Abstract Machines of Systems Biology

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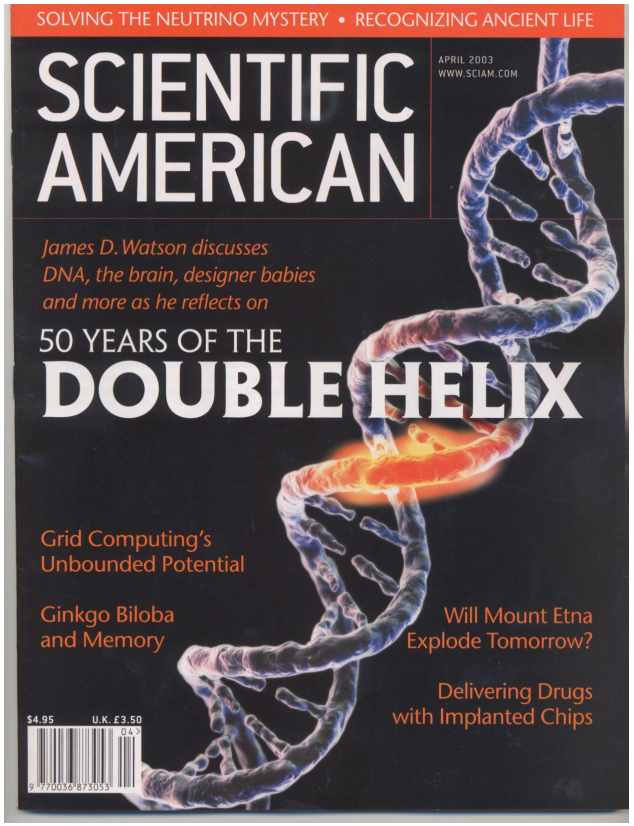
50 Years of Molecular Cell Biology

- Genes are made of DNA
  - Store digital information as sequences of 4 different nucleotides
  - Direct protein assembly through RNA and the Genetic Code

- Proteins (>10000) are made of amino acids
  - Process signals
  - Activate genes
  - Move materials
  - Catalyze reactions to produce substances
  - Control energy production and consumption

- Bootstrapping still a mystery
  - DNA, RNA, proteins, membranes are today interdependent. Not clear who came first
  - Separation of tasks happened a long time ago
  - Not understood, not essential



Towards Systems Biology

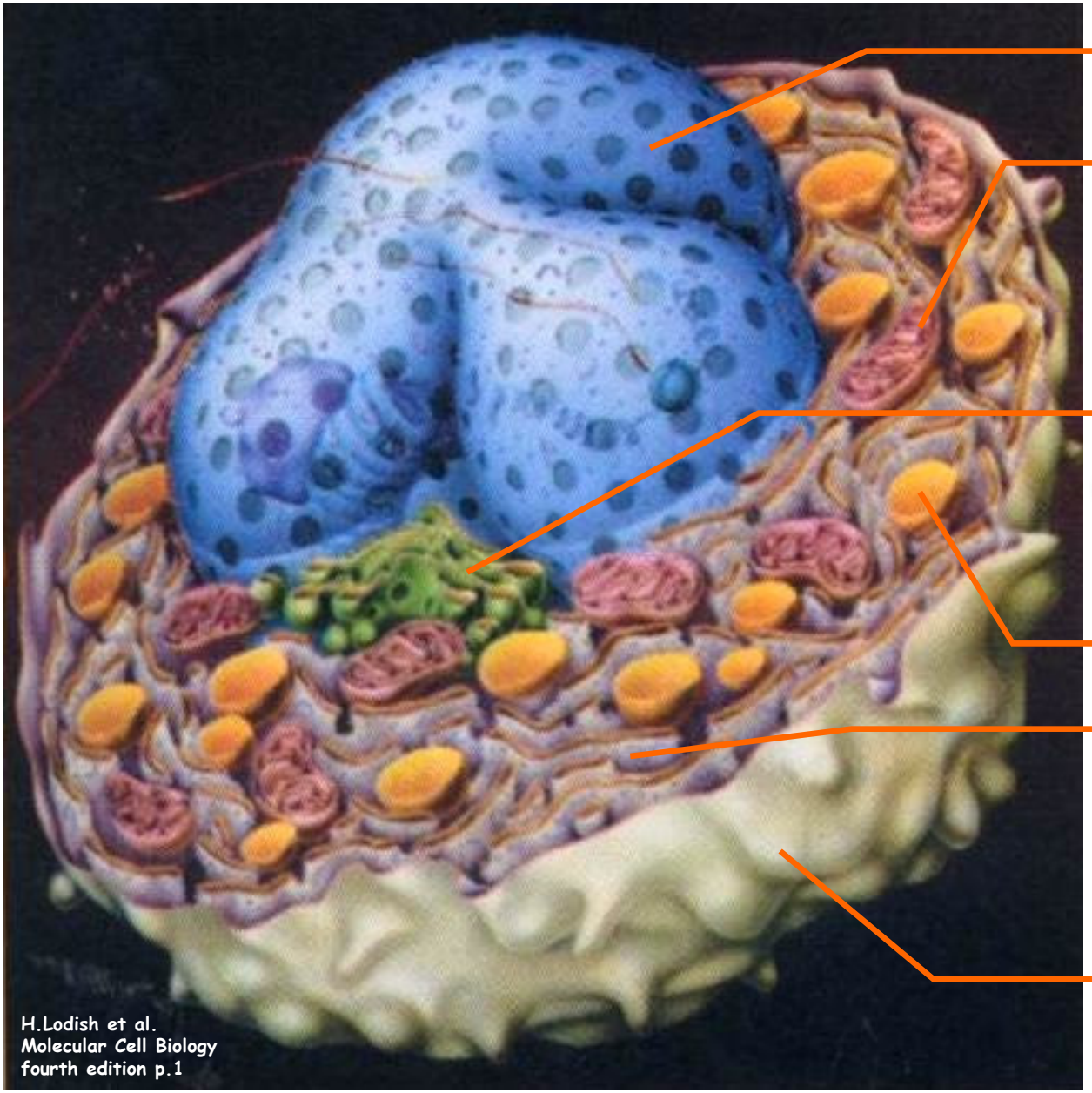
- Biologists now understand many of the cellular components
  - A whole team of biologists will typically study a single protein for years
  - Reductionism: understand the components in order to understand the system
- But this has not led to understand how "the system" works
  - Behavior comes from complex patterns of interactions between components
  - Predictive biology and pharmacology still rare
  - Synthetic biology still unreliable
- New approach: try to understand "the system"
  - Experimentally: massive data gathering and data mining (e.g. Genome projects)
  - Conceptually: modeling and analyzing networks (i.e. interactions) of components
- What kind of a system?
  - Just beyond the basic chemistry of energy and materials processing…
  - Built right out of digital information (DNA)
  - Based on information processing for both survival and evolution
  - *Highly* concurrent
- Can we fix it when it breaks?
  - Really becomes: How is information structured and processed?

Structural Architecture

Eukaryotic Cell

(10~100 trillion in human body)

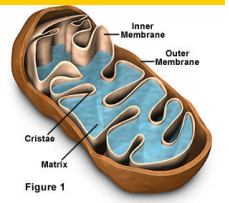
Membranes everywhere



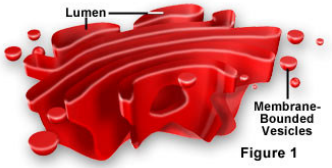
H.Lodish et al.
Molecular Cell Biology
fourth edition p.1

Nuclear membrane

Mitochondria

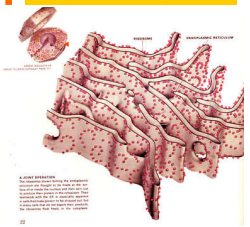


Golgi



Vesicles

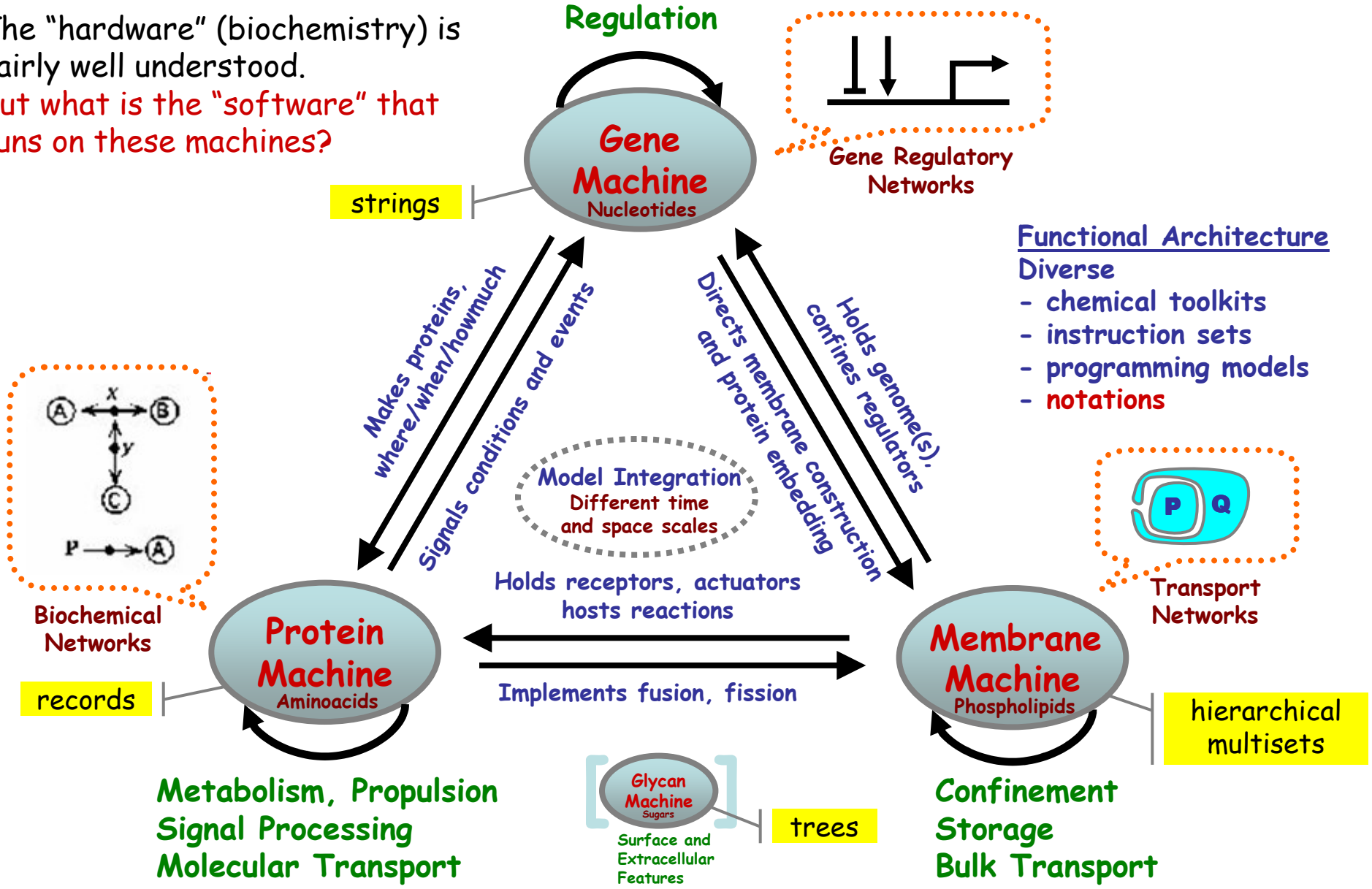
E.R.



Plasma membrane (<10% of all membranes)

Abstract Machines of Systems Biology

The "hardware" (biochemistry) is fairly well understood.
But what is the "software" that runs on these machines?



Methods

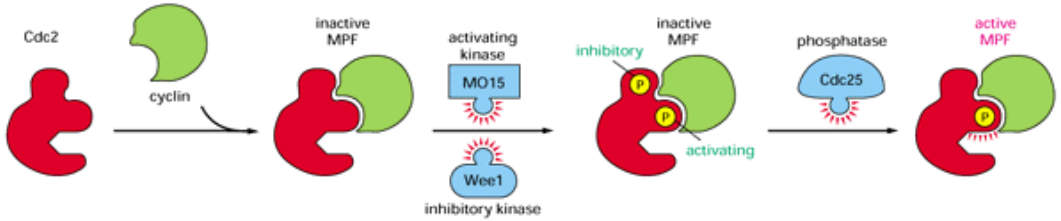
- Model Construction (writing things down precisely)
  - Formalizing the notations used in systems biology.
  - Formulating description languages.
  - Studying their kinetics (semantics).

- Model Validation (using models for postdiction and prediction)
  - Simulation from compositional descriptions
    - Stochastic: quantitative concurrent semantics.
    - Hybrid: discrete transitions between continuously evolving states.
  - "Program" Analysis
    - Control flow analysis
    - Causality analysis
  - Modelchecking
    - Standard, Quantitative, Probabilistic

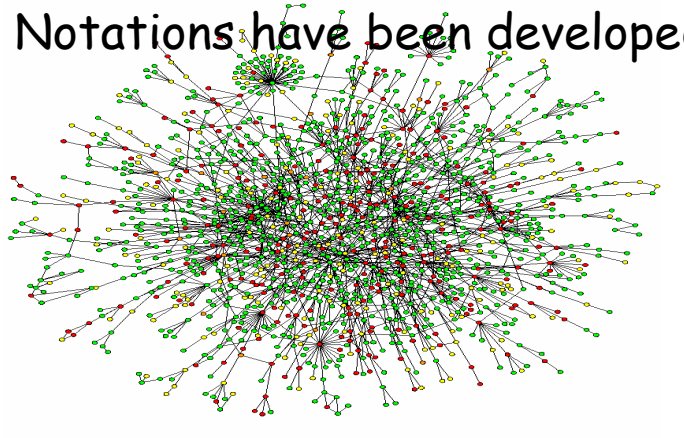
1. The Protein Machine

Very close to the atoms.

- Complex folded-up shapes that:
  - Fit together, dock, undock.
  - Excite/unexcite, warp each other.
  - Bring together, catalyze, transform materials.
  - Form complex aggregates and networks.



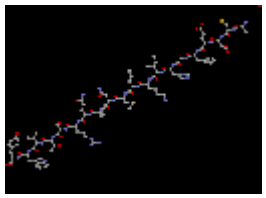
- Mapping out such networks:
  - In principle, it's "just" a very large set of chemical equations.
  - Notations have been developed to summarize and abstract.



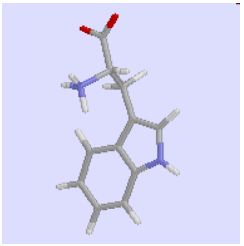
An actual molecular interaction network.
(Nodes are distinct protein kinds,
arcs mean that two kinds of proteins interact.)

Protein Structure

Primary

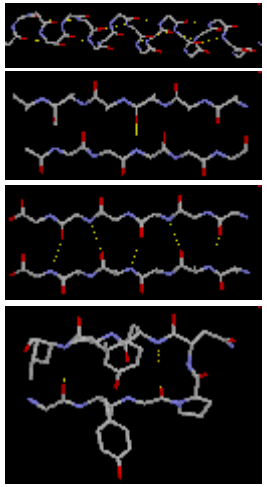


The 20 Aminoacids



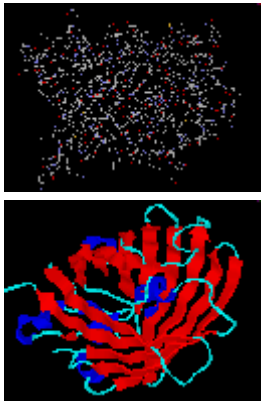
Tryptophan

Secondary



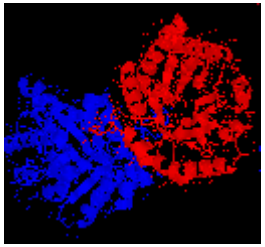
Alpha Helix, Beta Sheet

Tertiary



Green Fluorescent Protein

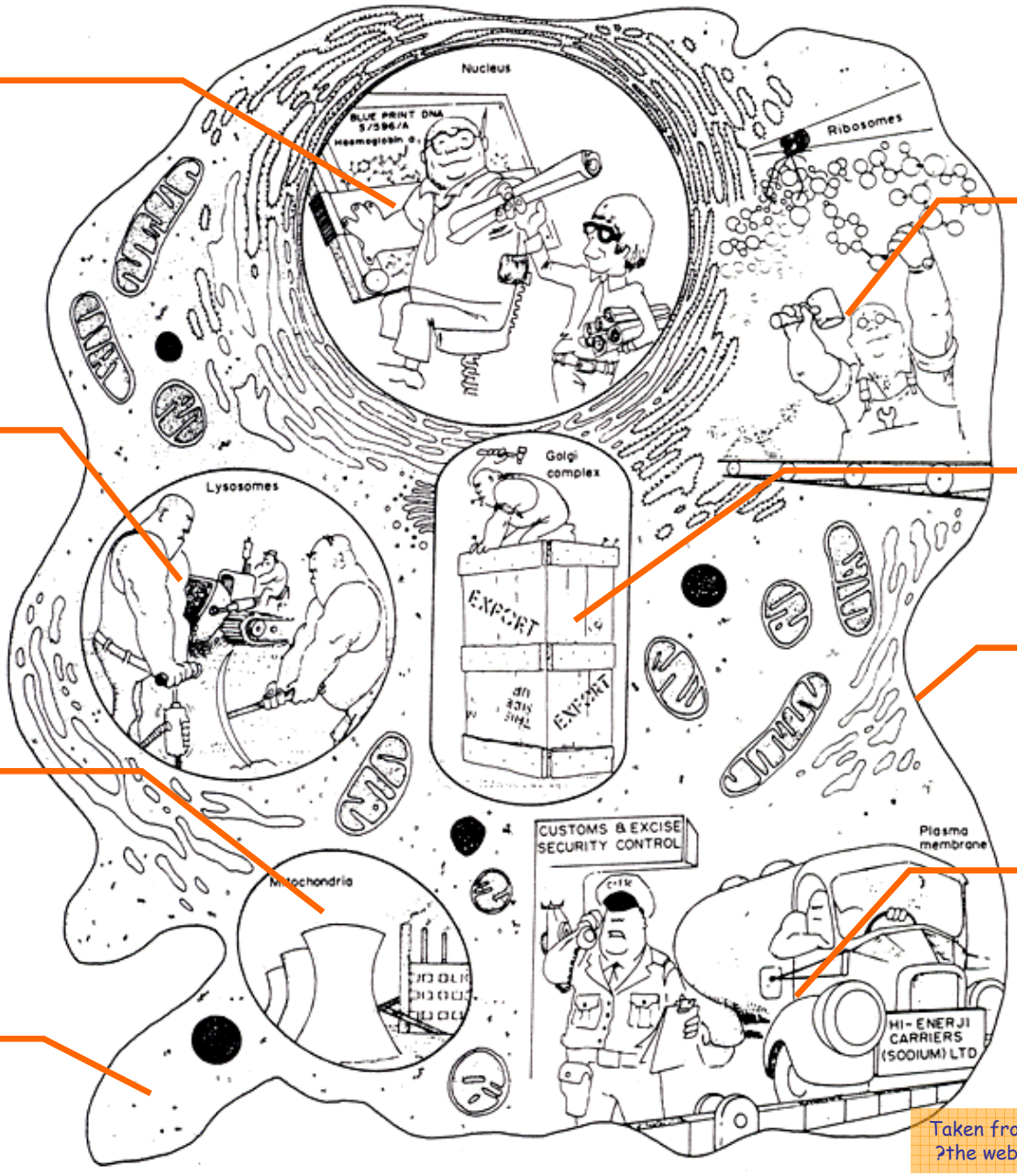
Quaternary



Triose Phosphate Isomerase

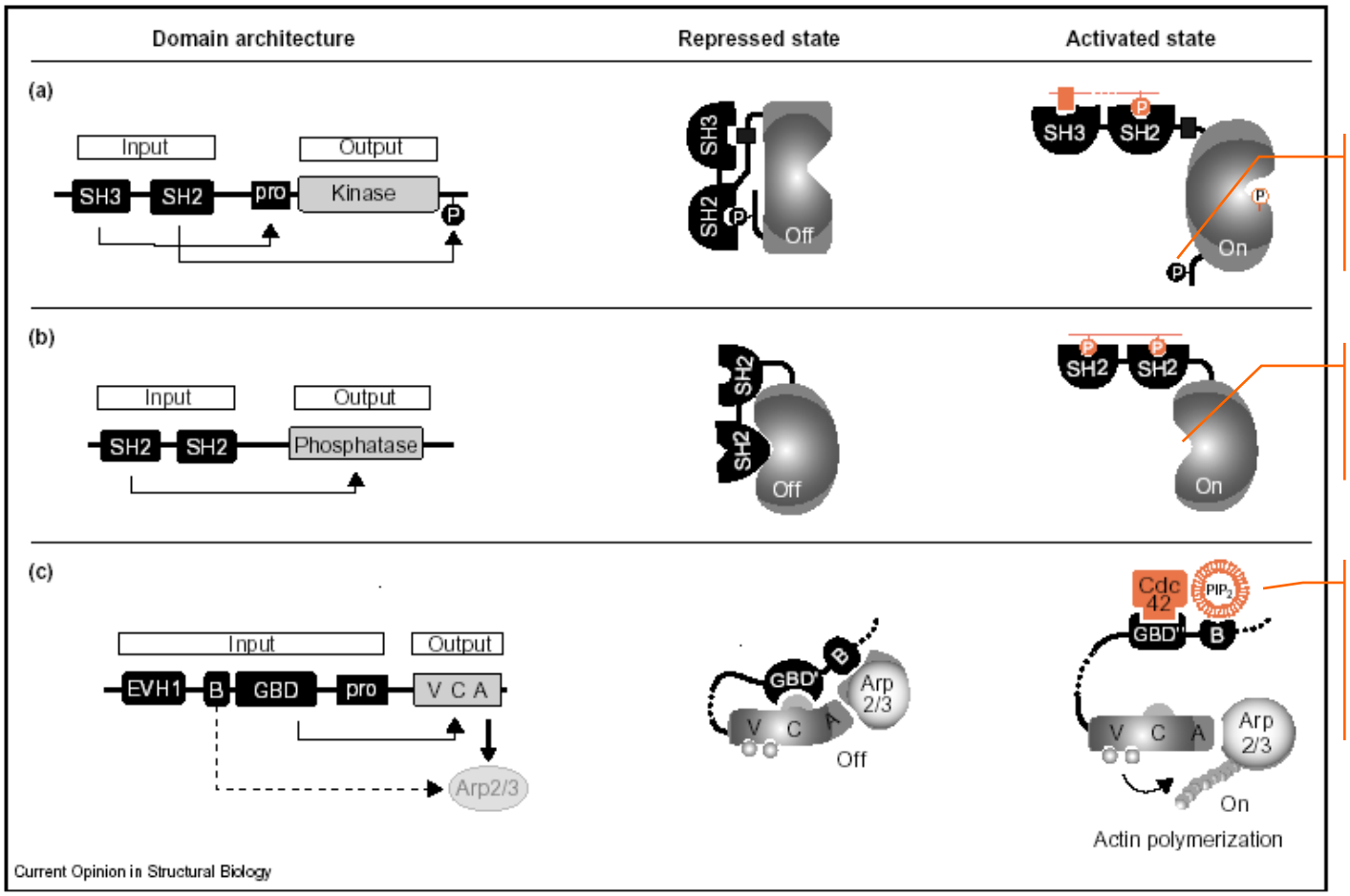
http://www.cmbi.kun.nl/gvteach/bioinformatica1/

Protein Function



Taken from ?the web?

Some Allosteric Switches



Domain architecture and autoinhibitory interactions in modular switch proteins. (a) Src family kinases contain N-terminal SH3 and SH2 domains, and a kinase domain flanked by intramolecular SH3-binding and SH2-binding sites (when the C-terminal motif tyrosine is phosphorylated by Csk). The crystal structures of several family members show that both intramolecular domain interactions function in concert to lock the kinase in an inactive conformation. Activating stimuli (red) include external SH2 or SH3 ligands. After initial activation, the kinase is maintained in an active state by autophosphorylation of its activation loop. (b) SHP-2 phosphatase contains two SH2 domains and a phosphatase domain. The crystal structure of the phosphatase shows that the N-terminal SH2 domain participates in an autoinhibitory interaction that directly blocks the phosphatase active site. Binding of external SH2 ligands activates by disrupting the autoinhibitory interaction. (c) N-WASP contains an Enabled VASP homology 1 (EVH1) domain, a B motif, a GBD, a proline-rich segment (pro) and an output region (VCA) that alone binds the Arp2/3 complex and stimulates its actin nucleation activity. The B and GBD motifs are required to repress activity and, by current models, are thought to participate in intracomplex interactions (only the structure of the GBD intramolecular complex for WASP is known). GTP-bound Cdc42 and PIP_2 synergistically activate N-WASP.

Allosteric ("other shape") reactions modify accessibility.

Kinase

= donates phosphate P
= phosphorilates other proteins

Phosphatase

= accepts phosphate P
= dephosphorilates other proteins

Logical AND

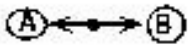
at equal concentrations of the individual input stimuli, activation is much higher if both stimuli are present

"Phosphatase Kinase Kinase" = a kinase that activates a kinase that activates a phosphatase that deactivates a protein.

Humans have the same number of modular protein *domains* (building blocks) as worms, but twice the number of multi-domain proteins.

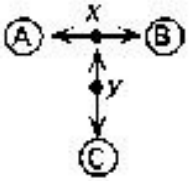
Taken from Wendell Lim

MIM: Molecular Interaction Maps (Kohn)

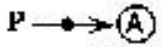


The double-arrowed line indicates that proteins **A** and **B** can bind to each other. The "node" placed on the line represents the **A:B** complex.

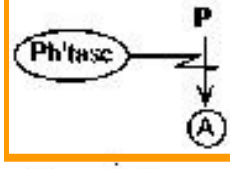
Asymmetric binding where protein **A** donates a peptide that binds to a receptor site or pocket on protein **B**.



Representation of multimolecular complexes: *x* is **A:B**; *y* is **(A:B):C**. This notation is extensible to any number of components in a complex.



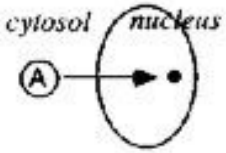
Covalent modification of protein **A**. The single-arrowed line indicates that **A** can exist in a phosphorylated state. The node represents the phosphorylated species.



Cleavage of a covalent bond: dephosphorylation of **A** by a phosphatase.

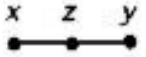
Proteolytic cleavage at a specific site within a protein.

Stoichiometric conversion of **A** into **B**.



Transport of **A** from cytosol to nucleus. The node represents **A** after it has been transported into the nucleus.

Formation of a homodimer. Filled circle on the right represents another copy of **A**. The node on the line represents the homodimer **A:A**.



z is the combination of states defined by *x* and *y*.

Enzymatic stimulation of a reaction.

General symbol for stimulation.
A bar behind the arrowhead signifies necessity.

General symbol for inhibition.

Shorthand symbol for transcriptional activation.

Shorthand symbol for transcriptional inhibition.

Degradation products

Taken from Kurt W. Kohn

Molecular Interaction Maps (Kohn)

http://www.cds.caltech.edu/~hsauro/index.htm

JDesigner

The p53-Mdm2 and DNA Repair Regulatory Network

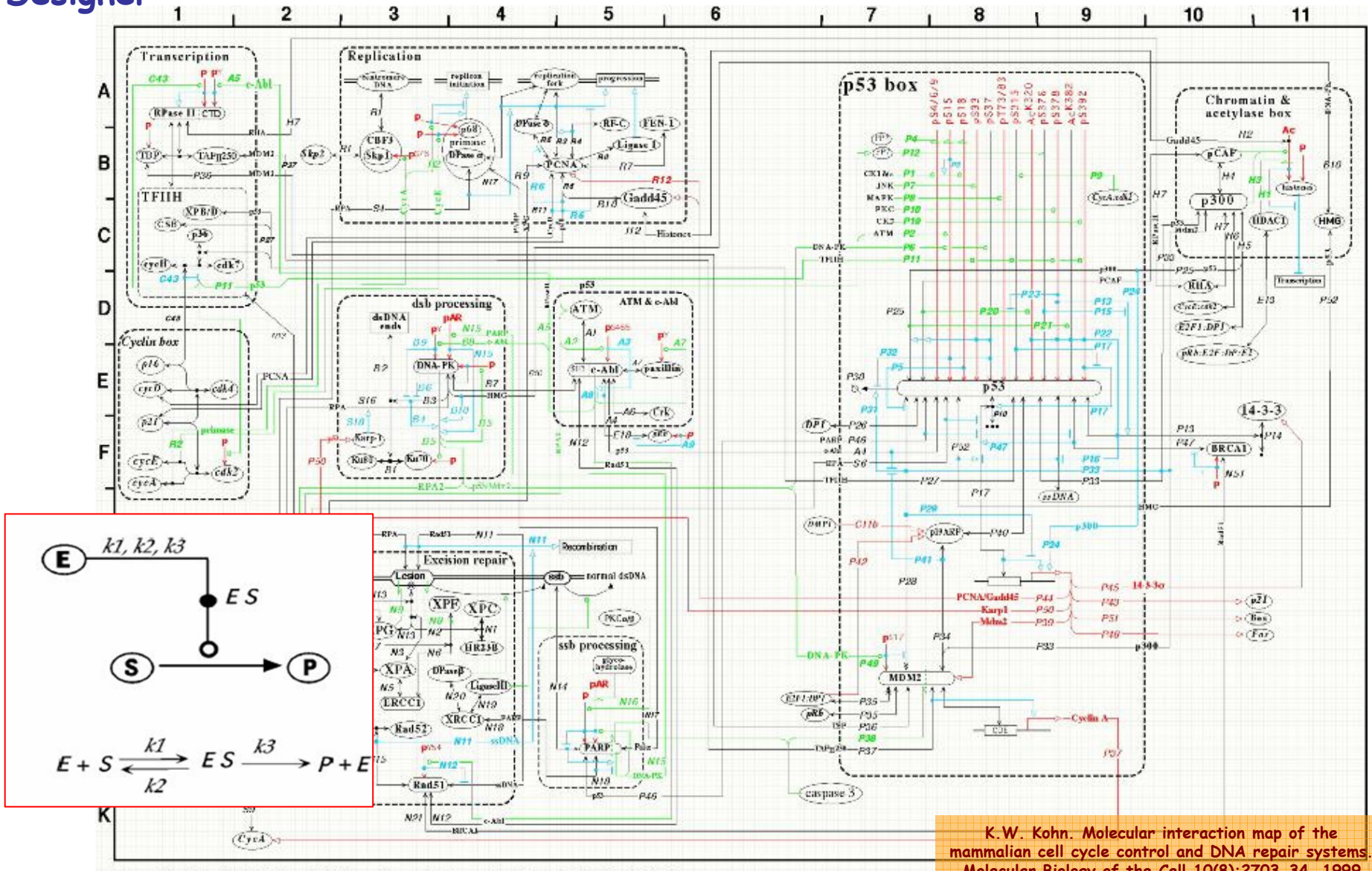
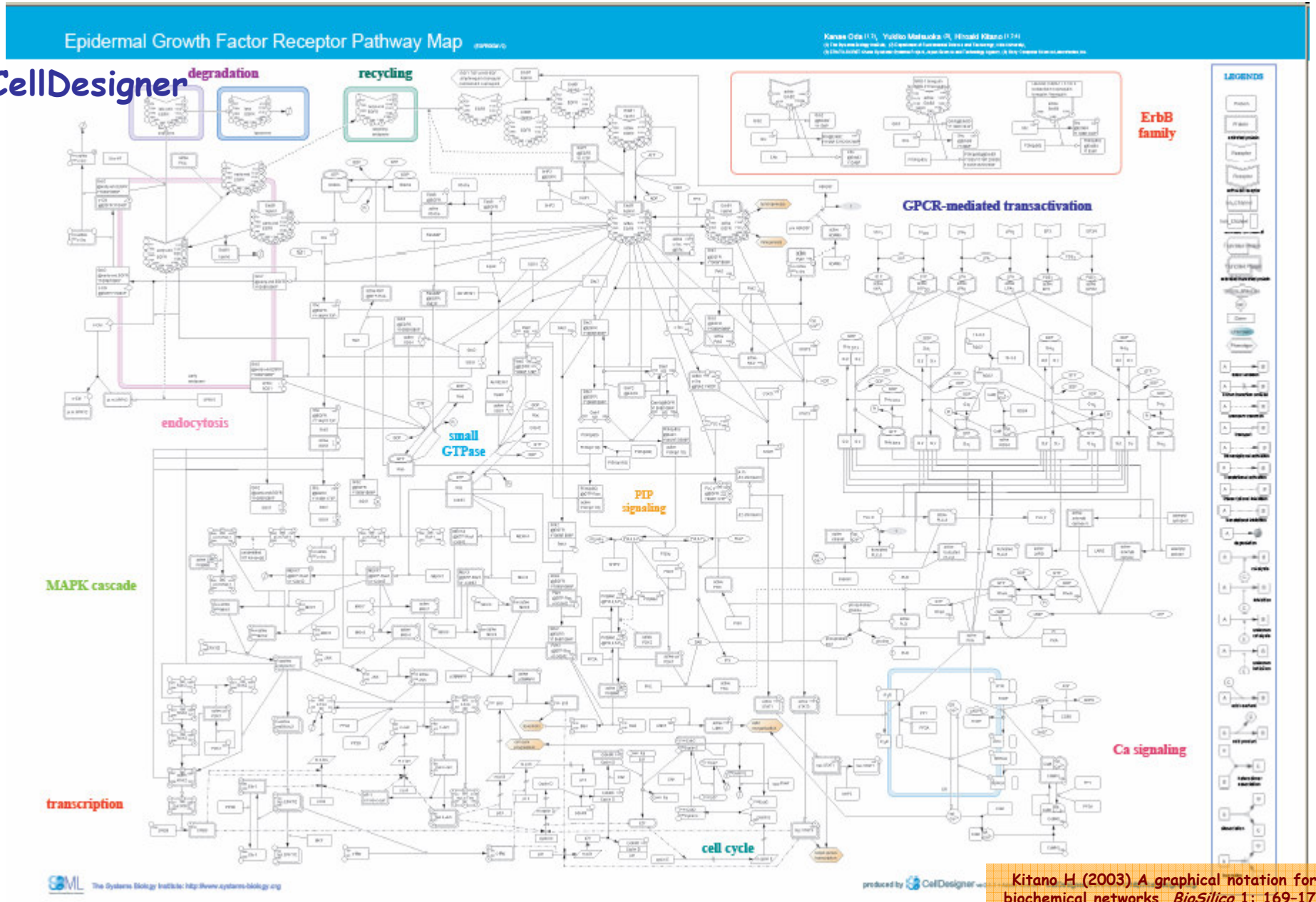


Figure 6B: The p53-Mdm2 and DNA repair regulatory network (version 2p - May 19, 1999)

K.W. Kohn. Molecular interaction map of the mammalian cell cycle control and DNA repair systems. Molecular Biology of the Cell 10(8):2703-34, 1999.

Molecular Interaction Maps (Kitano)



Kitano H (2003) *A graphical notation for biochemical networks. BioSilico* 1: 169-176

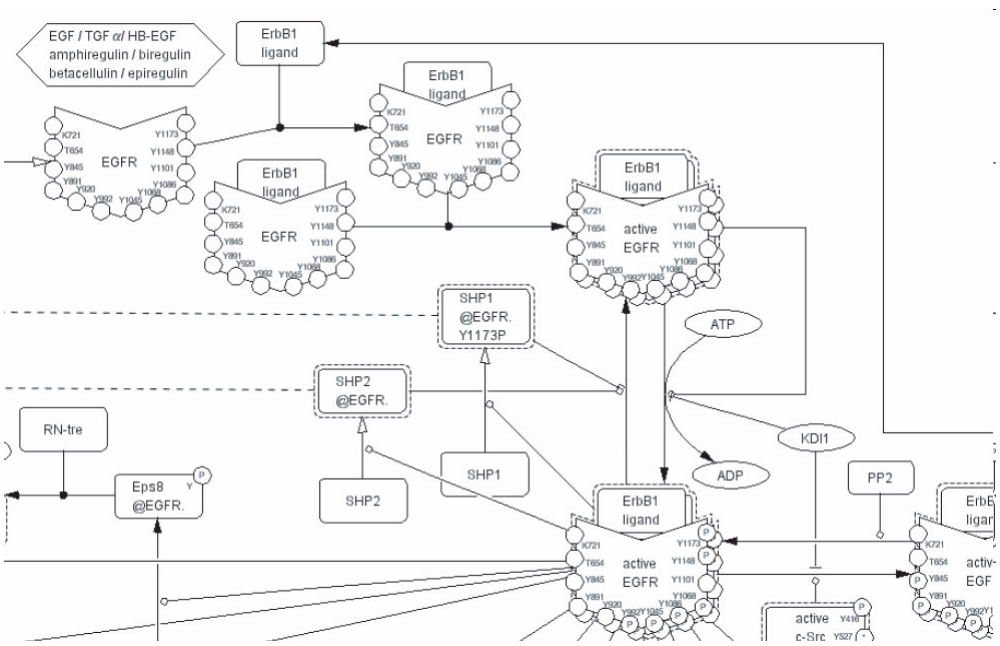
Molecular Interaction Maps (Kitano)

A comprehensive pathway map of epidermal growth factor receptor signaling

Kanae Oda[1,2], Yukiko Matsuoka[1,3], Akira Funahashi[1,3] and Hiroaki Kitano[1,2,3,4]

1. The Systems Biology Institute, Tokyo, Japan
2. Department of Fundamental Science and Technology, Keio University, Tokyo, Japan
3. ERATO-SORST Kitano Symbiotic Systems Project, Japan Science and Technology Agency, Tokyo, Japan
4. Sony Computer Science Laboratories, Inc., Tokyo, Japan

"The current EGFR map is essentially a state transition diagram, in which one state of the system is represented in one node, and an arc from one node to another node represents a transition of the state of the system. This class of diagrams is often used in engineering and software development, and the schema avoids using symbols that directly point to molecules to indicate activation or inhibition. "

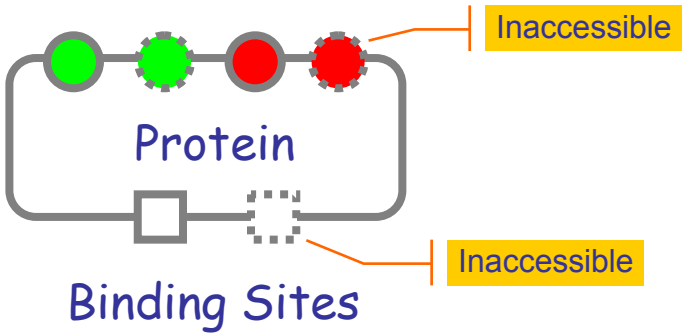


Kitano H (2003) *A graphical notation for biochemical networks. BioSilico* 1: 169–176

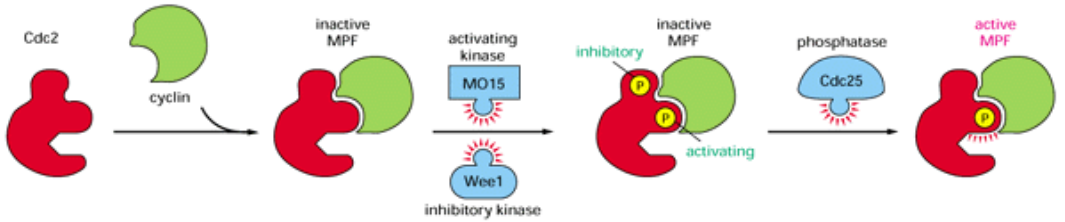
The Protein Machine "Instruction Set"

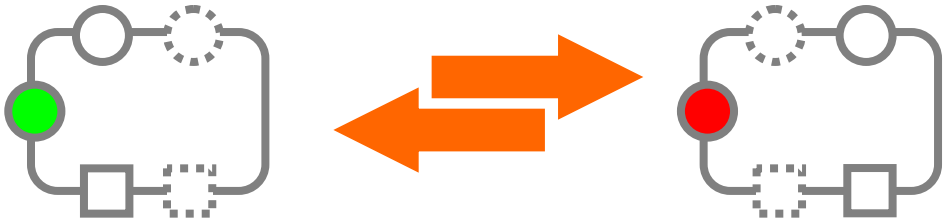
cf. BioCalculus [Kitano&Nagasaki], κ-calculus [Danos&Laneve]

On/Off switches



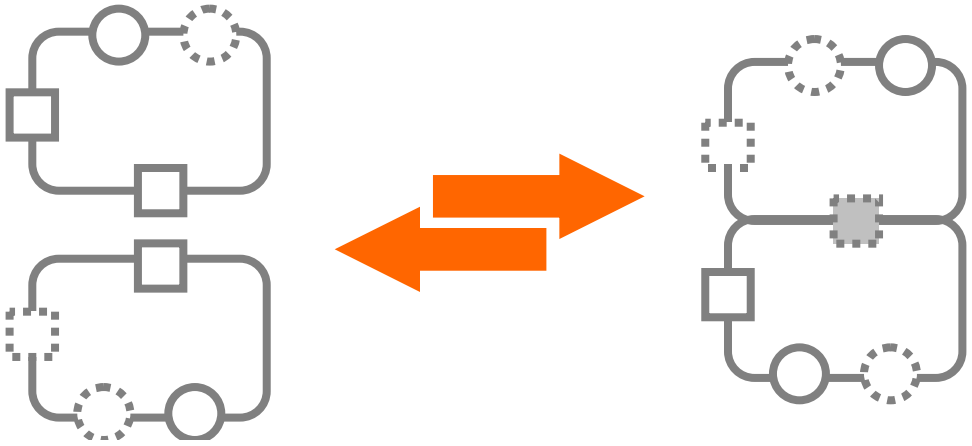
Each protein has a structure
of binary switches and binding sites.
But not all may be always *accessible*.





Switching of accessible switches.

- May cause other switches and
binding sites to become (in)accessible.
- May be triggered or inhibited by nearby specific
proteins in specific states.



Binding on accessible sites.

- May cause other switches and
binding sites to become (in)accessible.
- May be triggered or inhibited by nearby specific
proteins in specific states.

Notations for the Protein Machine

- Stochastic π-Calculus
  - Priami (following Hillston's PEPA) formalizes a stochastic version of p-calculus where channels have communication *rates*.
- BioSPi
  - Regev-Shapiro-Silverman propose modeling chemical interactions (exchange of electrons and small molecules) as "communication".
  - Standard stochastic simulation algorithms (Gillespie) can be used to run in-silico experiments.
  - Complex formation is encoded via p-restriction.
- PEPA
  - Calder Gilmore and Hillston model the ERK pathway.
- k-calculus
  - Danos and Laneve (following Kitano's BioCalculus) define a calculus where complex formation is primitive.
- (Stochastic) Petri Nets
  - S.Reddy'94 modeling pathways.
  - Srivastava Perterson and Bentley analyze and simulate E.coli stress response circuit.
- Bio State Charts
  - Harel uses State Charts to model biological interactions via a semi-graphical FSM notation.
- Pathway Logic
  - Talcott-Eker-Knapp-Lincoln use term-rewriting.
- BioCham
  - ChabrierRivier-Fages-Soliman use term-rewriting and CLT modelchecking.
- Kohn Diagrams, Kitano Diagrams
- SBML (Systems Biology Markup Language)
  - XML dialect for MIM's:
    - Compartments (statically nested)
    - Reagents with concentrations
    - Reactions with various rate laws
  - Read and written by many tools via the Systems Biology Workbench protocol

MAPK Cascade

Ultrasensitivity in the mitogen-activated protein **cascade**, Chi-Ying F. Huang and James E. Ferrell, Jr., 1996, *Proc. Natl. Acad. Sci. USA*, 93, 10078-10083.

Table 2. Predicted Hill coefficients for MAP kinase cascade components: Varying the assumed K_m values

Reaction	Range of assumed K_m values	Range of effective Hill coefficients (nH) predicted for MAPKKK	MAPKK	MAPK
1. MAPKKK → MAPKKK*	60–1500 nM	1.0	1.7	4.9
2. MAPKKK* → MAPKKK	60–1500 nM	1.0	1.7	4.9
3. MAPKK → MAPKK-P	60–1500 nM	1.0	1.3–2.3	4.0–5.1
4. MAPKK-P → MAPKK	60–1500 nM	1.0	1.5–1.9	3.6–6.7
5. MAPKK-P → MAPKK-PP	60–1500 nM	1.0	1.3–2.4	3.8–5.2
6. MAPKK-PP → MAPKK-P	60–1500 nM	1.0	1.7–1.8	4.1–6.4
7. MAPK → MAPK-P	60–1500 nM (300 nM†)	1.0	1.7	3.7–6.2
8. MAPK-P → MAPK	60–1500 nM	1.0	1.7	4.3–5.2
9. MAPK-P → MAPK-PP	60–1500 nM	1.0	1.7	3.4–6.1
10. MAPK-PP → MAPK-P	60–1500 nM	1.0	1.7	4.7–5.1

The assumed K_m values for each reaction were individually varied over the ranges shown, with the assumed K_m values for the other nine reactions held constant. The effective Hill coefficients were calculated from the steepness of the predicted stimulus/response curves, as described in the text.
†The K_m value for reaction 7 has been measured to be 300 nM for the phosphorylation of a mammalian MAPK by a MAPKK (N. Ahn, personal communication). All of the other K_m values were initially assumed to be 300 nM as well.

10 chemical reactions

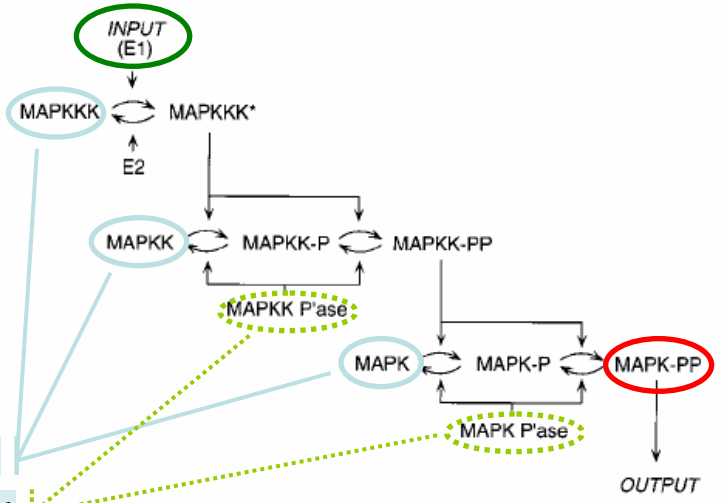


FIG. 1. Schematic view of the MAPK cascade. Activation of MAPK depends upon the phosphorylation of two conserved sites [Thr-183 and Tyr-185 in rat p42 MAPK/Erk2 (4, 5)]. Full activation of MAPKK also requires phosphorylation of two sites [Ser-218 and Ser-222 in mouse Mek-1/MKK1 (6–10)]. Detailed mechanisms for the activation of various MAPKKKs (e.g., Raf-1, B-Raf, Mos) are not yet established; here we assume that MAPKKKs are activated and inactivated by enzymes we denote E1 and E2. MAPKKK* denotes activated MAPKKK. MAPKK-P and MAPKK-PP denote singly and doubly phosphorylated MAPKK, respectively. MAPK-P and MAPK-PP denote singly and doubly phosphorylated MAPK. P′ase denotes phosphatase.

Calculations. Eqs. **1-10** represent the reactions of the MAPK cascade, which are shown schematically in Fig. 1. We have used Goldbeter and Koshland's nomenclature for the rate constants—the letter a denotes association, d denotes dissociation without catalysis, and k denotes product formation (11). KKK denotes MAPKKK; KK denotes MAPKK; and K denotes MAPK.

$$\mathrm{KKK} + \mathrm{E1} \underset{d_1}{\overset{a_1}{\rightleftharpoons}} \mathrm{KKK{\cdot}E1} \xrightarrow{k_1} \mathrm{KKK^*} + \mathrm{E1} \quad [1]$$

$$\mathrm{KKK^*} + \mathrm{E2} \underset{d_2}{\overset{a_2}{\rightleftharpoons}} \mathrm{KKK{\cdot}E2} \xrightarrow{k_2} \mathrm{KKK} + \mathrm{E2} \quad [2]$$

$$\mathrm{KK} + \mathrm{KKK^*} \underset{d_3}{\overset{a_3}{\rightleftharpoons}} \mathrm{KK{\cdot}KKK^*} \xrightarrow{k_3} \mathrm{KK\text{-}P} + \mathrm{KKK^*} \quad [3]$$

$$\mathrm{KK\text{-}P} + \mathrm{KK\ P'ase} \underset{d_4}{\overset{a_4}{\rightleftharpoons}} \mathrm{KK\text{-}P{\cdot}KK\ P'ase}$$

$$\mathrm{KK\text{-}P{\cdot}KK\ P'ase} \xrightarrow{k_4} \mathrm{KK} + \mathrm{KK\ P'ase} \quad [4]$$

$$\mathrm{KK\text{-}P} + \mathrm{KKK^*} \underset{d_5}{\overset{a_5}{\rightleftharpoons}} \mathrm{KK\text{-}P{\cdot}KKK^*} \xrightarrow{k_5} \mathrm{KK\text{-}PP} + \mathrm{KKK^*} \quad [5]$$

$$\mathrm{KK\text{-}PP} + \mathrm{KK\ P'ase} \underset{d_6}{\overset{a_6}{\rightleftharpoons}} \mathrm{KK\text{-}PP{\cdot}KK\ P'ase}$$

$$\mathrm{KK\text{-}PP{\cdot}KK\ P'ase} \xrightarrow{k_6} \mathrm{KK\text{-}P} + \mathrm{KK\ P'ase} \quad [6]$$

$$\mathrm{KK\text{-}PP} + \mathrm{K} \underset{d_7}{\overset{a_7}{\rightleftharpoons}} \mathrm{KK\text{-}PP{\cdot}K} \xrightarrow{k_7} \mathrm{KK\text{-}PP} + \mathrm{K\text{-}P} \quad [7]$$

$$\mathrm{K\text{-}P} + \mathrm{K\ P'ase} \underset{d_8}{\overset{a_8}{\rightleftharpoons}} \mathrm{K\text{-}P{\cdot}K\ P'ase} \xrightarrow{k_8} \mathrm{K} + \mathrm{K\ P'ase} \quad [8]$$

$$\mathrm{K\text{-}P} + \mathrm{KK\text{-}PP} \underset{d_9}{\overset{a_9}{\rightleftharpoons}} \mathrm{K\text{-}P{\cdot}KK\text{-}PP} \xrightarrow{k_9} \mathrm{K\text{-}PP} + \mathrm{KK\text{-}PP} \quad [9]$$

$$\mathrm{K\text{-}PP} + \mathrm{K\ P'ase} \underset{d_{10}}{\overset{a_{10}}{\rightleftharpoons}} \mathrm{K\text{-}PP{\cdot}K\ P'ase}$$

$$\mathrm{K\text{-}PP{\cdot}K\ P'ase} \xrightarrow{k_{10}} \mathrm{K\text{-}P} + \mathrm{K\ P'ase} \quad [10]$$

As 18 Ordinary Differential Equations Plus 7 conservation equations

$$\frac{d}{dt}[KKK] = -a_1[KKK][E1] + d_1[KKK{\cdot}E1] + k_2[KKK^*\cdot E2] \quad [11]$$

$$\frac{d}{dt}[KKK{\cdot}E1] = a_1[KKK][E1] - (d_1 + k_1)[KKK{\cdot}E1] \quad [12]$$

$$\frac{d}{dt}[KKK^*] = -a_2[KKK^*][E2] + d_2[KKK^*{\cdot}E2] + k_1[KKK\cdot E1] + (k_3 + d_3)[KK\cdot KKK^*] - a_3[KKK^*][KK] + (k_5 + d_5)[KK\text{-}P\cdot KKK^*] - a_5[KK\text{-}P][KKK^*] \quad [13]$$

$$\frac{d}{dt}[KKK^*{\cdot}E2] = a_2[KKK^*][E2] - (d_2 + k_2)[KKK^*{\cdot}E2] \quad [14]$$

$$\frac{d}{dt}[KK] = -a_3[KK][KKK^*] + d_3[KK{\cdot}KKK^*] + k_4[KK\text{-}P\cdot KK\ P'ase] \quad [15]$$

$$\frac{d}{dt}[KK{\cdot}KKK^*] = a_3[KK][KKK^*] - (d_3 + k_3)[KK\cdot KKK^*] \quad [16]$$

$$\frac{d}{dt}[KK\text{-}P] = -a_4[KK\text{-}P][KK\ P'ase] + d_4[KK\text{-}P{\cdot}KK\ P'ase] + k_3[KK\cdot KKK^*] + k_6[KK\text{-}PP\cdot KK\ P'ase] + d_5[KK\text{-}P\cdot KKK^*] - a_5[KK\text{-}P][KKK^*] \quad [17]$$

+ $d_5[KK\text{-}P\cdot KKK^*] - a_5[KK\text{-}P][KKK^*]$ [17]

$$\frac{d}{dt}[KK\text{-}P{\cdot}KK\ P'ase] = a_4[KK\text{-}P][KK\ P'ase] - (d_4 + k_4)[KK\text{-}P\cdot KK\ P'ase] \quad [18]$$

$$\frac{d}{dt}[KK\text{-}P{\cdot}KKK^*] = a_5[KK\text{-}P][KKK^*] - (d_5 + k_5)[KK\text{-}P\cdot KKK^*] \quad [19]$$

$$\frac{d}{dt}[KK\text{-}PP] = k_5[KK\text{-}P{\cdot}KKK^*] - a_6[KK\text{-}PP][KK\ P'ase] + d_6[KK\text{-}PP\cdot KK\ P'ase] - a_7[KK\text{-}PP][K] + (d_7 + k_7)[K\cdot KK\text{-}PP] + (d_9 + k_9)[K\text{-}P\cdot KK\text{-}PP] - a_9[K\text{-}P][KK\text{-}PP] \quad [20]$$

$$\frac{d}{dt}[KK\text{-}PP{\cdot}KK\ P'ase] = a_6[KK\text{-}PP][KK\ P'ase] - (d_6 + K_6)[KK\text{-}PP{\cdot}KK\ P'ase] \quad [21]$$

$$\frac{d}{dt}[K] = -a_7[K][KK\text{-}PP] + d_7[K{\cdot}KK\text{-}PP] + k_8[K\text{-}P\cdot K\ P'ase] \quad [22]$$

$$\frac{d}{dt}[K{\cdot}KK\text{-}PP] = a_7[K][KK\text{-}PP] - (d_7 + k_7)[K{\cdot}KK\text{-}PP] \quad [23]$$

$$\frac{d}{dt}[K\text{-}P] = k_7[K{\cdot}KK\text{-}PP] - a_8[K\text{-}P][K\ P'ase] + d_8[K\text{-}P\cdot K\ P'ase] - a_9[K\text{-}P][KK\text{-}PP] + d_9[K\text{-}P\cdot KK\text{-}PP] + k_{10}[K\text{-}PP\cdot K\ P'ase] \quad [24]$$

$$\frac{d}{dt}[K\text{-}P{\cdot}K\ P'ase] = a_8[K\text{-}P][K\ P'ase] - (d_8 + k_8)[K\text{-}P\cdot K\ P'ase] \quad [25]$$

$$\frac{d}{dt}[K\text{-}P{\cdot}KK\text{-}PP] = a_9[K\text{-}P][KK\text{-}PP] - (d_9 + k_9)[K\text{-}P\cdot KK\text{-}PP] \quad [26]$$

$$\frac{d}{dt}[K\text{-}PP] = -a_{10}[K\text{-}PP][K\ P'ase] + d_{10}[K\text{-}PP\cdot K\ P'ase] + k_9[K\text{-}P\cdot KK\text{-}PP] \quad [27]$$

$$\frac{d}{dt}[K\text{-}PP{\cdot}K\ P'ase] = a_{10}[K\text{-}PP][K\ P'ase] - (d_{10} + k_{10})[K\text{-}PP\cdot K\ P'ase] \quad [28]$$

$$[E1_{tot}] = [E1] + [KKK{\cdot}E1] \quad [30]$$

$$[E2_{tot}] = [E2] + [KKK^*{\cdot}E2] \quad [31]$$

$$[KK_{tot}] = [KK] + [KK\text{-}P] + [KK\text{-}PP] + [KK{\cdot}KKK^*] + [KK\text{-}P\cdot KKK^*] + [KK\text{-}P\cdot KK\ P'ase] + [KK\text{-}PP\cdot KK\ P'ase] + [KK\text{-}PP\cdot K] + [KK\text{-}PP\cdot K\text{-}P] \quad [32]$$

$$[KK\ P'ase_{tot}] = [KK\ P'ase] + [KK\ P'ase{\cdot}KK\text{-}P] + [KK\ P'ase\cdot KK\text{-}PP] \quad [33]$$

$$[K_{tot}] = [K] + [K\text{-}P] + [K\text{-}PP] + [KK\text{-}PP{\cdot}K] + KK\text{-}PP\cdot K\text{-}P] + [K\text{-}P\cdot K\ P'ase] + [K\text{-}PP\cdot K\ P'ase] \quad [34]$$

$$[K\ P'ase_{tot}] = [K\ P'ase] + [K\text{-}P{\cdot}K\ P'ase] + [K\text{-}PP\cdot K\ P'ase] \quad [35]$$

These equations were solved numerically using the Runge–Kutta-based NDSolve algorithm in Mathematica (Wolfram Research, Champaign, IL). An annotated copy of the Mathematica code for the MAPK cascade rate equations can be obtained from J.E.F.

The 10 reactions described above give rise to 18 rate equations.

One equation for each species (8) and complex (10), but not for constant concentration enzymes (4)

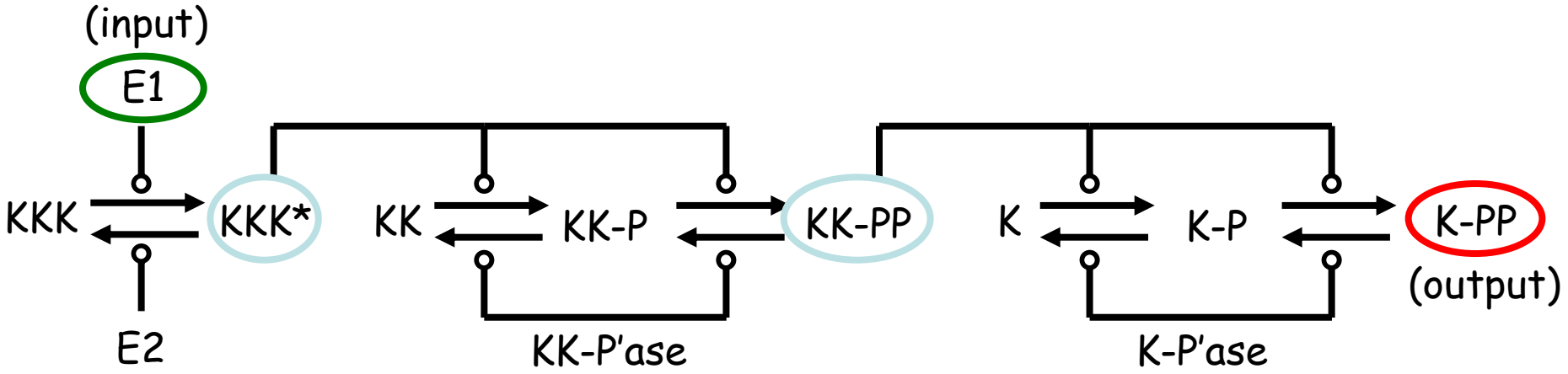
In addition, there are seven conservation equations (Eqs. 29-35).

$$[KKK_{tot}] = [KKK] + [KKK^*] + [KKK{\cdot}E1] + [KKK^*\cdot E2] + [KKK^*\cdot K] + [KKK^*\cdot K\text{-}P] \quad [29]$$

in exactly one state

Each molecule

The Circuit



As 12 processes (in SPiM)

```
let KKK() =
   (new u1@d1:Release new k1@r1:React
    !a1(u1,k1); (do !u1;KKK() or !k1;KKKst()))          [1]substrate
and KKKst() =
   (new u2@d2:Release new k2@r2:React
    do !a2(u2,k2); (do !u2;KKKst() or !k2;KKK())        [2]substrate
    or ?a3(u3,k3); (do ?u3;KKKst() or ?k3;KKKst())      [3]kinase
    or ?a5(u5,k5); (do ?u5;KKKst() or ?k5;KKKst()))     [5]kinase

let E1() =
   ?a1(u1,k1); (do ?u1;E1() or ?k1;E1())                [1]enzyme
let E2() =
   ?a2(u2,k2); (do ?u2;E2() or ?k2;E2())                [2]enzyme

let KK() =
   (new u3@d3:Release new k3@r3:React
    !a3(u3,k3); (do !u3;KK() or !k3;KK_P()))            [3]substrate

and KK_P() =
   (new u4@d4:Release new k4@r4:React
    new u5@d5:Release new k5@r5:React
    do !a4(u4,k4); (do !u4;KK_P() or !k4;KK())          [4]substrate
    or !a5(u5,k5); (do !u5;KK_P() or !k5;KK_PP()))      [5]substrate
```

KKK:E1 complex

E1:KKK complex

```
and KK_PP() =
   (new u6@d6:Release new k6@r6:React
    do !a6(u6,k6); (do !u6;KK_PP() or !k6;KK_P())       [6]substrate
    or ?a7(u7,k7); (do ?u7;KK_PP() or ?k7;KK_PP())      [7]kinase
    or ?a9(u9,k9); (do ?u9;KK_PP() or ?k9;KK_PP()))     [9]kinase

and KKPse() =
   do ?a4(u4,k4); (do ?u4;KKPse() or ?k4;KKPse())       [4]phtase
   or ?a6(u6,k6); (do ?u6;KKPse() or ?k6;KKPse())       [6]phtase

let K() =
   (new u7@d7:Release new k7@r7:React
    !a7(u7,k7); (do !u7;K() or !k7;K_P()))              [7]substrate

and K_P() =
   (new u8@d8:Release new k8@r8:React
    new u9@d9:Release new k9@r9:React
    do !a8(u8,k8); (do !u8;K_P() or !k8;K())            [8]substrate
    or !a9(u9,k9); (do !u9;K_P() or !k9;K_PP()))        [9]substrate

and K_PP() =
   (new u10@d10:Release new k10@r10:React
    !a10(u10,k10); (do !u10;K_PP() or !k10;K_P()))      [10]substrate

and KPse() =
   do ?a8(u8,k8); (do ?u8;KPse() or ?k8;KPse())         [8]phtase
   or ?a10(u10,k10); (do ?u10;KPse() or ?k10;KPse())    [10]phtase
```

One process for each component (12) including enzymes, but not for complexes.

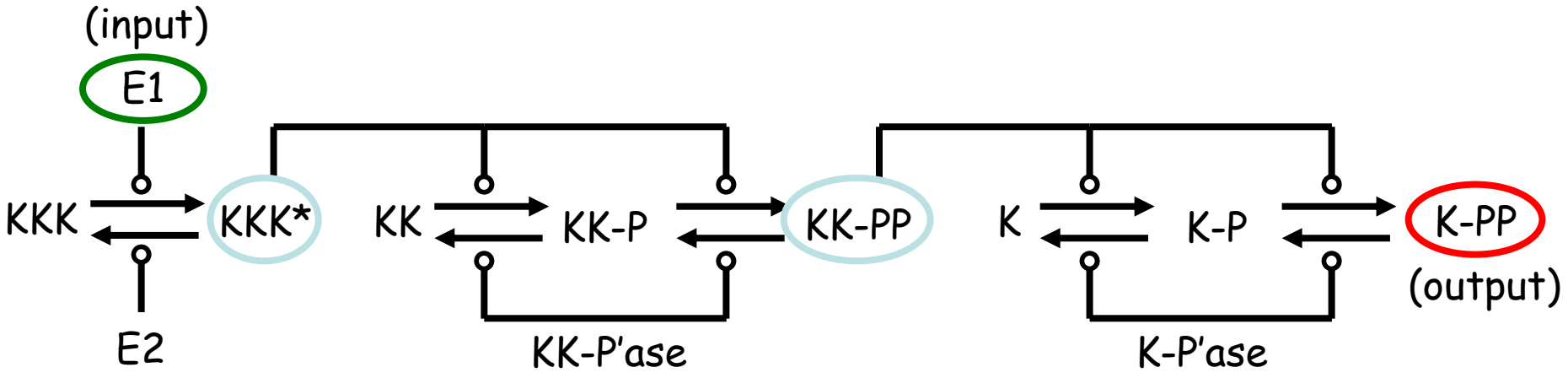
No need for conservation equations: implicit in "choice" operator in the calculus.

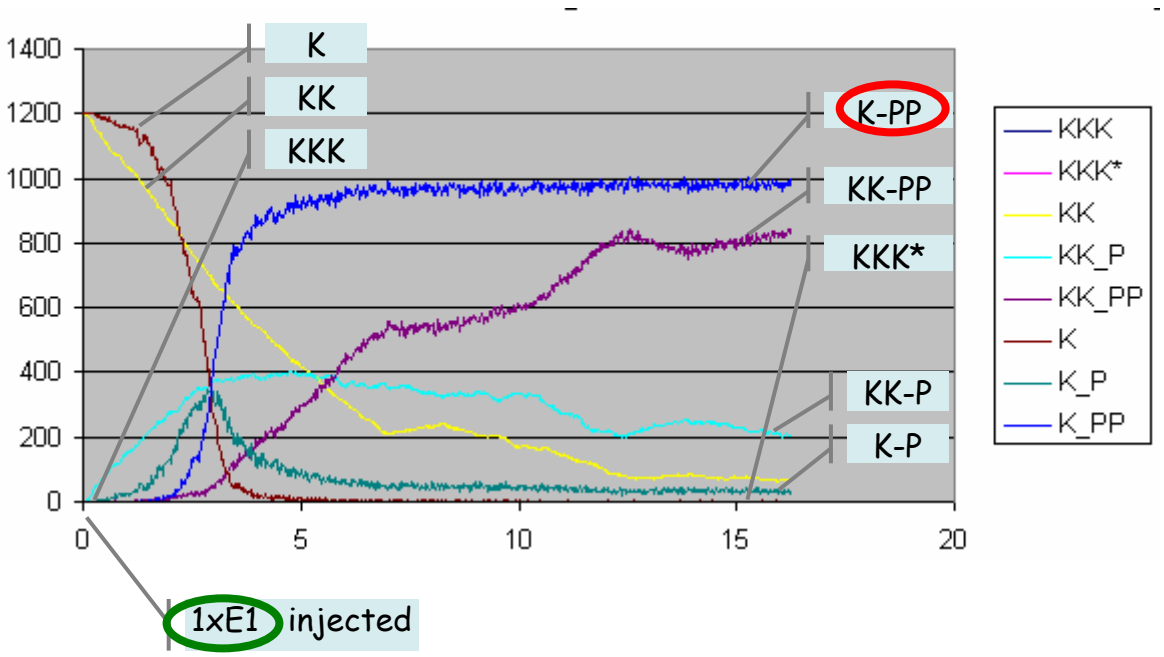
… and 30 Interaction Channels

```
type Release = chan()
type React = chan()
type Bond = chan(Release,React)

new a1@1.0:Bond val d1=1.0 val r1=1.0
new a2@1.0:Bond val d2=1.0 val r2=1.0
new a3@1.0:Bond val d3=1.0 val r3=1.0
new a4@1.0:Bond val d4=1.0 val r4=1.0
new a5@1.0:Bond val d5=1.0 val r5=1.0
new a6@1.0:Bond val d6=1.0 val r6=1.0
new a7@1.0:Bond val d7=1.0 val r7=1.0
new a8@1.0:Bond val d8=1.0 val r8=1.0
new a9@1.0:Bond val d9=1.0 val r9=1.0
new a10@1.0:Bond val d10=1.0 val r10=1.0

…

run 100 of KKK()  run 100 of KK()   run 100 of K()
run 1 of E2()  run 1 of KKPse()  run 1 of KPse()
run 1 of E1()
```

$a_i(u_i,k_i)$: release (u_i@d_i) and react (k_i@r_i) channels passed over bond (a_i) channel. (No behavior attached to channels except interaction rate.)

MAPK Cascade Simulation in SPiM





1st stage:
KKK* barely rises

2nd stage:
KK-PP rises, but is not stable

3rd stage:
K-PP flips up to max
even anticipating 2nd stage

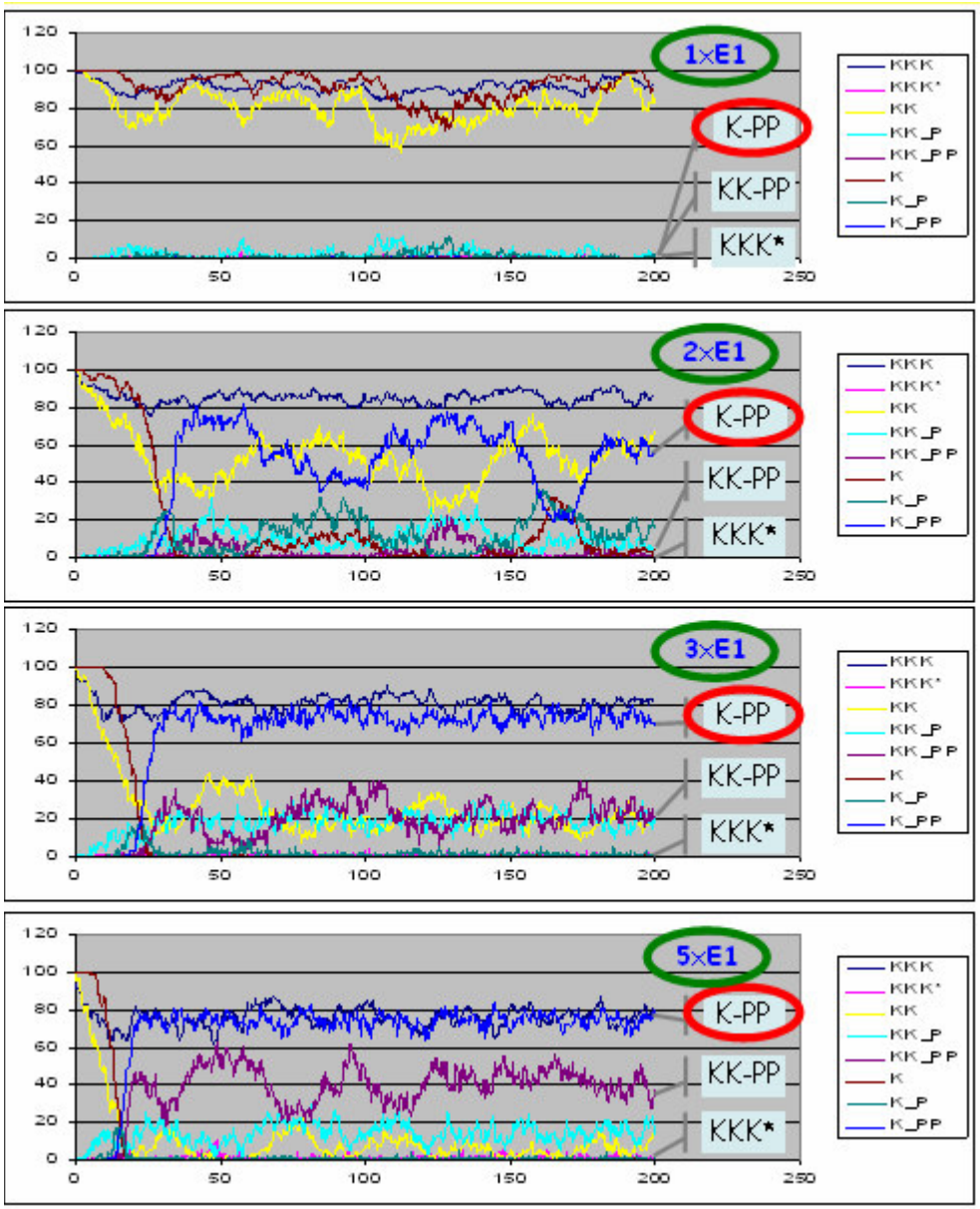
Rates and concentrations from paper:

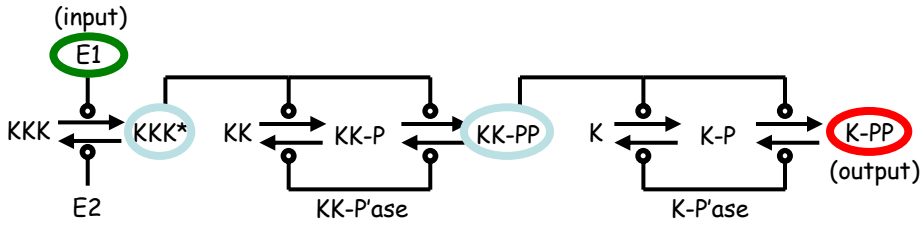
1xE2 (0.3 nM)
1xKKPase (0.3 nM)
120xKPase (120 nM)
3xKKK (3 nM)
1200xKK (1.2 uM)
1200xK (1.2 uM)

dx = rx = 150, ax = 1
(Kmx = (dx + rx) / ax, Km = 300 nM)

1xE1

MAPK Cascade Simulation in SPiM

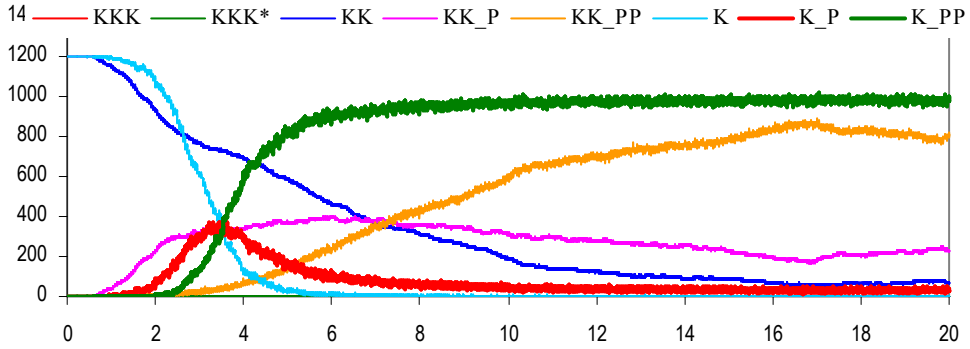




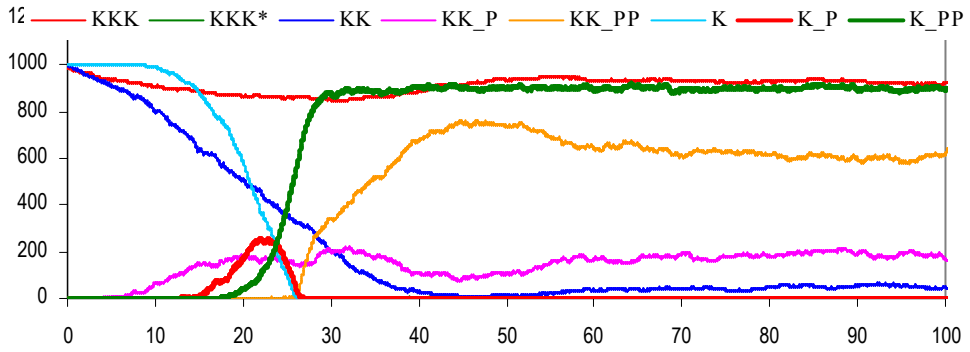
All coefficients 1.0 !!!
100xKKK, 100xKK, 100xK,
13xE2, 13xKKPse, 13xKPse.
nxE1 as indicated
(1xE1 is not sufficient to produce an output)

MAPK Cascade Simulation in SPiM

Parameters from paper
(wide rate range: 1-150, wide concentration range: 3nm – 1200nm)



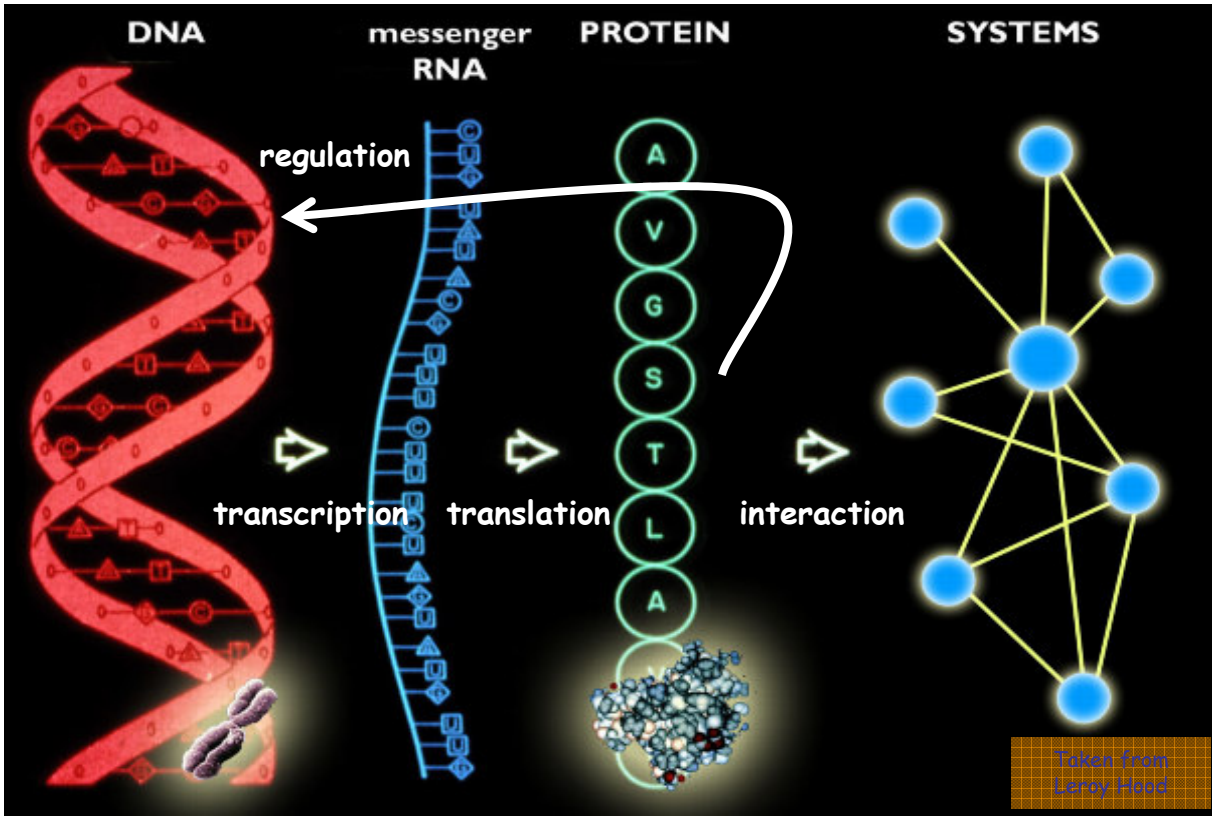
Artificial parameters
(all rates 1.0, all concentractions 1000)



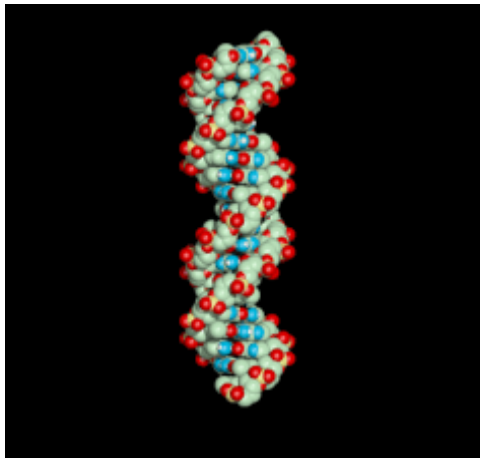
2. The Gene Machine

Pretty far from the atoms.

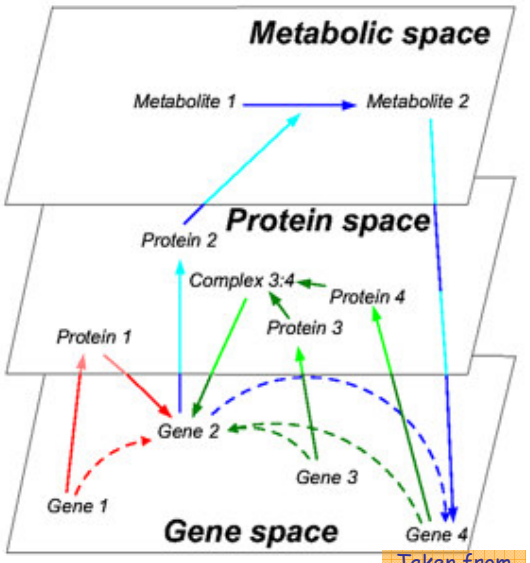
The "Central Dogma" of Molecular Biology

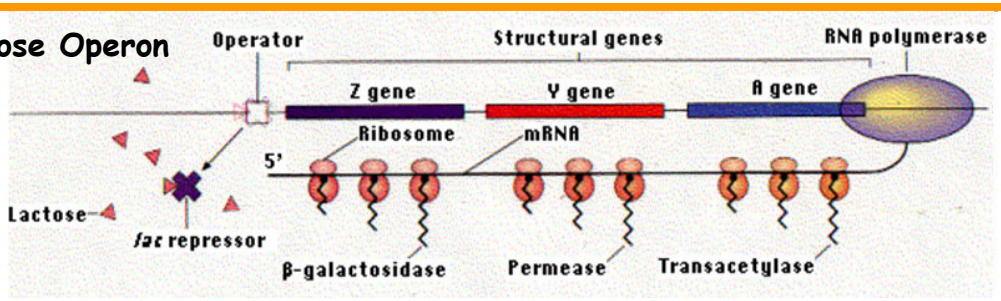


4-letter digital code | 4-letter digital code | 20-letter digital code | 50.000(?) shapes



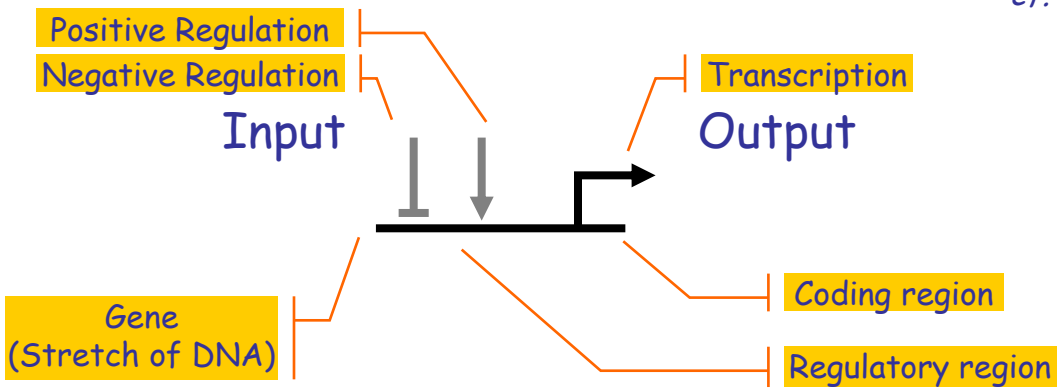
DNA Tutorial

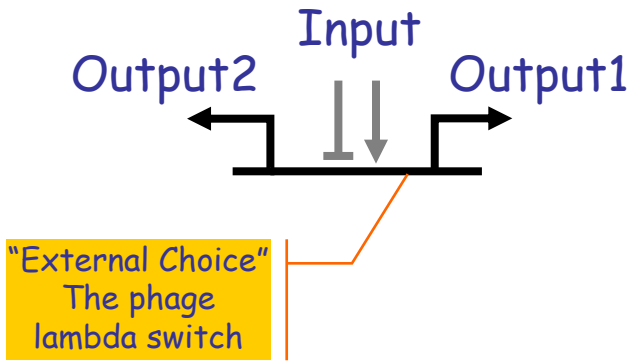




The Gene Machine "Instruction Set"

cf. Hybrid Petri Nets [Matsuno, Doi, Nagasaki, Miyano]





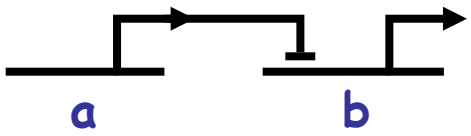
Regulation of a gene (positive and negative) influences transcription. The regulatory region has precise DNA sequences, but not meant for coding proteins: meant for binding regulators.

Transcription produces molecules (RNA or, through RNA, proteins) that bind to regulatory region of other genes (or that are end-products).

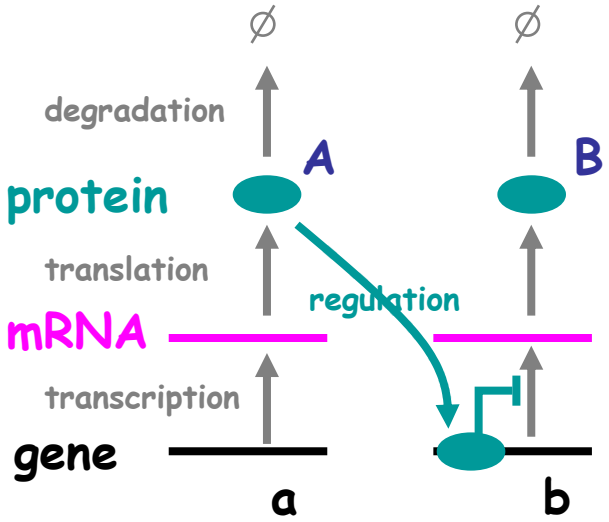
Human (and mammalian) Genome Size
3Gbp (Giga base pairs) 750MB @ 4bp/Byte (CD)
Non-repetitive: 1Gbp 250MB
In genes: 320Mbp 80MB
Coding: 160Mbp 40MB
Protein-coding genes: 30,000-40,000

M.Genitalium (smallest true organism)
580,073bp 145KB (eBook)
E.Coli (bacteria): 4Mbp 1MB (floppy)
Yeast (eukarya): 12Mbp 3MB (MP3 song)
Wheat 17Gbp 4.25GB (DVD)

Gene Composition



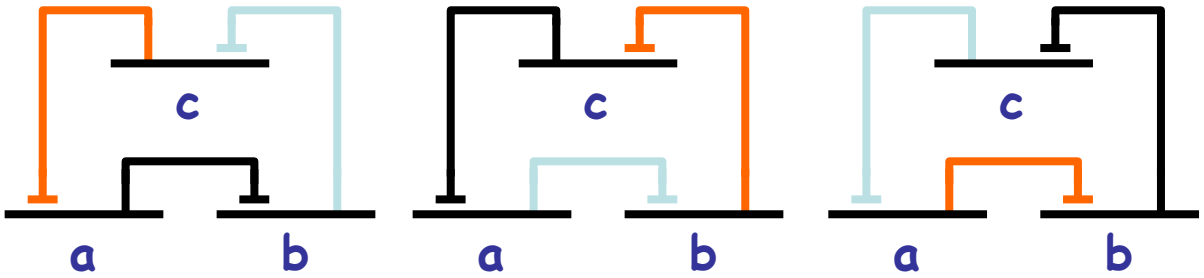
Is a shorthand for:



Under the assumptions [Kim & Tidor]
1) The solution is well-stirred
   (no spatial dependence on concentrations or rates).
2) There is no regulation cross-talk.
3) Control of expression is at transcription level only
   (no RNA-RNA or RNA-protein effects)
4) Transcriptions and translation rates monotonically
   affect mRNA and protein concentrations resp.

Ex: Bistable Switch

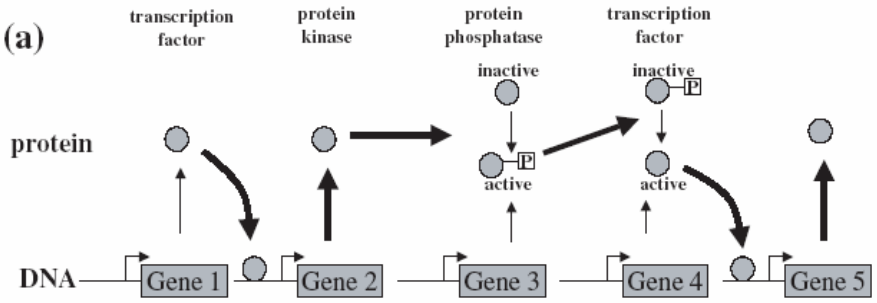
Ex: Oscillator



Expressed
Repressed
Expressing

Indirect Gene Effects

No combination of standard high-throughput experiments can reconstruct an a-priori known gene/protein network [Wagner].



(b)
Aspect of gene activity: mRNA expression
Genetic perturbation: gene deletion

G1:	G2, G5
G2:	G5
G3:	G5
G4:	G5
G5:	

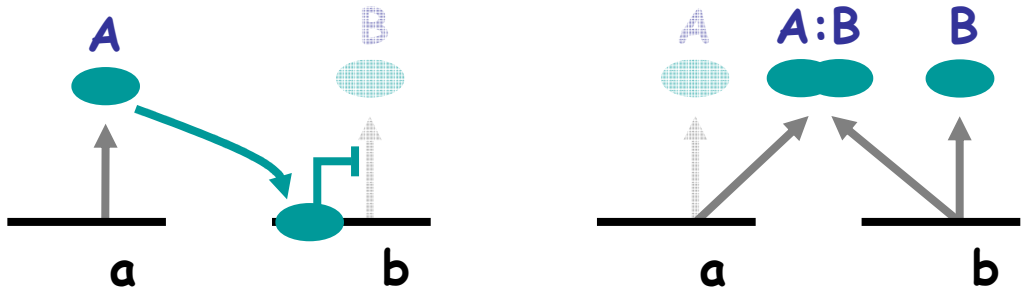
(c)
Aspect of gene activity: phosphorylation state
Genetic perturbation: gene deletion

G1:	G3, G4
G2:	G3, G4
G3:	G4
G4:	
G5:	

Taken from Andreas Wagner

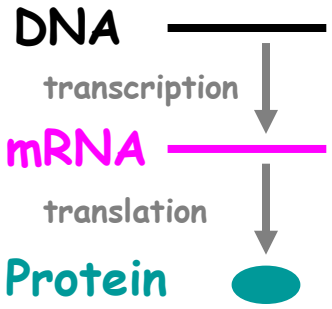
Fig. 1. The importance of specifiying gene activity when reconstructing genetic networks. (a) A hypothetical biochemical pathway involving two transcription factors, a protein kinase, and a protein phosphatase, as well as the genes encoding them. See text for details. (b) Shown is a list of perturbation effects for each of the five genes in (a), when perturbing individual genes by deleting them, and when using mRNA expression level as an indicator of gene activity. The left-most symbol in each line stands for the perturbed gene. To the right of each colon is a list of genes whose activity is affected by the perturbation. (c) Analogous to (b) but for a different notion of gene activity (phosphorylation state).

One of many bistable switches that cannot be described by pure gene regulatory networks [Francois & Hakim].

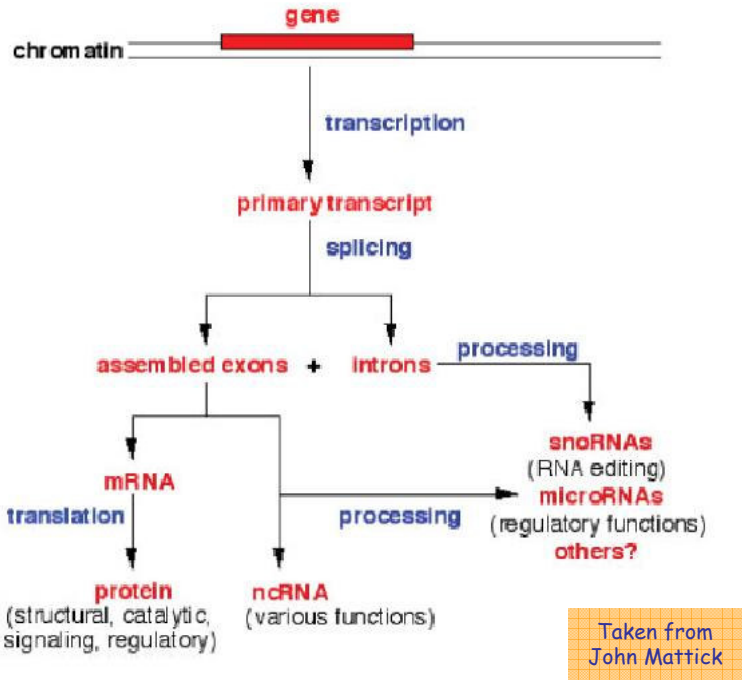


Structure of the Coding Region

The Central Dogma



Challenging the Dogma (in higher organisms)

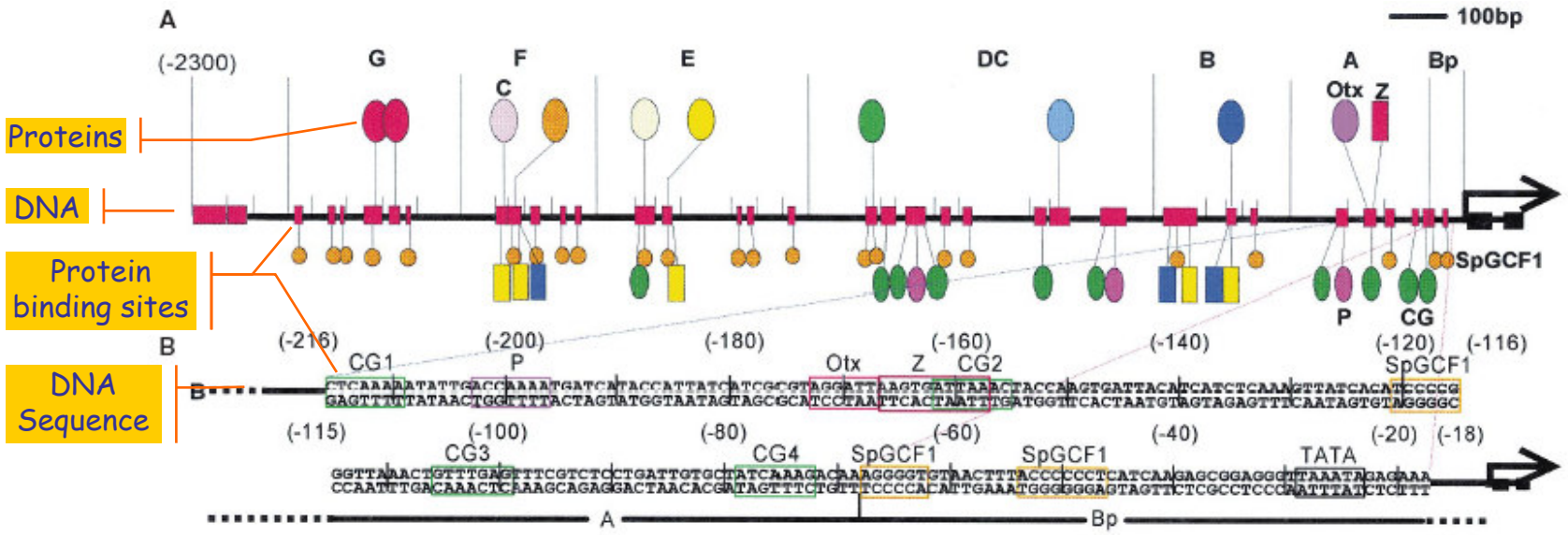


Taken from John Mattick

RNA is not just an intermediary; it can:
- **Fold-up like a protein**
- **Act like an enzyme**
- **Regulate other transcribed RNA**
- **Direct protein editing**
- **…**

97-98% of the transcriptional output of the human genome is non-protein-coding RNA.
30-40,000 "protein genes" (1.5% of genome)
60-100,000 "transcription units" (>30% of genome is transcribed)

Structure of a Regulatory Region



2300bp!

> average protein

C **Module A functions:**

Vegetal plate expression in early development:

Synergism with modules B and G enhancing endoderm expression in later development:

Repression in ectoderm (modules E and F) and skeletogenic mesenchyme (module DC):

Modules E, F and DC with LiCl treatment:

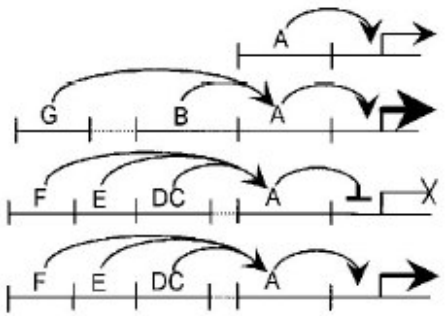
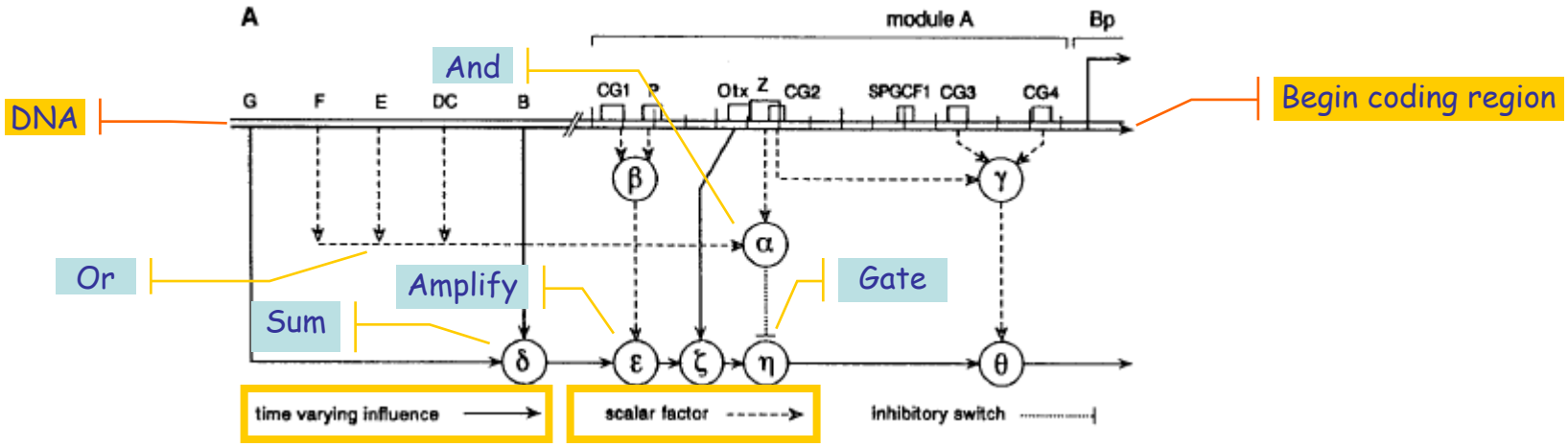


Fig. 1. *Endo16* cis-regulatory system and interactive roles of module A. (**A**) Diversity of protein binding sites and organization into modular subregions [modified from (*7*)]. Specific DNA binding sites are indicated as red blocks; modular subregions are denoted by letters G to A (Bp, basal promoter). Proteins binding at the target sites considered in this work are indicated: Otx, SpOtx-1 (*12*); SpGCF1 (*14*); the proteins CG, Z, and P, which are not yet cloned; and protein C [a CREB family protein (*18*)] in subregion F. Proteins for which sites occur in multiple regions of the DNA sequence (indicated by the black line) are shown beneath. (**B**) Sequence of module A and location of protein binding sites. Sites are indicated in the same colors as in (A). A fragment containing CG_3 and CG_4 sites as well as Bp has no endoderm-specific activity and services other upstream cis-regulatory systems promiscuously; similarly, the *Endo16* cis-regulatory system functions specifically with heterologous promoters substituted for Bp (*5, 8, 19*). Boxed sequences indicate conserved core elements of the target sites (*7, 12, 14*), not the complete target site sequences. (**C**) Integrative and interactive functions of module A (*5, 8*). Module A communicates the output of all upstream modules to the basal transcription apparatus. It also initiates endoderm expression, increases the output of modules B and G, and is required for functions of the upstream modules F, E, and DC. These functions are repression of expression in nonendodermal domains and enhancement of expression in response to LiCl.

Taken from Eric H Davidson

Function of a Regulatory Region



B

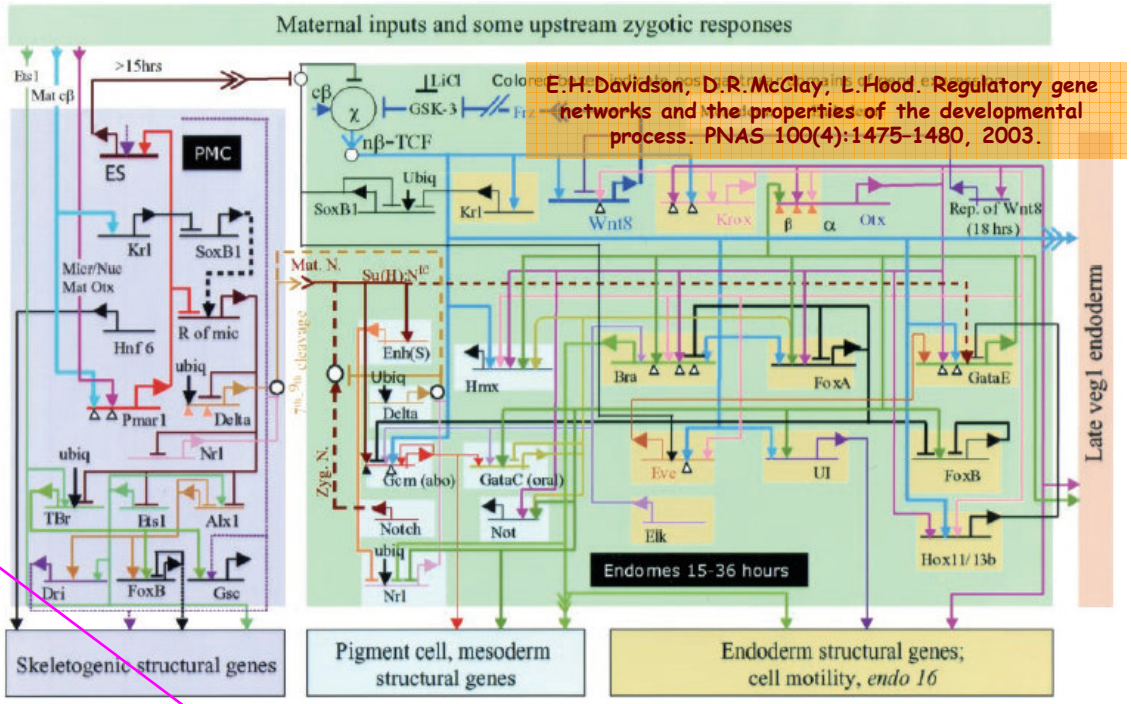
if (F = 1 or E = 1 or CD = 1) and (Z = 1)	Repression functions of modules F, E, and DC mediated by Z site
α = 1	
else α = 0	
if (P = 1 and CG_1 = 1)	Both P and CG_1 needed for synergistic link with module B
β = 2	
else β = 0	
if (CG_2 = 1 and CG_3 = 1 and CG_4 = 1)	Final step up of system output
γ = 2	
else γ = 1	
δ(t) = B(t) + G(t)	Positive input from modules B and G
ε(t) = β*δ(t)	Synergistic amplification of module B output by CG_1-P subsystem
if (ε(t) = 0)	Switch determining whether Otx site in module A, or upstream modules (i.e., mainly module B), will control level of activity
ξ(t) = Otx(t)	
else ξ(t) = ε(t)	
if (α = 1)	Repression function inoperative in endoderm but blocks activity elsewhere
η(t) = 0	
else η(t) = ξ(t)	
Θ(t) = γ*η(t)	Final output communicated to BTA

C-H.Yuh, H.Bolouri, E.H.Davidson. Genomic Cis-Regulatory Logic: Experimental and Computational Analysis of a Sea Urchin Gene. Science 279:1896-1902, 1998

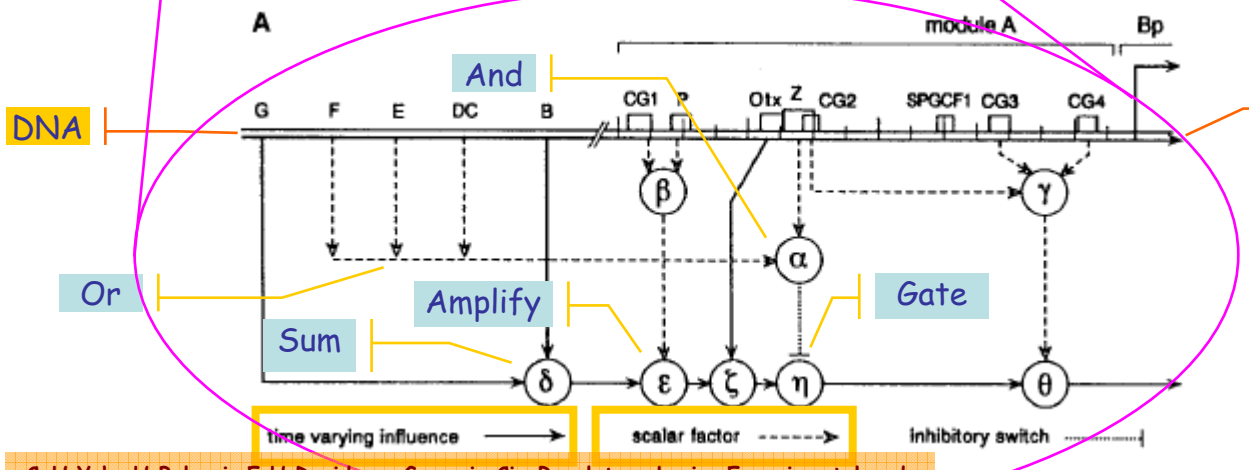
Gene Regulatory Networks

http://strc.herts.ac.uk/bio/maria/NetBuilder/

NetBuilder



E.H.Davidson, D.R.McClay, L.Hood. Regulatory gene networks and the properties of the developmental process. PNAS 100(4):1475-1480, 2003.



C-H.Yuh, H.Bolouri, E.H.Davidson. Genomic Cis-Regulatory Logic: Experimental and Computational Analysis of a Sea Urchin Gene. Science 279:1896-1902, 1998

The Programming Model

- Strange facts about genetic networks:
  - Not an operator algebra. The output of each gate is fixed and pre-determined; it is never a function of the input!
  - Not term-rewriting, nor Petri nets. Inhibition is widespread.
  - Not Communicating Sequential Processes. Feedback is widespread: asynchronous communication needed to avoid immediate self-deadlocks. Even the simplest gates cannot be modeled as a single synchronous automata.
  - Not Message-Passing between genes. Messages themselves have behavior (e.g., they stochastically decay and combine), hence messages are processes as well.
  - Not Data-Flow. Any attempt to use data-flow-style modeling seems doomed because of widespread loops that lead to deadlocks or unbounded queues. Data-flow tokens do not "decay" like proteins.

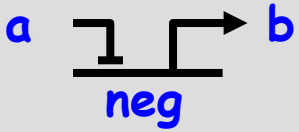
- How can it possibly work?
  - Stochastic broadcasting. The apparently crude idea of broadcasting a whole bunch of asynchronous decaying messages to activate a future gate, means there are never any "pipeline full" deadlocks, even in presence of abundant feedback loops.
  - Stochastic degradation. Degradation is fundamental for system stability, and at the same time can lead to sudden instability and detection of concentration levels.

Notations for the Gene Machine

- Many of the same techniques as for the Protein Machine apply.
  - Process Calculi, Petri Nets, Term-Rewriting Systems…
- But the "programming model" is different.
  - Asynchronous stochastic control.
  - Biologically poorly understood.
  - Network "motifs" are being analyzed.
- Specific techniques:
  - Hybrid Petri Nets
    - [Matsuno, Doi, Nagasaki, Miyano] Gene Regulation
    - Genomic Object Net www.genomicobject.net
- Gene Regulation Diagrams
- Mixed Gene-Protein Diagrams

Gene Gates and Circuits

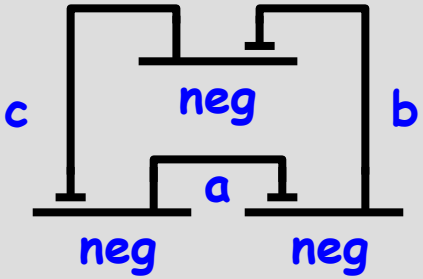
A gene gate



$neg(a,b) \triangleq$
$?a_r;\ \tau_\eta;\ neg(a,b) +$
$\tau_\varepsilon;\ (tr(b) \mid neg(a,b))$

$tr(p) \triangleq (!p_r;\ tr(p)) + \tau_\delta$

A genetic circuit (engineered in E.Coli)

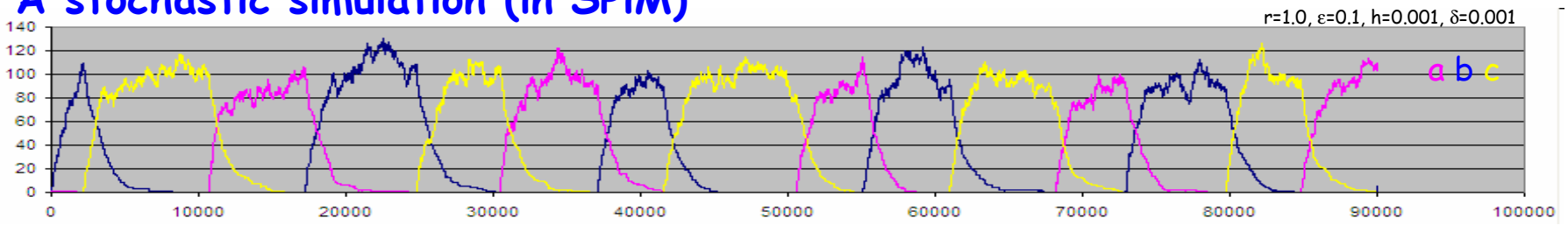


neg(a,b) |
neg(b,c) |
neg(c,a)

The stochastic-π program

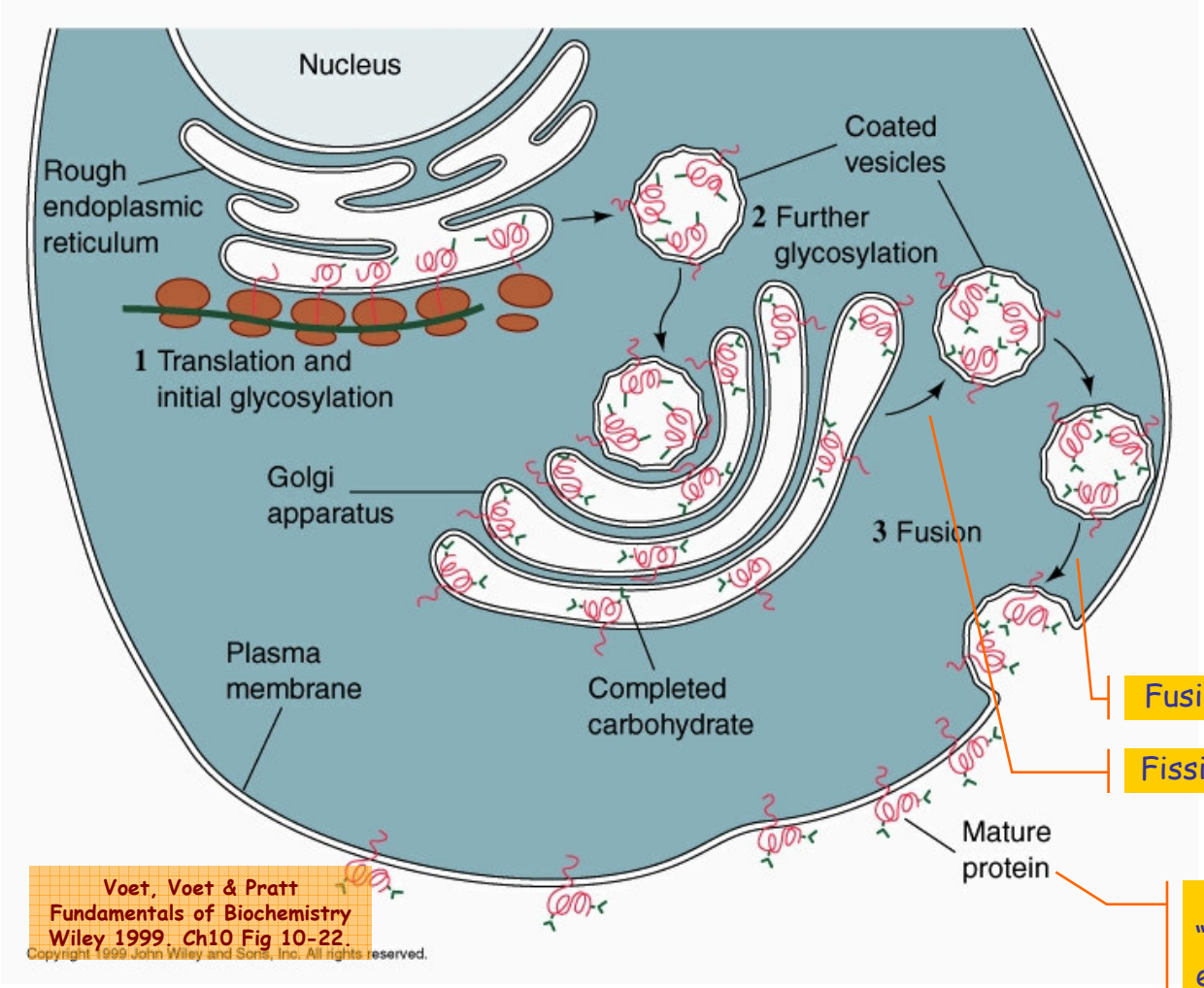
```
val dk = 0.001     (* Decay rate *)
val inh = 0.001    (* Inhibition rate *)
val cst = 0.1      (* Constitutive rate *)

let tr(p:chan()) =
    do !p; tr(p) or delay@dk

let neg(a:chan(), b:chan()) =
  do ?a; delay@inh; neg(a,b)
  or delay@cst; (tr(b) | neg(a,b))

(* The circuit *)
val bnd = 1.0      (* Protein binding rate *)
new a@bnd:chan() new b@bnd:chan() new c@bnd:chan()
run (neg(c,a) | neg(a,b) | neg(b,c))
```

A stochastic simulation (in SPiM)



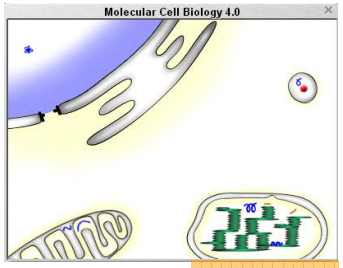
3. The Membrane Machine

Very far from the atoms.



Voet, Voet & Pratt
Fundamentals of Biochemistry
Wiley 1999. Ch10 Fig 10-22.

Molecular transport and transformation through dynamic compartment fusion and fission.



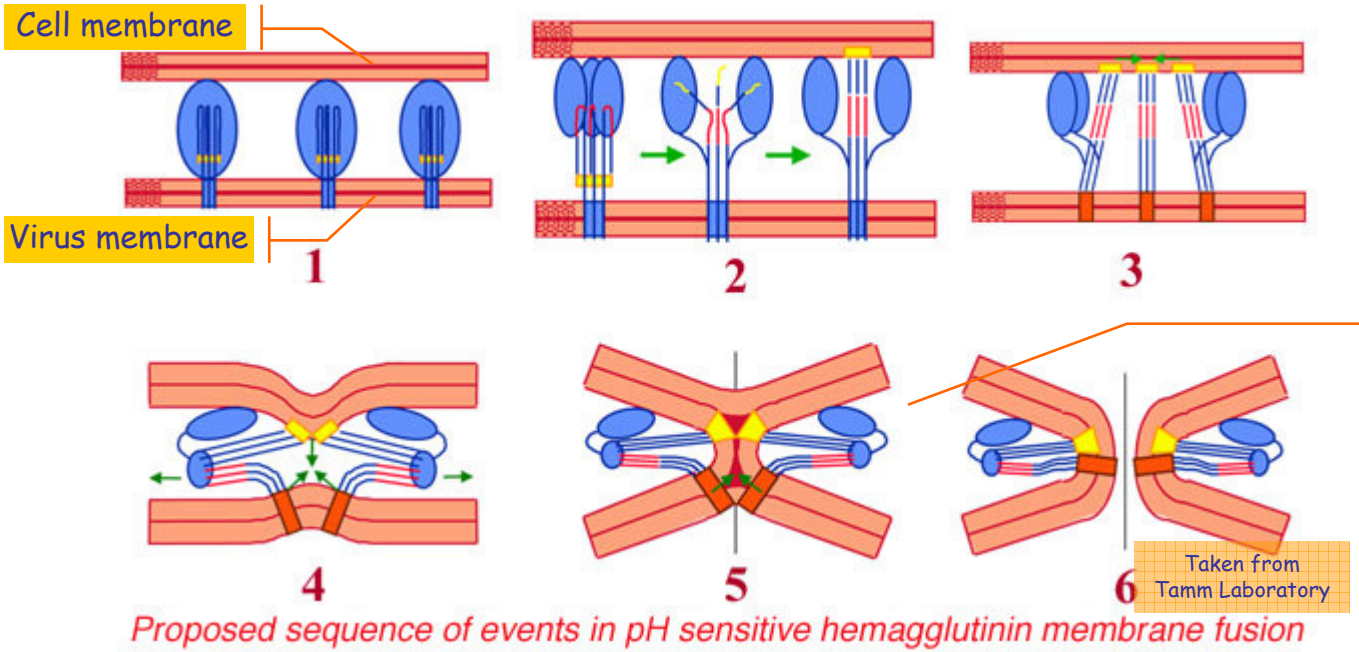
Taken from MCB CD

Fusion
Fission
} **The Instruction Set**

Well, what is all that for?
"Given the complicated pathways that have evolved to synthesize them, it seems likely that these [modified proteins] have important functions, but for the most part these functions are not known" [MBC p.609]

Membrane Fusion

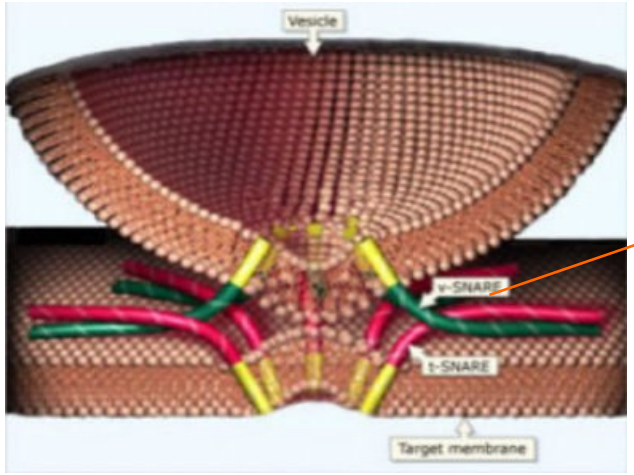
Positive curvature to Negative curvature transition in 3D



Proposed sequence of events in pH sensitive hemagglutinin membrane fusion

Aggressive fusion (virus)

By unknown mechanisms, the exoplasmic leaflets of the two membranes fuse" [MCB p745]

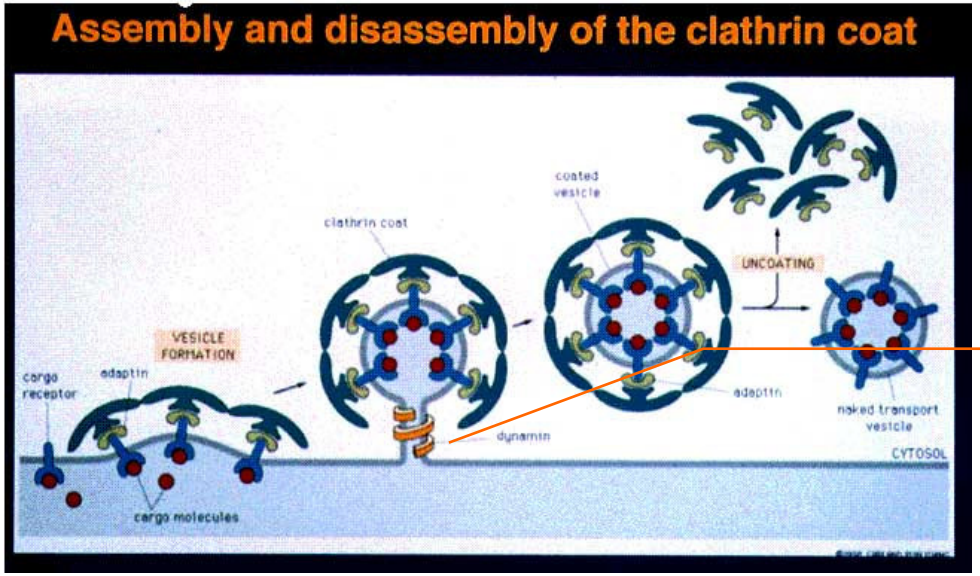


Cooperative fusion (vesicle)

"Fusion of the two membranes immediately follows prefusion, but precisely how this occurs is not known" [MCB p742]

Membrane Fission

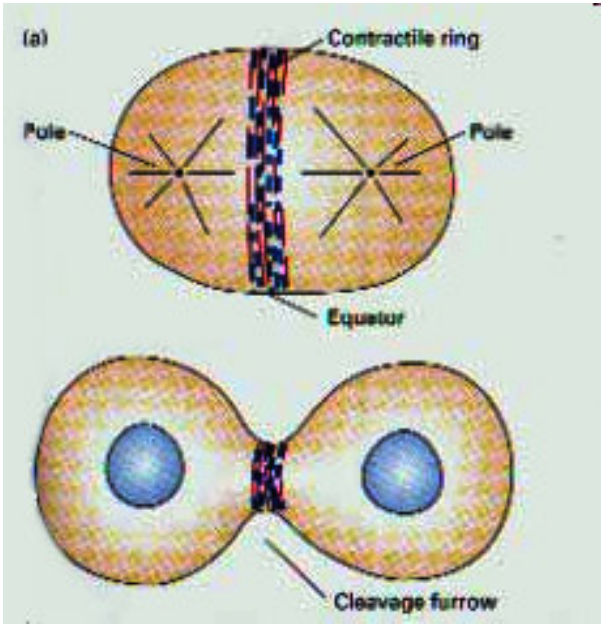
Negative curvature to Positive curvature transition in 3D



Vesicle Formation

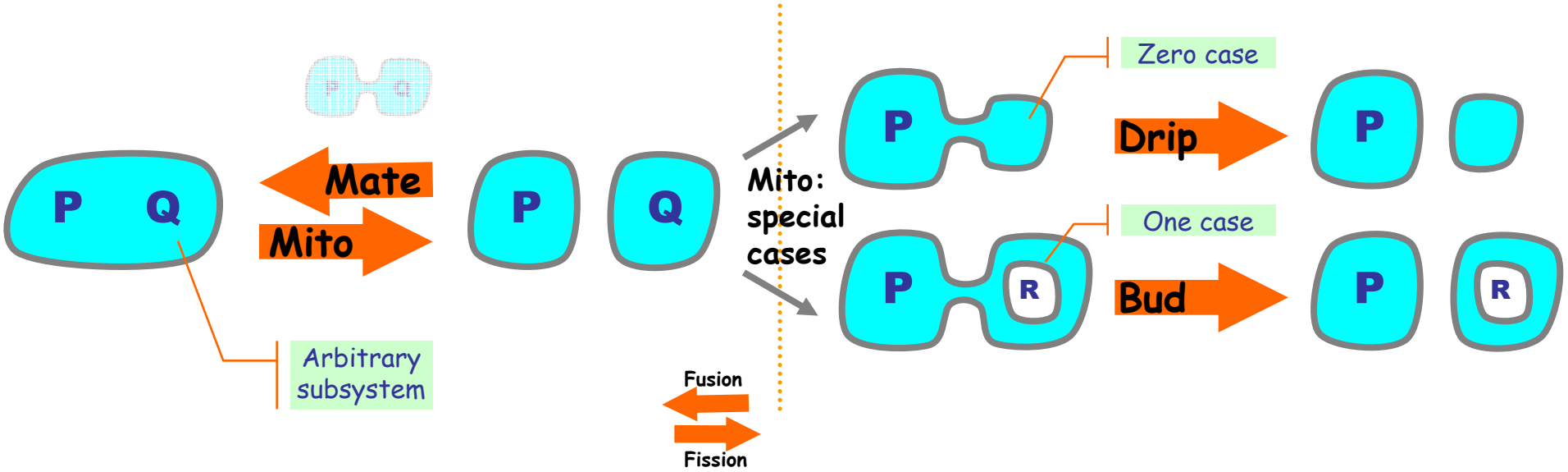
Movie by Allison Bruce

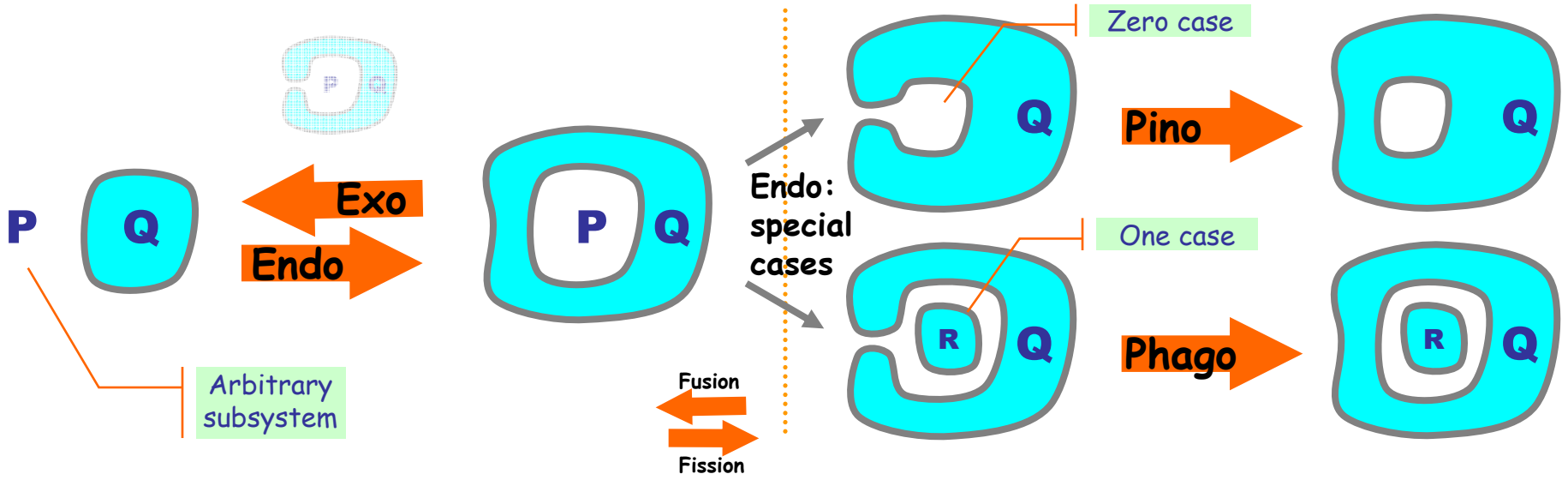
"Nonetheless, the actual process whereby a segment of phospholipid bilayer is 'pinched off' to form a pit and eventually a new vesicle is still not understood" [MCB p.746]



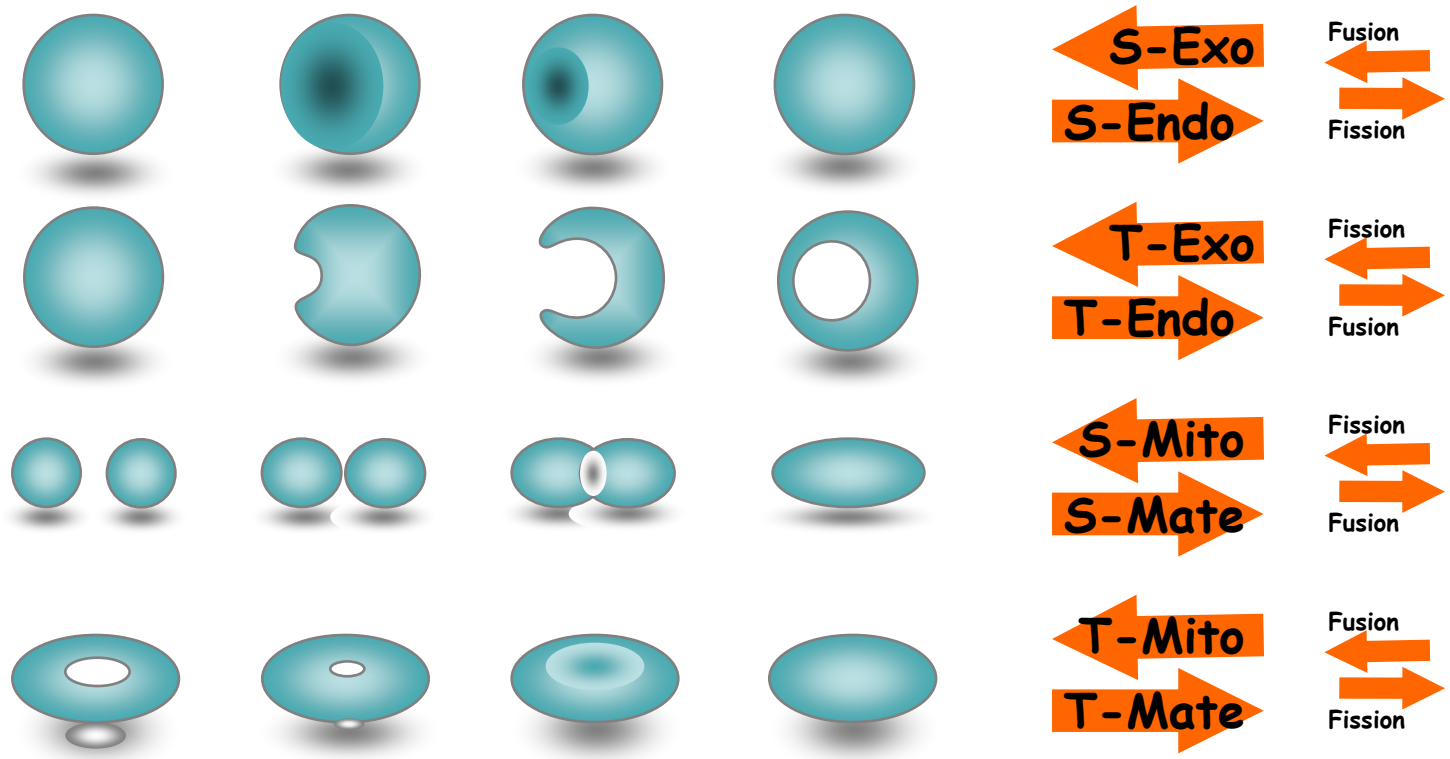
Cytokinesis (Mitosis)

The Membrane Machine "Instruction Set"



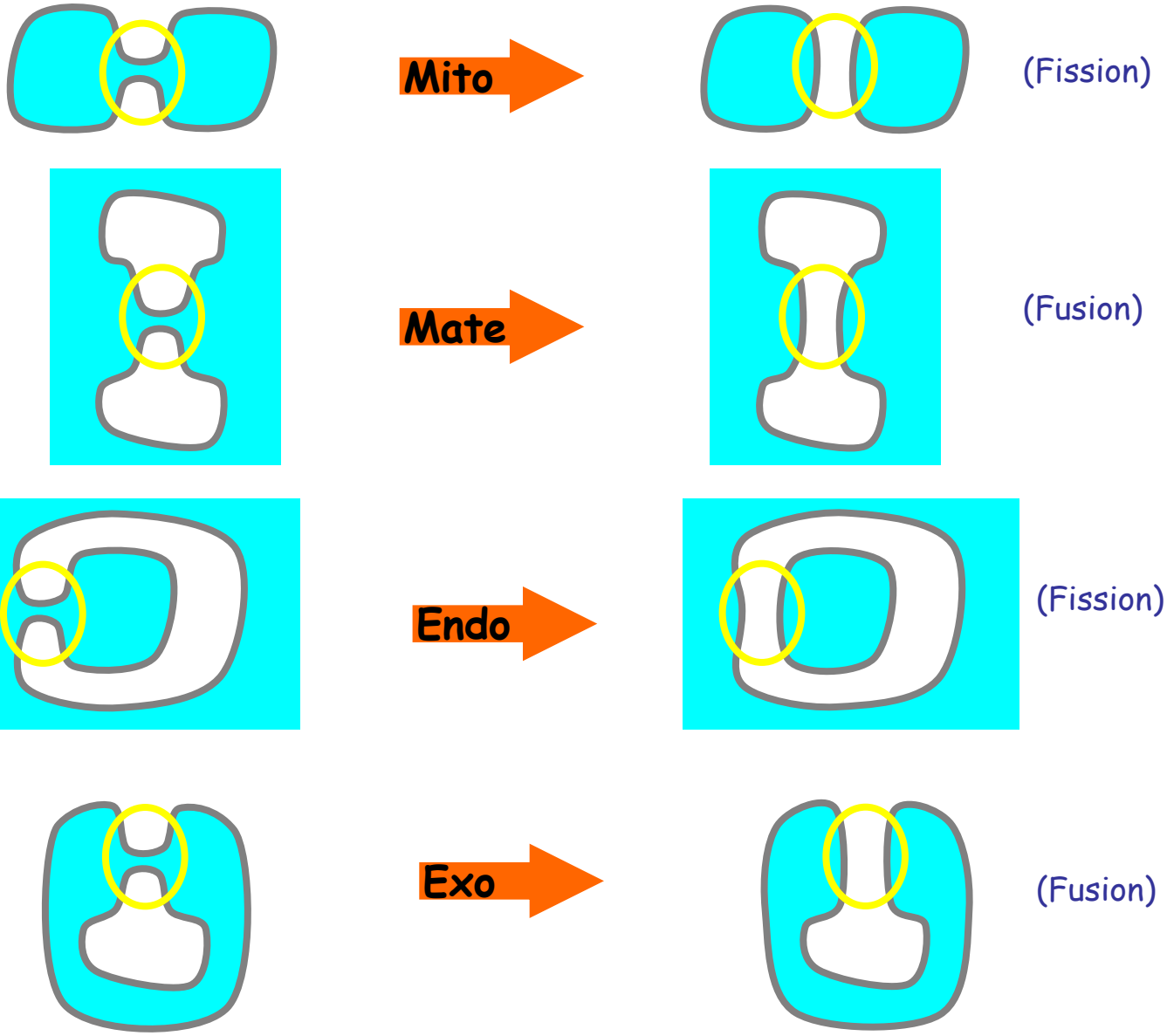


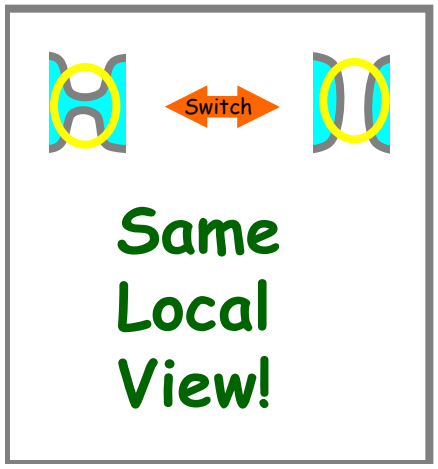
… in 3D


S-Exo
S-Endo
Fusion
Fission
T-Exo
T-Endo
Fission
Fusion
S-Mito
S-Mate
Fission
Fusion
T-Mito
T-Mate
Fusion
Fission

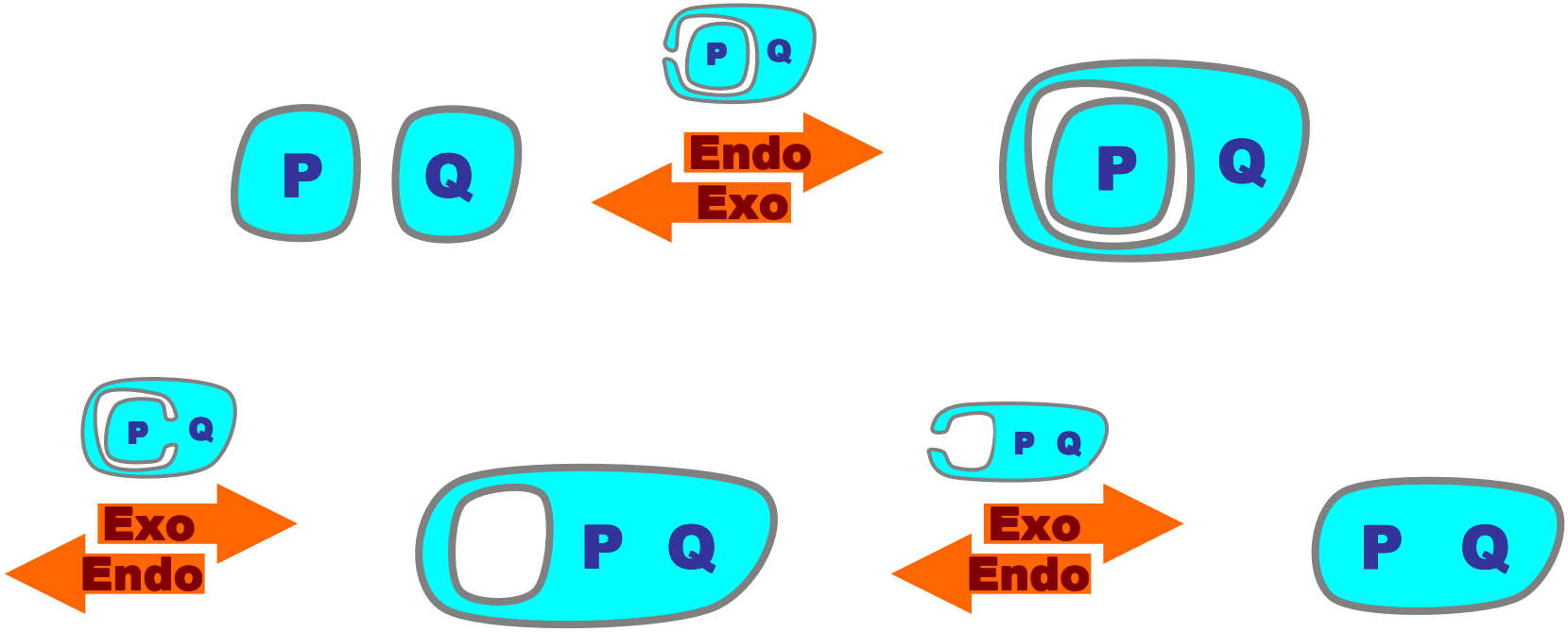
Locally Implementable!

Global Views





Mito/Mate by 3 Endo/Exo

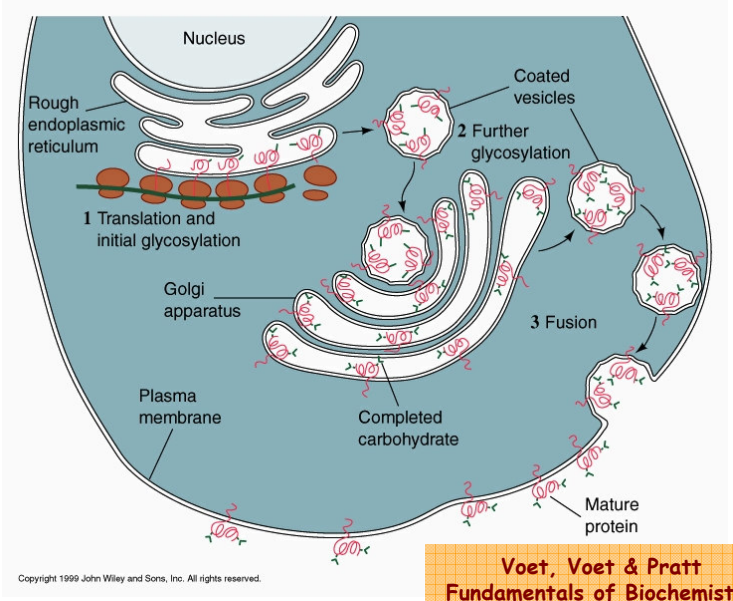


Notations for the Membrane Machine

- "Snapshot" diagrams
  - In biology literature.
- P-Systems
  - G.Paun uses ideas from the theory of grammars and formal languages to model "Membrane Computing" (book 2002).
    http://psystems.disco.unimib.it/.
- BioAmbients
  - An extension of BioSPI along Ambient Calculus lines (with more bio-relevant mobility primitives) to model dynamic compartments.
- Brane Calculi
  - Computation *on* the membrane…

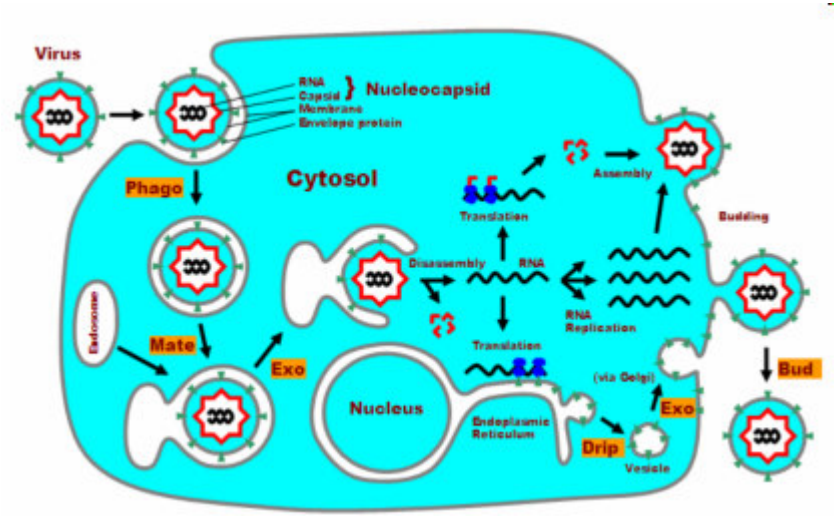
Membrane Algorithms

Protein Production and Secretion



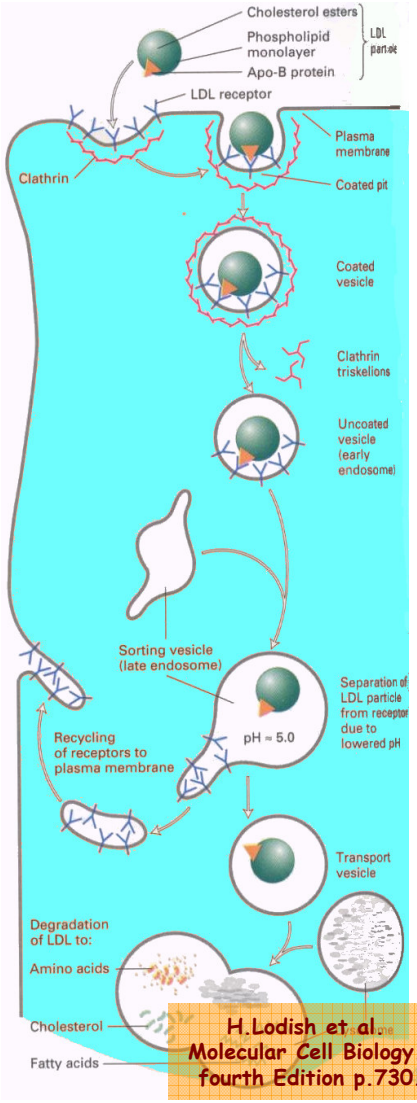
Voet, Voet & Pratt
Fundamentals of Biochemistry
Wiley 1999. Ch10 Fig 10-22.

Viral Replication



Adapted from: B.Alberts et al.
Molecular Biology of the Cell
third edition p.279.

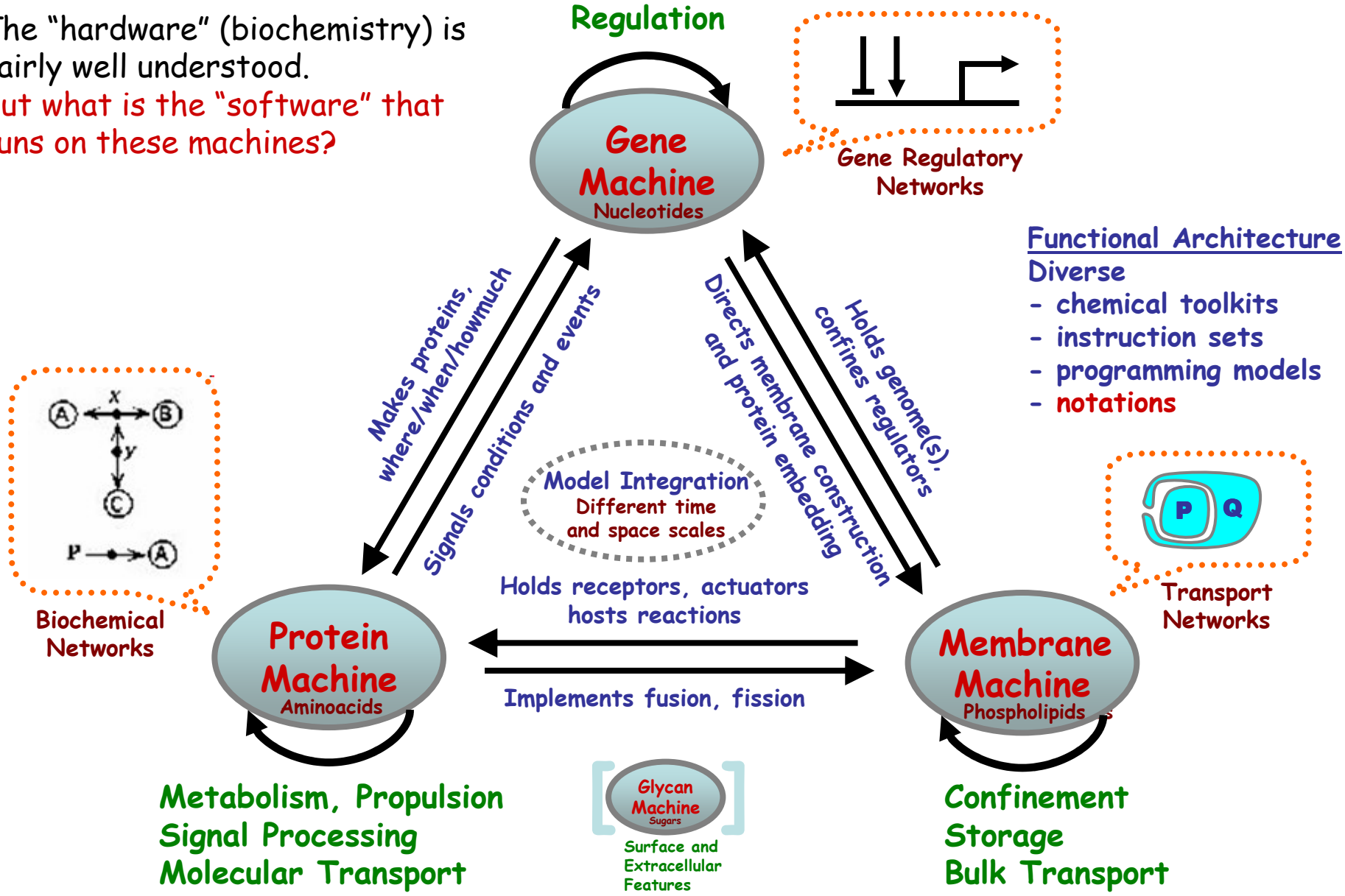
LDL-Cholesterol Degradation



H.Lodish et al.
Molecular Cell Biology.
fourth Edition p.730.

Abstract Machines of Systems Biology

The "hardware" (biochemistry) is fairly well understood.
But what is the "software" that runs on these machines?



Importance of Stochastic Effects

- *A* **deterministic** *system:*
  - May get "stuck in a fixpoint".
  - And hence **never oscillate**.

- *A similar* **stochastic** *system:*
  - May be "thrown off the fixpoint" by stochastic noise, entering a long orbit that will later bring it back to the fixpoint.
  - And hence **oscillate**.

Surprisingly enough, we have found that parameter values that give rise to a stable steady state in the deterministic limit continue to produce reliable oscillations in the stochastic case, as shown in Fig. 5. Therefore, the presence of noise not only changes the behavior of the system by adding more disorder but can also lead to marked qualitative differences.

Mechanisms of noise-resistance in genetic oscillators

Jose´ M. G. Vilar, Hao Yuan Kueh, Naama Barkai, Stanislas Leibler

PNAS **April 30, 2002** vol. 99 no. 9 p.**5991**

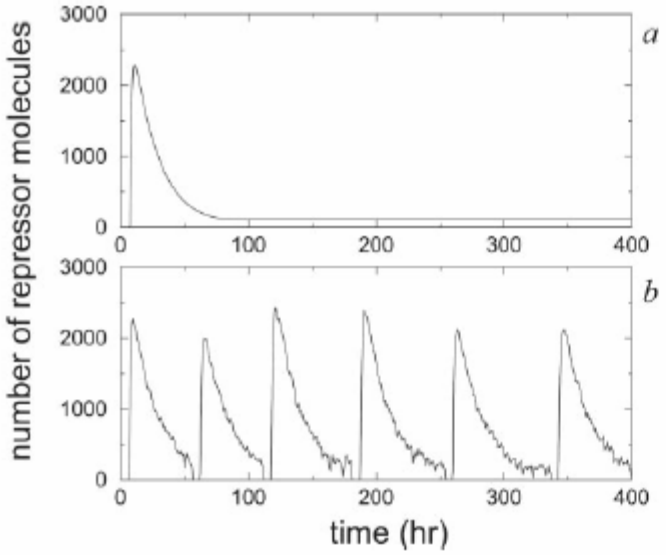


Fig. 5. Time evolution of R for the deterministic Eq. [1] (*a*) and stochastic (*b*) versions of the model. The values of the parameters are as in the caption of Fig. 1, except that now we set $\delta_R = 0.05\ h^{-1}$. For these parameter values, $\tau < 0$, so that the fixed point is stable.

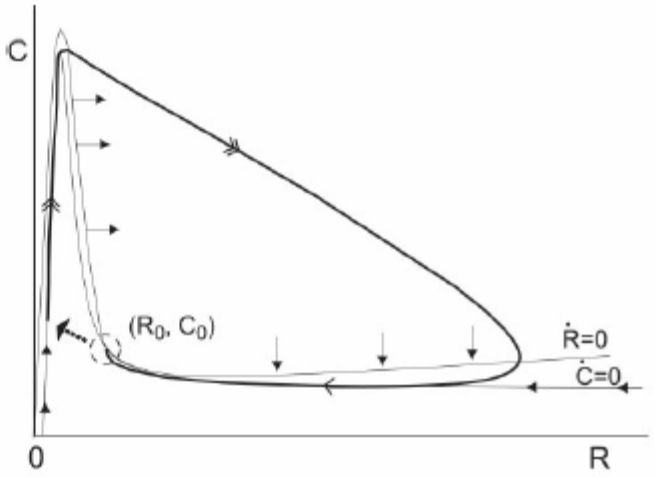
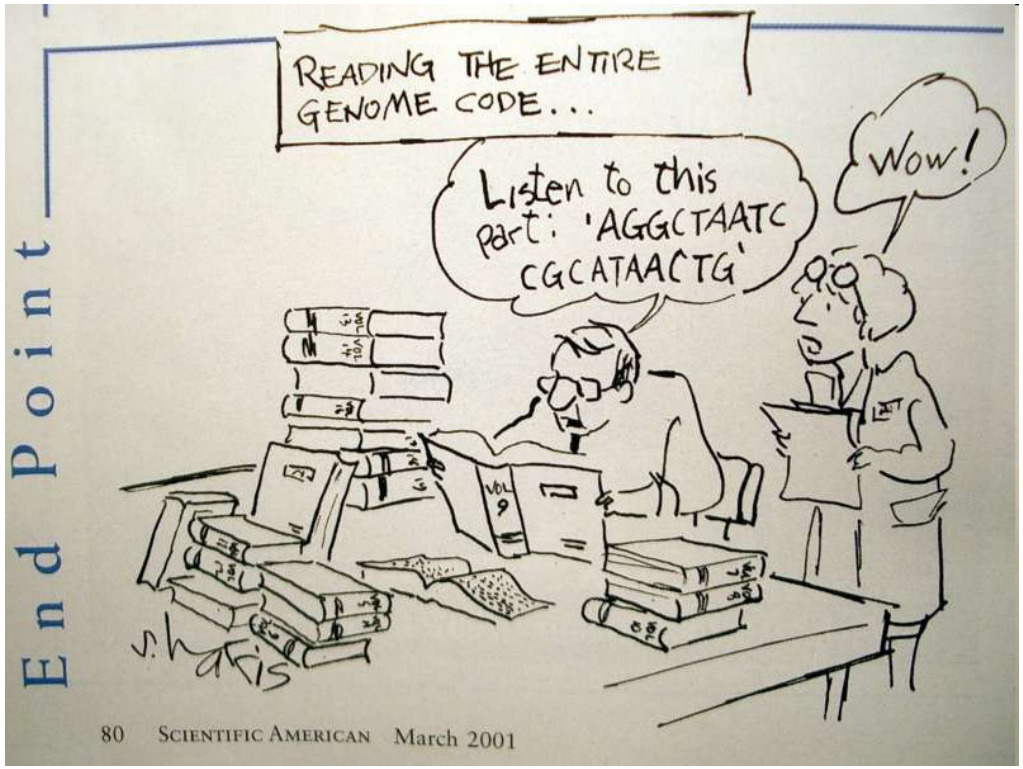


Fig. 6. Phase portrait as in Fig. 4 but for a situation in which the system falls into the stable fixed point (R_0,C_0). The dotted arrow to the left of the fixed point illustrates a perturbation that would initiate a single sweep of the (former) oscillatory trajectory.

Conclusions



Q: "The data are accumulating and the computers are humming, what we are lacking are the words, the grammar and the syntax of a new language..."

D. Bray (TIBS 22(9):325-326, 1997)

A: "The most advanced tools for computer process description seem to be also the best tools for the description of biomolecular systems."

E.Shapiro (Lecture Notes)

References

[MCB] Molecular Cell Biology, Freeman.

[MBC] Molecular Biology of the Cell, Garland.

[Ptashne] A Genetic Switch.

[Davidson] Genomic Regulatory Systems.

[Milner] Communicating and Mobile Systems: the Pi-Calculus.

[Regev] Computational Systems Biology: A Calculus for Biomolecular Knowledge (Ph.D. Thesis).

Papers

BioAmbients

a stochastic calculus with compartments.

Brane Calculi

process calculi with computation "on" the membranes, not inside them.

Bitonal Systems

membrane reactions and their connections to "local" patch reactions.

Abstract Machines of Systems Biology

the abstract machines implemented by biochemical toolkits.

www.luca.demon.co.uk/BioComputing.htm