Noise and Non-equilibrium in Nano-machine Operation:
A **Physics** Perspective

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Outline

1. Motor **Mechanics**: Forces on a Molecular Motor in Aqueous Medium

2. Motor **Thermodynamics**:
   bypassing laws & beating limits by operating far from equilibrium

3. **Stochastic (Chemo-)mechanical Kinetics** of a motor:
   hopping on a "network" of chemo-mechanical states

4. **Reverse Engineering** Natural Nano-mechines:
   extracting nodes and links of the network from experimental data

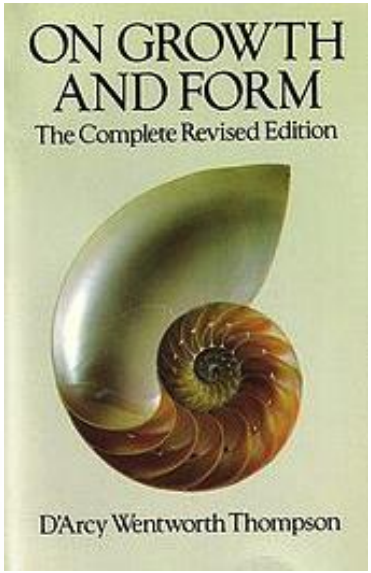
5. **Molecular Motor Traffic**:
   collective movement of motors on filamentous tracks

6. Summary and Conclusion

Motor Mechanics:
Forces on a Molecular Motor

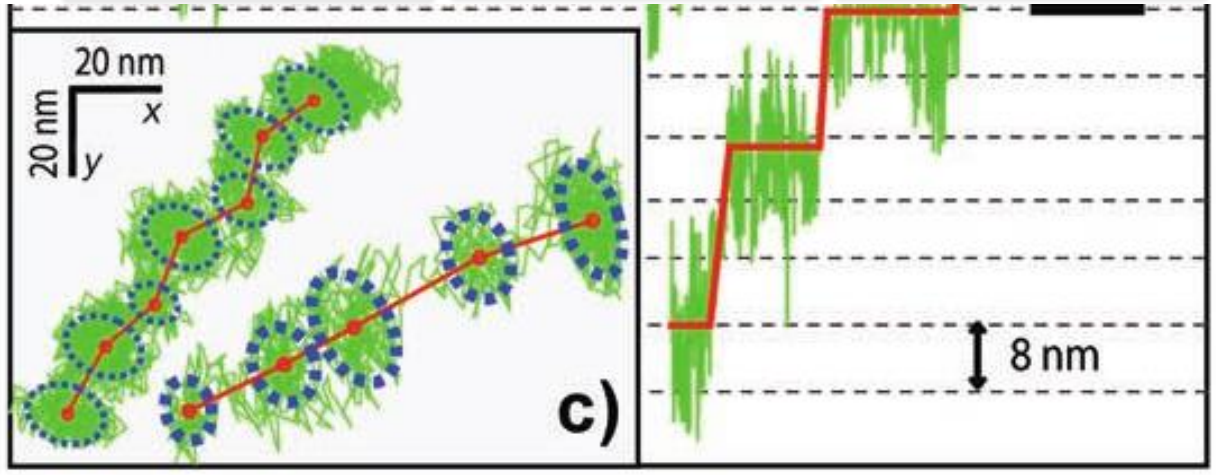
Forces on a Motor

"…gravitation is forgotten, and the viscosity of the liquid,…,the molecular shocks of the Brownian movement, …. Make up the physical environment….The predominant factor are no longer those of our scale; we have come to the edge of a world of which we have no experience, and where all our preconceptions must be recast". - **D'Arcy Thompson**.



Dominant Forces on a Motor

- External Force (Conservative)
- Viscous drag (Dissipative)
- Thermal (Random)



Nan et al. ChemPhysChem (2008)

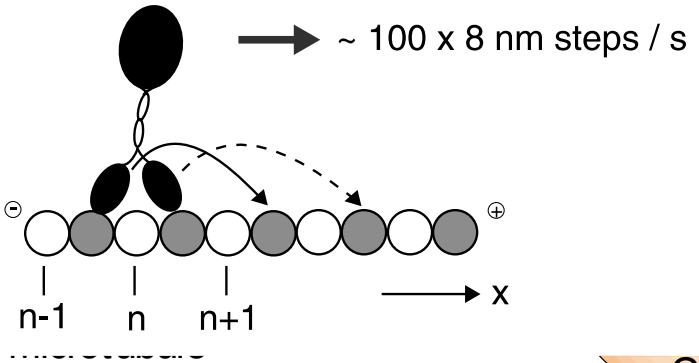
Motor Thermodynamics:
Bypassing Laws and Beating Limits
by operating
Far from Equilibrium

Fundamental Questions

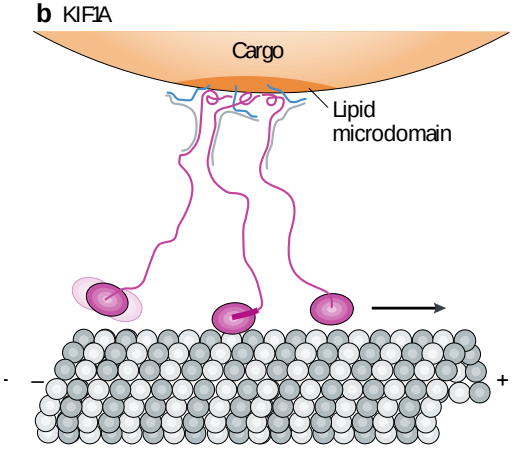
- How do force-generating molecular machines exploit noise for energy transduction without violating the **2nd Law** of thermodynamics?

- How do information-processing machines achieve accuracy far higher than the upper limits allowed by the laws of thermodynamics?

A 2-headed motor protein carrying `cargo' is like a `porter'



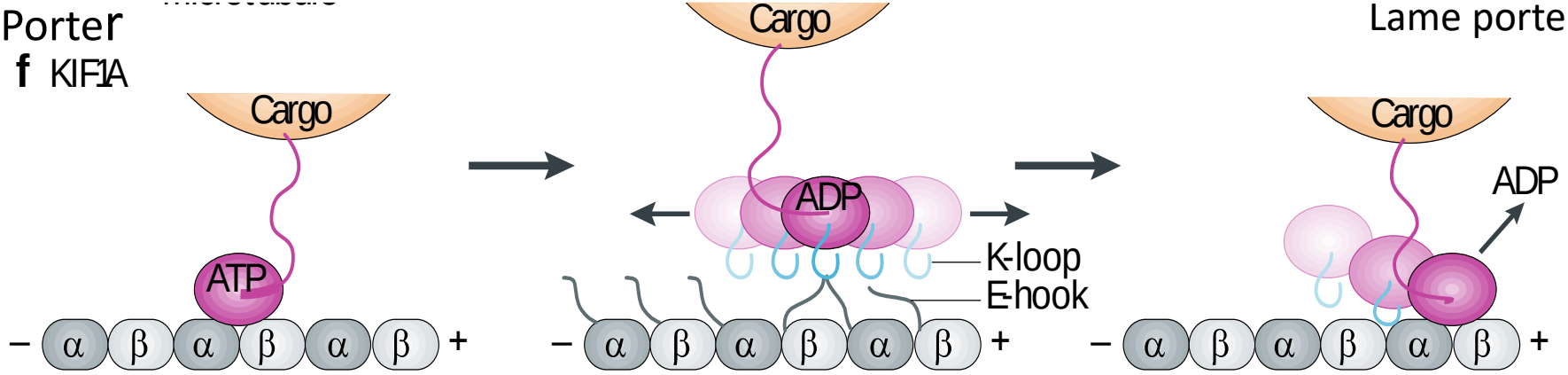
A single-headed motor protein is like a lame `porter'



Porter

f KIF1A

Lame porter



Hirokawa, Nitta, Okada, Nat. Rev. Mol. Cell Biol. 10, 877 (2009).

Genetically varied the number of charged amino acids on KIF1A and "digested" E-hook.

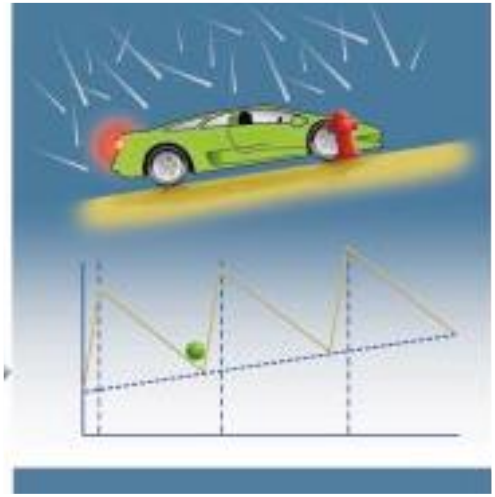
Conclusion: Electrostatic attraction between the K-loop of KIF1A and E-hook of MT acts as a tether and enables 1-dimensional diffusion of KIF1A along MT till release of the products of hydrolysis and re-binding to MT: ***BROWNIAN RATCHET* (EXPLOITING Noise** for Motility**)**

Nishinari, Okada, Schadschneider & Chowdhury, Phys. Rev. Lett (2005);
Greulich, Garai, Nishinari, Schadschneider, Chowdhury, Phys. Rev. E (2007).

Exploiting NOISE for Directed Movement of Motors

"*Sisyphus*" at the Nano-scale: Brownian Ratchet

A Brownian motor does not violate 2nd law of thermodynamics as it operates far from equilibrium where the 2nd law is not applicable.



R.D.Astumian , Scientific American, July, 2001

Feynman ratchet-and-pawl device

Stochastic (Chemo-)Mechanical kinetics of a motor:

Hopping on a “Network”

Master equation: general form for "***States and Rates***"

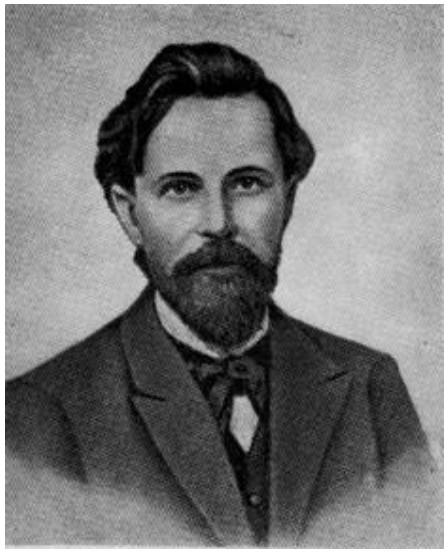
$P_n(t)$ = Probability of finding the "particle" in the discrete state n at time t.

$$dP_n(t)/dt = \sum_m P_m(t)\, W(m \rightarrow n) - \sum_{m'} P_n(t)\, W(n \rightarrow m')$$

GAIN terms Loss terms

$W(n \rightarrow m)$ = Probability of transition $n \rightarrow m$ per unit time ("rate constant").

Markov Process: Frog in a Lily Pond



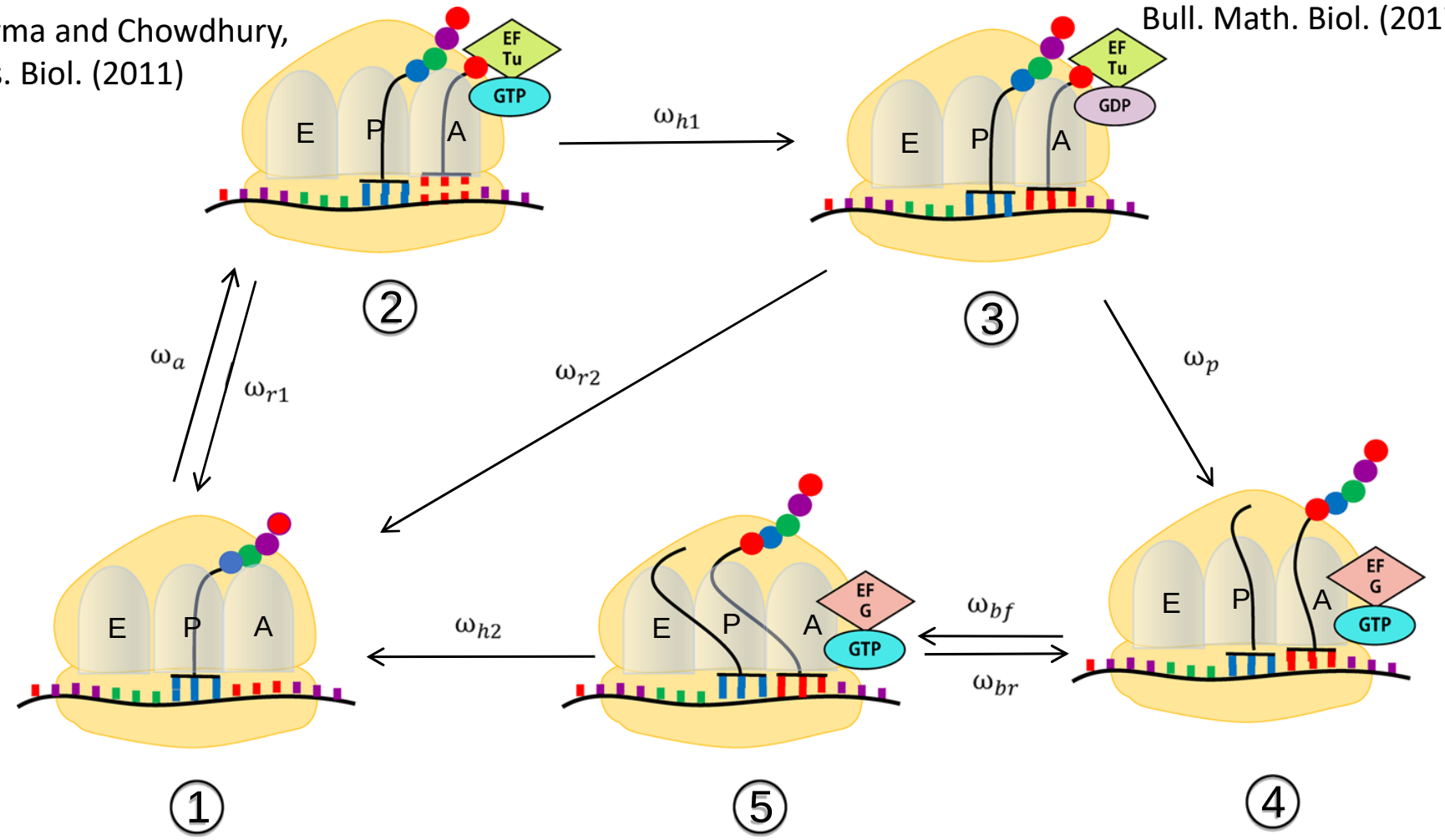
A. A. Markov

The discrete mechano-chemical states of the machine form the vertices of a ***network (graph)*** while the directed edges denote the allowed transitions.

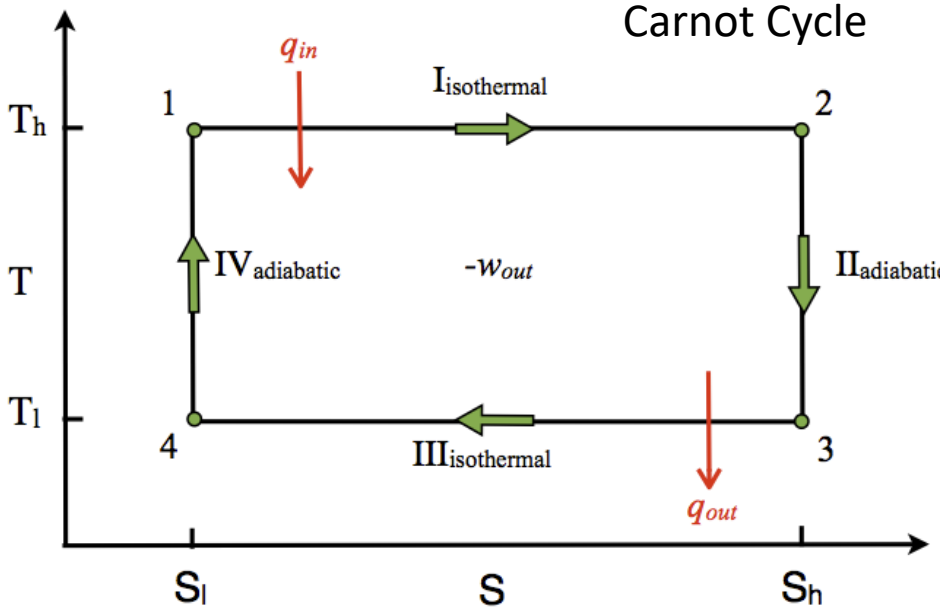
Machine operation, i.e., its stochastic kinetics, is modeled as a ***Markov process*** in a heat bath at a constant temperature and it is formulated in terms of ***master equations***.

Sharma and Chowdhury, Phys. Biol. (2011)

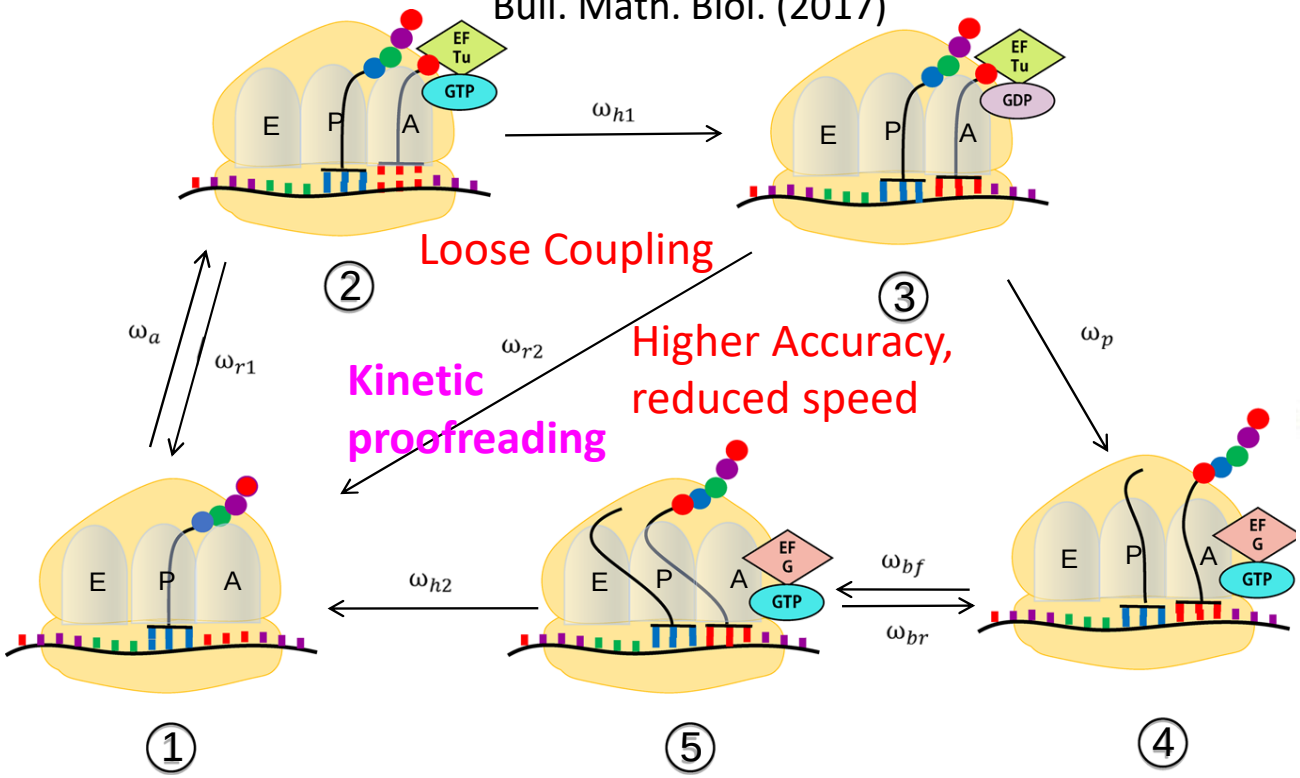
Dutta and Chowdhury, Bull. Math. Biol. (2017)

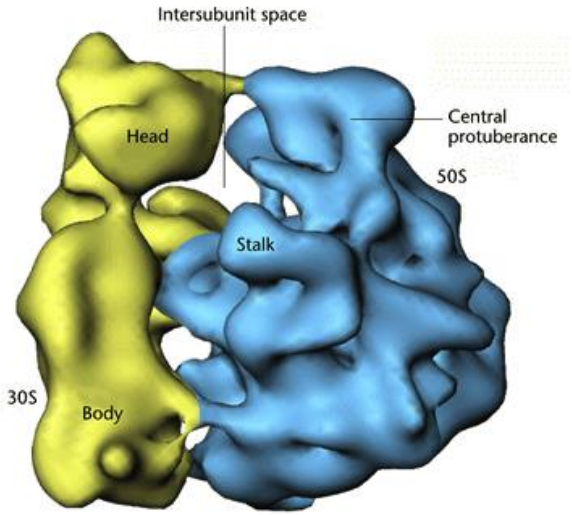


Sadi Carnot (1796-1832)



Dutta and Chowdhury, Bull. Math. Biol. (2017)

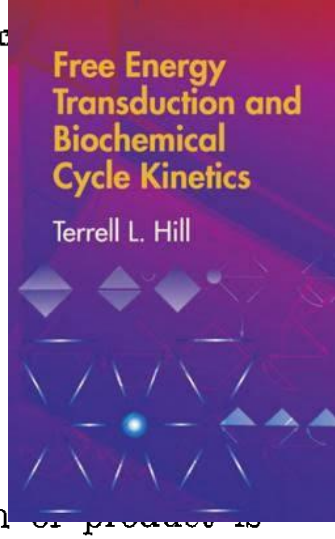




Ribosome

purpose of this article is to review some of the mathematical and physical techniques which have turned out to yield appropriate descriptions of such nonequilibrium situations. The starting point of all these techniques is a master equation formulation o
equation

time-dependent solutions in the physic
the region where



expression for $(EX_m)/[E]_0$ is the sum of the several different numerators.

A simple example will serve to illustrate this method. The mechanism involves 3 enzyme-containing species; thus each term in the expression for the relative concentration of E, ES or EP contains 2 rate constants and the appropriate

$$E + S \underset{}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_3}{\rightleftharpoons} EP \overset{k_5}{\rightleftharpoons} E + P$$

Since the net rate of production of product is

$$\frac{d(P)}{dt} = \frac{\{k_5(EP) - k_6(E)(P)\}[E]_0}{(E) + (ES) + (EP)}$$

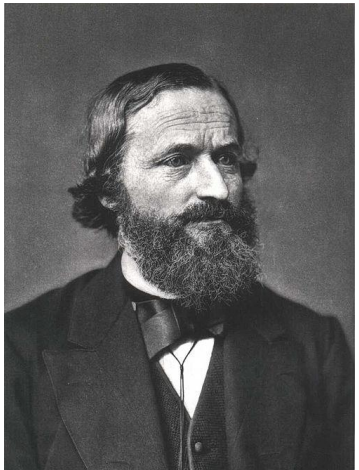
$$\frac{d(P)}{dt} = \frac{\{k_1k_3k_5(S) - k_2k_4k_6(P)\}[E]_0}{k_2k_5 + k_2k_4 + k_3k_5 + (S)(k_1k_5 + k_1k_4 + k_1k_3) + (P)(k_2k_6 + k_3k_6 + k_4k_6)}$$

It is to be noted that the patterns of line segments which are the schematic representations of the three numerator terms in the expressions for $(EX_i)/[E]_0$ are the same for each of the enzyme-

IRE TRANSACTIONS ON CIRCUIT THEORY *March* Gustav Kirchoff

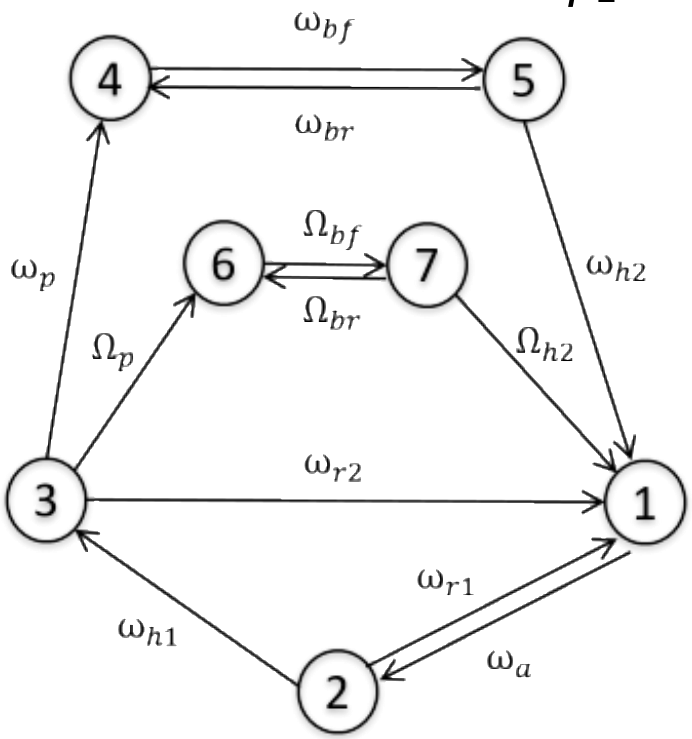
On the Solution of the Equations Obtained from the Investigation of the Linear Distribution of Galvanic Currents*

By G. KIRCHHOFF
Translated by J. B. O'TOOLE†



Elongation Cycle of Translation by RIBOSOME

$$p_1 = \frac{A(T_1^2(G)) + A(T_1^3(G)) + A(T_1^5(G)) + A(T_1^8(G)) + A(T_1^{10}(G)) + A(T_1^{11}(G))}{S}$$



$$T_i^{(\mu)}(G)$$

i-directed μ-th maximal tree on directed graph G

$$p_2 = \frac{A(T_2^2(G)) + A(T_2^{10}(G)) + A(T_2^{11}(G))}{S}$$

$$p_3 = \frac{A(T_3^9(G))}{S}$$

$$p_4 = \frac{A(T_4^6(G)) + A(T_4^{15}(G))}{S}$$

$$p_5 = \frac{A(T_5^{15}(G))}{S}$$

$$p_6 = \frac{A(T_6^{28}(G)) + A(T_6^{29}(G))}{S}$$

$$p_7 = \frac{A(T_7^{28}(G))}{S}$$

Wong, Dutta, Chowdhury, Gunawardena, to be published (2017)

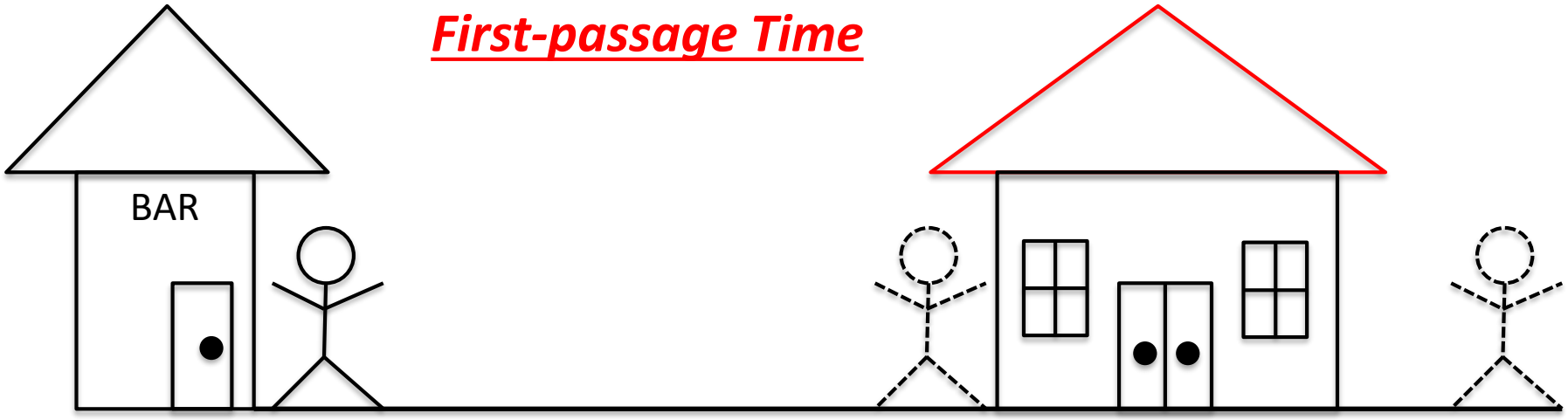
Fundamental Questions on **times taken by a motor** for **various stochastic chemo-mechanical kinetic processes: First-Passage times**

There are multiple pathways, each with multiple intermediate states with distinct transition rates, between the two arbitrary nodes of the directed network of mechano-chemical states.

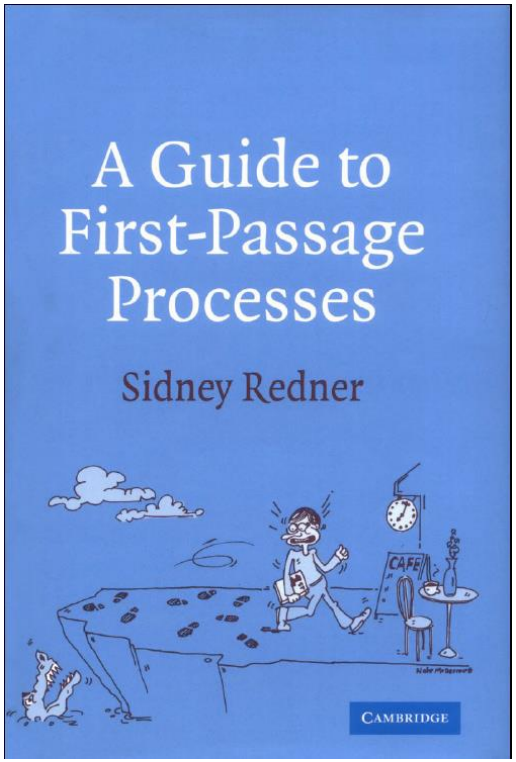
- What is the ***distributions of times taken for the transition between two given states (a First-Passage Time)*** and ***how does this time depend on the topology of the landscape/network***?

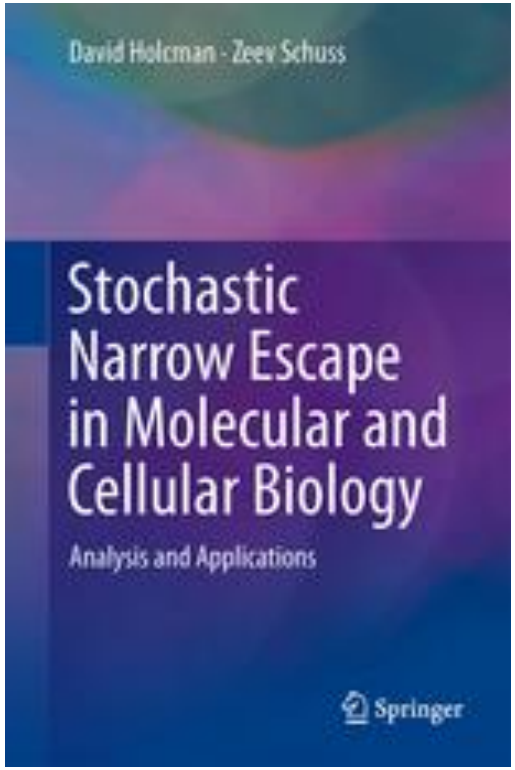
- How does the landscape / directed network get ***altered by physical (force) or chemical (ligand binding and/or reaction, particularly fuel `burning') perturbation*** and how does the ***average rate depend on the force and fuel/substrate concentration***?

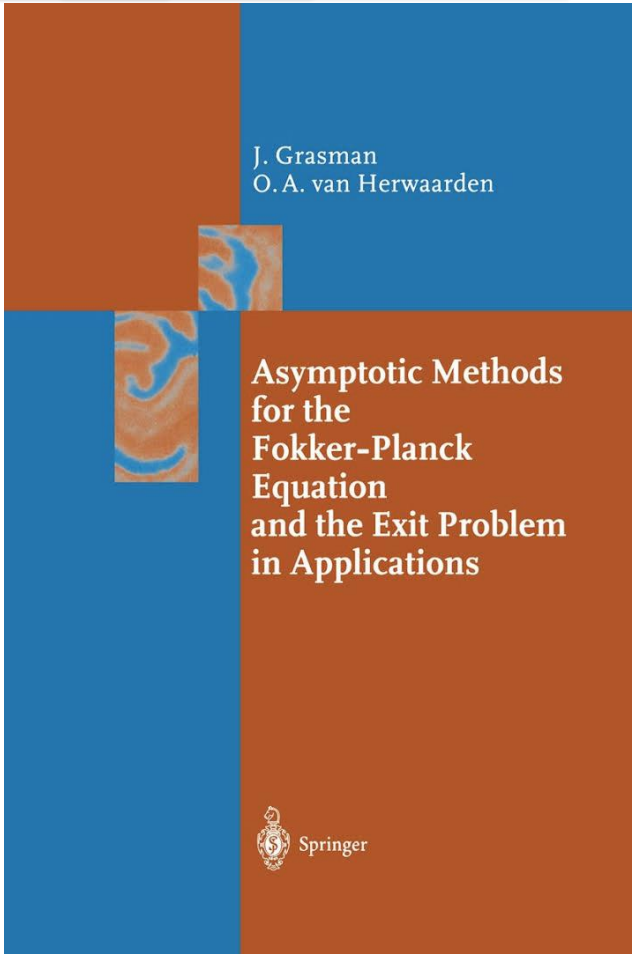
First-passage Time



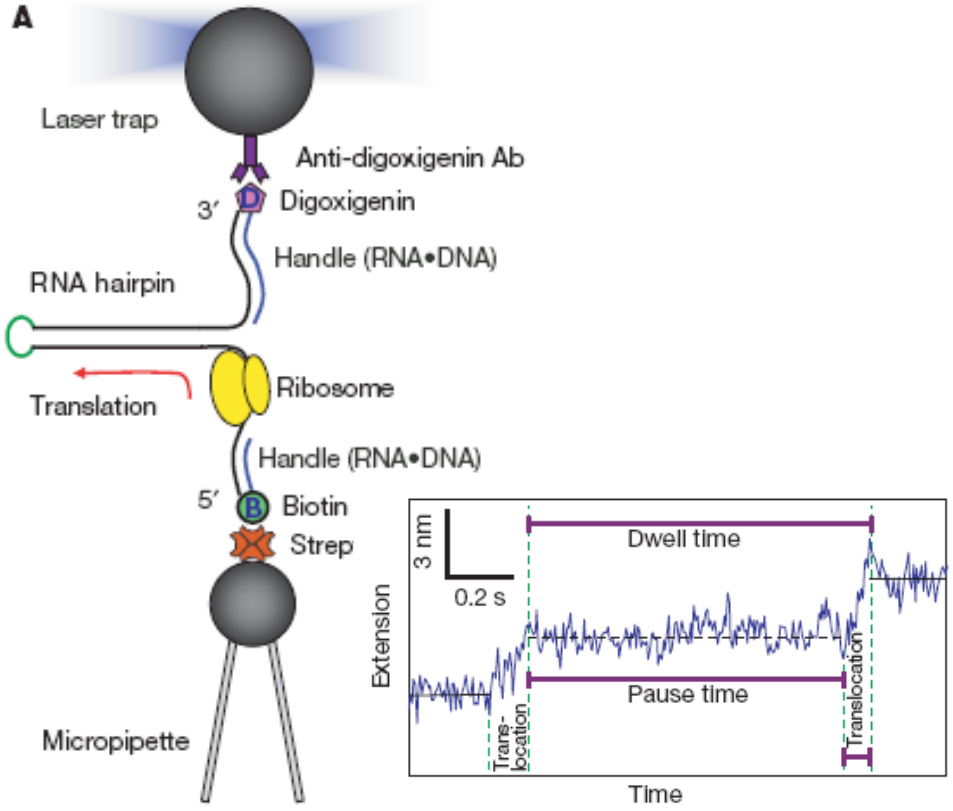
How much time does the drunkard take to reach the door of his ***house for the first time***?



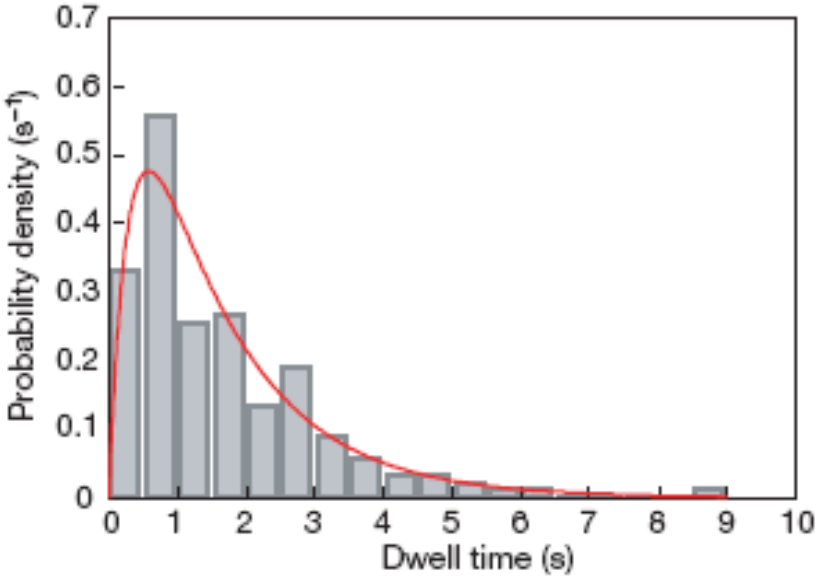




Stochastic pause-and-translocation of a ribosome: **DWELL TIME** Distribution



Wen,…, Noller, Bustamante and Tinoco Jr., Nature (2008)



Dwell Time

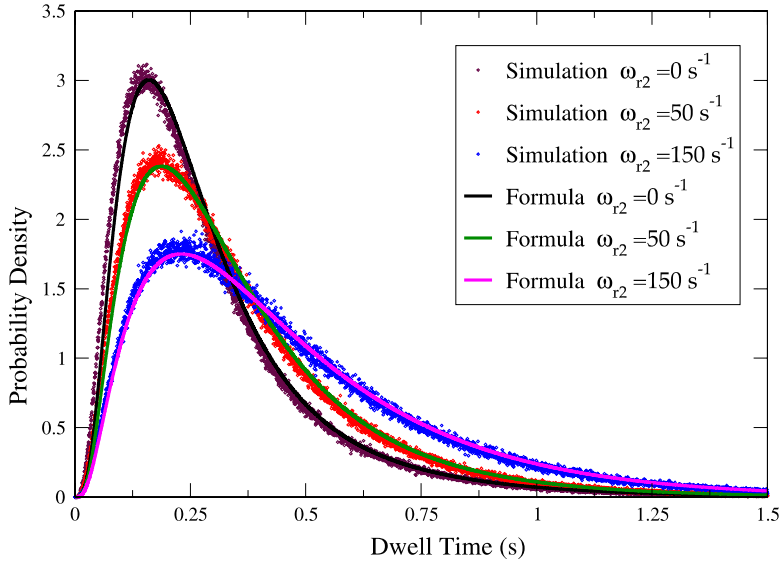
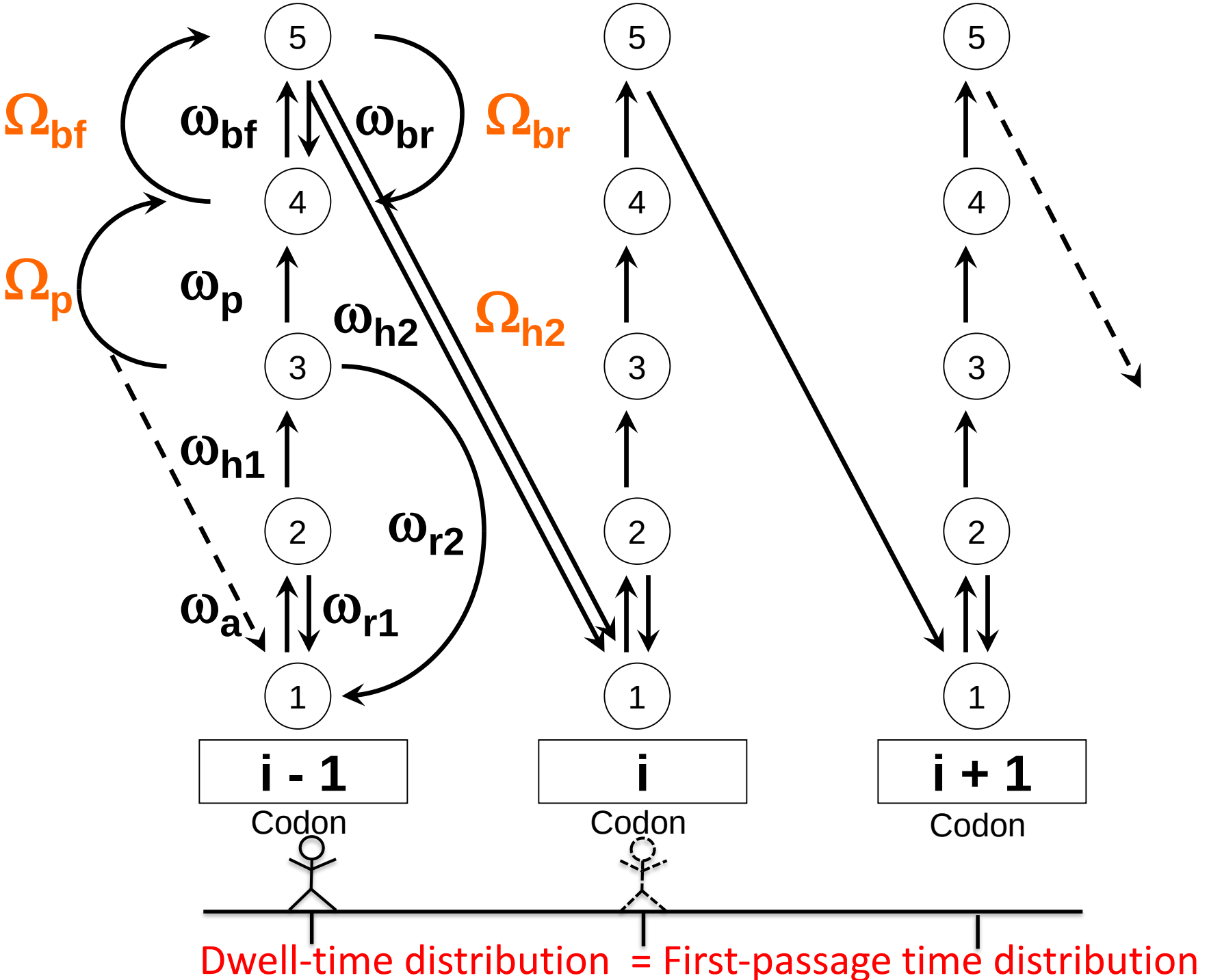


Figure 6. The probability density of the dwell times are plotted for a few different values of the parameter ω_{r2}.

Basu & Chowdhury, Phys. Rev. E (2007)
Garai, Chowdhury, Ramakrishnan, Phys. Rev E (2009)
Garai, Chowdhury, Chowdhury, Ramakrishnan, Phys. Rev. E (2009)
Sharma & Chowdhury, Phys. Rev. E (2010)
Sharma & Chowdhury, Phys. Biol. (2011)
Sharma & Chowdhury, J. Theor Biol. (2011)


5
4
3
2
1
Ω_{bf}
ω_{bf}
ω_{br}
Ω_{br}
Ω_{p}
ω_{p}
ω_{h2}
Ω_{h2}
ω_{h1}
ω_{r2}
ω_{a}
ω_{r1}
i - 1
i
i + 1
Codon
Codon
Codon
Dwell-time distribution = First-passage time distribution

Sharma & Chowdhury, Phys. Biol. (2011)

Average velocity of a Ribosome Motor: Dependence on substrate concentration

Garai, Chowdhury, Chowdhury and Ramakrishnan, PRE (2009)

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \frac{1}{[\mathrm{tRNA}]},$$

A Generalized Michaelis-Menten Eqn.

Leonor Michaelis

Maud Menten

$$K_M = \frac{\omega_{-1}^{\mathrm{eff}} + \omega_2^{\mathrm{eff}}}{\omega_a^0}$$

$$\omega_a = \omega_a^0 [\mathrm{tRNA}]$$

$$\omega_{-1}^{\mathrm{eff}} = \left(\frac{\omega_{r1}}{\omega_{h1}} + \frac{\omega_{r2}}{\omega_p + \Omega_p} + \frac{\omega_{r1}\omega_{r2}}{\omega_{h1}(\omega_p + \Omega_p)} \right) \omega_2^{\mathrm{eff}}$$

$$E + S \underset{\omega_{-1}}{\overset{\omega_{+1}}{\rightleftharpoons}} I_1 \xrightarrow{\omega_2} E + P$$

$$\frac{1}{V_{\max}} = \frac{1}{\omega_2^{\mathrm{eff}}} = \frac{1}{\omega_{h1}} \left(1 + \frac{\omega_{r2}}{\omega_p + \Omega_p} \right) + \frac{1}{\omega_p + \Omega_p}$$

$$+ \frac{\omega_p}{\omega_p + \Omega_p} \left[\frac{1}{\omega_{bf}} \left(1 + \frac{\omega_{br}}{\omega_{h2}} \right) + \frac{1}{\omega_{h2}} \right]$$

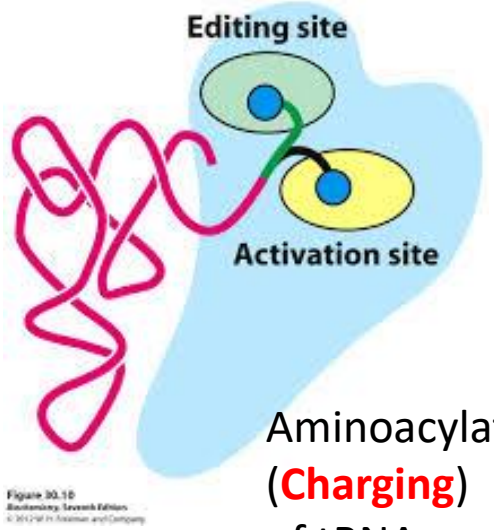
$$+ \frac{\Omega_p}{\omega_p + \Omega_p} \left[\frac{1}{\Omega_{bf}} \left(1 + \frac{\Omega_{br}}{\Omega_{h2}} \right) + \frac{1}{\Omega_{h2}} \right]$$

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \frac{1}{[S]}$$

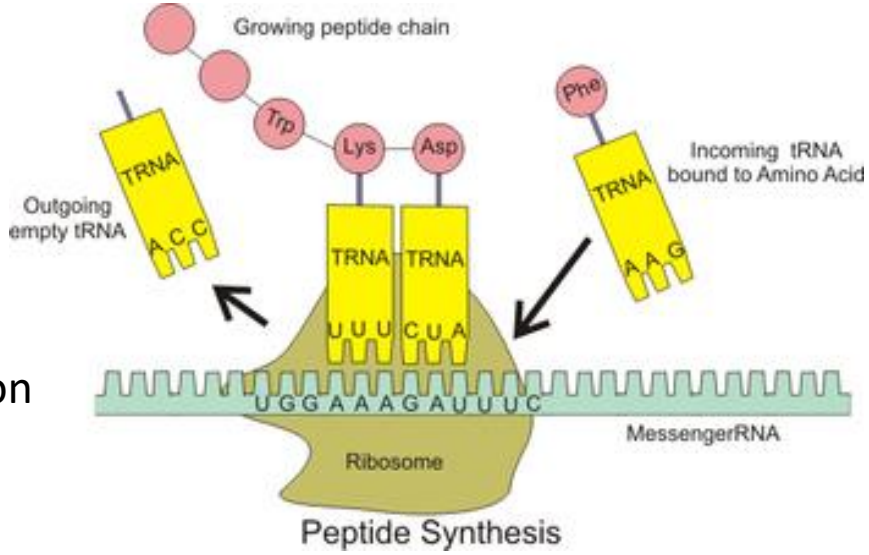
$$K_M = \frac{\omega_{-1} + \omega_{+2}}{\omega_{+1}}$$

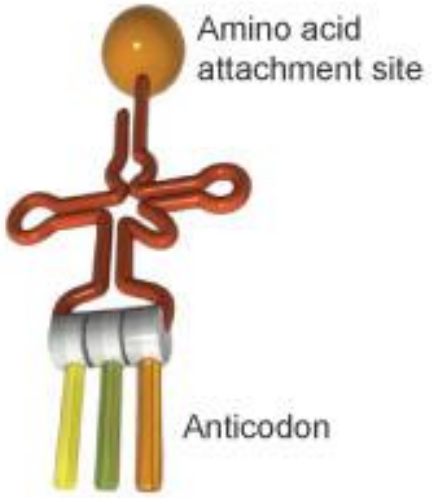
Chowdhury, FEBS J. 281, 601 (2014)

Effects of Random Translational error on the average speed



Aminoacylation (**Charging**) of tRNA

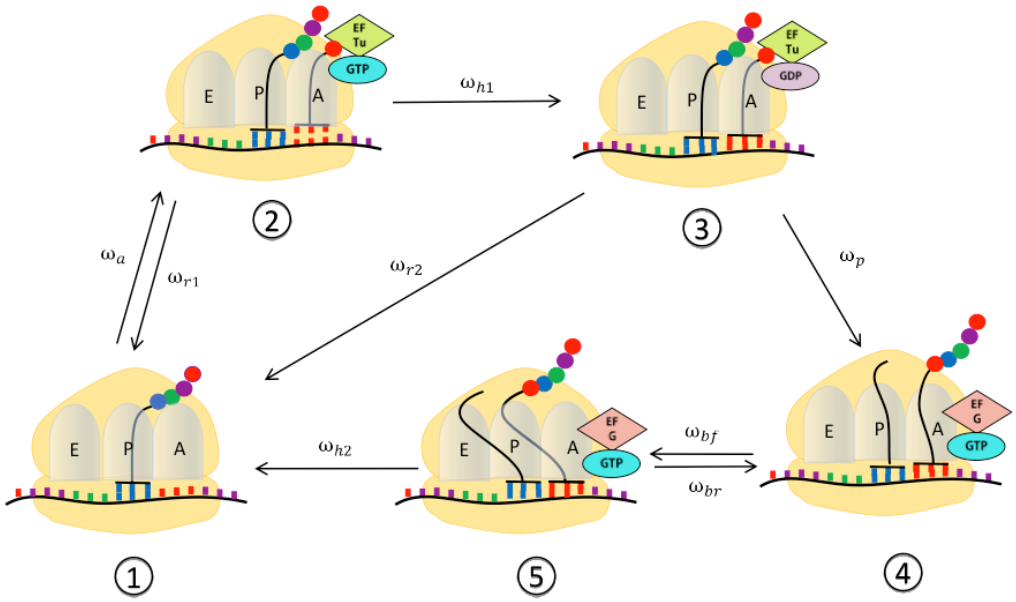




From: Nature Education

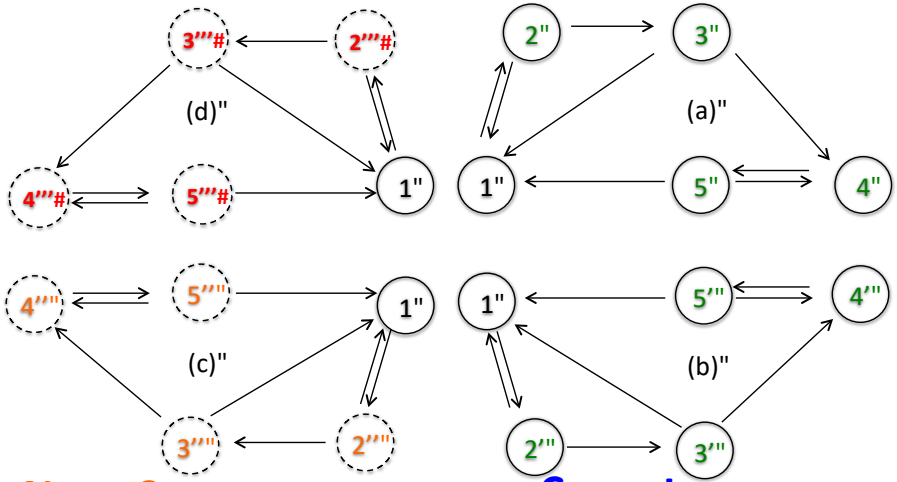
Charging error

Reading error



Non-Cognate, correctly charged

Cognate, Correctly charged



Near-Cognate, correctly charged

Cognate, mis-charged

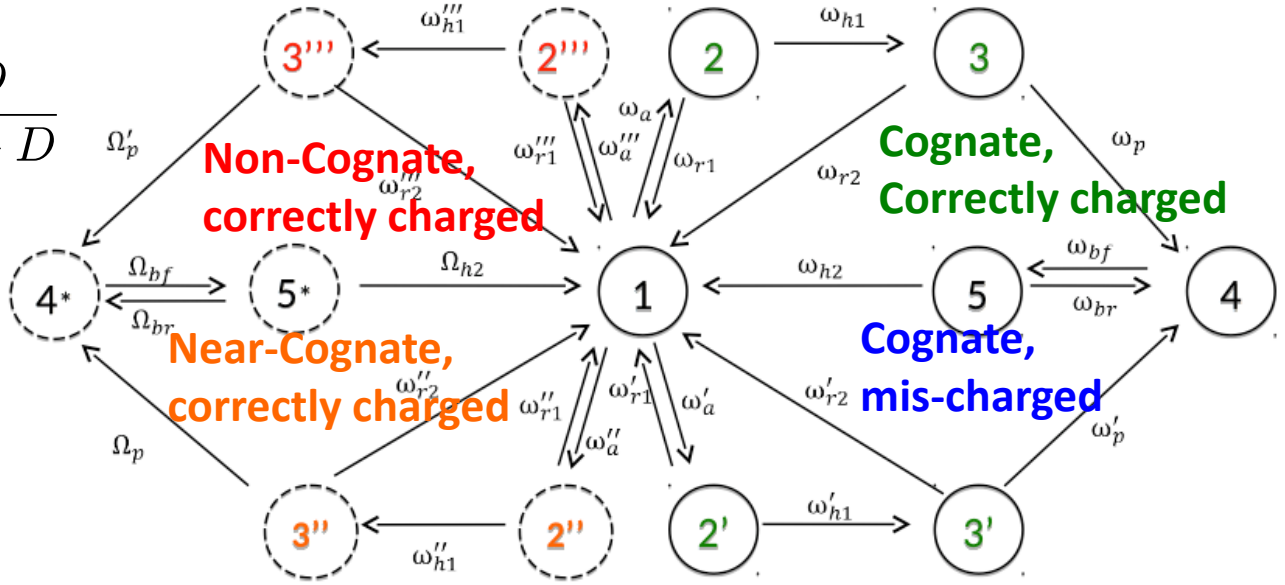
Random mis-sense ERRORS

$$E = 1 - \phi = \frac{B + C + D}{A + B + C + D}$$

$$E_{mc} = \frac{B}{A + B + C + D}$$

$$E_{mr} = \frac{C + D}{A + B + C + D}.$$

SPEED



Further Generalized form of *Michaelis-Menten Eqn*

Dutta & DC, Bull. Math. Biol. (2017)

$$\frac{1}{V} = \frac{1}{A + B + C + D} + \frac{A}{A + B + C + D}\left(\frac{1}{V_A}\right) + \frac{B}{A + B + C + D}\left(\frac{1}{V_B}\right) + \frac{C}{A + B + C + D}\left(\frac{1}{V_C}\right) + \frac{D}{A + B + C + D}\left(\frac{1}{V_D}\right)$$

where

$$\frac{1}{V_A} = \left[\frac{1}{\omega_{h1}}\left(1 + \frac{\omega_{r2}}{\omega_p}\right) + \frac{1}{\omega_p} + \frac{1}{\omega_{bf}}\left(1 + \frac{\omega_{br}}{\omega_{h2}}\right) + \frac{1}{\omega_{h2}}\right]$$

$$\frac{1}{V_B} = \left[\frac{1}{\omega'_{h1}}\left(1 + \frac{\omega'_{r2}}{\omega'_p}\right) + \frac{1}{\omega'_p} + \frac{1}{\omega_{bf}}\left(1 + \frac{\omega_{br}}{\omega_{h2}}\right) + \frac{1}{\omega_{h2}}\right]$$

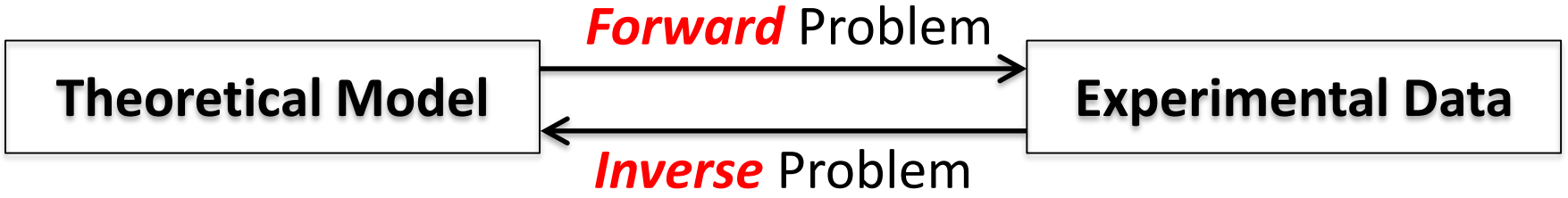
$$\frac{1}{V_C} = \left[\frac{1}{\omega''_{h1}}\left(1 + \frac{\omega''_{r2}}{\Omega_p}\right) + \frac{1}{\Omega_p} + \frac{1}{\Omega_{bf}}\left(1 + \frac{\Omega_{br}}{\Omega_{h2}}\right) + \frac{1}{\Omega_{h2}}\right]$$

$$\frac{1}{V_D} = \left[\frac{1}{\omega'''_{h1}}\left(1 + \frac{\omega'''_{r2}}{\Omega'_p}\right) + \frac{1}{\Omega'_p} + \frac{1}{\Omega_{bf}}\left(1 + \frac{\Omega_{br}}{\Omega_{h2}}\right) + \frac{1}{\Omega_{h2}}\right]$$

Reverse Engneering

Natural Nano-Machines:

Number of Nodes on the “Network”



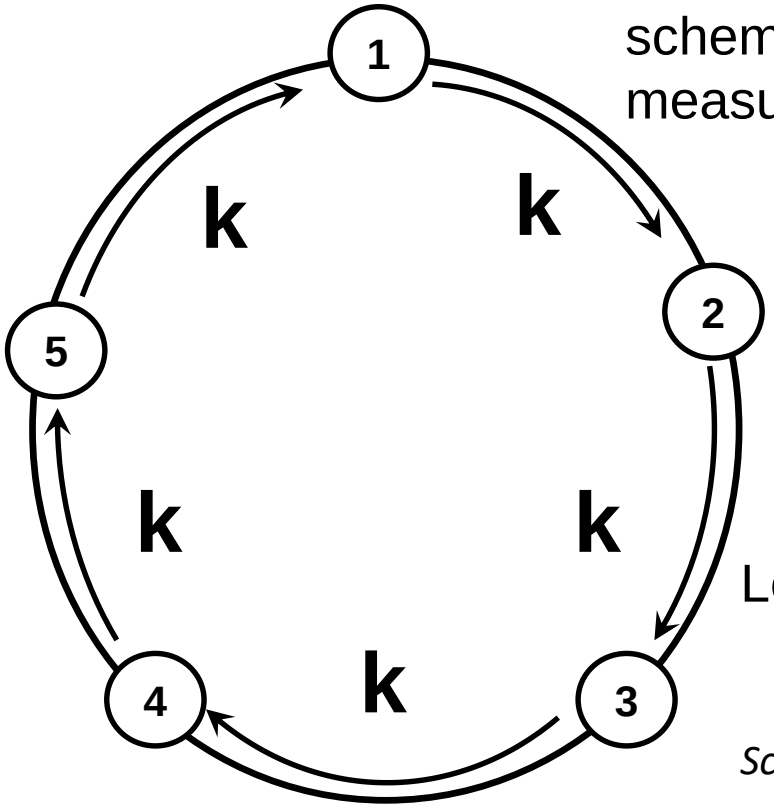
revised April 15, 1984 and October

The increased sophisticat
made more difficult the m
important so that o

sioned drawings for parts procurement. Document formats are described for recording information and data in the reverse engineering process that are synergistic with the method recommended for use in guiding that process. The documentation suggested directs the reverse engineering

How can the stochastic kinetic model of operation of a nano-machines, i.e., the **number of nodes** and the rates of transitions indicated by the **directed links** of the **network of states**, be extracted by the methods of reverse engineering?

Extracting Number of Nodes in the Network from Fluctuation Data: Reverse Engineering Molecular machines



Cycle Time (Dwell Time at each position on the track) is a random variable. What information on the kinetic scheme can we extract from the experimentally measured ***distribution of Cycle Times***?

As the simplest example, assume:

(i) Unbranched cycle,

(ii) Fully irreversible steps; and

(iii) Rates of all steps to be the same (k).

Let us calculate the randomness parameter

$$R = (\langle t^2 \rangle - \langle t \rangle^2)/\langle t \rangle^2$$

Schnitzer & Block, Cold Spring Harbor Symp. (1995)

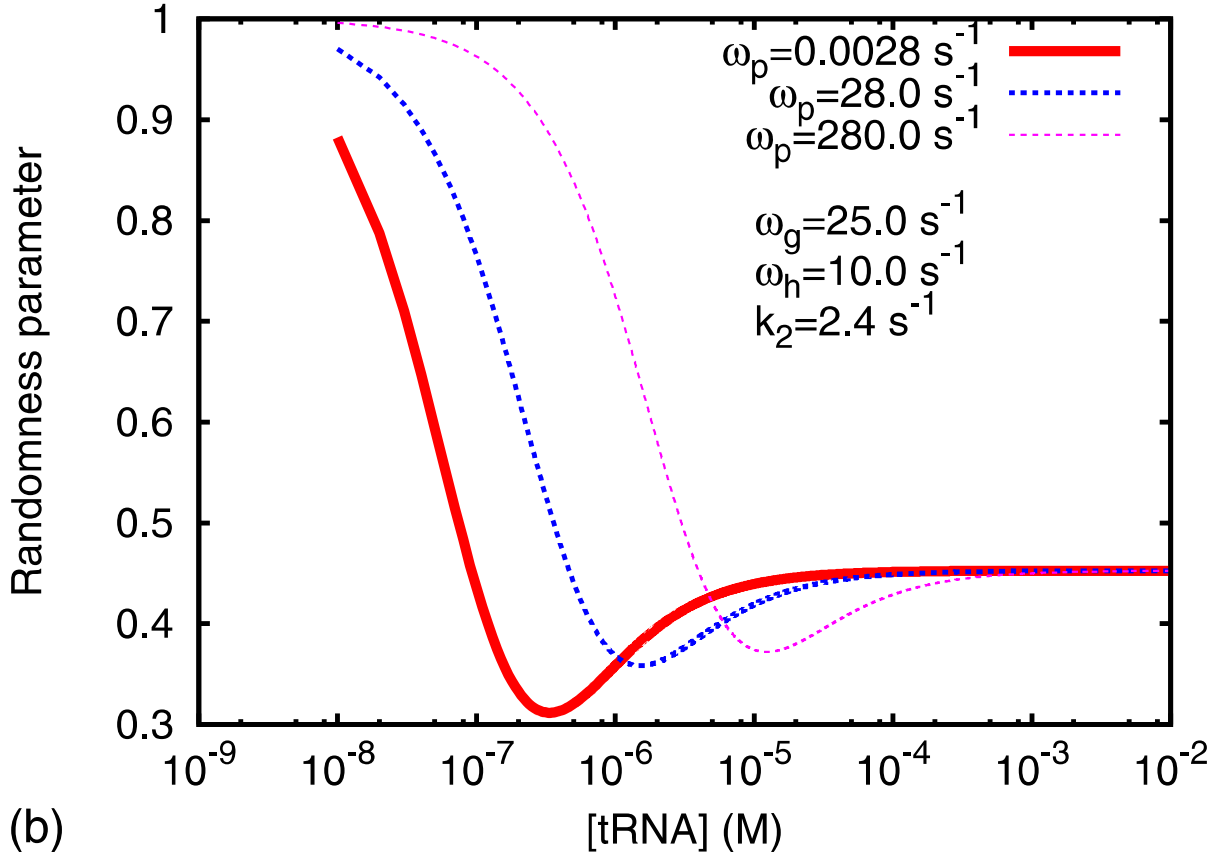
From the Cycle Time Distribution for such a cycle with N number of steps,

(Gamma distribution, also called Erlang Distribution in Queuing Theory)

$$f(t) = k^N t^{N-1} e^{-kt}/\Gamma(N), \qquad R = 1/N$$

Randomness parameter in a model of translation by a Ribosome

Garai, Chowdhury, Chowdhury and Ramakrishnan, Phys. Rev. E 80, 011908 (2009)

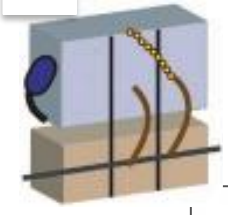


R would have regained its maximum value 1 at very high tRNA conc. if in that regime kinetics were dominated by a single rate-limiting step.

But, saturation of **R** at a value smaller than 1 at high tRNA concentration implies more than one rate-limiting steps when tRNA supply is no longer rate-limiting.

GSI: Classically- configured tRNA,
Non-rotated s.u.,
Open L1 stalk

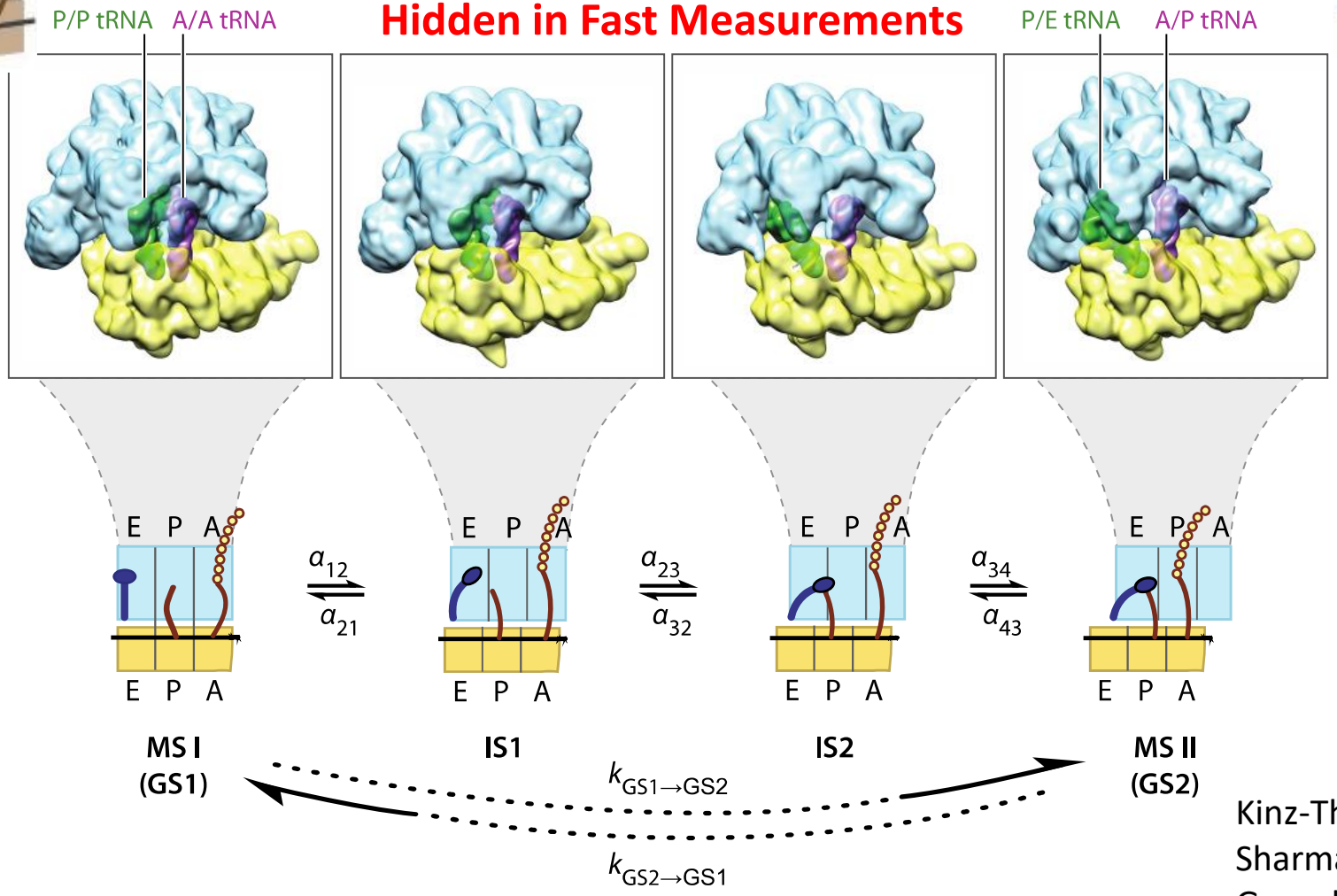
GSII: Hybrid- configured tRNA,
Non-rotated s.u.,
Closed L1 stalk



GS1

GS2

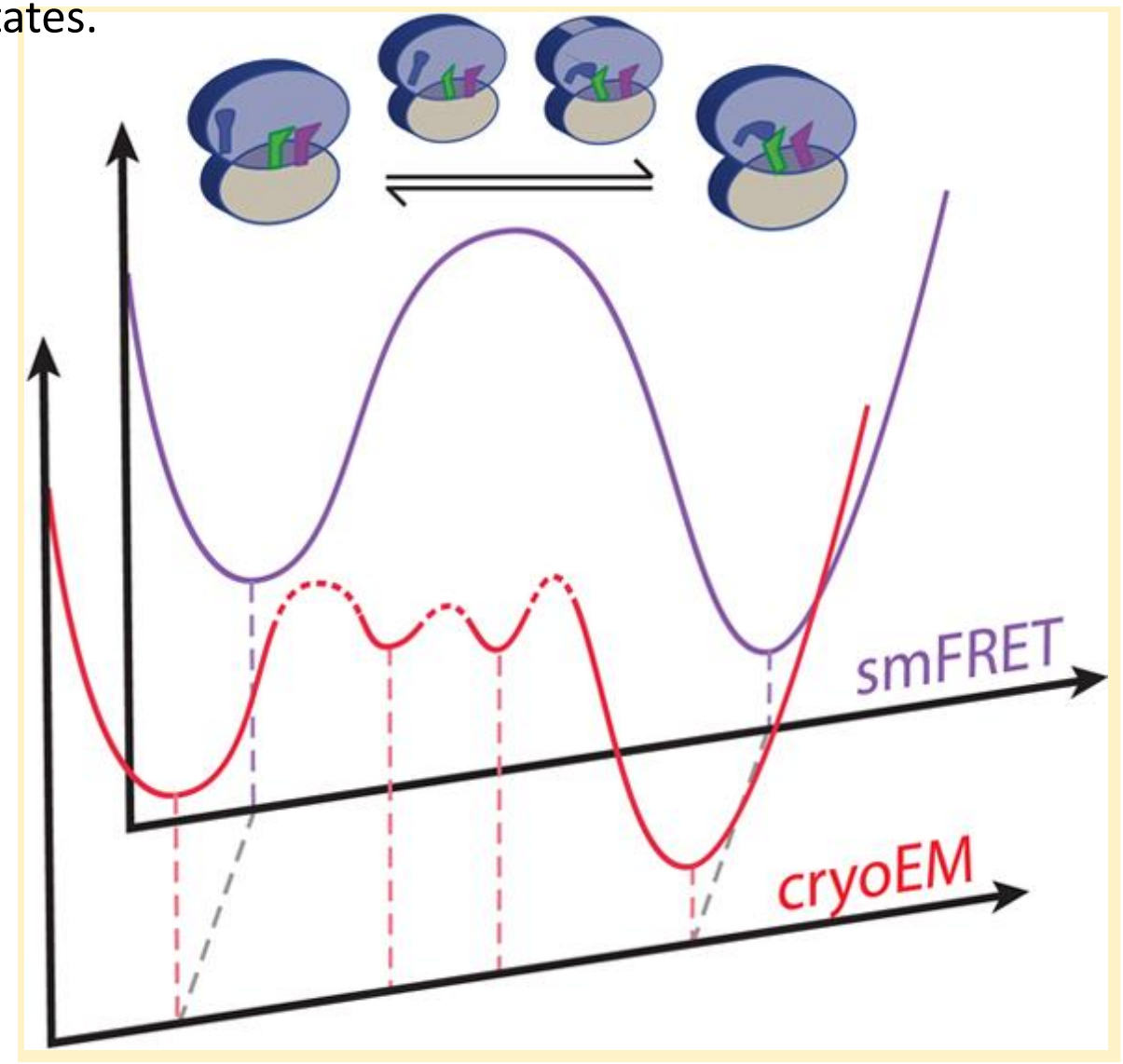
Detecting Nodes
Hidden in Fast Measurements



Fei et al.
Nat. Str.
Mol. Biol.
(2011)

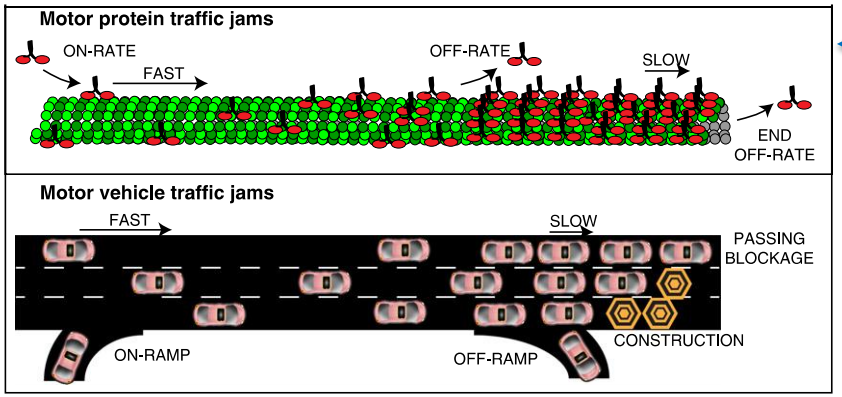
Kinz-Thmpson,
Sharma, Frank,
Gonzalez Jr.,
Chowdhury,
J. Phys. Chem. B
(2015)

Eq. occupation probabilities are obtained from cryo-EM data. The transition rates from 1 to 4 and 4 to 1 are extracted from smFRET. The corresponding exact expressions are derived using formalisms of **Eq. Stat. mech**. and ***mean First-Passage Time***, respectively. Analysis of the resulting equations establish existence of two short-lived intermediate states.



Kinz-Thmpson, Sharma, Frank, Gonzalez Jr., Chowdhury, J. Phys. Chem. B (2015)

Traffic-like Collective Movement
of
Molecular Motors on Filamentous Tracks

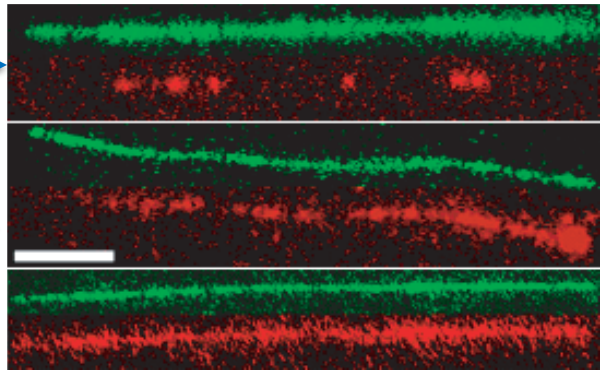


J.L. Ross, PNAS (2012)

Traffic of molecular motors on **MT track**

← MCAK

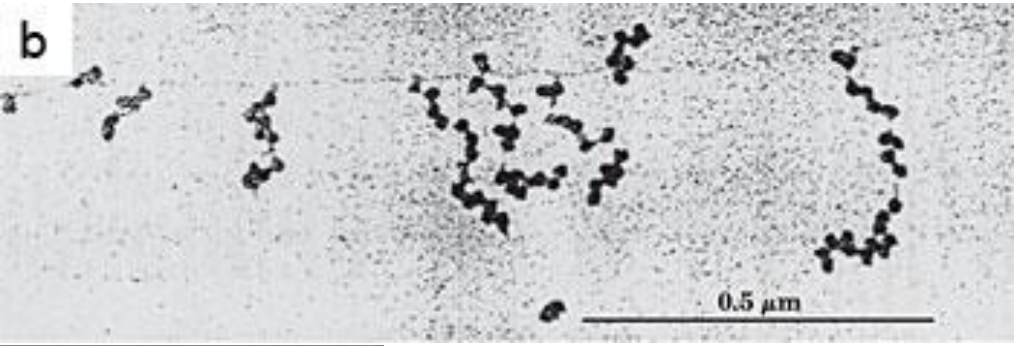
KIF1A →

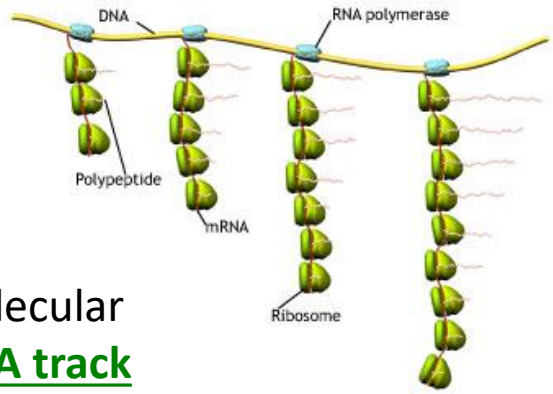


Nishinari, Okada, Schadschneider, Chowdhury. Phys. Rev. Lett (2005)

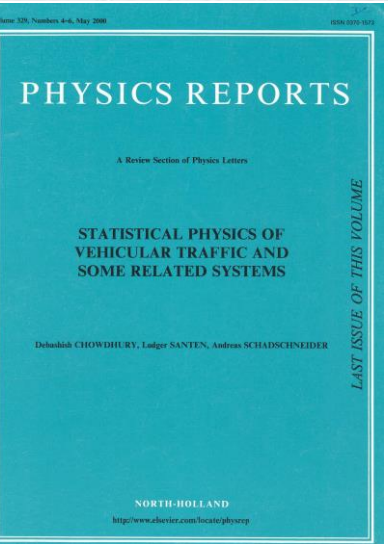
Non-equilibrium STEADY-STATE

NO underlying HAMILTONIAN, NO equilibrium exists!!



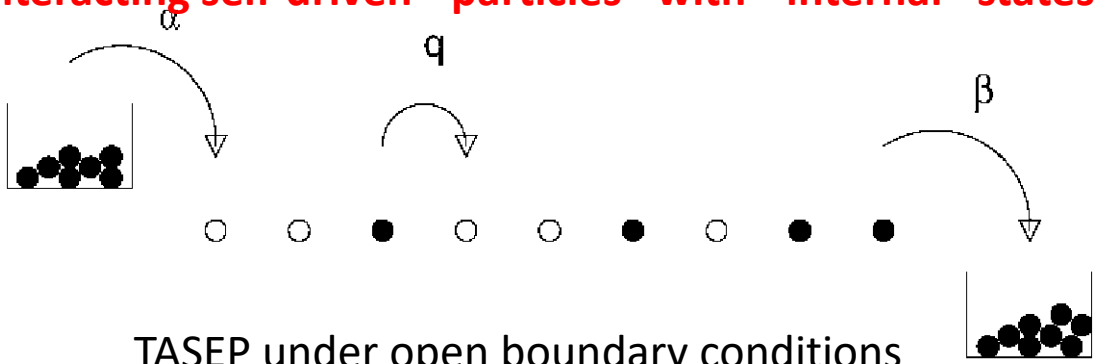


Traffic of molecular motors on **NA track**

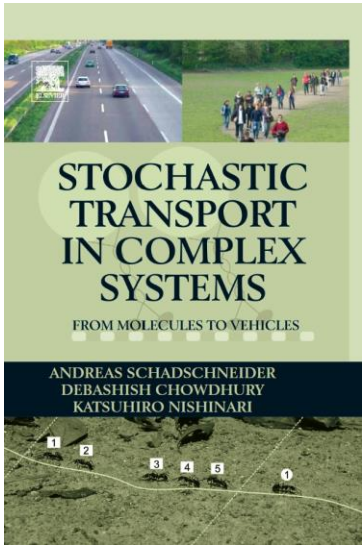


Phillips and Quake, Phys. Today, May 2006.

Fundamental principle: Traffic of interacting self-driven ``particles'' with ``internal'' states.



TASEP under open boundary conditions



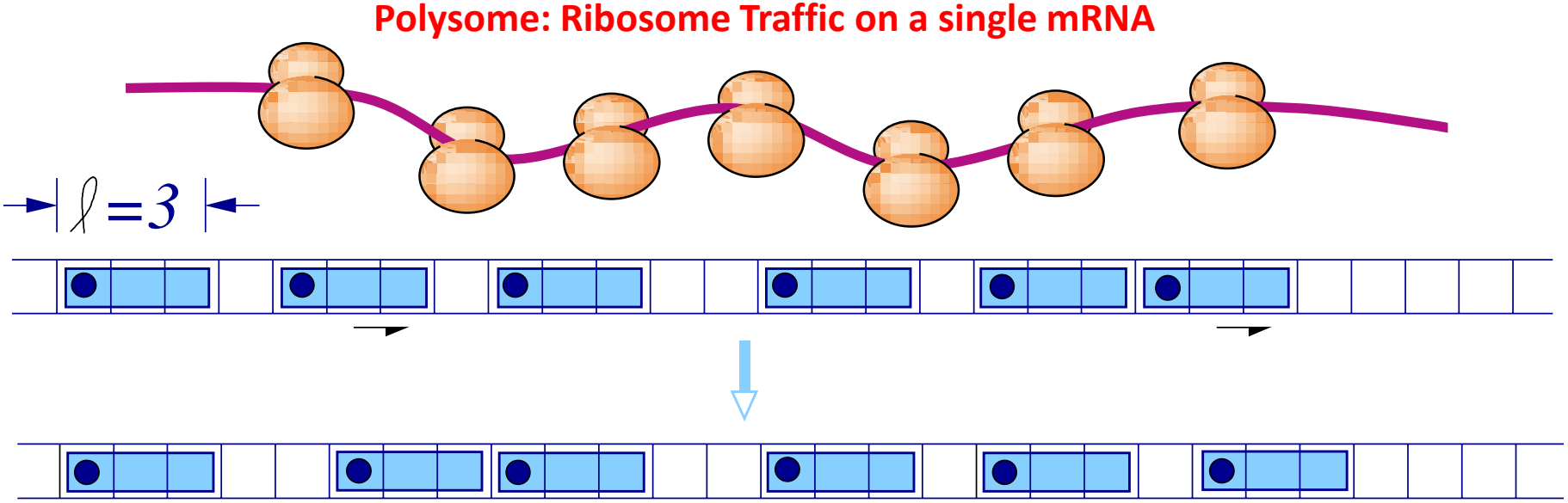
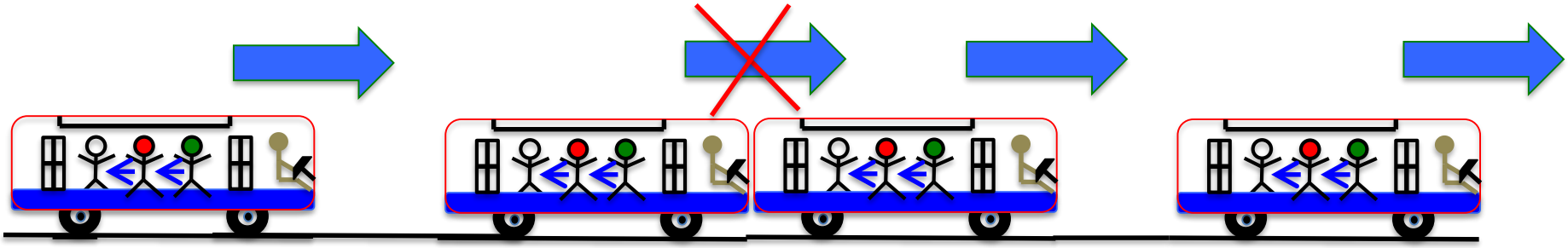


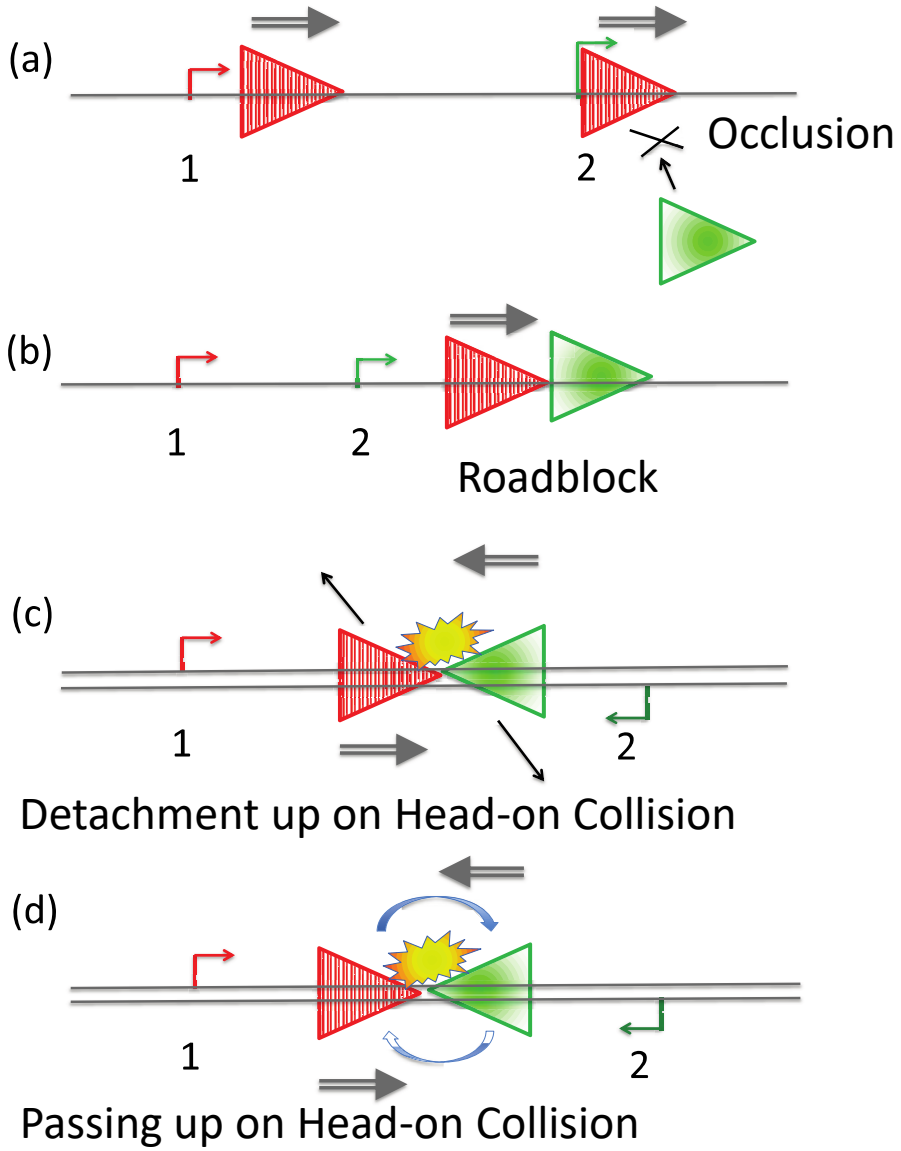
Figure 4. An interior section of an asymmetric exclusion process with extended particles of size $\ell = 3$. The individual particles occlude three of the lattice sites on which the hops occur.

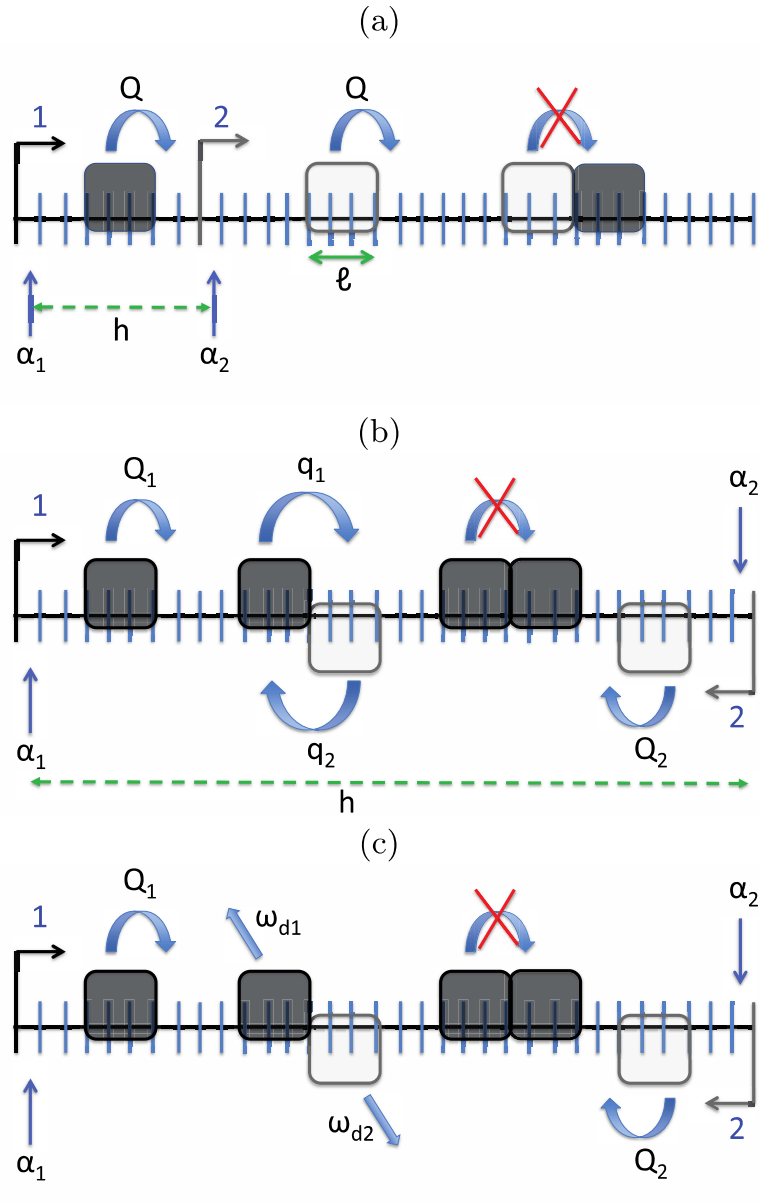


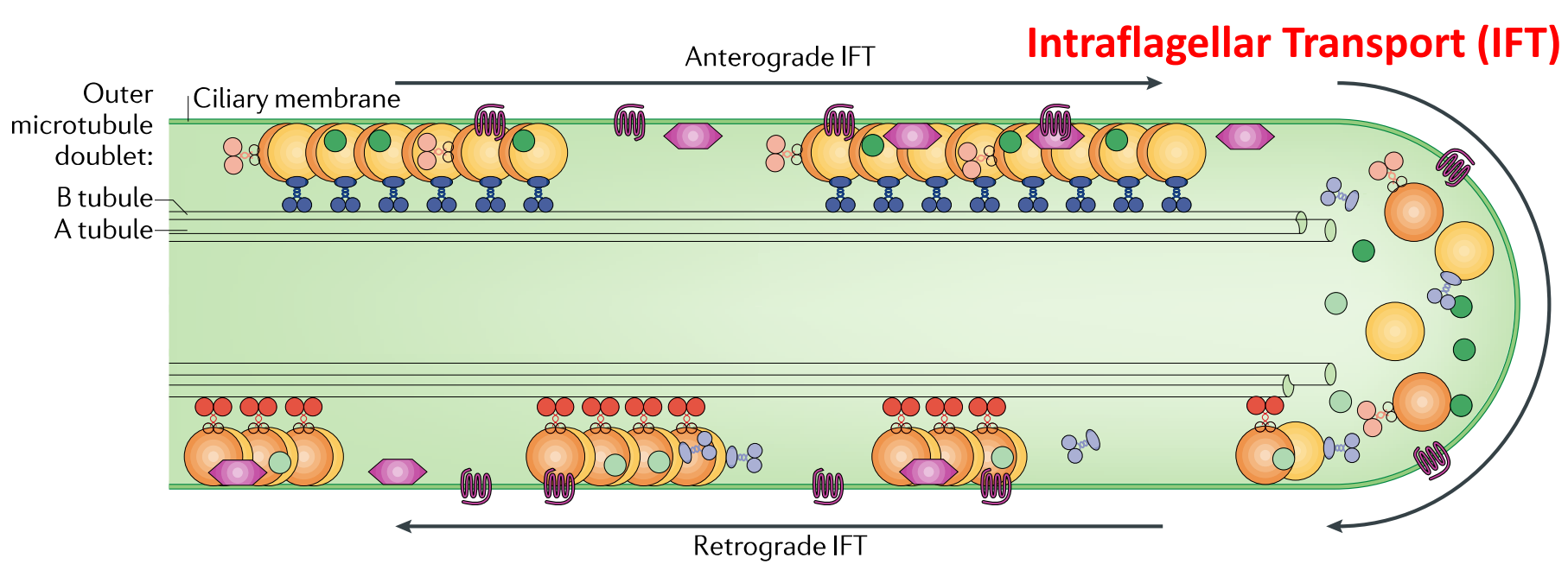
Basu & Chowdhury, Phys. Rev. E (2007)
Garai, Chowdhury, Chowdhury, Ramakrishnan, Phys. Rev. E (2009)
Sharma & Chowdhury, J. Theor. Biol. (2011)

Transcriptional Interference

Ghosh, Bameta, Ghanti, Chowdhury, J. Stat. Mech.: Theor. & Expt. (2016)

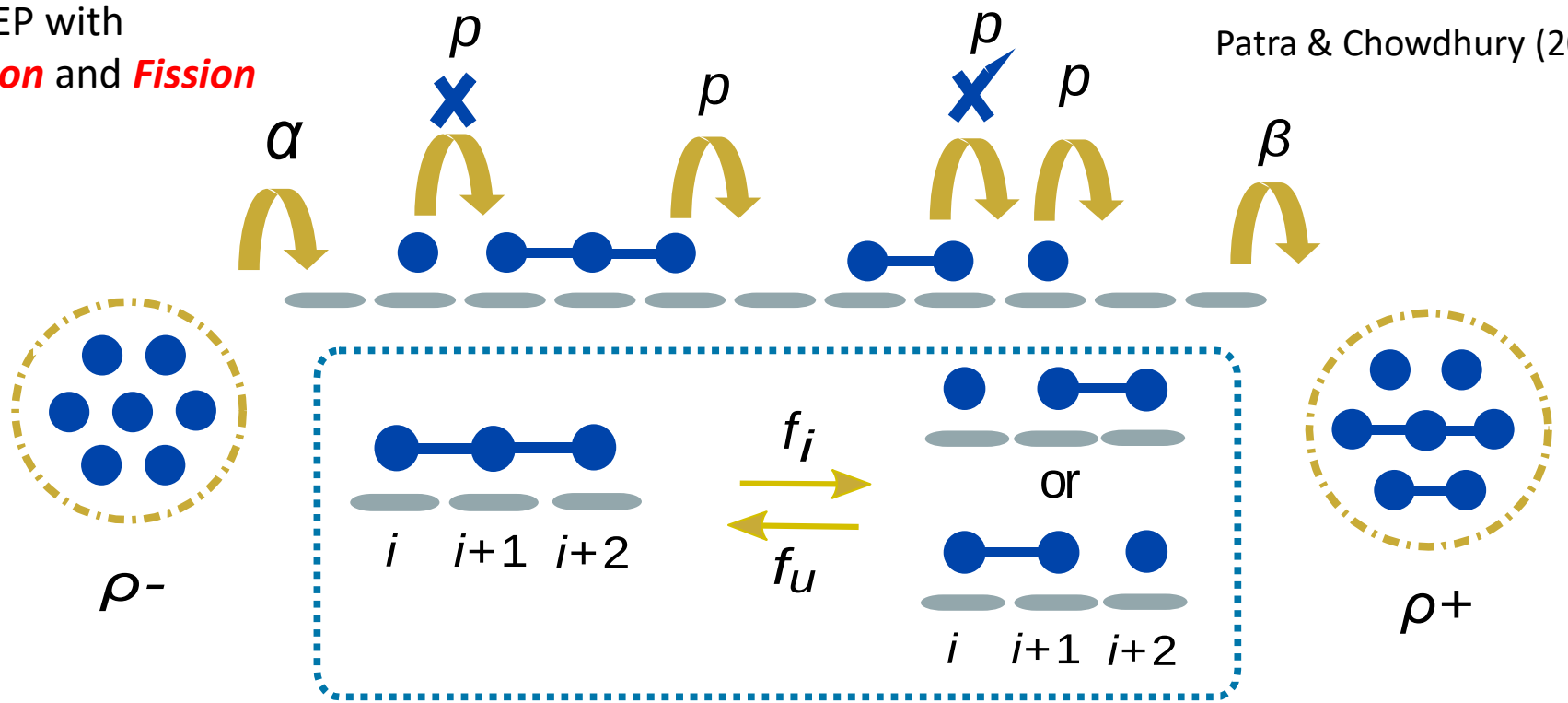






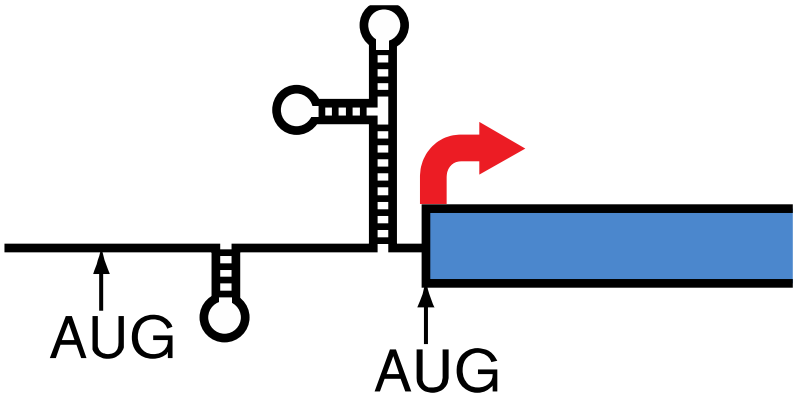
TASEP with
Fusion and ***Fission***

Patra & Chowdhury (2017)



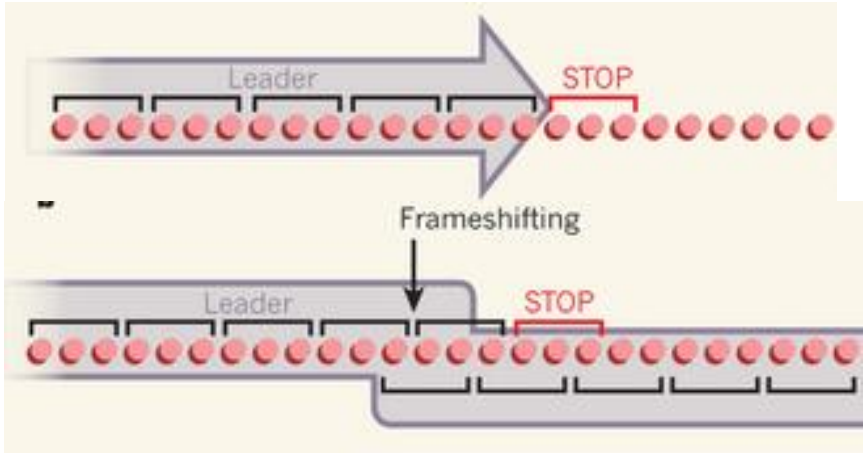
Traffic-like phenomena in Unconventional Translation:
Recoding by "***Programmed Error***"

Non-canonical Initiation:
Internal Ribosome Entry

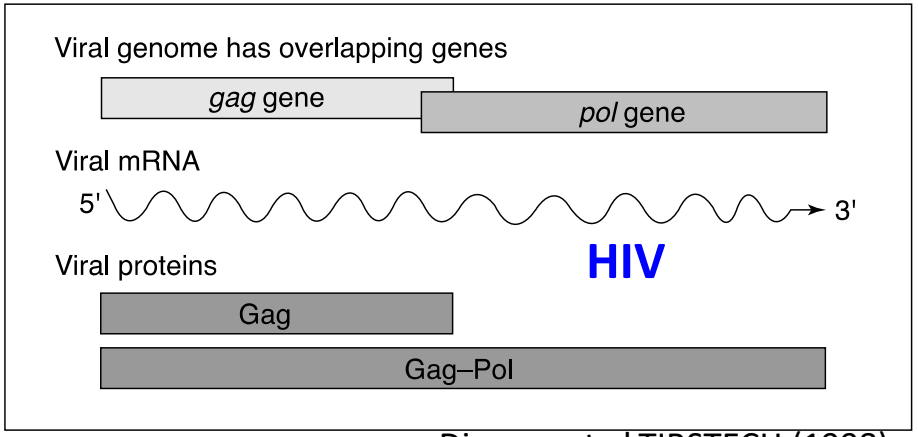


Mishra and Chowdhury, Phys. Rev. E 95, 062117 (2017)

Non-canonical Elongation: Frameshift



Atkins & Baranov, Nature 503, 478 (2013)



Dinman et al.TIBSTECH (1998)

Mishra, Schutz & Chowdhury EPL 114, 68005 (2016)

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