# Cells *in silico*: a Holistic Approach

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*joint work with a lot of nice BISCA people :-)*

*Bertinoro, 7th June 2007*

# Desiderata

Gaining knowledge about biological phenomena

- Understand the functionality of bio-components
  - assessment of known facts
  - discovery of new functionalities
- Investigate the underlying structure of biological complex systems
  - how genome, proteome and metabolome interact giving rise to *emergent properties*

# It’s a matter of language

Science is rough, language is subtle – d’après R. Barthes

Not only mathematics
[il mondo] è scritto in lingua matematica e i caratteri son triangoli, cerchi, .... – Galileo
but also executable, because

the closer the language to the described object, the more effective the description – Wittgenstein

# From Syntax to Semantics

*To understand function, study structure* – F. Crick

I've been told to work no longer in modern biology:

| STRUCTURE ***AND*** FUNCTION |
|---|

The genome as a 4-letters language — syntax

$\Downarrow$

what and how it expresses for — semantics "cells *as* computational devices"

Petri Nets, Rewrite Systems, Logics, ...

Pocess calculi

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Just as concurrent, distributed, mobile processes

# Processes

Concurrent, distributed, mobile processes are made of

- several components acting independently, interacting each other, distributed geographically
- interaction
  - is mainly binary
  - occurs on selected channels between components
  - is local, but affects the whole system globally

# Process calculi: primitives

Few basic primitives for

- sending $!a(v)$ and receiving $?a(v)$ the value $v$, if any, on channel $a$
  channels mimick *interaction* points, values the exchanged *information*
- performing non detailed activities $\tau$
  abstracting from, e.g., biochemical details
- creating/handling channels

composed with few operators ...

# Process calculi: composition

Among the few operators there are:

- parallel composition $P \mid Q$
  cells as processes, that may interact or proceed independently
- choice $P + Q$
  according to a *probabilistic distribution* — more to come

# Process calculi: semantics

How do systems evolve?

- Semantics is given through a logically based inference system, defining transitions — how a configuration changes into another
- Communication, i.e. interaction, is the basic computational step

# Process calculi: Semantics

Essentially, communication and asynchrony are ruled by:

- $?a(x).P \ \mid !a(v).Q \rightarrow P[x \mapsto v] \ \mid Q$

  the activity is local

- IF $P \rightarrow P'$ THEN $P \mid Q \rightarrow P' \mid Q$

  its effect is global — more to come

# Quantitative information

... otherwise "*stamp collection*" — Rutherford

- interactions occur at given rates – channels posses rates
- (often) interactions are reversible (possibly with different rates)
- the context affects the overall rates – not only temperature, pressure, etc, but also concentration – here the quantities of reactants per unit (typically, Gillespie's Stochastic Simulation Algorithm)

# Summing up

- molecules, metabolites, compounds, cells as processes
- (biochemical) interactions as communications
- affinity of interaction as communication capabilities

(other features, like membranes, geometry, time, ... often treated *ad hoc* or still under investigation)

**Process calculi specify and execute Bio-systems**

# What do we gain?

- run the model, and obtain virtual experiments — an integral abstract description of system behaviour: unexpected, global properties may emerge
- formally analyse the executions, collecting e.g. statistical data on behaviour, or causality among interactions, or similarities/differences between systems, model-checking properties ...
- compositionality — specify new components in isolation (e.g. active principles), put them aside the others with *no other change* and see (cf. ODE)

# A simple example

Consider the enzyme-catalysed production of a product $P$ from the substrate $S$:

$$E + S \rightleftharpoons^{K_{ES}}_{K_{ES}^{-1}} ES \rightharpoonup^{K_P} E + P$$

The corresponding processes are

$E = !a$ where $rate(a) = K_{ES}$

$S = ?a.ES$ where $rate(\tau_1) = K_{ES}^{-1}$

$ES = \tau_1.(E|P) + \tau_{-1}.(E|S)$ where $rate(\tau_{-1}) = K_P$

A computation is

$E = !a$ where $rate(a) = K_{ES}$

$S = ?a.ES$ where $rate(\tau_1) = K_{ES}^{-1}$

$ES = \tau_1.(E|P) + \tau_{-1}.(E|S)$ where $rate(\tau_{-1}) = K_P$

$$n \cdot E \mid m \cdot S \xrightarrow{r_0}$$

$$(n-1) \cdot E \mid (m-1) \cdot S \mid ES \xrightarrow{r_0'}$$

$$(n-2) \cdot E \mid (m-2) \cdot S \mid 2 \cdot ES \xrightarrow{r_1}$$

$$(n-1) \cdot E \mid (m-2) \cdot S \mid ES \mid P \xrightarrow{r_0''}$$

$$(n-2) \cdot E \mid (m-3) \cdot S \mid 2 \cdot ES \mid P \xrightarrow{r} \ldots$$

where the actual rates $r_0, r_0', \ldots$ are typically computed with Gillespie's SSA and depend on the rates of channels and on the number of reactants.

# Other approaches

- Petri nets
- formal languages (P systems, ...)
- rewriting systems ($\kappa$-calculus, Biocham, calculus of looping sequences, ...)
- logically based formalisms (Pathway logic, ...)
- reactive systems (Statecharts...)
- ....

# Work within BISCA

- VIrtual CEll:
  artificial ur-cell, from a simplified prokaryote
  — with a variant of the $\pi$-calculus
- E. Coli:
  the whole metabolic pathways, with knock-outs
  — with a very fast (subset of) the $\pi$-calculus
- Calix of Held: a neuronal synapsis

Towards a holistic model of a ***whole*** cell: all interactions among metabolic pathways (properties emerge), the whole movie not only snapshots

# Building up VICE: the genome

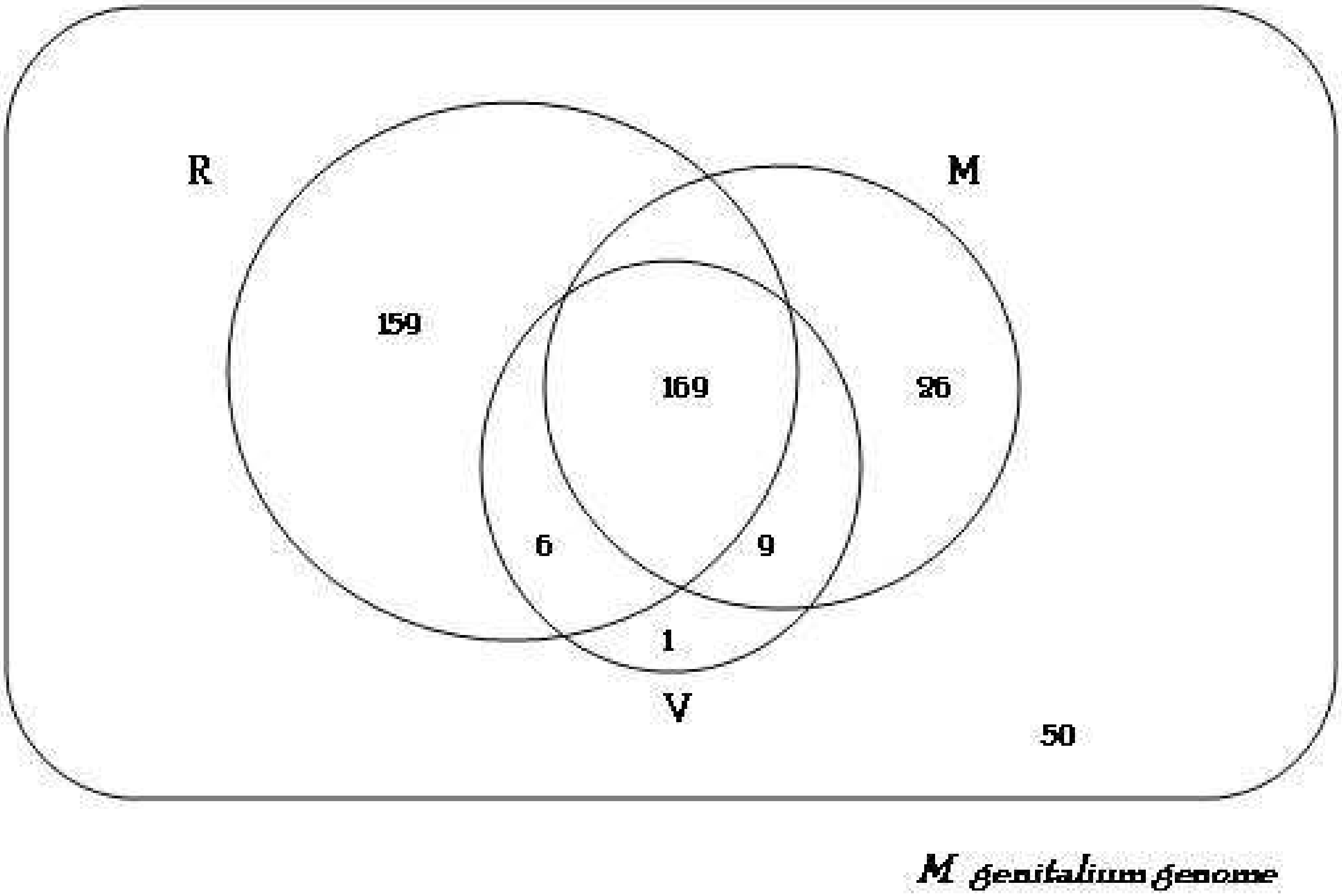
Problems:

- not an arbitrary list of genes
- *small* enough for the sake of computability

Our choice: The "Minimal Gene Set"

- from *Haemophylus influenzae, Mycoplasma genitalium*
- cf. Glass et al. – gene KO *in vitro*

# Genome comparison



# Building up VICE: hypothesis

Reduction and update of the *Minimal Gene Set*, based on a functional analysis.

- selection of basic activities (*eating*, production of energy, synthesis of basic structural components, reproduction)
- choice of the 187 genes involved
- design of the metabolic pathways needed (presently only for *survival*)

# VICE: Validation

- Check on biological consistency:
  - all the pathways selected have been taken: *sufficient*
  - no genes are left inactive: *necessary*
- Comparison with real results:
  - confirm basic modelling choice
  - calls for deeper analysis and more features

# Activities

Group pathway (and reactions) in the standard biochemical manner:

**Oxidations:** extraction of energy from nutrients:

Glycolysis→Pyruvate→. . .

**Lipid metabolism:** synthesis of structural components from monomers: fatty acids. . .

**Nucleotide metabolism:** building DNA/RNA bases, no *de novo* synthesis

**DNA/RNA synthesys:** RNA for building proteins, DNA for reproduction – not yet available

**Protein synthesis:** no amino acids

**Uptake:** Glycerol, amino acids, nitrous bases, fatty acids. . .

. . . plus a few other pathways.

# Virtual experiments

Through runs of the $\pi$-specification of VICE

- in presence of different quantities of food (VICE in parallel with different numbers of glucose processes – naïve)
- for different periods of time (computations of different length)

Under the assumption on the environment:

- enough nutrients (water, sugar, phosphates, amino acids, nitrous bases. . . )
- no toxics
- no competing organisms (a single VICE)
- right temperature, pressure, ...

# Results

Data are collected from $10^3$ computations, made of $10^4$ transitions, involving $10^6$ different processes ($\sim 12$ hours each)

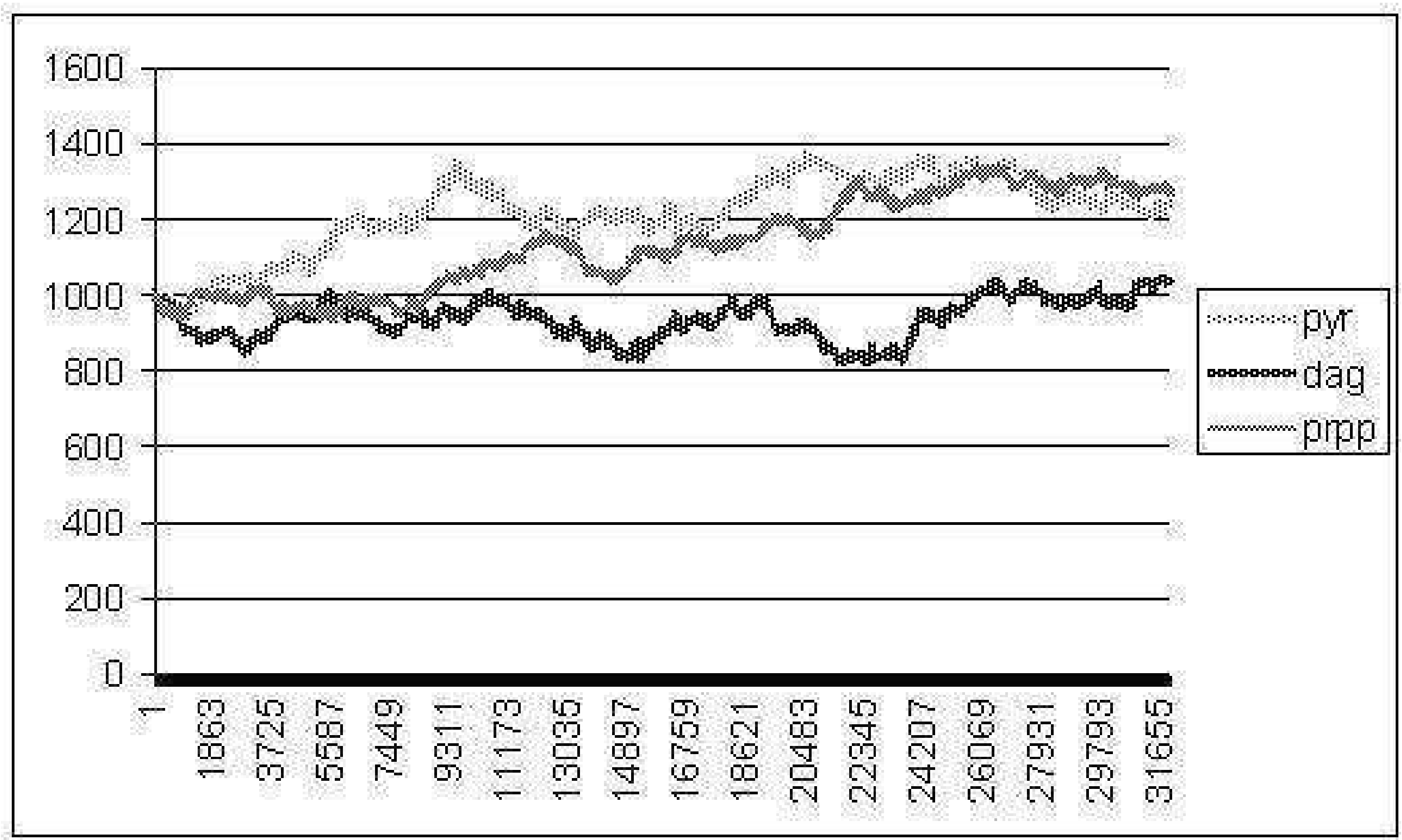
*Throughput*:

- Production of energy and metabolites, through oxidation of glucose, shows homeostasis
- biomass produced as expected

*Distribution of metabolites over Glycolysis pathway*:

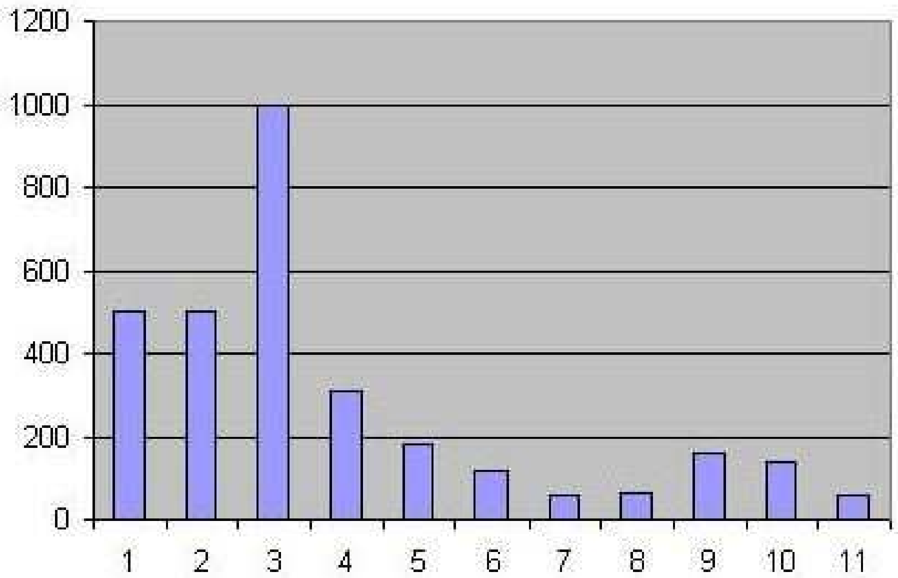
- Like in real prokaryotes (in their steady state)
- The distributions agree with those computed in vitro.

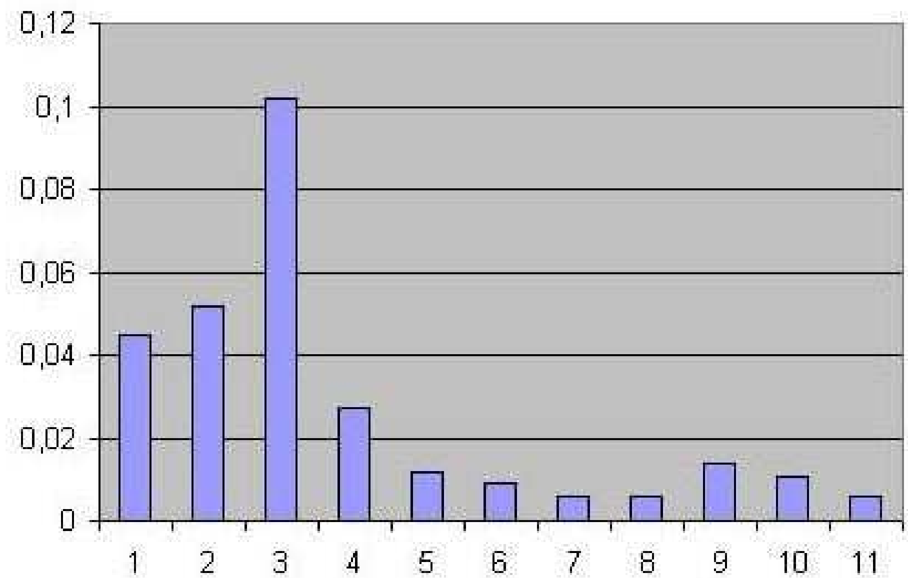
# Steady state




pyruvate, diacilglycerol, phosphoribosylpyrophosphate

# Usage of enzymes



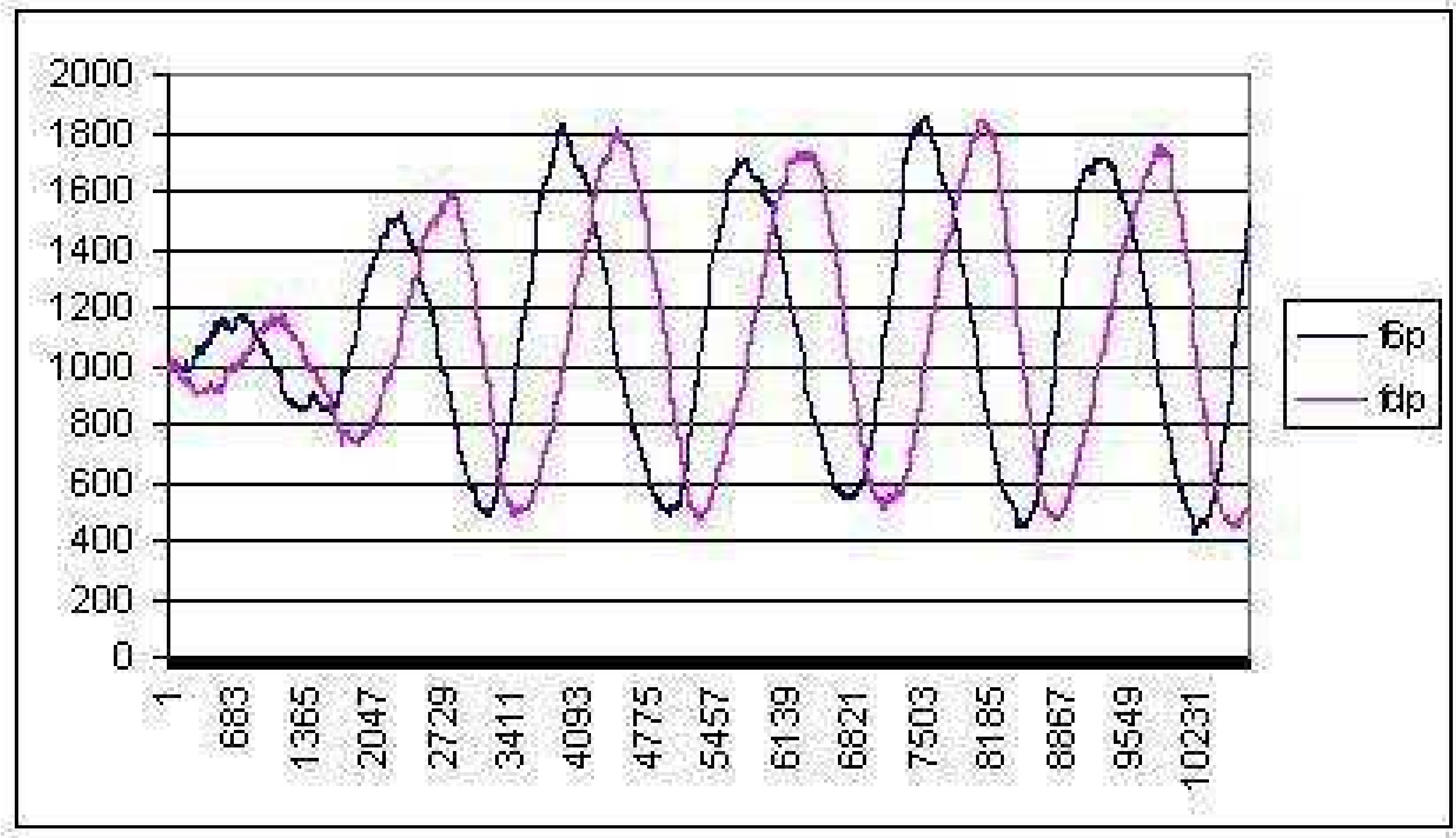




| | | | | | |
|---|---|---|---|---|---|
| 1 | mg111 | 5 | mg300 | 9 | compl. pyr. dehydrogenase |
| 2 | mg215 | 6 | mg430 | 10 | mg299 |
| 3 | mg023 | 7 | mg407 | 11 | mg357 |
| 4 | mg031 | 8 | mg216 | | |

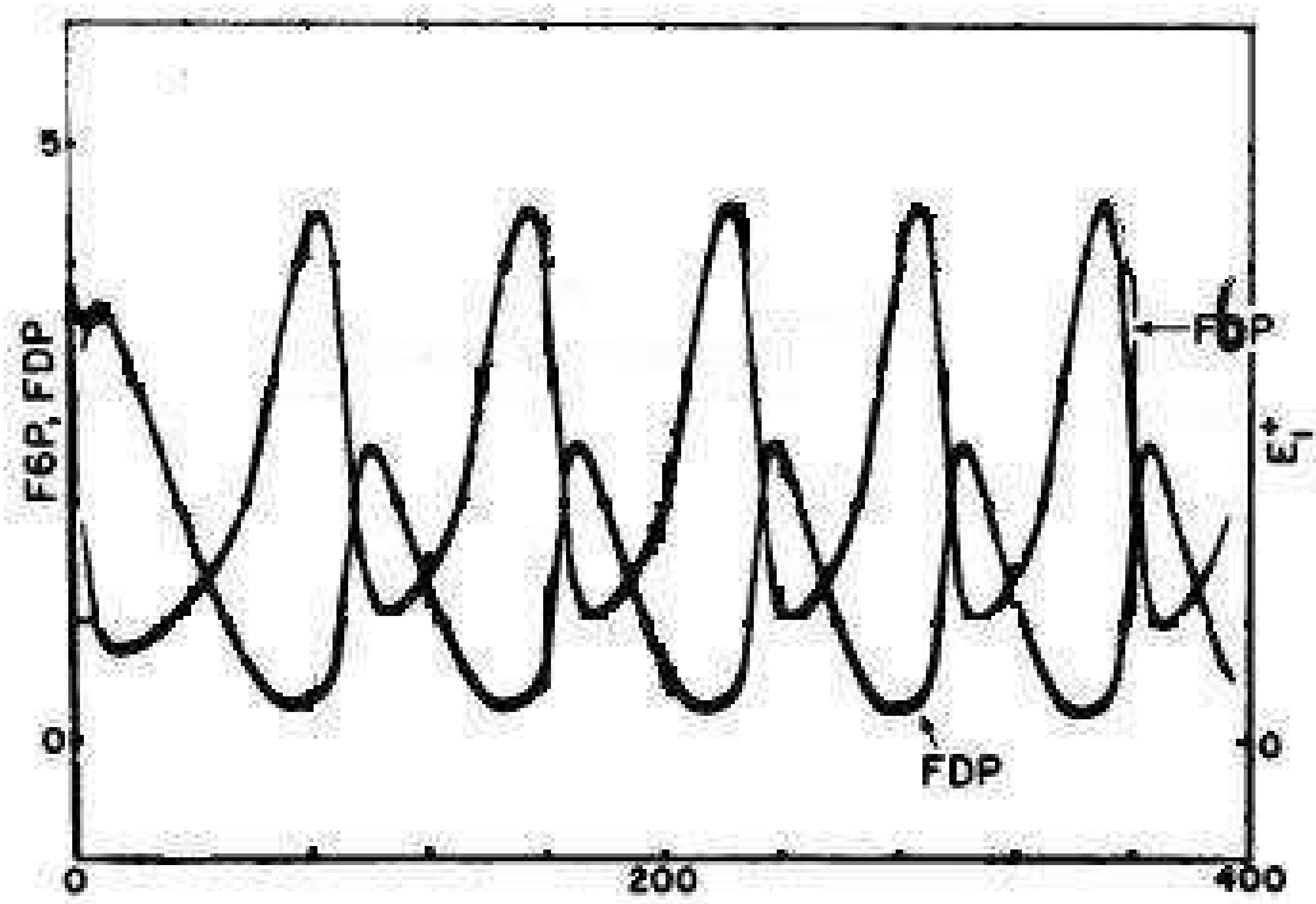
# Something emerges

- Add the specification of a regulatory feedback circuit on the enzyme phosphofructokinase (the more ADP the faster the phosphorilation of fructose-6-phosphate).
  Look then at the time course of fructose-6-phosphate and fructose-1.6-bephosphate
- Change the feeding regimen by supplying the sugar:
  - all at the beginning, a huge quantity — no oscillations
  - at a constant rate — oscillations show up!!

# Oscillations



# the real ones ...



# Other case studies ...

- Specify and run the metabolome of E. coli
- Because of efficiency problems, a new implementation
  - a subset of CCS (fast also with name passing)
  - essentially multiplication of stoichiometric matrices - allowing for either stochastic or deterministic simulations
  - many orders of magnitude faster than the previous one ($10^8$ transitions involving $10^7$ different kinds of processes in few minutes)

# E. Coli

- The virtual behaviour “matches” the real one
- Knock out some genes
  - agrees on known KO (ppc, pgi, zwf)
  - a new KO (rpe) – no data in the literature

# Calix of Held

- A large synapsis in the mammalians
- A first step to studying plasticity and memory
- Pre-synaptic and post;synaptic mechanisms of neuro-transmitter release and reception
- Executable model (in Spim) + a bit of spatiality
- Results agree with other deterministic, non executable models:

  - high sensitivity to low concentration of Ca++
  - adaptive response of vescicle turn-over

# Conclusions

- Cells as processes $\Rightarrow$ "virtual" living matter
- Formal, mathematical theory $\Rightarrow$ mechanical analysis tools
  - constructive and executable
  - compositional, with different abstraction levels
- Quantities crucial for behavioural descriptions
- New computational models (e.g. new interation mechanisms) $\Rightarrow$ new semantics
- "Virtual" experiments as computations $\Leftarrow$ not enough!! wet experiments ...

# To Do

Far from satisfactory languages! New challenges:

- membranes, compartments and the like
- geometrical issues
- more faithful (and efficient) bio-chemistry
- causality
- usability (graphich interfaces, fast interpreters, specification generators from data bases, ...)
- new analysis techniques (static vs dynamic) and tools

Towards ...

# Bio-calculus environment

Towards uniform (families of) environments

- sharing formal grounds and tools
- providing the user with mechanisms for describing systems at different levels of abstraction

$$\Uparrow$$

More fundamental research and more case studies