Integrated Scientific Modeling and Lab Automation

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APLAS 2020-11-30

Outline

- The Scientific Method
  - and its eventual automation
- Models
  - that know nothing about protocols

  Chemical Reaction Networks
- Lab Protocols
  - that know nothing about models

  Digital Microfluidics
- Integration

  Closed-loop modeling and protocol execution

  The Kaemika App

Discovery through Observation

The Scientific Method ~ 1638

1 Guy


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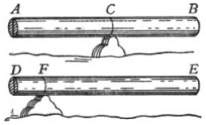
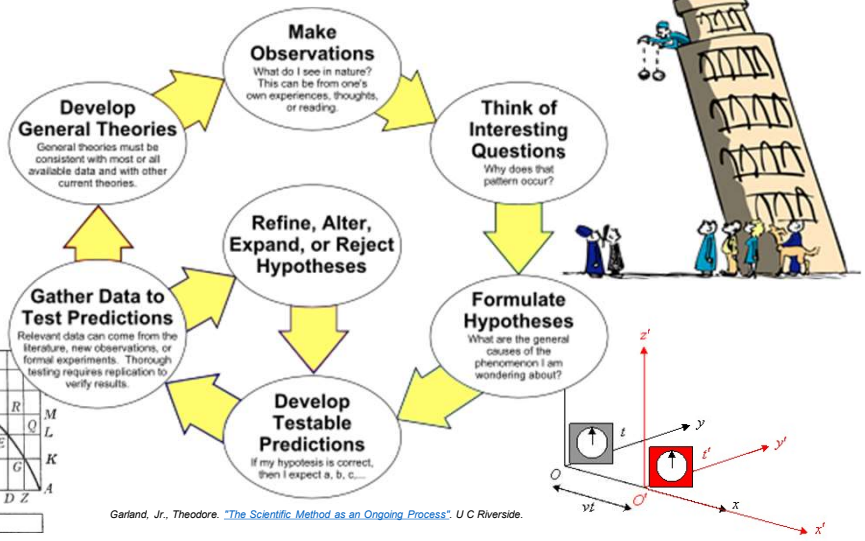
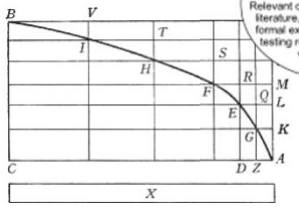
DIMOSTRAZIONI
MATEMATICHE,
Mecanica & i Movimenti Locali,
GALILEO GALILEI LINCEO,
IN LEIDA.







Garland, Jr., Theodore. "The Scientific Method as an Ongoing Process". U C Riverside.

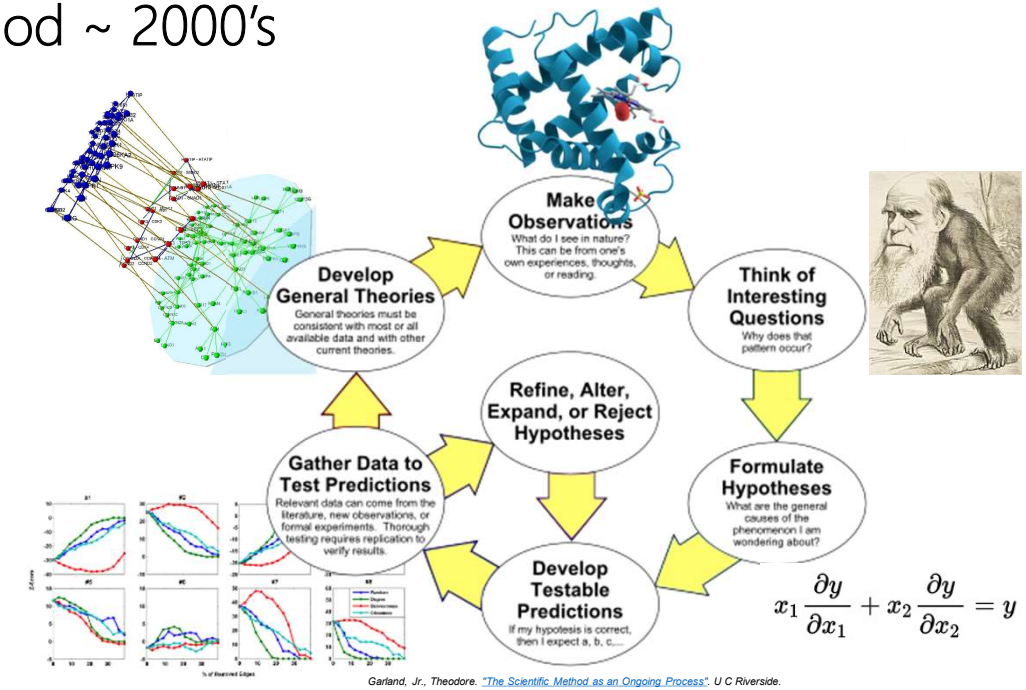
Discovery through Collaboration

The Scientific Method ~ 2000's

1 Lab

1 protein = 30 people / 30 years

Humans have >250,000 proteins ☹

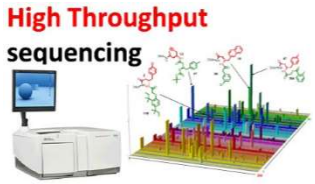


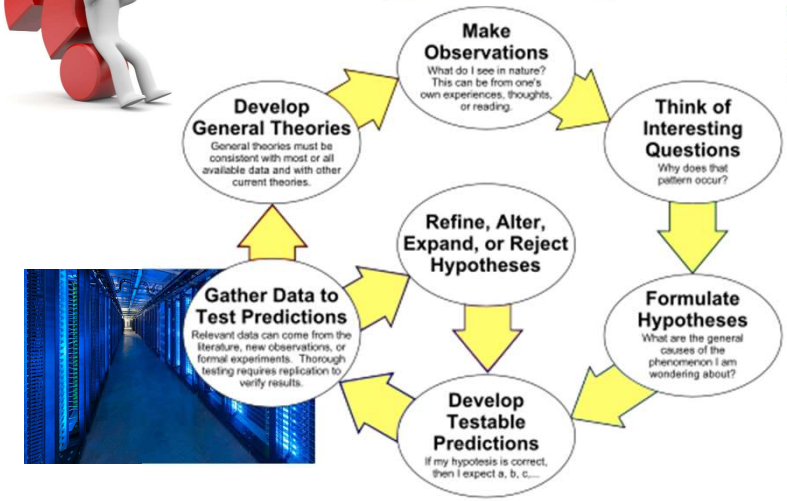
Garland, Jr., Theodore. "The Scientific Method as an Ongoing Process". U C Riverside.

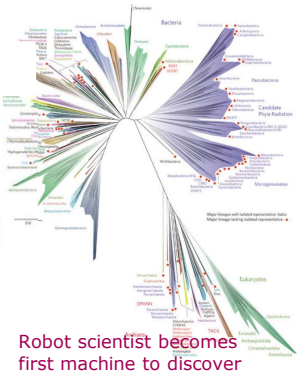
Discovery through Automation

The Scientific Method ~ 2020's


```
while (true) {
    predict();
    falsify();
}
```







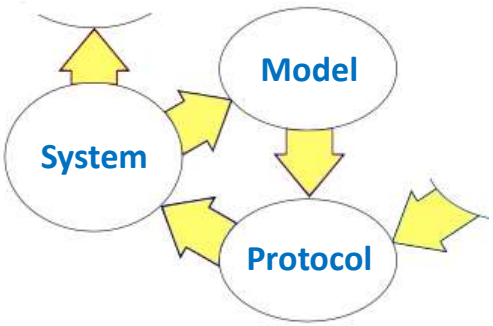
Robot scientist becomes first machine to discover new scientific knowledge

Ross King

Garland, Jr., Theodore. "The Scientific Method as an Ongoing Process". U C Riverside.

The Inner Loop

- A *model* is refined by testing a (fixed) *protocols* against a *systems*
- A *protocol* is refined by testing a (fixed) *model* against a *systems*



- Today: publication does not accurately reflect execution
  - Model: poorly-maintained matlab script
  - Protocol: poorly-described manual steps in the lab
  - System: poorly-characterized and hardly "resettable"
  - ⇒ Crisis in biology: experiments are done once and are hard to reproduce

    http://www.nature.com/news/reproducibility-1.17552

The Inner Loop

- Tomorrow, automation

Nodes

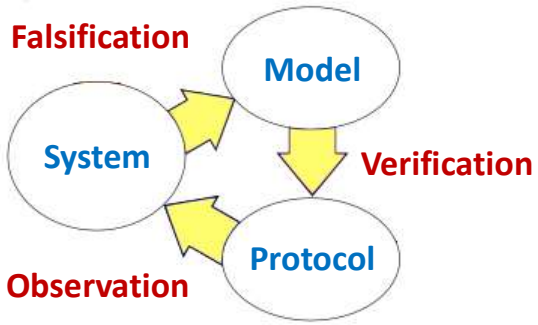
- Model: unambiguous (mathematical) description (CompBio)
- Protocol: standardized (engineered) parts and procedures (SynthBio)
- System: characterized (biological) organism and foundries (SysBio)

Arcs

- Verification: simulation / analysis / model checking / theorem proving
- Observation: lab automation
- Falsification: statistical inference / model reduction

Lifecycle

- Performance evaluation/optimization: of model+protocol+system combined
- Management: version control, equipment monitoring, data storage



The Inner Loop

via Molecular Programming

- Tomorrow, automation

Nodes
- Model: unambiguou… …l) description (CompBio)
- Protocol: standardized (engineered) parts and procedures (SynthBio)
- System: characterized (biological) organism and foundries (SysBio)

Arcs
- Verification: simulation / analysis / model checking / theorem proving
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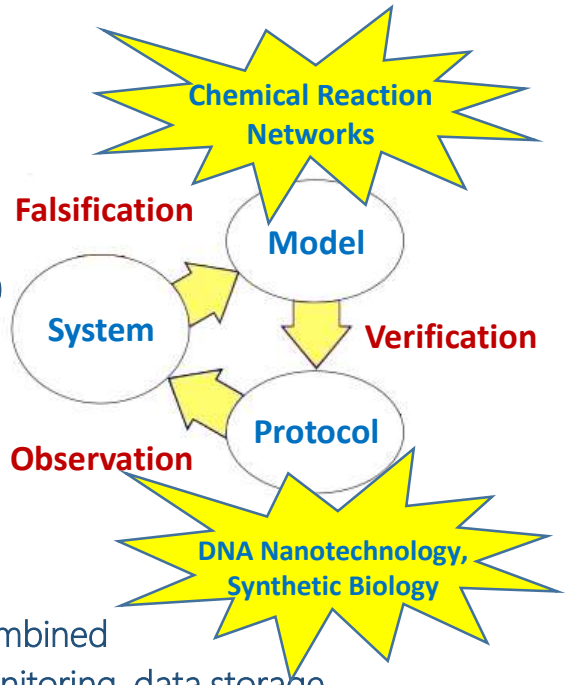


Why are chemical reactions interesting?

$$X + Y \xrightarrow{r} Z + W$$

- A *phenomenological model* of kinetics in the natural sciences
  - By *(only) observing* naturally occurring reactions
- A *programming language*, *finitely* encoded in the genome
  - By which living things manage the *unbounded* processing of matter and information
- A *mathematical structure*, rediscovered in many forms
  - Vector Addition Systems, Petri Nets, Bounded Context-Free Languages, Population Protocols, …
- A description of *mechanism* ("instructions" / "interactions") rather than *behavior* ("equations" / "approximations")
  - Although the two are related in precise ways
  - Enabling, e.g., the study of the evolution of *mechanism* through unchanging *behavior*

100 years of chemical infinite loops

It is, therefore, somewhat contrary to his first expectations that the writer now finds the conditions for undamped oscillations may occur in the absence of any geometrical causes in a homogeneous system.
[Lotka, 1920]

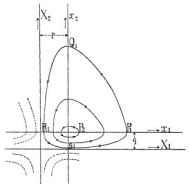
Chemical reaction networks are interesting independently of actual chemical substances:

UNDAMPED OSCILLATIONS DERIVED FROM THE LAW OF MASS ACTION.

By Alfred J. Lotka.

Received June 2, 1920.

$$\frac{dX_1}{dt} = a_1X_1 - b_1X_1X_2$$
$$\frac{dX_2}{dt} = a_2X_1X_2 - b_2X_2.$$



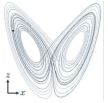
2 ordinary differential equations (ODEs) with chemical reaction network (CRN) interpretation, but no actual chemicals.

1920 First theoretical proof of chemical oscillation [Lotka]
1921 First experimental (accidental) chemical oscillator [Bray]
1926 Predatory-prey interpretation (independent discovery) [Volterra]
1958 Bray ignored until the (accidental) BZ oscillator [Belousov–Zhabotinsky]
1963 Lorenz chaotic oscillator (3 ODEs, can be adapted to mass action)
1981 First intentionally-designed chemical oscillator [De Kepper]
2005 First biochemical protein/ATP oscillator (circadian clock) [Nakajima et al.]
2017 First DNA-only oscillator [Srinivas et al.] (a version of Lotka's)

The first single, homogeneous oscillating chemical reaction was discovered accidentally by Bray [6] in 1921.

OSCILLATIONS AND CHAOS IN CHEMICAL SYSTEMS

Irving R. EPSTEIN



Chemical algorithms

$$\frac{dX_1}{dt} = a_1X_1 - b_1X_1X_2$$
$$\frac{dX_2}{dt} = a_2X_1X_2 - b_2X_2.$$

prey population x1

predator population x2

(A1) $\frac{dN_1}{dt}=(\varepsilon_1-\gamma_1N_2)N_1$ (A2) $\frac{dN_2}{dt}=(-\varepsilon_2+\gamma_2N_1)N_2$ [Volterra 1926]

- Hungarian Lemma: ODE -> CRN

Lotka-Volterra
Population ODEs

```
∂ x1 = a1 · x1 - b1 · x1 · x2
∂ x2 = a2 · x1 · x2 - b2 · x2
```

Hungarization / Mass Action

Population algorithm (*)

```
x1 -> 2 · x1                {a1}
x1 + x2 -> x2               {b1}
x1 + x2 -> x1 + 2 · x2      {a2}
x2 -> Ø                     {b2}
```

preys increase
predators decrease preys by b1
preys increase predators by a2
predators decrese (without preys)

By turning each ODE monomial into one reaction

(Restricted to "Hungarian ODEs" such that all negative monomials have their l.h.s. differential variable as a factor. But by variable doubling this covers w.l.o.g. the solutions of all polynomial ODEs.)

- From populations to individuals (agents)

The interaction between prey population and predator population is indirect (2 separate reactions)
We can change it to a direct interaction between 1 prey agent and 1 predator agent, but we need to take a2 = b1. Consider the two x1 + x2 reactions:

```
x1 + x2 -> x2               {b1}
x1 + x2 -> x1 + 2 · x2      {b1}
```

predators *decrease* preys (predators stay the same)
preys *increase* predators (preys stay the same)

??? →

```
x1 + x2 -> 2 · x2   {b1}
```

predator agent *eats* prey agent and *reproduces*

(*) Although [Lotka 1920] intuitively describes just 3 reactions, b1≠a2 requires interpreting the ODEs as 4 reactions. Lotka's motivation for b1≠a2, or rather for b1>a2, is: "We may, however, make the more general supposition that along with x2 any other substances are formed (further depleting x1)".

[Volterra 1926, eqn A1,A2] instead says that b1 is the prey's defensive ability, and a2 is the predator's offensive ability, so the intuition here is what happens when a population of prey meets a population predators. This again is incompatible with a 3-reaction 1-prey vs. 1-predator CRN.

- Law of Mass Action: CRN -> ODE

Agent algorithm (*)

```
x1 -> 2 · x1                {a1}
x1 + x2 -> 2 · x2           {b1}
x2 -> Ø                     {b2}
```

Mass Action →

Agent ODEs

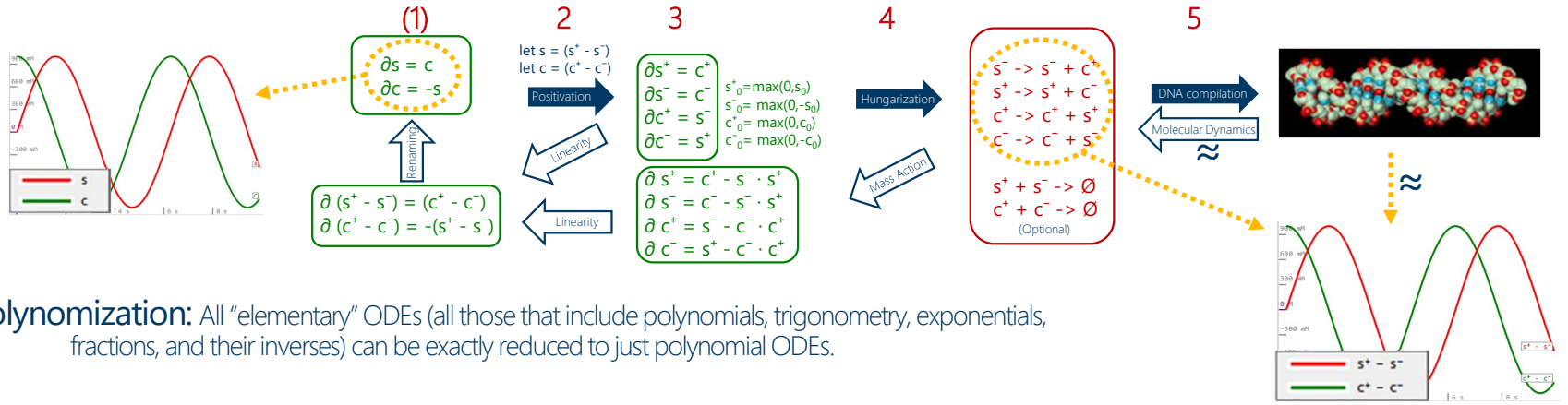
```
∂ x1 = a1 · x1 - b1 · x1 · x2
∂ x2 = b1 · x1 · x2 - b2 · x2
```

PolulationAlg = AgentAlg, when a2=b1

There can be multiple algorithms (CRNs) for the same behavior (ODEs).
CRNs are *programs*.
ODEs support program equivalence.

Programming *any* dynamical system as a CRN

For example, take *the* canonical oscillator: sine/cosine



1. **Polynomization:** All "elementary" ODEs (all those that include polynomials, trigonometry, exponentials, fractions, and their inverses) can be exactly reduced to just polynomial ODEs.

2. **Positivation:** All polynomial ODEs can be exactly reduced to polynomial ODEs in the positive quadrant (as differences).

3. **All positivized ODEs are Hungarian:** I.e., all negative monomials have their l.h.s. differential variable as a factor.

4. **Hungarization:** All Hungarian ODEs can be exactly reduced to mass action CRNs.

5. **Molecular Programming:** All mass action CRNs, up to time rescaling, can be arbitrarily approximated by engineered DNA molecules.

Chemistry is also a formal language that we can use to implement *any* dynamical system with *real* (DNA) molecules

- ANY collection of abstract chemical reactions can be implemented with specially designed DNA molecules, with accurate kinetics (up to time scaling).
- Approaching a situation where we can "systematically compile" (synthesize) a model, run an (automated) protocol, and observe (sequence) the results in a closed loop.

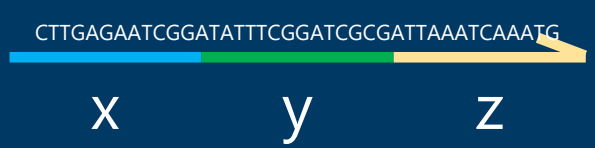
DNA as a universal substrate for chemical kinetics

David Soloveichik, Georg Seelig, and Erik Winfree

PNAS March 23, 2010 107 (12) 5393-5398; https://doi.org/10.1073/pnas.0909380107

Domains

- Subsequences on a DNA strand are called domains
  - *provided* they are "independent" of each other

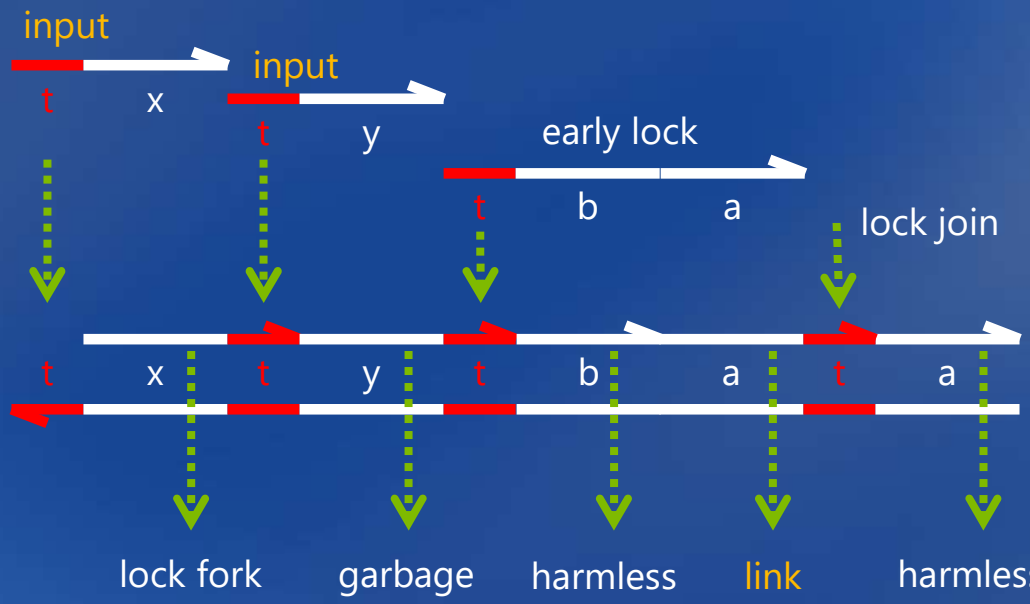


oriented DNA single strand

- Differently named domains must not hybridize
  - With each other, with each other's complement, with subsequences of each other, with concatenations of other domains (or their complements), etc.

Reaction $x + y \rightarrow z + w$

reactants
half

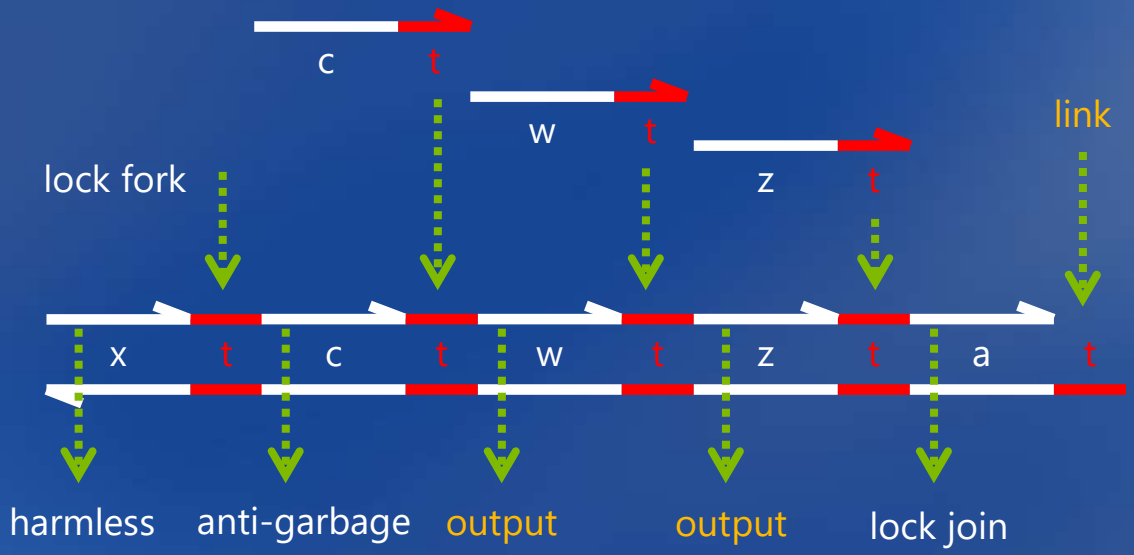


"join" structure

(2-input 2-output reactions are universal)

Reaction $x + y \rightarrow z + w$

products
half

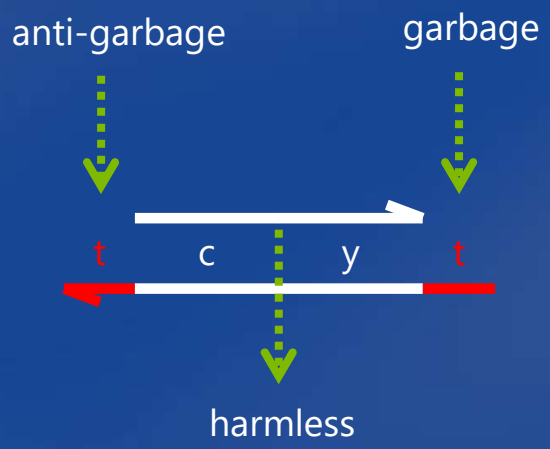


"fork" structure

(2-input 2-output reactions are universal)

Reaction $x + y \rightarrow z + w$

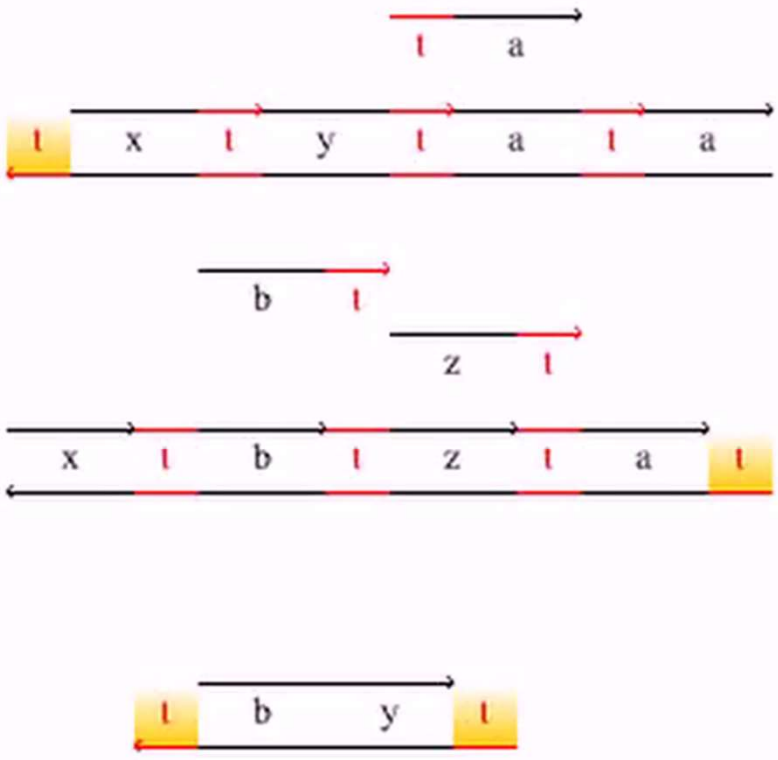
garbage collection



(2-input 2-output reactions are universal)

Powered by Sothink

Join x+y → z



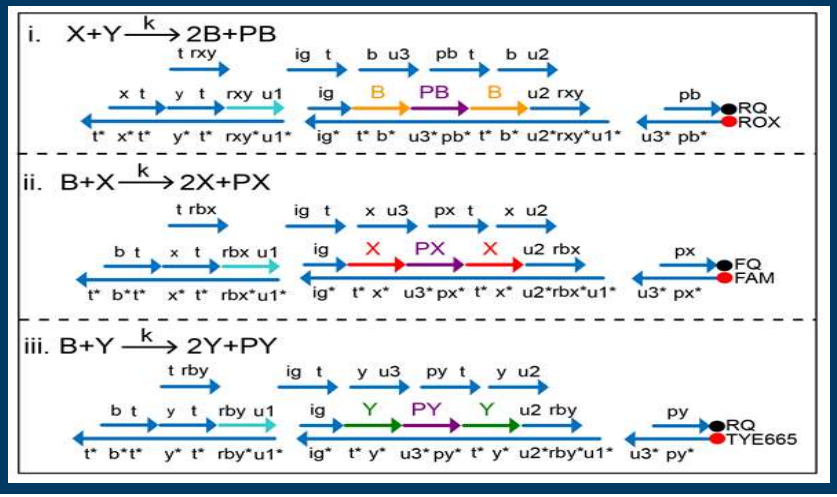
DNA Implementation of the Approximate Majority Algoithm

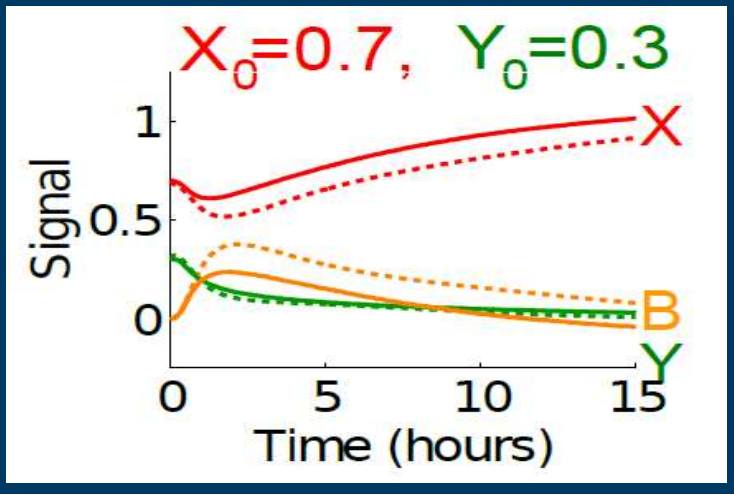
$$X + Y \rightarrow 2B$$
$$B + X \rightarrow 2X$$
$$B + Y \rightarrow 2Y$$

nature nanotechnology

Programmable chemical controllers made from DNA

Yuan-Jyue Chen, Neil Dalchau, Niranjan Srinivas, Andrew Phillips, Luca Cardelli, David Soloveichik & Georg Seelig





Experimental-Protocol Languages for Chemical Reaction Networks

- Overview and Reaction sublanguage

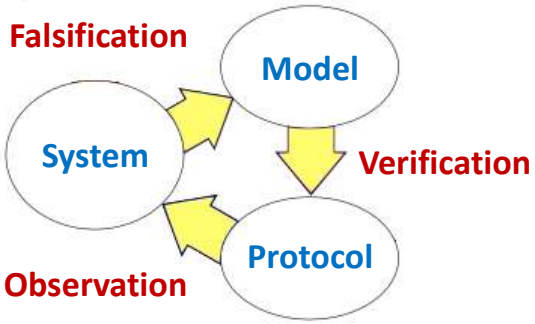
Automating "the whole thing"

- Protocols: sets of steps to direct lab machinery (or people)
  - Published in specialized journals. With varying accuracy.
- Models: sets of equations to predict the results of lab experiments
  - Published in Auxiliary Online Materials. With lots of typos.

- Protocols know nothing about models
  - What hypothesis is the protocol trying to test? It is not written in the protocol.
- Models know nothing about protocols
  - What lab conditions are being used to test the model? It is not written in the model.
- While presumably talking about the same system
  - Through the experiment.

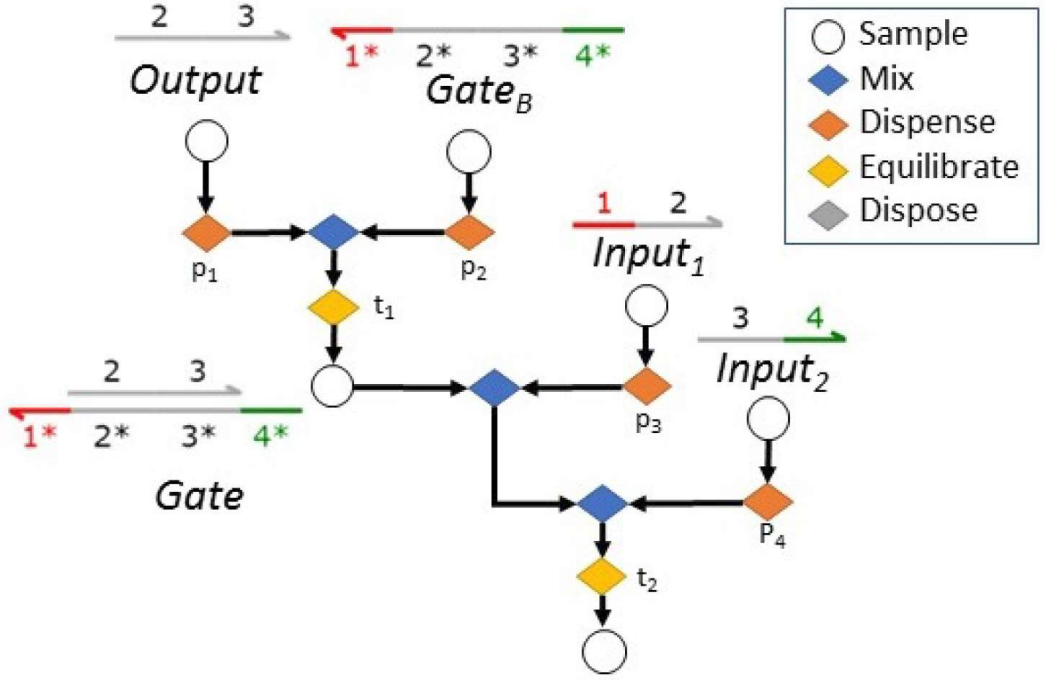
- Reproducibility crisis
  - Experiments are hard to reproduce.
  - Even models are hard to reproduce!

- Similar to classical lifecycle problems in C.S.
  - Documentation (model) gets out of step from code (protocol) if their integration is not automated.



A Protocol

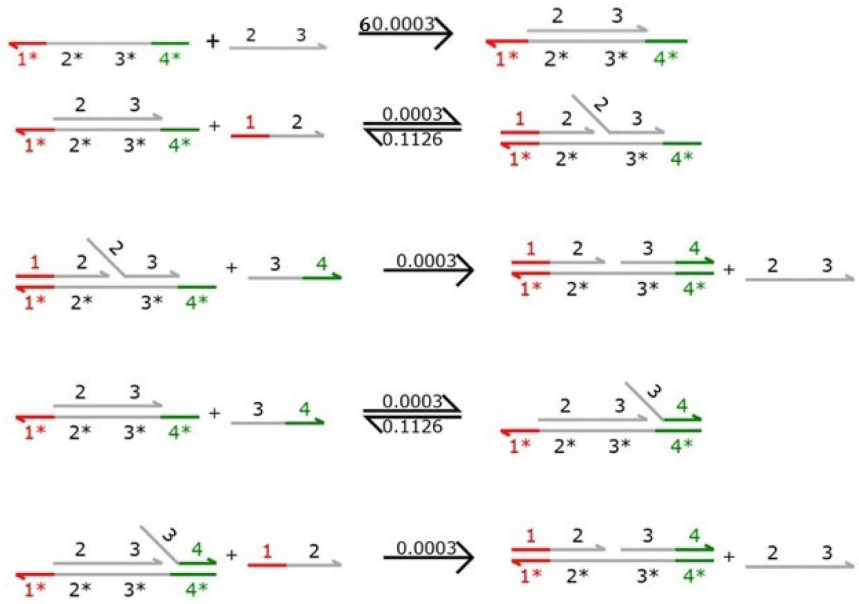
For DNA gate assembly and activation in vitro



Protocol steps (liquid handing)

A Model

A Chemical Reaction Network, provided explicitly or (in this case) generated from a higher-level description of the initial strands, according to the DNA strand displacement rules



An Integrated Description

Samples: containers with volume, temperature, concentrations

$$
\begin{aligned}
P = \quad & x && (a\ sample\ variable)\\
& (x_0, V, T) && (initial\ condition)\\
& let\, x = P_1\, in\, P_2 && (define\ local\ variable)\\
& Mix(P_1, P_2) && (mix\ samples)\\
& let\, x, y = Split(P_1, p)\, in\, P_2 && (split\ samples)\\
& Equilibrate(P, t) && (equilibrate\ sample\ for\ t\ seconds)\\
& Dispose(P) && (discard\ sample)
\end{aligned}
$$

each sample evolves (via *Equilibrate*) according to a given overall CRN:

$\mathcal{C} = (\mathcal{A}, \mathcal{R})$ (species, reactions)

Experimental Biological Protocols with Formal Semantics

Alessandro Abate[2], Luca Cardelli[1,2], Marta Kwiatkowska[2], Luca Laurenti[2], and Boyan Yordanov[1]

[1] Microsoft Research Cambridge
[2] Department of Computer Science, University of Oxford

Protocol + Model =

Joint script

$Input_1 =< 1^*\ 2 >\quad Output =< 2\ 3 >$
$Input_2 =< 3\ 4^* >\quad Gate = \{1^*\}[2\ 3]\{4^*\}$

$$
\begin{aligned}
P_1 = & let\ In1 = ((Input1, 100.0nM), 0.1mL, 25.0^oC)\ in\\
& let\ In2 = ((Input2, 100.0nM), 0.1mL, 25.0^oC)\ in\\
& let\ GA = ((Output, 100.0nM), 0.1mL, 25.0^oC)\ in\\
& let\ GB = ((Gate_B, 100.0nM), 0.1mL, 25.0^oC)\ in\\
& let\ sGA,_= Dispense(GA, p_1)\ in\\
& let\ sGB,_= Dispense(GB, p_2)\ in\\
& let\ sIn1,_= Dispense(In1, p_3)\ in\\
& let\ sIn2,_= Dispense(In1, p_4)\ in\\
& Observe(Equilibrate(Mix(Mix(Equilibrate(\\
& \quad Mix(sGA, sGB), t_1), sIn1), sIn2), t_2), idn).
\end{aligned}
$$

Language Semantics (deterministic)

The deterministic case is a warm-up exercise, simpler to explain.
Each program denotes a *final* state <concentrations, volume, temperature>

$[\![P]\!]^\rho$ is the final state produced by a protocol P for a fixed CRN $\mathcal{C} = (\mathcal{A}, \mathcal{R})$:

$$
\begin{aligned}
&[\![x]\!]^\rho = \rho(x)\\
&[\![x_0, V, T]\!]^\rho = (x_0, V, T)\\
&[\![Mix(P_1, P_2)]\!]^\rho =\\
&\quad let\,(x_0^1, V_1, T_1) = [\![P_1]\!]^\rho\\
&\quad let\,(x_0^2, V_2, T_2) = [\![P_2]\!]^\rho\\
&\quad (\frac{x_0^1 V_1 + x_0^2 V_2}{V_1 + V_2}, V_1 + V_2, \frac{T_1 V_1 + T_2 V_2}{V_1 + V_2})\\
&[\![let\,x = P_1\,in\,P_2]\!]^\rho =\\
&\quad let\,(x_0, V, T) = [\![P_1]\!]^\rho\\
&\quad let\,\rho_1 = \rho\{x \leftarrow (x_0, V, T)\}\\
&\quad [\![P_2]\!]^{\rho_1}
\end{aligned}
$$

$$
\begin{aligned}
&[\![let\,x, y = Split(P_1, p)\,in\,P_2]\!]^\rho =\\
&\quad let\,(x_0, V, T) = [\![P_1]\!]^\rho\\
&\quad let\,\rho_1 = \rho\{x \leftarrow (x_0, V \cdot p, T), y \leftarrow (x_0, V \cdot (1-p), T)\}\\
&\quad [\![P_2]\!]^{\rho_1}\\
&[\![Equilibrate(P, t)]\!]^\rho =\\
&\quad let\,(x_0, V, T) = [\![P]\!]^\rho\\
&\quad [\![(\mathcal{A}, \mathcal{R}, x_0), V, T]\!](H)(t)\\
&[\![Dispose(P)]\!]^\rho = (0^{|\mathcal{A}|}, 0, 0),
\end{aligned}
$$

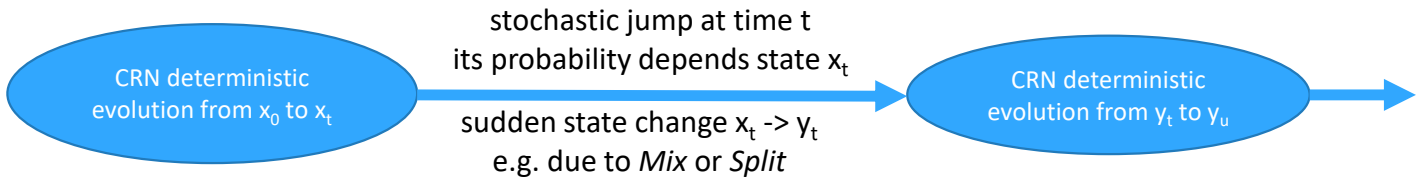
State produced by CRN $\mathcal{C} = (\mathcal{A}, \mathcal{R})$ with flux *F* at time t:

$$
\begin{aligned}
&[\![((\mathcal{A},\mathcal{R}, x_0), V, T)]\!](H)(t) =\\
&\quad let\,G : [0...H) \to \mathbb{R}^{|\mathcal{A}|}\ be\ the\ solution\ of\ G(t') = x_0 + \int_0^{t'} F(V,T)(G(s))ds\\
&\quad (G(t), V, T)
\end{aligned}
$$

Language Semantics (stochastic)

Split has a volume uncertainty.
Equilibrate has a time uncertainty.
Reactions have rate uncertainty and/or intrinsic molecular noise.

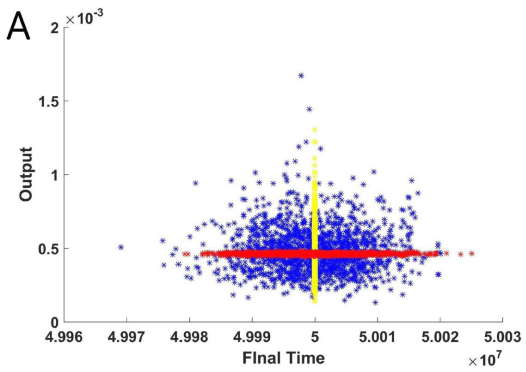
Each program now represents a Hybrid System with stochastic jumps between deterministic evolutions:



Which in turn denotes a *Piecewise Deterministic Markov Process (PDMP)*

Stochastic Analysis

- We can ask: what is the probability of a certain outcome given uncertainties in *both the protocol and the model*?
- Conversely: which parameters of *both the protocol and the model* best fit the observed result?



1500 executions including protocol uncertainty due timing and pipetting errors (red).
1500 executions including only model uncertainty about rates of the CRN (yellow).
1500 executions including both sources of uncertainty (blue).

We may estimate by Statistic Model Checking, e.g. the probability that Output will fall in a certain range, given distributions over uncertain model and protocol parameters.

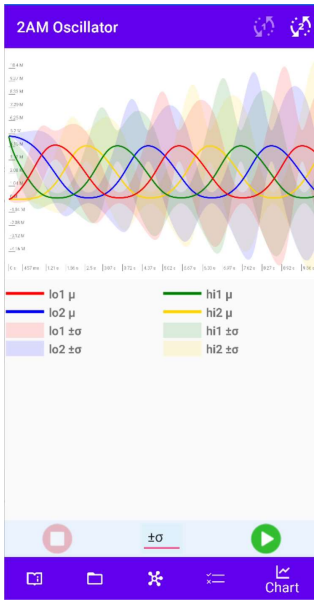
Kaemika

- A prototype language for chemical models & protocols

- http://lucacardelli.name/kaemika.html

- Search "Kaemika" in the App stores

macOS
iOS
Android
Windows
GitHub



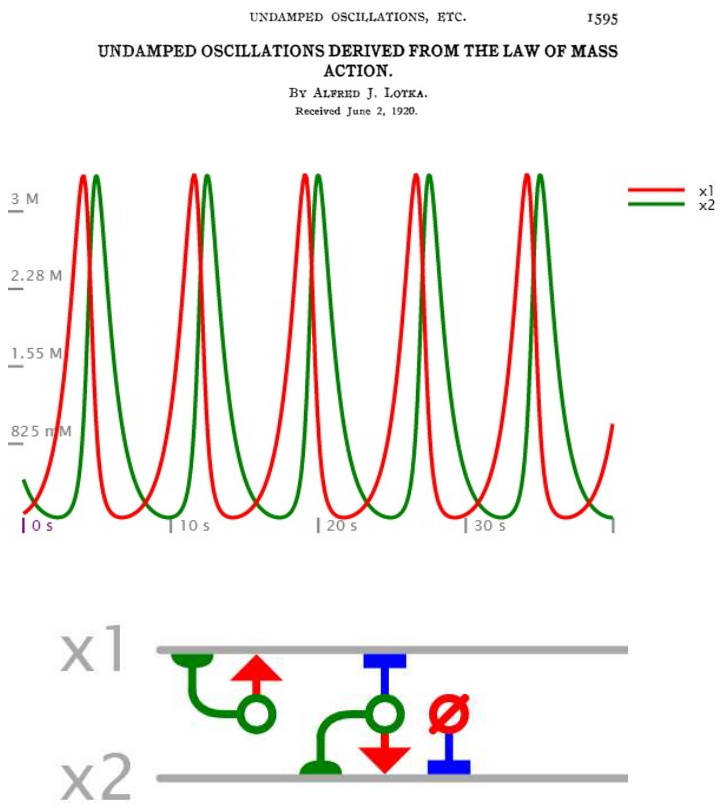
- CRN simulation
- Microfluidics simulation
- Reaction graphs
- ODE equations
- Stochastic noise (LNA)

Main features

- *Species* and *reactions*
  - Characterized by initial values and rates
- *"Samples" (compartments) and Protocols*
  - Isolate species and reactions in a compartment, and mix compartments
- *Kinetics (simulation)*
  - Deterministic (ODE) or stochastic (LNA) for chemical models
  - Digital microfluidics for chemical protocols
- *Programming abstractions*
  - Assemble models and protocols as compositions of modules

Species and Reactions

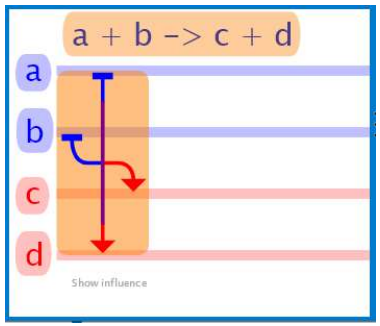
```
//=======================================
// Lotka 1920, Volterra 1926
// (simplified with all rates = 1)
//=======================================

number x1₀ <- uniform(0,1) // random x1₀
number x2₀ <- uniform(0,1) // random x2₀

species x1 @ x1₀ M      // prey
species x2 @ x2₀ M      // predator

x1 -> x1 + x1        {1} // prey reproduces
x1 + x2 -> x2 + x2   {1} // predator eats prey
x2 -> Ø              {1} // predator dies

equilibrate for 40
```



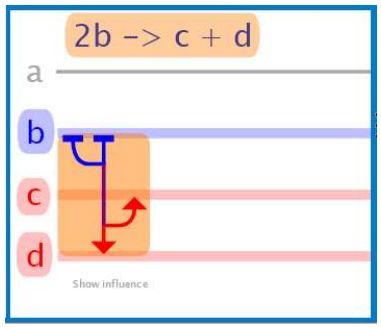
Reaction scores (graphical representation of reaction networks)

Horizonal lines: *species*. Vertical stripes: *reactions*. Blue: reagents. Red: products. Green: catalysts.

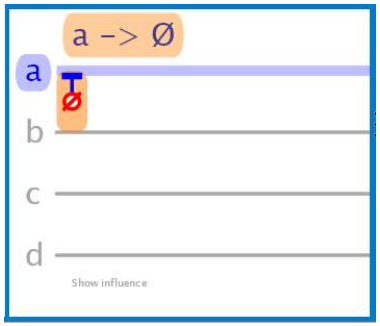
Reactants and products



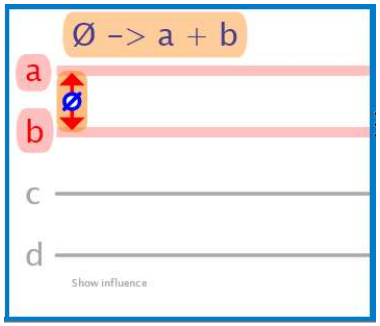
Repeated species



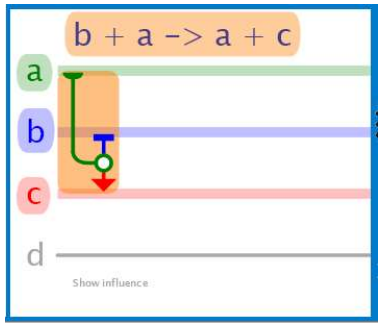
Reactants but no products



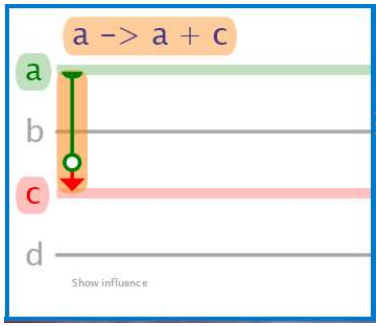
Products but no reactants



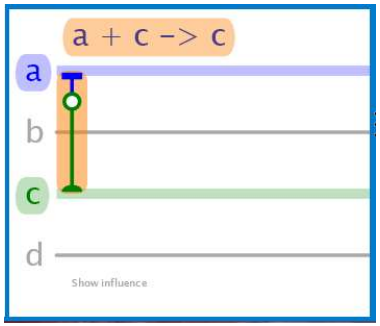
Catalyst



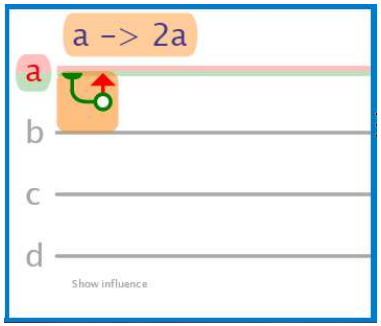
Catalyst but no reactants



Catalyst but no products



Autocatalyst



Writing Models Compositionally

- Models are generated by programs

  Freely containing both chemical reactions and control flow
  Can generate unbounded-size reaction networks

- Rich data types

  numbers, ***species***, *functions*, ***networks***, *lists*, ***flows*** *(time-courses)*
  flows are composable functions of time used in ***rates***, ***plotting***, and ***observation***

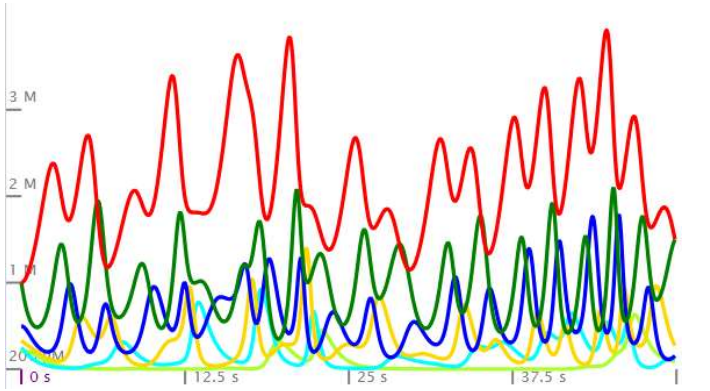
- Modern abstractions

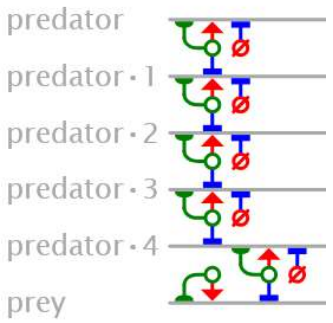
  Functional: programs take *data* as parameters and produce *data* as results
  Monadic: programs also produce *effects* (*species*, *reactions*, *liquid handling*)
  Nominal: *lexically scoped* chemical species (species are not "strings")

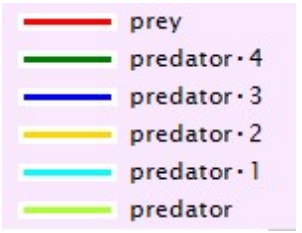
Ex: Predatorial

```
function Predatorial(number n) {
  if n = 0 then
    define species prey @ 1 M
    prey -> 2 prey // prey reproduces
    report prey
    yield prey
  else
    define species predator @ 1/n M
    species prey = Predatorial(n-1)
    prey + predator ->{n} 2 predator // predator eats
    predator -> ∅ // predator dies
    report predator
    yield predator
  end
}

species apexPredator = Predatorial(5)
equilibrate for 50
```

```
//=====================================
// Creates a stack of predator-prey
// relationships in Lotka-Volterra style,
// and returns the apex predator.
//=====================================
```







Demo

- Lotka-Volterra
- Predatorial

Experimental-Protocol Languages for Chemical Reaction Networks

- *Protocol sublanguage and Microfluidics*

Describing a Protocol

- *Samples* (e.g. test tubes)
  - Are characterized by a volume and a temperature
  - Contain a specified set of species
  - Evolve according to reactions that operates on those species
  - Isolate species and reactions

- *Protocol Operations* (e.g. liquid handling)
  - Accept and produce samples
  - Accepted samples are *used up* (they can only be operated-on once)

Samples

- Samples contain concentrations of species, acted over by reactions.
- Each sample has a fixed volume and a fixed temperature through its evolution.
- Sample concentrations are in units of molarity M = mol/L.
- The default implicit sample is called the vessel {1 mL, 20 C}

```
species {c} // a species for multiple samples

sample A {1μL, 20C}     // volume and temperature
species a @ 10mM in A   // species local to A
amount c @ 1mM in A     // amount of c in A
a + c -> a + a

sample B {1μL, 20C}
species b @ 10mM in B   // species local to B
amount c @ 1mM in B     // amount of c in B
b + c -> c + c
```

```
An amount can also be given in
grams (if molar mass is specified).
The resulting concentration is then
relative to sample volume.

species {NaCl#58.44}

sample C {1mL, 20C}
amount NaCl @ 8g in C
```

```
Reactions can be specified with
Arrhenius parameters {collision
frequency, activation energy}.
The reaction kinetics is then
relative to sample temperature T.

a + c ->{2, 5} a + a
// rate is 2*e^(-5/(R*T))
```

Liquid Handling

```
Mix two samples into one

        mix A = B, C

Split a sample into two

        split B,C = A by 0.5

Let a sample evolve by its reactions

        equilibrate A = B for 3

Throw away a sample

        dispose C


Change sample temperature (heat or cool)

          regulate A = B to 37C

Change sample volume (concentrate or dilute)

          concentrate A = B to 1mL
```

Experimental Biological Protocols with Formal Semantics

Alessandro Abate[2], Luca Cardelli[1,2], Marta Kwiatkowska[2], Luca Laurenti[2], and Boyan Yordanov[1]

[1] Microsoft Research Cambridge
[2] Department of Computer Science, University of Oxford

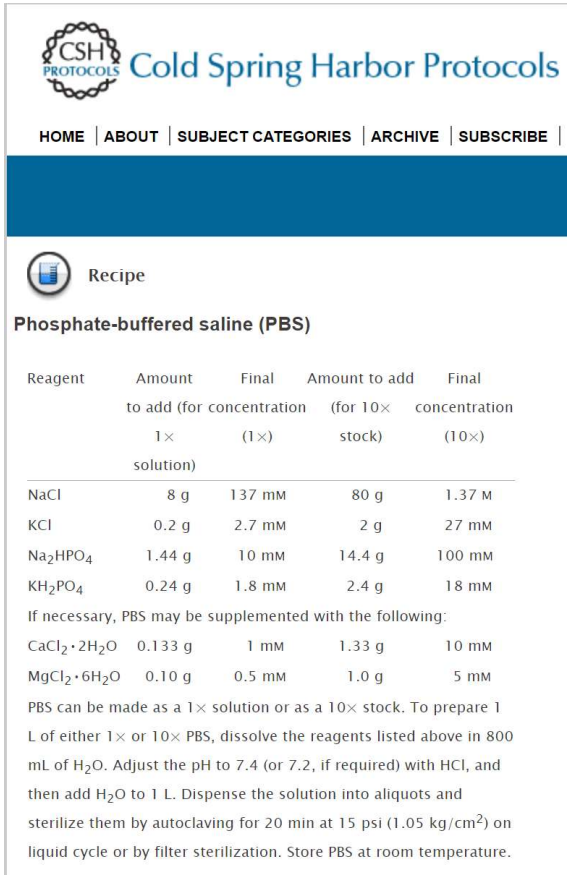
Demo: Sample Manipulation

```
species {c}

sample A
species a @ 1M in A
amount c @ 0.1M in A
a + c -> a + a
equilibrate A1 = A for 1

sample B
species b @ 1M in B
amount c @ 0.1M in B
b + c -> c + c
equilibrate B1 = B for 1

split C,D = A1 by 0.5
dispose C

mix E = D with B1
a + b -> b + b

equilibrate F = E for 20
dispose F
```

Multiple equilibration (simulation) steps

Ex: Phosphate-buffered saline (PBS)

```
species {NaCl#58.44, KCl#74.5513, NA2HPO4#141.96, KH2PO4#136.086}
report NaCl, KCl, NA2HPO4, KH2PO4

function Autoclave(sample PBS, number t) {
   define
      // increase temperature, preserve volume:
      regulate hot = PBS to 121C
      // bake
      equilibrate hot for t
      // decrease temperature, preserve volume:
      regulate PBS = hot to 20C
   yield PBS
}

function MakePBS() {
   define
      sample PBS {800mL, 20C}
      amount NaCl @ 8g in PBS
      amount KCl @ 0.2g in PBS
      amount NA2HPO4 @ 1.44g in PBS
      amount KH2PO4 @ 0.24g in PBS

      sample topup {200mL, 20C}
      mix PBS = PBS,topup
   yield Autoclave(PBS, 20*60)
}

sample PBS = MakePBS()
```



Cold Spring Harbor Protocols

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Recipe

Phosphate-buffered saline (PBS)

Reagent	Amount to add (for 1× solution)	Final concentration (1×)	Amount to add (for 10× stock)	Final concentration (10×)
NaCl	8 g	137 mM	80 g	1.37 M
KCl	0.2 g	2.7 mM	2 g	27 mM
Na_2HPO_4	1.44 g	10 mM	14.4 g	100 mM
KH_2PO_4	0.24 g	1.8 mM	2.4 g	18 mM
If necessary, PBS may be supplemented with the following:				
$CaCl_2 \cdot 2H_2O$	0.133 g	1 mM	1.33 g	10 mM
$MgCl_2 \cdot 6H_2O$	0.10 g	0.5 mM	1.0 g	5 mM

PBS can be made as a 1× solution or as a 10× stock. To prepare 1 L of either 1× or 10× PBS, dissolve the reagents listed above in 800 mL of H_2O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H_2O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle or by filter sterilization. Store PBS at room temperature.

http://cshprotocols.cshlp.org/content/2006/1/pdb.rec8247

Ex: Serial Dilution (recursive protocol)

```
network SerialDilution(number count, sample s, network f) {
  if count > 0 then
    sample solvent {9*observe(volume,s) L, observe(kelvin,s) K}
    mix s = s, solvent
    split s, dilution = s by 0.1, 0.9
    f(dilution)
    SerialDilution(count-1, s, f)
  end
}

//initial sample to be diluted:

sample init {1mL, 25C}
species A @ 1M in init
species B @ 1M in init
A + B ->{20} A
A -> ∅

//apply this network to each dilution;
//note that this invokes a simulation
//each time in each solution

network test(sample s) {
   equilibrate s for 10
   dispose s
}

//dilute 4 times

SerialDilution(4, init, test)
```

Prepare a series of increasingly diluted solutions and apply a network f to each (f can add species and reactions to the solutions)

```
RESULT:
sample init {1mL, 298.2K} {A = 1M, B = 1M}
sample s2 {1mL, 298.2K} {A = 100mM, B = 100mM}
sample s4 {1mL, 298.2K} {A = 10mM, B = 10mM}
sample s7 {1mL, 298.2K} {A = 1mM, B = 1mM}
sample s10 {1mL, 298.2K} {A = 100uM, B = 100uM}
```

Executing the protocols

- We have seen that *reactions can be executed* by DNA
- But how can we *execute the protocols*, so that we can execute the whole thing together?
- -> Digital Microfluidics Compiler

Digital Microfluidics

- A general, *programmable*, platform to execute the main liquid-handling operations

- To close the cycle, it can support many automated observation techniques on-board or off-board via peripheral pumps (sequencing, mass spec, …) although these are all very hardware-dependent.

Digital Microfluidics

OpenDrop

https://www.youtube.com/watch?v=ncfZWqPm7-4

Speed test

https://www.youtube.com/watch?v=pSls9L_h3Q0

Digital Microfluidics

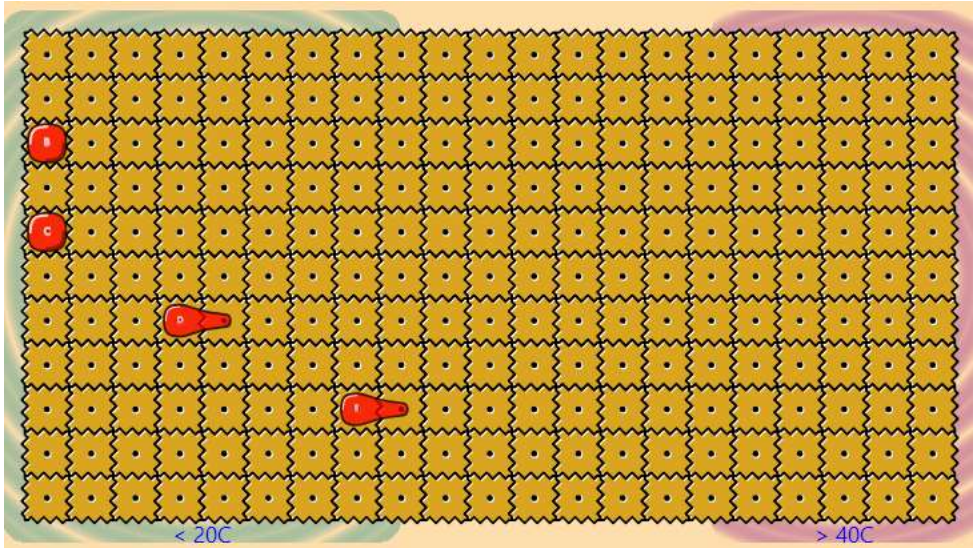
- A general, *programmable*, platform to execute the main liquid-handling operations

- To close the cycle, it can support many automated observation techniques on-board or off-board via peripheral pumps (sequencing, mass spec, …) although these are all very hardware-dependent.

Digital Microfluidics Compiler

- Mix, split, equilibrate, dispose
- Automatic routing – no geometrical information
- Hot/cold zones

```
sample A {3µL, 20C}

split B,C,D,E = A

mix F = E,C,B,D

dispose F
```



Demo

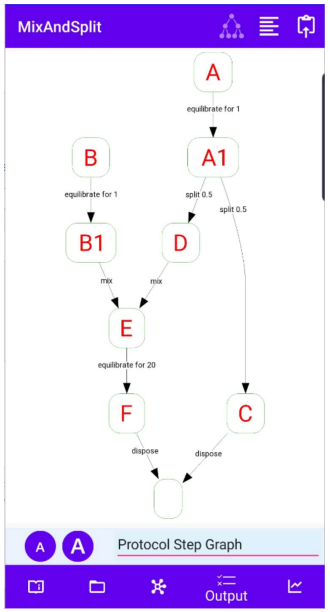
- Mix and Split

Extracting the Model and the Protocol

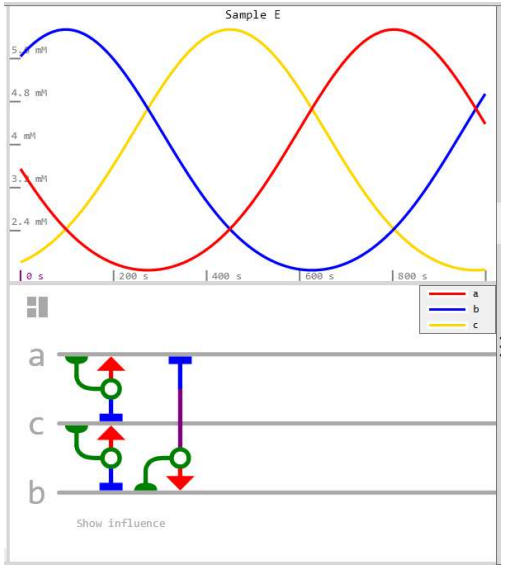
From the script

```
species {c}

sample A
species a @ 1M in A
amount c @ 0.1M in A
a + c -> a + a
equilibrate A1 = A for 1

sample B
species b @ 1M in B
amount c @ 0.1M in B
b + c -> c + c
equilibrate B1 = B for 1

split C,D = A1 by 0.5
dispose C

mix E = D with B1
a + b -> b + b

equilibrate F = E for 20
dispose F
```

The protocol



The (final) model (sample E)



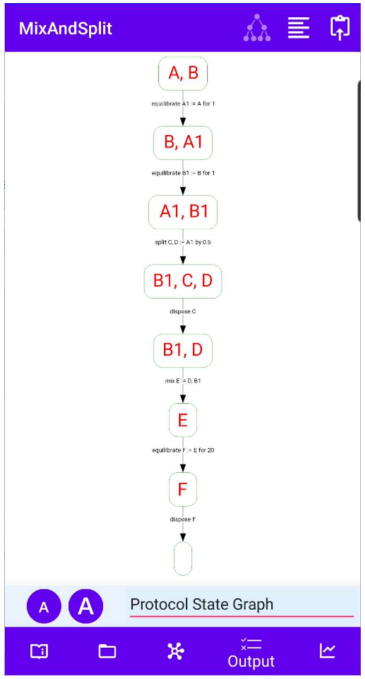

```
STATE_5
sample E {1.5mL, 293.2K} {
   a = 354.5mM
   c = 178mM
   b = 0.5674M
   consumed
   a + c -> a + a
   b + c -> c + c
   a + b -> b + b
}

KINETICS for STATE_5 (sample E) for 20 time units:
∂a = a * c - a * b
∂c = c * b - a * c
∂b = a * b - c * b
```

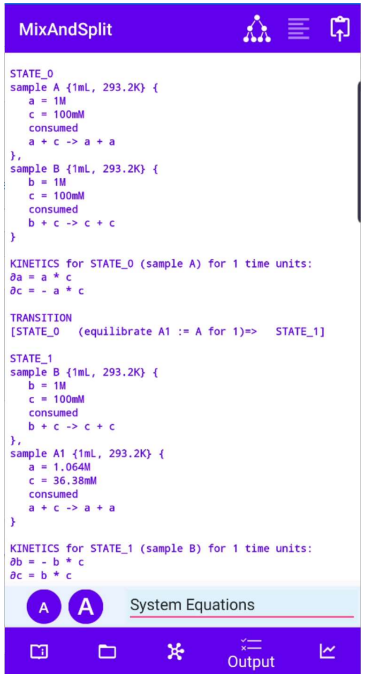
Extracting the Hybrid Transition System

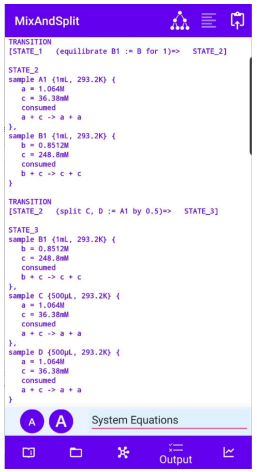
From the script

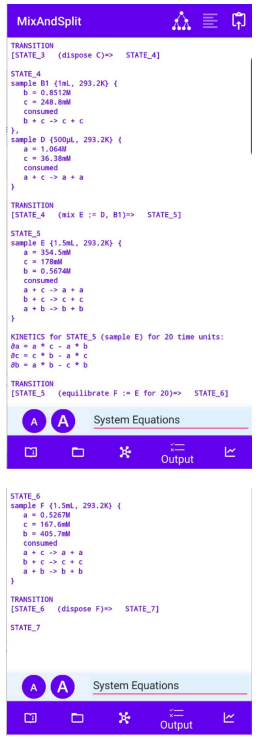
```
species {c}

sample A
species a @ 1M in A
amount c @ 0.1M in A
a + c -> a + a
equilibrate A1 = A for 1

sample B
species b @ 1M in B
amount c @ 0.1M in B
b + c -> c + c
equilibrate B1 = B for 1

split C,D = A1 by 0.5
dispose C

mix E = D with B1
a + b -> b + b

equilibrate F = E for 20
dispose F
```

The full story (Hybrid system)









Conclusions

Experimental biological protocols with formal semantics
Alessandro Abate, Luca Cardelli, Marta Kwiatkowska,
Luca Laurenti, Boyan Yordanov. CMSB 2018.

Kaemika app - Integrating protocols and chemical simulation
Luca Cardelli. CMSB 2020.

Integrated modeling

- Of chemical reaction networks and protocols
- How the Kaemika app supports it

Closed-loop modeling, experimentation and analysis

- For complete lab automation
- To “scale up” the scientific method