Protein Synthesis by Ribosomes

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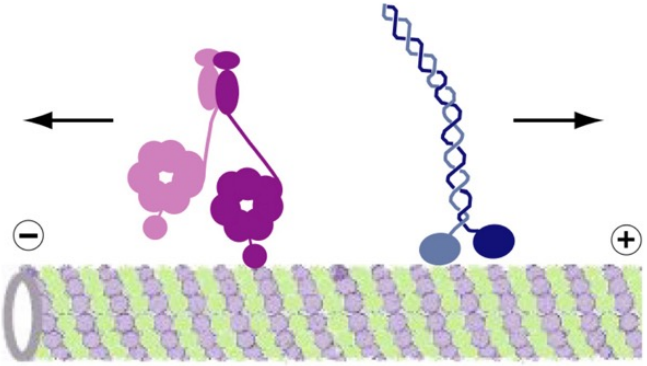
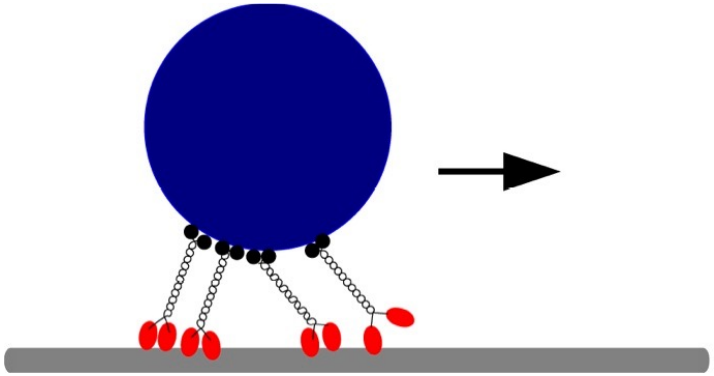
- Intro I: Biomolecular Machines
- Intro II: Stochastic Processes
- Protein Synthesis by Ribosomes
- Kinetics In Vivo and In Vitro
- Kinetic Distance Minimization
- Outlook

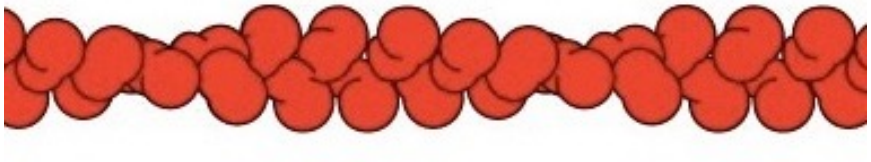
Biomolecular Machines



• Stepping motors



• Motor teams

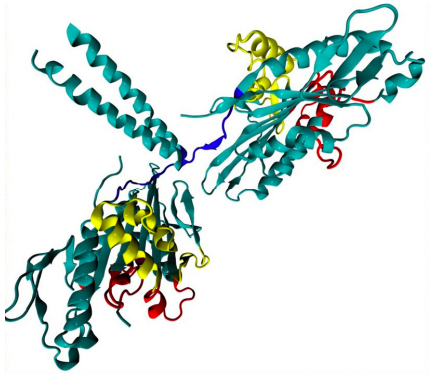


• Actin filaments

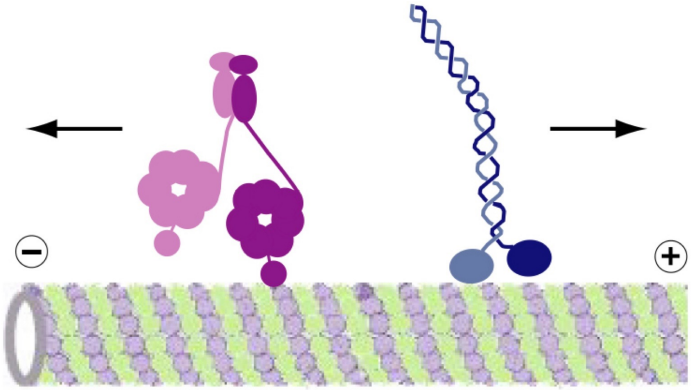
• Ribosomes

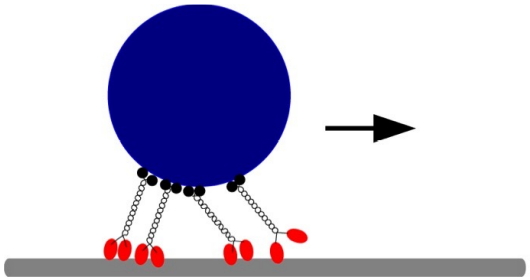
Multiscale Motor Systems

- ATP hydrolysis ~ 1 nm



- Mechanical steps ~ 10 nm





- Cargo transport by motor teams ~ 100 μm

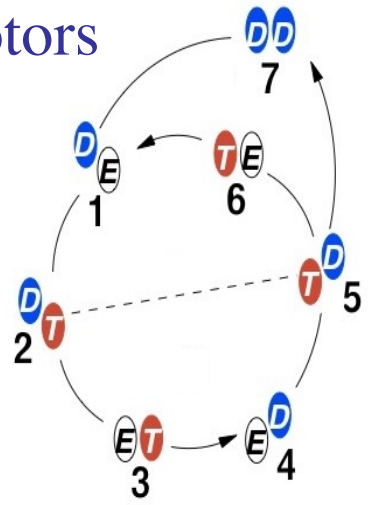
- Traffic of many motors/cargos and traffic phase transitions

Stochastic Modelling I

- Stochastic processes on discrete state spaces

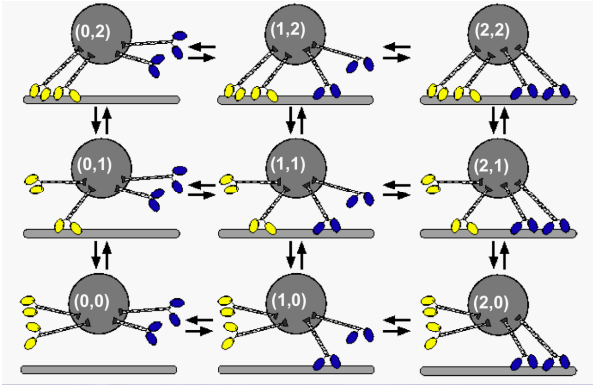
- Single Motors

Liepelt, RL
PRL (2007)



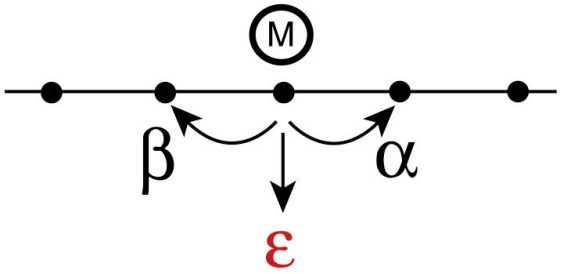
- Motor Teams

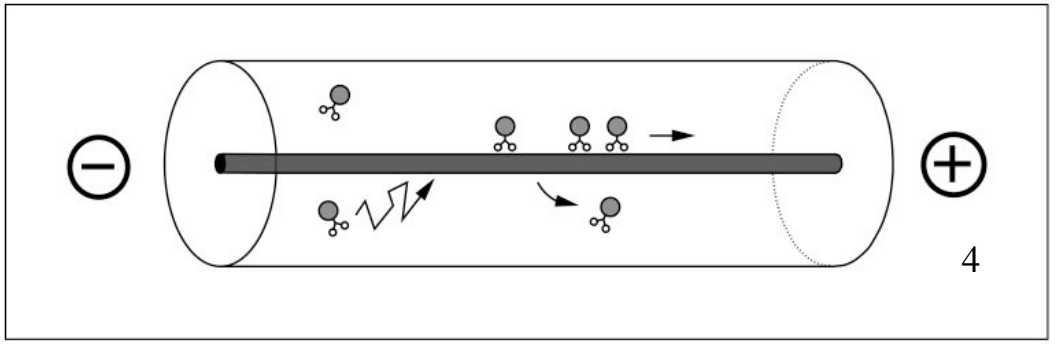
Klumpp, RL, *PNAS* (2005)
Müller ... RL, *PNAS* (2008)



- Motor Traffic as ASEPs
  + Diffusion

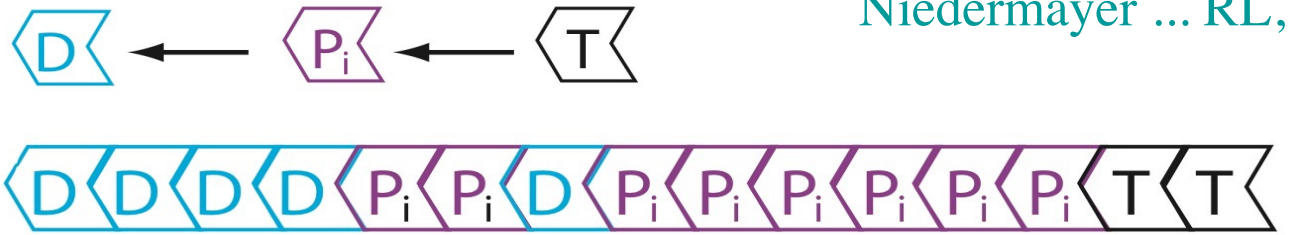
RL ... Nieuwenhuizen, *PRL* (2001)





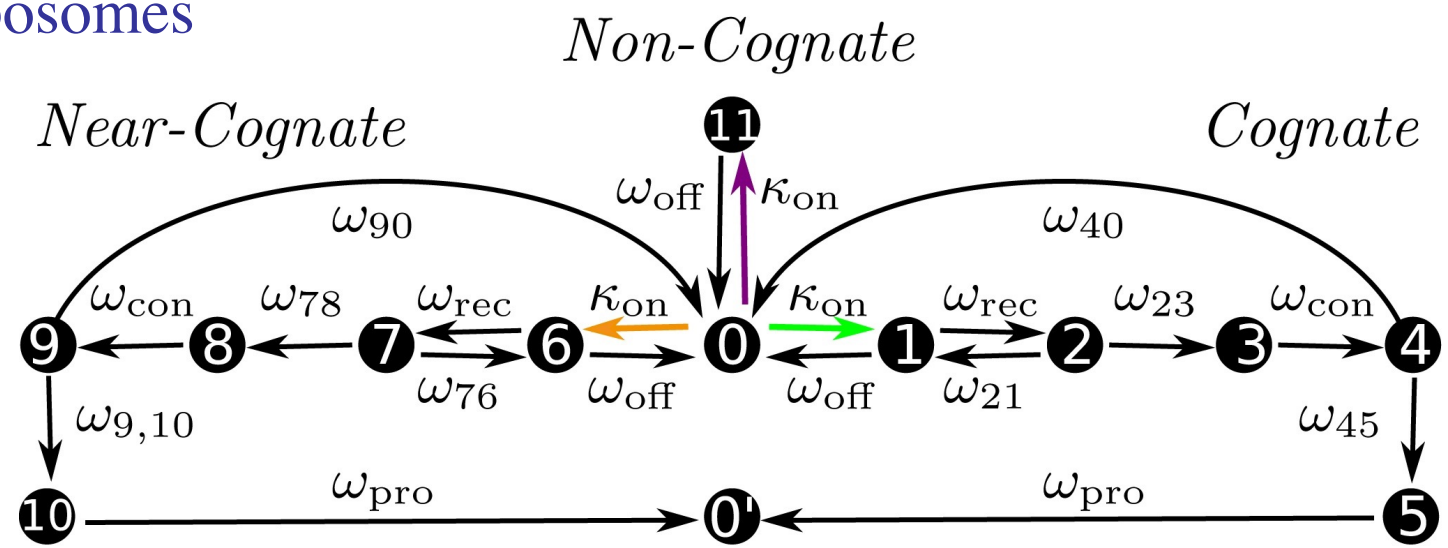
Stochastic Modelling II

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



Niedermayer ... RL, *PNAS* (2012)

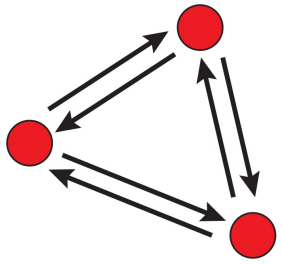
- Ribosomes



Continuous Time Markov Processes

- Discrete state space with states i
- Transitions |ij> from state i to state j with rate ω_{ij}
- Transition rates ω_{ij} can be measured
- State space + rates: continuous time Markov process (CTMP)
- Time evolution for probabilities P_i :

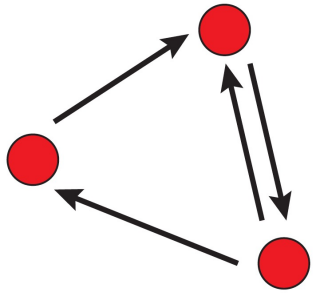
$$d P_i / dt = - \sum_j [P_i \omega_{ij} - P_j \omega_{ji}]$$



- In general, backward transitions |ji> with rates ω_{ji}
- CTMPs provide general theoretical framework
- In practice, identify states and transitions, specify rates

(Ir)Reversible Transitions

- Reversible transition |ij>: appreciable rate ω_{ji} for |ji>
- Irreversible transition |ij> : negligible rate ω_{ji} , put $\omega_{ji} = 0$
- Thermodynamics: no irreversible transitions
- Biochemistry: rates ω_{ji} too small to be measurable, put $\omega_{ji} = 0$



- Steady state: $d P_i / dt = 0$
- 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

Sophia Rudorf

- Molecular Components
- Elongation Cycle
- Competition between tRNAs
- In Vivo from In Vitro rates:

  Similarity measure = kinetic distance

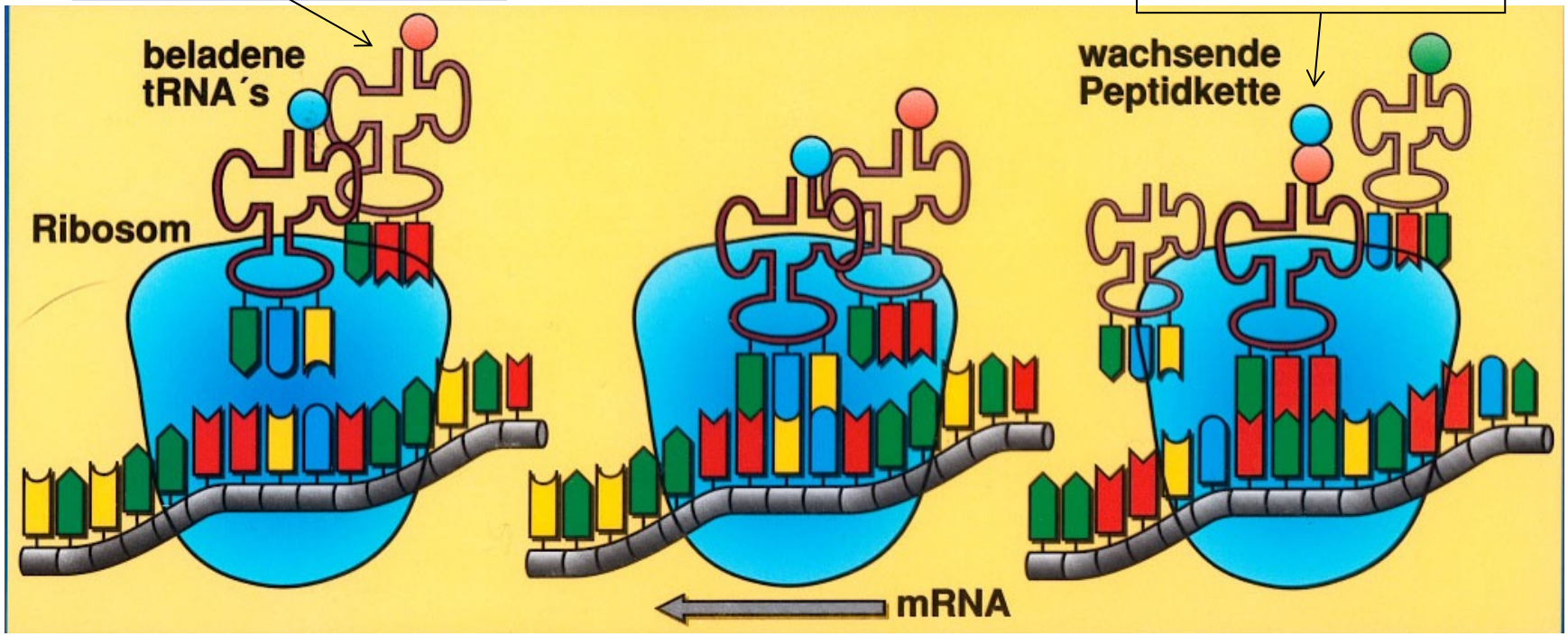
  Minimization of kinetic distance

  Validation of predicted rates

Speed of Ribosomes

tRNAs charged with amino acids

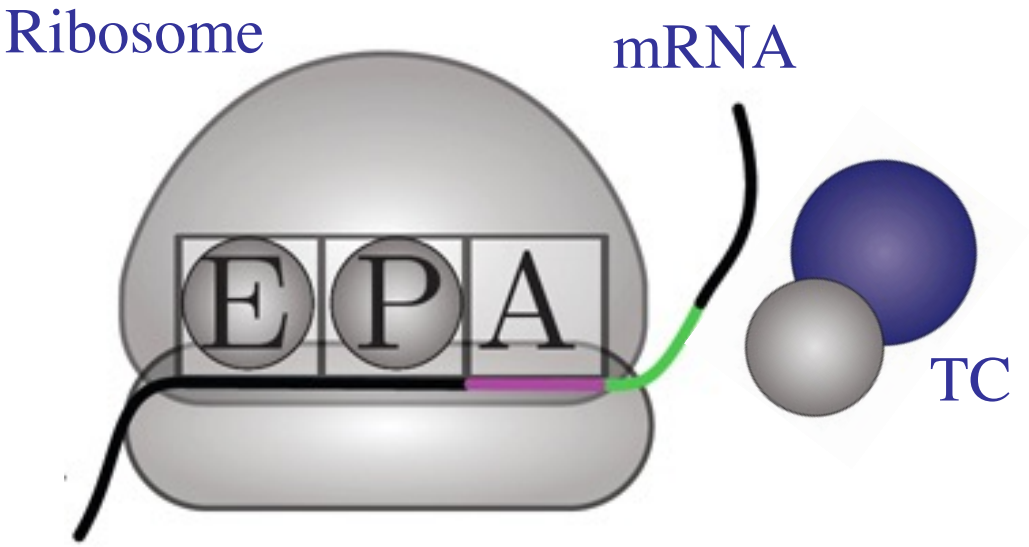
growing peptide chain



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



TC = ternary complex =
tRNA + EF-Tu + GTP

EF-Tu =
most abundant protein

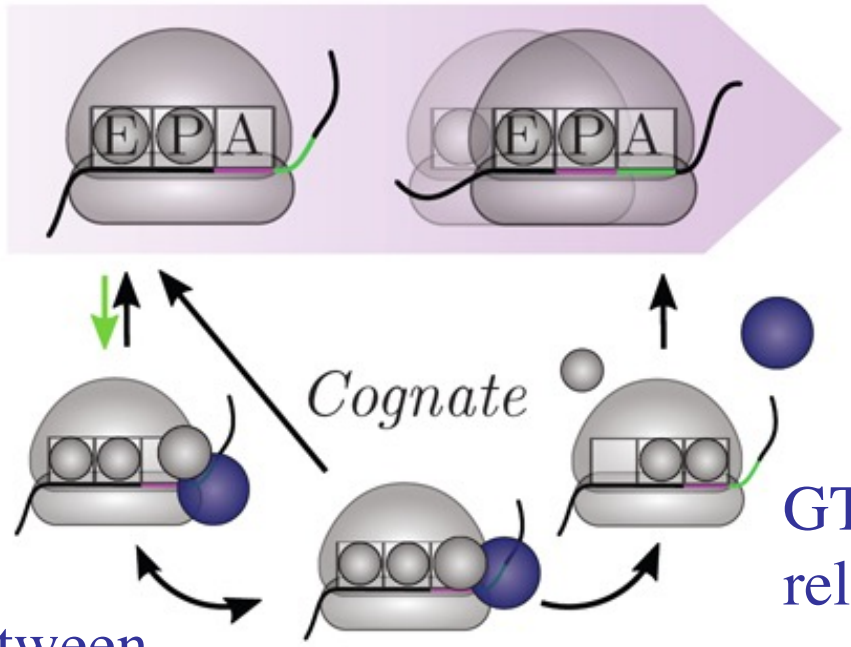
- Ribosome steps along codons of mRNA (purple -> green) consuming one ternary complex at each codon
- Elongation cycle during one step:

  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

Translocation

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

Recognition between codon and anti-codon

- Complexity of decoding:

  61 different codons and 43 elongator tRNA species (E. coli)

Codon-tRNA Relationships

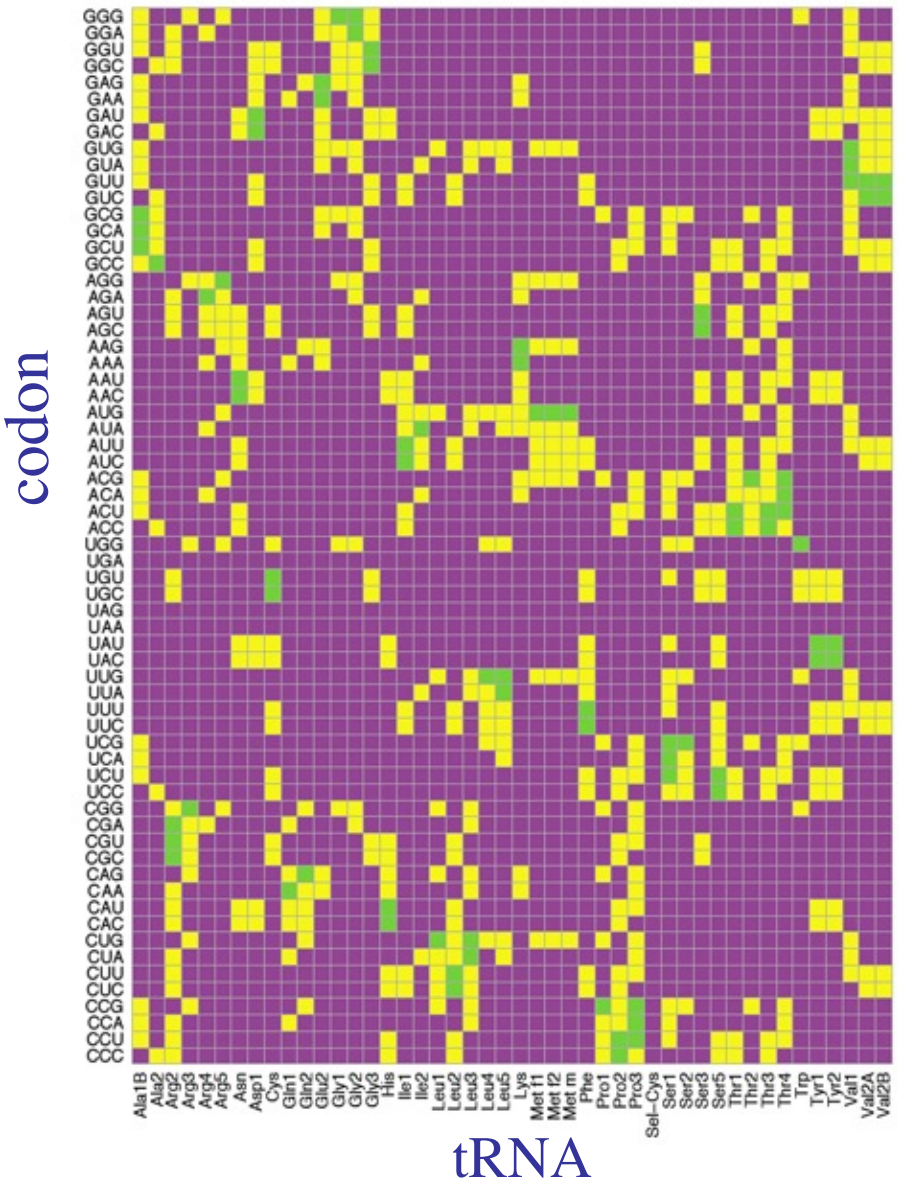
- cognate = green
- near-cognate = yellow
- non-cognate = red/purple

- cognate decoding
  => correct amino acid

- near-cognate decoding
  => incorrect amino acid

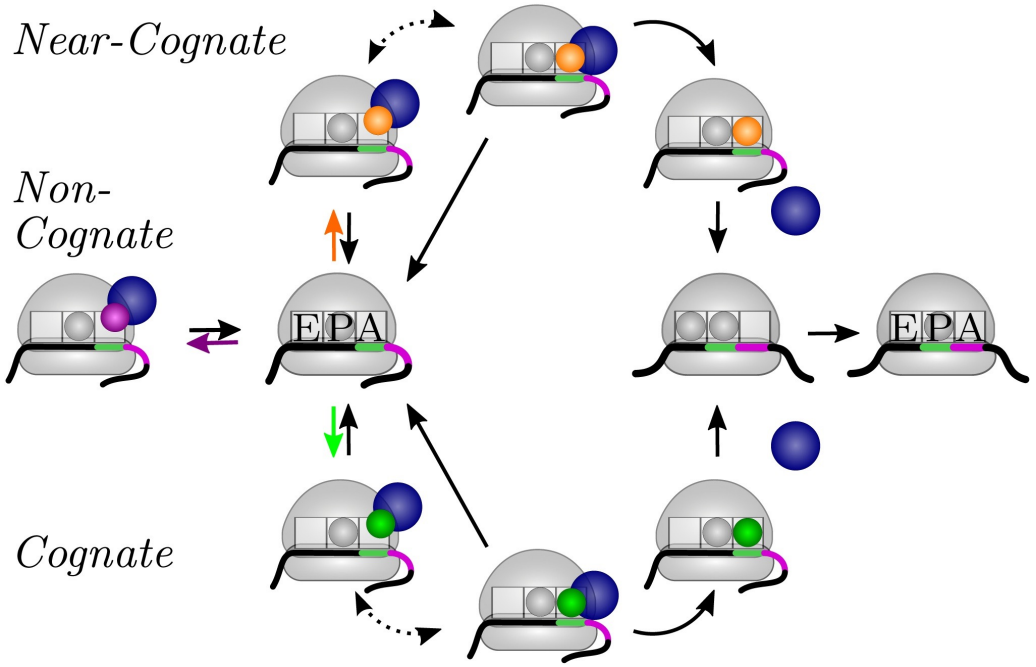
- non-cognate tRNAs are released after initial binding

Decoding pattern



Single Elongation Cycle - Refined

- Possible binding of cognate/near-cognate/non-cognate tRNAs:



- Accommodation of near-cognate tRNA

  => error rate

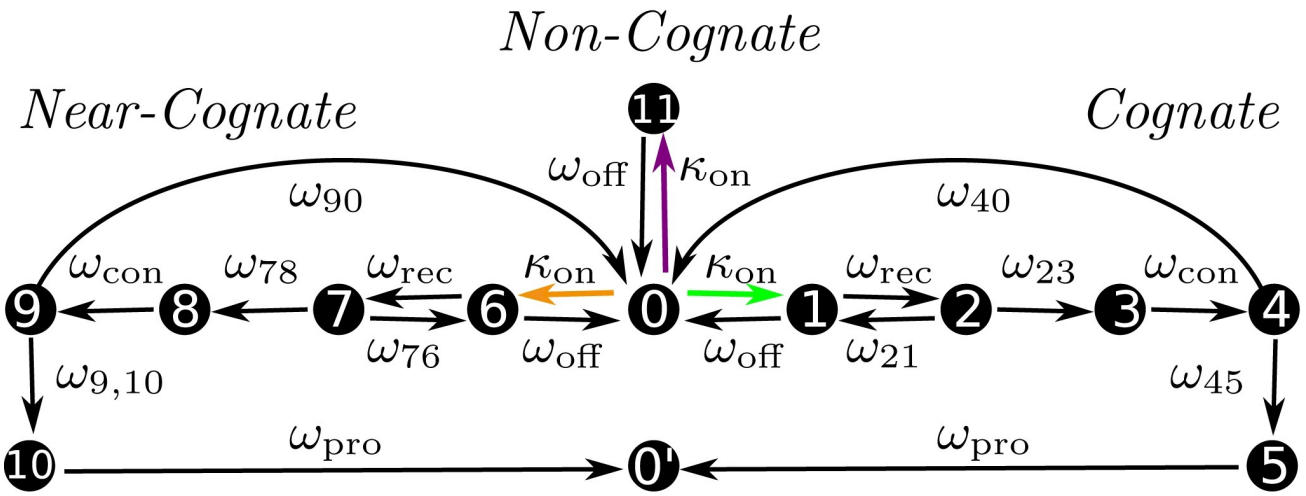
- Accommodation of cognate tRNA

- Competition between cognate, near-cognate, and non-cognate tRNAs

Markov Process

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

- Map cartoon of multistep process onto Markov chain:



- Individual transitions:

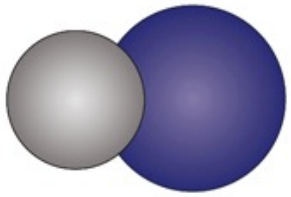
  initial binding, recognition, initial selection, GTP hydrolysis, phosphate release, proof reading, full accommodation

- All transition rates ω_{ij} have been measured in vitro
- Some rates identical for cognates and near-cognates

Transition Rates in vitro

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

Ribosome + mRNA + tRNAs



tRNA + EF-Tu + GTP
= ternary complex

- 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 ω_{ij}

  Wohlgemuth ... Rodnina, *Phil. Trans. Roy. Soc. B* (2011)

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

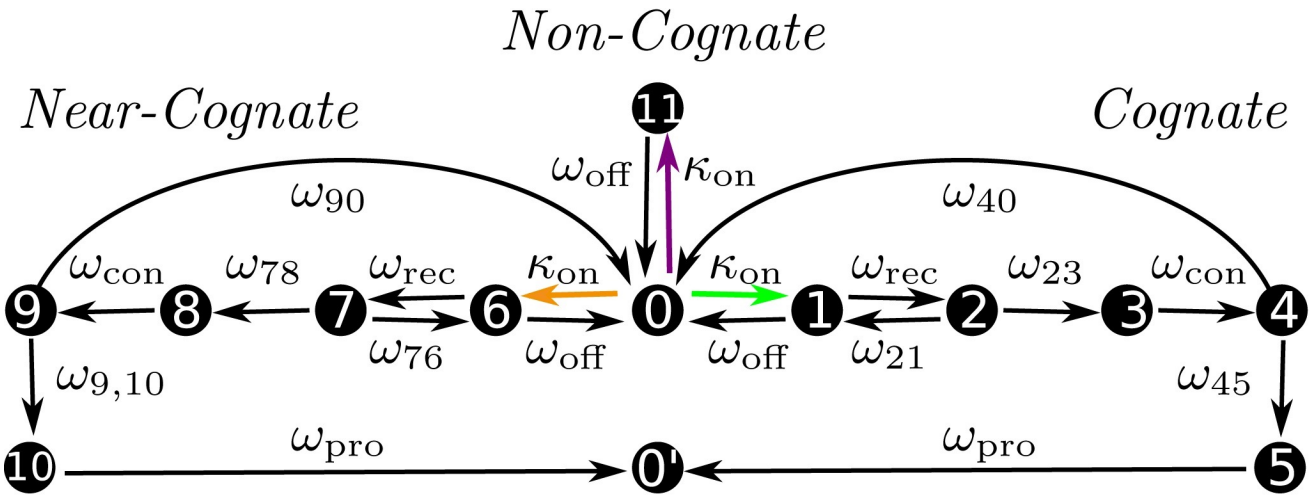
- Discrepancy for ribosome speed = peptide synthesis rate:
  In vivo value >> in vitro value

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

  Johansson ... Ehrenberg, *Curr. Op. Microbiology* (2008)

‘Similarity’ of In Vitro and In Vivo ?

- Multistep process with many individual transitions



- Set of in-vitro rates ω_{ij} ⇔ Set of in-vivo rates ω_{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 ω_{ij} , in-vivo value ω^*_{ij}
- Naive distance: Absolute value of $\omega_{ij} - \omega^*_{ij}$
- But: could equally well consider transition times

$$\tau_{ij} = 1/\omega_{ij} \quad \text{and} \quad \tau^*_{ij} = 1/\omega^*_{ij}$$

- 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

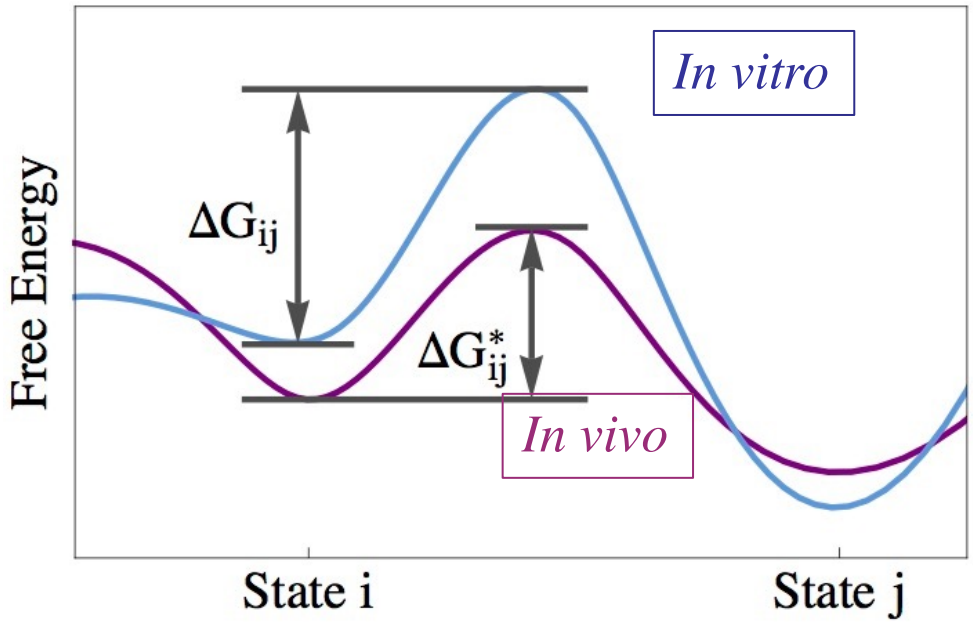
- Arrhenius form of transition rates:

$$\omega_{ij} = \nu_{ij} \exp[- \Delta G_{ij} / kB\ T\ ]$$

attempt frequency

free energy barrier

- Coordinates $\Delta_{ij} = \ln(\omega_{ij} / \omega^*_{ij})$ represent 'single barrier shifts'

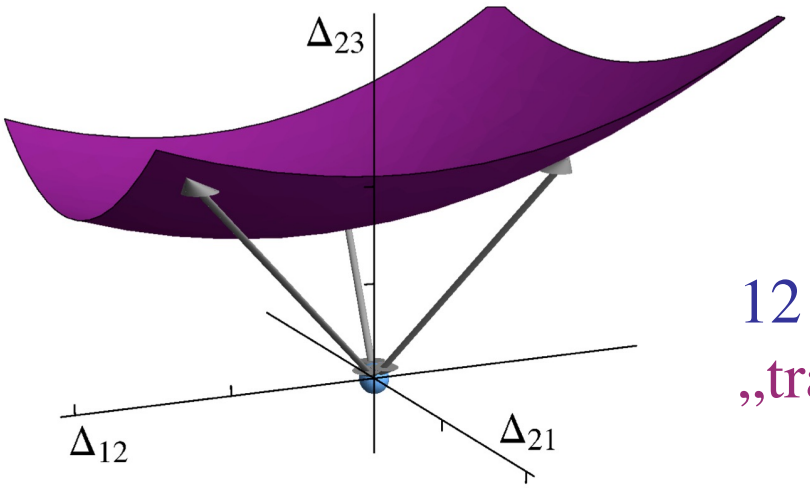


Kinetic Distance: Multistep Process

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

in vivo in vitro

- Multi-dimensional space with coordinates Δ_{ij}



3-dimensional subspace corresponding to three individual rates

12 distinct rates for elongation
„translation in 12 dimensions"

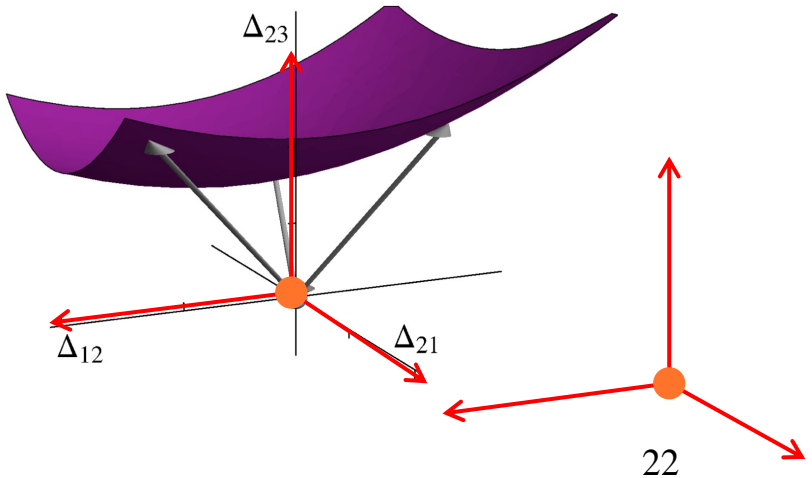
Kinetic Distance: Multistep Process

- Kinetic distance = Euclidean distance in Δ_{ij} –space:

$$\mathcal{D} \equiv \sqrt{\sum \Delta_{ij}^2} = \sqrt{\sum \left[\ln(\omega_{ij}^{\star}/\omega_{ij})\right]^2}$$

- What about ‚weight factors‘? Δ_{ij} replaced by $u_{ij}\ \Delta_{ij}$
- Limit of single transition => all $u_{ij} = 1$

- Two different assays, A1 and A2
- Change from A1 to A2 leads to simple coordinate transformation = shift of origin

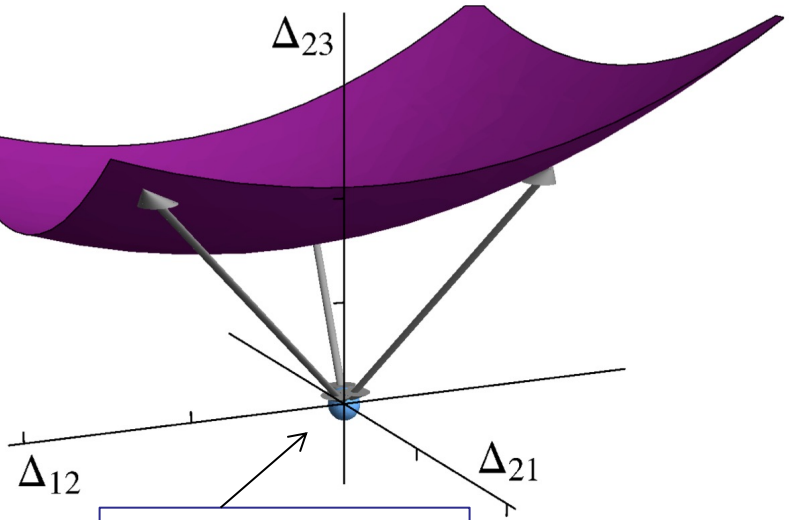


Minimization of Kinetic Distance

- 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 Δ_{ij} - space:

constraint => hypersurface with possible in-vivo points



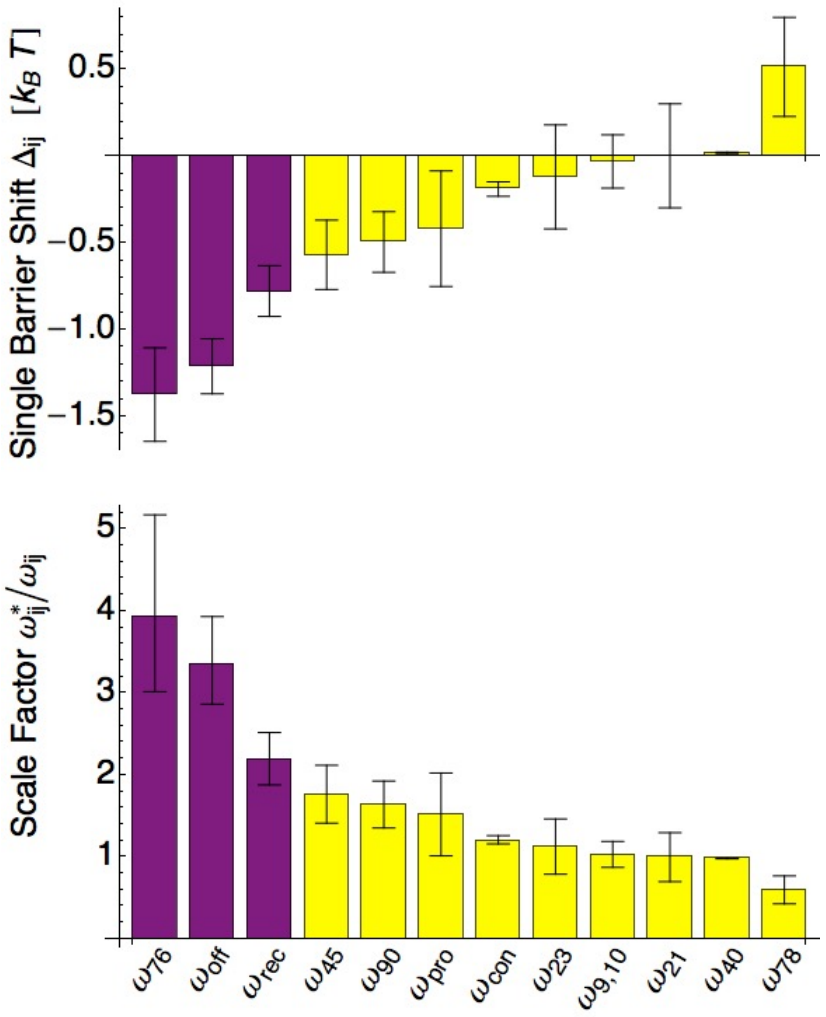
Predicted In-Vivo Point

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

- Single barrier shifts

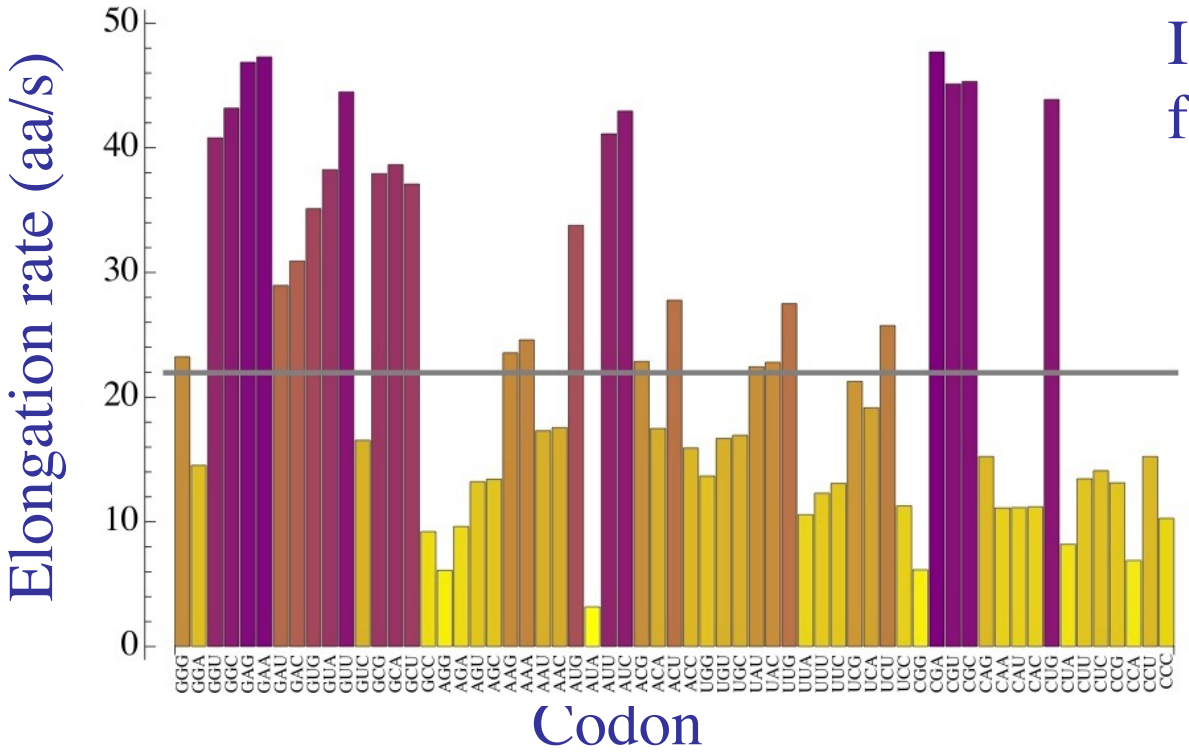
$$\Delta_{ij} = \ln(\omega^*_{ij}/\omega_{ij})$$

- 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:



In vivo profile, fast growth

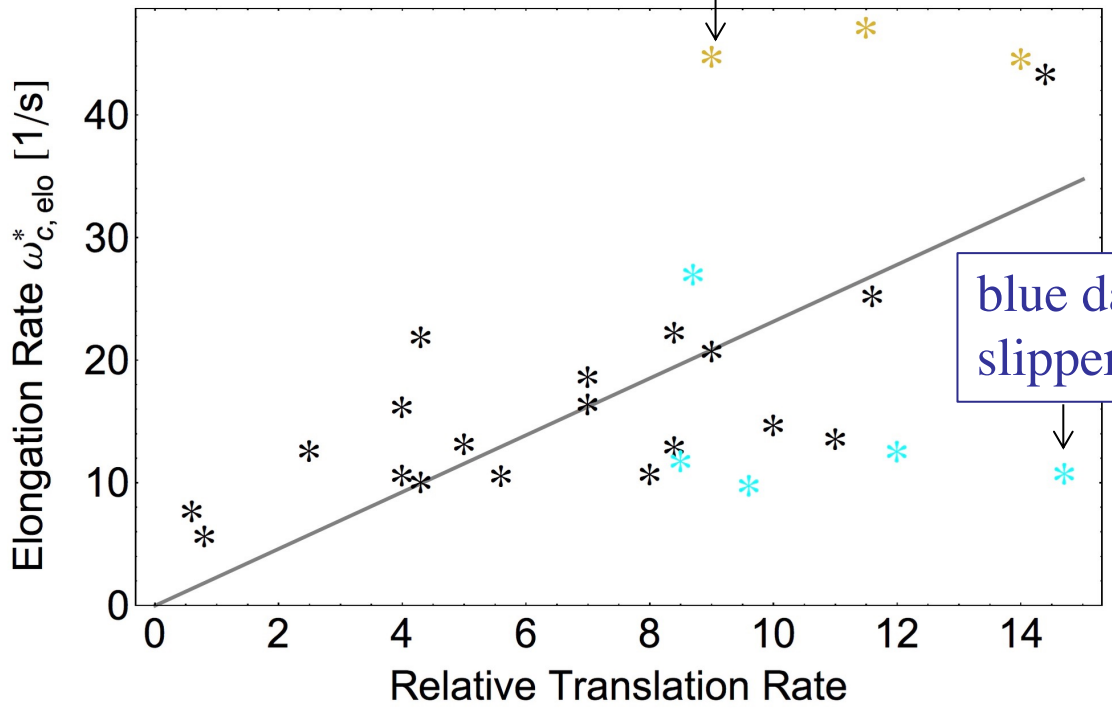
Validation by In-Vivo Data I

- Relative translation rates

Curran + Yarus, *J. Mol. Biol.* (1989)

yellow data: anticodons with wobble

Predicted c-specific elong. rates



blue data: slippery sequences

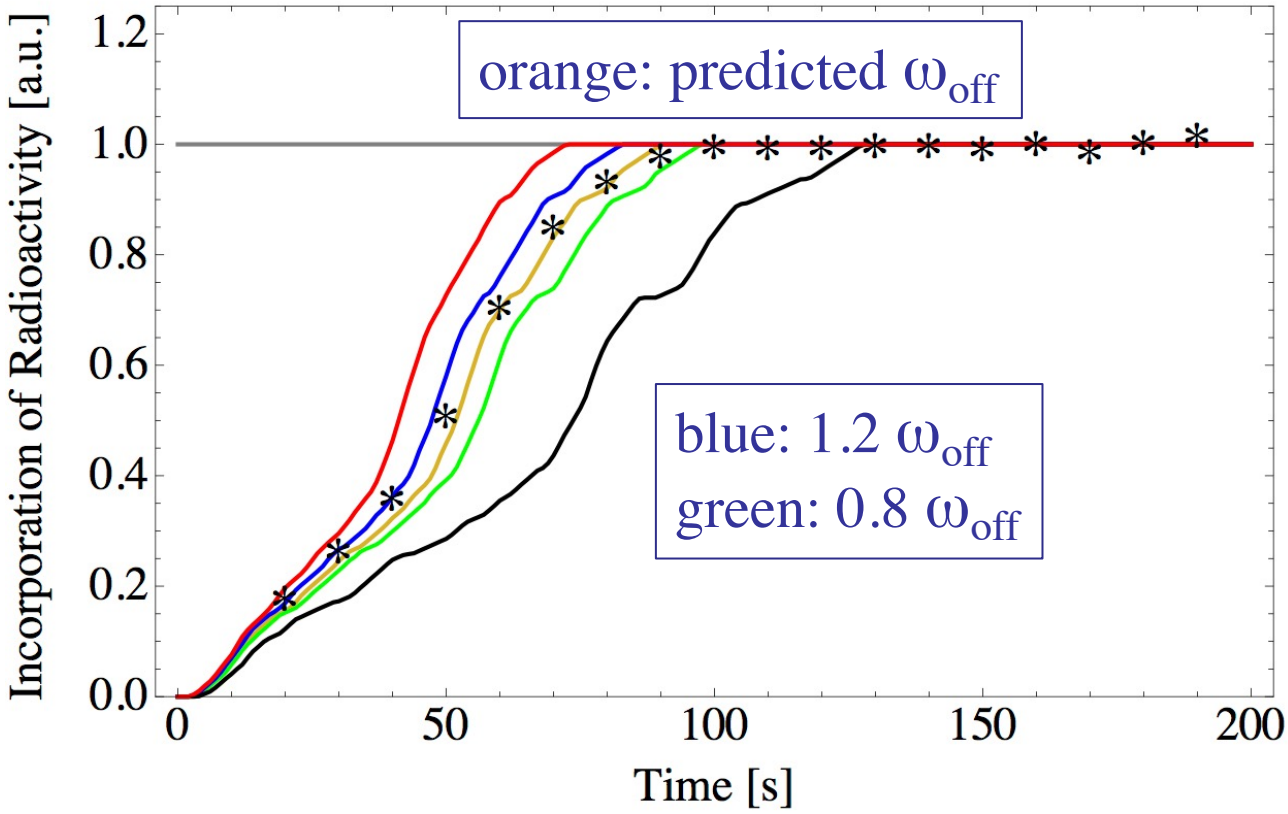
Experimental estimates

- Pearson correlation coefficent = 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



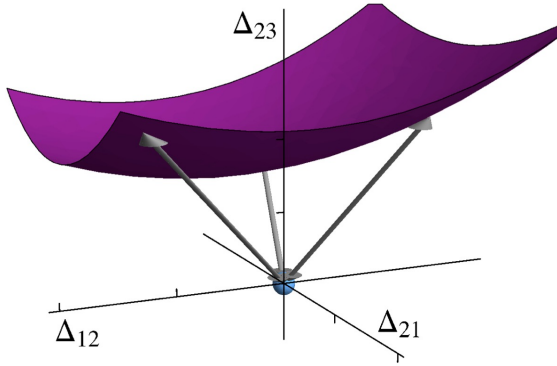
Validation by In-Vivo Data III

Kramer + Farabough, *RNA* (2007)

- 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 2×10^{-4}
- Predicted in vivo rates lead to 3×10^{-4}

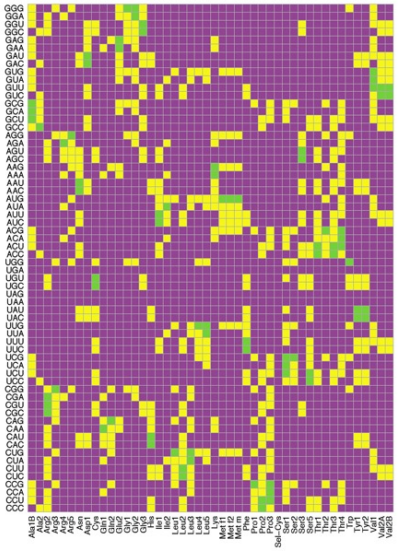
Good agreement with three independent sets of in vivo data without any fit parameter !

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 Δ_{ij} – space but no distance

Summary

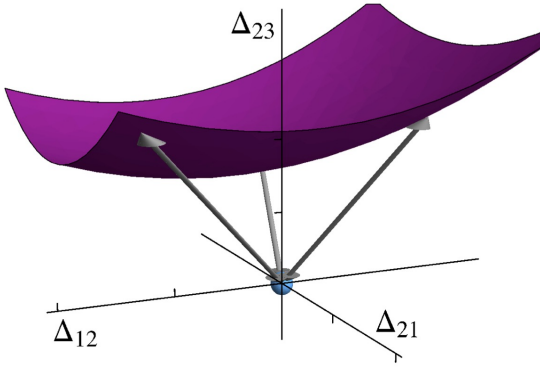


- Protein Synthesis by Ribosomes
  Competition between different tRNA species
- Kinetics in vivo and in vitro
  Long controversy about (dis)similarity
- Kinetic distance
  (Dis)similarity measure
  for multi-step process

$$\Delta_{ij} = \ln(\omega_{ij}^{*}/\omega_{ij})$$

in vivo in vitro

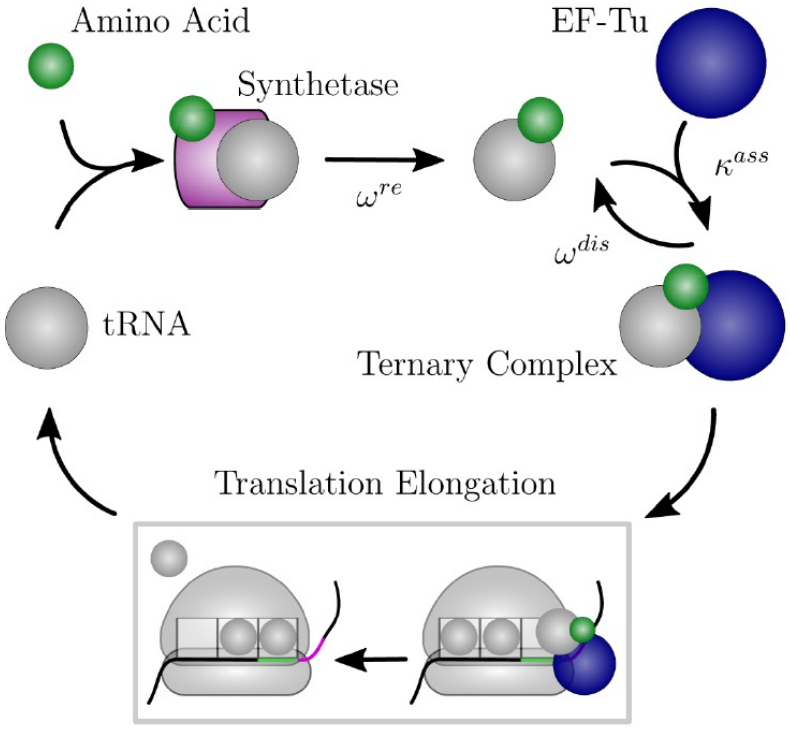
- Kinetic distance minimization
  Minimal distance of in-vitro point
  from in-vivo hyper-surface
- General computational method



Refined Modelling

Rudorf, RL, *PLoS ONE* (2015)

- Distinguish TC concentration from total tRNA concentration
- Important subpopulation: uncharged but bound to ribosome
- Include recharging cycle of tRNA



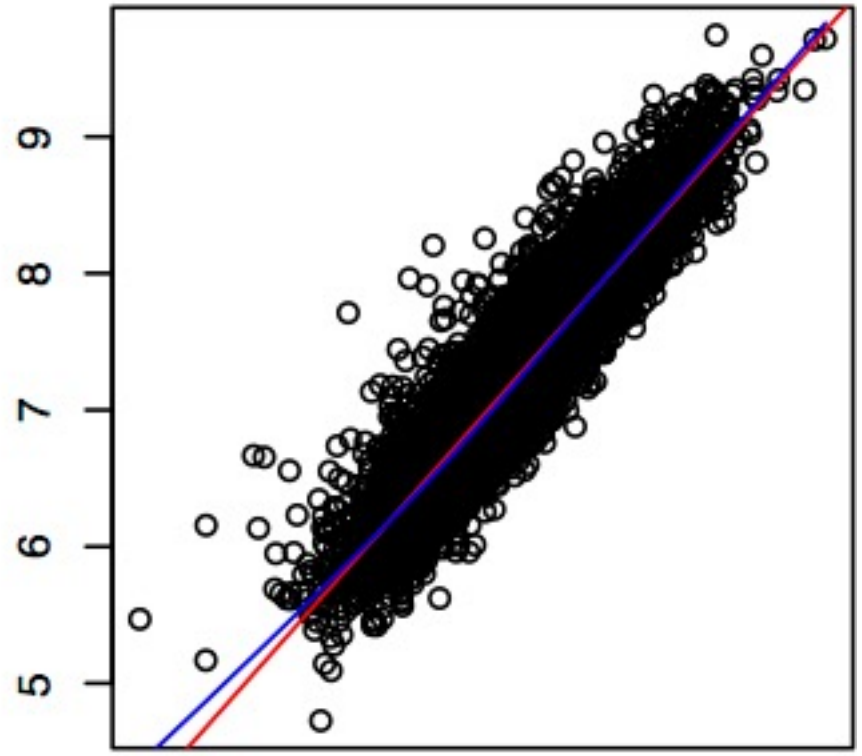
- Release of tRNA from E site
- Immediate release: 2-1-2 process
- Delayed release: 2-3-2 process

Extension to Human Cells

- Scatter plot for 7500 genes of Hela cells:

Sophia Rudorf
Jan Trösemeier
Christel Kamp

Total amount of protein per unit time



Average elongation rate for corresponding mRNA

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!