1. CRN Syntax

Let $S = \{x_1, \ldots, x_s\}$ be a finite set of molecular species names.

Def. A reaction is a quadruple (R, I, P, f), also noted $R\ /\ I \xrightarrow{f} P$
where R (resp. I, P) is a multiset of reactant species (resp. inhibitor, product species)
and $f: \mathbb{R}_+^s \to \mathbb{R}_+$ is a rate function (kinetic expression).

- Multisets are represented by linear expressions with integer stoichiometric coefficients
- A reaction catalyst is a molecular species that is both a reactant and a product (can also be an inhibitor).

Def. A CRN is a finite set of reactions.

E.g. reactions with

- Mass action law kinetics $\sum_j n_j \times x_j \xrightarrow{k \times \prod_j x_j^{n_j}} p$
- Michaelis-Menten kinetics $x \xrightarrow{V \times x/(K+x)} y$
- Hill kinetics $x \xrightarrow{V \times x^n/(K^n+x^n)} y$ Negative Hill kinetics $\emptyset / x \xrightarrow{V/K^n+x^n} y$

Well-formed Reactions

Def. A reaction (R, I, P, f) is well-formed if

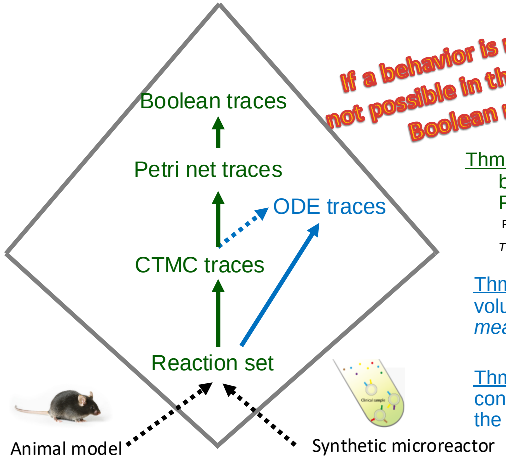
- $f: \mathbb{R}_+^s \to \mathbb{R}_+$ is a partially differentiable function
- $x_i \in R$ if and only if $\frac{\partial f}{\partial x_i}(x) > 0$ for some value $x \in \mathbb{R}_+^s$
- $x_i \in I$ if and only if $\frac{\partial f}{\partial x_i}(x) < 0$ for some value $x \in \mathbb{R}_+^s$.

Def. A reaction is strict if $R(x_i) > 0$ implies $f(x_1, \ldots, x_s) = 0$ whenever $x_i = 0$.

Prop. The ODE associated to a well-formed and strict reaction system (CRN) defines a positive system.

Fages, Gay, Soliman. Inferring Reaction Systems from Ordinary Differential Equations. *Theoretical Computer Science*, 599:64–78, 2015.

Hierarchy of Semantics


Boolean traces

Petri net traces

ODE traces

CTMC traces

Reaction set

Animal model

Synthetic microreactor

If a behavior is not possible in the Boolean semantics, it is not possible in the stochastic semantics for any reaction rates. Boolean model-checking can exhibit rare events

Thm. (abstract interpretation) Galois connections between the domains of syntactical, stochastic, Petri net and Boolean trace semantics

Fages, Soliman. Abstract Interpretation and Types for Systems Biology. *Theoretical Computer Science*, 403(1):52–70, 2008.

Thm. (approximation) [Gillespie 1971 Kurtz 1978, 1992] When the volume tends to infinity the ODE trace approximates the *mean* stochastic trace

Thm. (equality) [Buscemi Fages CMSB 2024] Under graphical conditions on the ancestors of polymolecular reactions, the ODE trajectory equals the *mean* stochastic trace.

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2. Computable Real Numbers and Functions

Classical definitions of *computable analysis* based on Turing machines

Definition. A real number *r* is computable if there exists a Turing machine with
Input: precision $p \in \mathbb{N}$
Output: rational number $q \in \mathbb{Q}$ with $|r-q| < 2^{-p}$

Examples. Rational numbers, limits of computable Cauchy sequences ($\lim_{p,q\to\infty} |x_p - x_q| = 0$), π, e, …

Definition. A real function $f: R \to R$ is computable if there exists a Turing machine that computes $f(x)$ with an oracle (Turing machine) for x.

Examples. Polynomials, trigonometric functions, analytic functions ($f(x) = \sum_{n=0}^{+\infty} a_n (x - x_0)^n$) …

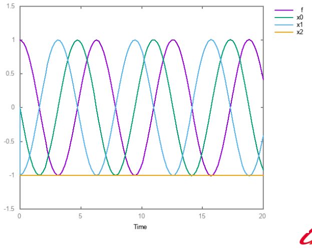
Counter-examples. x=0, $\lceil x \rceil$ are not computable (undecidable on x=0.000…) discontinuous functions are not computable

Decision problem $w \in \mathcal{L}$: analog encoding by a real function $f: R \to R$?
Input encoding $e: \mathcal{L} \to R$ problem encoding by f: accept w if $f(e(w)) > 1$ reject if < -1

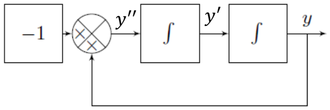
General Purpose Analog Computer [Shannon 1941]

Shannon's formalization of the Differential Analyser by GPAC circuits
A time function if GPAC-generated if it is the output of some unit of a GPAC circuit built from:

1. Constant unit
2. Sum unit
3. Product unit
4. Integral $\int y\,dx$ unit (dt by default)



What does this GPAC circuit compute ?



$$y' = \frac{dy}{dt}$$

$$y'' = \frac{d^2y}{dt^2} = -y$$

$y(t) = cos(t) \quad y'(t) = -sin(t)$
if $y(0) = 1,\ y'(0) = 0$

CRN Implementation of GPAC Units

Mass action law kinetics reaction network with output concentration stabilizing on the result of the operation applied to the input concentrations

Positive constant units: molecular concentrations

Product unit $z = x.y$

$$x + y \xrightarrow{k.x.y} x + y + z$$
$$z \xrightarrow{k.z} \_$$
$$\frac{dz}{dt} = k(xy - z)$$
$$= 0 \text{ when } z = x.y$$

Sum unit $z = x + y$

$$x \xrightarrow{k.x} x + z$$
$$y \xrightarrow{k.y} y + z$$
$$z \xrightarrow{k.z} \_$$
$$\frac{dz}{dt} = k(x + y - z)$$
$$= 0 \text{ when } z = x + y$$

Time integral $z = \int x\,dt$ unit

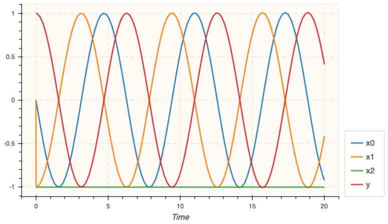
$$x \xrightarrow{x} x + z$$
$$\frac{dz}{dt} = x$$
$$z = \int_0^T x\,dt$$

Polynomial ODE Initial Value Problems (PIVP)

Graça and Costa 2003's formalization of GPAC generated functions

Definition. A real time function $f:R_+ \to R$ is PIVP-generable iff there exist a vector of polynomials $p \in R^n[R^n]$ and of initial values $y(0) \in R^n$ and a solution function $y:R_+ \to R^n$ such that $y'(t)=p(y(t))$ and $f(t)=y_1(t)$

Example. y=cos(t)

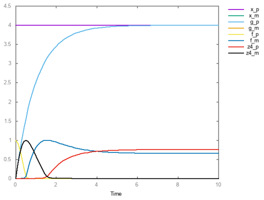


Closure properties:
f+g, f-g, f.g, 1/f, ,f ∘g, y s.t. y' =f(y) are GPAC-generable if f, g are.

PIVP-Computable Function f(x)

Definition. [Graça Costa 03 J. Complexity] A real function $f:R \to R$ is PIVP-computable if there exists vectors of polynomials $p \in R^n[R^n]$ and $q \in R^n[R]$ and a function $y: R^n \to R^n$ such that $y'(t)=p(y(t))$, $\mathbf{y(0)=q(x)}$ and $\mathbf{|y_1(t)-f(x)|<y_2(t)}$ with $y_2(t) \geq 0$ decreasing for t>1 and $\lim_{t\to\infty} y_2(t) = 0$

Example. y=cos(4)



Reconciles Digital and Analog Computation !

Theorem (analog characterization of Turing computability).

[Bournez Campagnolo Graça Hainry 07 J. Complex]

A real function is computable (by Turing machine) iff it is PIVP-computable.

Normal Form Theorem

Theorem (abstract CRN normal form)

A real function is computable if and only if it is computable by a system of elementary reactions of the form

_ => z or x => x+z or x+y => x+y+z

plus annihilation reactions x+y => _ all with mass action law kinetics

Realistic CRN:

- formal annihilations by complexations (e.g. in a stable inactive complex)
- formal syntheses by modifications (e.g. phosphorylation with kinases)

Concrete CRN: search mapping with real enzymes (e.g. Brenda database)

- Easier for CRN with rate independence property
- Robustness w.r.t. parameter perturbations (extrinsic variability)
- Robustness w.r.t. stochastic simulations (intrinsic variability)

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5. Logical Gates

Assuming concentrations in [0, 1]

And: C = A /\ B

[C] = min([A],[B]) `A+B => C` (destructive on A, B, rate-independent)

or

[C]=[A]*[B] $\frac{dC}{dt} = A * B - C$ (non-destructive on A, B)

`MA(k) for A+B => A+B+C`

`MA(k) for C => _` (any rate constant k but the same for both reactions)

Or: C = A \/ B

[C]=[A]+[B]-[A]*[B] $\frac{dC}{dt} = A + B - A * B - C$ (non-destructive on A, B)

`MA(k) for A => A+C`

`MA(k) for B => B+C`

`k*A*B for A+B+C => A+B` (not well-formed, should use C+ C-)

`MA(k) for C => _`

Not: C = ¬ A

[C]=1-[A] $\frac{dC}{dt} = 1 - A - C$

`k for - => C`

`k*A for A+C => A` (not well-formed, should use C+ C-)

`MA(k) for C => _`

---

1. Chemical Reaction Kinetics

Molecular species: $A_1, \dots, A_m$

|A|=Number of molecules A

[A]=Concentration of A in the solution: [A] = |A| / Volume

dimension volume^{-1} , e.g. unit ML^{-1}, *noted also A by abuse of notation*

Molecular solution: multiset of molecules S, S', …

linear expression with stoichiometric coefficients $S = k1 * A_1 + \dots + c_n * A_n$

Reaction: multiset rewriting rule given with a rate function f for S => S'

Rate function f gives the number of reactions per time and volume units: dimension volume^{-1}time^{-1}

determines the velocity of our « chemical computer »

Well-formed reaction: $A \in S \iff \frac{\partial f}{\partial A} \neq 0$ (catalyst if >0, inhibitor if <0) and $A \in S \wedge A = 0 \Rightarrow f(A) = 0$

Reaction Rate Functions

Guldberg and Waage, 1864

Mass action law kinetics (proportionality)

k*A for A => B

k*A*B for A+B => C

k*A^m*B^n for m*A + n*B => R

Victor Henry (X) 1903

Michaelis and Menten 1913

Henri-Michaelis-Menten kinetics (saturation)

Vm*A/(Km+A) for A => B

Archibald Hill 1910

Hill kinetics (cooperativity, sigmoid velocity)

Vm*A^n/(Km+A^n) for A => B

Origin and justification of these other rate functions?

By model reduction of a detailed mass action CRN, e.g. by elimination of the enzyme variables

ODE Semantics of a CRN

To a set of species $\{A_1, \ldots, A_m\}$ with real valued concentrations
and a set of reactions $\{ f_j \text{ for } l_j => r_j \}_{j=1,\ldots,n}$ given with rate functions f_j

one associates the Ordinary Differential Equations (ODE) over $\{A_1, \ldots, A_m\}$

$$dA_i/dt = \Sigma^n_{j=1} f_j . (r_j(A_i) - l_j(A_i)) = \Sigma^n_{j=1} f_j . v_j(A_i)$$

where $l_j(A_i)$ is the stoichiometric coefficient of A_i in l_j
$r_j(A_i)$ is the stoichiometric coefficient of A_i in r_j
$v_j=r_i - l_j$ is the net stoichiometric change vector of reaction j
f_j is the rate function of dimension volume^{-1} time^{-1}

In matrix form: $\dot{A} = V.f(A)$

$$\begin{pmatrix} v_{11} & \cdots & v_{1n} \\ \vdots & \ddots & \\ v_{m1} & & v_{mn} \end{pmatrix} . \begin{pmatrix} f_1 \\ \cdots \\ f_n \end{pmatrix}$$

Variable Elimination by Conservation Laws

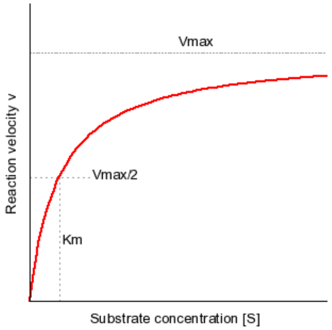
$E+S \rightarrow^{k1} C \rightarrow^{k3} E+P$
$E+S \leftarrow^{k2} C$

$dE/dt = -k1.E.S+(k2+k3).C$
$dS/dt = -k1.E.S+k2.C$
$dC/dt = k1.E.S-(k3+k2).C$
$dP/dt = k3.C$

A conservation law is a set of species {Mi} than remains with same total amount
i.e. a *Petri net place invariant*, or equivalently a *structural ODE invariant* $\Sigma^n_{i=1} dMi/dt = 0$

Here two invariants: $E+C=E_0+C_0$,
$S+C+P=S_0+C_0+P_0$
We can thus eliminate variables **$E= E_0+C_0-C$** and $P= S_0+C_0+P_0-C-P$
and get the algebra-differential system **$E= E_0-C$** assuming $C_0=0$, $P_0=0$,
$dS/dt = -k1.(E_0 - C).S + k2.C$
$dC/dt = k1.E_0.S - (k1.S+k2+k3).C$

Model Reduction by Quasi-Steady State Approximation (QSSA)



After short time assume $dC/dt \simeq 0 \simeq k1E_0S-(k1S+k2+k3)C$
Then $C = k1E_0S/(k1S+k2+k3)$
$= E_0S/(S+(k2+k3) /k1)$
$= E_0S/(K_m+S)$ with $K_m=(k2+k3)/k1$
K_m is substrate concentration with half maximum velocity

We get $dP/dt = -dS/dt = -k1(E_0-C)S+k2C$
$= -k1E_0S + (k1S+k2) E_0 S / (K_m+S)$
$= V_m S / (K_m+S)$ where $V_m= k3E_0$
V_m is maximum velocity at saturing substrate concentration

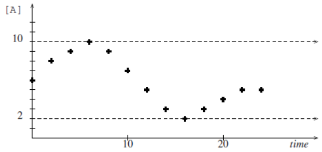
Michaelis-Menten kinetics: **`Vm S/(Km+S) for S => P`** *justified when* ***E<<S***

C and E are eliminated but well sometimes E can be re-injected as a slow variable in Vm …

`k3*E*S /(Km+S) for S+E => E+P`

Closed LTL($\mathbb{R}$) Formulae over Finite Traces

A trace (of experiment or simulation) gives concentration values at discrete time points:



State variables: concentrations $A, B, \ldots.$, possibly real time $Time$
Arithmetic expressions over state variables (no free variable in closed formulae)
Temporal operators of LTL: **X** next, **F** finally, **G** globally, **U** until, **R** release.

Reachability of minimum value: $\mathbf{F}(A > 0.2)$
Global minimum value: $\mathbf{G}(A > 0.2)$
Reachability of global minimum value: $\mathbf{FG}(A > 0.2)$
Peak: $A < 0.4 \wedge \mathbf{F}(A > 0.4 \wedge \mathbf{F}(A < 0.4))$
Curve fitting: $\mathbf{F}(Time == 1 \wedge M == 8.1 \wedge \mathbf{F}(Time == 2 \wedge M == 9 \wedge \ldots$
$==$ provides equality between discrete time points by interpolation

Semantics of Closed LTL(ℝ) over Infinite Traces

Completion of finite traces with an infinite loop on the last state.

$\pi \models \phi$ for a closed proposition ϕ if ϕ holds in the first state of π
$\pi \models \mathbf{X}\phi$ if $\pi^1 \models \phi$
$\pi \models \mathbf{F}\phi$ if $\exists k \geq 0\ \pi^k \models \phi$
$\pi \models \mathbf{G}\phi$ if $\forall k \geq 0\ \pi^k \models \phi$
$\pi \models \phi\ \mathbf{U}\ \psi$ if $\exists k \geq 0\ \pi^k \models \psi \wedge \forall j < k\ \pi^j \models \phi$
$\pi \models \phi\ \mathbf{R}\ \psi$ if $\forall k \geq 0\ \pi^k \models \psi \vee \exists j < k\ \pi^j \models \phi$
ϕ releases ψ if ψ is always true or until ϕ becomes true

Duality:
$\neg\mathbf{X}\phi = \mathbf{X}\neg\phi$,
$\neg\mathbf{F}\phi = \mathbf{G}\neg\phi$,
$\neg(\phi\ \mathbf{U}\ \psi) = \neg\phi\ \mathbf{R}\ \neg\psi$.

First-Order FO-LTL($\mathbb{R}_{lin}$) Constraints with Free Variables

- Free variables $x, y, \ldots$ in addition to state variables $A, B, \ldots$
- Linear constraints over free and state variables as atomic propositions
- Logical quantifiers $\forall x\ \exists y$
- Temporal operators: **X**, **F**, **G**, **U**, **R**

maximum(A,x): $\mathbf{G}(A <= x) \wedge \mathbf{F}(A >= x)$

local_maximum(A,x): $\mathbf{F}(A < x \wedge \mathbf{X}(A >= x \wedge \mathbf{X}(A <= x)))$

decrease(A): $\exists x\ A >= x \wedge \mathbf{X}(A < x)$

peak(A,x,t)): $A < x \wedge \mathbf{X}(A >= x \wedge \mathbf{X}(A <= x) \wedge Time = t)$

Minimal Set of CTL* Operators

Minimal set of operators:		Other operators defined by abbreviations:	
Logical connectives:	$\vee$ $\neg$	$\phi \wedge \psi = \neg(\phi \vee \psi)$ $\phi \Rightarrow \psi = \neg \phi \vee \psi$	
Path quantifier:	**E** “exists”	$\mathbf{A}\phi = \neg\ \mathbf{E}\ \neg\ \phi$	“always”
Temporal operators:	**X** “next”	$\mathbf{F}\phi = \text{true}\ \mathbf{U}\ \phi$	“finally”
	U “until”	$\mathbf{G}\phi = \neg\ \mathbf{F}\ \neg\ \phi$	“globally”
		$\phi_1\ \mathbf{R}\ \phi_2 = \neg\ (\neg\ \phi_1\ \mathbf{U}\ \neg\ \phi_2)$	“release”

CTL Fragment of CTL*

In CTL fragment, each temporal operator must be preceded by a path quantifier

Basis of three operators: **EX, EG, EU**

- **EF** ϕ = **E**(true **U** ϕ) s $\models$ **EF** ϕ if $\exists\ \pi$ from s $\exists$ k $\geq$ 0 $\pi^k \models \phi$
- **AX** ϕ = $\neg$ **EX** $\neg\ \phi$ s $\models$ **AX** ϕ if $\forall\ \pi$ from s $\pi^1 \models \phi$
- **AF** ϕ = $\neg$ **EG** $\neg\ \phi$ s $\models$ **AF** ϕ if $\forall\ \pi$ from s $\exists$ k $\geq$ 0 $\pi^k \models \phi$
- **AG** ϕ = $\neg$ **EF** $\neg\ \phi$ s $\models$ **AG** ϕ if $\forall\ \pi$ from s $\forall$ k $\geq$ 0, $\pi^k \models \phi$
- Etc…

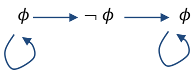
Any CTL formula is thus a state formula
and can be identified to the set of states that satisfy it
$\phi \simeq \{s \in S : s \models \phi\}$ [Emerson 90]

LTL Fragment of CTL*

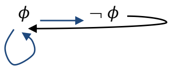
Linear Time Logic (LTL) formulae are of the form **A**ϕ (noted just ϕ without the **A**) where ϕ contains no path quantifier, only temporal operators: **X, U** and their duals

- The LTL formula **FG** ϕ is not expressible in CTL

Stronger CTL formula ? ***AF(AG*** ϕ***)*** *false on*



Weaker CTL formula ? ***AF(EG*** ϕ***)*** *true on*



- The CTL formula **EF**(**AG** ϕ) **AF**(**AG** ϕ) are not expressible in LTL
- LTL and CTL are strict fragments of CTL*

Biochemical Reachability Properties in CTL (from some initial state)

Initial state = initial biological conditions = molecules present / absent (/ undetermined)

- Can the cell produce some protein P (from initial state) ?
  - $\mathbf{EF}(P) \triangleq \mathbf{reachable}(P)$
- Can the cell produce P, Q and not R?
  - $\mathbf{reachable}(P \wedge Q \wedge \neg R)$

About *pathways*:

- Can the cell reach a given set s of states while passing by another set of states s_2?
  - $\mathbf{EF}(s_2 \wedge \mathbf{EF} s)$
- Is it possible to produce P without Q before ?
  - $\mathbf{E}(\neg Q \ \mathbf{U}\ P)$
- If not, this gives a *phenomenological non-causal notion of checkpoint*
  - $\neg\mathbf{E}(\neg s_2 \ \mathbf{U}\ s) \triangleq \mathbf{checkpoint}(s_2, s)$

Cum hoc sed non propter
Correlation is not causality

Biochemical Reachability Properties in CTL (from some initial state)

- Is a given set of states s a stable state set *(infinite loop with no escaping possibility)*?
  - $\mathbf{stable}(s) \triangleq \mathbf{AG}(s)$
- Is s a steady state *(infinite loop with escaping possibility)* ?
  - $\mathbf{steady}(s) \triangleq \mathbf{EG}(s)$
- Can the cell reach a given stable state s?
  - $\mathbf{reachable}(\mathbf{stable}(s))$

  alternance of path quantifiers **EF AG** ϕ *(not expressible in LTL)*
- Must the cell reach a given stable state s?
  - $\mathbf{AF}(\mathbf{stable}(s))$
- What are the stable states?
  - *Not expressible in CTL.*

  needs to combine CTL with enumeration, see Biocham `generate_ctl(stable(s))`