Tractable Kinetics of RNA–Ligand Interaction

On the Folding Kinetics of Riboswitches

Felix Kühnl

Bioinformatics, Leipzig University
felix@bioinf.uni-leipzig.de

RNA structure, design and interaction with proteins
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Simulating RNA Folding

RNA sequence $\rightarrow$ (secondary) structure(s) $\rightarrow$ function

Types of folding simulations:

- thermodynamic:
  - structure probabilities *in equilibrium*
  - fast (use e. g. `ViennaRNA`)
- kinetic:
  - yields distribution of structures over time
  - computationally harder, size restrictions (use e. g. `treekin`)

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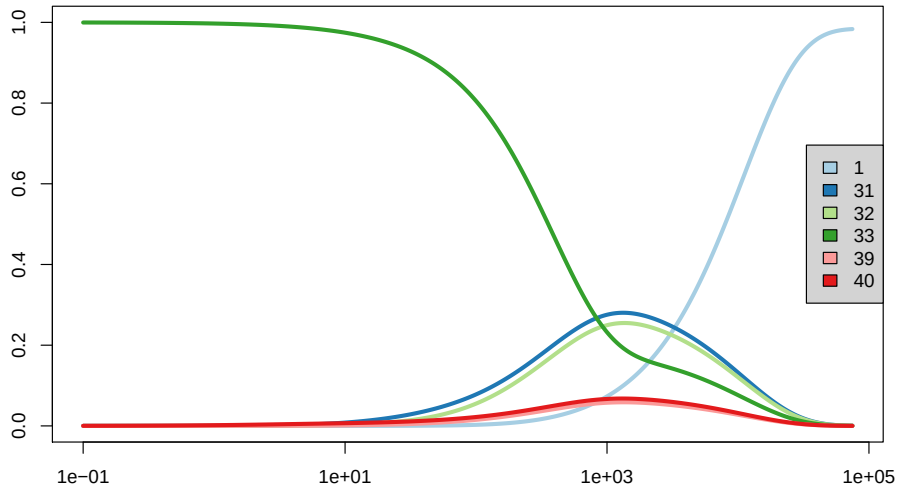
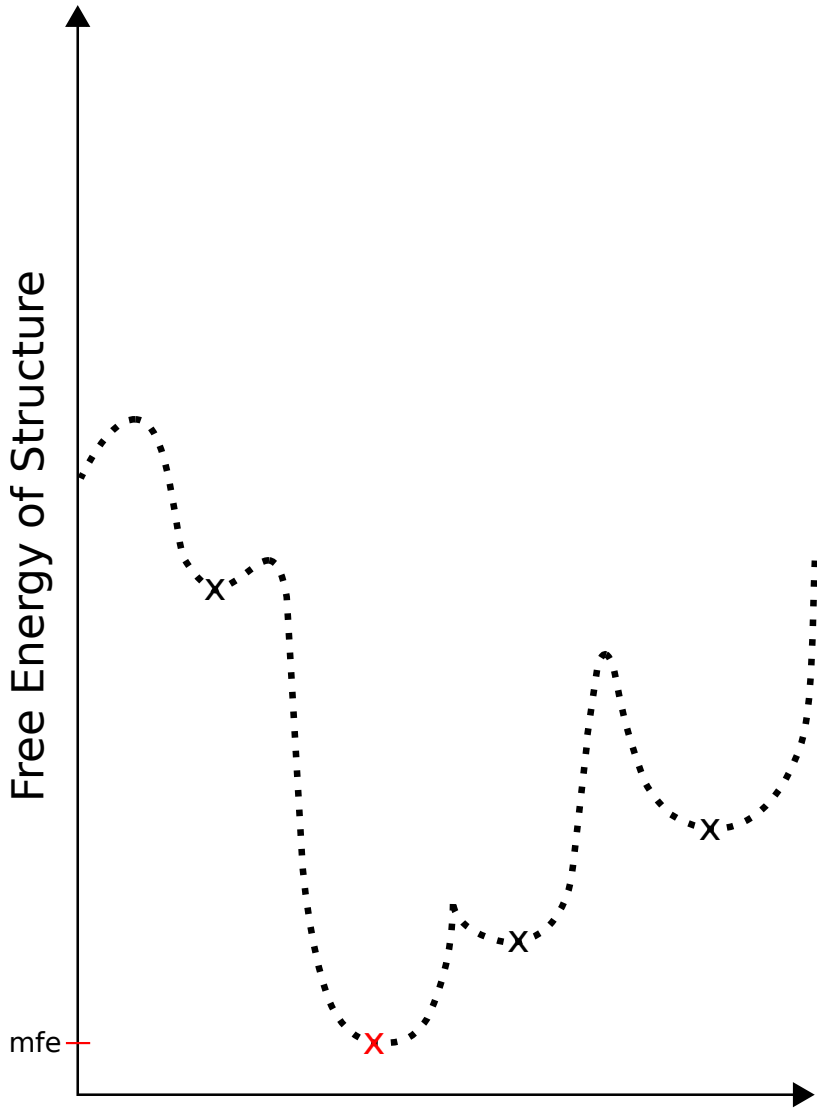


RNA Folding Kinetics: Energy Landscapes

- goal: model folding process
- structure $\varphi \mapsto$ energy value E_φ (TURNER energy model)
- *transitions* between structures
  - insertion, deletion, shift
  - only if distance is 1
  - rate constants $r_{\chi \leftarrow \varphi}(E_\varphi, E_\chi)$
- $\#_{\text{structures}}(s) \in \mathcal{O}(2.62^{|s|})$
  - coarse graining necessary
  - *gradient basins* as macrostates
  - *macrorate* constants $r_{j \leftarrow i}$



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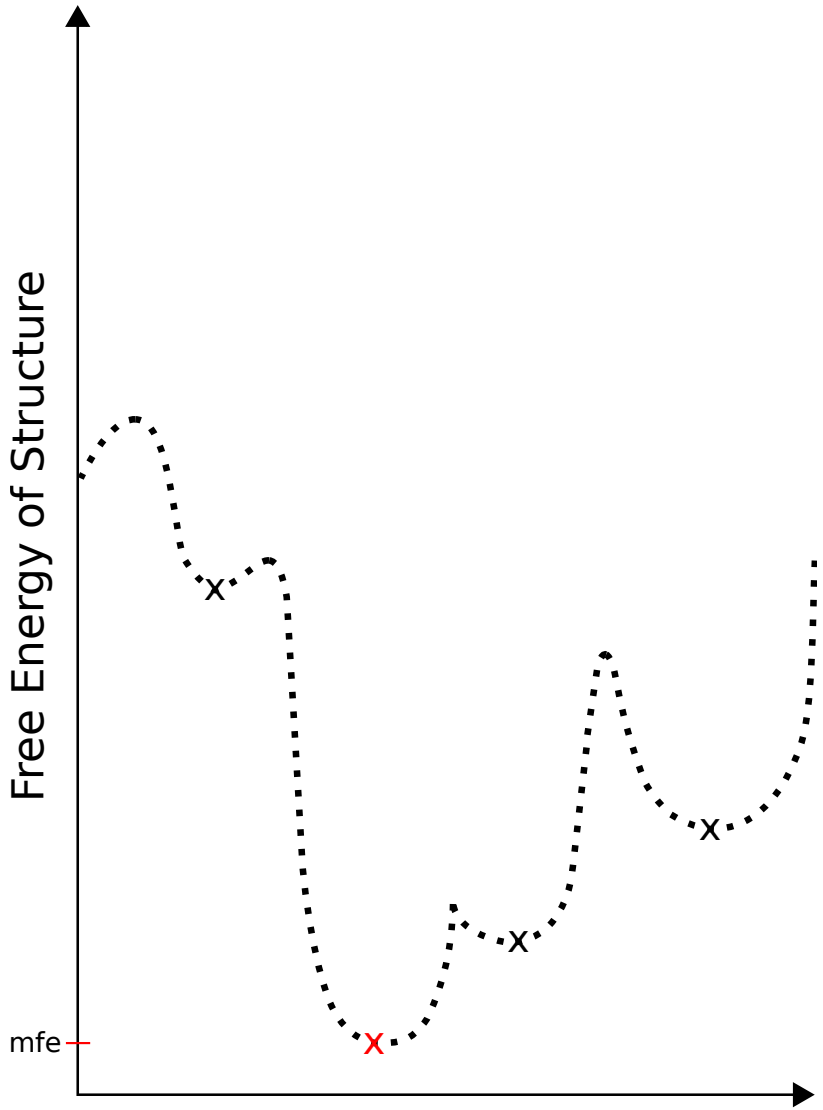

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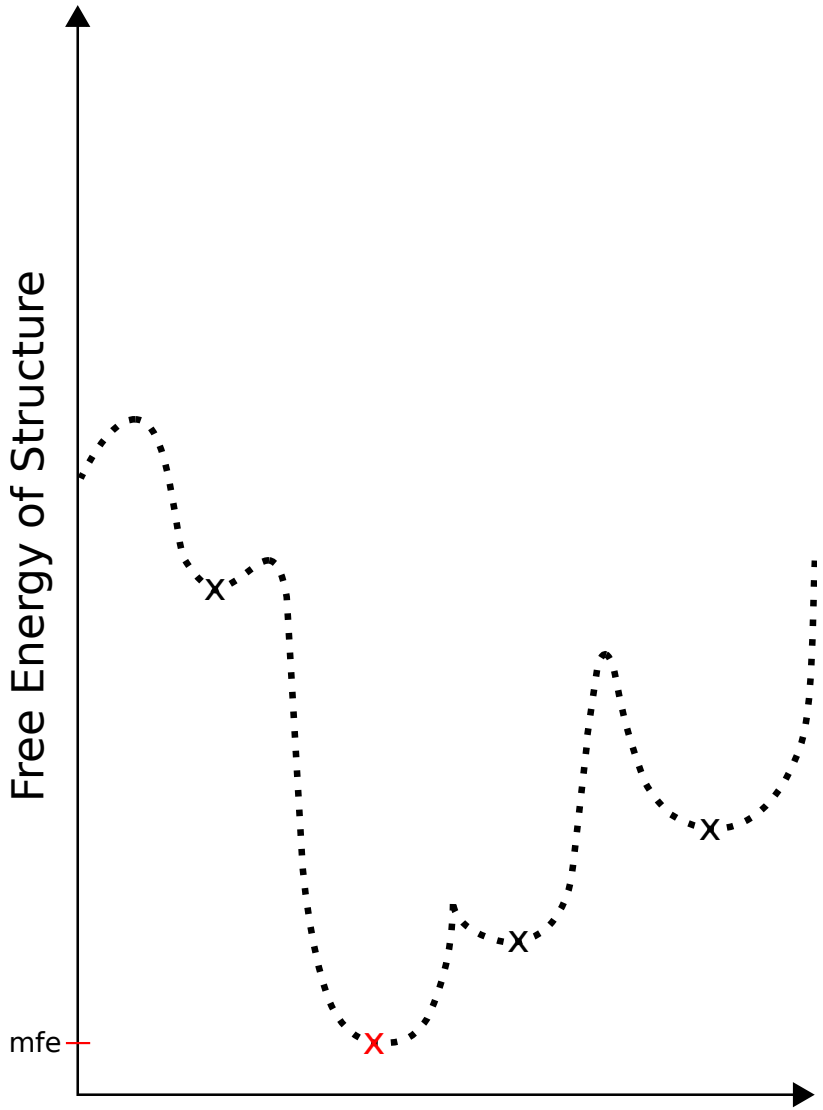

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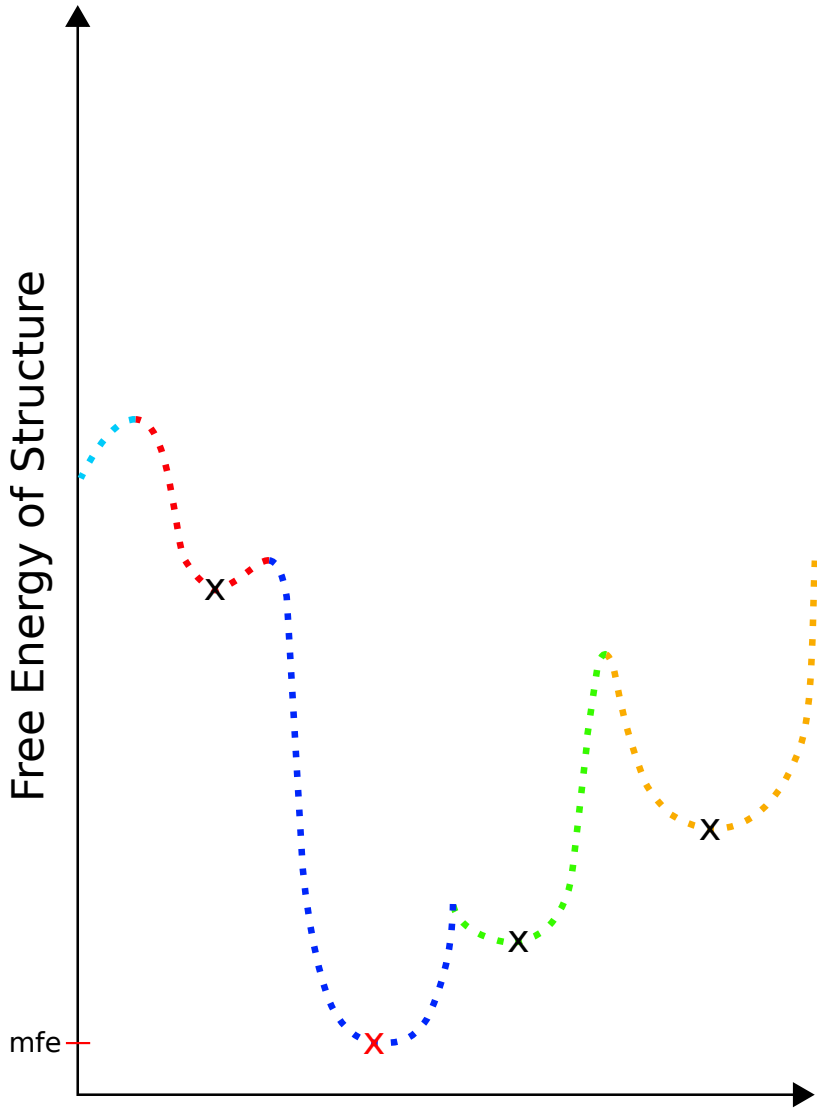

Rate Coefficients

- general form (Arrhenius equation):

$$r_{\chi \leftarrow \varphi} = a_{\mathrm{RNA}} \cdot \exp\left(-\frac{E_a(\chi \leftarrow \varphi)}{RT}\right)$$

  for RNA structures φ and χ
  (E_a – activation energy R – gas constant T – temperature)
- a_{RNA} – "collision rate" (from experiment)
- activation energy (Metropolis rule):

$$E_a(\chi \leftarrow \varphi) = \begin{cases} \max(E_\varphi, E_\chi) - E_\varphi & \text{if } \varphi \text{ and } \chi \text{ are neighbors} \\ \infty & \text{else} \end{cases}$$

- macrorates for basins i, j (Wolfinger et al.):

$$r_{j \leftarrow i} = \sum_{\substack{\varphi \in i \\ \chi \in j}} \underbrace{\Pr[\,\varphi \mid i\,]}_{= \frac{Z[\varphi]}{Z[i]}} \cdot r_{\chi \leftarrow \varphi}$$

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Riboswitches . . .

- . . . are regulatory RNA elements in 5′-UTR of genes
- regulate transcription or translation depending on a *ligand*
- consist of . . .
  - an *aptamer*: contains *binding pocket* for ligand L
  - an *actuator*, e. g. a *terminator hairpin*
- binding of ligand yields *energy bonus* $\theta_L < 0$
  $\Longrightarrow E_{L\varphi} = E_\varphi + \theta_L$
- time-critical interaction with RNA polymerase / ribosome

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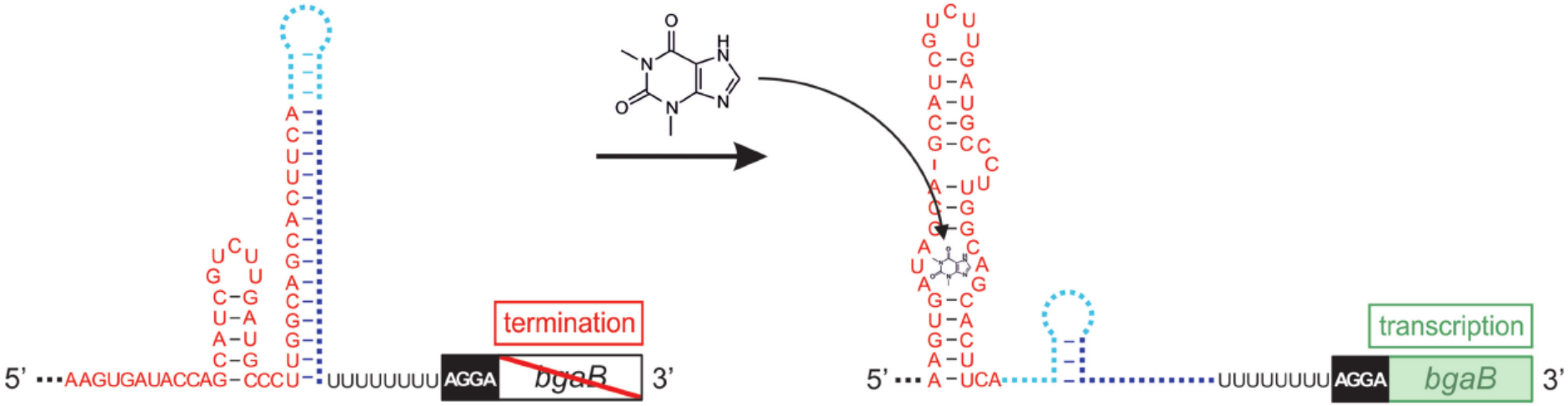

transcriptional, theophylline-controlled ON switch (figure: Wachsmuth et al. 2012)

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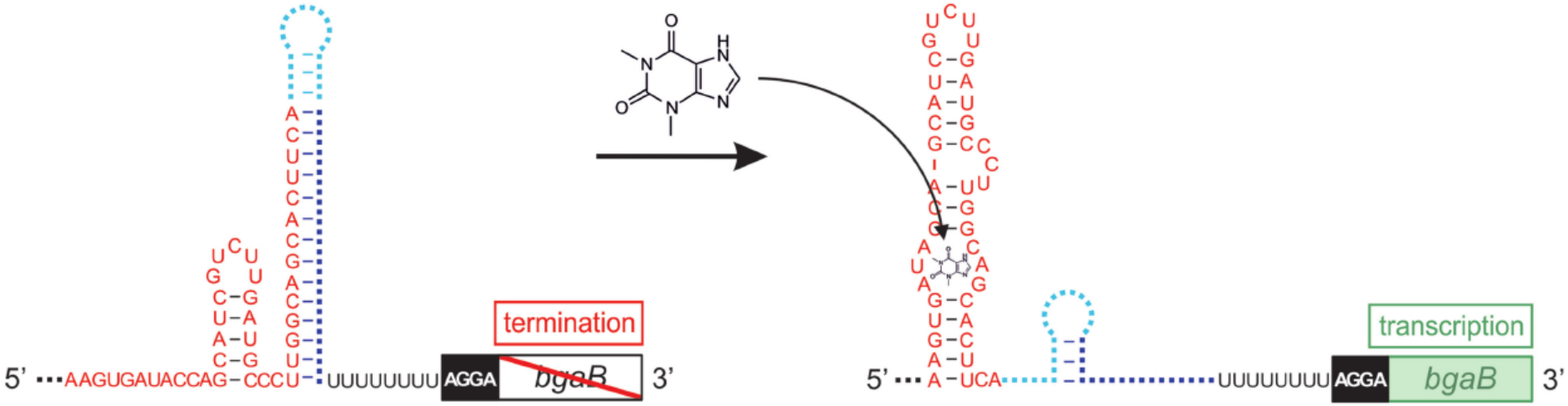

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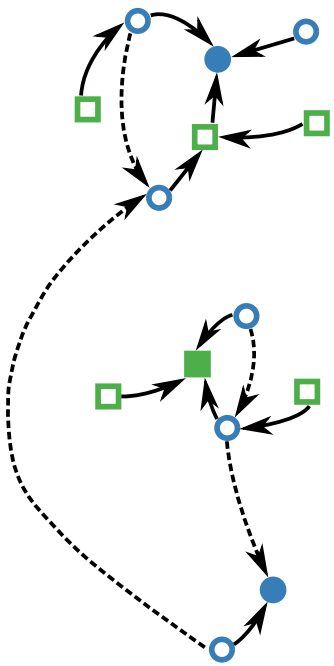

monomers

dimers

$R_i \rightleftharpoons R_j$ $L + R_i \rightleftharpoons LR_j$ $LR_i \rightleftharpoons LR_j$

(R – RNA L – ligand LR – dimer)

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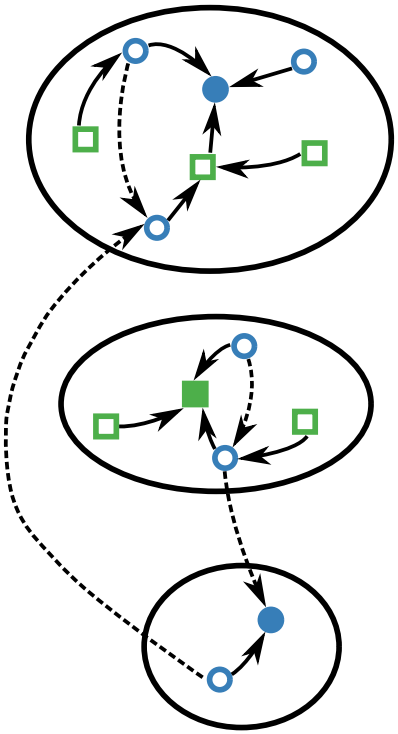

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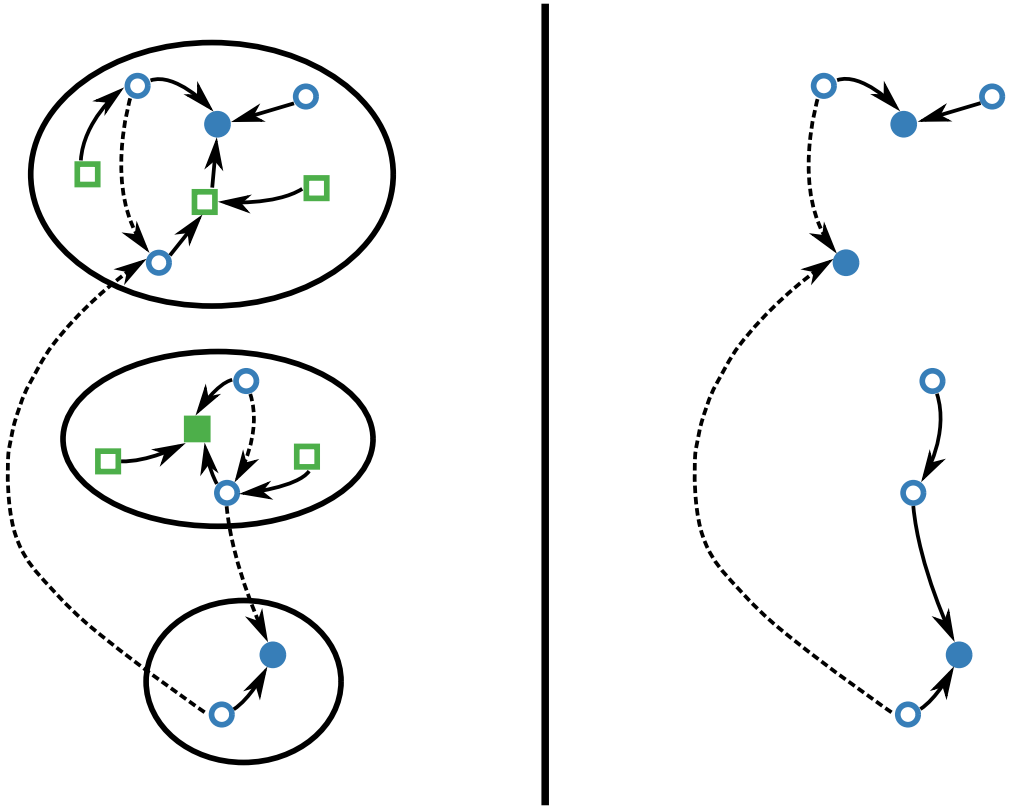

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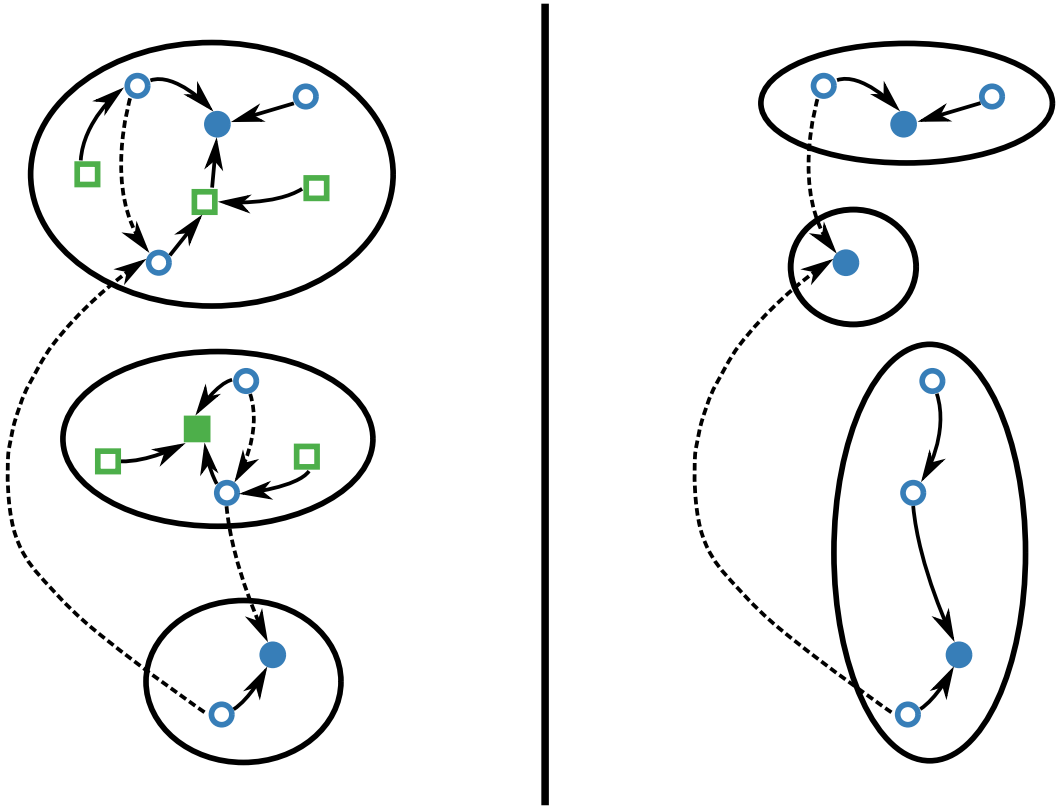

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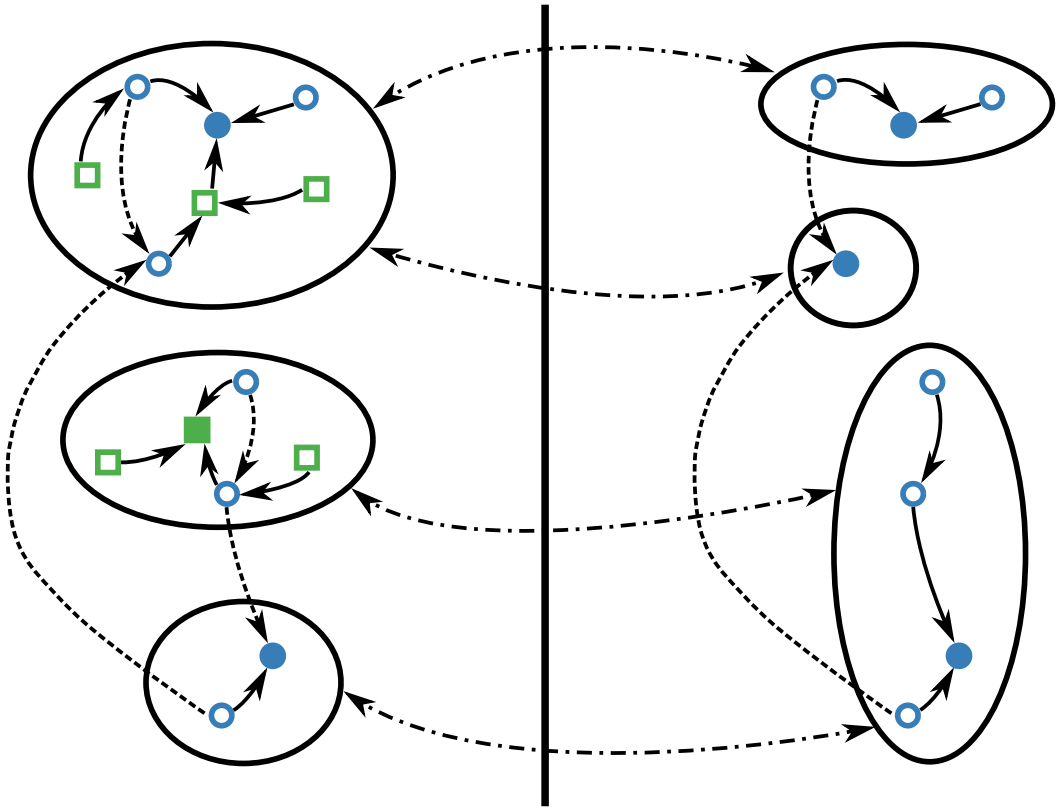

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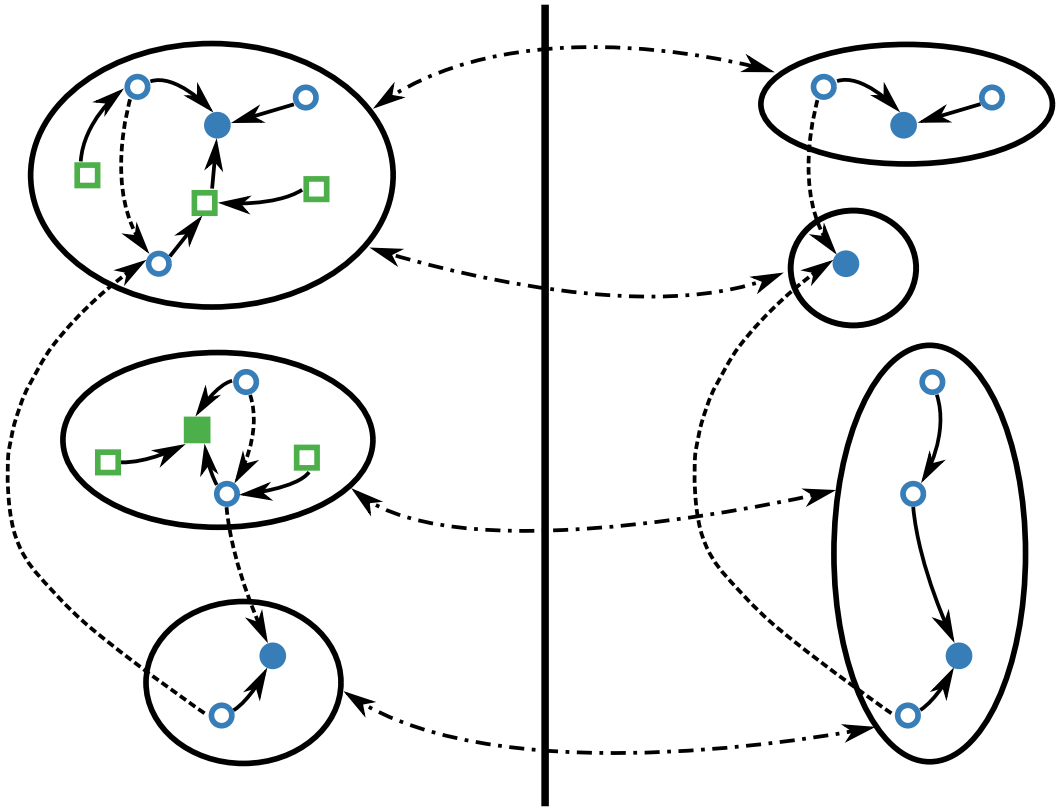

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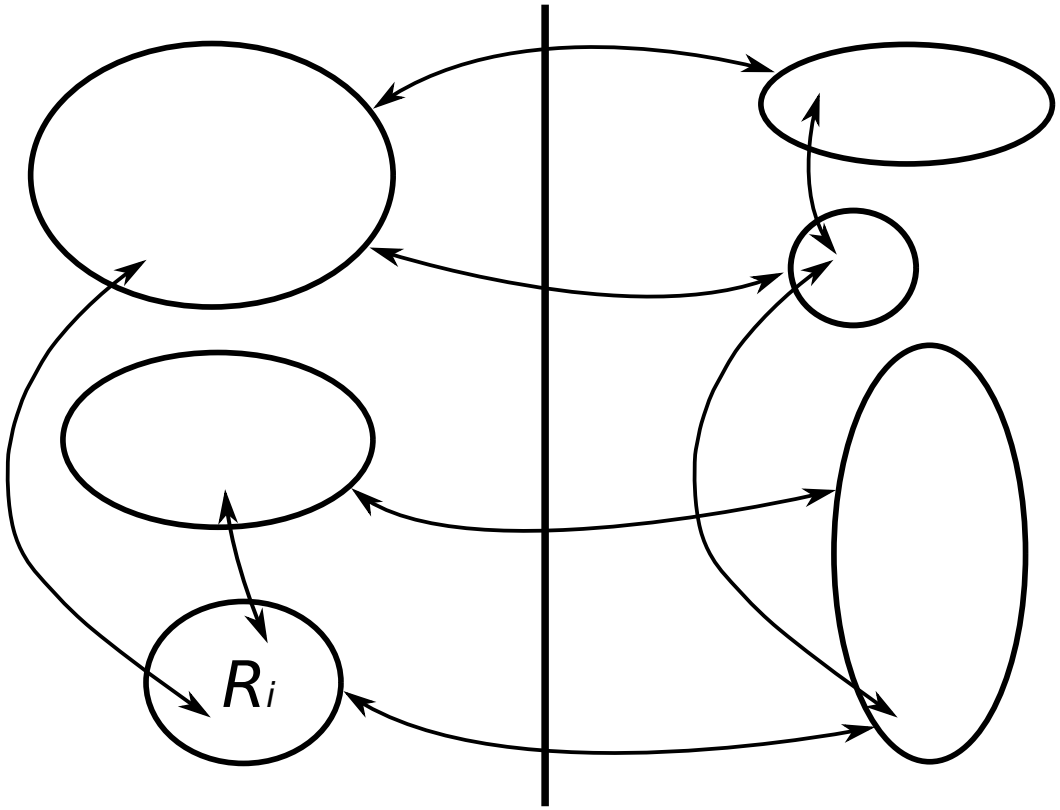

By first-order rate laws: (assumption: $[L] = l_0 = \text{const}$)

$$[\dot{R}_i] = \sum_{\substack{1 \le k \le n \\ k \neq i}} r_{i \leftarrow k}[R_k] + \sum_{1 \le k \le m} r_{i \leftarrow Lk}[LR_k] - \sum_{\substack{1 \le k \le n \\ k \neq i}} r_{k \leftarrow i}[R_i] - \sum_{1 \le k \le m} r_{Lk \leftarrow i} \cdot l_0 \cdot [R_i]$$

for n monomer and m dimer basins.

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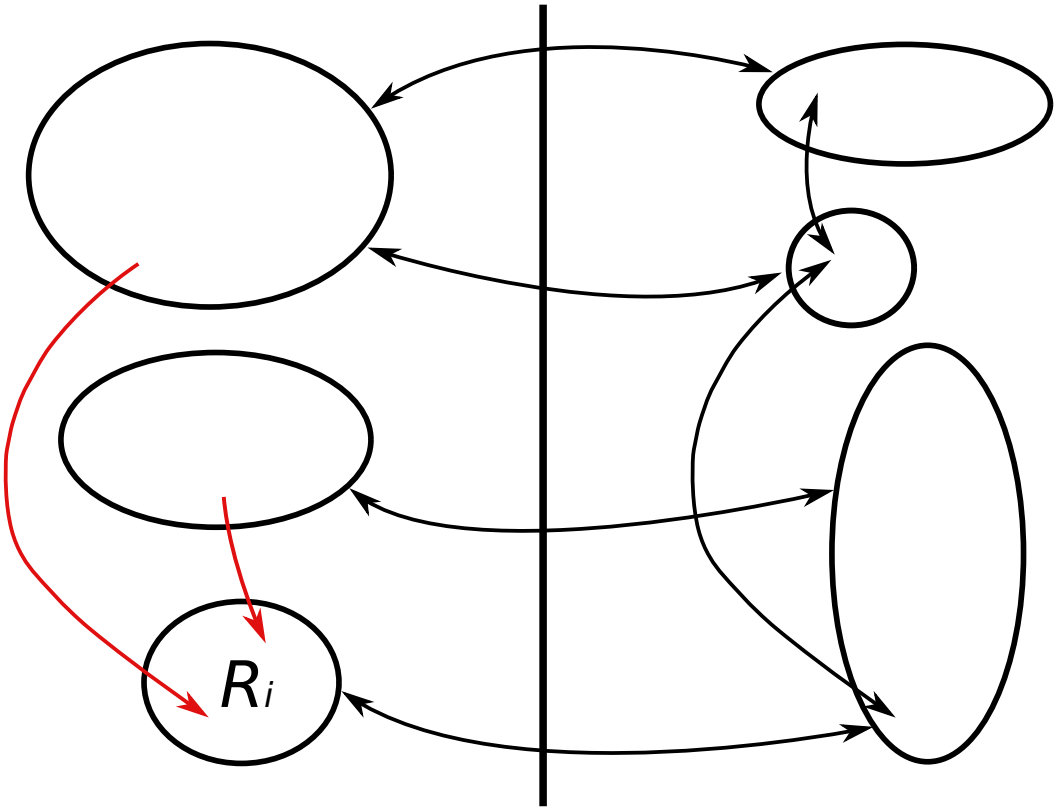

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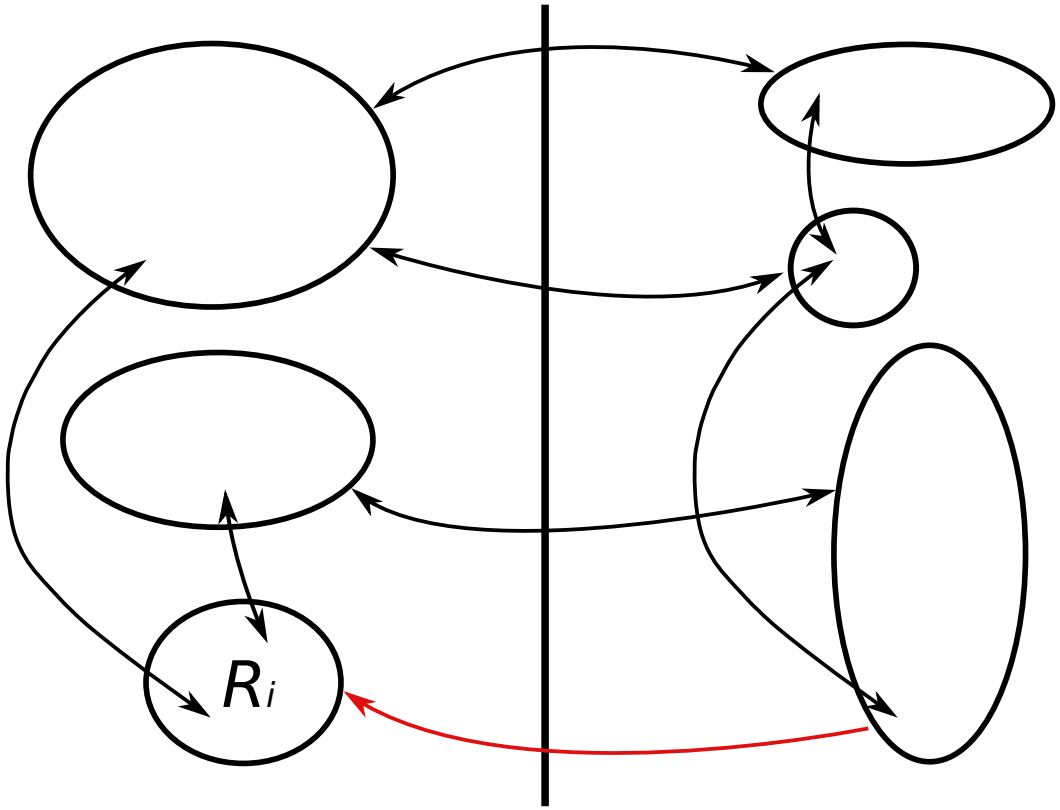

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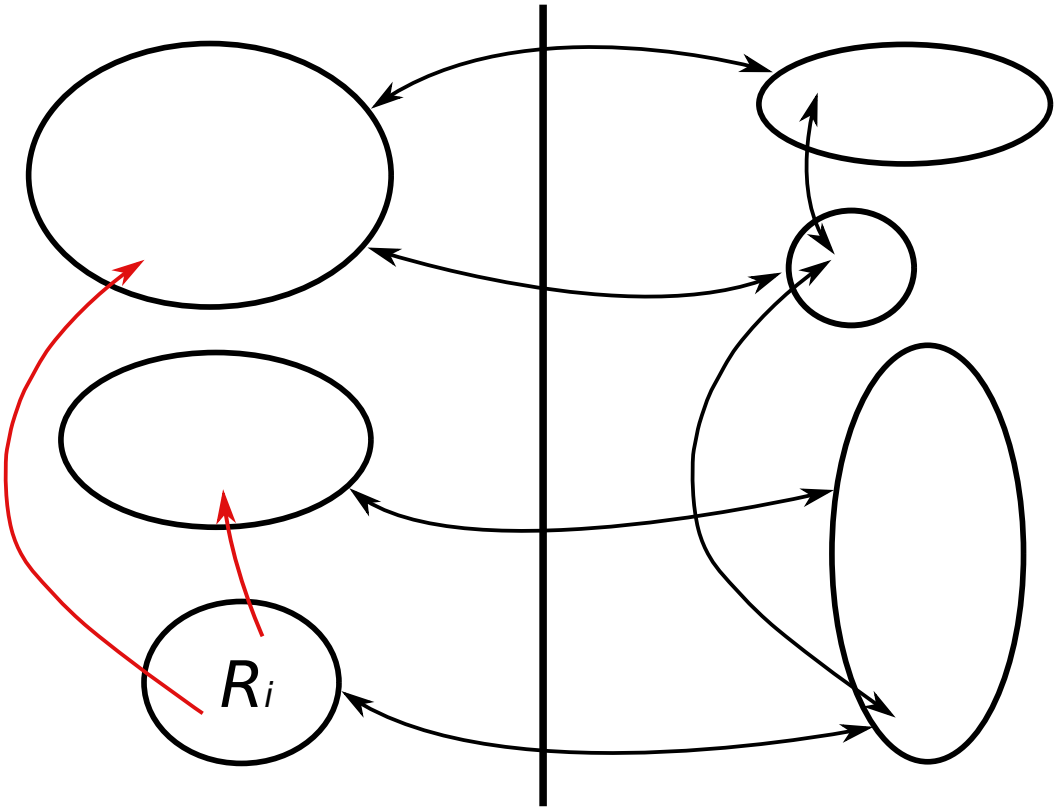

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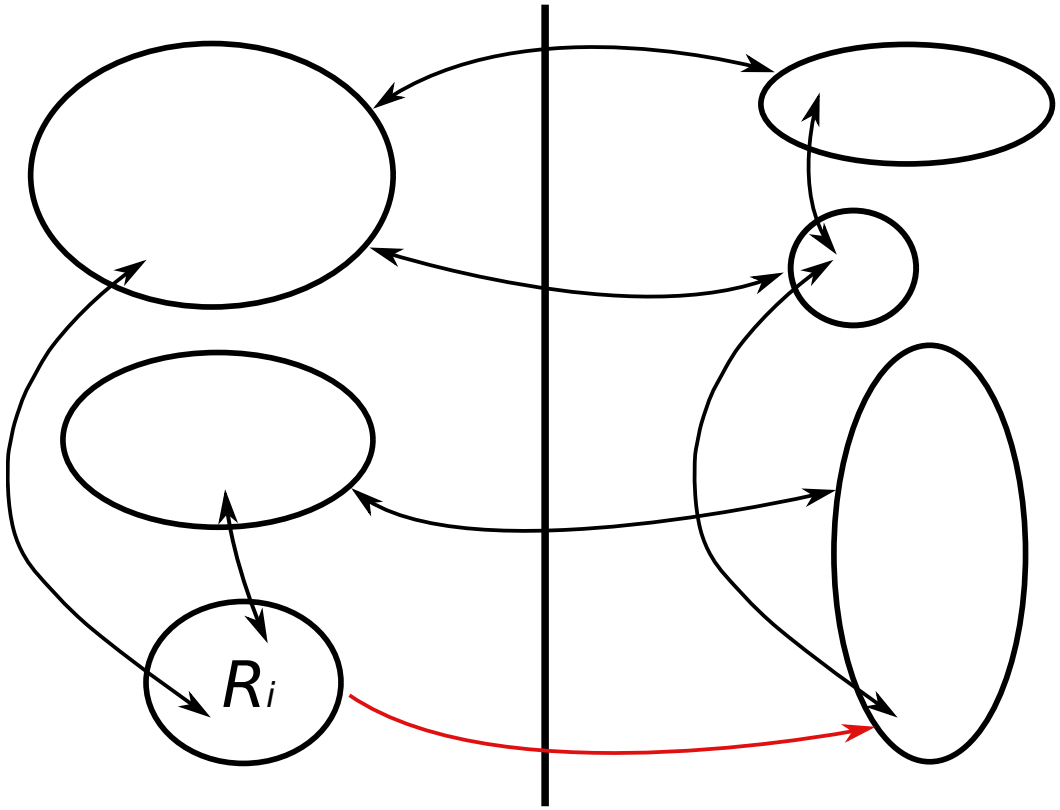

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Modelling the System ctd.

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$$(j = 1, \ldots, m) \qquad [\dot{LR}_j] = + \sum_{1 \le k \le n} r_{Lj \leftarrow k} \cdot l_0 \cdot [R_k] + \sum_{\substack{1 \le k \le m \\ k \neq j}} r_{Lj \leftarrow Lk}[LR_k] - \sum_{1 \le k \le n} r_{k \leftarrow Lj}[LR_j] - \sum_{\substack{1 \le k \le m \\ k \neq j}} r_{Lk \leftarrow Lj}[LR_j].$$

for n monomer and m dimer basins.

Simple, isn't it?

After some clever rewriting ...

$$\dot{x} = R(l_0) \cdot x$$

with ...

- $x = ([R_1], \ldots, [R_n], [LR_1], \ldots, [LR_m])^T$ – vector of concentrations
- $R(l_0)$ – (constant) rate coefficient matrix, more detailed:

$$R(l_0) = \begin{pmatrix} A & C \\ l_0 \cdot D & B \end{pmatrix}$$

where

- A – monomer rate coefficients, $(a_{ji}) = r_{j \leftarrow i}$
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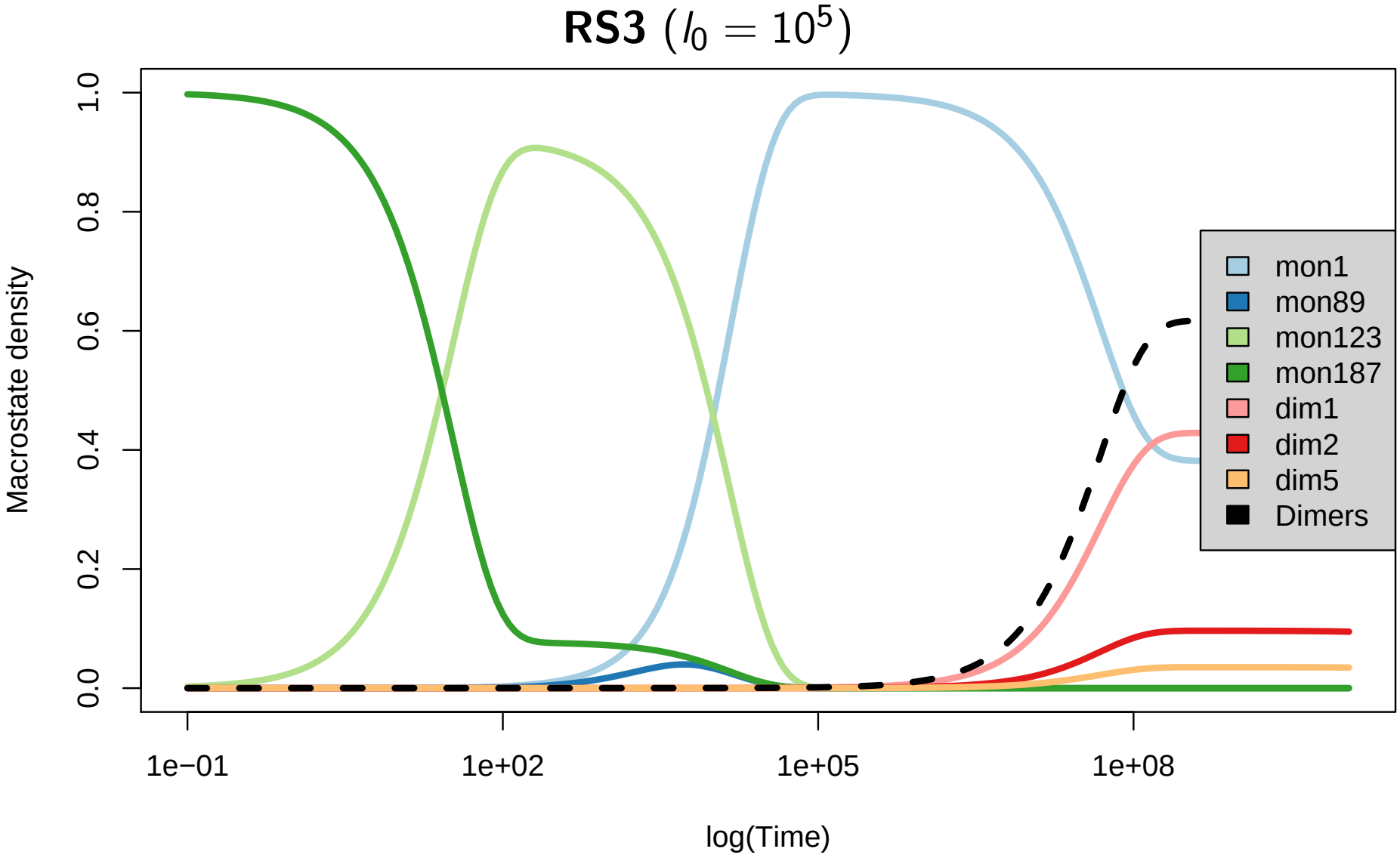
Kinetics of Theophylline Riboswitch RS3

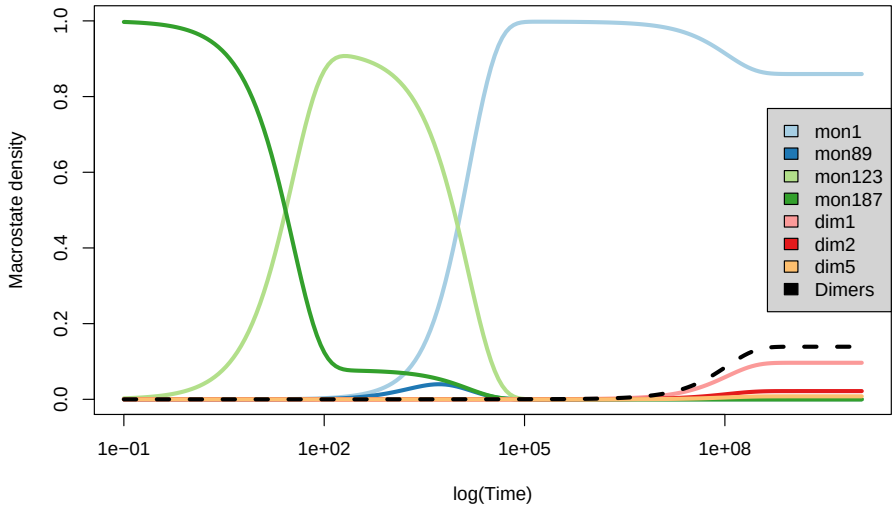
`RNAsubopt` → `barriers` + "monomer↔dimer" rates → `treekin`



Concentration-dependent Behavior

RS3

$I_0 = 10^4$



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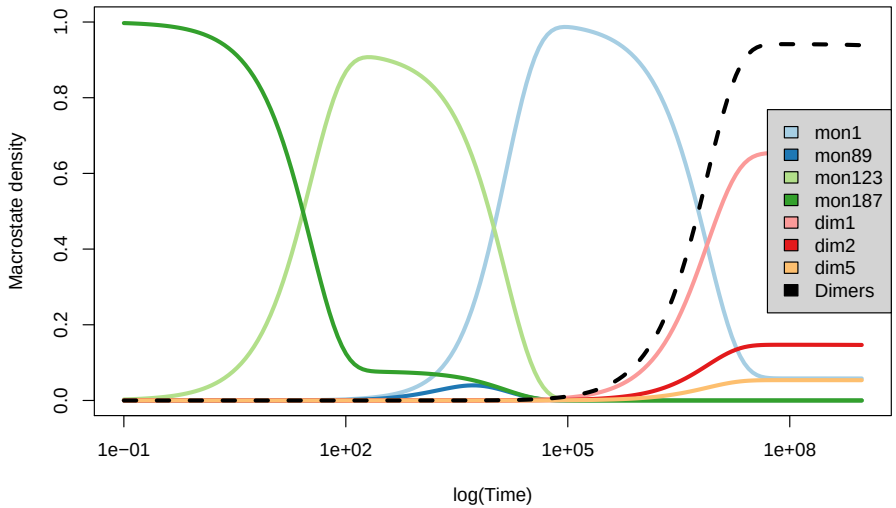

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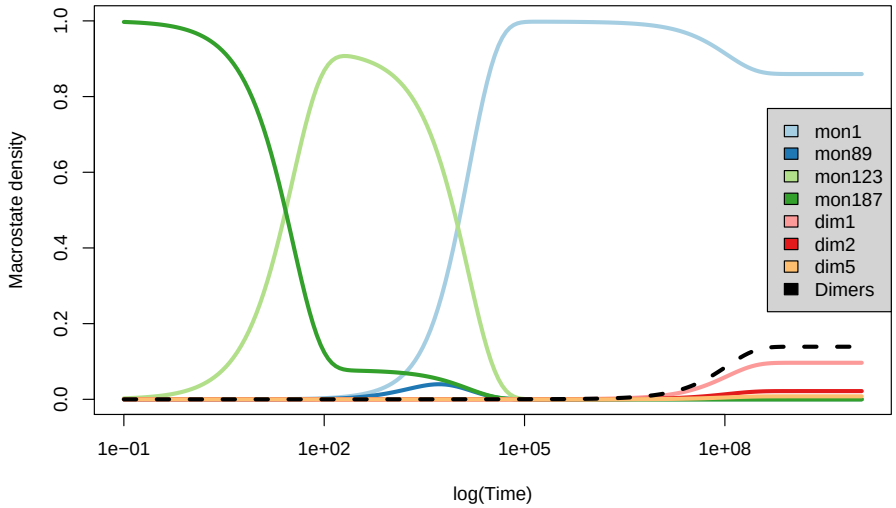
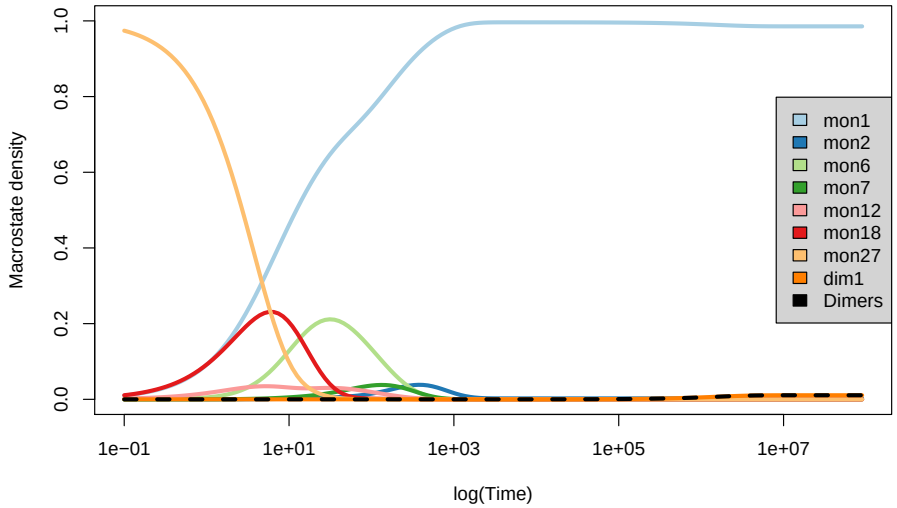


RS3 shortened

$I_0 = 10^{-8}$



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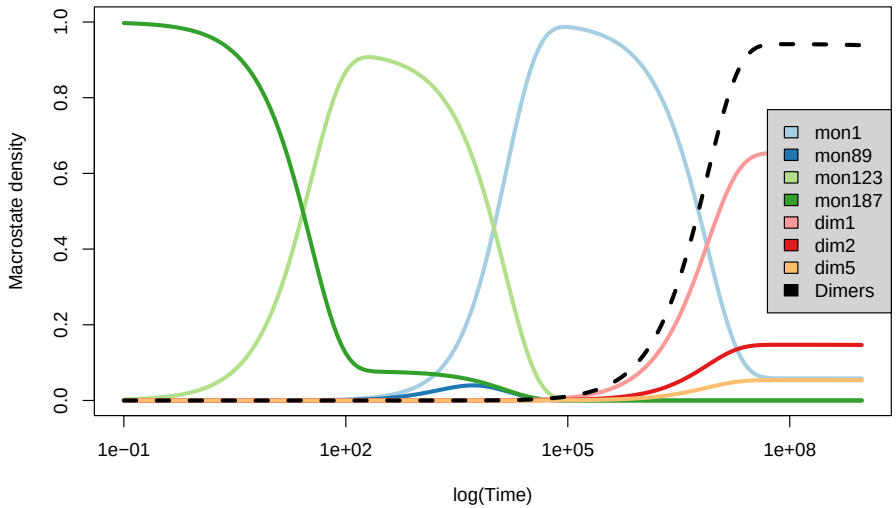

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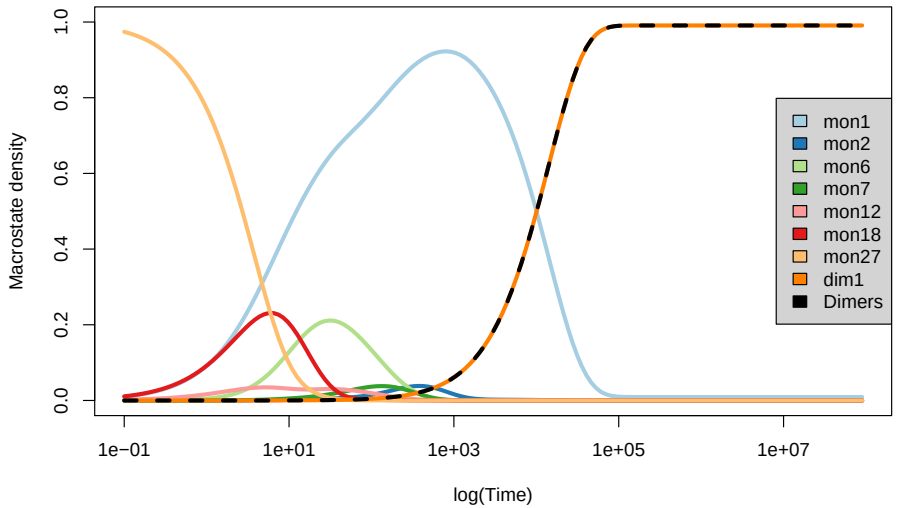

Cotranscriptional Folding

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Applications: cotranscriptional folding, RNA thermometers, ...

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`BarMap-QA`: ready-to-use pipeline utilizing `BarMap` for co-trascriptional folding (quality control statistics, result visualizations, Docker container)

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  - considers (constant) **ligand concentration**
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- enables *in silico* **testing of designed riboswitches**
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Appendix

Additional Material

Rate Coefficients in Riboswitch Landscapes

Lemma

1. *coefficient for $LR_i \to LR_j$ (dimer–dimer rate):*

$$r_{L\chi \leftarrow L\varphi} = r_{\chi \leftarrow \varphi} \quad \Longrightarrow \quad r_{Lj \leftarrow Li} = r_{j \leftarrow i}$$

2. *coefficient for $L + R_i \to LR_j$ (dimerization rate):*

$$r_{L\varphi \leftarrow \varphi} = a_{dim} \quad \Longrightarrow \quad r_{Lj \leftarrow i} = a_{dim} \cdot \frac{Z[i \cap j]}{Z[i]}$$

3. *coefficients for $LR_i \to L + R_j$ (dissociation rate):*

$$r_{\varphi \leftarrow L\varphi} = a_{dim} \cdot \exp\left(\frac{\theta_L}{RT}\right) \Longrightarrow r_{j \leftarrow Li} = a_{dim} \cdot \frac{Z[i \cap j]}{Z[i]} \cdot \exp\left(\frac{\theta_L}{RT}\right)$$

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