Predicting Kinetics of RNA–RNA Interaction

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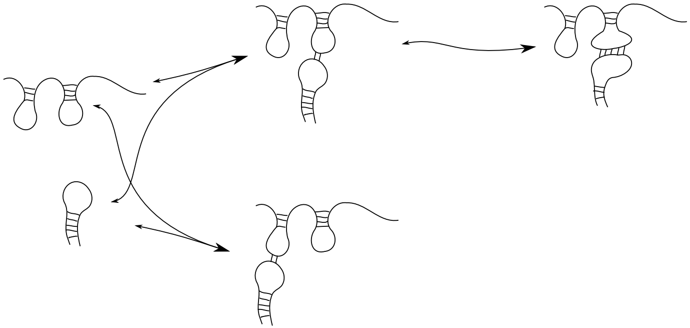
Benasque'22

RNA–RNA interaction is a dynamic process



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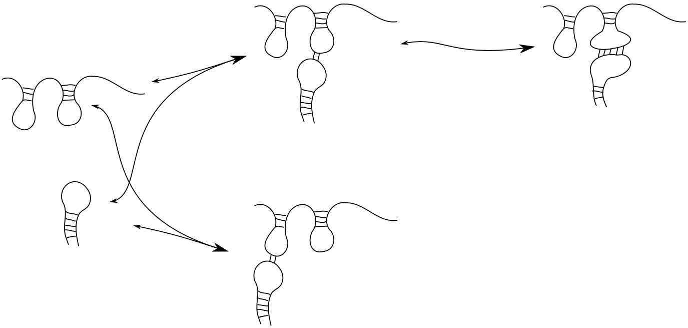


In principle, the interaction process can be studied:

1. *Model* as **Continuous-time Markov Process** (by **States** and **Transition rates**)
2. *Solve* **Master Equation**

Final objective: kinetics of interaction process

Definition (Markov process)

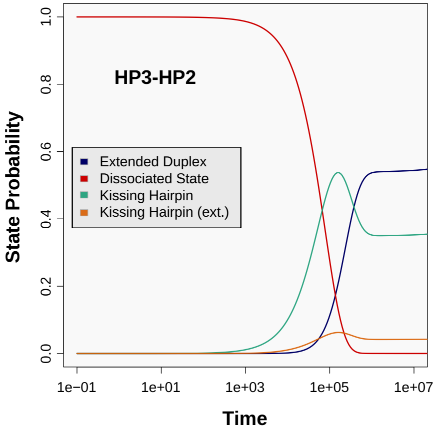
The **Markov process** $(\mathcal{X}, R, P_0)$ is the stochastic process governed by the **master equation**

$$\frac{dP}{dt} = RP,$$

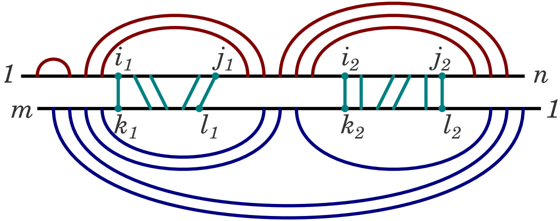
Solve as

$$P_t = \exp(tR)P_0.$$

$$\boxed{P_t(x) = \text{probability of } x \text{ at } t}$$

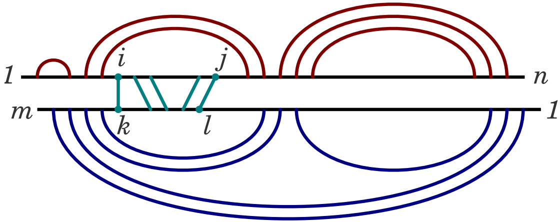


Joint (secondary) structures



Hybridization sites i_1, j_1, k_1, l_1 and i_2, j_2, k_2, l_2

Joint (secondary) structures



Single hybridization site i, j, k, l

E(**joint structure**) =
E(**structure 1**) + E(**hybridization**) + E(**structure 2**)[1]

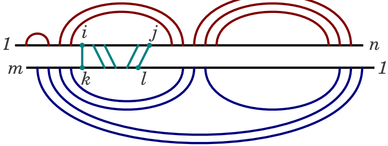
[1] $+E_{\text{duplex init}}$ if hybridization is non-empty

The joint secondary structure landscape

Given two RNAs A and B.

Energy landscape $\mathcal{L}^{\mathsf{j}} = (\mathcal{X}, \mathcal{N}, \mathsf{E})$

- state space $\mathcal{X}$ = set of joint secondary structures (of A and B)
- neighborhood $\mathcal{N}$ defined by single base pair moves;
  i.e. neighbors differ by 1 base pair
- energy function E on jss



The joint structure state space explodes quickly

Example: CGCAAUGCGAAUGCC and CGCGAUUCG

```
CGCAAUGCGAAUGCC
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CGCGAUUCG
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CGCAAUGCGAAUGCC&CGCGAUUCG
.......(((((((.&.)).)))))
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            ⋮
```
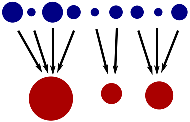

⇒ **Apply coarse graining (here, even several levels)**

Energy landscapes and general coarse graining

Coarse graining acts on landscapes $\mathcal{L}$ and yields macro-landscape $\mathcal{L}'$.

Coarse graining assigns states $x \in \mathcal{X}$ to macro-states $\alpha \in \mathcal{X}'$.



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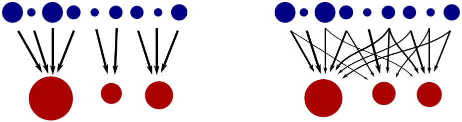
discrete



continuous

Definition (Coarse graining matrix)

$\mathbf{C} \in \mathbb{R}^{|\mathcal{X}'| \times |\mathcal{X}|}$; $\quad \mathbf{C}_{\alpha x} = Pr[\, x \text{ assigned to } \alpha \,]$

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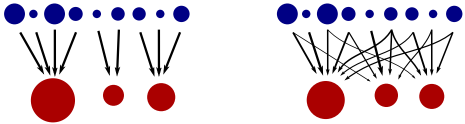
discrete *continuous*



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Remarks:

- in discrete case, $\mathbf{C} \in \{0,1\}^{|\mathcal{X}'|\times|\mathcal{X}|}$.
- columns of $\mathbf{C}$ sum to 1 (stochastic)

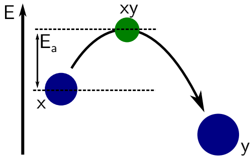
General coarse graining II

(Re)define **energy landscape** as $\mathcal{L} =: (\mathcal{X}, \tilde{Z}, Z)$, where

- $\mathcal{X}$ is a set of states x
- Z_x are Boltzmann weights of states $\exp(-\mathrm{E}(x)/RT)$
- $\tilde{Z}_{xy}$ are Boltzmann weights of transition states

Then,

- states x and y are connected if $Z_{xy} > 0$.
- rate (constant) from y to x: $r_{x \leftarrow y} = \tilde{Z}_{xy}/Z_y$ **(Arrhenius rate).**



$$r_{x \leftarrow y} = \exp(-\mathrm{E}_a/RT) = \exp(-(\mathrm{E}_{xy} - \mathrm{E}_x)/RT)$$

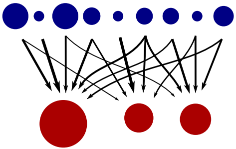
Example:

Metropolis rates

$\mathrm{E}_{xy} = max(E_x, E_y)$

General coarse graining III

General coarse graining: $\mathcal{L} = (\mathcal{X}, \tilde{Z}, Z) \longrightarrow_{\mathbf{C}} \mathcal{L}' = (\mathcal{X}', \tilde{Z}', Z')$



CG matrix $\mathbf{C} \in \mathbb{R}^{|\mathcal{X}'|\times|\mathcal{X}|}$

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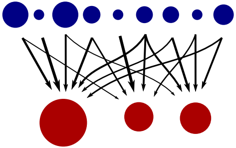

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Then:

- CG by $\mathbf{C}$ determines state weights:

$$Z' = \mathbf{C}Z \qquad \equiv \qquad Z'_\alpha = \sum_{x \in \mathcal{X}} \mathbf{C}_{\alpha x} Z_x$$

- CG by $\mathbf{C}$ determines transition weights:

$$\tilde{Z}'_{\alpha\beta} = \sum_{x,y \in \mathcal{X}} \mathbf{C}_{\alpha x} \cdot \mathbf{C}_{\beta y} \cdot \tilde{Z}_{xy} \qquad \text{(canonical CG)}$$

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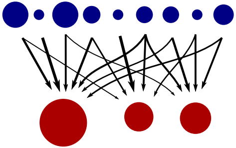

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discrete case: $r_{\alpha \leftarrow \beta} = \sum_{x \in \alpha, y \in \beta} Pr[y \mid \beta] r_{x \leftarrow y}$

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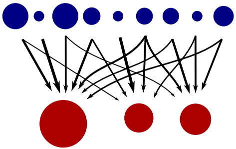

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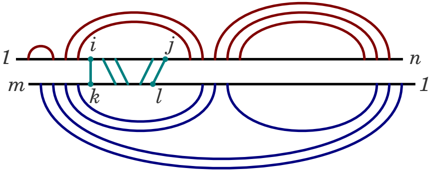
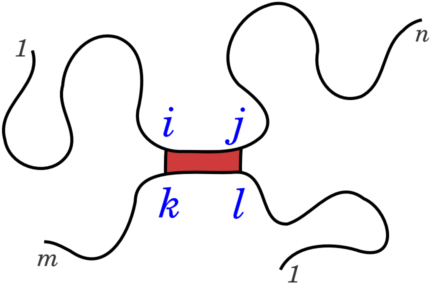
general case: $r_{\alpha \leftarrow \beta} = \sum_{x,y \in \mathcal{X}} \mathbf{C}_{\alpha x} \cdot \mathbf{C}_{\beta y} Pr[y \mid \beta] r_{x \leftarrow y}$

RNAup-like model



joint structure
with hybridization site (i,j,k,l)

RNAup-like model



hybdridization site state (i,j,k,l)

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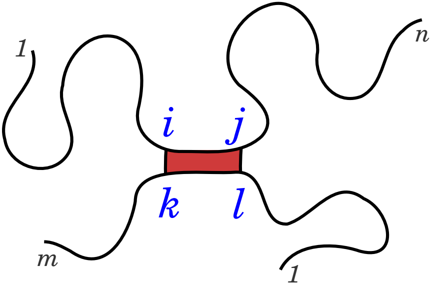

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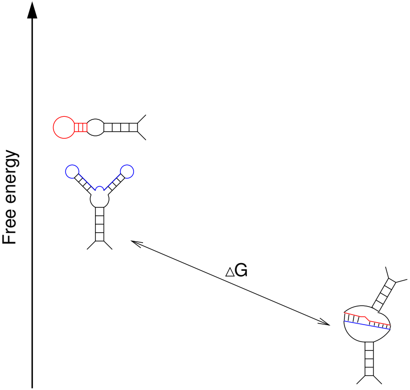

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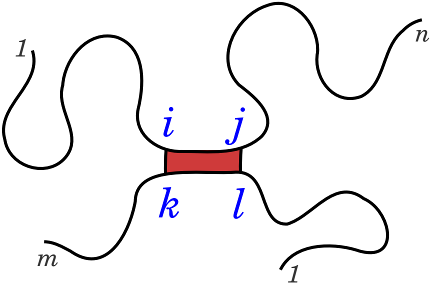

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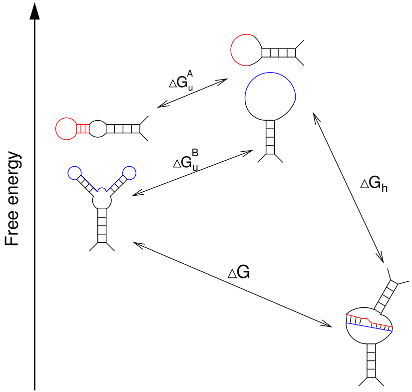

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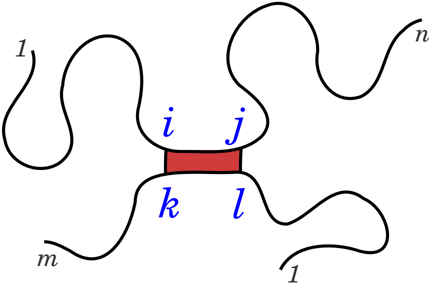

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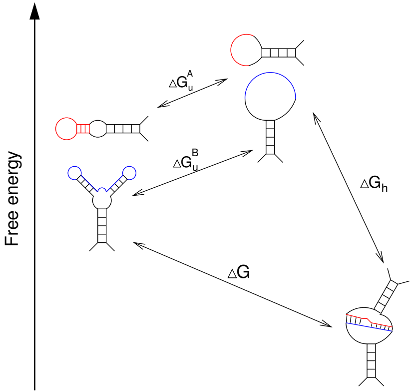
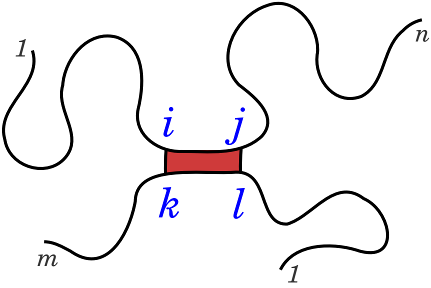


$$\Delta G(i,j,k,l) := \Delta G_u^A(i,j) + \Delta G_u^B(k,l) + \Delta G_h(i,j,k,l)$$

RNAup-like model



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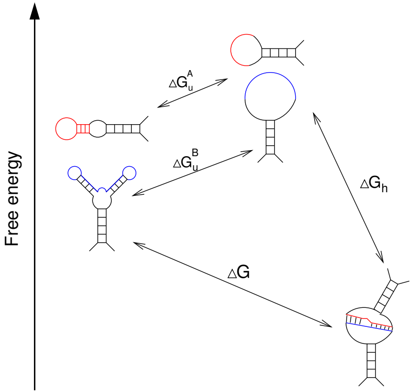
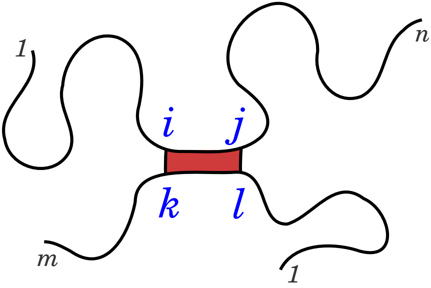
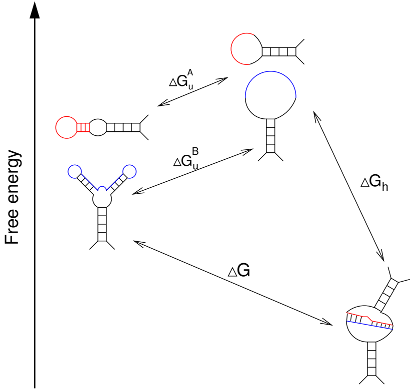


$$Z^{\mathrm{h}}(i,j,k,l) := \sum_{\text{joint structure } x \text{ with site } (i,j,k,l)} \exp(-E(x)/RT)$$

RNAup-like model

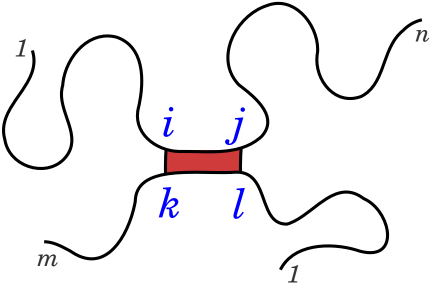


hybdridization site state (i,j,k,l)



$$Z^{\mathrm{h}}(i,j,k,l) := Z^{A}(i,j) \cdot Z^{B}(k,l) \cdot Z^{\mathrm{hyb}}(i,j,k,l)$$

RNAup-like model



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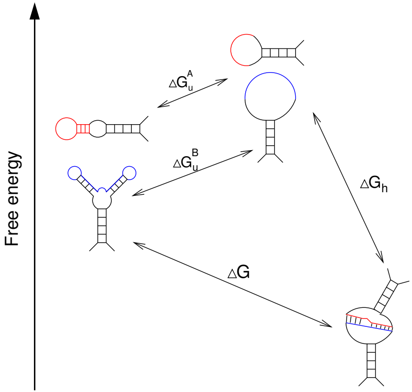

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Hybridization site states **coarse grain** joint structures: $Z^{\mathrm{h}} = \mathbf{C}^{\mathrm{h}} Z^{\mathrm{j}}$

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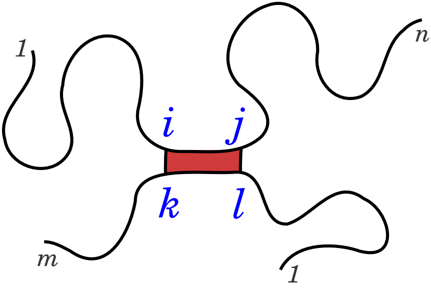

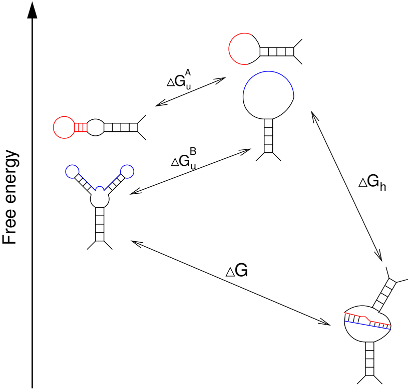

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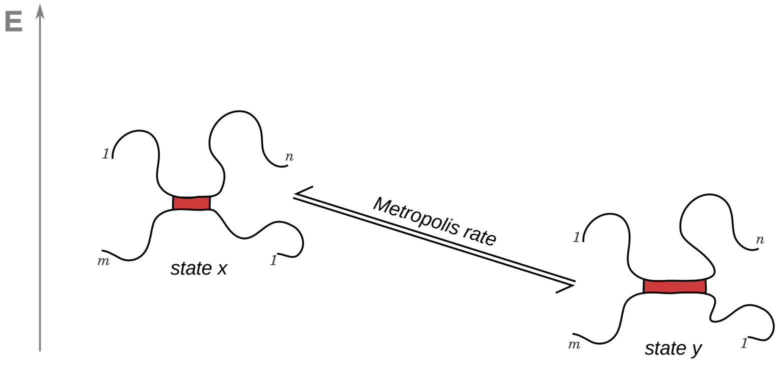
$$Z^{\mathrm{h}}(i,j,k,l) := Z^{A}(i,j) \cdot Z^{B}(k,l) \cdot Z^{\mathrm{hyb}}(i,j,k,l)$$

+ Fewer States **+ Efficient** **+ Complex interactions**

Transition rates for the hybridization site states



Transition rates defined by weights of (complex) transition states

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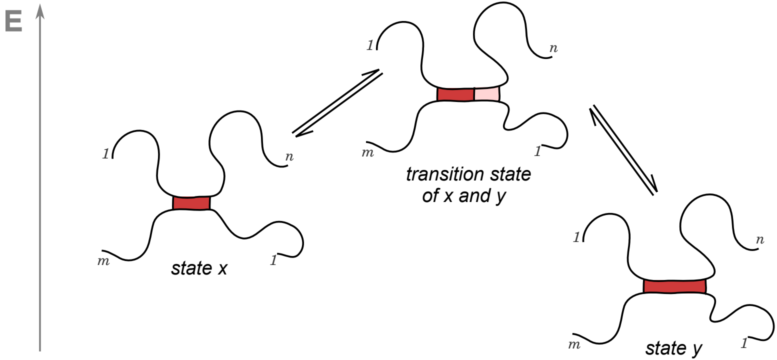

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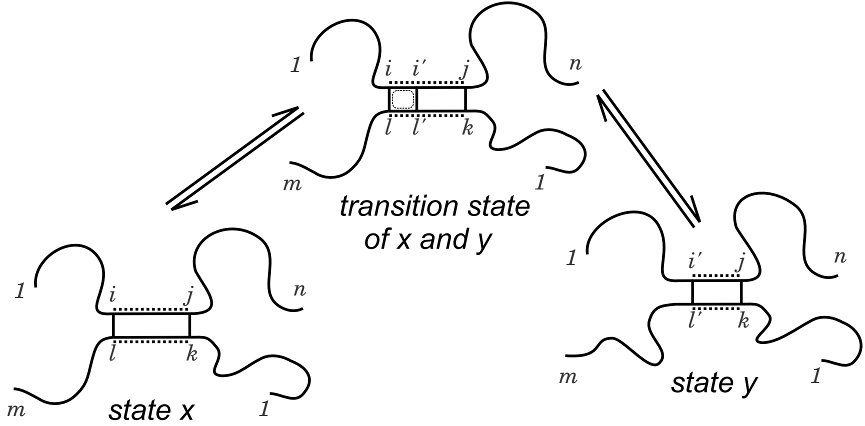
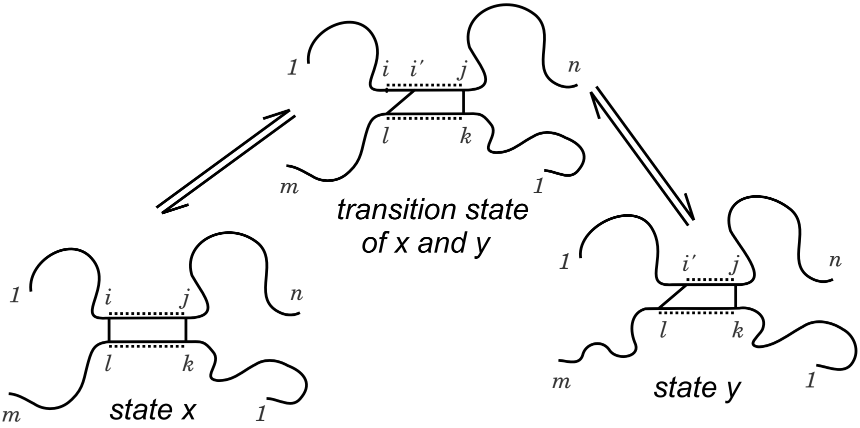
Grow and Shrink Moves



Shift Moves


1
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of x and y
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state x
1
1
i′
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k
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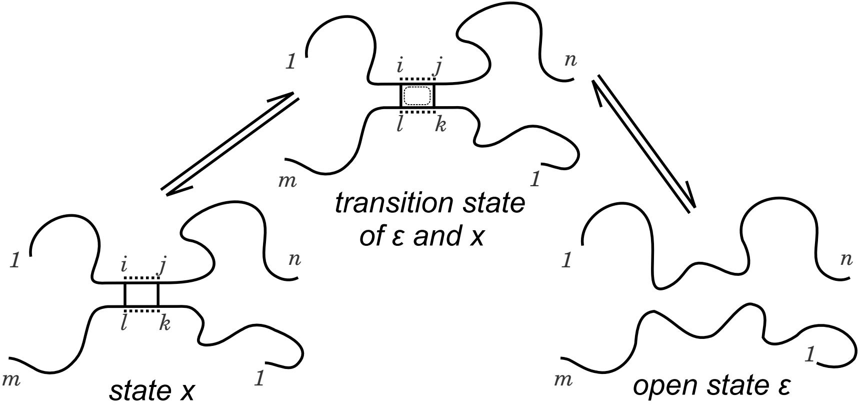
state y
1

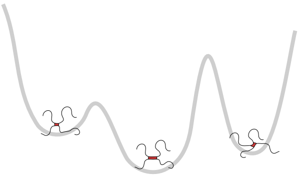
Association and Dissociation



Further coarse graining

Continous CG with 'traditional' discrete CG as pre-processing

1. **Discrete:** partition into *gradient basins*



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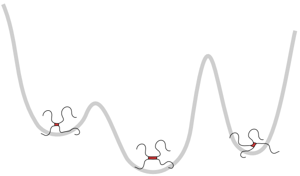
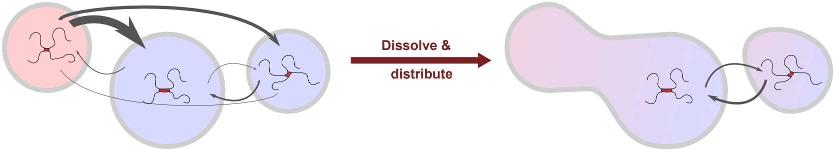


2. **Continous:** dissolve 'shallow' basins; distribute proportional to **rate** (or equivalently, transition state weight)



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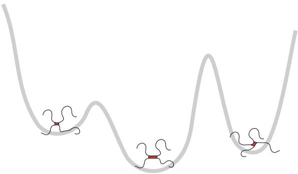

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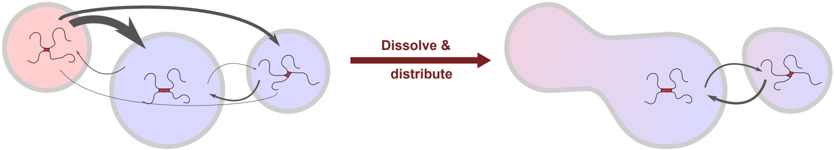

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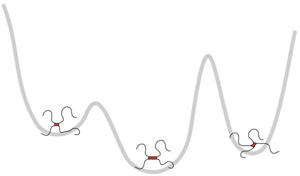

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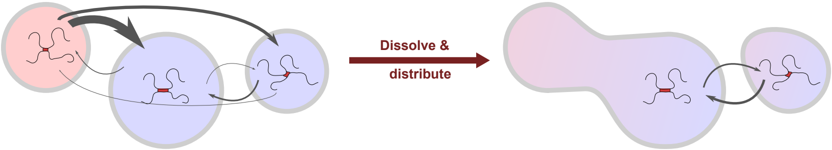
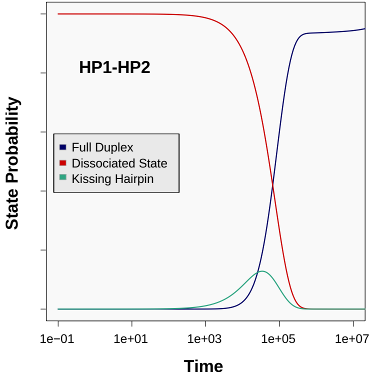


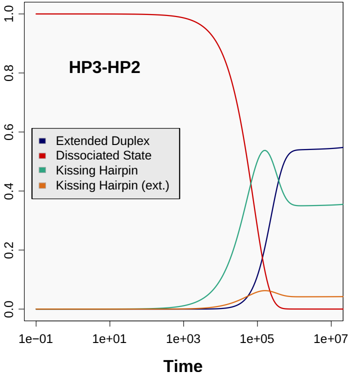
Both steps fit the concept of **general coarse graining** w/ corresp. matrices $\mathbf{C}^{gb}$ and $\mathbf{C}^{c}$; landscapes $\mathcal{L}^{gb}$ and $\mathcal{L}^{c}$

Quick validation w/ FRET experiments

- kinetic mutation study: Salim et al., Biophys J, 2012
- **3 RNA fragments**: **HP1**, **HP2** from DsrA–rpoS; **HP3** = HP1-variant
- **HP1–HP2** can form kissing hairpin *and* full duplex
- **HP3–HP2** *cannot* form complete duplex

side remark: reproducing the results works only at *correct temperature*





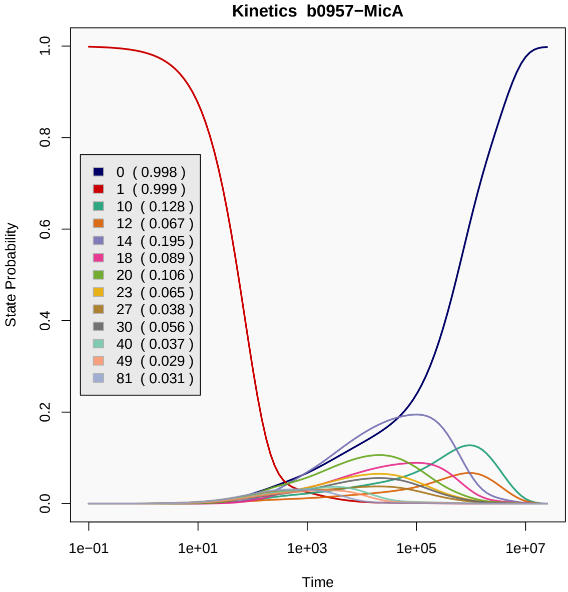
Example: E. coli MicA–ompA

```
>Mic A
GAAAGACGCGCAUUUGUUAUCAUCAUCCCUGAAUUCAGAGAUGAAAUUUUGGCCACUCACGAGUGGCCUUUU
>ompA 5'UTR
CUUUUUUUUCAUAUGCCUGACGGAGUUCACACUUGUAAGUUUUCAACUACGUUGUAGACUUUACAUCGCCAG
GGGUGCUCGGCAUAAGCCGAAGAUAUCGGUAGAGUUAAUAUUGAGCAGAUCCCCCGGUGAAGGAUUUAACCG
UGUUAUCUCGUUGGAGAUAUUCAUGGCGUAUUUUGGAUGAUAACGAGGCGCAAAAAAUGAAAAAGACAGCUA
UCGCGAUUGCAGUGGCACUGGCUGGUUUCGCUACCGUAGCGCAGGCCGCUCCGAAAGAUAACACCUGGUACA
CUGGUGCUAAAC
```

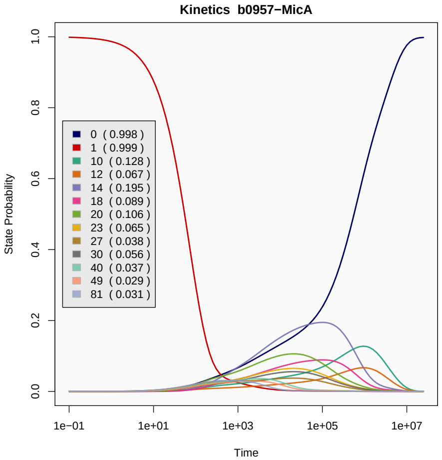
- **Enumerate** hybridization site states — 416992 states
- **discrete coarse graining** — 40772 gradient basins
- **continuous coarse graining** — 255 continuous macro states
- **Solve** macro-transition system

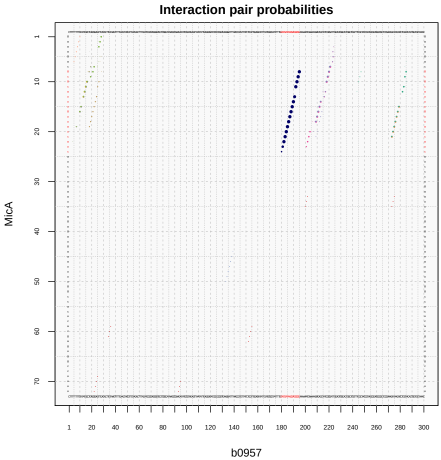
Total computation time: *several minutes*

Interpretation of results (MicA–ompA)



Interpretation of results (MicA–ompA)



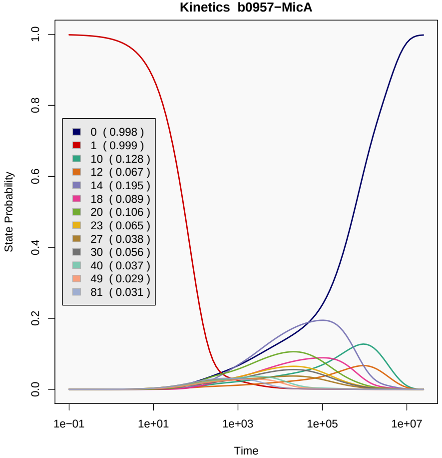


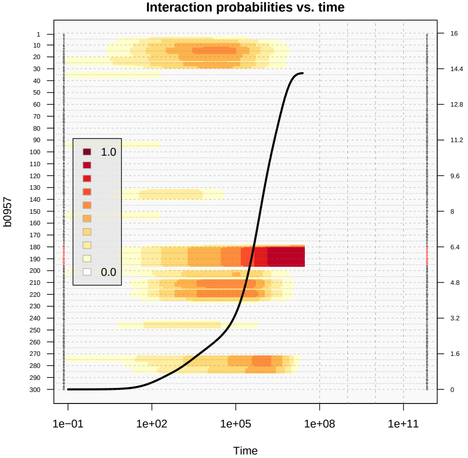
Probabiltities of interaction base pairs:

$$Pr[(i,j) \mid t] = \sum_{\substack{x^{\mathrm{c}} \in \mathcal{X}^{\mathrm{c}}, x^{\mathrm{gb}} \in \mathcal{X}^{\mathrm{gb}} \\ x^{\mathrm{h}} \in \mathcal{X}^{\mathrm{h}}, x^{\mathrm{j}} : (i,j) \in x^{\mathrm{j}}}} Pr[\alpha \mid t] \cdot \mathbf{C}^{\mathrm{c}}_{x^{\mathrm{c}} x^{\mathrm{gb}}} \cdot \mathbf{C}^{\mathrm{gb}}_{x^{\mathrm{gb}} x^{\mathrm{h}}} \cdot \mathbf{C}^{\mathrm{h}}_{x^{\mathrm{h}} x^{\mathrm{j}}}$$

Interaction probability of mRNA positions: $Pr[i \mid t] = \sum_{\text{sRNA pos. } j} Pr[(i,j) \mid t]$

Interpretation of results (MicA–ompA)





Probabiltities of interaction base pairs:

$$Pr[(i,j) \mid t] = \sum_{\substack{x^{c}\in\mathcal{X}^{c},x^{gb}\in\mathcal{X}^{gb} \\ x^{h}\in\mathcal{X}^{h},x^{j}:(i,j)\in x^{j}}} Pr[\alpha \mid t] \cdot \mathbf{C}^{c}_{x^{c}x^{gb}} \cdot \mathbf{C}^{gb}_{x^{gb}x^{h}} \cdot \mathbf{C}^{h}_{x^{h}x^{j}}$$

Interaction probability of mRNA positions: $Pr[i \mid t] = \sum_{\text{sRNA pos. } j} Pr[(i,j) \mid t]$

Take home

- **Kinetics model** for fairly complex RNA–RNA interaction
- Kinetic analysis of sRNA–mRNA 5'UTR interaction "in minutes"
- Tailored coarse graining enables **feasible computation**
- Procedure for **continuous coarse graining**
- Generalized coarse graining offers **unified perspective**: from discrete CG (e.g. gradient basin) to continuous CG (e.g. Stadler&Stadler, 2010)
- ... and allows **'back propagation'**
- Interpretation at **base pair resolution**
- **[WIP]** software will be made availabe