Protein Synthesis by Ribosomes

Reinhard Lipowsky
Theory & Biosystems, MPI-KG, Potsdam-Golm

• Intro I: Biomolecular Machines
• Intro II: Stochastic Processes
• Protein Synthesis by Ribosomes
• Kinetics In Vivo and In Vitro
• Kinetic Distance Minimization
• Outlook

Biomolecular Machines

• Stepping motors
• Motor teams
• Actin filaments
• Ribosomes
Multiscale Motor Systems

- ATP hydrolysis ~ 1 nm
- Mechanical steps ~ 10 nm
- Cargo transport by motor teams ~ 100 μm
- Traffic of many motors/cargos and traffic phase transitions

Stochastic Modelling I

- Stochastic processes on discrete state spaces
- Motor Traffic as ASEPs + Diffusion: RL ... Nieuwenhuizen, \textit{PRL} (2001)
Stochastic Modelling II

- Stochastic processes on discrete state spaces
- Actin Filaments, space of patterns of T, P, and D subunits

\[
\begin{align*}
&D \rightarrow P \rightarrow T \\
&D \cdots D \cdots P_i \cdots D \cdots P_i \cdots D \cdots T \cdots T
\end{align*}
\]

Niedermayer ... RL, PNAS (2012)

- Ribosomes

\[
\begin{array}{c}
\text{Near-Cognate} \\
\begin{array}{c}
\omega_{on} \\
\omega_{off} \\
\omega_{78} \\
\omega_{76}
\end{array}
\end{array}
\]

\[
\begin{array}{c}
\text{Non-Cognate} \\
\begin{array}{c}
\omega_{on} \\
\omega_{off} \\
\omega_{rec}
\end{array}
\end{array}
\]

\[
\begin{array}{c}
\text{Cognate} \\
\begin{array}{c}
\omega_{on} \\
\omega_{off} \\
\omega_{23} \\
\omega_{21} \\
\omega_{40}
\end{array}
\end{array}
\]

Continuous Time Markov Processes

- Discrete state space with states i
- Transitions |ij> from state i to state j with rate \( \omega_{ij} \)
- Transition rates \( \omega_{ij} \) can be measured
- State space + rates: continuous time Markov process (CTMP)
- Time evolution for probabilities \( P_i \):
  \[
  \frac{d P_i}{dt} = - \sum_j \left[ P_i \omega_{ij} - P_j \omega_{ji} \right]
  \]
- In general, backward transitions |ji> with rates \( \omega_{ji} \)
- CTMPs provide general theoretical framework
- In practice, identify states and transitions, specify rates
(Ir)Reversible Transitions

- Reversible transition \( |i\rangle \rightarrow |j\rangle \): appreciable rate \( \omega_{ji} \) for \( |ji\rangle \)
- Irreversible transition \( |i\rangle \rightarrow |j\rangle \): negligible rate \( \omega_{ji} \), put \( \omega_{ji} = 0 \)
- Thermodynamics: no irreversible transitions
- Biochemistry: rates \( \omega_{ji} \) too small to be measurable, put \( \omega_{ji} = 0 \)

- Steady state: \( dP_i/dt = 0 \)
- Local excess fluxes \( \Delta J_{ij} = P_i \omega_{ij} - P_j \omega_{ji} \)
- Local detailed balance: \( \Delta J_{ij} = P_i \omega_{ij} - P_j \omega_{ji} = 0 \)

Protein Synthesis by Ribosomes

- Molecular Components
- Elongation Cycle
- Competition between tRNAs
- In Vivo from In Vitro rates:
  - Similarity measure = kinetic distance
  - Minimization of kinetic distance
  - Validation of predicted rates

Sophia Rudorf
How long does it take for the ribosome to move to the next codon? How long does it take to add a single amino acid to the chain?

Ribosome + mRNA + tRNAs

- Ribosome steps along codons of mRNA (purple -> green) consuming one ternary complex at each codon
- Elongation cycle during one step:
  Decoding of codon by binding/accommodation of tRNA
  Elongation of growing peptide chain by one amino acid
  Translocation of mRNA together with two tRNAs
Single Elongation Cycle

Initial binding of ternary complex

Recognition between codon and anti-codon

Translocation

GTP hydrolysis, release of EF-Tu and E-site tRNA, peptide bond formation

- Complexity of decoding:
  61 different codons and 43 elongator tRNA species (E. coli)

Codon-tRNA Relationships

- cognate = green
- near-cognate = yellow
- non-cognate = red/purple

- cognate decoding => correct amino acid
- near-cognate decoding => incorrect amino acid
- non-cognate tRNAs are released after initial binding
Single Elongation Cycle - Refined

- Possible binding of cognate/near-cognate/non-cognate tRNAs:
  - Accommodation of near-cognate tRNA => error rate
  - Accommodation of cognate tRNA
  - Competition between cognate, near-cognate, and non-cognate tRNAs

Markov Process


- Map cartoon of multistep process onto Markov chain:

- Individual transitions:
  - initial binding, recognition, initial selection, GTP hydrolysis, phosphate release, proof reading, full accommodation
- All transition rates $\omega_{ij}$ have been measured in vitro
- Some rates identical for cognates and near-cognates
**Transition Rates in vitro**

<table>
<thead>
<tr>
<th>Rates</th>
<th>$k$-not.</th>
<th>20 °C</th>
<th>37 °C</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{on}$</td>
<td>$k_1$</td>
<td>140 ± 20</td>
<td>175 ± 25</td>
<td>$\frac{1}{\mu M \text{ s}}$</td>
</tr>
<tr>
<td>$\omega_{off}$</td>
<td>$k_{-1}$</td>
<td>85 ± 25</td>
<td>700 ± 270</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{rec}$</td>
<td>$k_2$</td>
<td>180 ± 30</td>
<td>1500 ± 450</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{21}$</td>
<td>$k_{-2,co}$</td>
<td>0.2 ± 0.03</td>
<td>2 ± 0.6</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{23}$</td>
<td>$k_{3,co}$</td>
<td>190 ± 30</td>
<td>1500 ± 450</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{con}$</td>
<td>$k_4$</td>
<td>50</td>
<td>450</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{45}$</td>
<td>$k_{5,co}$</td>
<td>22 ± 4</td>
<td>200 ± 40</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{40}$</td>
<td>$k_{7,co}$</td>
<td>0.1</td>
<td>1</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{76}$</td>
<td>$k_{-2,nr}$</td>
<td>140 ± 20</td>
<td>1100 ± 330</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{78}$</td>
<td>$k_{3,nr}$</td>
<td>0.6 ± 0.1</td>
<td>7 ± 2</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{9,10}$</td>
<td>$k_{5,nr}$</td>
<td>0.06 ± 0.006</td>
<td>0.26 ± 0.04</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{90}$</td>
<td>$k_{7,nr}$</td>
<td>0.84 ± 0.08</td>
<td>4 ± 0.7</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{pro}$</td>
<td></td>
<td>3 ± 1</td>
<td>150 ± 50</td>
<td>1/s</td>
</tr>
<tr>
<td>$\omega_{elo}$</td>
<td></td>
<td>0.8 ± 0.2</td>
<td>6.9 ± 2.3</td>
<td>aa/s</td>
</tr>
</tbody>
</table>

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**Ribosome + mRNA + tRNAs**

- Ribosome steps along codons of mRNA (purple -> green)
- Elongation cycle during one step:
  - Decoding of codon by binding/accommodation of tRNA
  - Elongation of growing peptide chain by one amino acid
  - Translocation of mRNA together with two tRNAs
**In Vitro versus In Vivo**

- *In vitro*: Set of individual transition rates $\omega_{ij}$
  

- *In vivo*: Individual rates cannot be measured but overall speed of ribosomes can be determined

- Discrepancy for ribosome speed = peptide synthesis rate:
  
  In vivo value $>>$ in vitro value

- Ehrenberg group concludes from *competition* effect:
  
  in vivo values of $\omega_{off}$ must be 10000 times larger than in vitro values!
  

**‘Similarity’ of In Vitro and In Vivo?**

- Multistep process with many individual transitions

  ![Diagram of multistep process](image)

- Set of in-vitro rates $\omega_{ij}$ $\not\equiv$ Set of in-vivo rates $\omega^*_{ij}$

- How ‘similar’ or ‘close’ are the in-vivo to the in-vitro rates?

- Quantitative measure for such a ‘similarity’?
Kinetic Distance: Single Transition

Rudorf ... RL, *PLOS Comp Biol* (2014)

- Consider single transition from state i to state j
- Transition rates: In-vitro value $\omega_{ij}$, in-vivo value $\omega_{ij}^*$
- Naive distance: Absolute value of $\omega_{ij} - \omega_{ij}^*$
- But: could equally well consider transition times

\[
\tau_{ij} = \frac{1}{\omega_{ij}} \quad \text{and} \quad \tau_{ij}^* = \frac{1}{\omega_{ij}^*}
\]

- Kinetic distance $D_{ij}$ for single transition:

\[
D_{ij} (\omega_{ij}, \omega_{ij}^*) = D_{ij} (\tau_{ij}, \tau_{ij}^*) = D_{ij} (1/\omega_{ij}, 1/\omega_{ij}^*)
\]

- Simplest expression that fulfills this requirement:

\[
D_{ij} (\omega_{ij}, \omega_{ij}^*) = | \ln(\omega_{ij}^*/\omega_{ij}) | = | \Delta_{ij} |
\]

Kinetic Distance: Interpretation

- Arrhenius form of transition rates:

\[
\omega_{ij} = \nu_{ij} \exp[- \frac{\Delta G_{ij}}{k B T}]
\]

- Coordinates $\Delta_{ij} = \ln(\omega_{ij}/\omega_{ij}^*)$ represent ‘single barrier shifts’
Kinetic Distance: Multistep Process

- Set of in-vitro rates $\omega_{ij}$, set of in-vivo rates $\omega_{ij}^*$
- Define ‘single barrier shifts’
  \[ \Delta_{ij} = \ln(\omega_{ij}^*/\omega_{ij}) \]

- Multi-dimensional space with coordinates $\Delta_{ij}$
  - 3-dimensional subspace corresponding to three individual rates
  - 12 distinct rates for elongation, “translation in 12 dimensions”

Kinetic Distance: Multistep Process

- Kinetic distance = Euclidean distance in $\Delta_{ij}$–space:
  \[ D = \sqrt{\sum \Delta_{ij}^2} = \sqrt{\sum \left[\ln(\omega_{ij}^*/\omega_{ij})\right]^2} \]
- What about ‘weight factors’? $\Delta_{ij}$ replaced by $u_{ij} \Delta_{ij}$
- Limit of single transition $\Rightarrow$ all $u_{ij} = 1$
- Two different assays, A1 and A2
  - Change from A1 to A2 leads to simple coordinate transformation $= \text{shift of origin}$
Minimization of Kinetic Distance

- Individual transition rates are not known *in vivo* but overall *in-vivo* speed is known (for different conditions)
- Minimize kinetic distance between known *in-vitro* rates and unknown *in-vivo* rates under overall constraint

- Multi-dimensional $\Delta_{ij}$ - space:

Predicted In-Vivo Point

- Single barrier shifts
  \[ \Delta_{ij} = \ln\left(\frac{\omega_{ij}^*}{\omega_{ij}}\right) \]

- Scale factors $\omega_{ij}^*/\omega_{ij}$
Codon-specific Elongation Rates

- Each codon characterized by a different set of cognate/near-cognate/non-cognate tRNAs
- Initial binding leads to codon-specific elongation rates:

![Elongation Rate Graph]

In vivo profile, fast growth

Validation by In-Vivo Data I

- Relative translation rates
  - yellow data: anticodons with wobble
  - blue data: slippery sequences

![Correlation Graph]

- Pearson correlation coefficient = 0.73 (or 0.56)
Validation by In-Vivo Data II

Sorensen + Pedersen, *J. Mol. Biol.* (1991)

- Uptake of radioactive S-methionine into β-galactosidase
- Simulation with codon-specific elongation rates

![Graph showing incorporation of radioactivity over time](image)

- orange: predicted $\omega_{\text{off}}$
- blue: 1.2 $\omega_{\text{off}}$
- green: 0.8 $\omega_{\text{off}}$

Validation by In-Vivo Data III


- Missense error frequency = probability to fully accommodate certain tRNA at one of its near-cognate codons
- Error frequency depends on codon usage $p_c$
- Error frequency for tRNA-Lys measured to be $2 \times 10^{-4}$
- Predicted in vivo rates lead to $3 \times 10^{-4}$

Good agreement with three independent sets of in vivo data without any fit parameter!
General Computational Method

- Applicable to any multistep process
- Global method with unique solution or discrete set of solutions (bifurcation)
- Applicable to highly nonlinear constraints
- No a priori bias about importance of different transitions („Principle of least prejudice“)
- Comparison with Flux Control Analysis (FCA): FCA is local, restricted to linear response, no metric, i.e., provides only direction in $\Delta_{ij}$ – space but no distance

Summary

- Protein Synthesis by Ribosomes
  Competition between different tRNA species
- Kinetics in vivo and in vitro
  Long controversy about (dis)similarity
- Kinetic distance
  (Dis)similarity measure for multi-step process
  $\Delta_{ij} = \ln(\omega_{ij}^*/\omega_{ij})$
- Kinetic distance minimization
  Minimal distance of in-vitro point from in-vivo hyper-surface
- General computational method
Refined Modelling

- Distinguish TC concentration from total tRNA concentration
- Important subpopulation: uncharged but bound to ribosome
- Include recharging cycle of tRNA
- Release of tRNA from E site
  - Immediate release: 2-1-2 process
  - Delayed release: 2-3-2 process

Extension to Human Cells

- Scatter plot for 7500 genes of Hela cells:

Sophia Rudorf
Jan Trösemeier
Christel Kamp
Coworkers

**Single Motors**
- Steffen Liepelt
- Aliaksei Krukau
- Volker Knecht

**Actin Filaments**
- Thomas Niedermayer
- Jan Kierfeld
- Marie-France Carlier
- G. Romet-Lemonne

**Ribosomes**
- Sophia Rudorf
- Marina Rodnina
- Michael Thommen
- Jan Trösemeier
- Christel Kamp

**Motor Teams**
- Stefan Klumpp
- Melanie Müller
- Corina Keller
- Florian Berger
- Gero Steinberg
- Martin Schuster

Active nightlife in Golm-Potsdam!