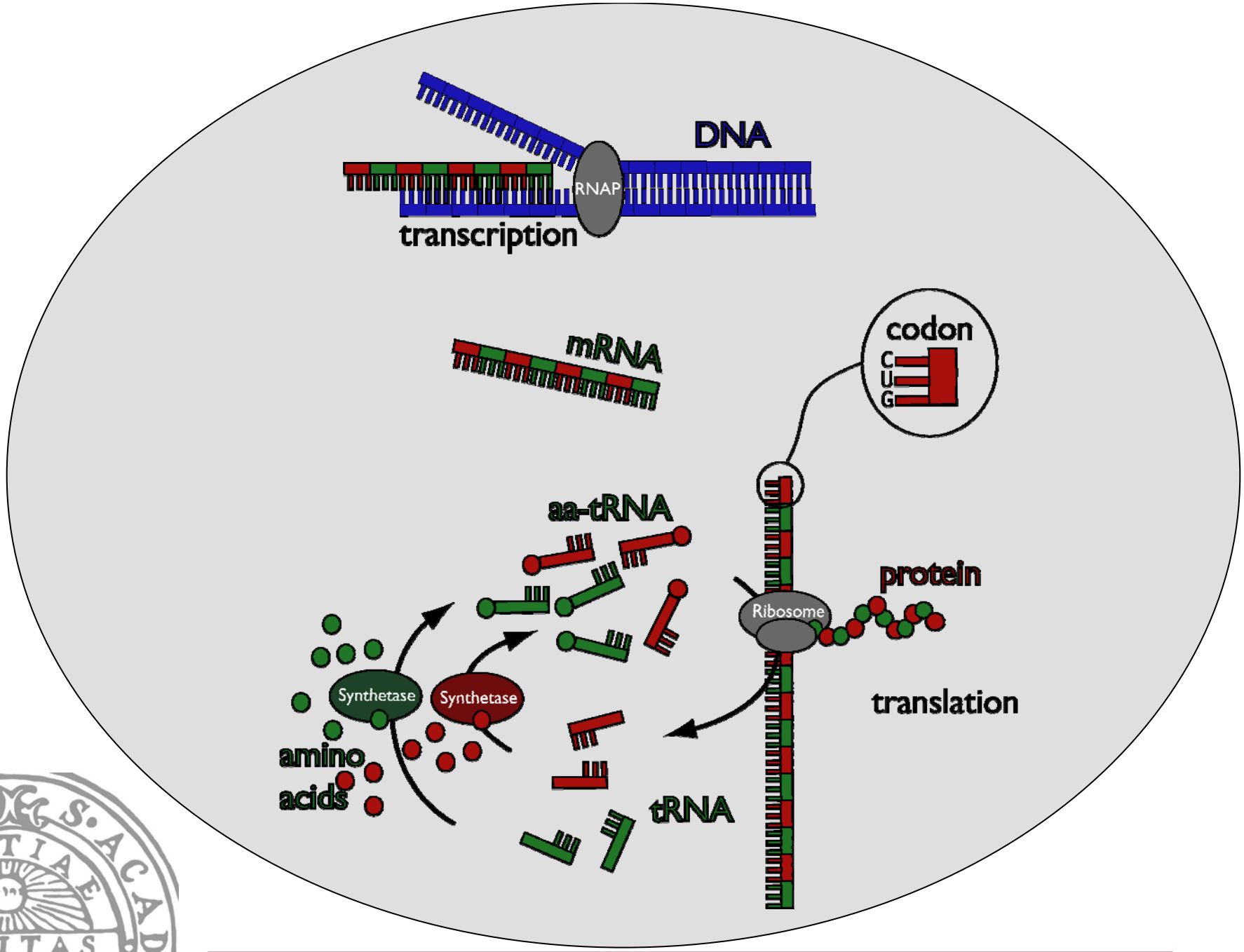
Transcriptional regulation of amino acid synthetic operons



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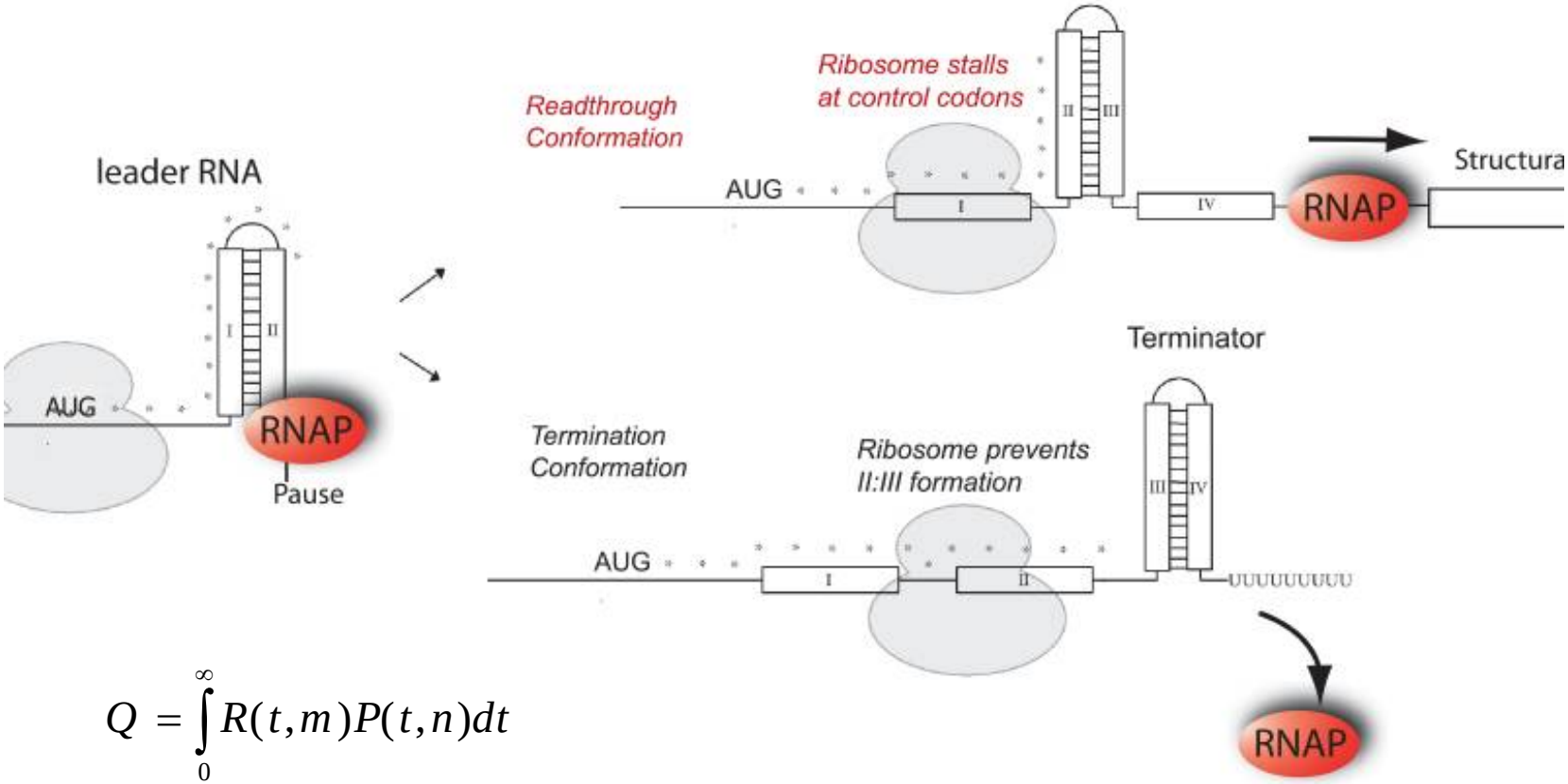
Transcriptional Attenuation



$$Q = \int_0^{\infty} R(t,m)P(t,n)dt$$

$$R(t,m) = \sum_{i=0}^{m-1} \mathrm{Po}(i, mkt)$$

$$P(t,n) = q \cdot \mathrm{Po}(n-1, nqt)$$

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Mechanistic sensitivity of attenuation: Hyperbolic control

The sensitivity, a_{rk}, of the regulation response, r, to the rate, k, of control codon reading, when

$$m=1,$$

$$n=1$$

$$Q=\int_0^\infty R(t)\cdot P(t)dt=\int_0^\infty e^{-kt}qe^{-qt}dt=\frac{1}{1+k/q}=r(k)$$

$$a_{rk}=\frac{d\log r}{d\log k}=-\frac{k/q}{1+k/q}$$

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Mechanistic sensitivity:exponential control

$m = 1$, step rate for translation mk=k, average time to finish stoch. proc. 1/k

$n >> 1$, step rate for transcription nq, average time to finish stoch. proc. 1/q

in the limit $n \to \infty$

$$Q = \int_0^\infty e^{-kt} \delta(t - 1/q) dt = e^{-k/q} = r(k)$$

$$a_{rk} = \frac{d \log r}{d \log k} = -k/q = \ln(r)$$

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Mechanistic sensitivity: Boolean control

$m >> 1$, step rate mk

$n >> 1$, step rate nk

in the limit m,k $\rightarrow \infty$

$r(k) = 1$ when k<q, $r(k) = 0$ when k>q

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System sensitivity of attenuation

Synthesis and consumption of limiting amino acid are equal

$$k_E[E] = f[R]v = \frac{f[R]}{\frac{1-f}{k_R} + \frac{f}{k}}$$

$$s = \frac{k_E[E]}{f[R]k_R}$$

Rate, k, of reading rare codon (f<<1) is hypersensitive ($|a_{ks}|$>>1) to amir limitation just below (s<1) balance point (s=1) of supply and demand:

$$a_{ks} = \frac{d\log k}{d\log s} = \frac{1}{1-s+fs}$$

$$f << 1 \Rightarrow a_{ks} >> 1 \text{ when } s \approx 1$$

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Overall sensitivity of attenuation may be numerically very large

The overall sensitivity of attenution is the mechanistic sensitivity a_{rk} multiplied by the system sensitivity a_{ks}

$$a_{rs} = \frac{d \log r}{d \log s} = \frac{d \log r}{d \log k} \cdot \frac{d \log k}{d \log s} = a_{rk} \cdot a_{ks}$$

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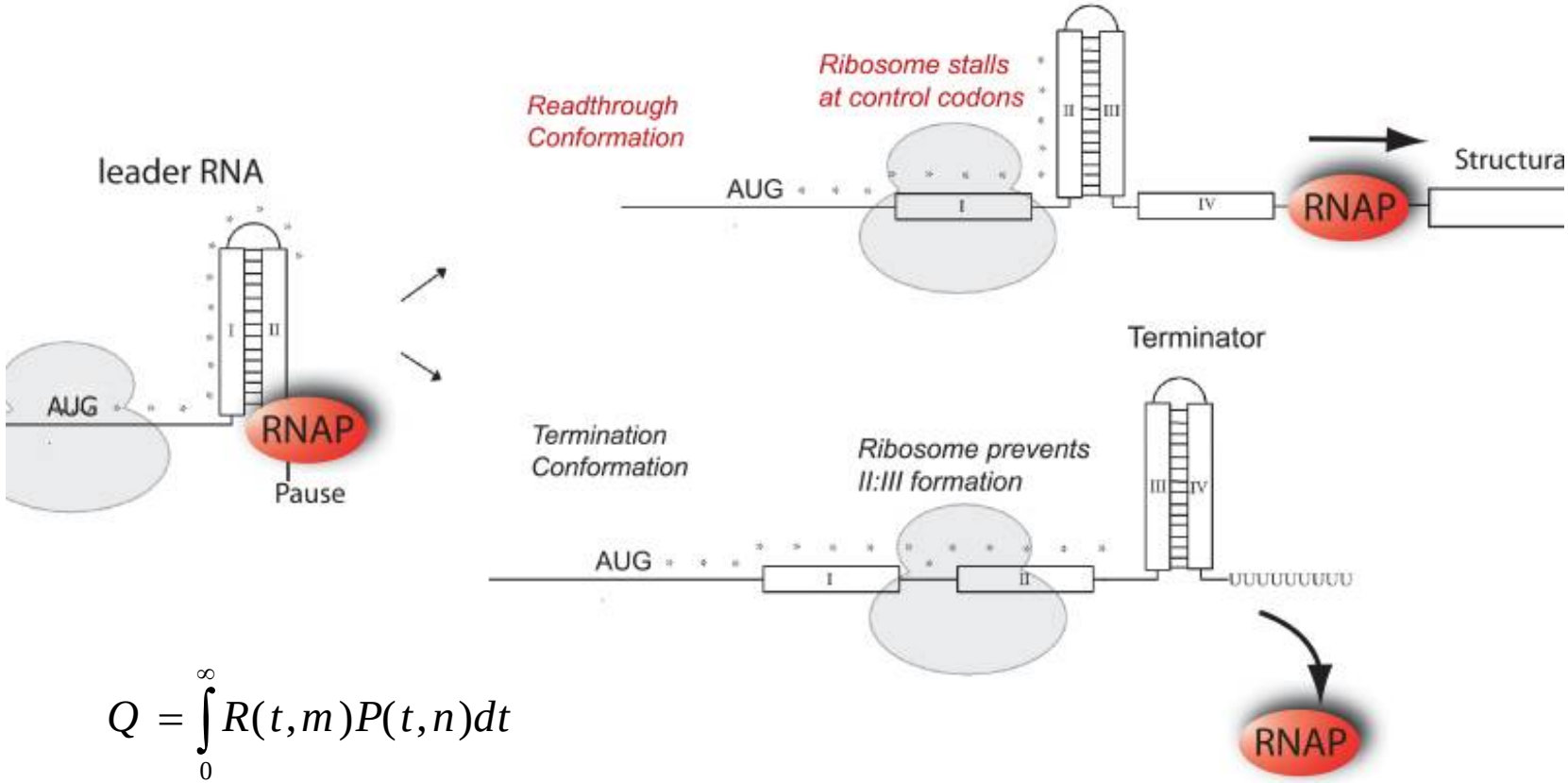
Codon usage in attenuation leaders is highly biased. Why?



$$Q = \int_0^\infty R(t,m)P(t,n)dt$$

$$R(t,m) = \sum_{i=0}^{m-1} \mathrm{Po}(i,kt)$$

$$P(t,n) = q \cdot \mathrm{Po}(n-1,qt)$$

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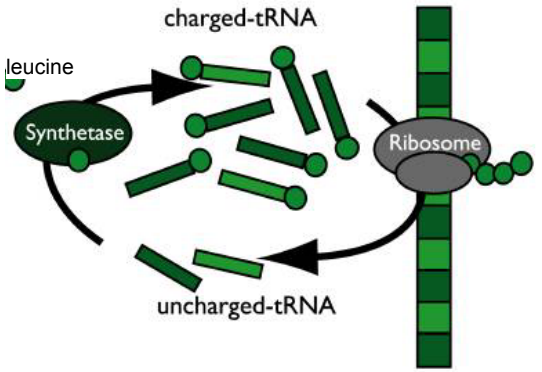
The genetic code

Second base in codon

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A

Third base in codon

tRNA isoacceptor	Codonrecognition (5'-3')	
Leu1	CUG	
Leu2	CUC	CUU
Leu3	CUA	CUG
Leu4	UUG	
Leu5	UUA	UUG



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Codon usage in attenuation leaders

Very highly favored codons in attenuation leaders:

Leu (CUA), Thr (ACC), Val (GUC), Ile (AUU, AUC).

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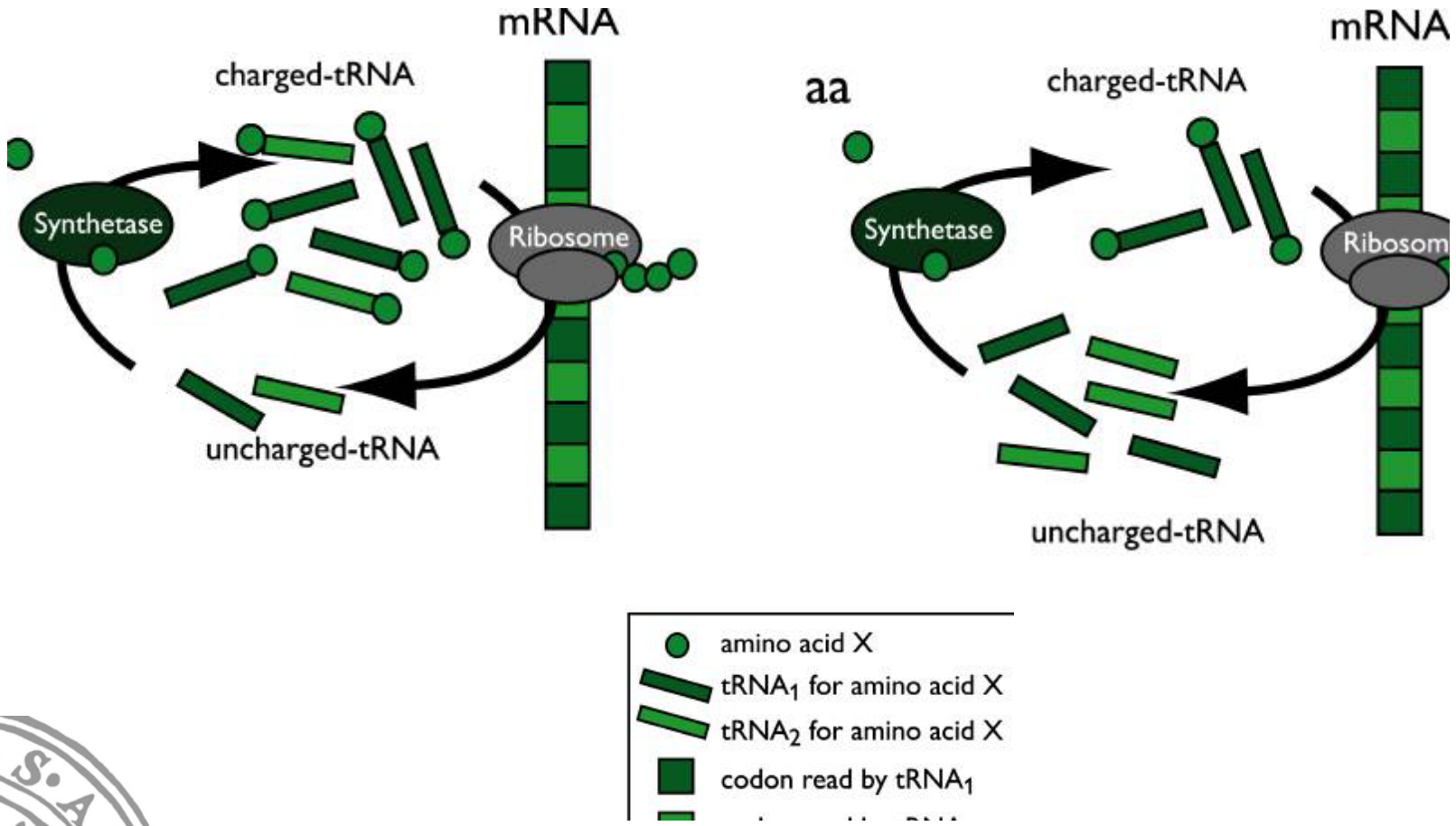
Theory: Selective Charging of tRNA isoacceptors



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Two isoacceptors – one codon each

$$\frac{(1-\alpha)a}{(1-\beta)b} = \frac{f_A}{f_B} \Leftrightarrow \alpha = \left(1-(1-\beta)\frac{f_A}{a}\Big/\frac{f_B}{b}\right)$$

$$\textit{if} \quad \frac{f_A}{a} < \frac{f_B}{b} \quad \text{and } \beta \to 0 \Rightarrow \alpha = \left(1-\frac{f_A}{a}\Big/\frac{f_B}{b}\right)$$

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Flow coupling relations

The two types of tRNAs are either in amino acid lacking form, *i.e.* $tRNA_A$ or $tRNA_B$ **or in aminoacylated form, *i.e.*** T_{3A} or T_{3B}

Define

$$[tRNA_A]+[T_{3A}]=a \qquad [tRNA_B]+[T_{3B}]=b$$

and

$$[tRNA_A]=(1-\alpha)\cdot a \qquad [tRNA_B]=(1-\beta)\cdot b$$

$$[T_{3A}]=\alpha\cdot a \qquad [T_{3B}]=\beta\cdot b$$

Flow relations:

$$j_A=[AA][E](1-\alpha)a=f_A[R]v_R$$
$$j_B=[AA][E](1-\beta)b=f_B[R]v_R$$

So that

$$\frac{j_A}{j_B}=\frac{a(1-\alpha)}{b(1-\beta)}=\frac{f_A}{f_B}\Rightarrow\frac{(1-\alpha)}{(1-\beta)}=\frac{f_A/a}{f_B/b}$$

Furthermore $j=j_A+j_B=[R]v_R=[R]/\tau$ **where** $\tau=\frac{1}{v_{\max}}\left[f_A\left(1+\frac{K_m}{a\cdot\alpha}\right)+f_B\left(1+\frac{K_m}{b\cdot\beta}\right)\right]$

When $j\to 0$ **then** $\tau\to\infty$ **and** $\beta\to 0,\ \alpha\to 1-\frac{b/f_B}{a/f_A}$

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Experimental data

For *E. coli*, total concentrations of all tRNAs and translation frequencies of all codons have been estimated.

From this information the aminoacylated fraction of each member in any family of isoacceptors can be estimated for any degree of amino acid limitation

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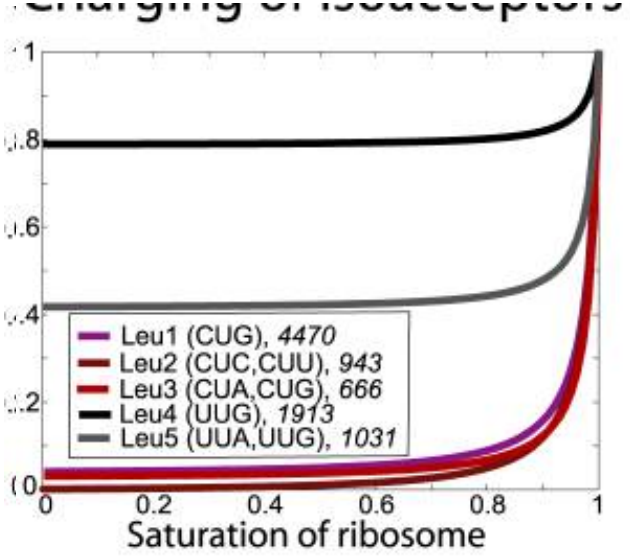
Predictions for the leucine family



Codons sensitivity to starvation

Amino Acid	Codon	Sensitivity	Codon Usag
Leucine	UUG	0.59	6.3
	UUA	3.0	6.1
	CUG	5.5	60.1
	CUC	24.8	6.2
	CUU	24.8	5.7
	CUA	26.9	2.2

Codon used in tmRN

Codon used in attenuation leader

Codon usage in *leu* genes

Genes	Codon groups	Occurrences	Expe
leuABCD	insensitive (UUG,UUA)	28	8-2
	intermediate (CUG)	87	73-9

Explaining codon usage in attenuation leaders and amino acid synthesis

By selecting the codon for which the rate of translation is most sensitive to amino acid deficiency, the codon usage in attenuators for the Leu (CUA), Thr (ACC), Val (GUC), Ile (AUU, AUC) cases were rationalized.

Frequencies of codons sensitive or insensitive to amino acid limitation are under or over represented, respectively, in genes encoding amino acid synthesizing enzymes.

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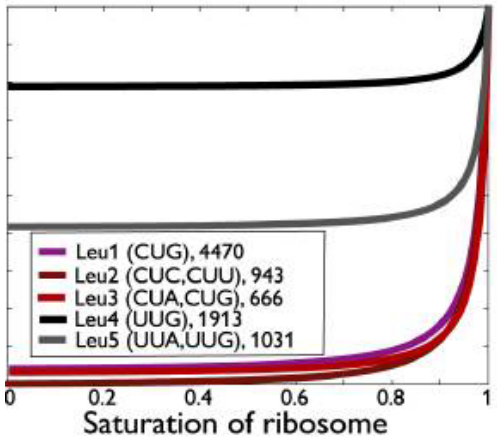
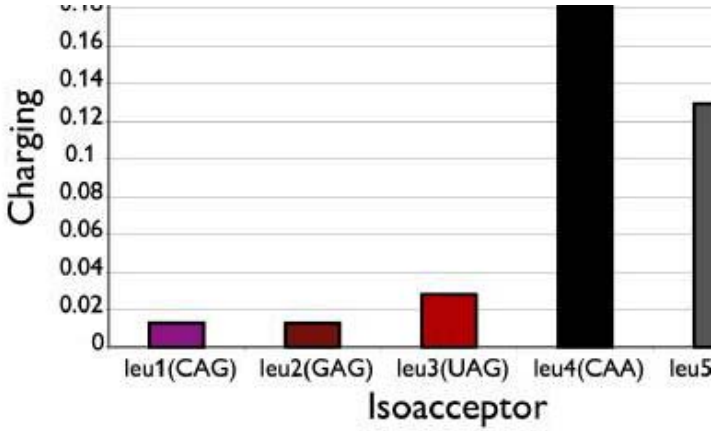
Selective Charging Test 1:
Direct Charging measurement

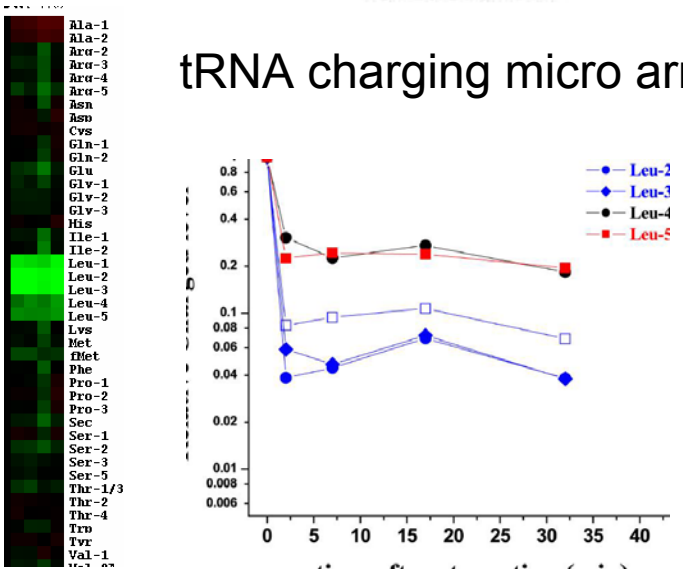
Prediction



Northern hybridization



tRNA charging micro array



with
Tao Pan & Kimberly Dittmar, Chicago
Michael Sörensen, Copenhagen

Dittmar et al. EMBO reports,2005

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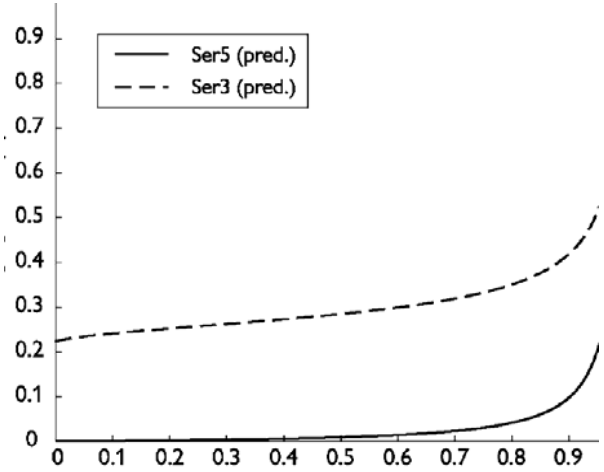
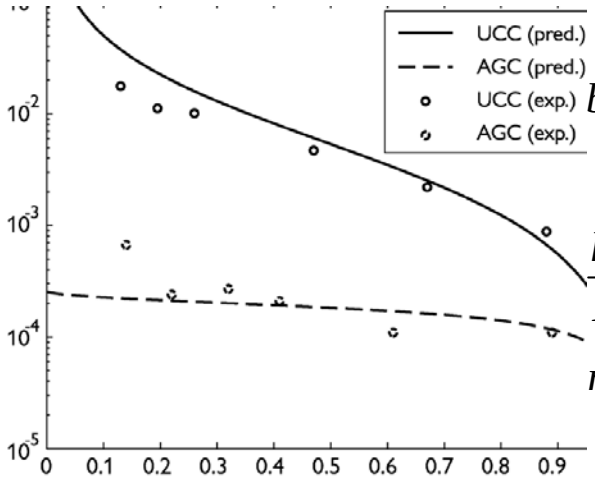
Selective Charging Test 2:
Ribsomal Bypassing at Serine codons

Bypassing Construct: ATG…… **TTC** [TCC or AGC] ATC *TAG* C *TAA* **TTT** →→lacZ

Control Construct: ATG…….TTC TCC GTC TAC CAG TTC →→lacZ





$$bypassfreq. = \frac{r_{bp}}{r_{bp} + \frac{k_{cat}}{K_m}[aa - tRNA]}$$

$$\frac{k_{cat}}{K_m} = 10^{7} M^{-1} s^{-1}$$

$$r_{bp} = 10^{-3} s^{-1} \text{ (fitted)}$$

with
Jonathan Gallant, Dale Lindsley, Paul Bonthuis,Teodora Tofoleanu, Seattle

Lindsey et al. EMBO reports,2005

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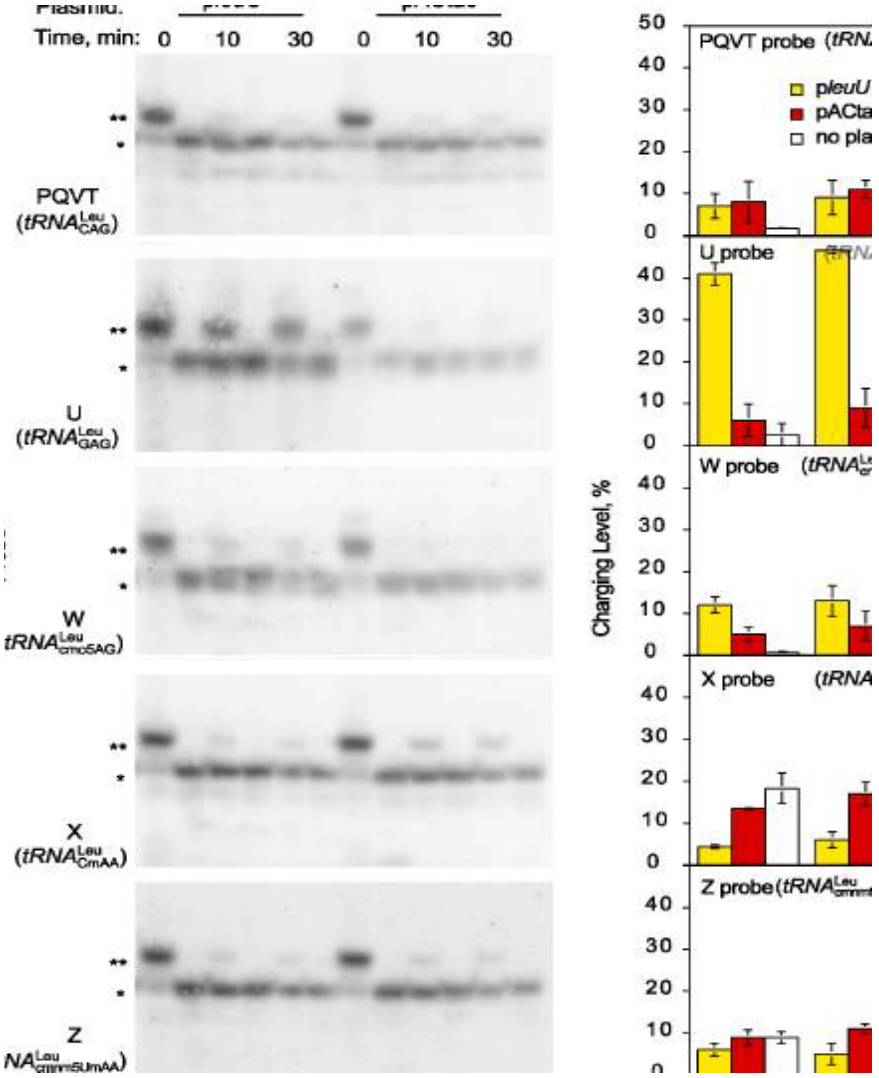
Selective Charging Test 3: Perturbation of tRNA concentration



with
M. Sörensen, Copenhagen
T. Tenson, Tartu

Sörensen, Elf, Bouakaz et al. submitted

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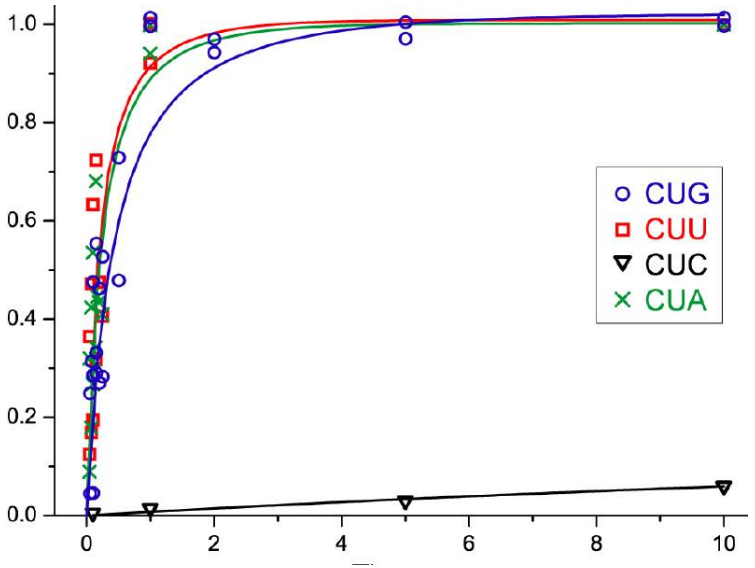
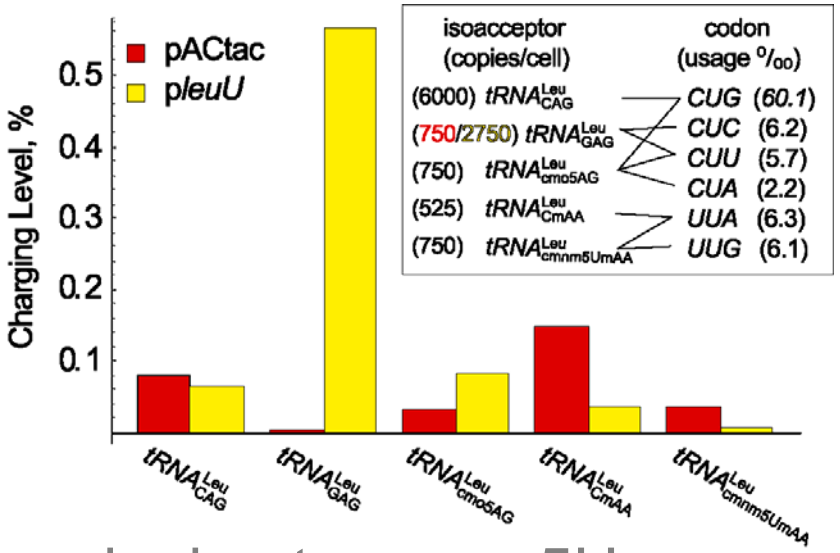
New codon reading is predicted, verified and explained





The extended reading pattern is due to a cmo5U modification of the wobble position

with
G. Björk, Umeå
M. Sörensen, Copenhagen
T. Tenson, Tartu

Sörensen, Elf, Bouakaz et al. *In press*

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Summary

1. Prediction of very uneven fractions of aminoacylated tRNAs in a family of isoacceptors upon limited supply of their common amino acid.
2. Successful predictions of codon usage among control codons in leaders of mRNAs for operons controlled by ribosome dependent attenuation
3. Succesful predictions of codon usage in amino acid biosynthetic genes
4. Theory confirmed for four isoacceptors families with Northerns and microarrays
5. Successful prediction of by-passing frequencies for two tRNASer isoacceptors upon inhibition of SerRS.
6. Prediction and verification of novel codon assignment.

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On method I

Global mathematical modeling of growing bacteria based on careful biochemistry has also explained

1. The *E. coli* protein elongation rate and its variation with growth condition
2. The missense error level in *E. coli*
3. The sensitivity of attenuation of transcription, i.e. 1/f effect
4. Bi-stability in growth rate for pathogens exposed to antibiotics
5. Modes of action of erythromycin and josamycin
6. Resistance against erythromycin and josamycin by expression of mini genes for cis acting peptides
7. Control of initiation of chromosome replication in *E. coli*

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On Method II

The above examples high-light the power of integrating biochemistry and microbiology through mathematical modeling.

This type of modeling will also create a basis for population genetics in cell physiology

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People involved

Uppsala:
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Umeå:
Glenn Björk
Copenhagen:
Michael Sörensen
Tartu:
Tanel Tenson
Chicago:
Kimberly Dittmar,Tao Pan
Seattle:
John Gallant, Dale Lindsley, Paul Bonthuis,Teodora Tofoleanu

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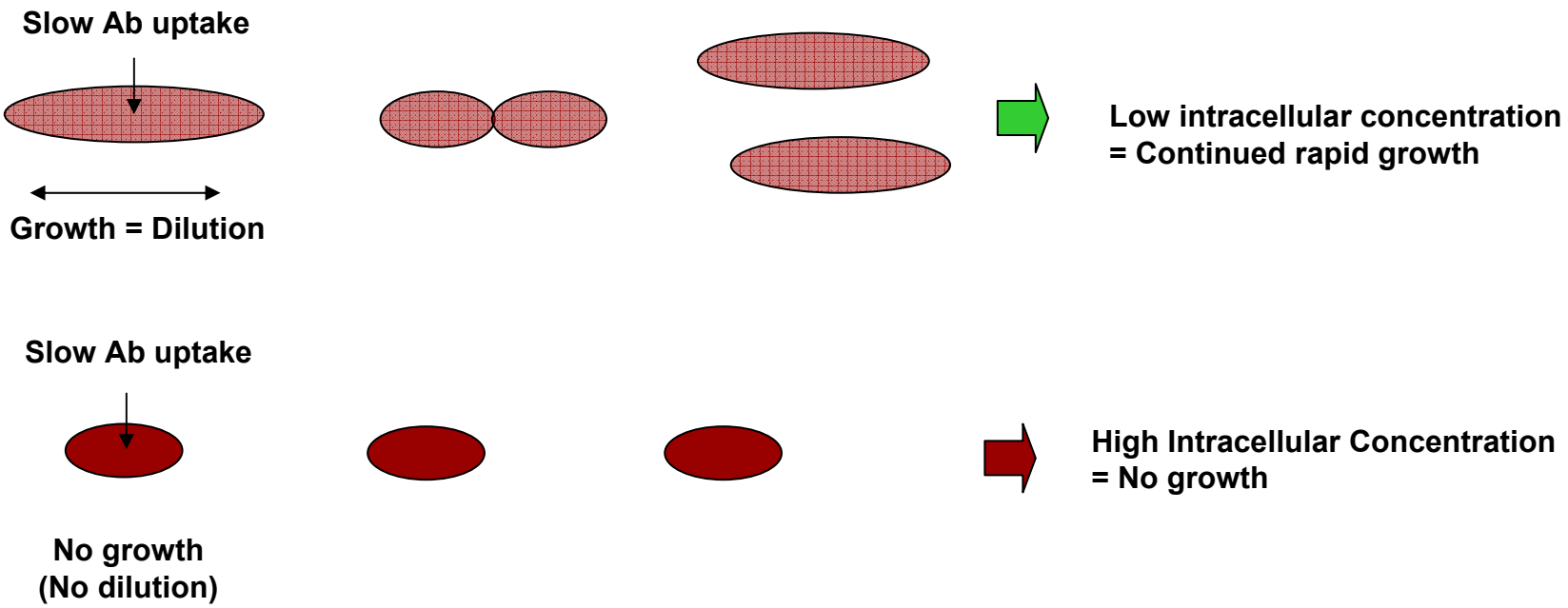
Part II: Bistability and Memory in Bacterial Responses to Antibiotics

The basic idea



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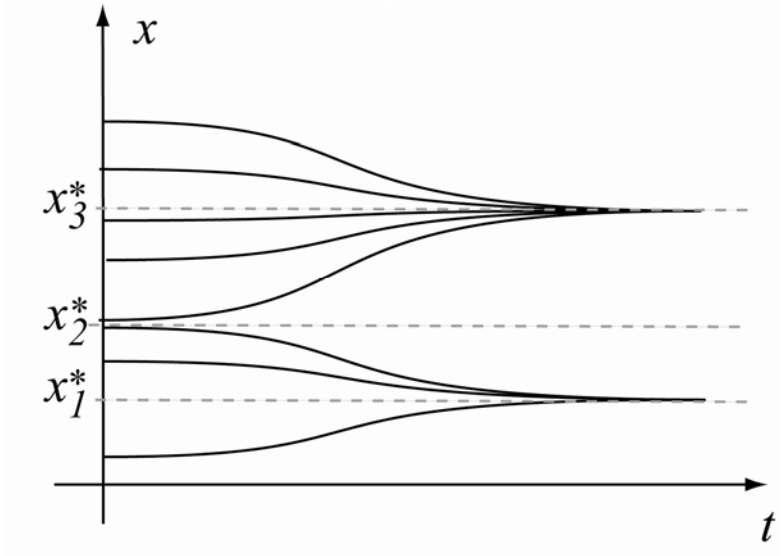
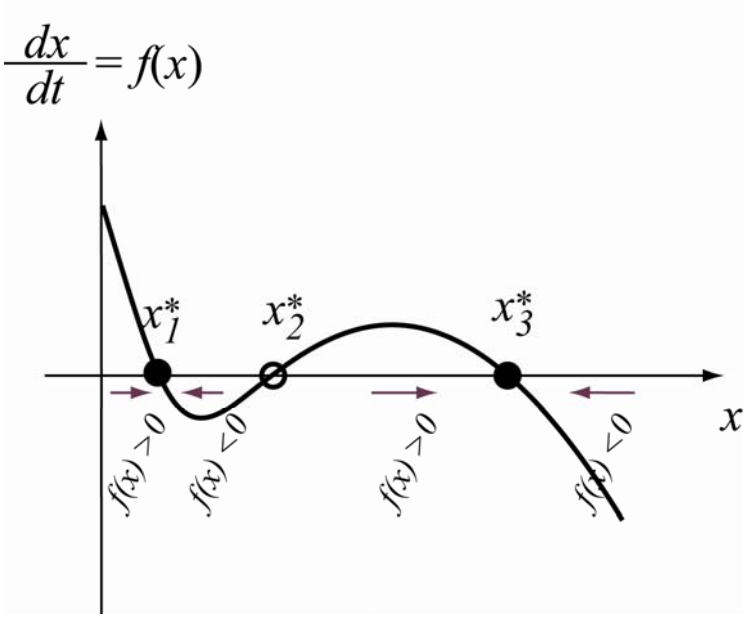
Bistability





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General model for antibiotic uptake in growing cells

$$\frac{da}{dt} = \underbrace{c \cdot \left(a_{ex} - a_{fr} \right)}_{J_{mem}} - \underbrace{\mu \cdot a}_{J_{gr}}$$

a	intracellular concentration
c	permeability
a_{ex}	external ab concentration
a_{fr}	free ab conc. (depends on a)
m	growth rate (depends on a)

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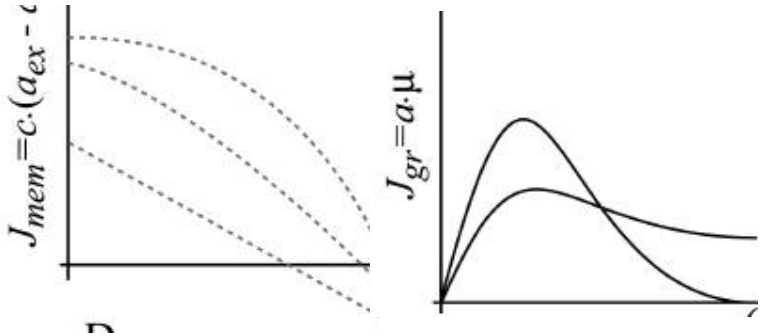
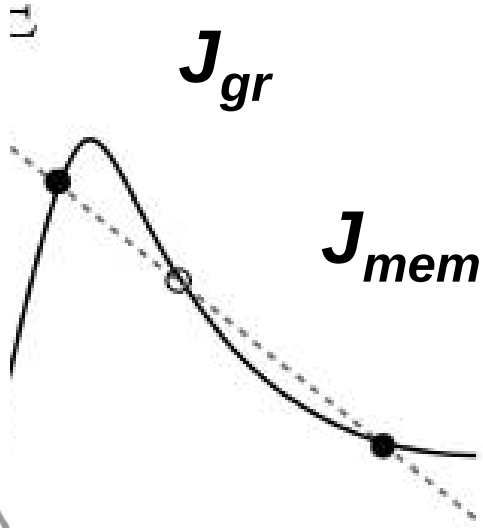
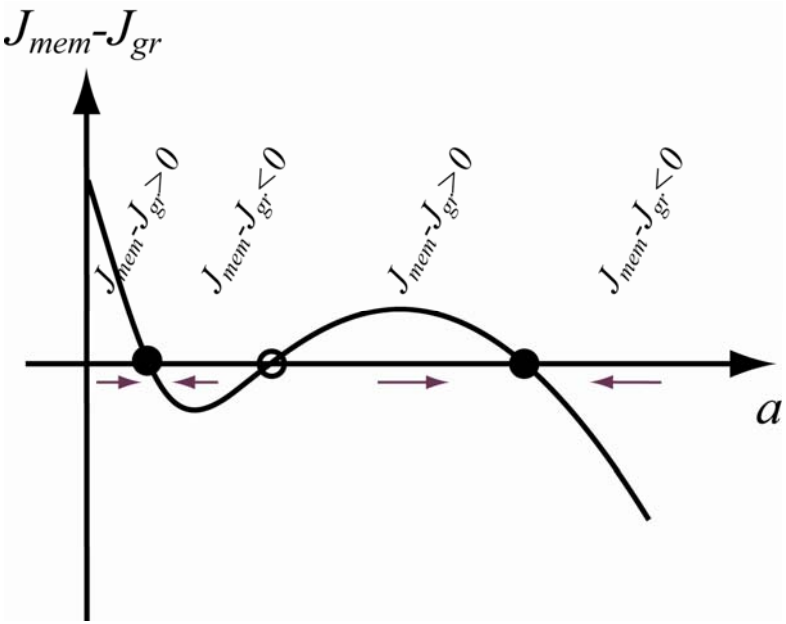
Bistability in intracellular antibiotic concentration

$$\frac{da}{dt} = \underbrace{c \cdot \left(a_{ex} - a_{fr} \right)}_{J_{mem}} - \underbrace{\mu \cdot a}_{J_{gr}}$$







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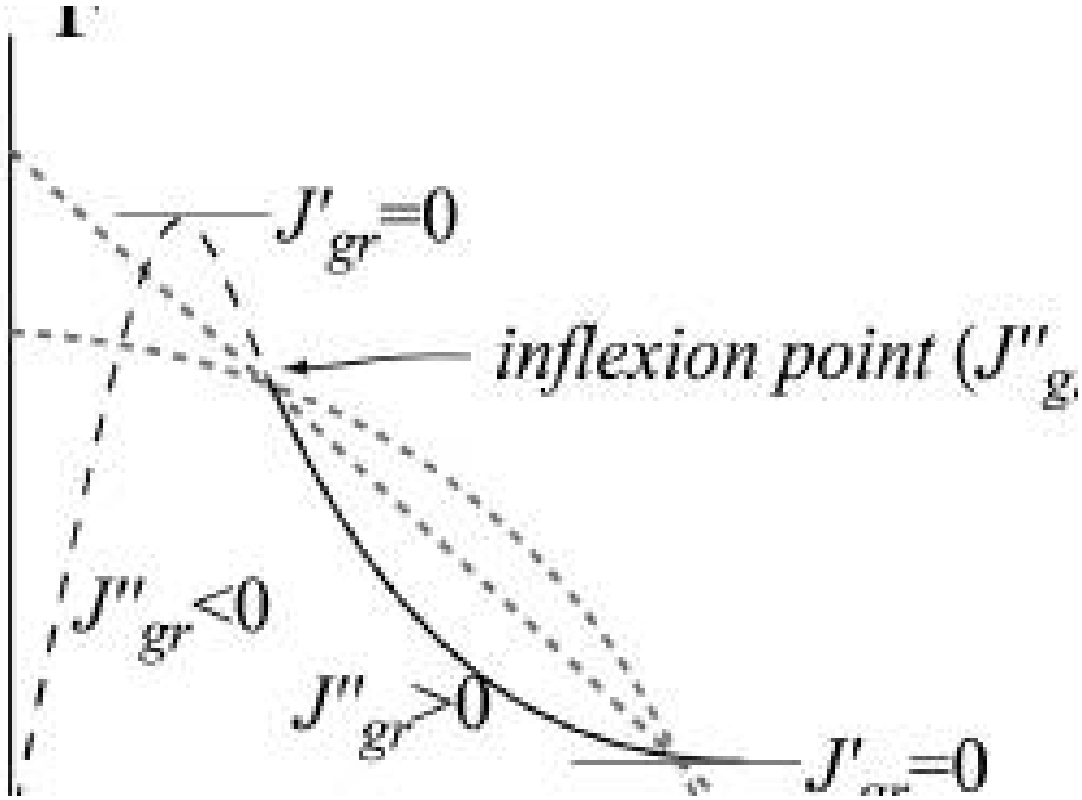

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$a_{fr}(a)$ and $\mu(a)$ for ribosome inhibitors (simple model)

1. Free intracellular antibiotic is in equilibrium with ribosomes → $a_{fr}(a)$

$$a_{fr} \cdot \left(r_t - \left(a - a_{fr} \right) \right) = K_d \cdot \left(a - a_{fr} \right)$$

a intracellular concentration
a_{fr} free Ab conc. (depends on a)
K_d dissociation constant
r_t total ribosome concentration

2. Free ribosomes translate at full rate → $m\,(a)$

$$\mu(a) = \frac{v_m \overbrace{\left(r_t - \left(a - a_{fr} \right) \right)}^{\text{uninhibited ribsomes}}}{\rho_0}$$

v_m uninhibited protein synthesis rate
r_0 concentration of amino acids in proteins

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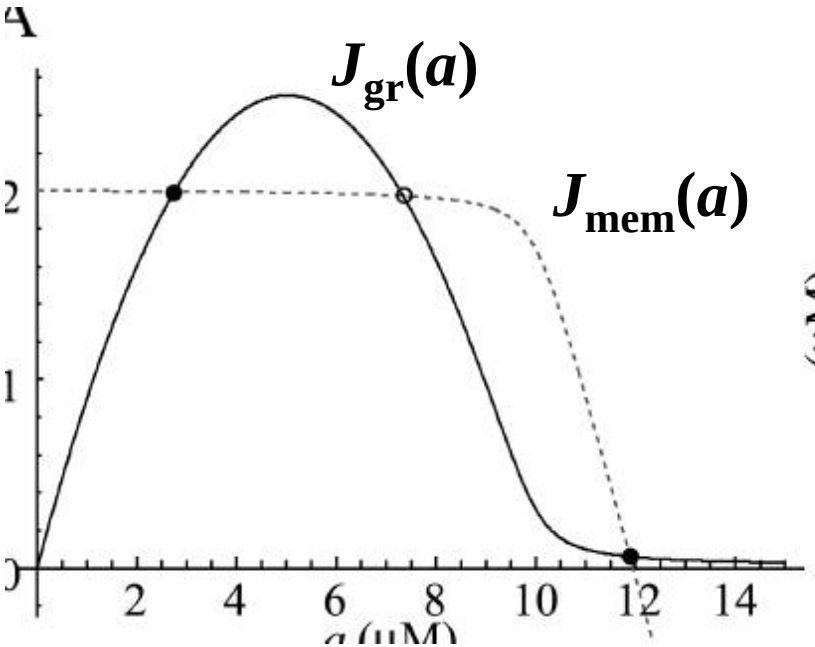
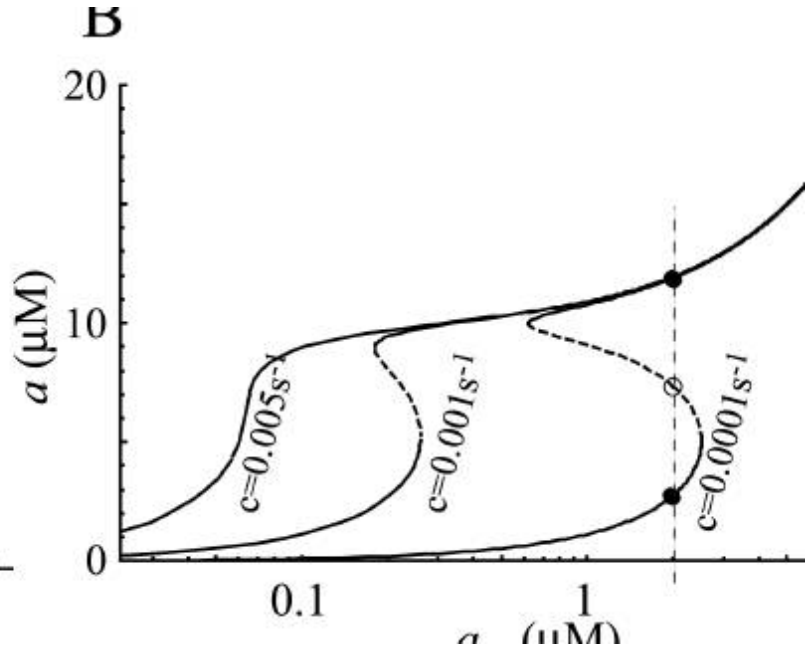
Bistability in ribsome inhibitor model





Steady states for different permeability values (*c*)

$K_d = 10^{-8}$ M

$v_m = 20 s^{-1}$

$r_t = 10 \mu M$

$c = 0.0001 s^{-1}$

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The critical cell wall permeability

$$c^* = \frac{v_m}{27\rho_0}\frac{\left(r_t - K_d\right)^3}{\left(r_t K_d\right)} \approx \frac{v_m}{27\rho_0}\frac{r_t^2}{K_d}$$

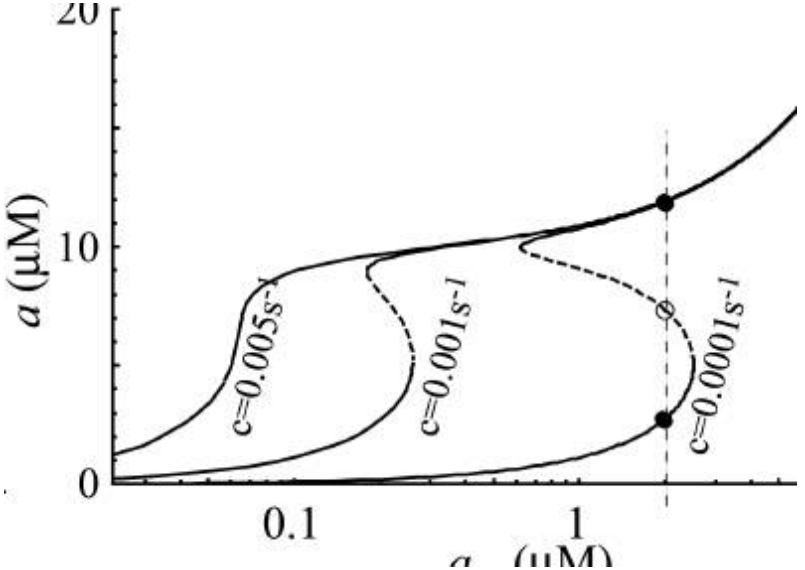
K_d=10^{-8} M

v_m=20s^{-1}

r_t=10µM

Rho=2M

$\rightarrow c^*=0.037s^{-1}$



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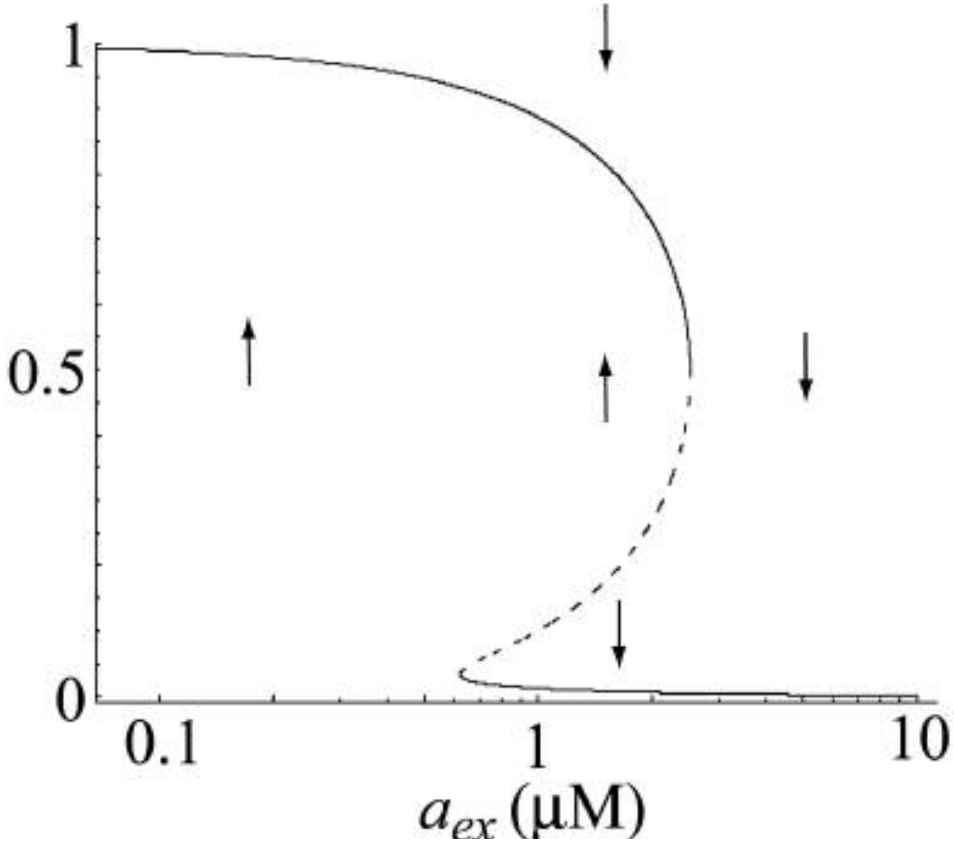
Hysteresis



What about the MIC?

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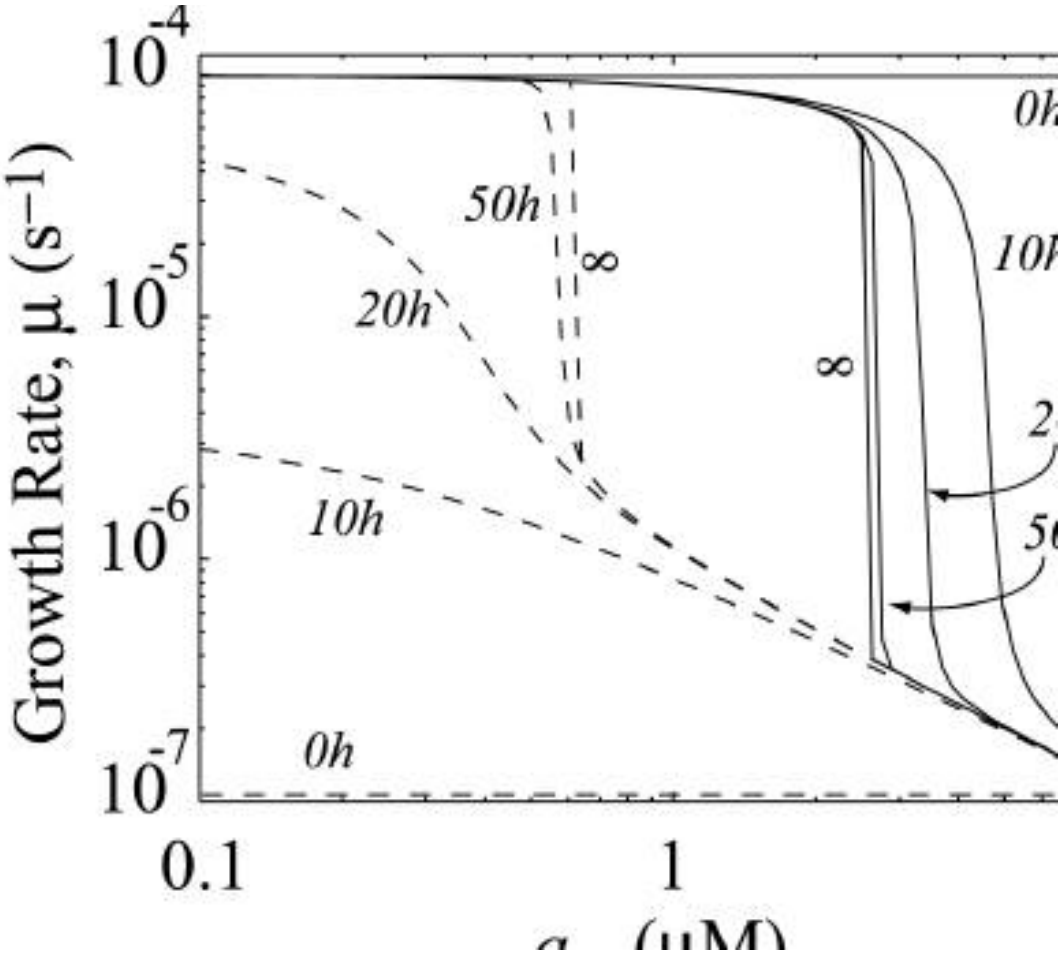
How long time does it take to reach steady state?



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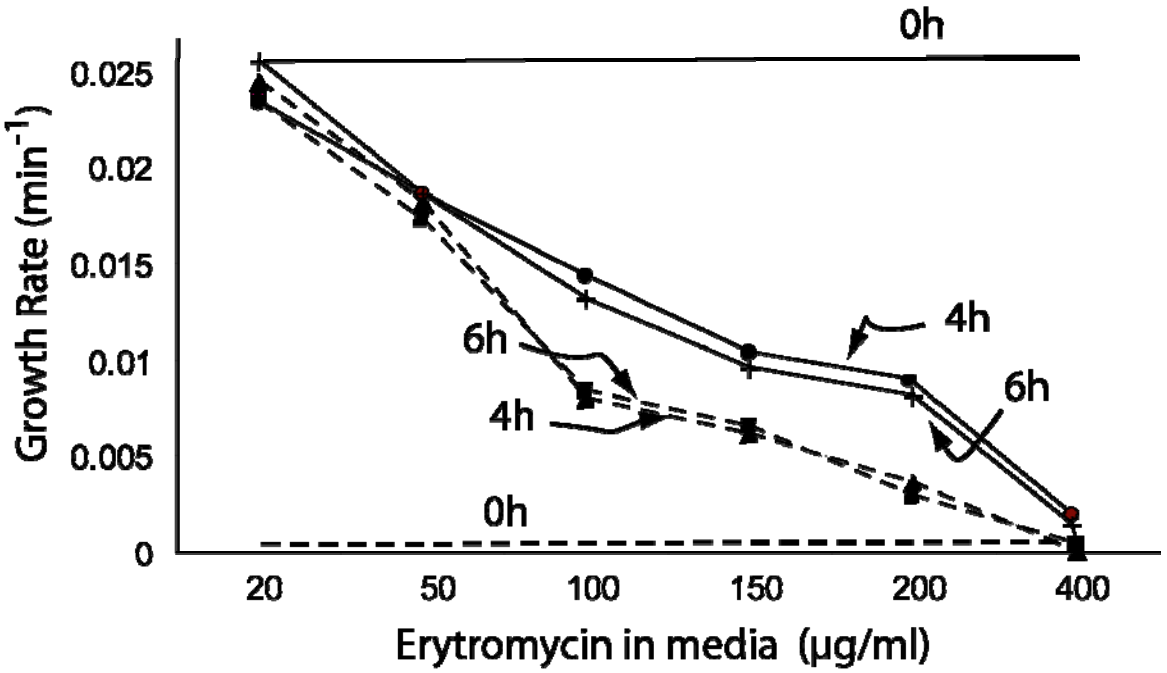
Preliminary experimental results



Experiment by Liina Kosenkranius, Tartu

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Collaborators

- **Johan Elf & Karin Nilsson (modeling, Uppsala)**
- **Tanel Tenson, Liina Kosenkranius (Experiments, Tartu)**
- **Arvi Jõers, Martin Lovmar (Experiments, Uppsala)**

VETENSKAPSRÅDET
SWEDISH RESEARCH COUNCIL

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Non-competitive Inhibitors

$$E + I + S \underset{q}{\overset{k}{\rightleftarrows}} ES + I \xrightarrow{k_c} E + P$$

$$q_I \uparrow\downarrow k_I \qquad\qquad q_I \uparrow\downarrow k_I$$

$$EI + S \underset{q}{\overset{k}{\rightleftarrows}} ESI$$

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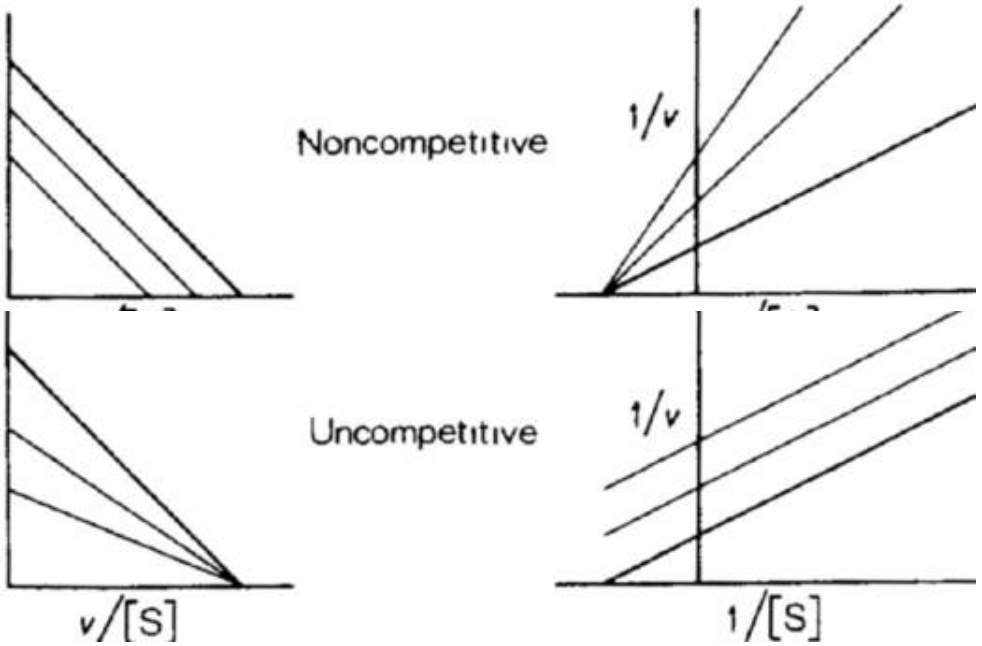
Non- or uncompetitive Inhibitors

$$E + I + S \underset{q}{\overset{k}{\rightleftarrows}} ES + I \xrightarrow{k_c} E + P$$

$q_I \uparrow\downarrow k_I$ $\qquad$ $q_I \uparrow\downarrow k_I$

$$EI + S \underset{q}{\overset{k}{\rightleftarrows}} ESI$$

If substrate binding is in equilibrium.



Picture from "Structure and Mechanism in Protein Science", Alan Fersht (1999)

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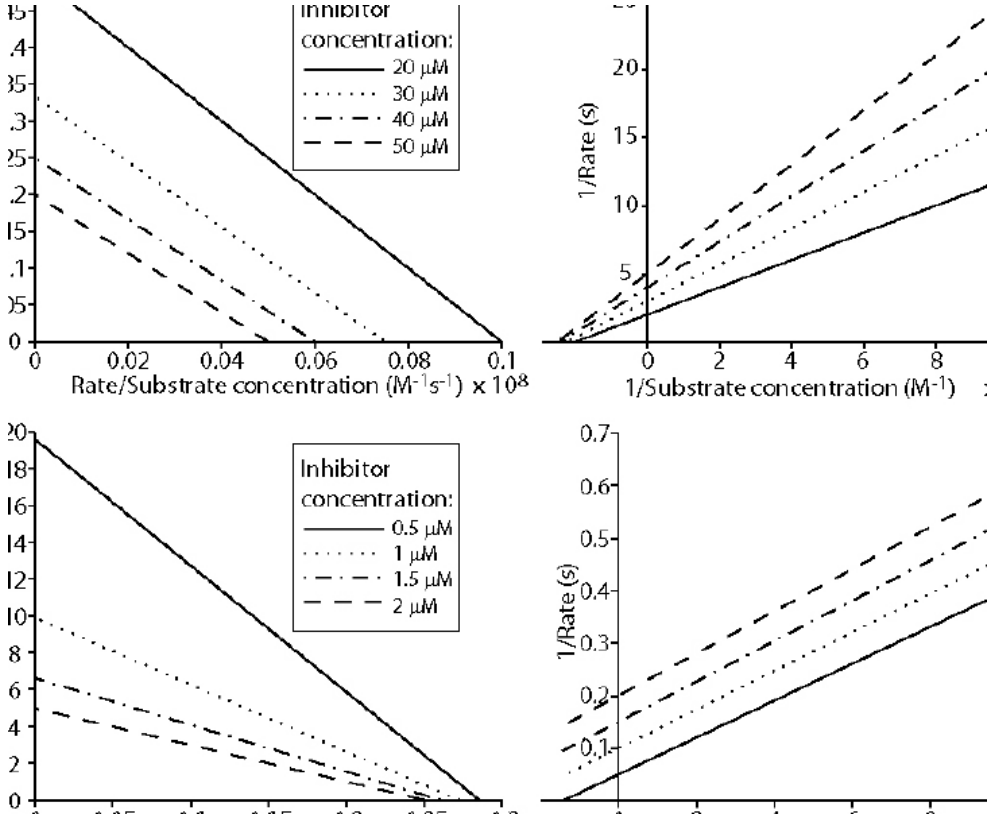
Non-competitive Inhibitors

$$E + I + S \underset{q}{\overset{k}{\rightleftharpoons}} ES + I \xrightarrow{k_c} E + P$$

$q_I \uparrow\downarrow k_I$ $\qquad$ $q_I \uparrow\downarrow k_I$

$$EI + S \underset{q}{\overset{k}{\rightleftharpoons}} ESI$$

- Substrate binding out of equilibrium.
- Inhibitor binding equilbrated.



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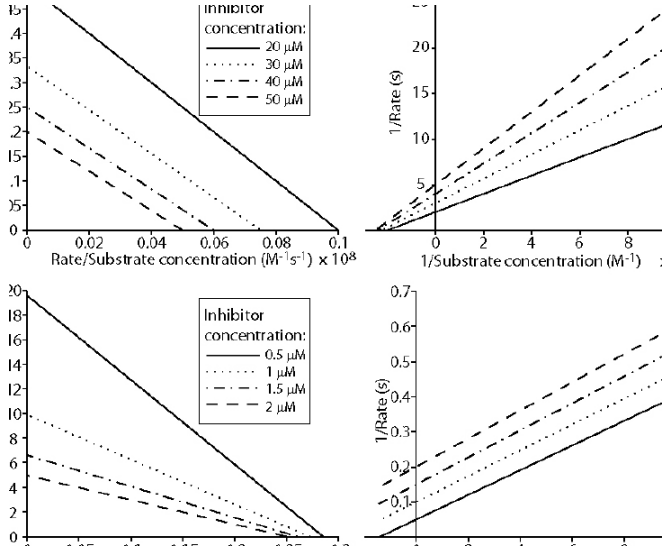
Non-competitive inhibition out of equilibrium

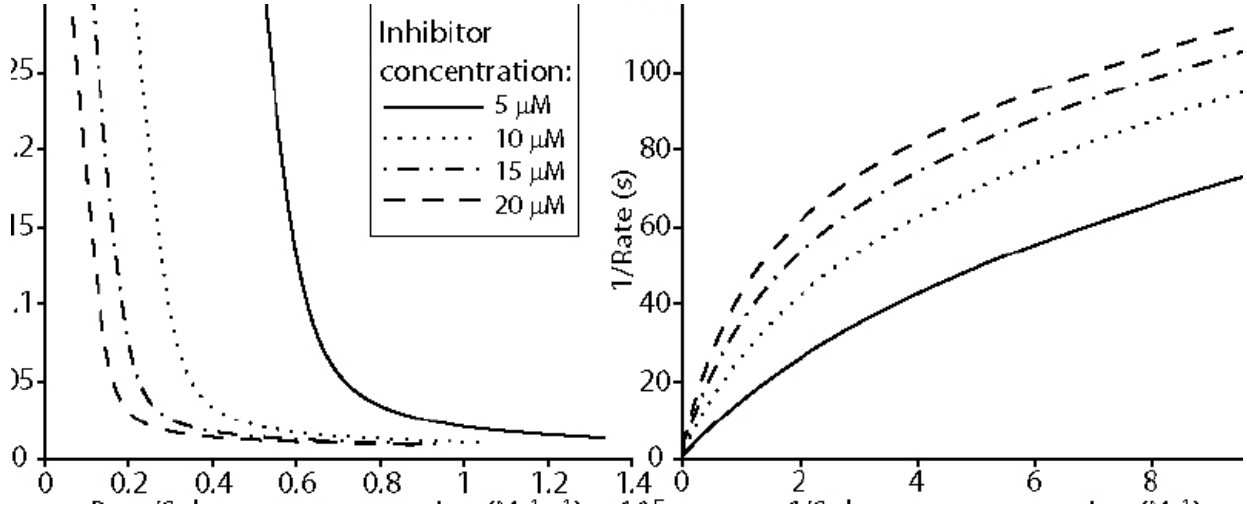
$$E + I + S \underset{q}{\overset{k}{\rightleftarrows}} ES + I \xrightarrow{k_c} E + P$$

$$q_I \uparrow\downarrow k_I \qquad q_I \uparrow\downarrow k_I$$

$$EI + S \underset{q}{\overset{k}{\rightleftarrows}} ESI$$

If the binding kinetics is like for erythromycin...





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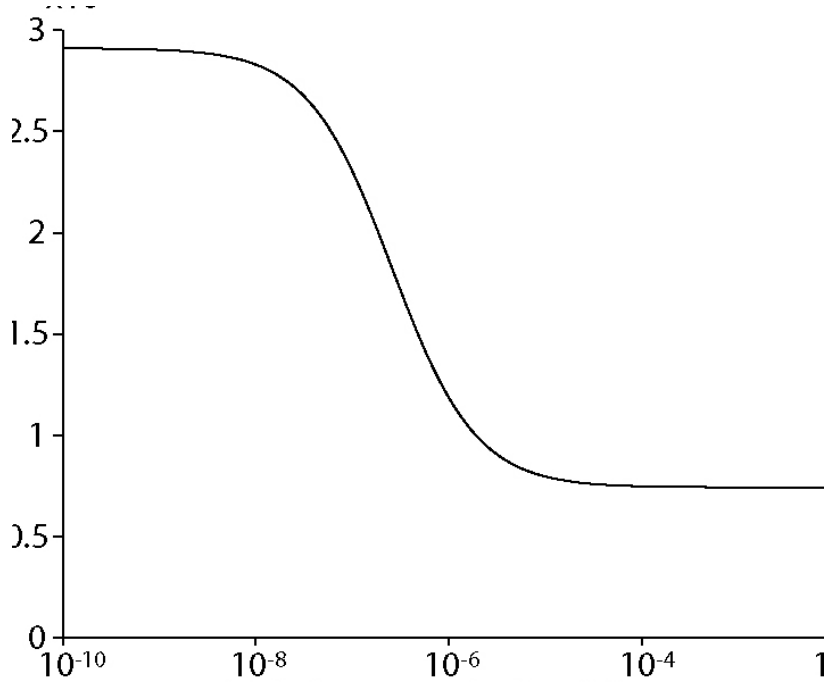
Inhibition of protein Synthesis

$$50S + I \xrightarrow{k_{Init}} R + I \xrightarrow{k_e} R_e \xrightarrow{k_{e1}}$$

$$q_I \uparrow\downarrow k_I \qquad q_I \uparrow\downarrow k_I$$

$$50S \cdot I \xrightarrow{k_{Init}} R \cdot I$$

With an inhibitor that has binding kinetics like erythromycin



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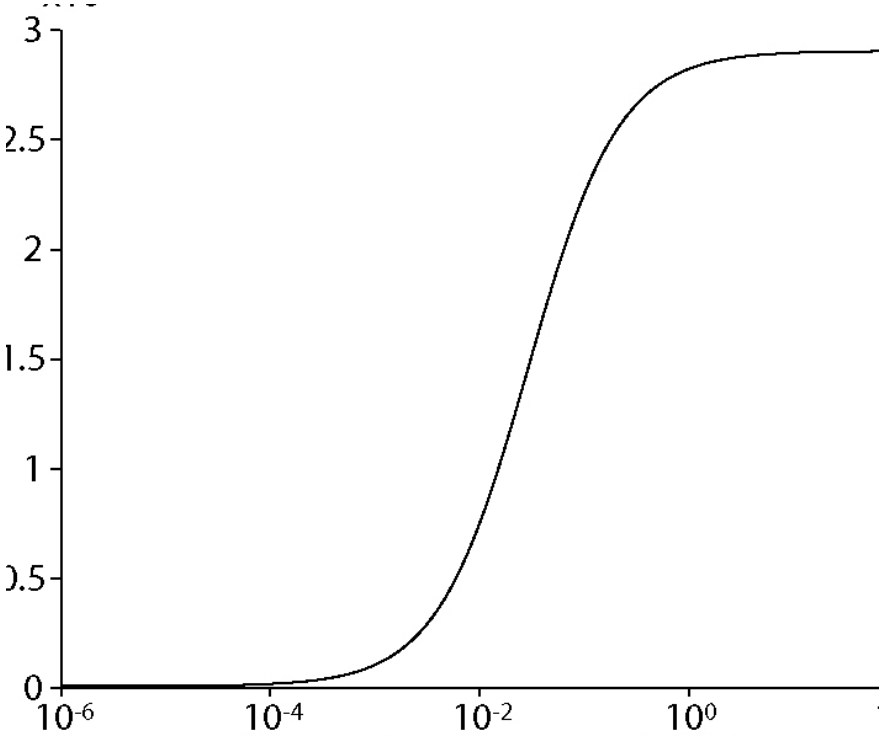
Inhibition of Protein Synthesis in the test tube

$$50S + I \xrightarrow{k_{Init}} R + I \xrightarrow{k_e} R_e \xrightarrow{k_{e1}}$$

$$q_I \uparrow\downarrow k_I \qquad q_I \uparrow\downarrow k_I$$

$$50S \cdot I \xrightarrow{k_{Init}} R \cdot I$$

Constant binding constant, but different rate constants



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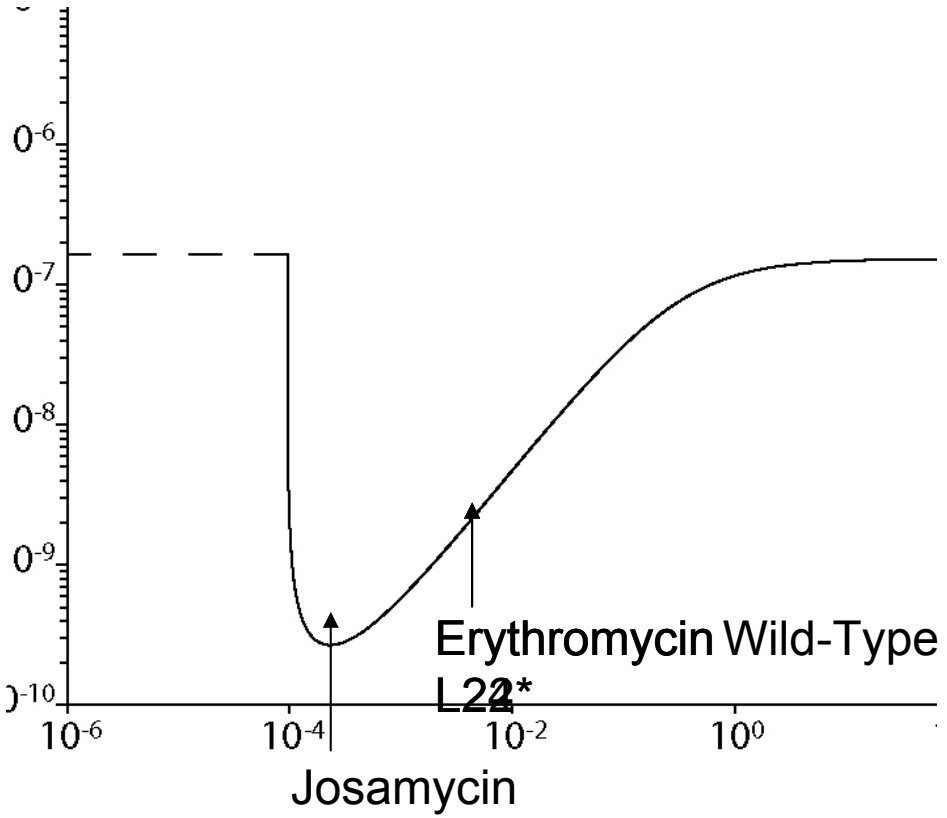
A Kinetic Window for Inhibition of Protein Synthesis in the Living Cell

$$50S + I \xrightarrow{k_{Init}} R + I \xrightarrow{k_e} R_e \xrightarrow{k_{e1}}$$

$$q_I \uparrow\downarrow k_I \qquad q_I \uparrow\downarrow k_I$$

$$50S \cdot I \xrightarrow{k_{Init}} R \cdot I$$

Dilution due to growth of the bacteria



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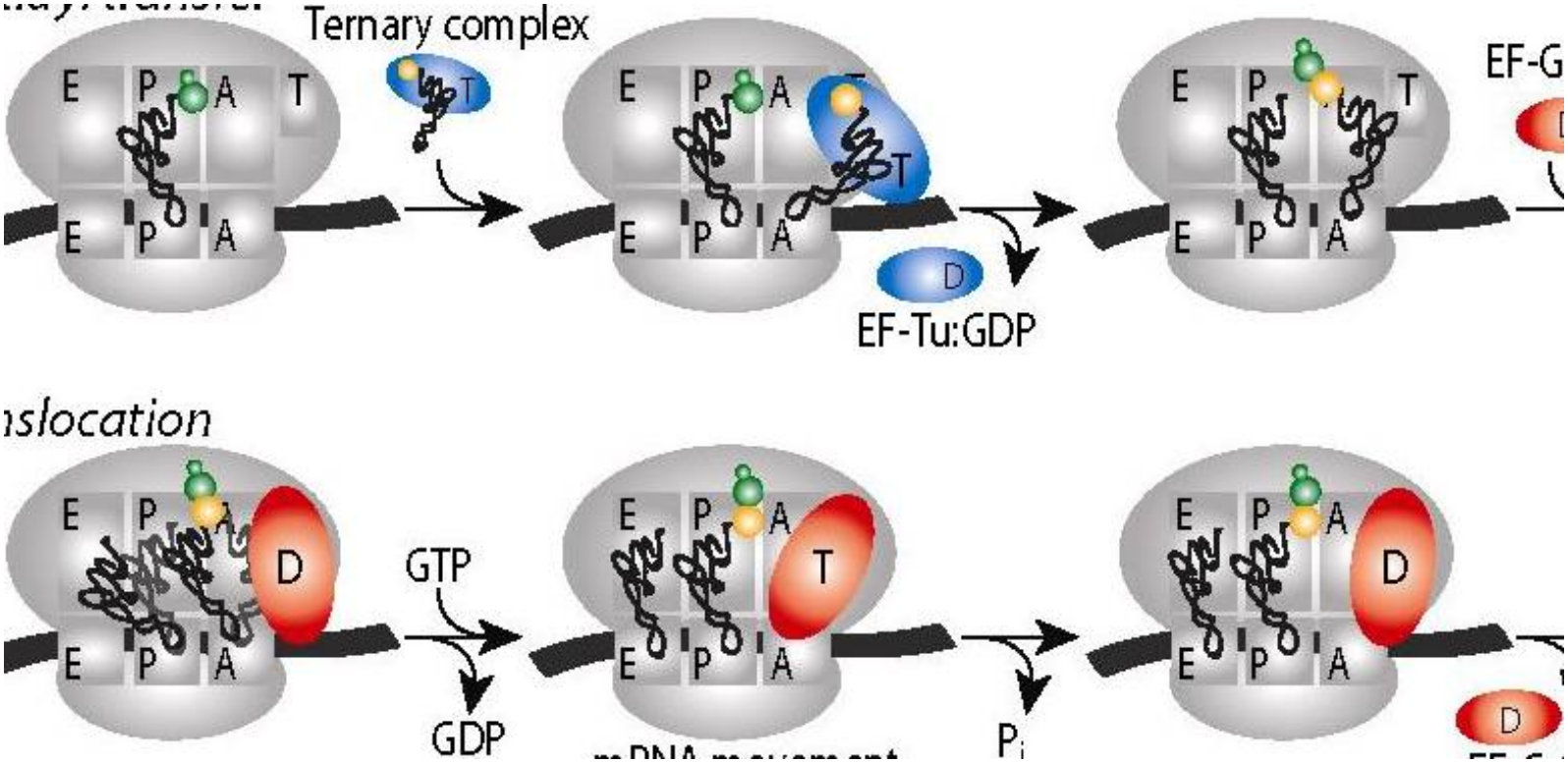
Dichotomous codon reading during amino acid starvation

Peptide chain elongation on ribosomes



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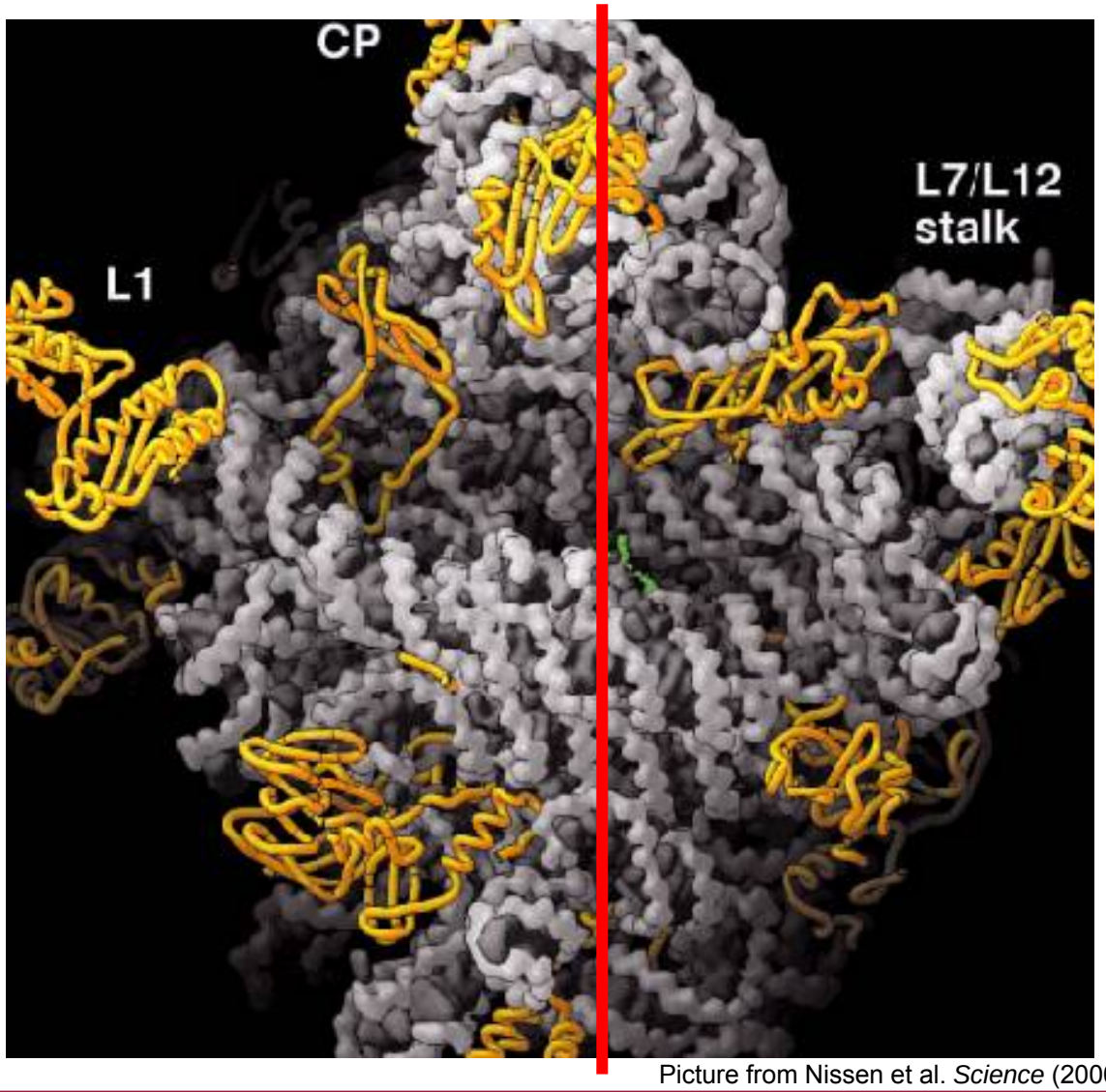
The large ribosomal subunit viewed from the the small subunit interface



Picture from Nissen et al. *Science* (2000) **289**, 920-930

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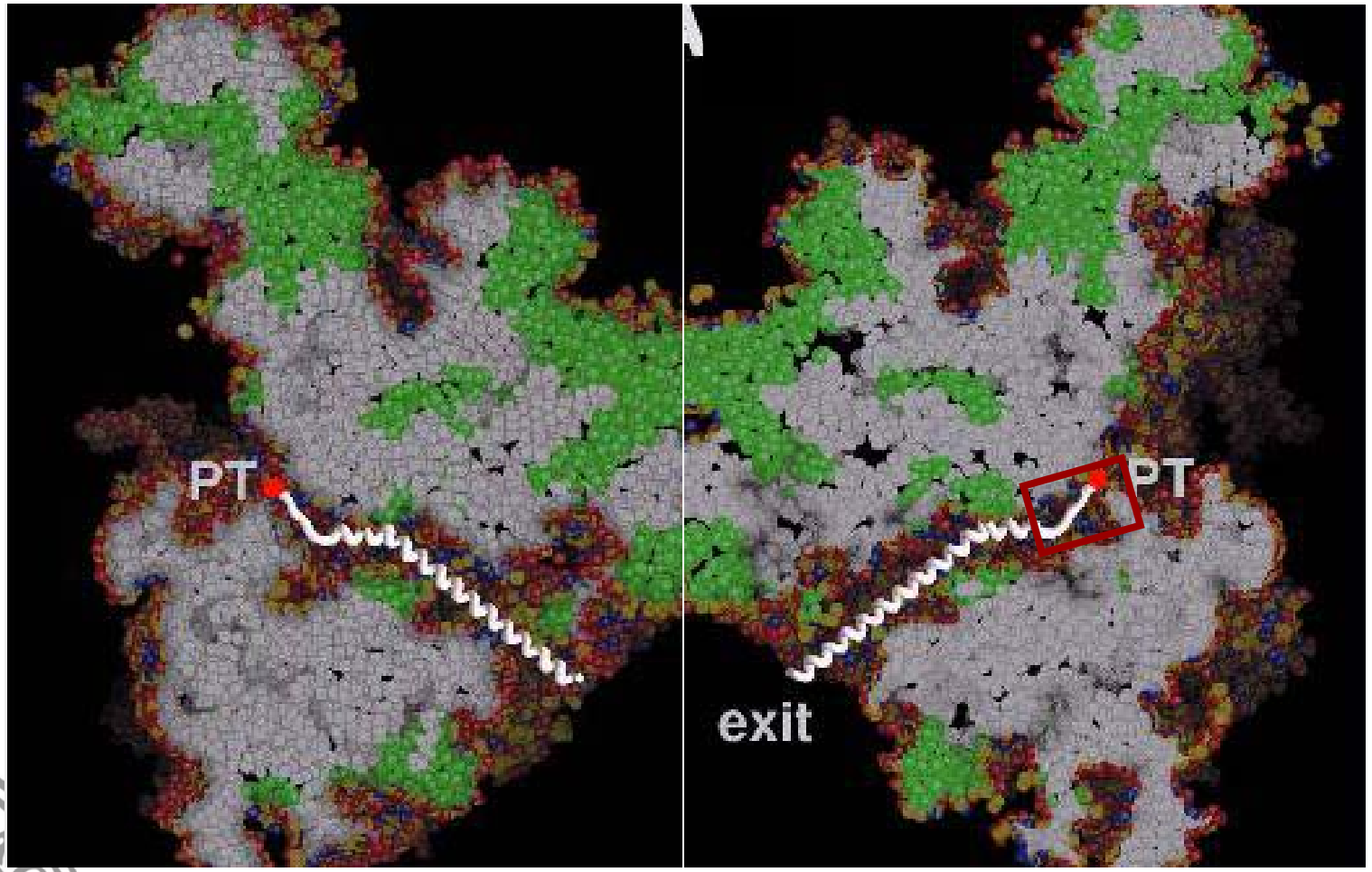
The large ribosomal subunit cleaved along the nascent peptide exit tunnel



Picture from Nissen et al. *Science* (2000) **289**, 920-930

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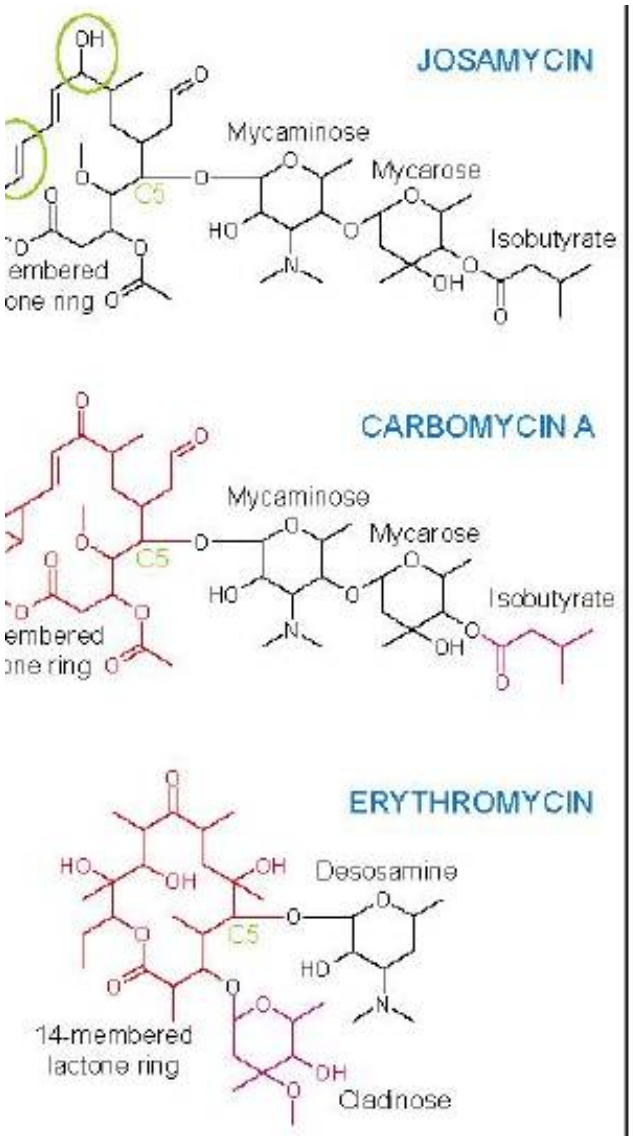
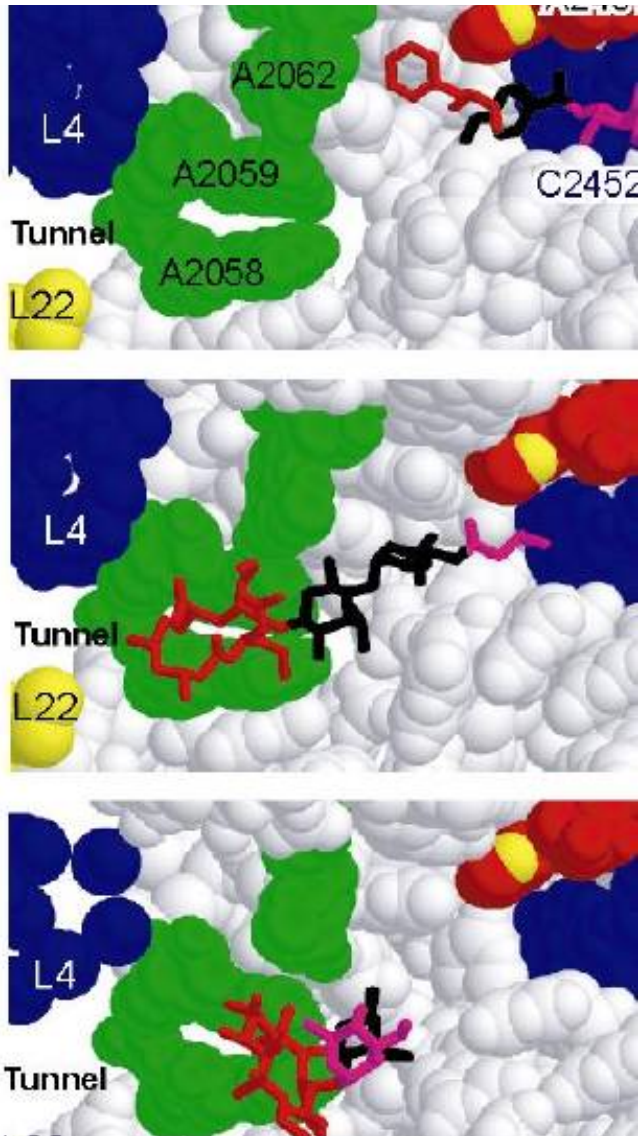
Macrolides block the peptide exit tunnel





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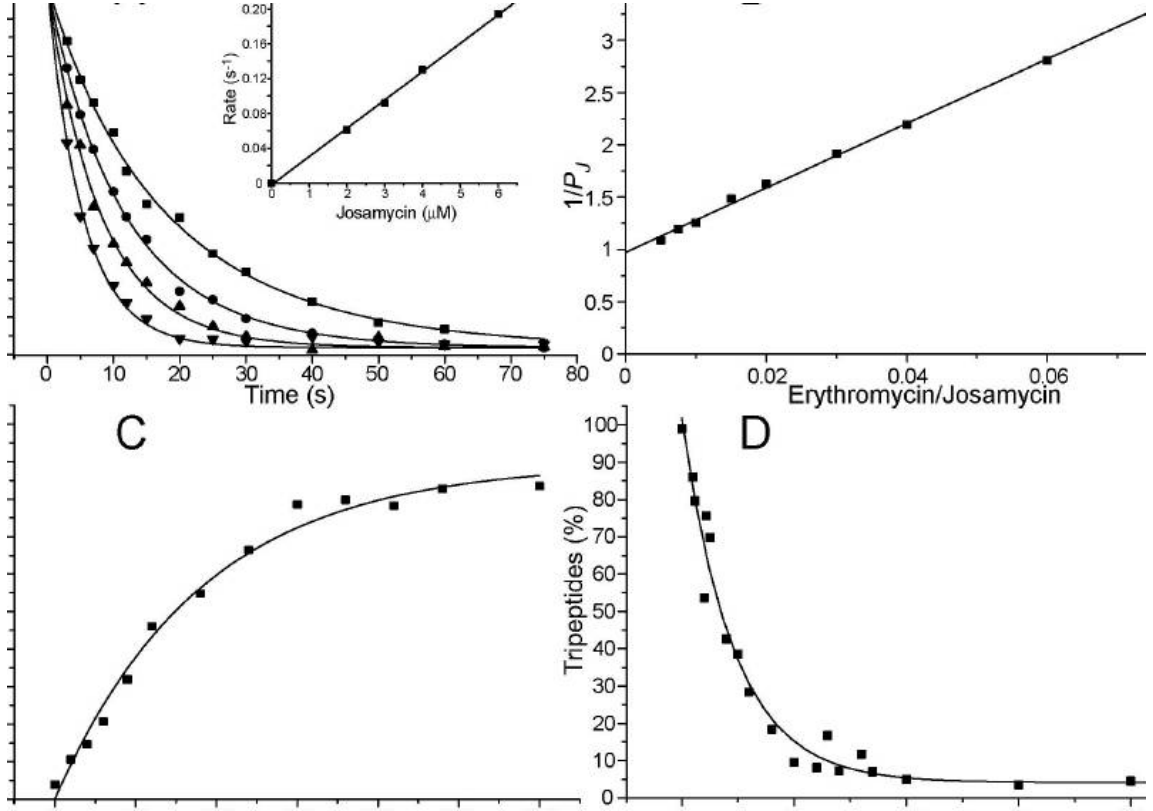
Rate constants for erythromycin and josamycin interactions with the ribosome



	Josamycin	Erythromycin
k_a ($\mu M^{-1}s^{-1}$)	0.0325 ± 0.0007	1.00 ± 0.04
k_d (s^{-1})	$0.18 \cdot 10^{-3} \pm 0.02 \cdot 10^{-3}$	$10.8 \cdot 10^{-3} \pm 0.7 \cdot 10^{-3}$
K_D (nM)	5.5 ± 0.5	10.8 ± 0.3

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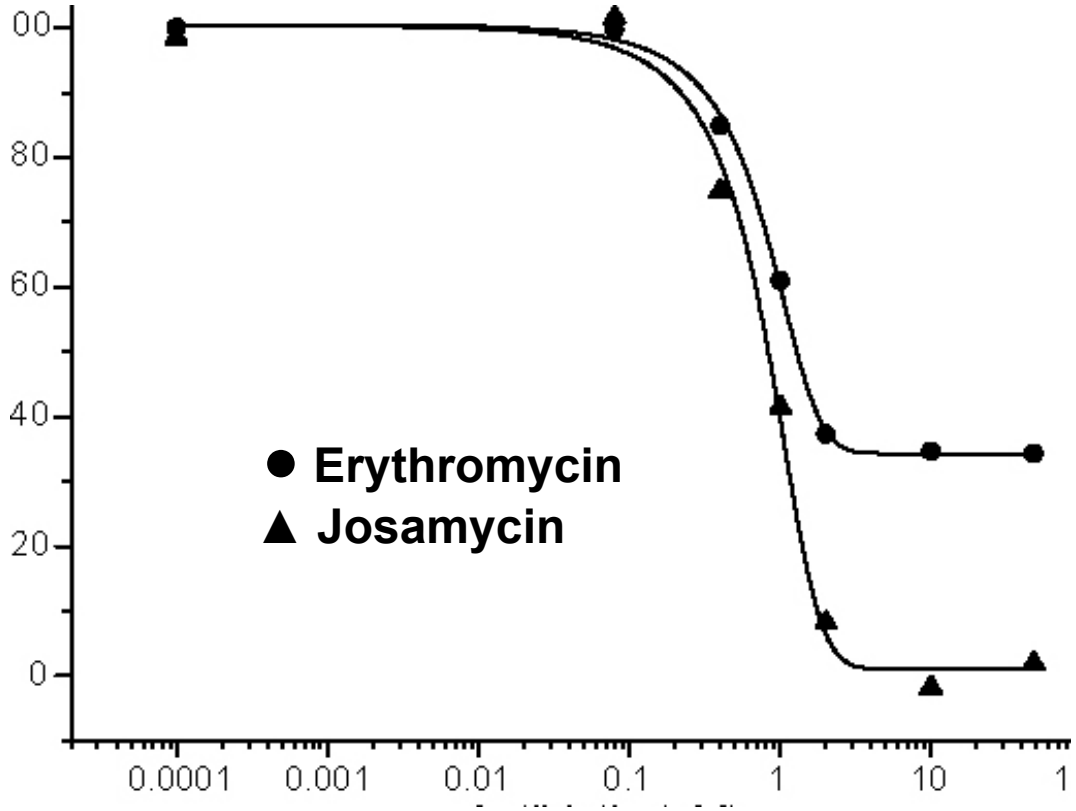
Inhibition of protein synthesis by erythromycin and josamycin



	Josamycin	Erythromycin
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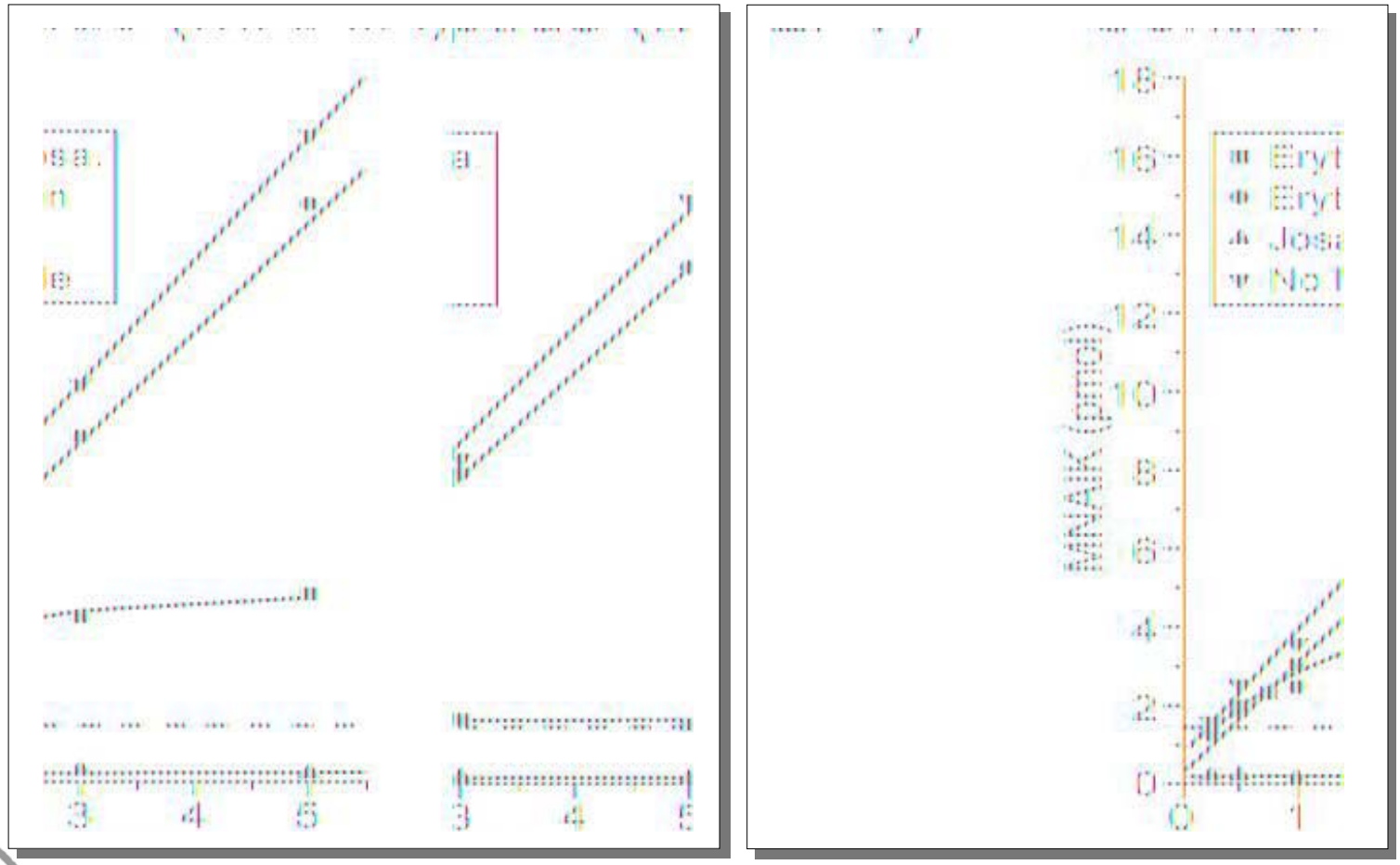
Systems Biology of Antibiotic Action and Resistance

Part II: Cis-acting pentapeptides conferring erythromycin resistance

Erythromycin chased by josamycin



Erythromycin dissociates with a probability close to 1 when the resistance peptide is expressed.

Lovmar, M. *et al.* (2006), *J Biol Chem* **286**, 6742-6750

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Resistance peptide expression increases the erythromycin dissociation rate constant

Translated Peptide	Erythromycin dissociation rate constant (s^{-1})
Init. complex	0.011 ±0.001
MR	0.017 ±0.005
MRL	0.025 ±0.004
MRLF	0.051 ±0.004
MRLFV	**0.068 ±0.006**
MRLFVA	0.014 ±0.001
MRLFVAN	0.014 ±0.001
MN	0.011 ±0.001
MNA	0.015 ±0.001
MNAI	0.016 ±0.001
MNAIK	0.017 ±0.001
MNAIK	0.015 ±0.001

Lovmar, M. *et al.* (2006), *J Biol Chem* **286**, 6742-6750

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Erythromycin and peptide dissociation rate constants

Translated Peptide	Releasing agent	Erythromycin dissociation rate constant (s^{-1})[a]	Peptide dissociation rate constant (s^{-1})[a]	Peptidyl-tRNA hydrolysis rate constant (s^{-1})[b]
MRLFV	RF1	0.14 ±0.02	0.073 ±0.007	
MRLFV	RF1	No Erythromycin	0.22 ±0.02	
MRLFV	**RF2**	**0.13 ±0.01**	**0.074 ±0.01**	**0.10 ±0.01**
MRLFV	**RF2**	**No Erythromycin**	**0.25 ±0.01**	**0.26 ±0.03**
MRLFV	Puromycin	0.067 ±0.009	Not determined[c]	0.23 ±0.02
MRLFV	Puromycin	No Erythromycin	Not determined[c]	0.31 ±0.03
MRLFVA	RF2	0.014 ±0.001	0.015 ±0.002	0.015 ±0.001
MRLFVA	RF2	No Erythromycin	0.29 ±0.03	0.32 ±0.03
MRLFVAN	RF2	0.014 ±0.001	0.006 ±0.0004	0.004 ±0.0004
MRLFVAN	RF2	No Erythromycin	0.27 ±0.03	0.44 ±0.03
MNAIK	**RF2**	**0.015 ±0.001**	**0.26 ±0.03**	**0.28 ±0.02**
MNAIK	RF2	No Erythromycin	0.25 ±0.02	0.27 ±0.02

[a]Measured by nitro-cellulose filter binding
[b]Measured by formic acid precipitation
[c]Could not be determined because fMRLFV-Puromycin bind to NC-filters

Lovmar, M. *et al.* (2006), *J Biol Chem* **286**, 6742-6750

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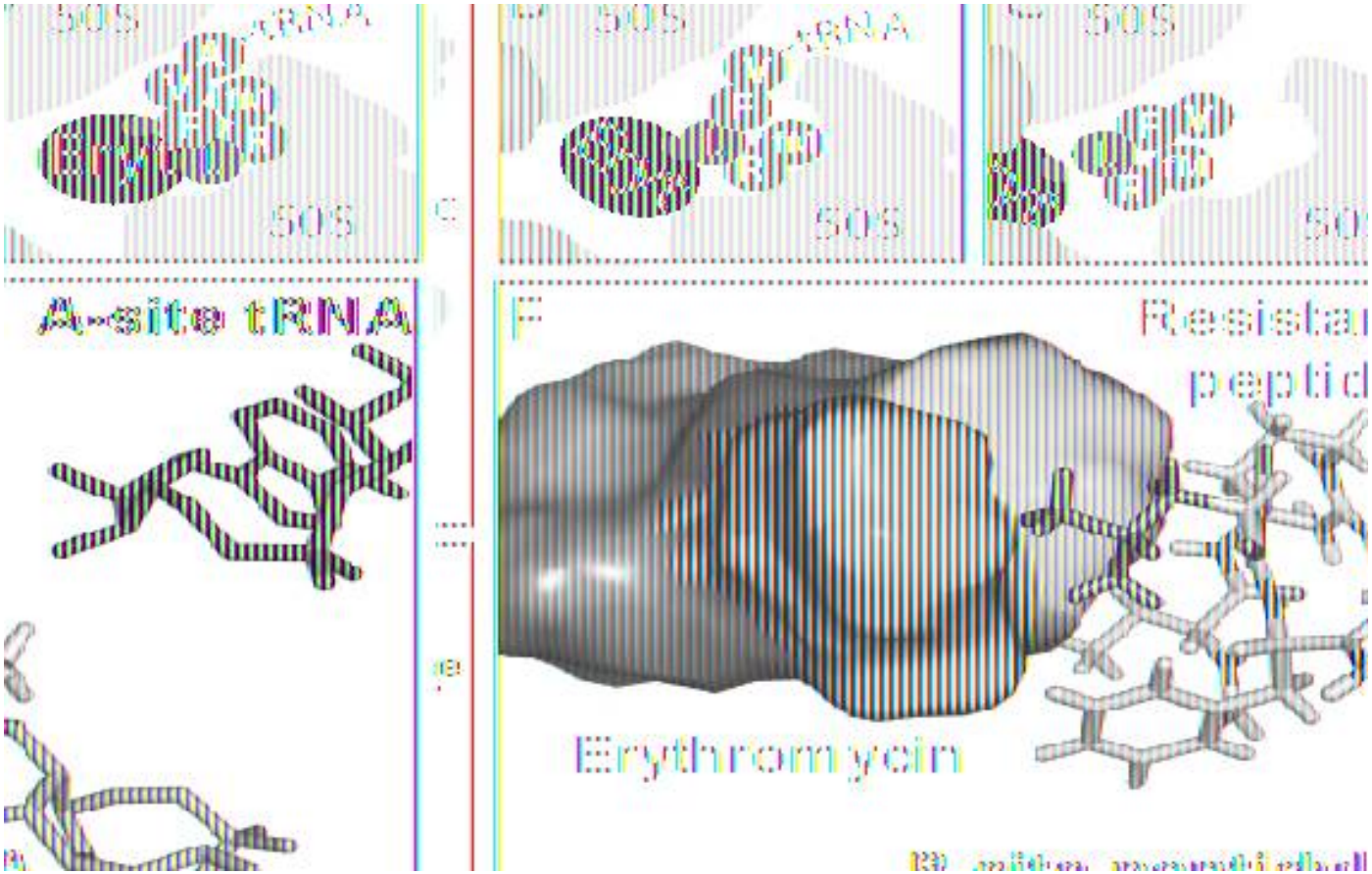
A molecular model for the mechanism of peptide mediated resistance supported by structural simulations



Lovmar, M. *et al.* (2006), *J Biol Chem* **286**, 6742-6750

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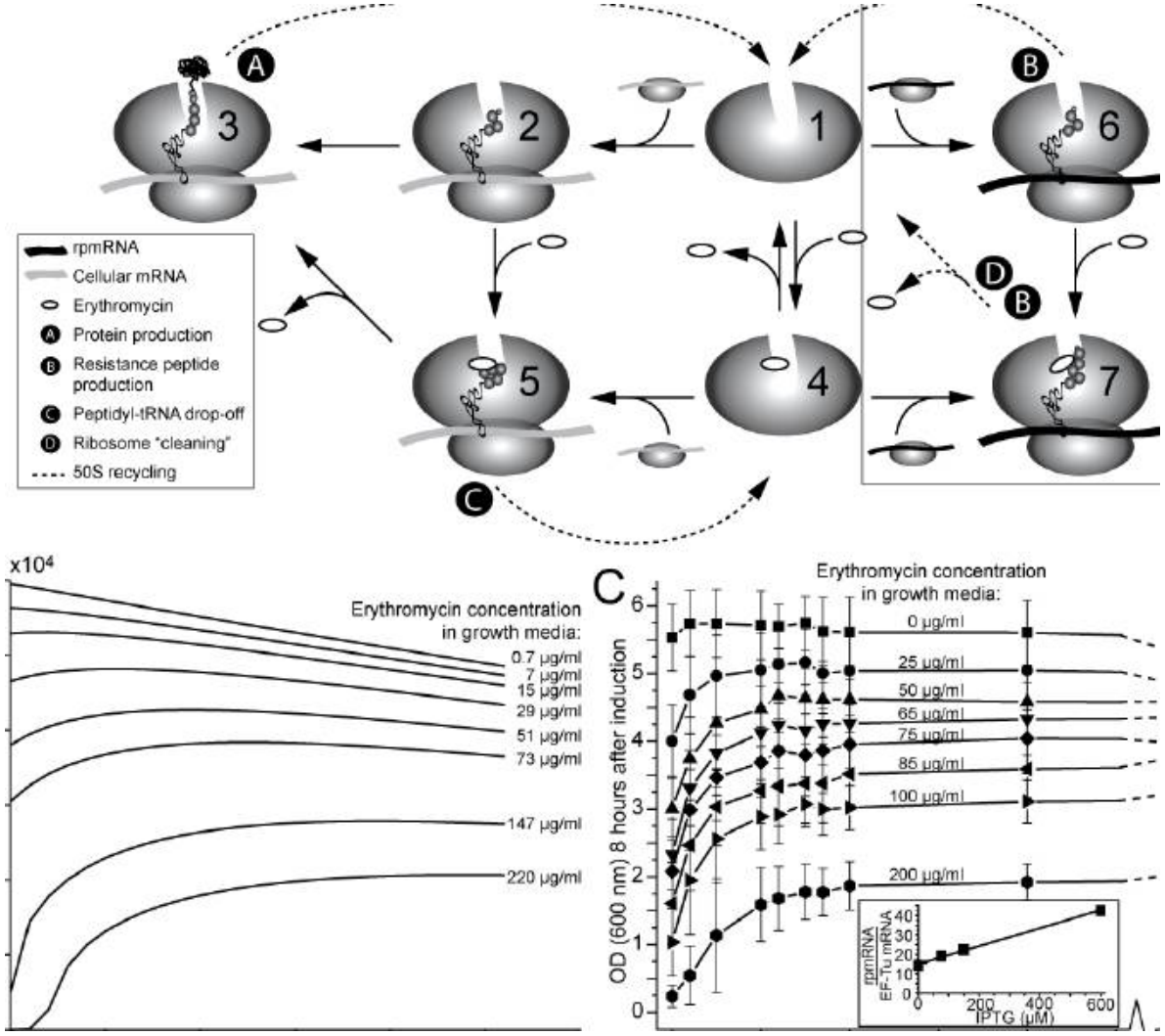
Systems Biology of Antibiotic Action and Resistance

Part III: Modeling erythromycin action and resistance peptide action validated in vivo

Model of erythromycin and resistance peptide action tested *in vivo*



Lovmar, M. *et al.* (2006), *J Biol Chem* **286**, 6742-6750

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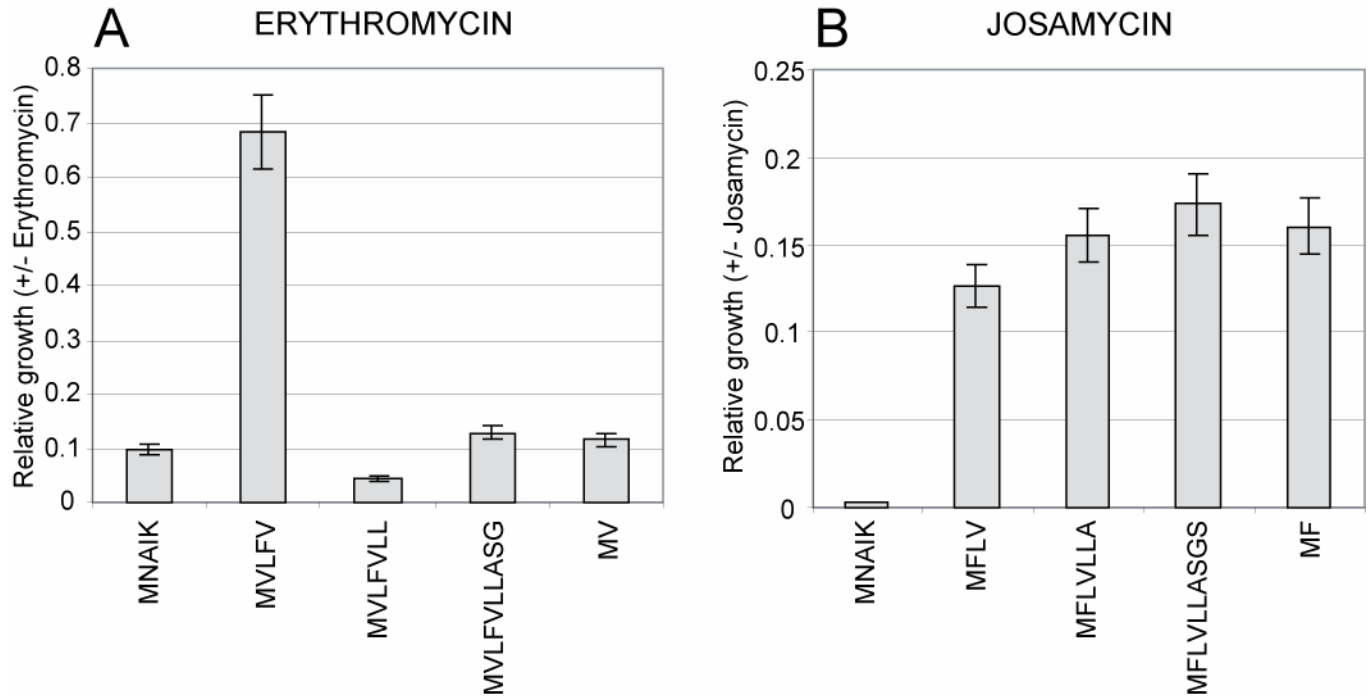
Systems Biology of Antibiotic Action and Resistance

Part IV: Josamycin action and josamycin resistance peptides

Jocamycin and erythromycin resistance peptides differ in mode of action



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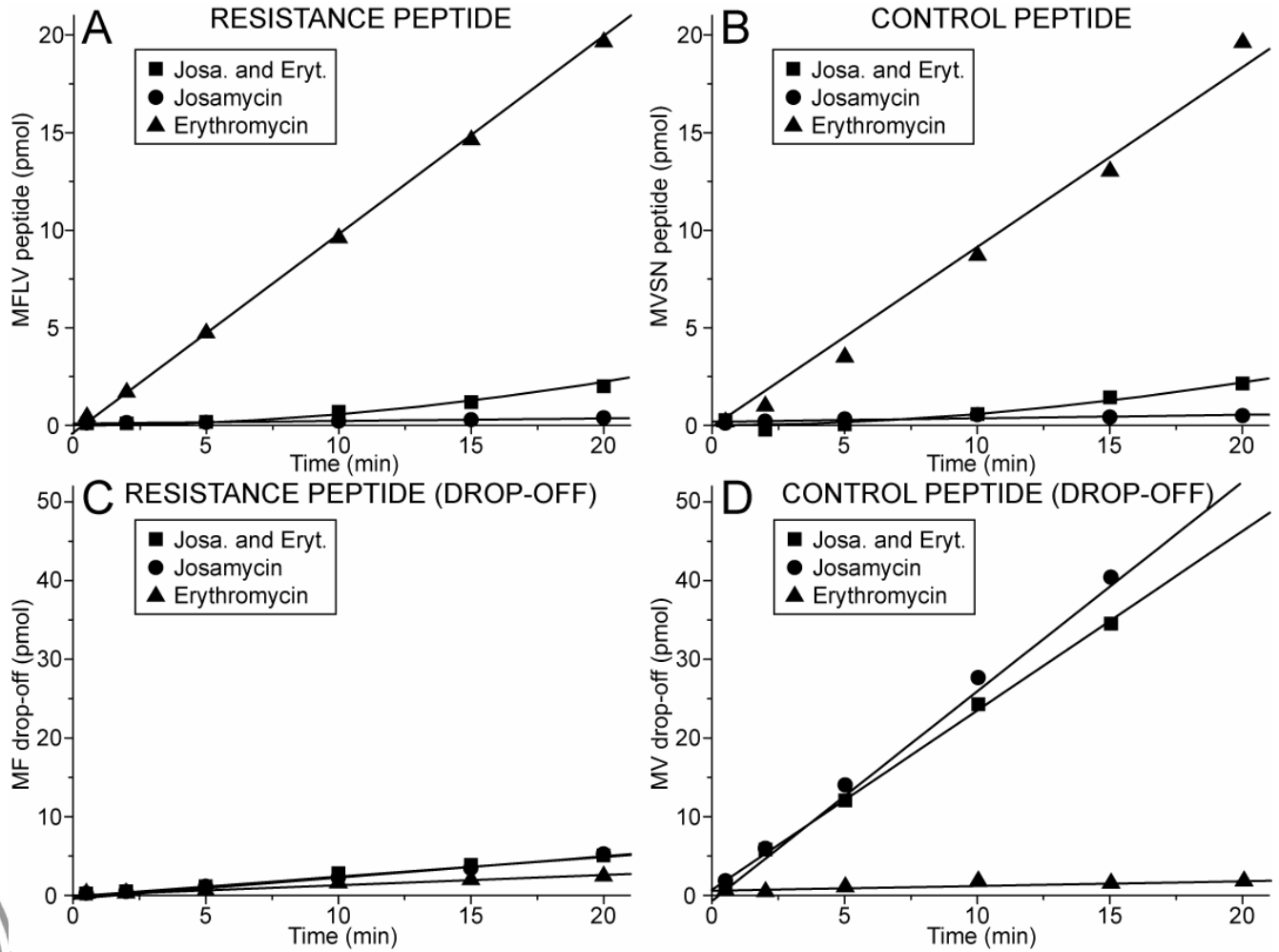
Josamycin resistance peptide does not eject josamycin. Peptidyl-tRNA drop-off



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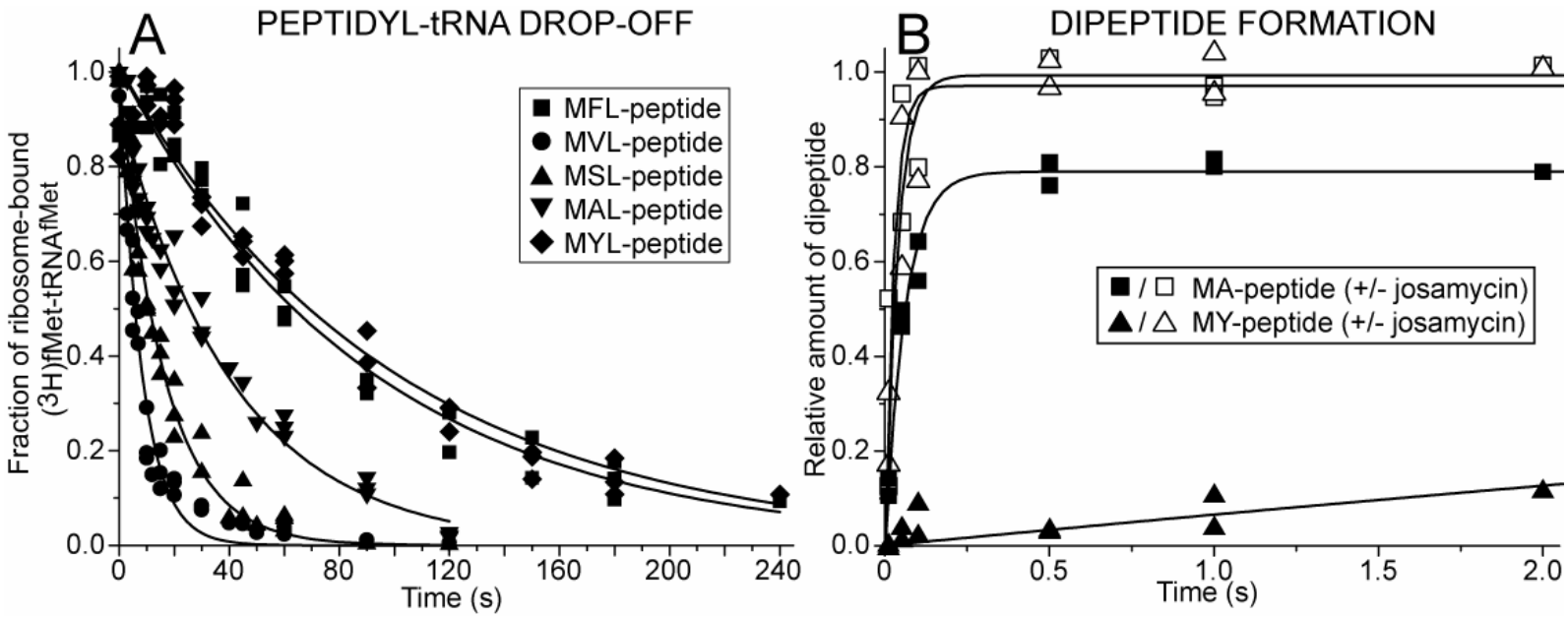
Rapid josamycin induced drop-off of control, but not resistance peptidyl-tRNA



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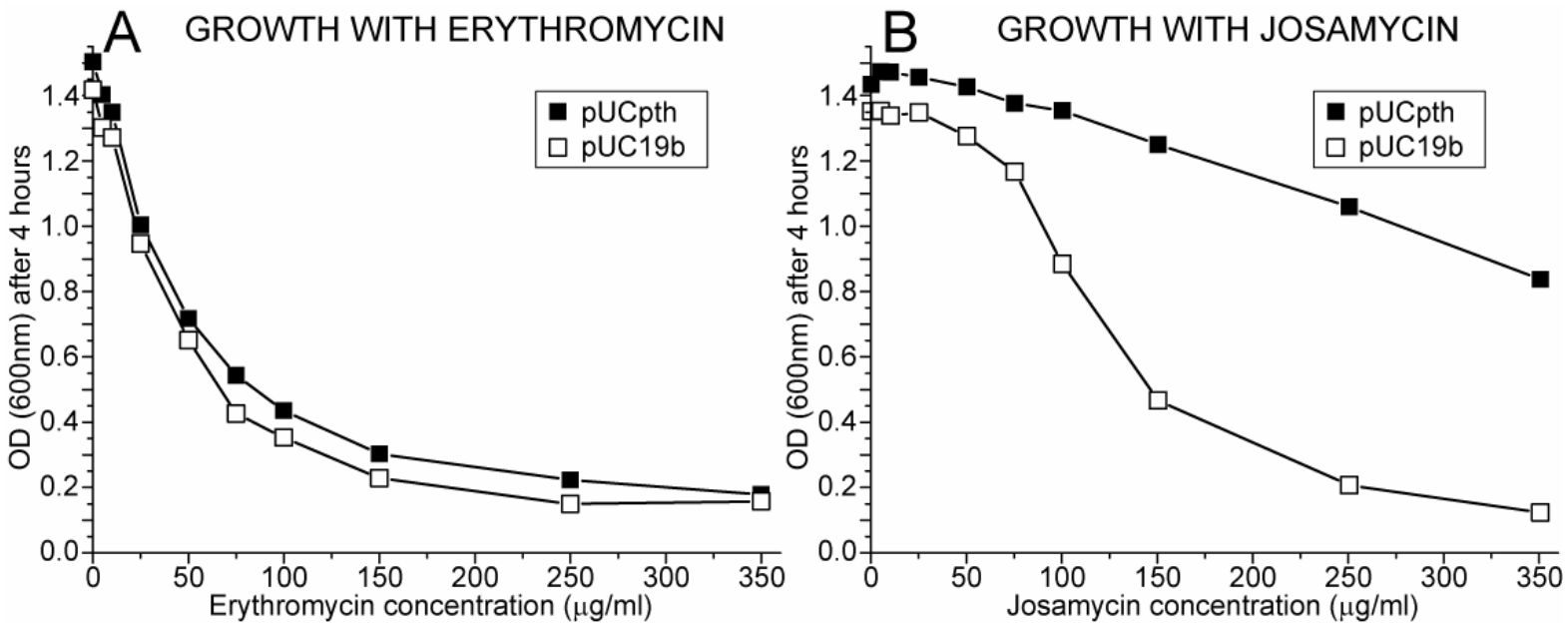
Peptidyl-tRNA hydrolase over expression confers josamycin, but not erythromycin, resistance



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Systems Biology of Antibiotic Action and Resistance

Part V: Modeling josamycin action and resistance *in vivo*

(in progress)

Modeling in progress

1. Primary effect of josamycin is induction of dipeptidyl-tRNA drop-off from a small fraction of josamycin bound ribosomes
2. Primary effect of josamycin resistance peptides is to sequester josamycin bound 50S subunits in the cell, thereby attenuating dipeptidyl-tRNA drop-off
3. The model predicts that the tRNA isoacceptor (i) with the largest ratio (f_{2i}/[$tRNA_i$] between second position codon usage on ribosomes (f_{2i}) and total intracellular concentration ([$tRNA_i$]) will become sequestered as dipeptidyl-tRNA and rate limiting for bacterial growth
4. The model predicts that over expression of the limiting tRNA will make the tRNA with the next largest such ratio rate limiting for bacterial growth, etc etc
5. Accordingly, peptidyl-tRNA sequestering occurs according to a mechanism similar to that at work in selective charging of tRNA isoacceptors during starvation of their common amino acid

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- Tanel Tenson, Tartu

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