Beyond Numbers: Physical Simulation with Complex States

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Algorithmic Nature of Biology

"Computers are to Biology as Mathematics is to Physics."

Harold Morowitz

- Biological systems are highly organized
- We recognize data structures and programs
- Nature Computes!
- Especially true for Synthetic Biology

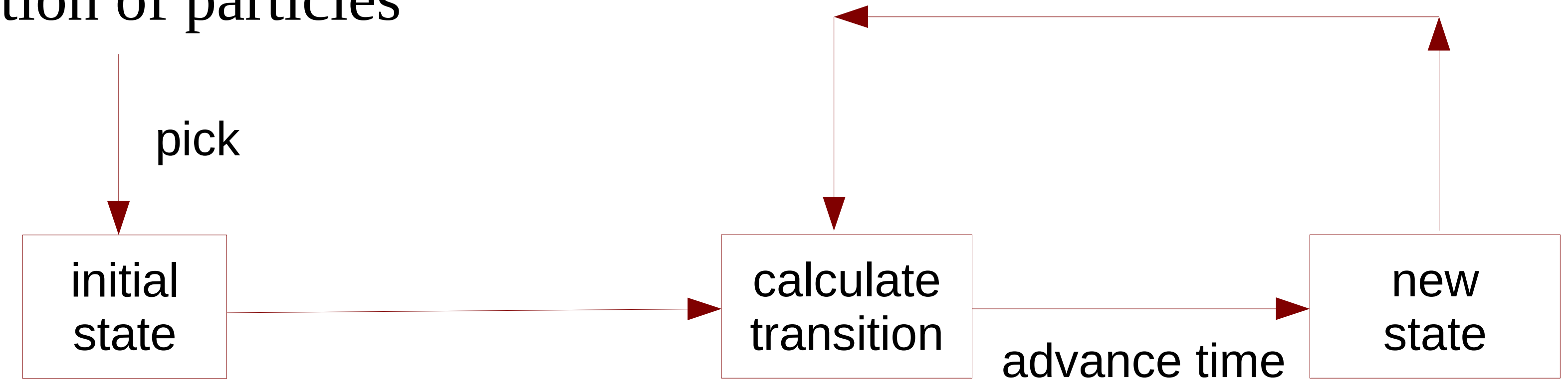
Outline

- **Physical simulation for Synthetic Biology**
  - Self-replicating emulsion compartments
  - Molecular DNA/RNA replicators
  - DNA assembly and computing
- **Formal calculi for Synthetic Biology**
  - Molecular DNA/RNA replicators
  - Compartmented reaction systems
  - Reconfiguring biological DNA
- **Conclusion**

Simulation – The Mathematics of Time

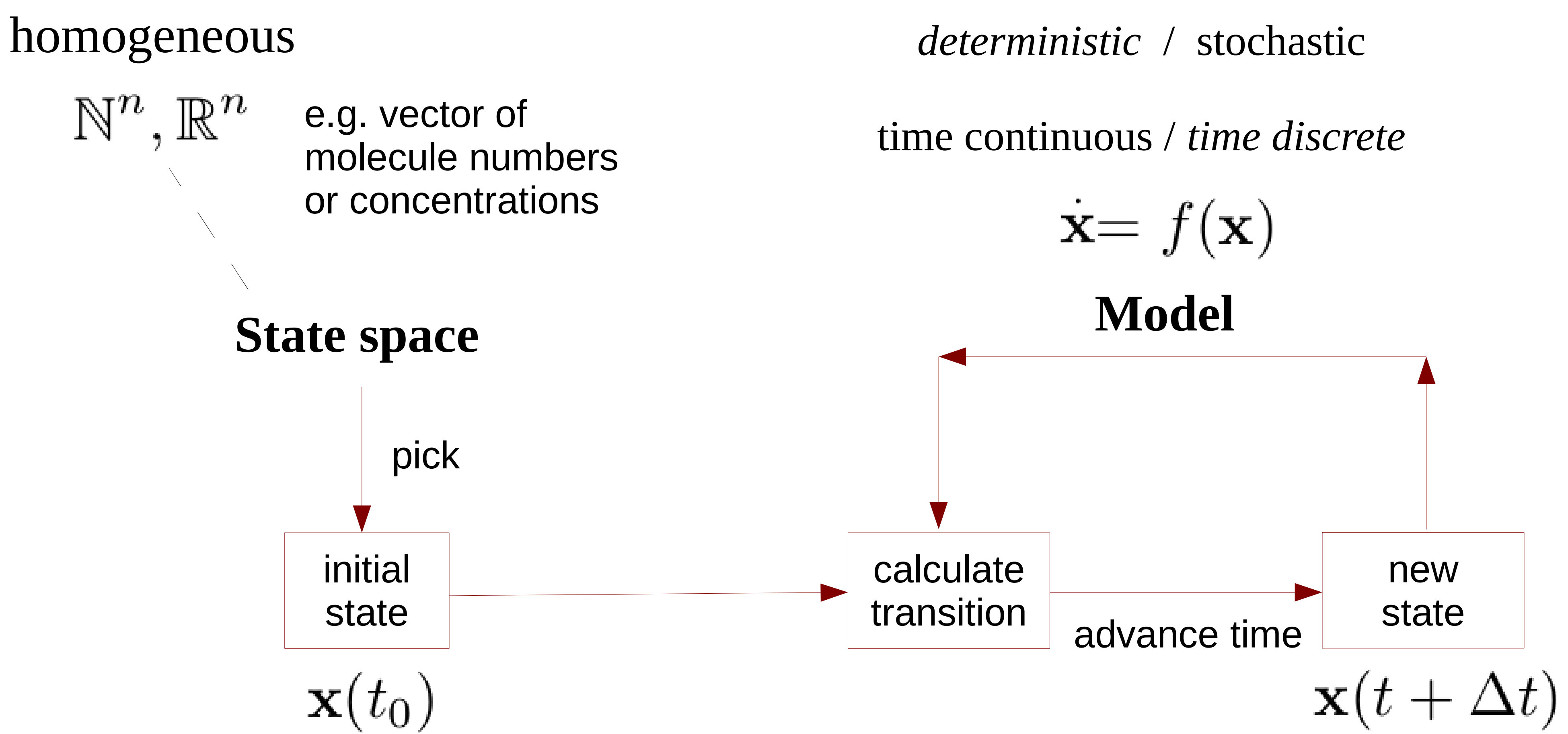
State space,e.g.
- mol. concentrations
- position of particles

Model
defines interactions
(domain expertise enter here!)

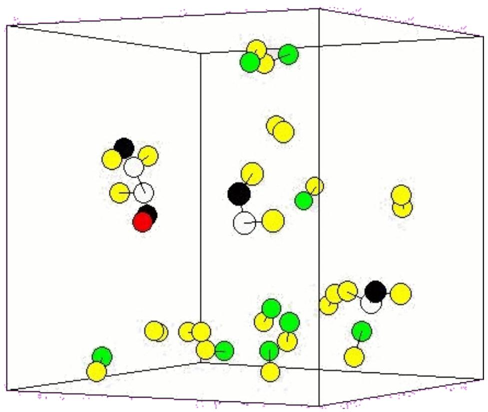


→ predict, explain, guide experiments, illuminate uncertainties, ...

Simulation – The Mathematics of Time



Simulation – The Mathematics of Time



particle based

$\mathbb{R}^{3N}$

State space

deterministic / stochastic

equation of motion:

$$\dot{\mathbf{x}}_i\ (t) = \mathbf{v}_i(t)$$

$$\dot{\mathbf{v}}_i\ (t) = -\nabla F_i(\mathbf{x}(t))$$

F defines interactions

Model

pick

initial state

$\mathbf{x}(t_0)$

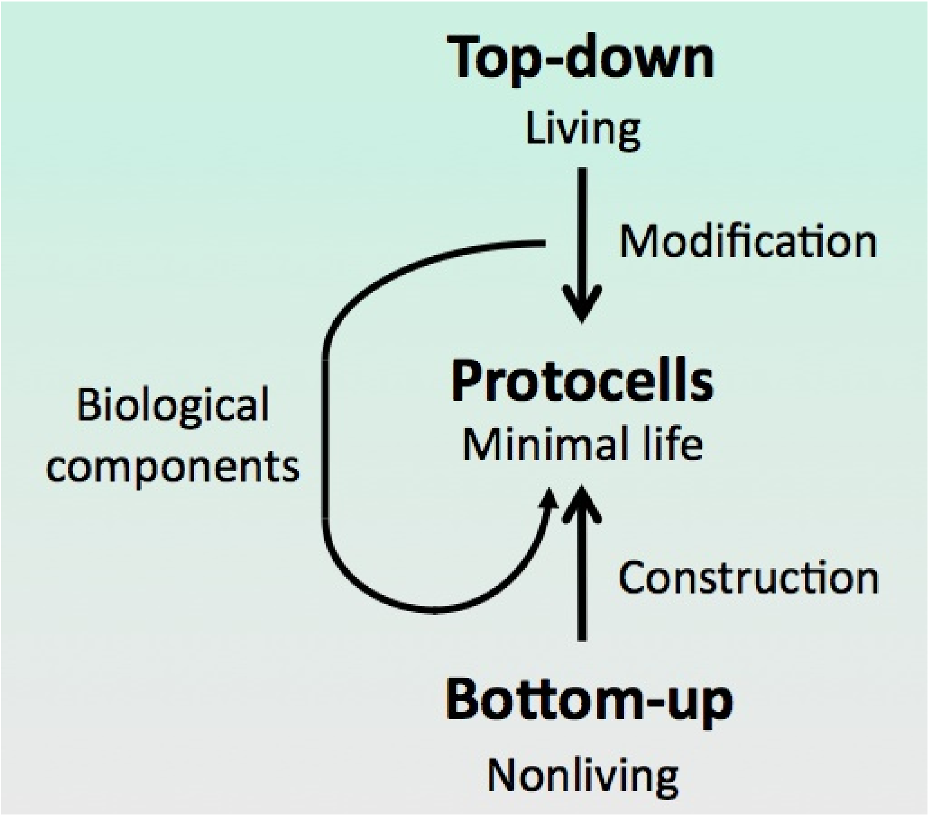
calculate transition

advance time

new state

$\mathbf{x}(t + \Delta t)$

Top-Down & Bottom-Up Synthetic Biology



This talk is about bottom-up approaches:

Biomolecules are used to assemble biomimetic system:
Amphiphiles (lipids), DNA/RNA, proteins

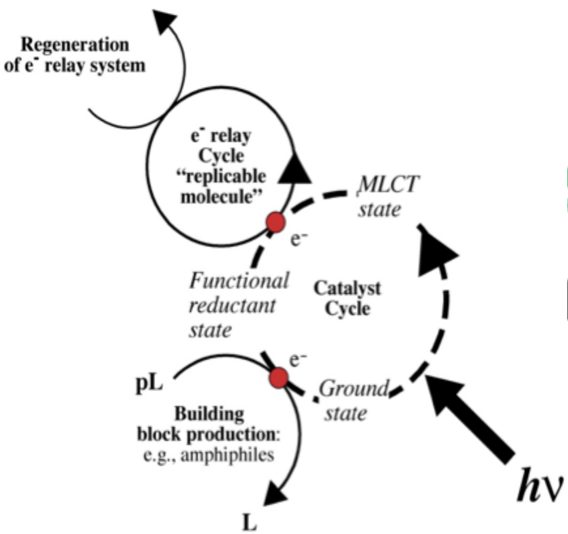
Protocells: Bottom-Up Synthetic Biology

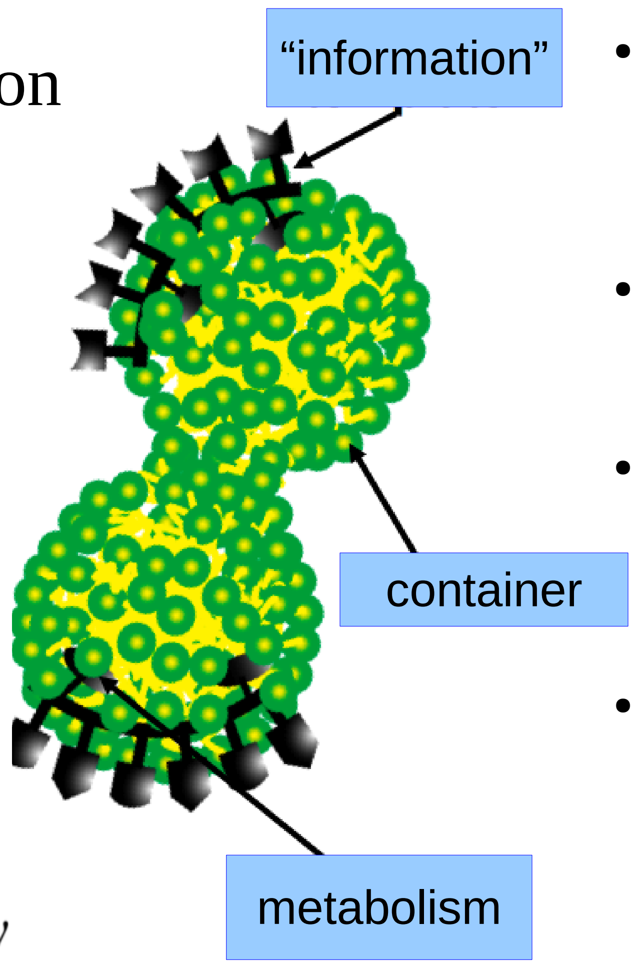
Danmarks Grundforskningsfond
Danish National Research Foundation

Aim:

De novo creation of chemical aggregates able to

- metabolize
- replicate
- undergo evolution



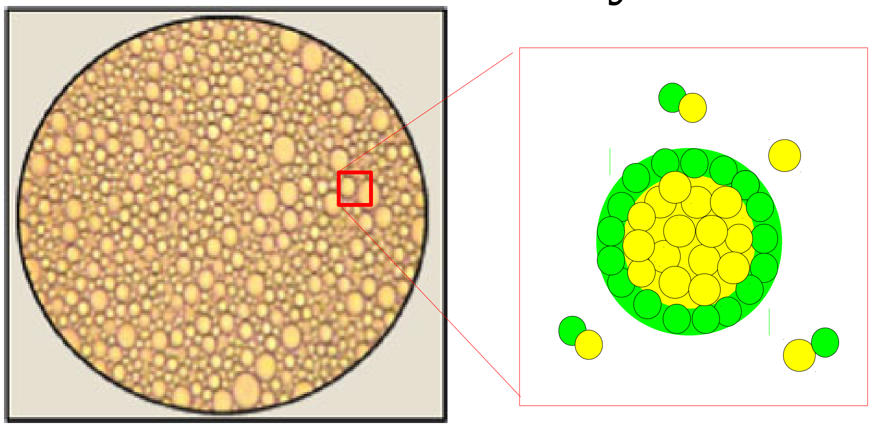


- Everything attached to the **external interface** of a lipid aggregate
- A **single reaction mechanism** for all metabolic reactions
- **direct participation of information** molecules in the metabolic reaction (no proteins!)
- Information is sequence-dependent but **not encoding**

Rasmussen et al., *Artif. Life*, 2003

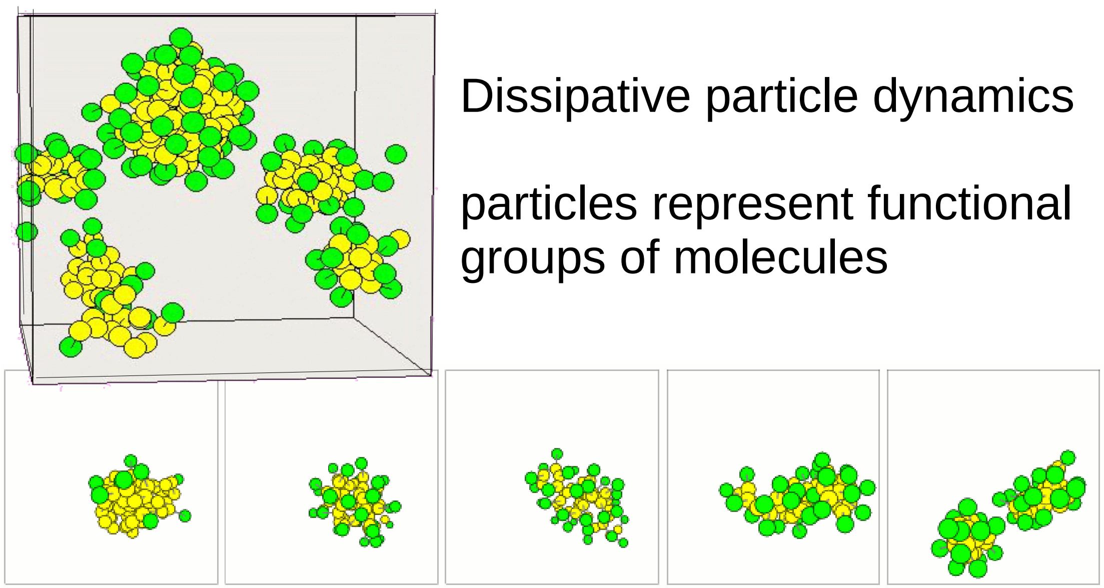
Protocells: Replicating Compartments

Oil-water-surfactant systems form emulsion compartments



Metabolism that can transform
oily precursor into functional surfactant:

“Coarse-grained” simulation



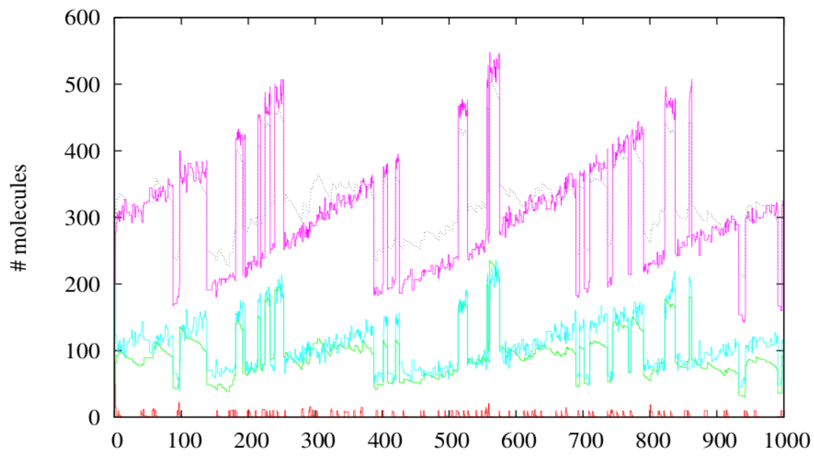
Dissipative particle dynamics

particles represent functional groups of molecules

Fellermann & Sole, *Phil. Trans. R. Soc., B*, 2007

Non-Spatial simulation

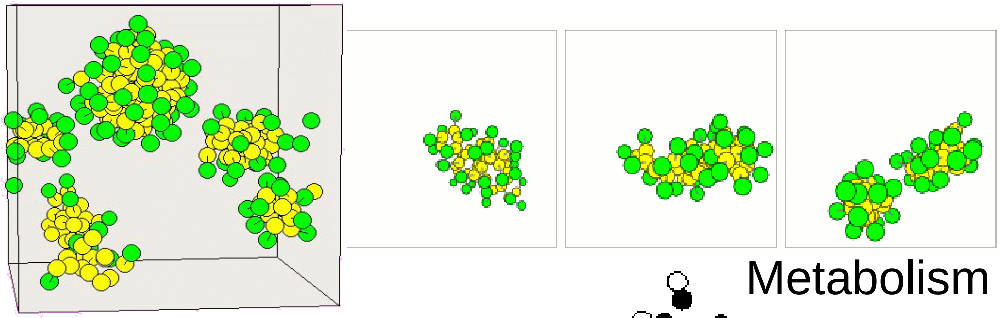
$$(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \in \mathbb{N}^4$$



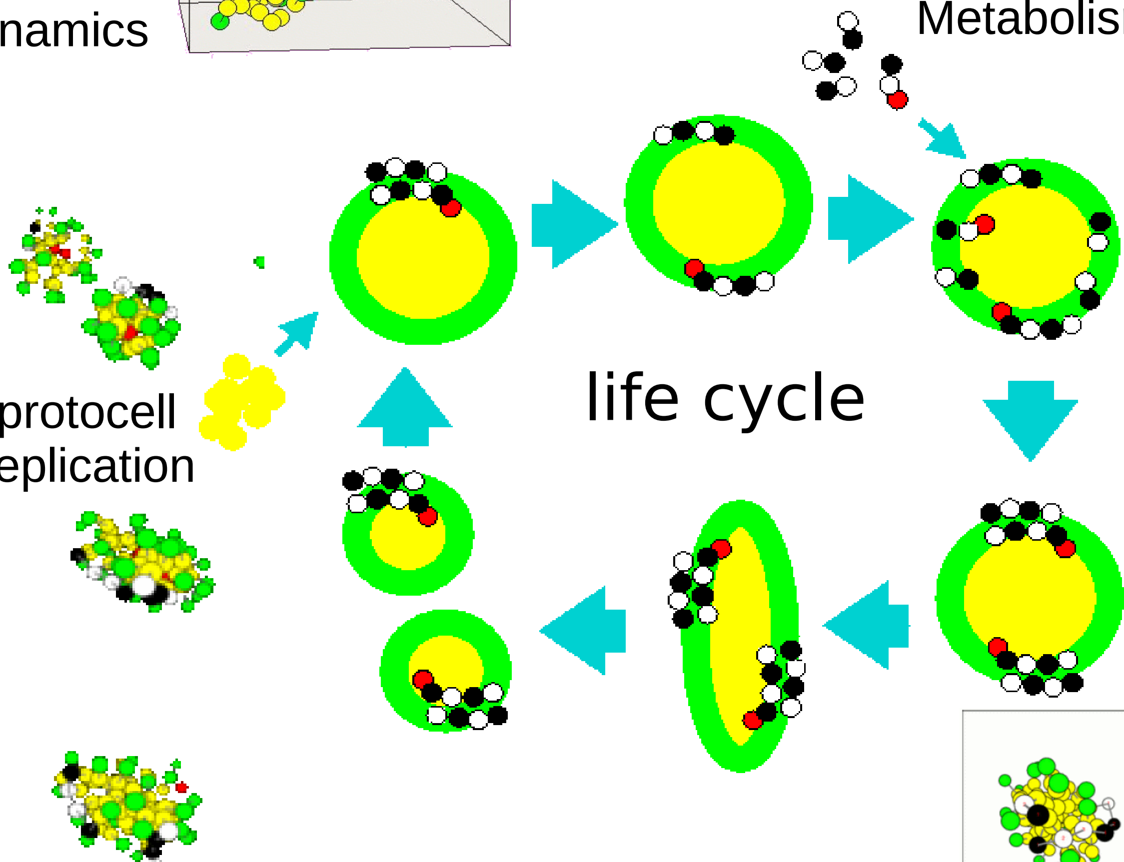
stochastic (Gillespie) simulation

Protocells: Physical Simulation

Container dynamics

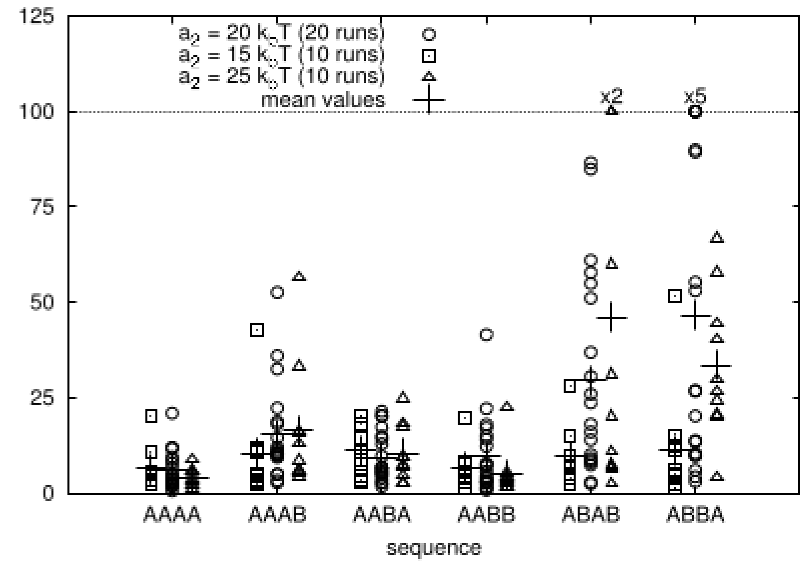


Metabolism

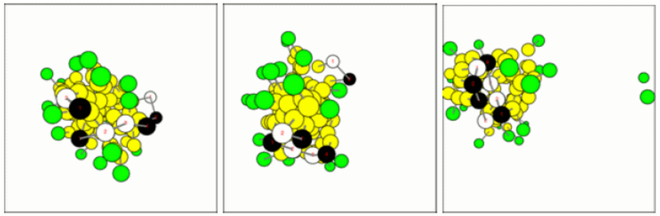


protocell replication

Emergent fitness function



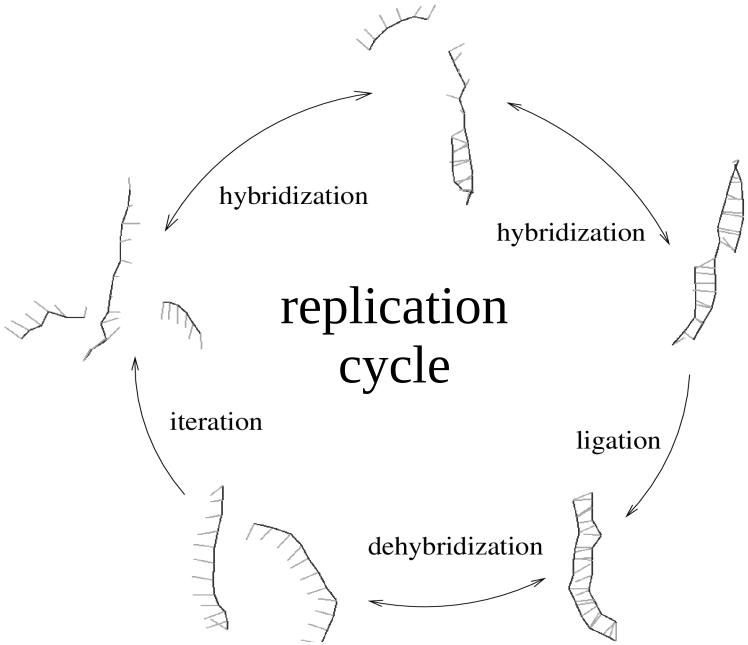
DNA replication

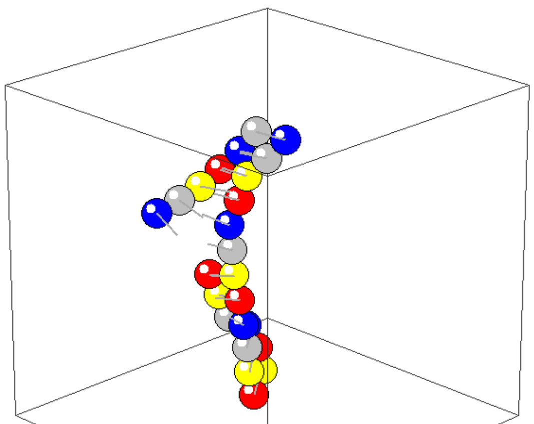


Fellermann & Sole, *Phil. Trans. R. Soc., B*, (2007) **362:** 1803

Non-enzymatic DNA/RNA replication

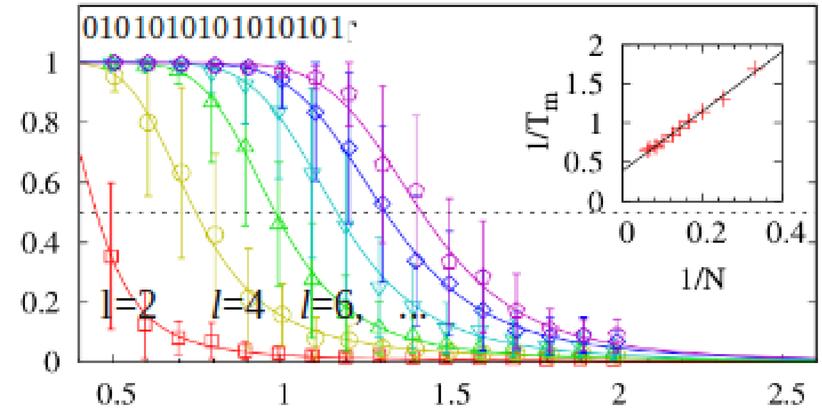
Template directed replication reaction



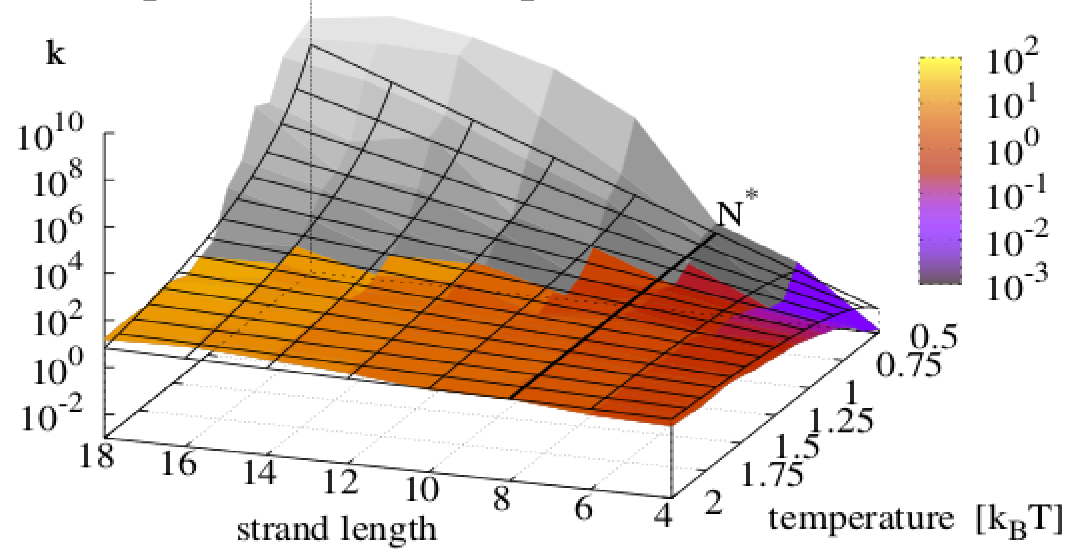


3D constrained Langevin dynamics simulation

melting curve measurements



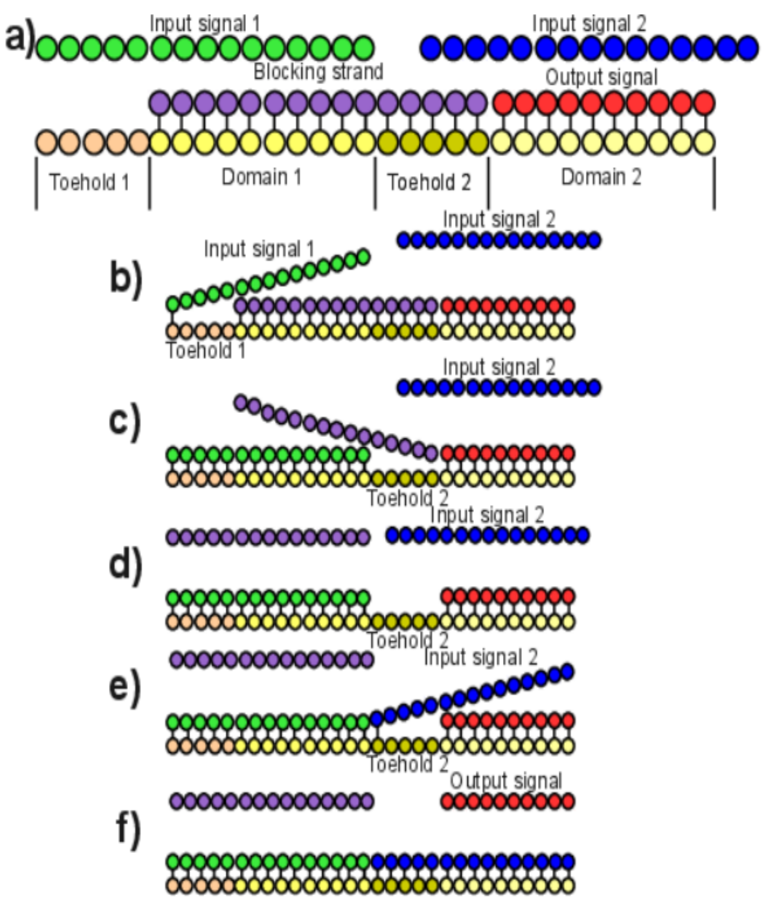
replication rate dependence

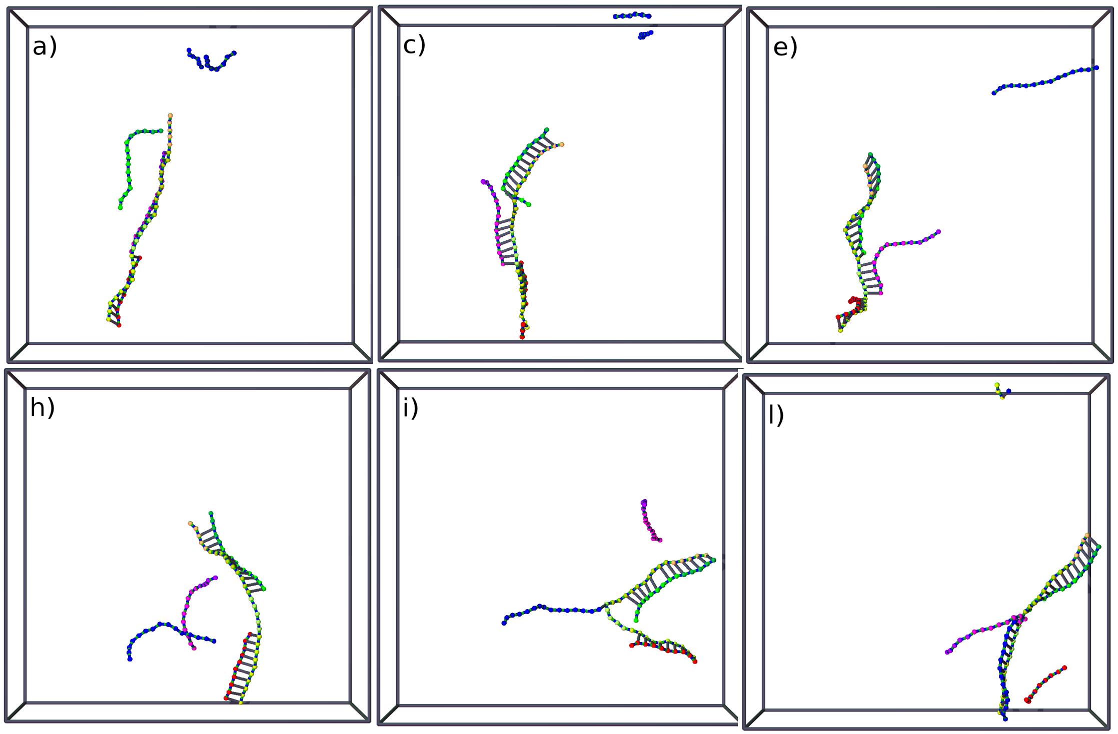


Fellermann, Rasmussen, *Entropy* 2011

DNA Strand Displacement Computing

DNA strand displacement *join* gate (Cardelli, 2010)





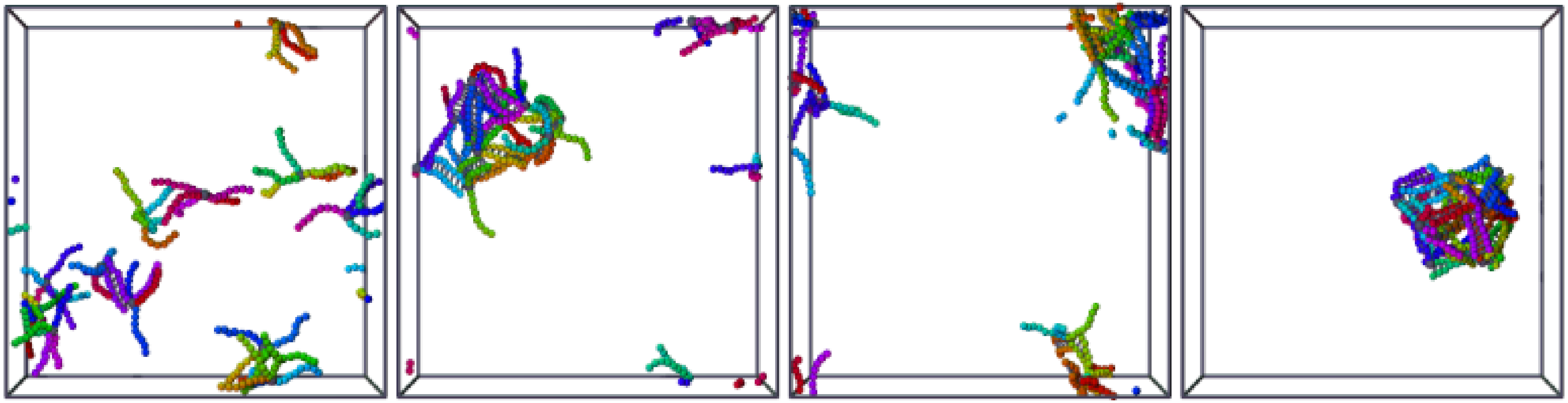
Fidelity of the gate:

11	0.95 ± 0.05
10	0.02 ± 0.01
01	$\boxed{0.58 \pm 0.15}$
00	0.00 ± 0.00

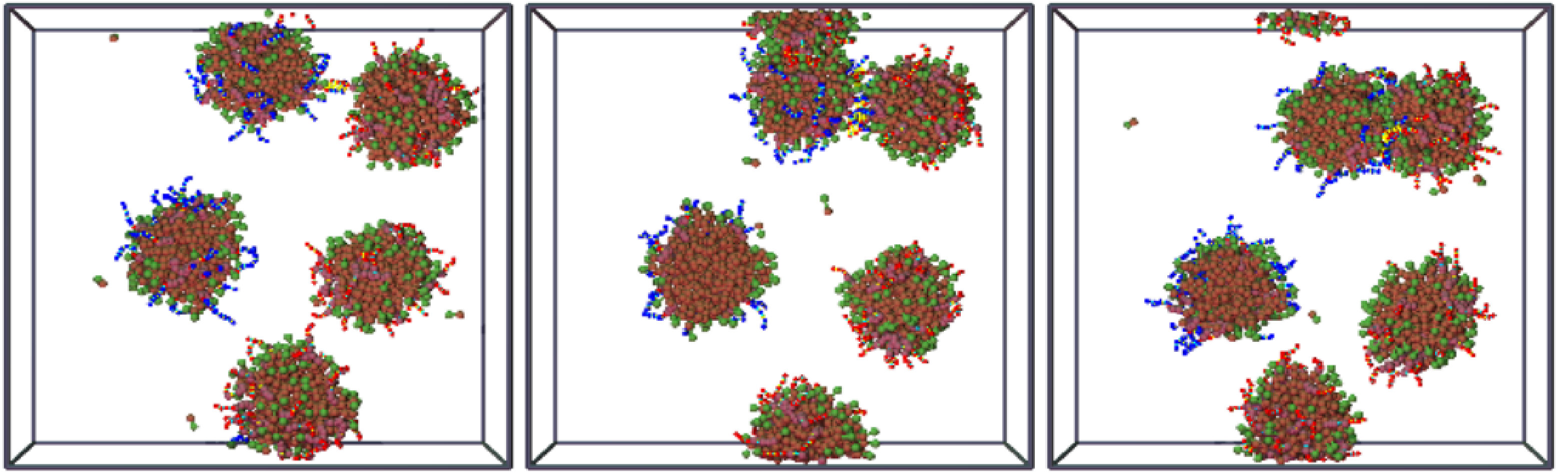
Svaneborg, Fellermann, Rasmussen *Lect. Notes Comput. Sc.* 2012

Physical Simulation of DNA Assembly

DNA assembly of an icosahedron from trisoligomers



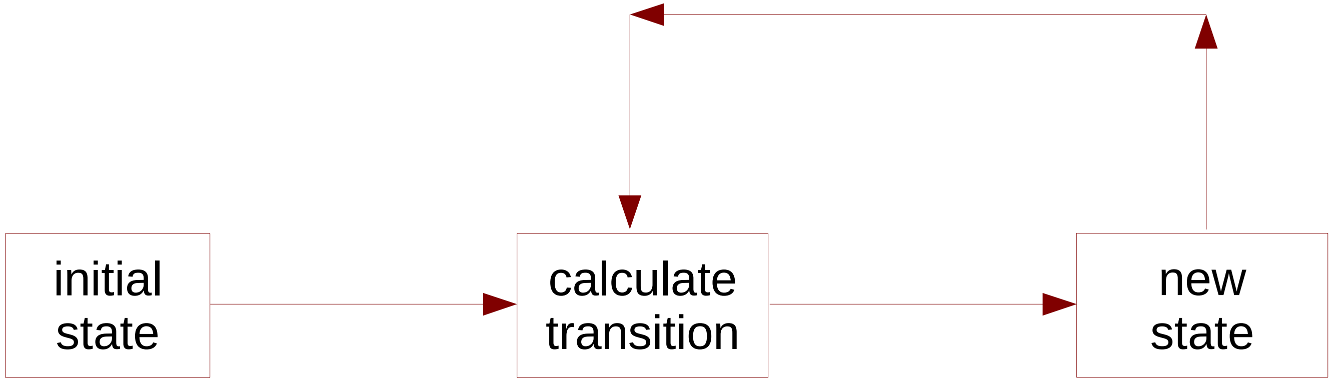
DNA induced association and fusion of oil-in-water compartments



Svaneborg, Fellermann, Rasmussen *Lect. Notes Comput. Sc.* 2012

Simulation Beyond Numbers

- Most simulation frameworks operate over a **simple, static** and relatively **small** state space.



$$(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \in \mathbb{N}^4$$

$$(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \xrightarrow{k_{\mathrm{L}}^{+} L^{\mathrm{b}}} (L+1, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})}$$

$$(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \xrightarrow{k_{\mathrm{L}}^{-} L} (L-1, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})}$$

$$(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \xrightarrow{k_{\mathrm{L_P}}^{+} L_{\mathrm{P}}^{\mathrm{b}}} (L, L_{\mathrm{P}}+1)_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})}$$

$$(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \xrightarrow{k_{\mathrm{L_P}}^{-} L_{\mathrm{P}}} (L, L_{\mathrm{P}}-1)_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})}$$

The state is dynamic, but it is embedded in a static state space.

Simulation Beyond Numbers

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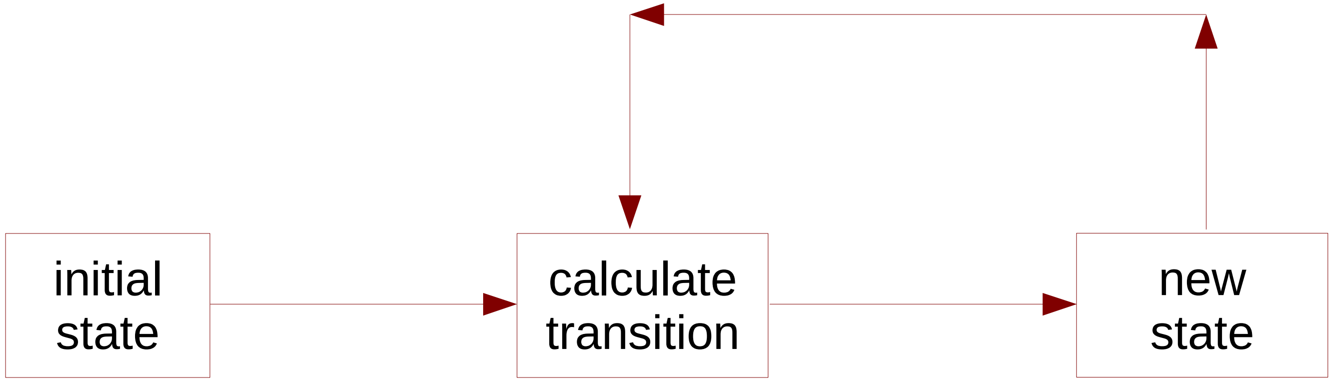


$$(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \in \mathbb{N}^4$$

$$
\begin{aligned}
(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} &\xrightarrow{k_{\mathrm{L}}^{+} L^{\mathrm{b}}} (L+1, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \\
(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} &\xrightarrow{k_{\mathrm{L}}^{-} L} (L-1, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \\
(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} &\xrightarrow{k_{\mathrm{L_P}}^{+} L_{\mathrm{P}}^{\mathrm{b}}} (L, L_{\mathrm{P}}+1)_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} \\
(L, L_{\mathrm{P}})_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})} &\xrightarrow{k_{\mathrm{L_P}}^{-} L_{\mathrm{P}}} (L, L_{\mathrm{P}}-1)_{(L^{\mathrm{tot}}, L_{\mathrm{P}}^{\mathrm{tot}})}
\end{aligned}
$$

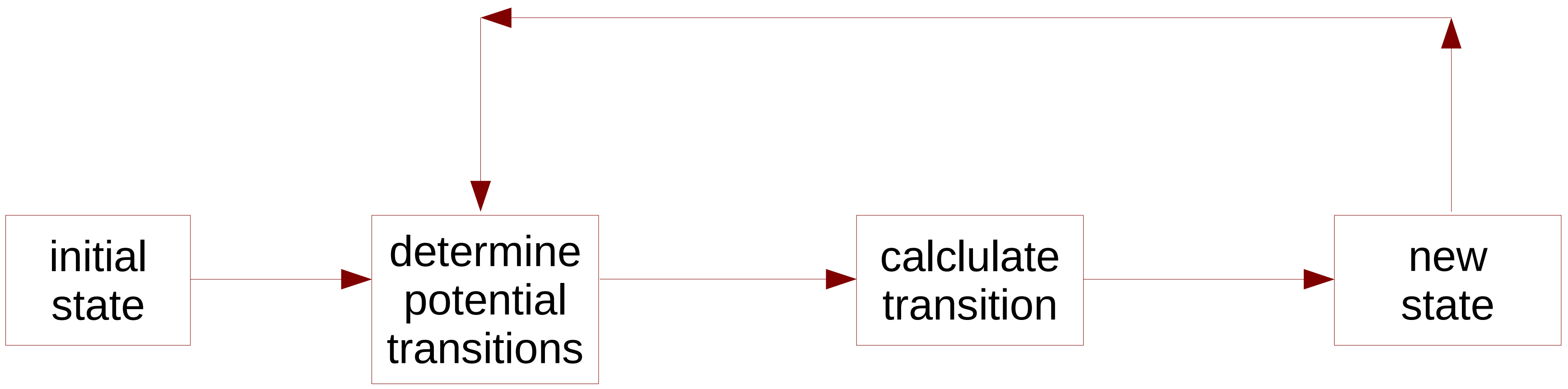
The state is dynamic, but it is embedded in a static state space.

Simulation Beyond Numbers

- Most simulation frameworks operate over a **simple, static** and relatively **small** state space.
- Biological state spaces are often **dynamic, complex**, and can be **arbitrarily large**.
- Examples:
  - Reconfiguring polymers (RNA, DNA, oligosaccharides)
  - Protein complexes
  - Compartment structures

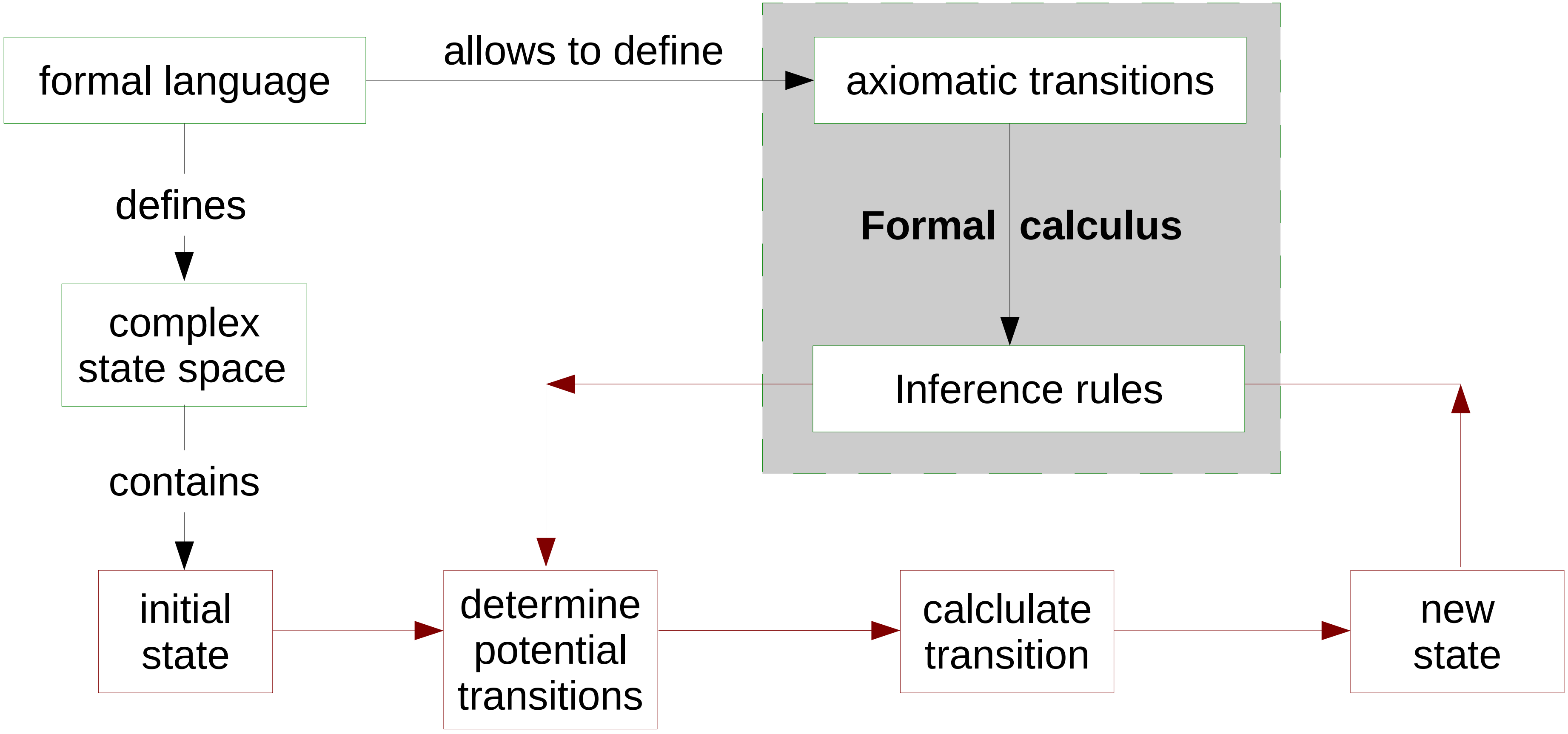
Simulation Beyond Numbers

- Most simulation frameworks operate over a **simple, static** and relatively **small** state space.
- Biological state spaces are often **dynamic, complex**, and can be **arbitrarily large**.
- CS offers tools to operate over “dynamic” state spaces.



Simulation Beyond Numbers

- CS offers tools to operate over dynamic state spaces.



Example: Self-Replicating Polymers

Alphabet of monomers: $A = \{0,1\} = \{$○, ●$\}$

Polymers are strings over A^*

We assume the following processes:

1. Degradation: $l.m \longrightarrow l + m$ 

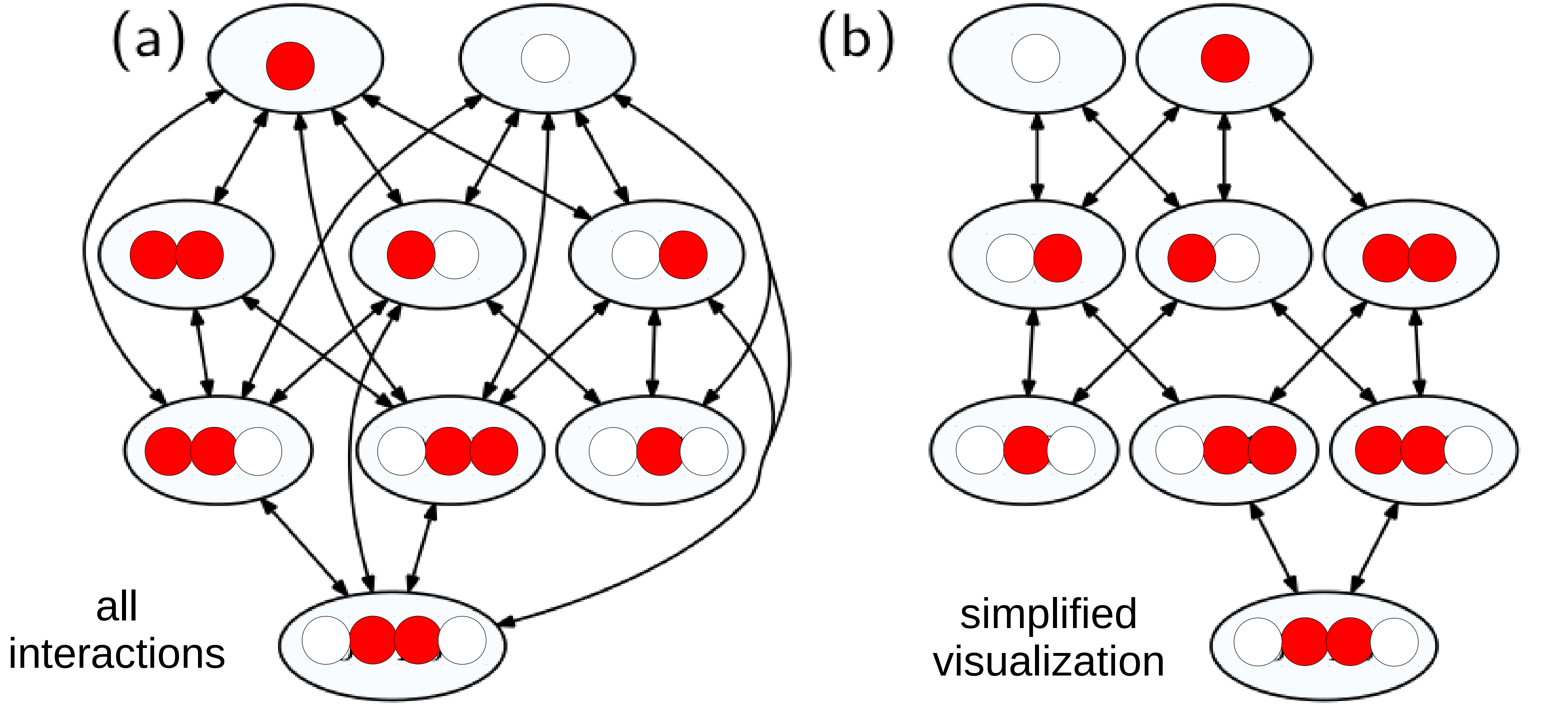
2. Random ligation: $l + m \longrightarrow l.m$ 

3. Autocatalysis: $l + m + l.m \longrightarrow 2\, l.m$ 

The number of possible species A^* is infinite and scales exponential with strand length.

Tanaka, Fellermann, Rasmussen. *Euro Phys Lett*, (submitted)

Example: Self-Replicating Polymers



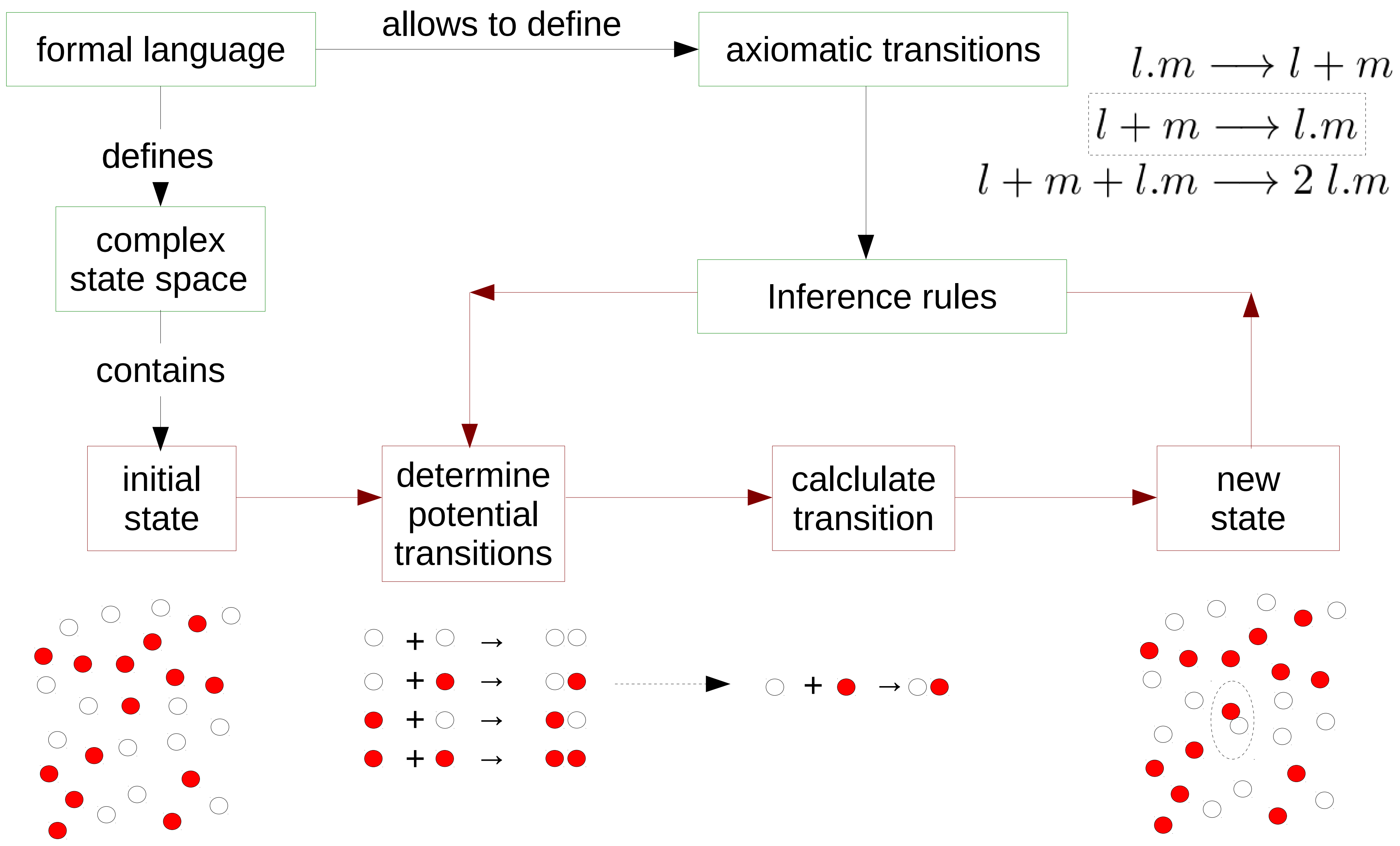
Replicators compete for resources.

They are each other's reaction and degradation products.

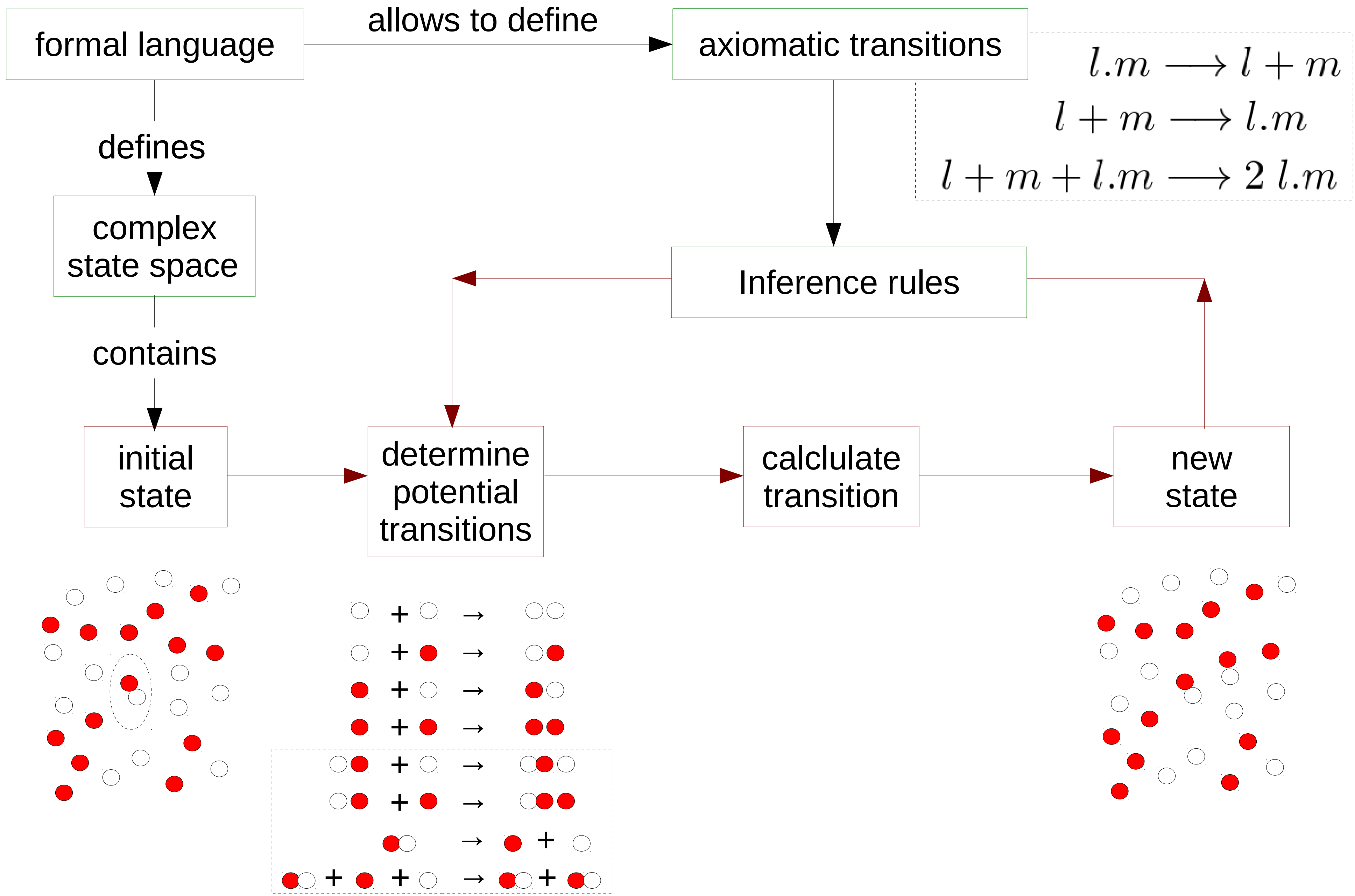
System is closed but energy flow is assumed.

What happens in a pool of monomers?

Example: Self-Replicating Polymers

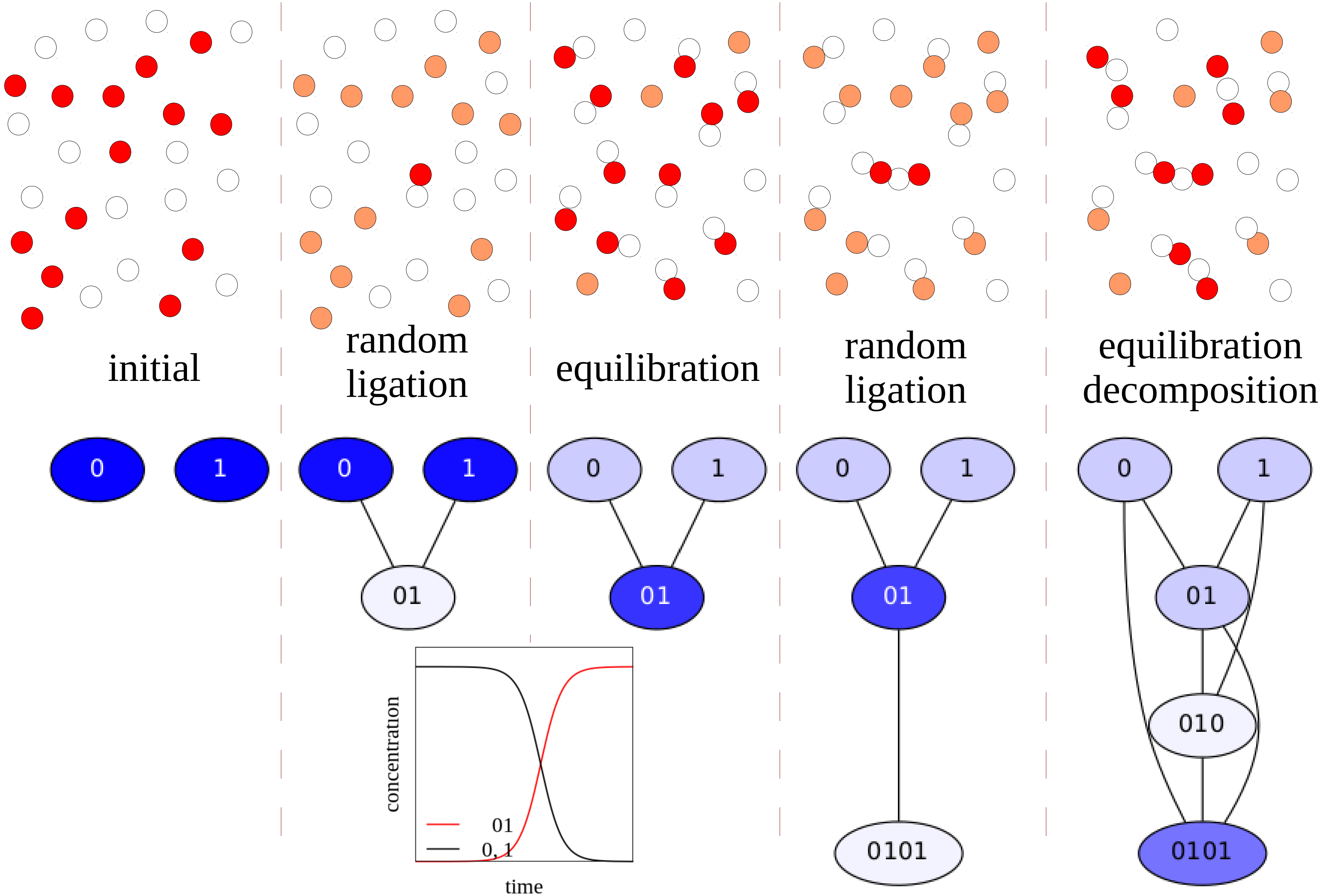


Example: Self-Replicating Polymers



Example: Self-Replicating Polymers

If random ligation is rare

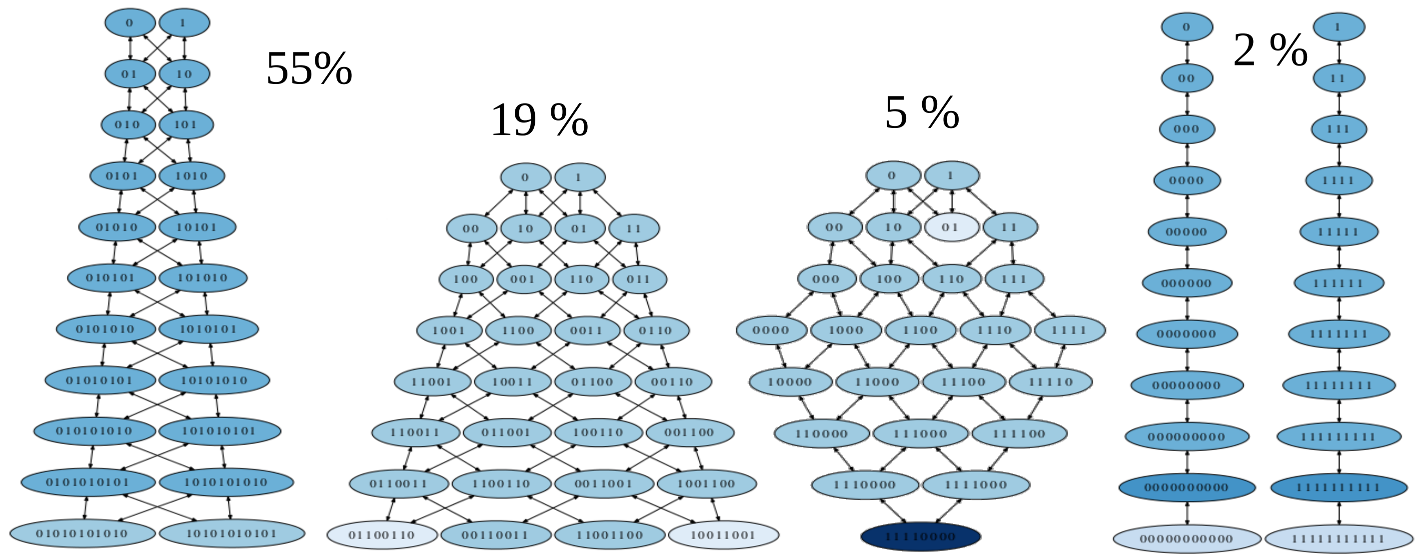


Example: Self-Replicating Polymers

If random ligation is rare, highly ordered sequence patterns emerge:

Gillespie simulation over
infintie dimensional
state space

HCA of final states



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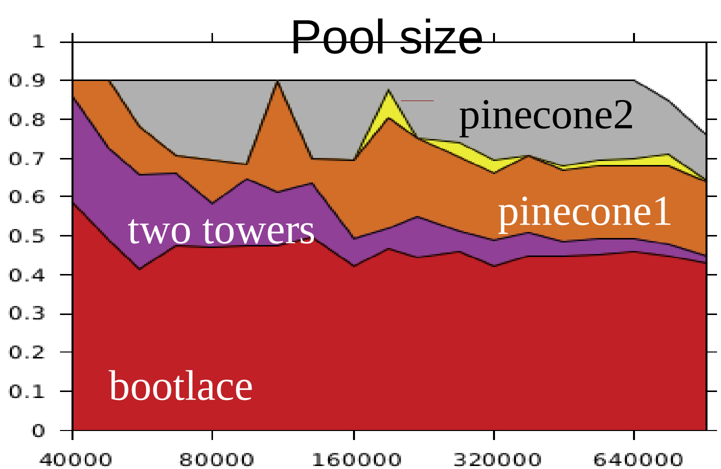
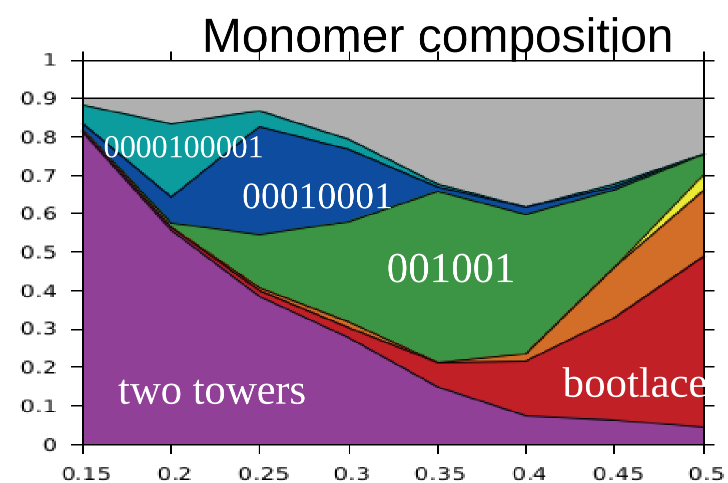
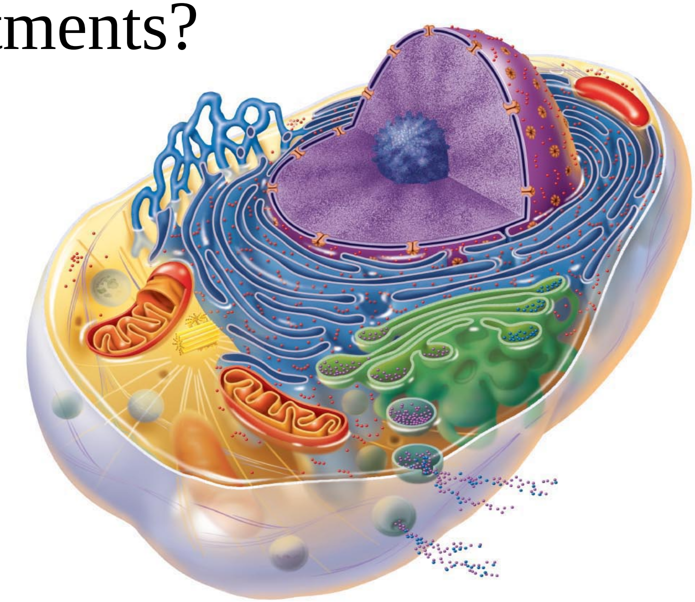




Tanaka, Fellermann, Rasmussen. *Euro Phys Lett*, (submitted)

Compartment Dynamics

Biological systems are commonly compartmentalized.
How to capture dynamics in <u>and of</u> compartments?



Language of nested parentheses:

Recursive grammar:

$$\mathsf{P} := \emptyset \;\mid\; \mathsf{P} + \mathsf{P} \;\mid\; \llparenthesis\, \mathsf{P}\, \rrparenthesis \;\mid\; m_j$$

empty state   composition   compartment   mol. cargo

Transitions among compartments:

$$\llparenthesis\, P \,\rrparenthesis + \llparenthesis\, Q \,\rrparenthesis \longrightarrow \llparenthesis\, P + Q \,\rrparenthesis$$

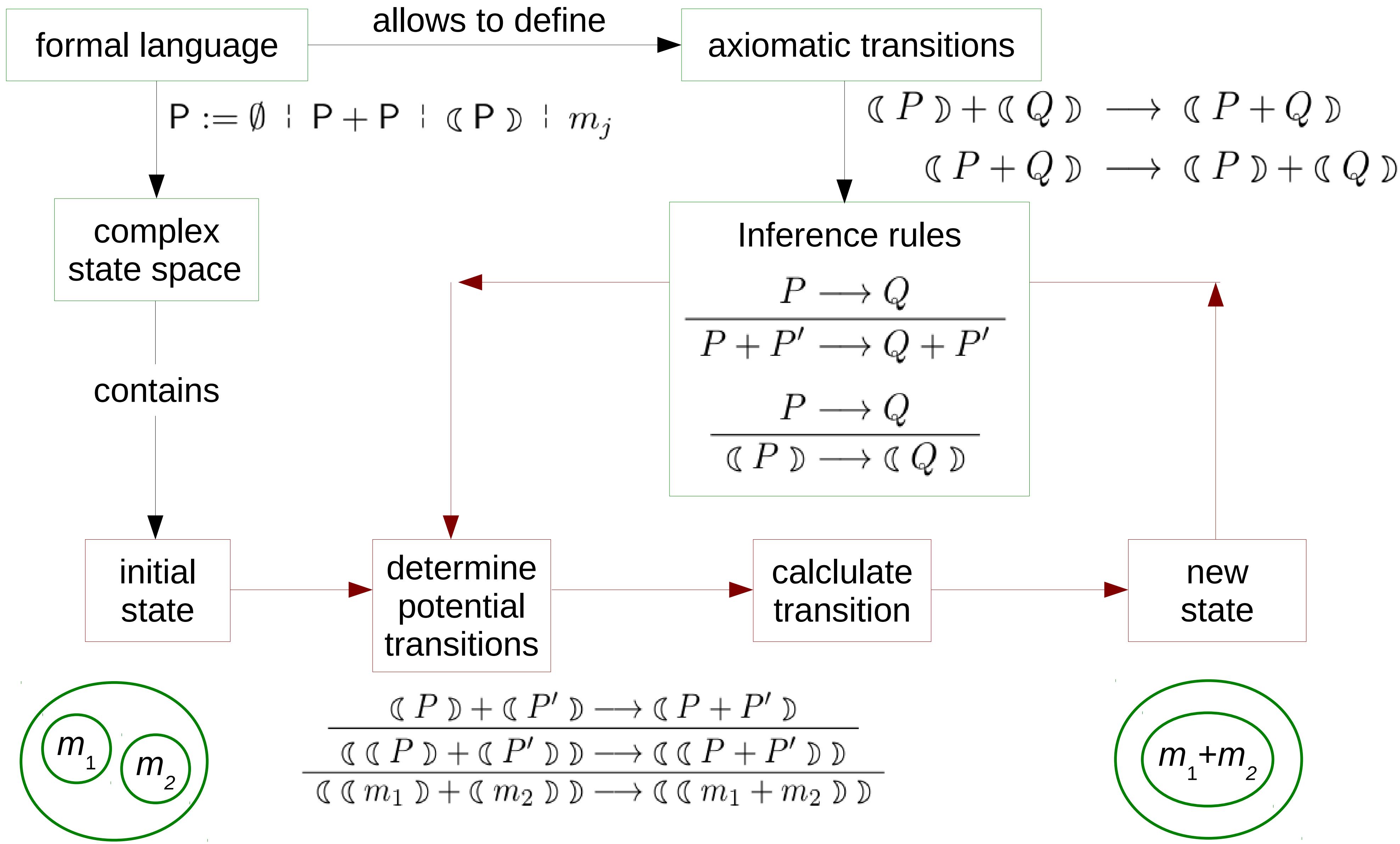
$$\llparenthesis\, P + Q \,\rrparenthesis \longrightarrow \llparenthesis\, P \,\rrparenthesis + \llparenthesis\, Q \,\rrparenthesis$$

e.g. *brane calculus*:

$$\mathrm{mate}_i \llparenthesis\, P \,\rrparenthesis + \mathrm{mate}_i^{\top} \llparenthesis\, Q \,\rrparenthesis \longrightarrow \llparenthesis\, P + Q \,\rrparenthesis$$

Cardelli, 2005

Example: Compartment Dynamics



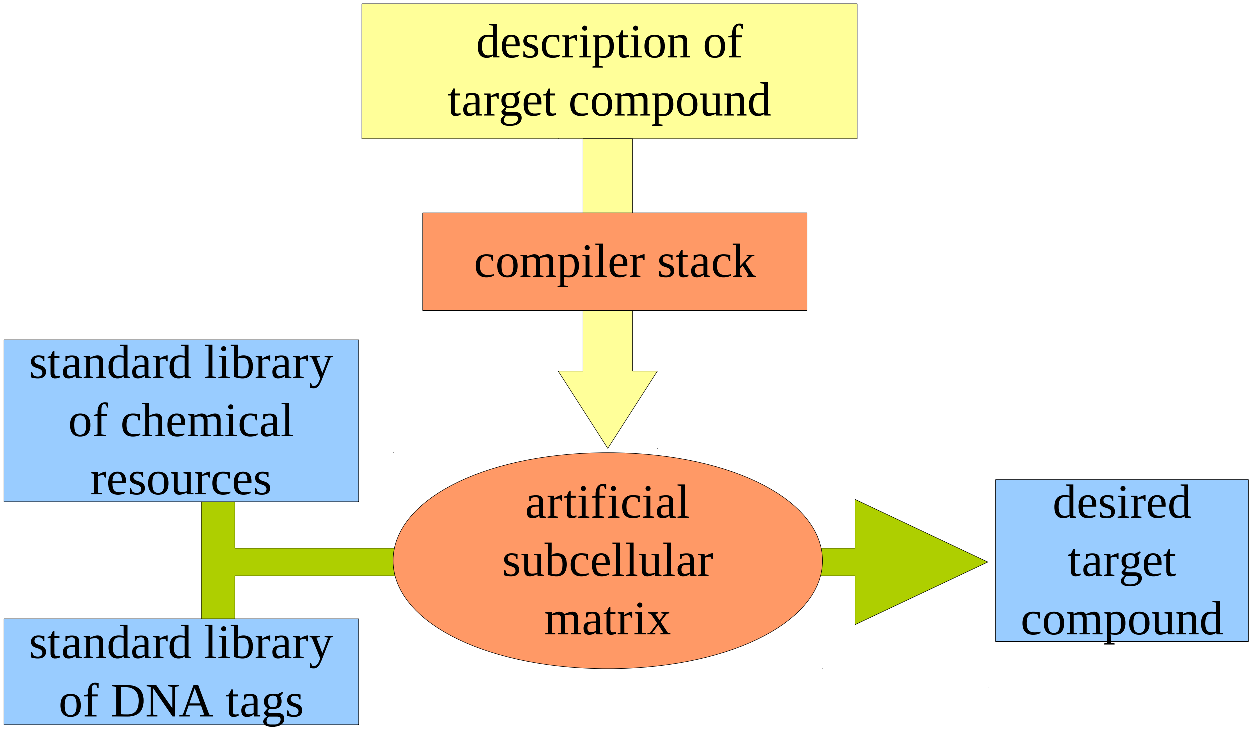
MATCHIT – Matrix for Chemical IT

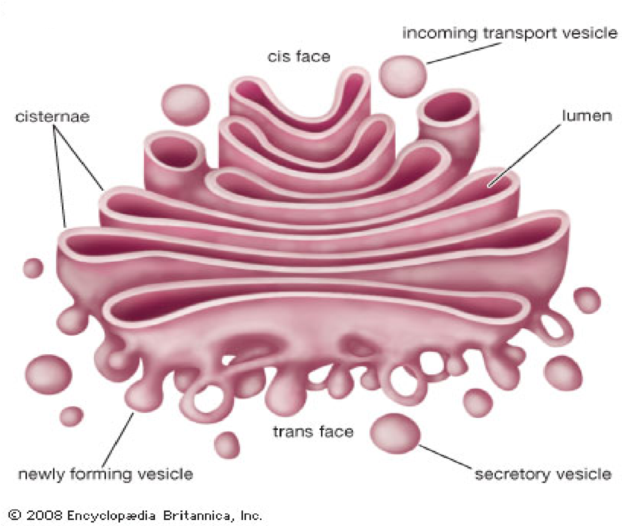
Aim:

Toward personal chemical manufactoring in an “artificial subcellular matrix”.

Methodology:

Integrating molecular computing and chemical production in microfluidic environments.

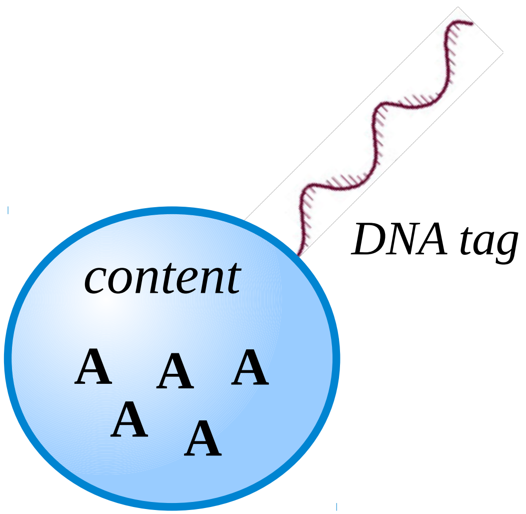




Golgi apparatus

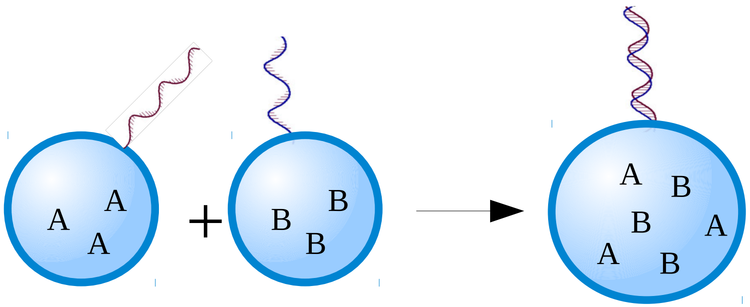
Programming Chemistry in Addressable Microcompartments

"Chemtainer" approach

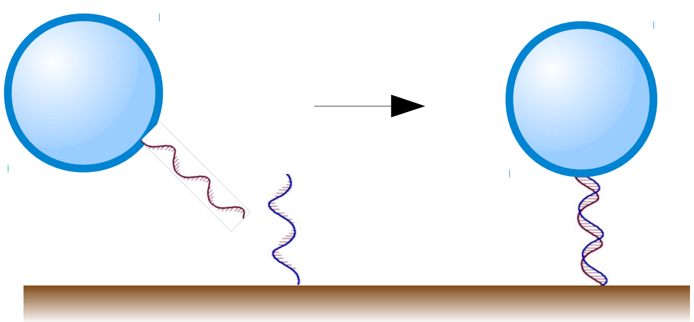


e.g. lipid vesicles,
oil droplets,
DNA nanocages...

DNA programmable chemtainer interactions

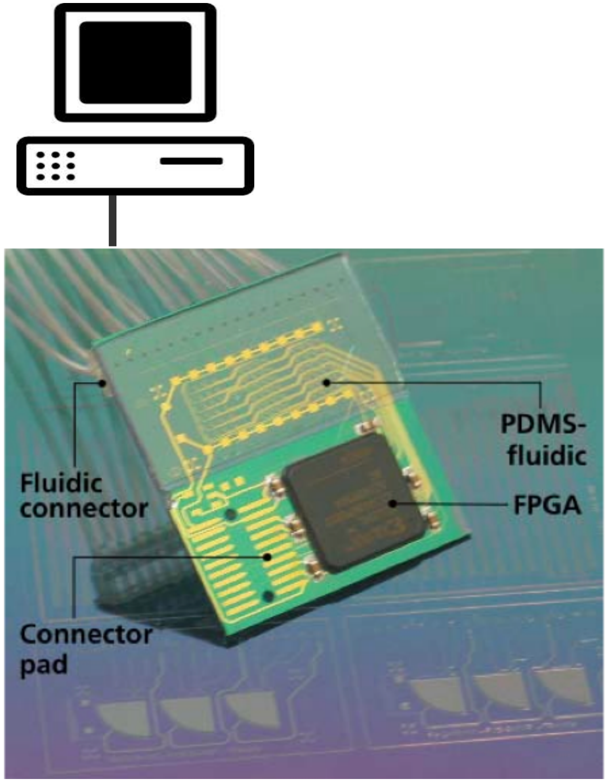


Chemtainer-chemtainer interaction



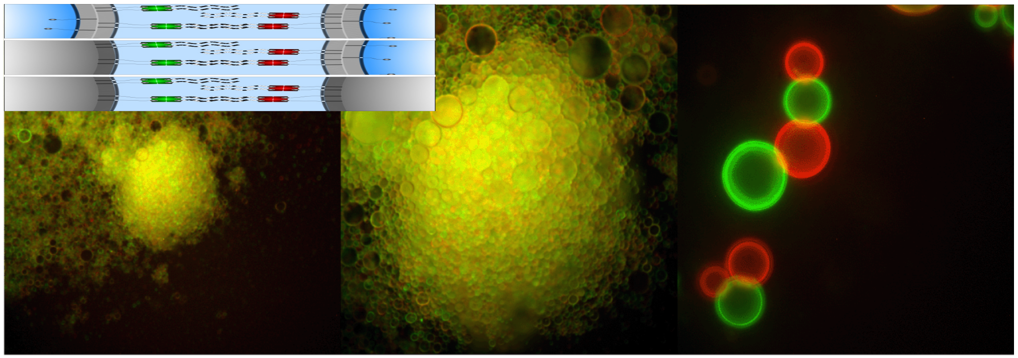
Chemtainer-matrix interaction

microfluidic embedding and computer control

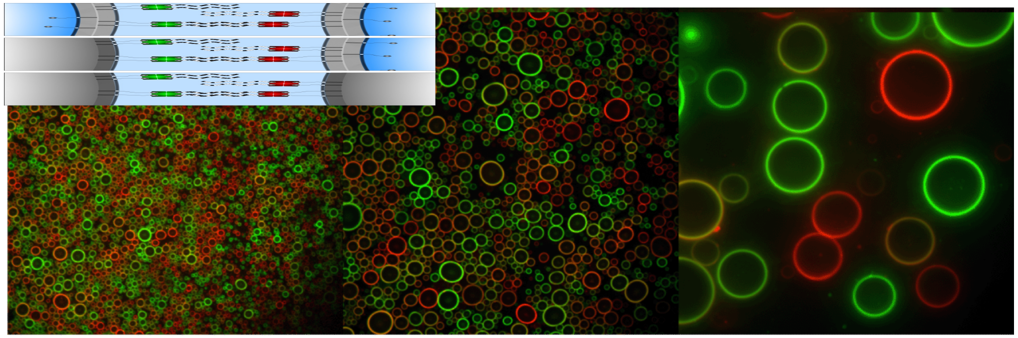


Experimental Chemtainer Interactions

Complementary tags

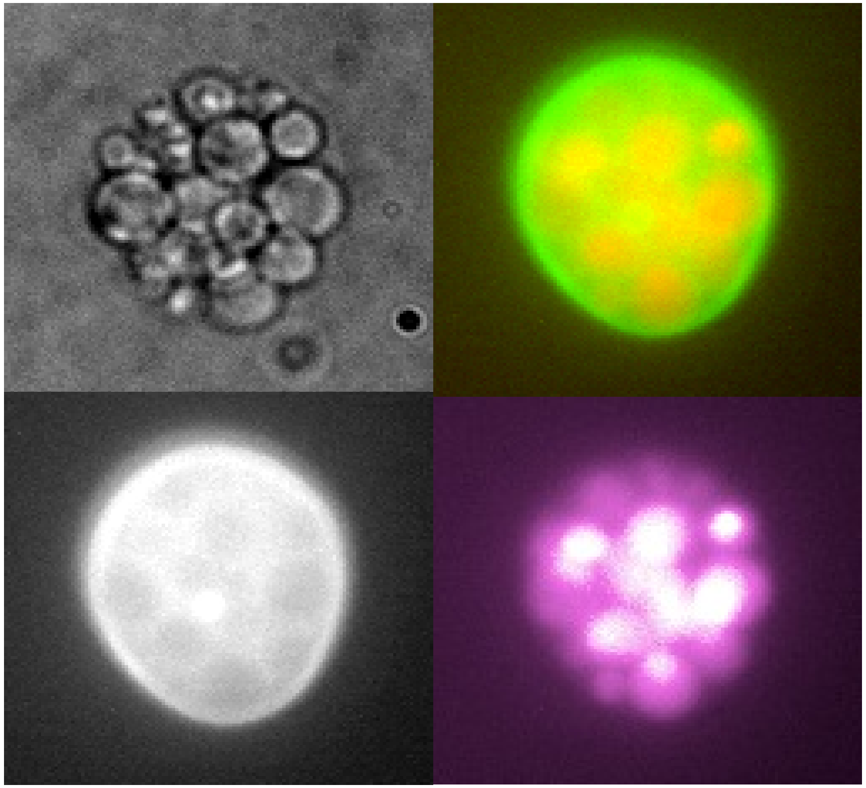


Non-complementary tags



4x 10x 100x

Hierarchical encapsulation

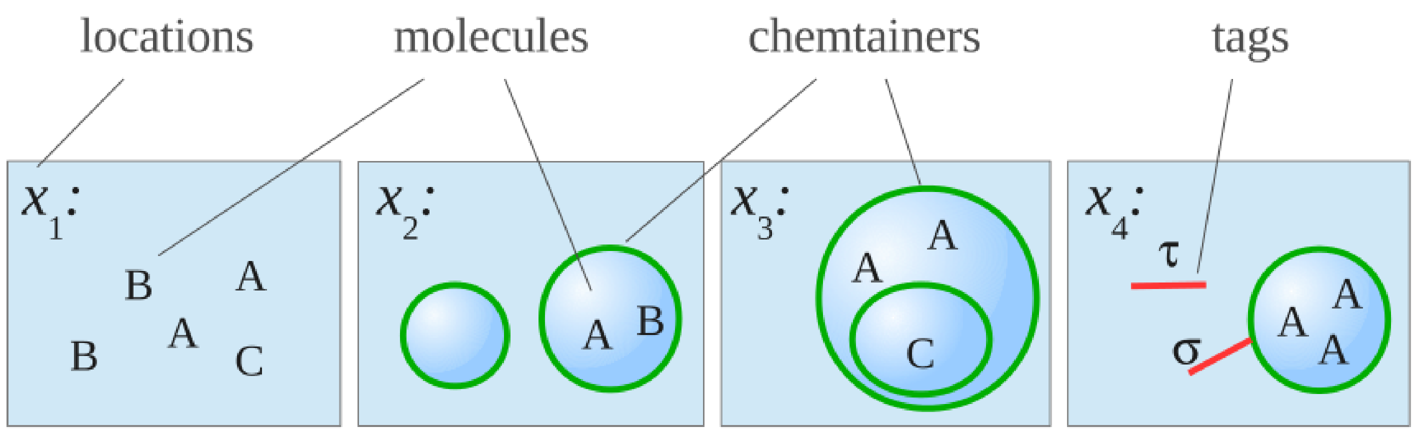


Hadorn, Bönzli, Sørensen, Fellermann, Eggenberger Hotz, Hanczyc; *PNAS* 109(47) 2012
Hadorn, Bönzli, Hanczyc, Eggenberger-Hotz; *PLOS One* 2012

Chemtainer Calculus: Grammar

System states are arrangements of localized molecules, chemtainers, and DNA tags

Example:



$$x_1 : 2A + 2B + C \circ x_2 : \llparenthesis\ \rrparenthesis + \llparenthesis A + B \rrparenthesis \circ x_3 : \llparenthesis 2A + \llparenthesis C \rrparenthesis \rrparenthesis \circ x_4 : \tau + \sigma \llparenthesis 3A \rrparenthesis$$

Grammar:

global state	$\mathsf{S} := \emptyset \mid \mathsf{S} \circ \mathsf{S} \mid x_i : \mathsf{P}$
local state	$\mathsf{P} := 0 \mid \mathsf{P} + \mathsf{P} \mid \mathsf{q}^* \llparenthesis \mathsf{P} \rrparenthesis \mid \mathsf{q} \mid m_j$
tag	$\mathsf{q} := \mathsf{s} \mid \mathsf{s}^* \triangleright \mathsf{s}^*$

Fellermann & Cardelli, *Interface* 2014 (in press)

Chemtainer Calculus: Transitions

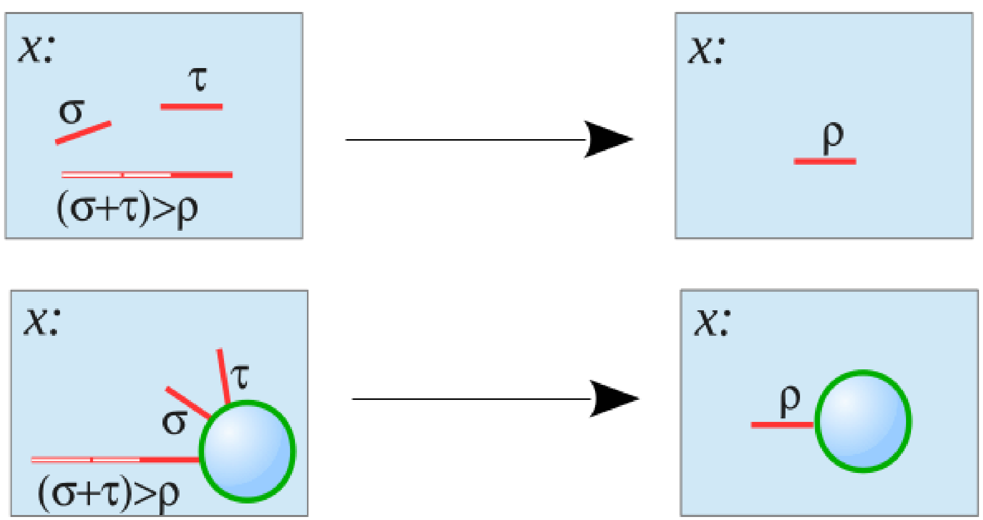
Autonomous transitions:

1. Application chemistry

$$P_i + M_i E^*_{x_i} \longrightarrow P_{i+1} + E_{x_i}$$

2. DNA *join & fork* gates $s_1^* \triangleright s_2^*$

$$s_1^* \triangleright s_2^* \; + \; s_1^* \longrightarrow s_2^*$$



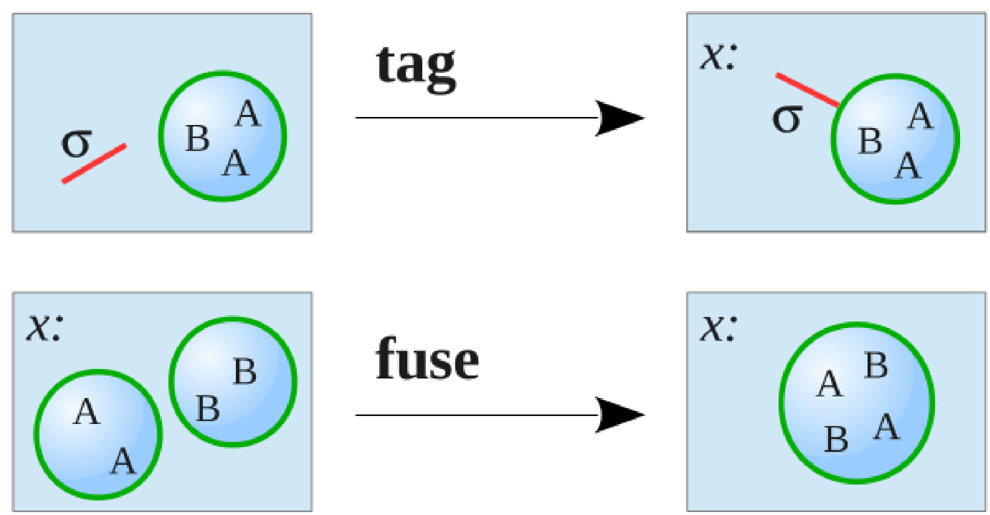
Induced transitions:

Triggered by microfluidic control

6 - 8 operations, e.g.

tag:

$$x : s + q^* \llparenthesis\, P \,\rrparenthesis \longrightarrow x : (s + q^*) \llparenthesis\, P \,\rrparenthesis$$



Fellermann & Cardelli, *Interface* 2014 (in press)

Chemtainer Calculus: Language

- Domain specific language
  specified in non-deterministic structural operational semantics

- Sequential imperative language

$$\frac{\langle \pi, S'' \rangle \longrightarrow S \qquad I : S' \longrightarrow S''}{\langle I; \pi, S' \rangle \longrightarrow S}$$

- Parallel composition

$$\frac{\langle \pi', S' \rangle \longrightarrow \bar{S}' \qquad \langle \pi'', S'' \rangle \longrightarrow \bar{S}''}{\langle \pi' | \pi'', S' \circ S'' \rangle \longrightarrow \bar{S}' \circ \bar{S}''}$$

- Spontaneous transitions may occurr any time during execution

$$\frac{\langle \pi, S \rangle \longrightarrow S' \qquad S' \longrightarrow S''}{\langle \pi, S \rangle \longrightarrow S''}$$

Fellermann & Cardelli, *Interface* 2014 (in press)

Chemtainer Calculus: Programming

Program set $\ni$ **Program**

Π_{min} Π_{wrap} Π_{burst}

Instruction
Instruction
Instruction
Instruction

I_{min} I_{wrap} I_{burst}

Instruction sets $\ni$ $\boxed{\text{Instruction}}$

Instruction
Instruction

State n $\xrightarrow{\text{Transitions}}$ States n+1

Initial state $\xrightarrow{\text{Program Set}}$ $\omega^+(\Pi_X)$ Constructive closure

$$\omega^+(\Pi_{\mathbf{min}}) \subset \omega^+(\Pi_{\mathbf{wrap}}) \subset \omega^+(\Pi_{\mathbf{burst}}) = L(G_S)$$

Constructive proof.

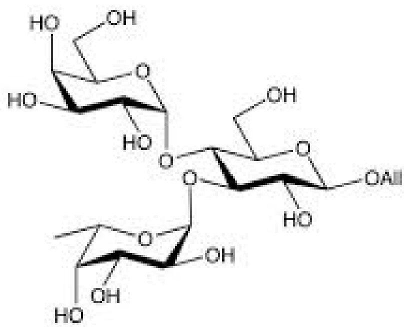
We can *automatically derive programs* to build any given target state.

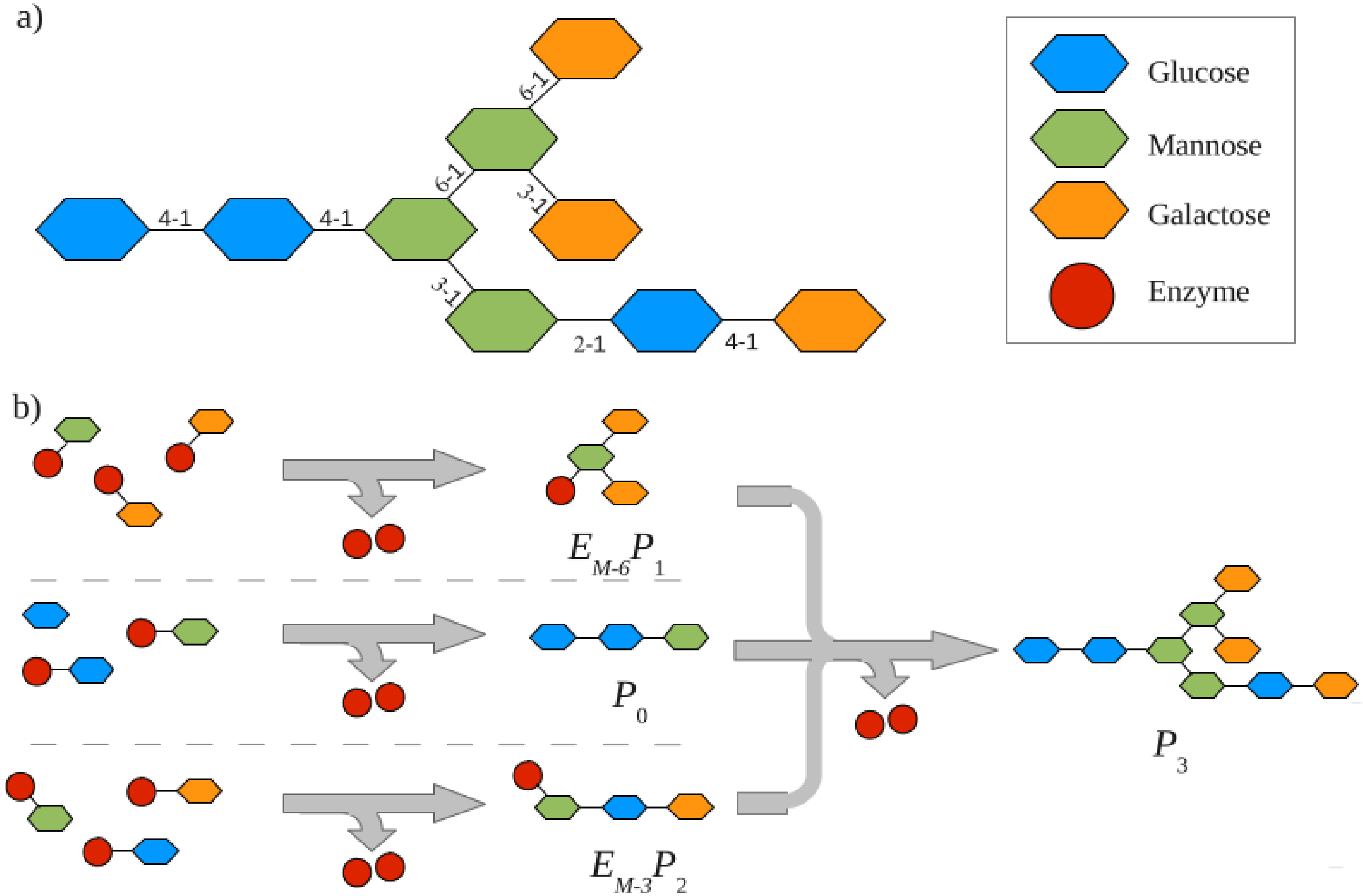
Fellermann & Cardelli, *Interface* 2014 (in press)

Branches oligo-saccharides (e.g. antibodies)

Limited number of monomers and linking sites

Combinatorial explosion of targets – and side reactions :-(





Weyland, Füchslin, Sorek, Lancet, Fellermann, Rasmussen, *Comp. Math. Meht. Med.* 2013

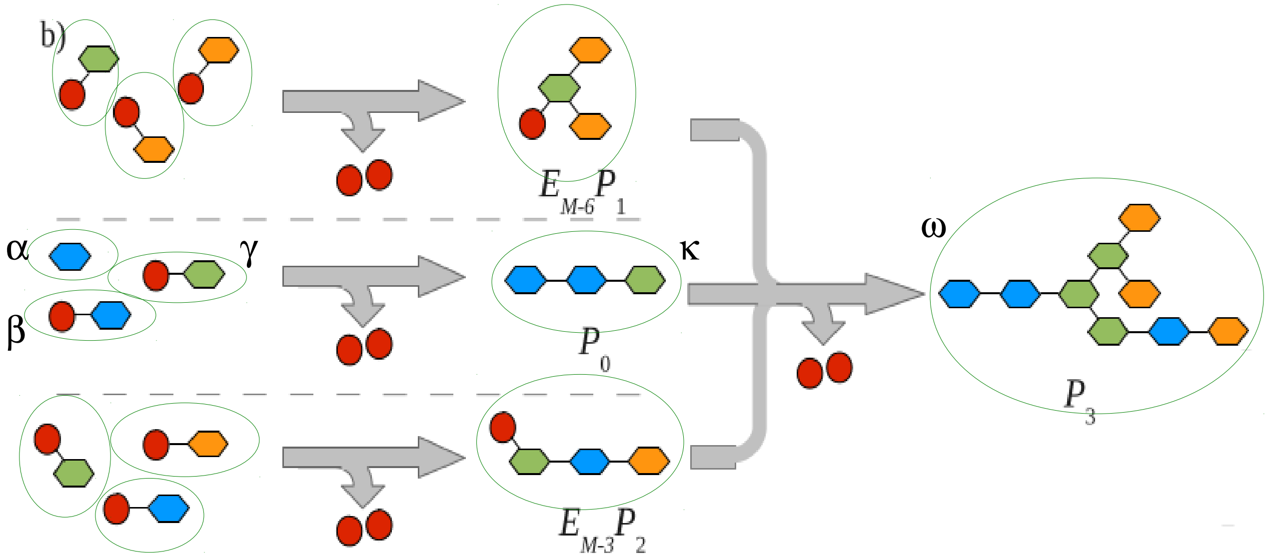
Chemtainer Calculus: Programmable Synthesis

Reaction cascades

$$P_i + M_i E^*_{x_i} \longrightarrow P_{i+1} + E_{x_i}$$

are encoded in DNA gates:

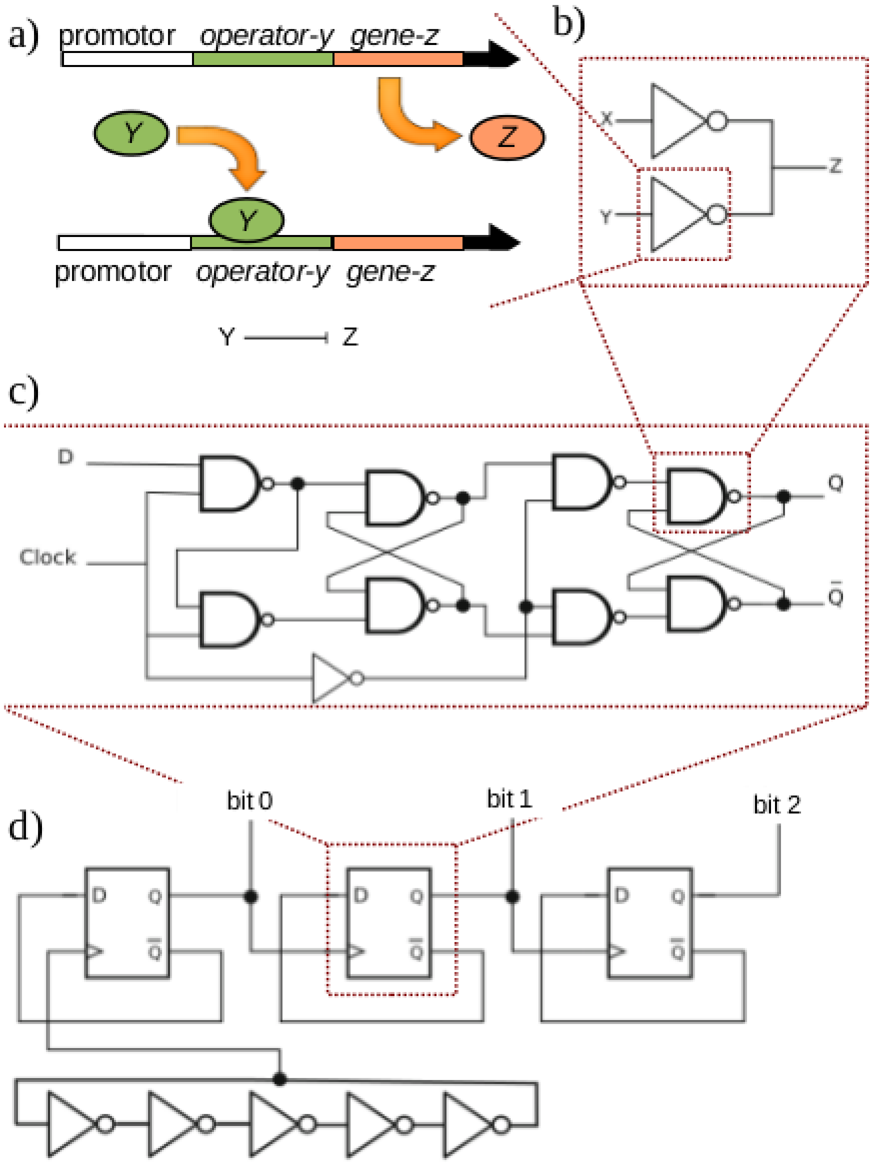
$$\begin{aligned}
(\alpha + \beta + \gamma) &\rhd \kappa \\
(\delta + \epsilon + \zeta) &\rhd \lambda \\
(\eta + \theta + \iota) &\rhd \mu \\
(\kappa + \lambda + \mu) &\rhd \omega
\end{aligned}$$



DNA computing reports fusion-induced chemical reactions on the chemtainer surface:

$$\begin{array}{ll}
 & x_0 : (\alpha+\beta+\gamma) \rhd \kappa \llparenthesis\ \rrparenthesis \circ\ x_S : \alpha \llparenthesis \mathrm{Gal} \rrparenthesis + \beta \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Gal} \rrparenthesis + \gamma \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Man} \rrparenthesis \\
\mathbf{move}(\alpha, x_S, x_0) & x_0 : (\alpha+\beta+\gamma) \rhd \kappa \llparenthesis\ \rrparenthesis + \alpha \llparenthesis \mathrm{Gal} \rrparenthesis \circ\ x_S : \beta \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Gal} \rrparenthesis + \gamma \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Man} \rrparenthesis \\
\mathbf{move}(\beta, x_S, x_0) & x_0 : (\alpha+\beta+\gamma) \rhd \kappa \llparenthesis\ \rrparenthesis + \alpha \llparenthesis \mathrm{Gal} \rrparenthesis + \beta \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Gal} \rrparenthesis \circ\ x_S : \gamma \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Man} \rrparenthesis \\
\mathbf{move}(\gamma, x_S, x_0) & x_0 : (\alpha+\beta+\gamma) \rhd \kappa \llparenthesis\ \rrparenthesis + \alpha \llparenthesis \mathrm{Gal} \rrparenthesis + \beta \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Gal} \rrparenthesis + \gamma \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Man} \rrparenthesis \\
\mathbf{fuse}(x_0) & x_0 : (\alpha + (\alpha+\beta+\gamma) \rhd \kappa) \llparenthesis \mathrm{Gal} \rrparenthesis + \beta \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Gal} \rrparenthesis + \gamma \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Man} \rrparenthesis \\
\mathbf{fuse}(x_0) & x_0 : (\alpha + \beta + (\alpha+\beta+\gamma) \rhd \kappa) \llparenthesis \mathrm{Gal} + \mathrm{E}^*_{\text{Gal-4}}\mathrm{Gal} \rrparenthesis + \gamma \llparenthesis \mathrm{E}^*_{\text{Gal-4}}\mathrm{Man} \rrparenthesis \\
\mathbf{fuse}(x_0) & x_0 : (\alpha + \beta + \gamma + (\alpha+\beta+\gamma) \rhd \kappa) \llparenthesis \mathrm{Gal} + \mathrm{E}^*_{\text{Gal-4}}\mathrm{Gal} + \mathrm{E}^*_{\text{Gal-4}}\mathrm{Man} \rrparenthesis \\
 & x_0 : \kappa \llparenthesis P_0 + 2\,\mathrm{E}_{\text{Gal-4}} \rrparenthesis \\
\mathbf{move}(\kappa, x_0, x_S) & x_S : \kappa \llparenthesis P_0 + 2\,\mathrm{E}_{\text{Gal-4}} \rrparenthesis
\end{array}$$

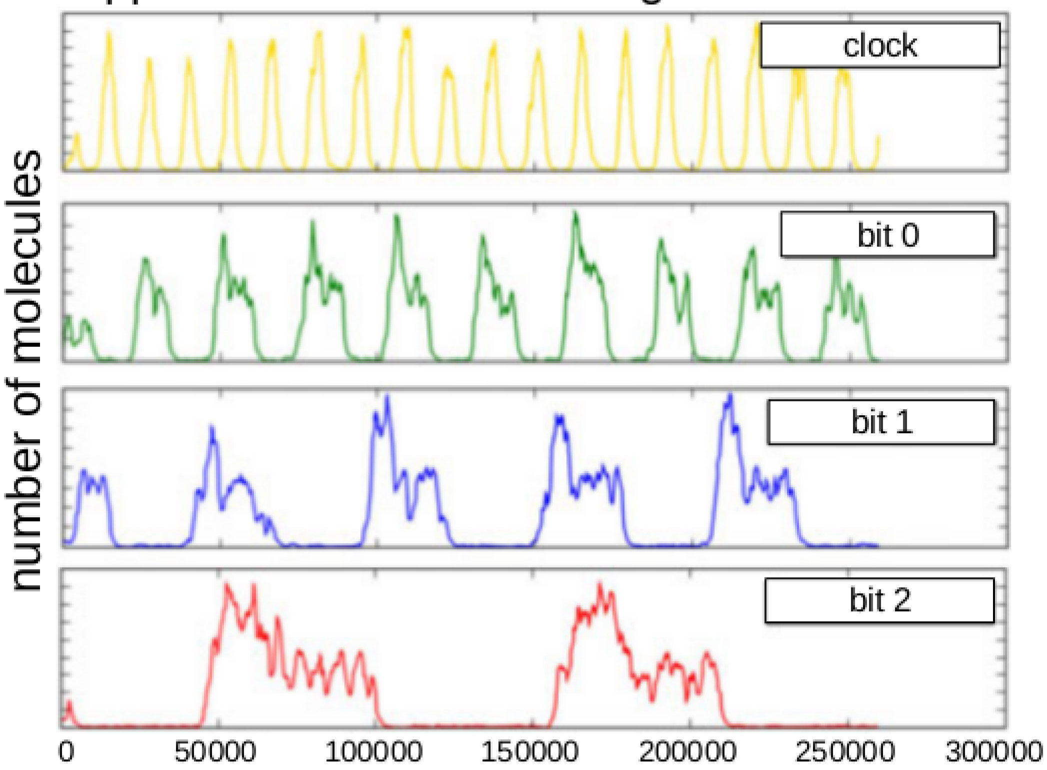
Distributed Molecular Computing



Complex circuits builts from gene regulatory networks

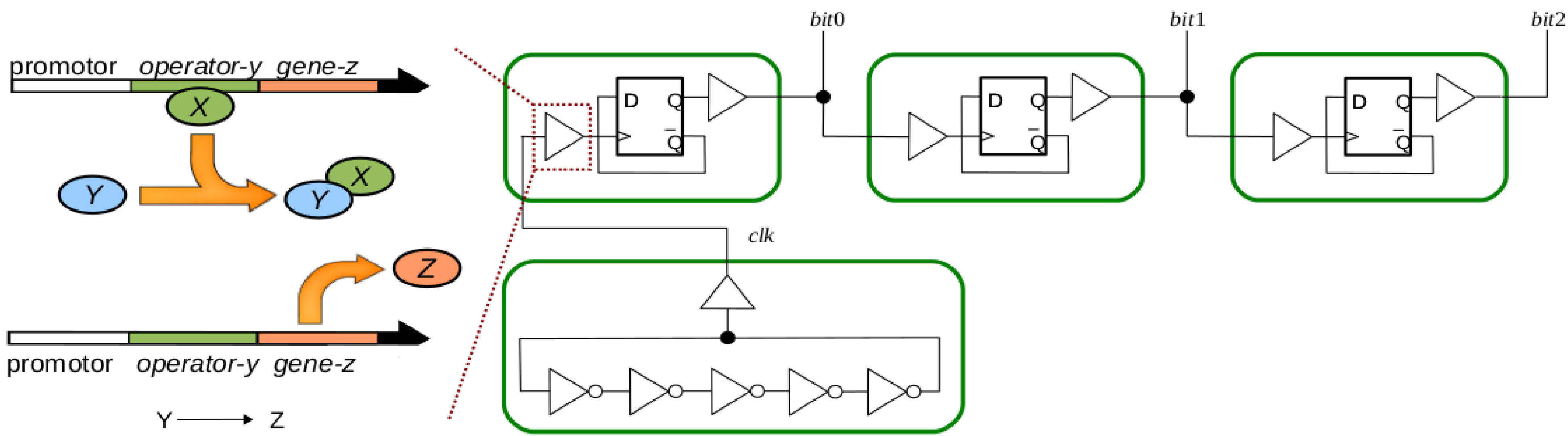
Smaldon et al. *Syst. Synth. Biol.* **4**(3), 2010

Ripple counter with 5-bit ring oscillator clock



Distributed Molecular Computing

Using chemtainer calculus, we compile and wire a distributed implementation from few standard parts.



Example wiring of a flipflop by fusing transducers:

$$ {}^{\alpha^\top+\beta^\top+\gamma^\top}\llparenthesis\ \rrparenthesis + {}^{\alpha}\llparenthesis\, F(x,y)\,\rrparenthesis + {}^{\beta}\llparenthesis\, T(clk,x)\,\rrparenthesis + {}^{\gamma}\llparenthesis\, T(y,bit0)\,\rrparenthesis $$

$$ \longrightarrow {}^{\alpha^{||}+\beta^{||}+\gamma^{||}}\llparenthesis\, F(clk,bit0)\,\rrparenthesis $$

Fellermann, Krasnogor, CiE proceedings 2014 (accepted)
Fellermann, Hadorn, Füchslin, Krasnogor, *JETC* 2014 (submitted)

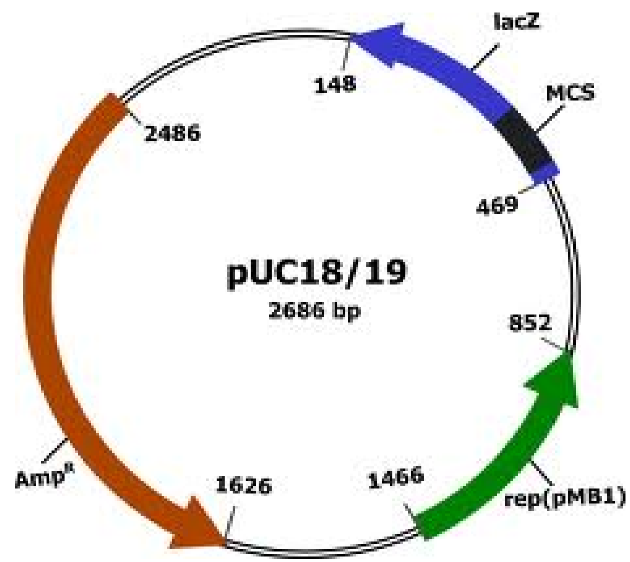
Calculus for DNA manipulation

Formal language to denote domains on plasmids/chromosome:

$$
\begin{aligned}
\mathsf{STATE} &:= 0 \mid \mathsf{STATE} + \mathsf{STATE} \mid \mathsf{DNA} \mid \mathsf{RNA} \mid \mathsf{PROT} \\
\mathsf{DNA} &:= [\mathsf{DSEQ}] \mid < \mathsf{DSEQ} > \\
\mathsf{DSEQ} &:= \epsilon \mid \mathsf{DSEQ.DSEQ} \mid \mathsf{DSEQ\hat{}} \mid \{\mathsf{RNA}\}\mathsf{DSEQ} \mid \mathsf{DOM} \\
\mathsf{RNA} &:= [\mathsf{SEQ}] \mid < \mathsf{SEQ} > \\
\mathsf{SEQ} &:= \epsilon \mid \mathsf{SEQ.SEQ} \mid \{\}\mathsf{SEQ} \mid \mathsf{DOM} \\
\mathsf{DOM} &:= \mathsf{TYPE} : \mathsf{IDENT}
\end{aligned}
$$

Domain can be promoters, operators, terminators, introns, restriction sites, etc.

```
pUC19 = <P:x.G:lacZ.G:AmpR.T:y.P:z.pMB1.T:y>
```



Calculus for DNA manipulation

Axiomatic transitions for

- Translation

$$\texttt{P:}x.s \longrightarrow \texttt{P:}x.\{\epsilon\}s$$
$$\{r\}\texttt{O:}x.s \longrightarrow \texttt{O:}x.\{r\}s$$
$$\{r\}\texttt{T:}x \longrightarrow \texttt{T:}x + [r]$$

- Transcription

$$\texttt{B:}x.r \longrightarrow \texttt{B:}x.\{\}r$$
$$\{\}\texttt{G:}x.r \longrightarrow \texttt{G:}x.\{\}r + x$$

- Splicing

$$r.\texttt{I:}x.r' \longrightarrow r.r'$$

- Operon regulation

$$x + [s.\texttt{O:}x.s'] \longrightarrow [s.\emptyset\texttt{:}x.s']$$

- Restriction
- Recombination

- Transposons, etc.

Conclusion

- Physical simulations give detailed insight into biological systems
  - prediction
  - verification
  - explaining
  - design of experiments
  - design of systems

- Formal calculi allow to apply physical simulations to complex states
  - applicable to unbounded state spaces
  - capture logical organization
  - allow for analytic treatment (proofs!)

Thanks for you attention!

Questions?