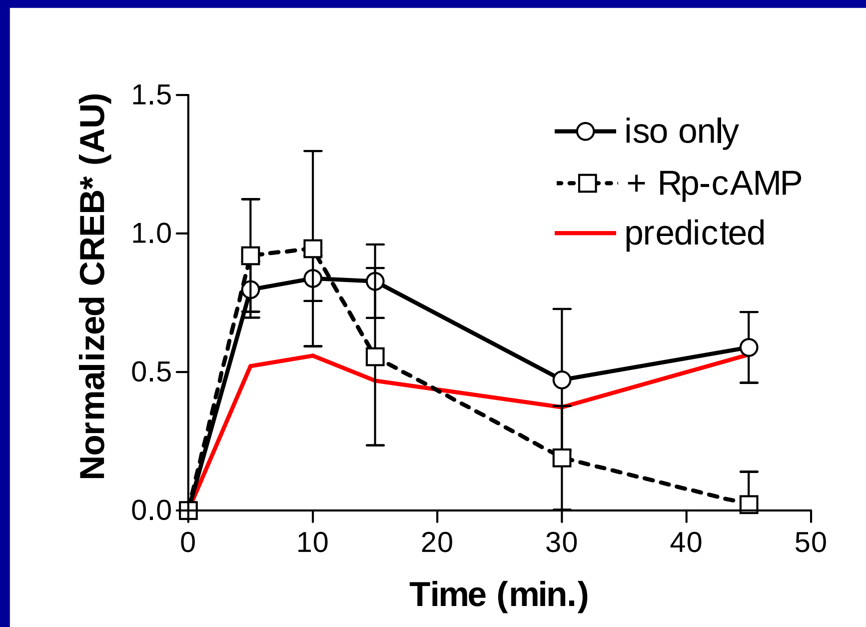




MEK Inhibition Blocks Sustained CREB Activation

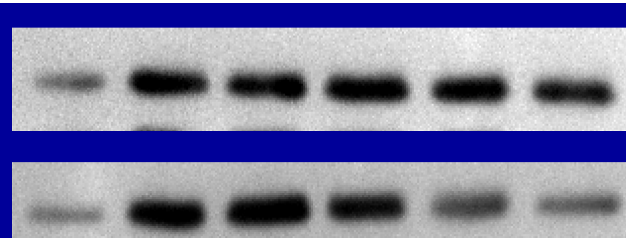


PKA inhibition indicated a more complex network



iso

iso + Rp-cAMP

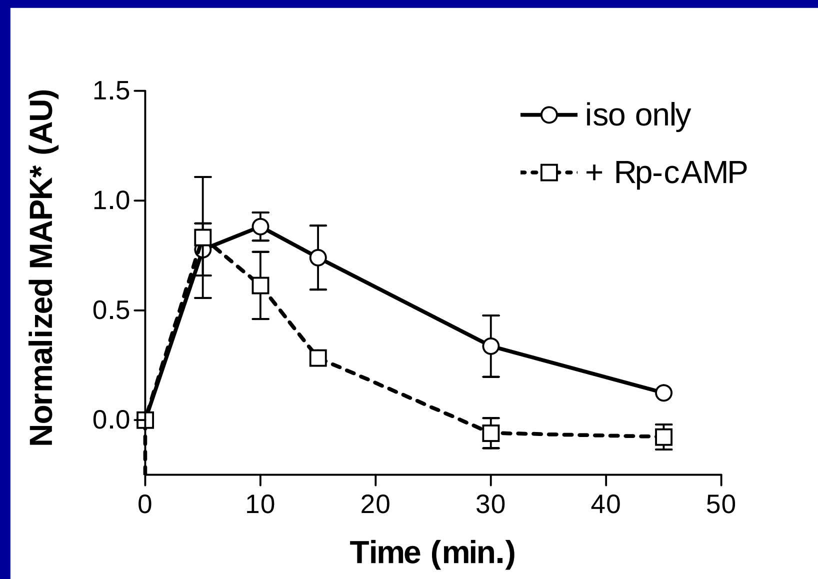


0' 5' 10' 15' 30' 45'

Initial Conclusions

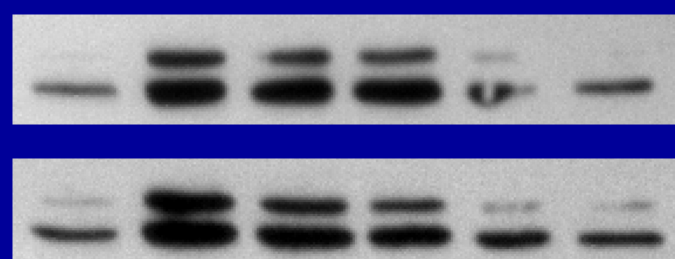
- FFM can prolong CREB signaling in kidney podocytes
- Sustained signaling requires both PKA and MAPK signaling (“coincidence detection”)
  - *Synergy between pathways, not simply additive*
- Experiments indicate the following:
  - PKA-independent mechanism of MAPK activation
  - Potential interactions between PKA and MAPK pathways in prolonging output signals

MAPK Can be Activated via a PKA-independent Mechanism



iso

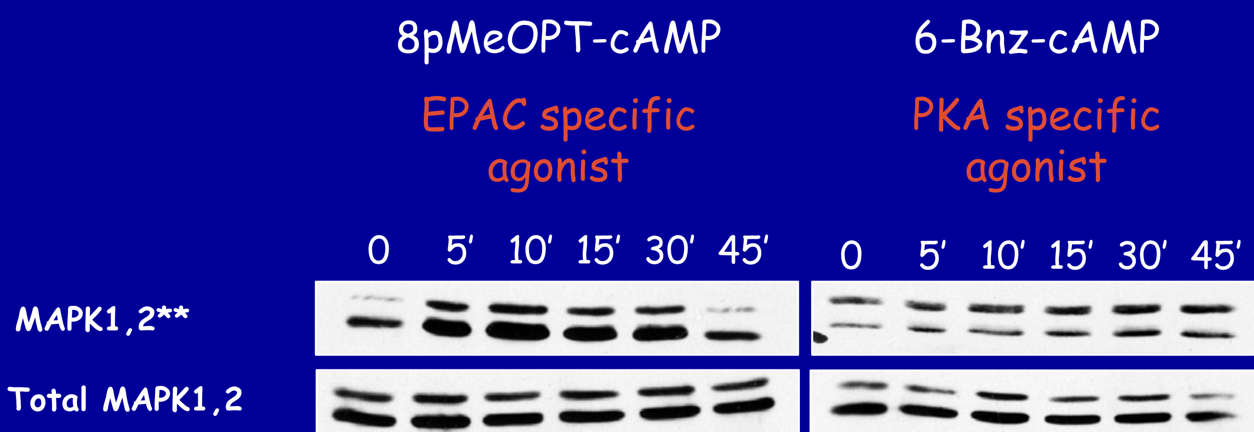
iso + Rp-cAMP



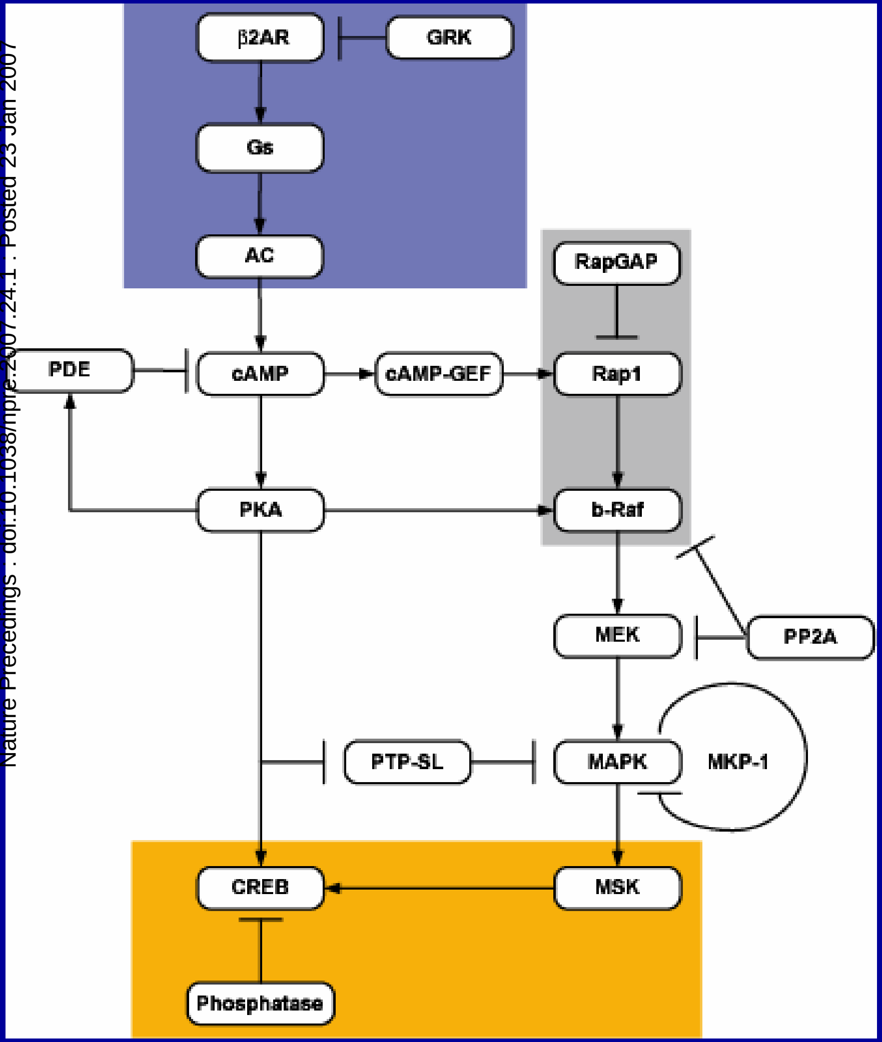
0′ 5′ 10′ 15′ 30′ 45′

Role of the cAMP/EPAC/Rap1 pathway?

EPAC Agonist Activates MAPK in Kidney Podocytes



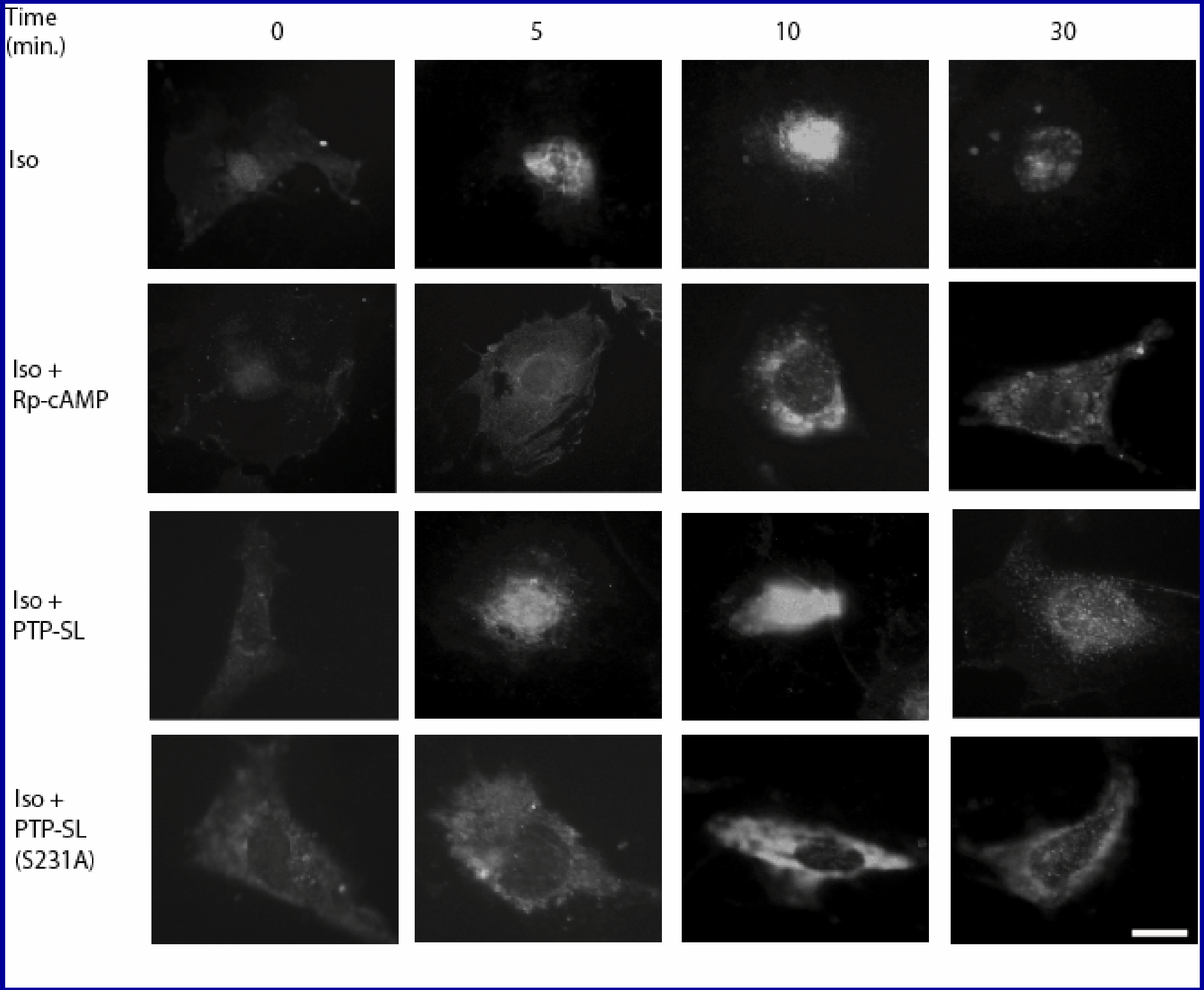
Modulation of MAPK activity via a Spatially Specific Feedback Loop



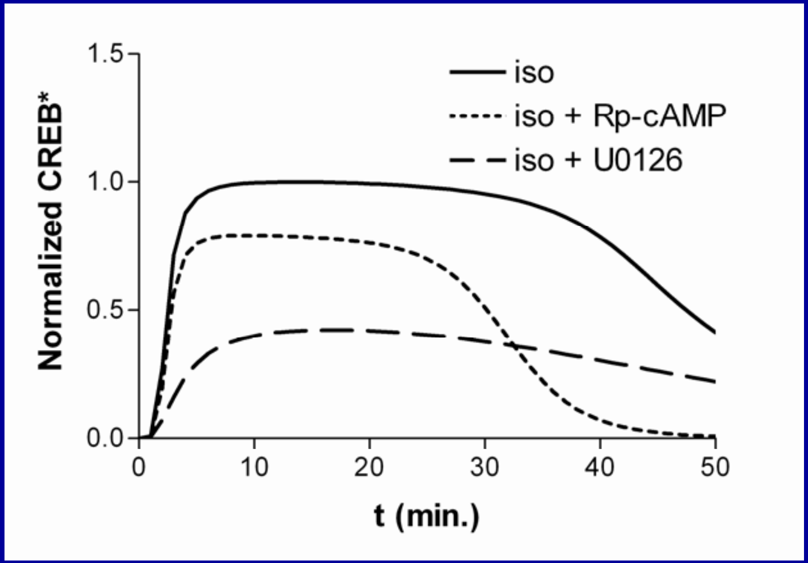
- PTP-SL contains a substrate recognition domain (KIM) in its noncatalytic region.
- Phosphorylation of the KIM of PTP-SL by PKA inhibits PTPSL's association with and the tyrosine dephosphorylation of ERK1/2 and p38.
- Nuclear translocation of ERK1/2 and p38 , in the presence of PTP-SL, is favored upon activation of PKA.
- We hypothesize that Rp-cAMP treatment lifts PKA-dependent inhibition.

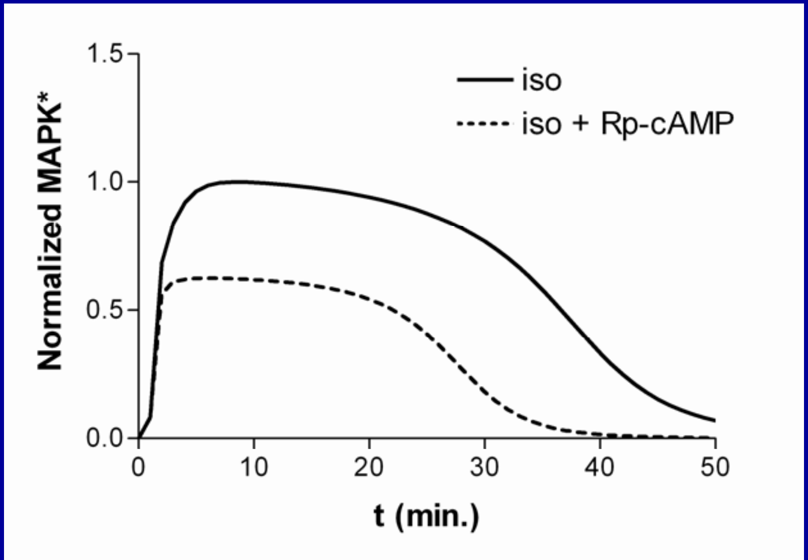
Thus, PTP-SL can retain MAPK in the cytoplasm and prevent its translocation to the nucleus.

PKA regulates nuclear localization of MAPK* by PTP-SL Inhibition



Nested Feedforward loops regulate the duration of CREB activation





Conclusions from 2nd round models

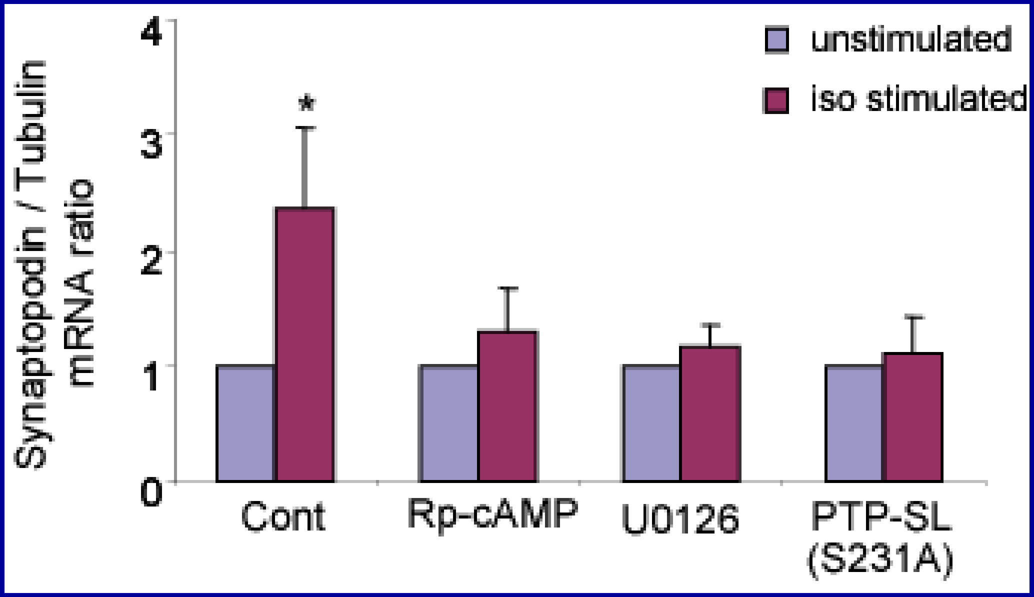
- Nesting results in CREB control of CREB activation by time-dependent AND and OR gates
- Iso stimulation activates MAPK through a PKA-independent mechanism (Epac/Rap1/BRaf)
- Rp-cAMP treatment did not strongly affect total cellular MAPK activity. However, persistent activation of CREB is strongly inhibited (due the spatial regulation)
- PKA modulates nuclear localization of MAPK* through inhibition of PTP-SL.

  Immunofluorescence experiments indicate that PKA dynamically regulates the cellular location of MAPK signaling
- Simulation of the nested FFM network qualitatively captures the salient features of CREB activation seen in the experiments

Nature Precedings : doi:10.1038/npre.2007.24.1 : Posted 23 Jan 2007

What is the physiological function of FFM?

Modulation of Podocytes Differentiation by PTP-SL



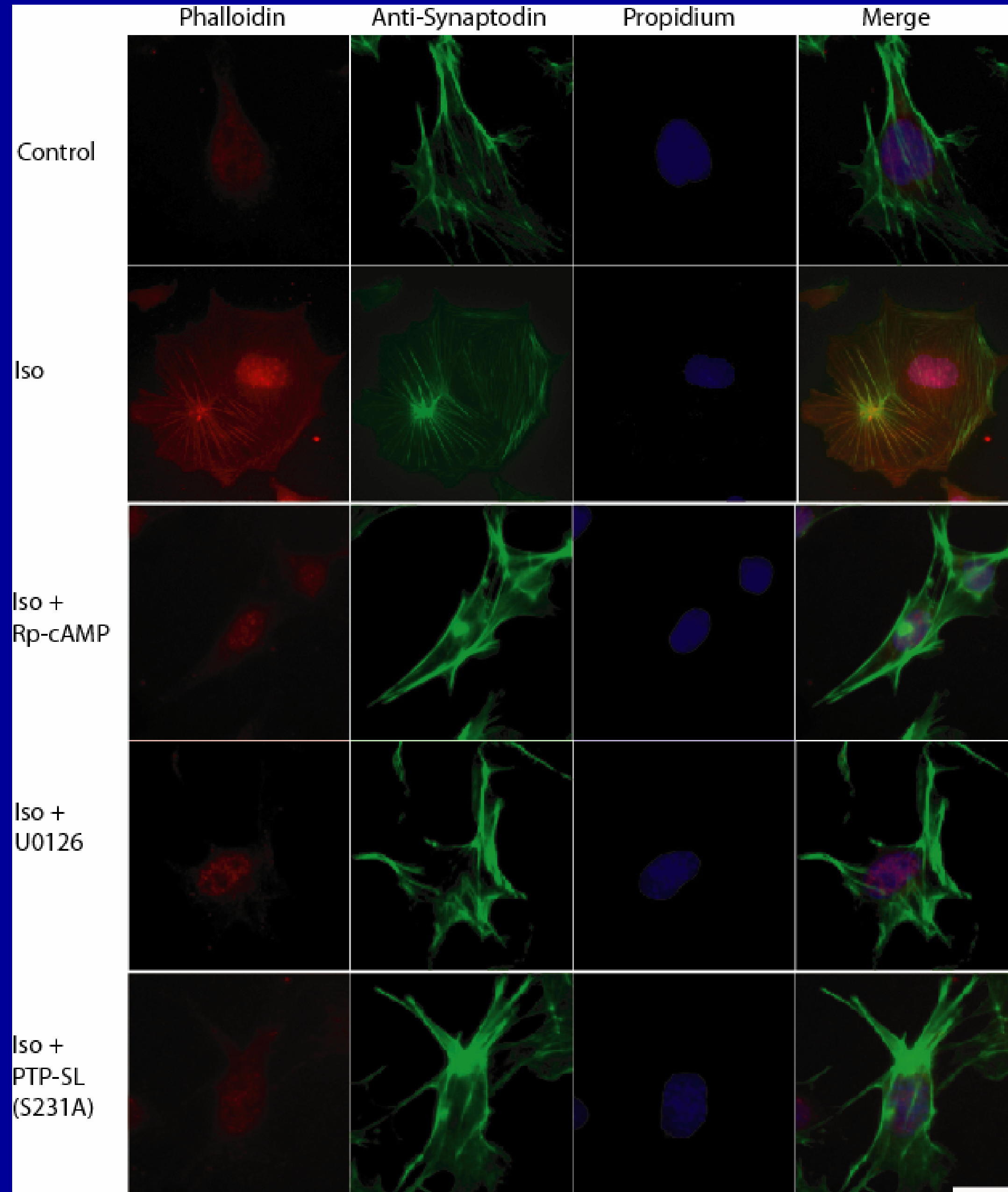
Synaptopodin is a marker for the differentiated state of podocytes

as the name suggests synaptopodin is required for the formation of the foot processes

Primary Kidney podocytes

In the differentiated state Synaptopodin colocalizes with the actin filaments

Inhibitors that block the FFL also block colocalization of synaptopodin with the actin bundles



Conclusions
Functions of feedforward motifs

- FFM can prolong signal output in a mammalian signaling network
- Coincidence detection and synergy between short and long paths emerge as a result of spatially specific nesting (via PTP-SL)
- Nested FFM modulates the proliferation-differentiation switch in kidney podocytes, as measured by synaptopodin expression.

Overall Conclusions

Regulatory motifs possess considerable information processing capabilities

Spatial specification of motifs may be critical in understanding their functional capabilities

Combining motifs by stacking and nesting can lead to complex behaviors that underlie decisions regulating changes in cell state