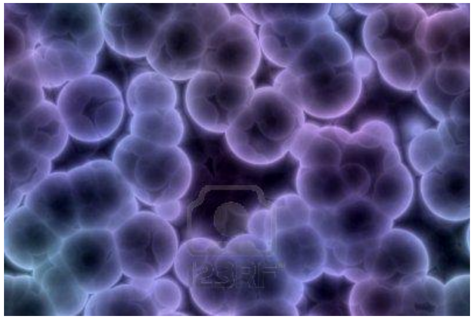
Lecture 3:
Bridging scales: From Biophysics to Populations

1. **Evolution at multiple scales**

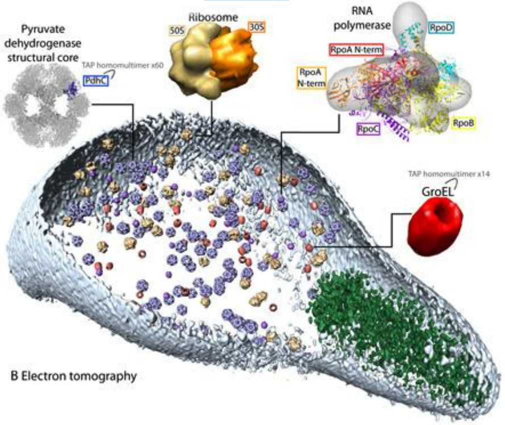
2. **Emerging “universals” from biophysics and genomics**

1. A unified framework of molecular evolution

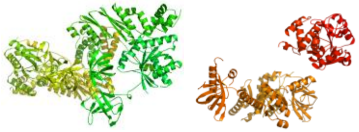
Length and Time Scales in Evolution



POPULATION



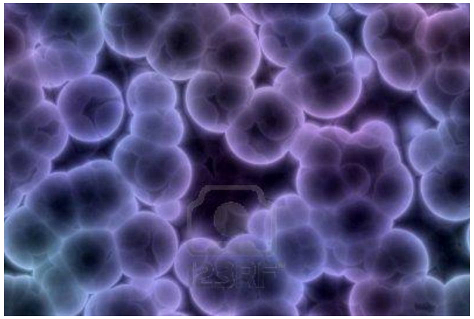
ORGANISM



BIOMOLECULES

ATTGCCATAACGGATGTAAATTGCCATAACGGGCTAA

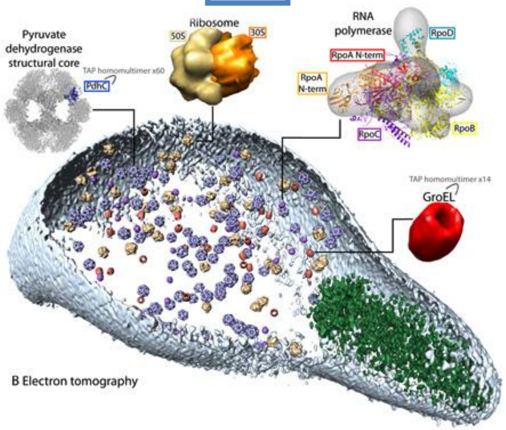
Length and Time Scales in Evolution



POPULATION

Population Genetic/ Ecological variable

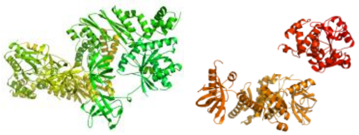
N Population size



ORGANISM

Genomic variables

C protein copy numbers
dN, dS molecular clock rates
PPI protein-protein interaction
μ mutation rate



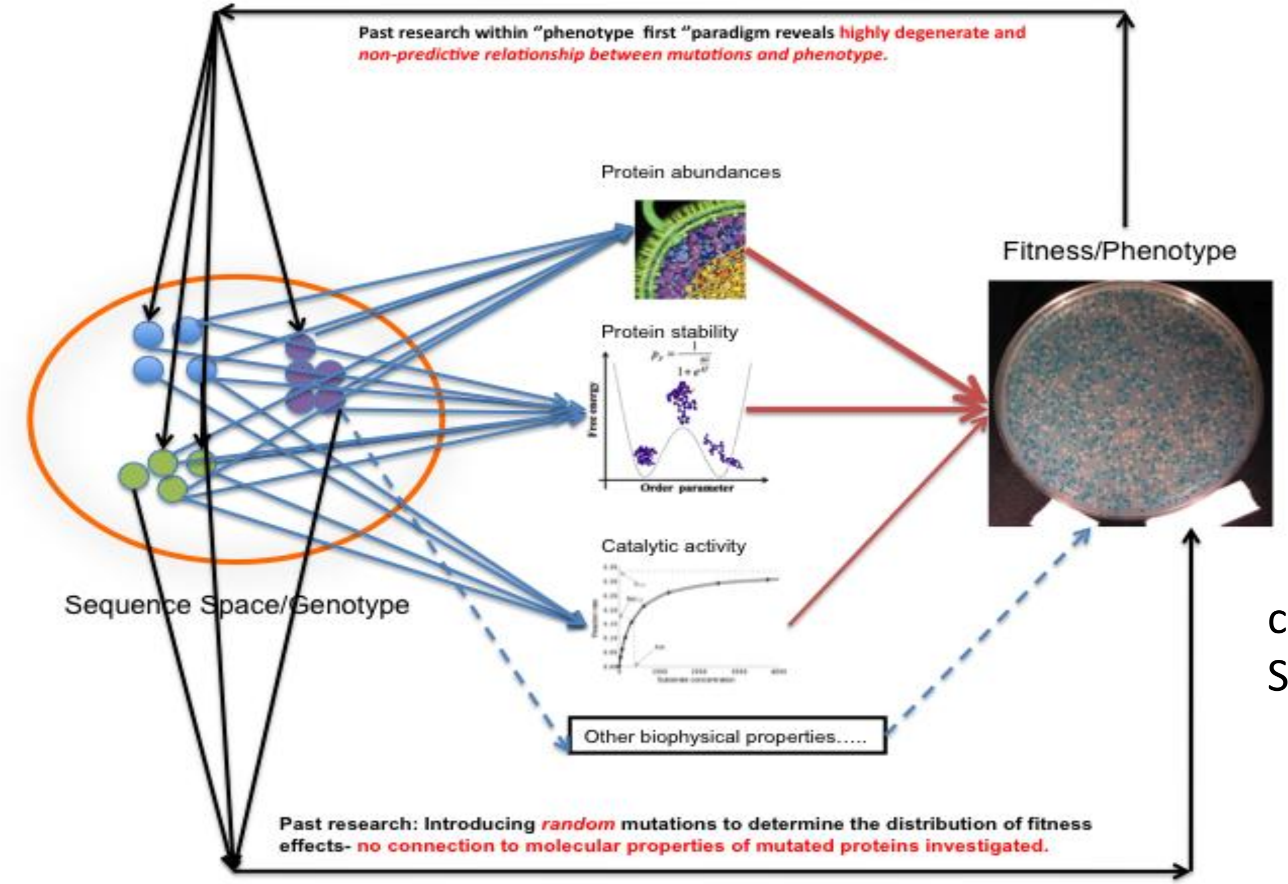
ATTGCCATAACGGATGTAAATTGCCATAACGGGCTAA

BIOMOLECULES

Molecular variables

ΔG Stability
SAS Surface accessible areas
D Fold designability
AA Amino acid composition
ΔG_{pd} Protein-DNA interaction

Hard to Bridge Scales, or Ruggedness of Fitness Landscape..



Past research within "phenotype first "paradigm reveals highly degenerate and *non-predictive relationship between mutations and phenotype.*

Past research: Introducing *random* mutations to determine the distribution of fitness effects- no connection to molecular properties of mutated proteins investigated.

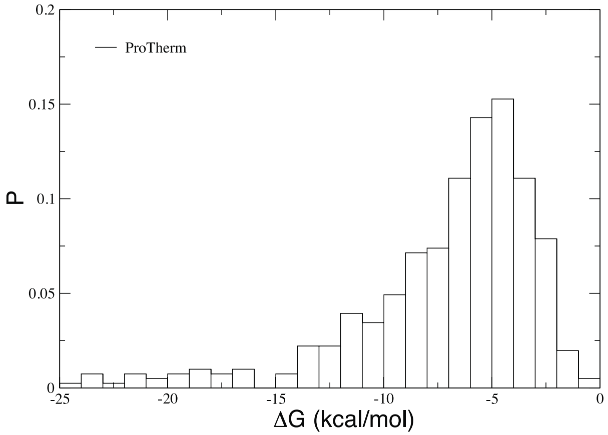
cf: Order parameters in StatMech

Protein Folding Stability

Distribution

Range

Various organisms



-20 – 0 kcal/mol

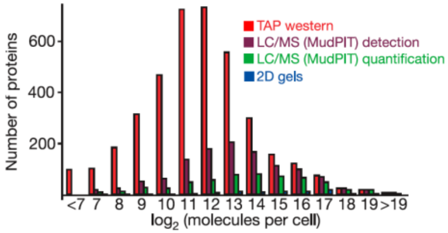
(PROTHERM Database, 2010)

Protein Abundance in the Cell

Distribution

Range

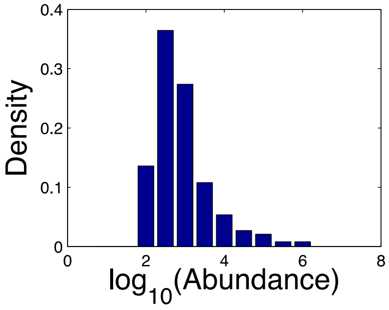
Yeast



~50 - 10^6

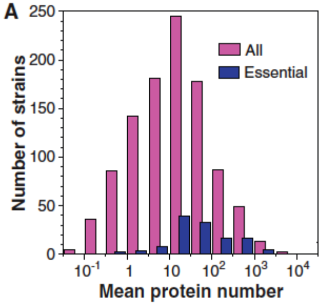
(Ghemmagami S, et al., *Nature* 2003)

E. coli



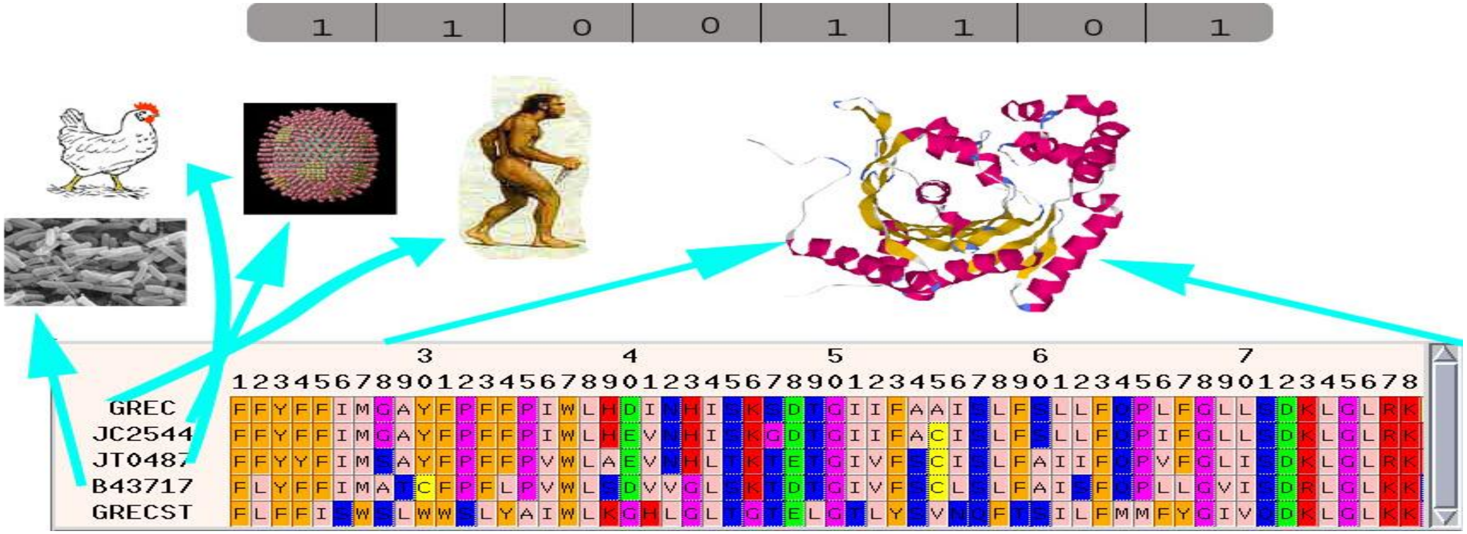
~65 - 10^9

(Ishihama/Frishman, *BMC Genomics* 2008)



(Taniguchi/Xie, *Science* 2010)

Evolutionary rates: Universal Observables in Biology



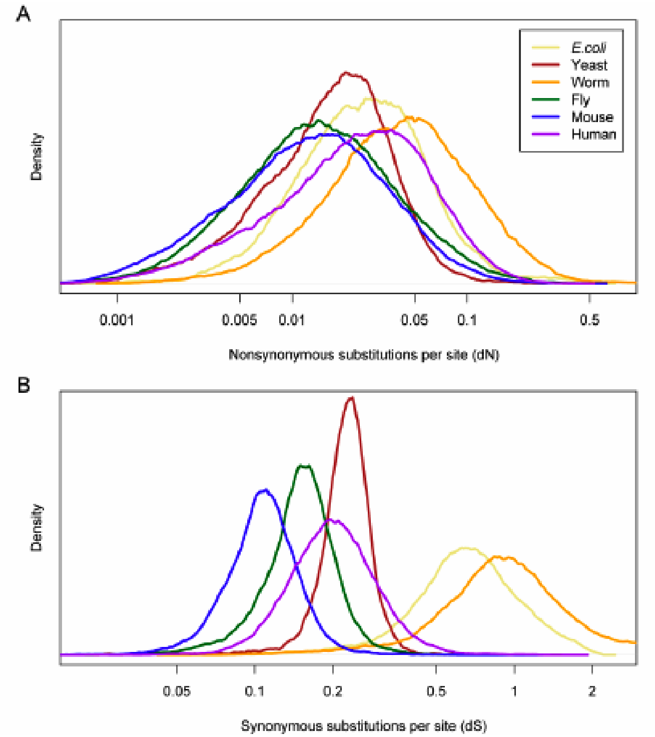
```
                  3         4         5         6         7
        1234567890123456789012345678901234567890123456789012345678
GREC    FFYFFIMGAYFPFFPIWLHDINHISKSDTGIIFAAISLFSLLFQPLFGLLSDKLGLRK
JC2544  FFYFFIMGAYFPFFPIWLHEVNHISKGDTGIIFACISLFSLLFQPIFGLLSDKLGLRK
JT0487  FFYYFIMSAYFPFFPVWLAEVNHLTKTETGIVFSCISLFAIIFQPVFGLISDKLGLRK
B43717  FLYFFIMATCFPFLPVWLSDVVGLSKTDTGIVFSCLSLFAISFQPLLGVISDRLGLKK
GRECST  FLFFISWSLWWSLYAIWLKGHLGLTGTELGTLYSVNQFTSILFMMFYGIVQDKLGLKK
```

The number of differences (''Hamming distance'') between aligned sequences of **orthologous** proteins - number of non-synonymous substitutions Na - is a measure of evolutionary divergence.
Na per unit time (i.e. normalized by number of synonymous substitutions) presents **evolutionary rates.**

Orthologous proteins: Proteins from different species that have same function

Evolutionary Rates:
Some proteins evolve **much** faster than others

Log-normal Distribution-



Range

~2- to 3-orders of magnitude

Pal, et al. *Nat Genetics*, 2003
Drummond DA et al. *Cell* 2008.
Wolf Y, et. al *PNAS* 2009

Overdispersion of molecular clock

Population Size

Distribution

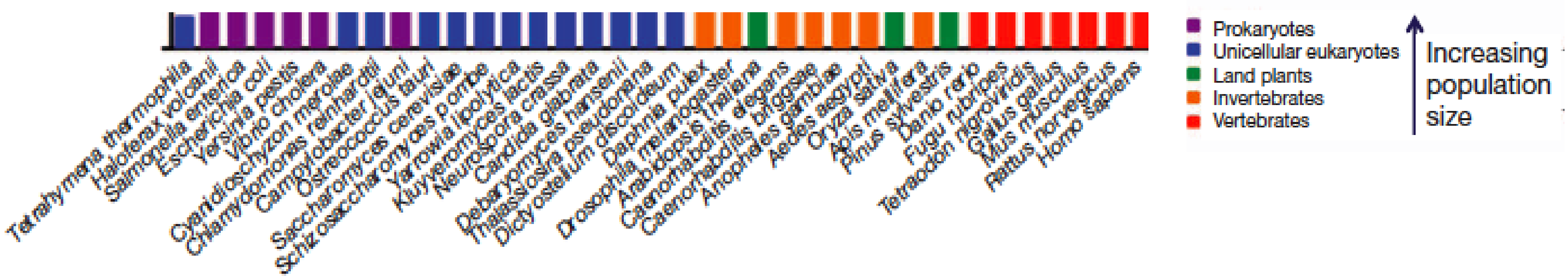
Various organisms

?

Range

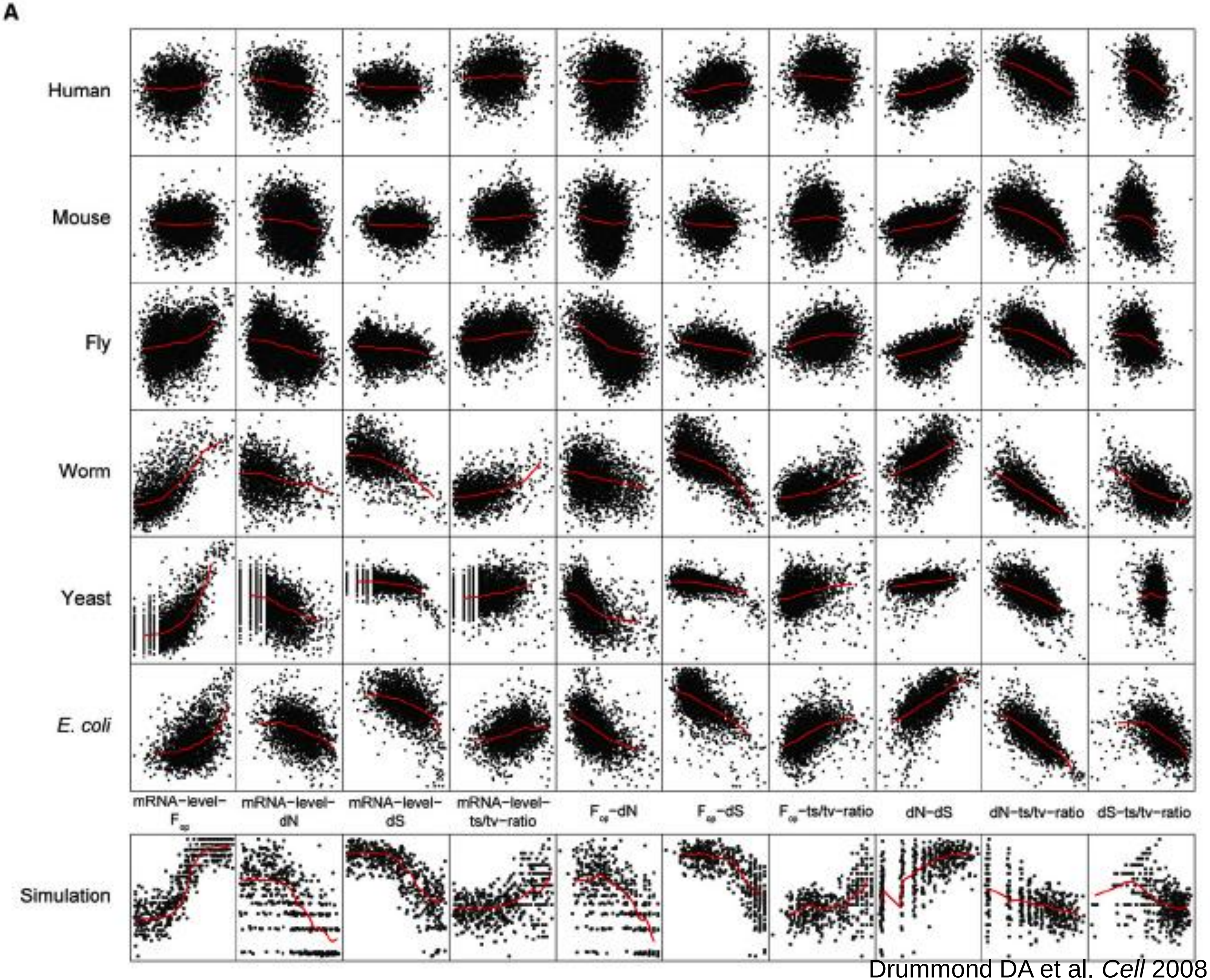
10^8	Prokaryotes
$10^7 – 10^8$	Unicellular Eukaryotes
$\sim 10^5 – 10^6$	Invertebrates
$10^4 – 10^5$	Vertebrates

(Lynch & Connery, *Science* 2003)



(Fernandez & Lynch, *Nature* 2011)

Universal Patterns in Molecular Evolution: Correlation between rates and abundances



Drummond DA et al. *Cell* 2008.

Patterns of molecular evolution

Stability vs. Expression Level

Stability vs. Evolutionary Rate

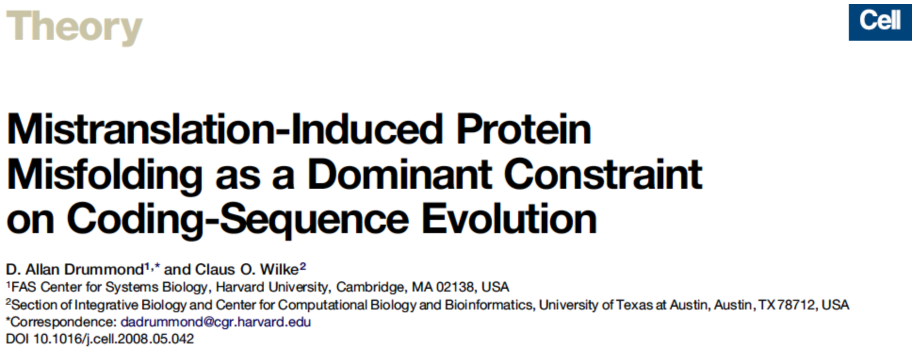
Stability vs. Structure

Expression Level vs. Structure

- **What is the underlying mechanism of evolutionary dynamics?**
- **What is the relative contribution of these variables to evolutionary dynamics?**
- **How do ecological parameters modulate these patterns?**

Emerging constraints in molecular evolution

Selection against misfolding …



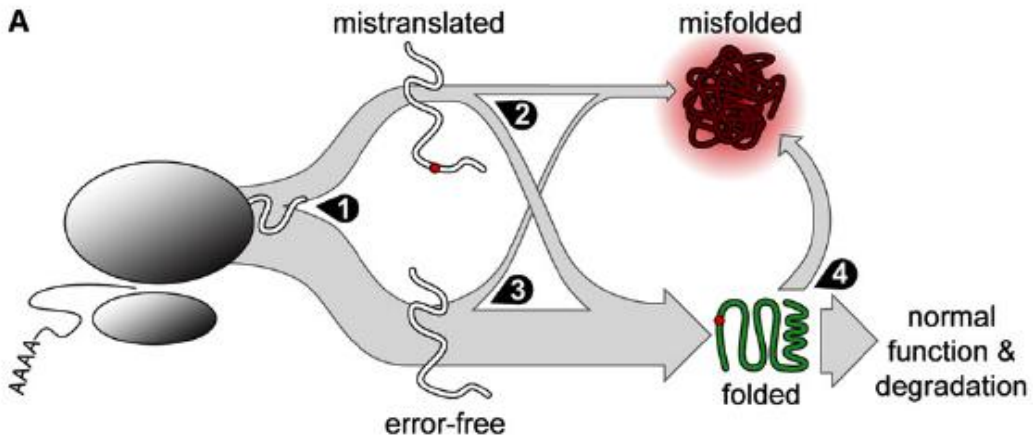
Theory

Cell

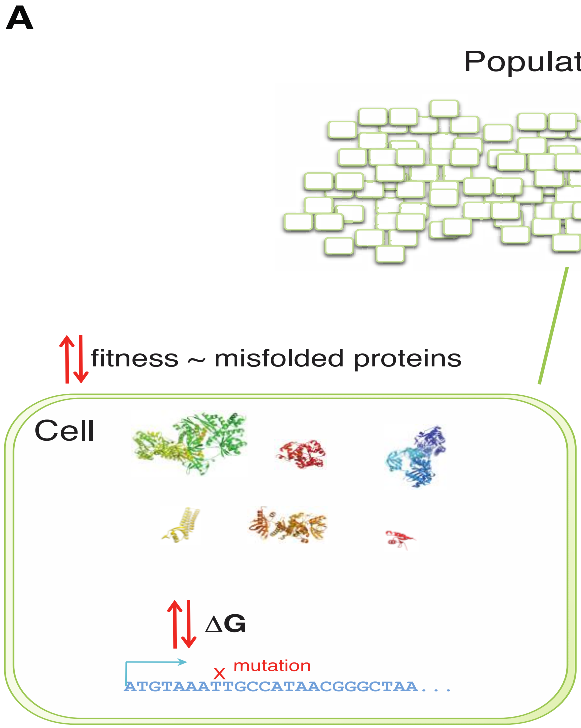
Mistranslation-Induced Protein Misfolding as a Dominant Constraint on Coding-Sequence Evolution

D. Allan Drummond[1,*] and Claus O. Wilke[2]
[1]FAS Center for Systems Biology, Harvard University, Cambridge, MA 02138, USA
[2]Section of Integrative Biology and Center for Computational Biology and Bioinformatics, University of Texas at Austin, Austin, TX 78712, USA
*Correspondence: dadrummond@cgr.harvard.edu

DOI 10.1016/j.cell.2008.05.042



Multiscale Evolutionary Framework



Assumption: Unfolded proteins are toxic.

Fitness ~ 1/(number of unfolded proteins)

Death ~ (number of unfolded proteins)

Misfolded proteins:

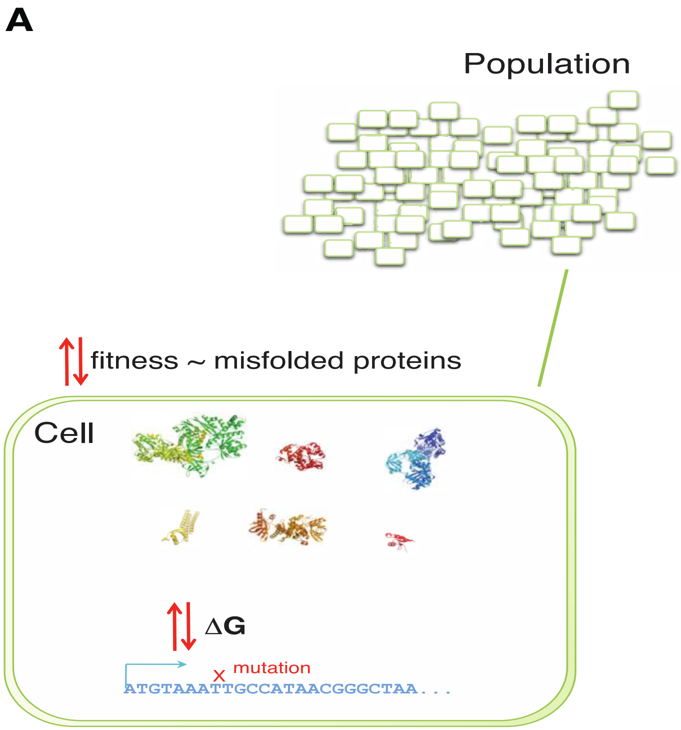
$$d = d_0 \sum_{i=1}^{G} c_i \frac{e^{bDG_i}}{1+e^{bDG_i}},$$

Fitness (Wright-Fisher sense):

$$w_{SS} = \exp(-d) = \exp\left(- d_0 \sum_{i=1}^{G} c_i \frac{e^{bDG_i}}{1+e^{bDG_i}}, \right)$$

(Drummond & Wilke, *Cell* 2008)
(Lobgovsky/Koonin, *PNAS* 2010)

Multiscale Evolutionary Framework



For a given mutation:

$$\Delta G_{k,mut} = \Delta G_{k,wildtype} + \Delta\Delta G$$

Fitness effect:

$$s = \ln w_A - \ln w_B = d_0 c_k \left[\frac{1}{1+e^{-\beta(\Delta G_i + \Delta\Delta G)}} - \frac{1}{1+e^{-\beta \Delta G_i}} \right],$$

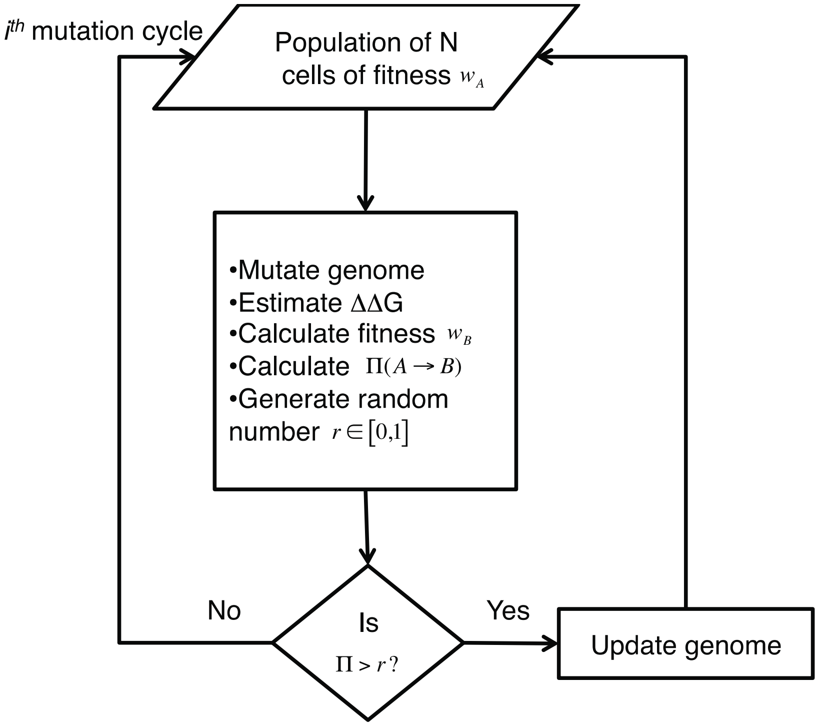
$$s \approx - d_0 c_k e^{\beta \Delta G_k} \left(e^{\beta \Delta\Delta G_k} - 1 \right), \ \ \Delta G_k < -3 \text{ kcal/mol}$$

(Sella & Hirsh, *PNAS* 2005)

Fixation probability (Nμ<<1):

$$\mathrm{P}(A \rightarrow B) = \frac{1 - e^{-2s}}{1 - e^{-2Ns}},$$

Multiscale Evolutionary Framework



For a given mutation:

$$DG_{k,\,mut} = DG_{k,\,wildtype} + DDG$$

Fitness effect:

$$s = \ln w_A - \ln w_B = d_0 c_k \left[\frac{1}{1+e^{-b(DG_i + DDG)}} - \frac{1}{1+e^{-bDG_i}} \right],$$

$$s \approx - d_0 c_k e^{bDG_k}\left(e^{bDDG_k} - 1\right),\ \ DG_k < -3 \text{ kcal/mol}$$

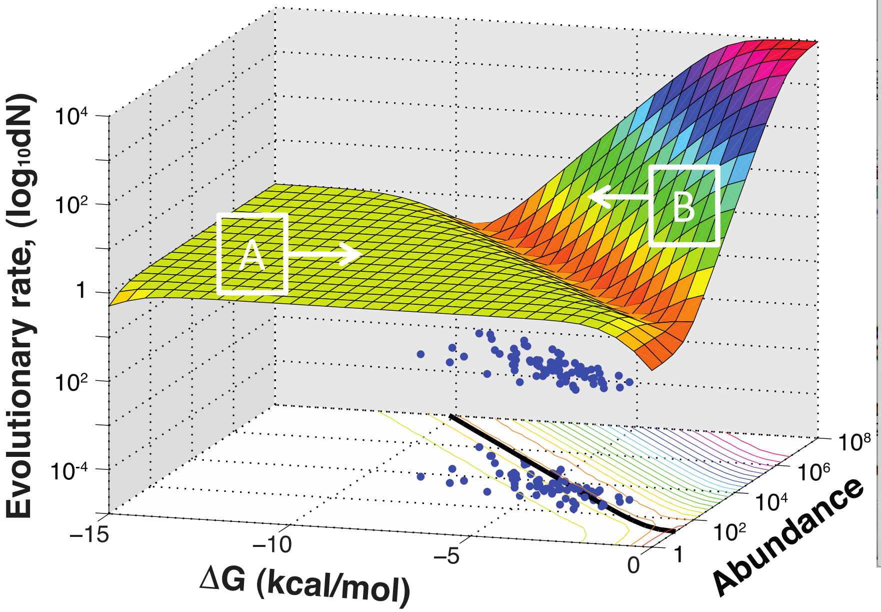
(Sella & Hirsh, *PNAS* 2005)

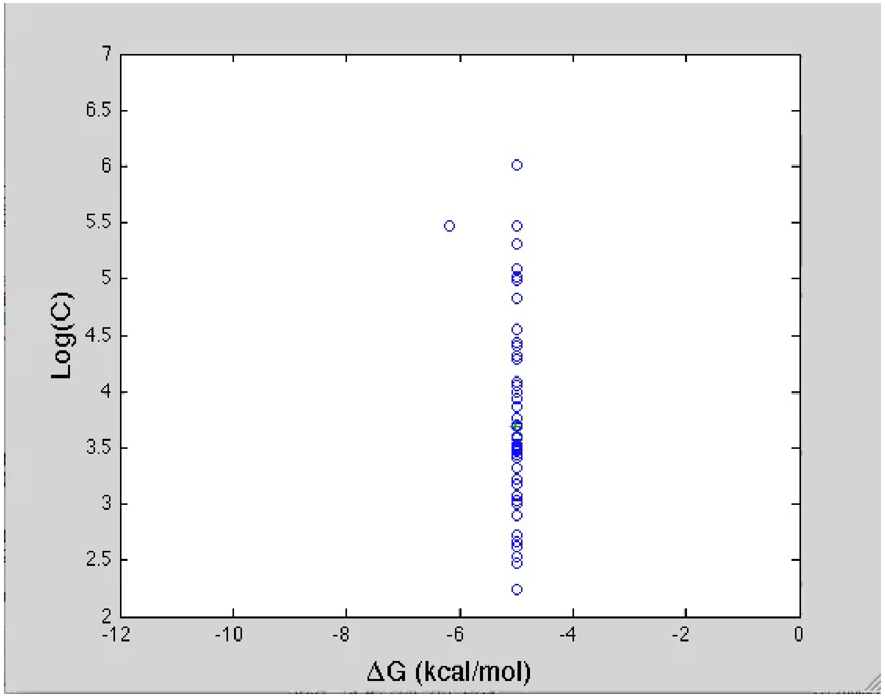
Fixation probability (Nμ<<1):

$$P(A \to B) = \frac{1 - e^{-2s}}{1 - e^{-2Ns}},$$

Average fixation probability: 1/Rate

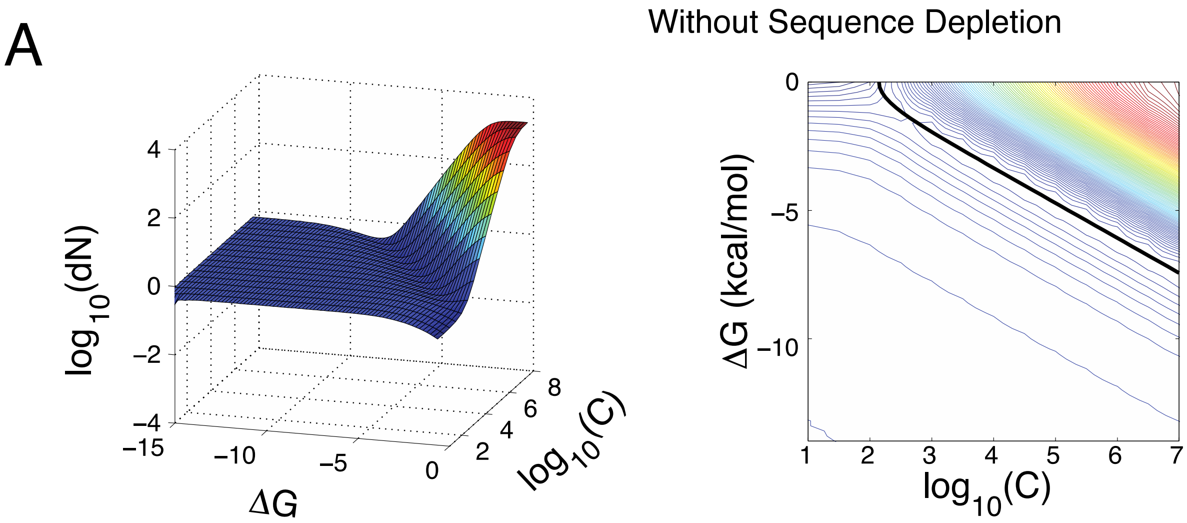
$$\langle \Pi_{i\to f} \rangle = \int_{-\infty}^{-\infty} d(\Delta\Delta G) \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(\frac{-(\Delta\Delta G - \mu)^2}{2\sigma^2} \right) \frac{1-\exp(-2s)}{1-\exp(-2Ns)}$$





Settling in the ‘’gulley’’

Bridging molecular, organismal and population scales: **Highly expressed proteins are more stable and so are proteins in large populations**



Approximation of the minimum:

$$C = \frac{\mu}{\beta\sigma^2}\frac{1}{(N-1)h}\frac{\left(1+e^{-\beta\Delta G}\right)^2}{e^{-\beta\Delta G}}, \quad \beta = 1/k_B T$$

$$\sim \frac{\mu}{\beta\sigma^2}\frac{1}{Nh}e^{-\beta\Delta G}, \quad \Delta G < -3 \text{ kcal/mol}$$

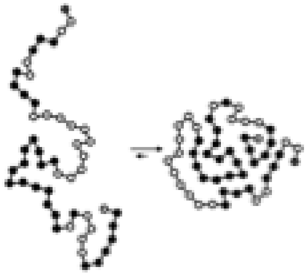
$$\Delta G_k \approx -k_B T\ln N - k_B T\ln C_k - k_B T\ln h - k_B T\ln\left(\frac{1}{k_B T}\frac{\sigma^2}{\mu}\right)$$

Scaling between evolutionary parameters

$$\Delta G_k \approx - k_B T \ln N - k_B T \ln C_k - k_B T \ln h - k_B T \ln\left(\frac{1}{k_B T}\frac{\sigma^2}{\mu}\right)$$

20 kcal/mol	N~10^4-10^8	C~10^1-10^6	h~10^{-6}	σ~1.7 kcal/mol; μ~ 1kcal/mol
	7 kcal/mol	**6 kcal/mol**	**6 kcal/mol**	**1 kcal/mol**

Two-state protein folding paradigm

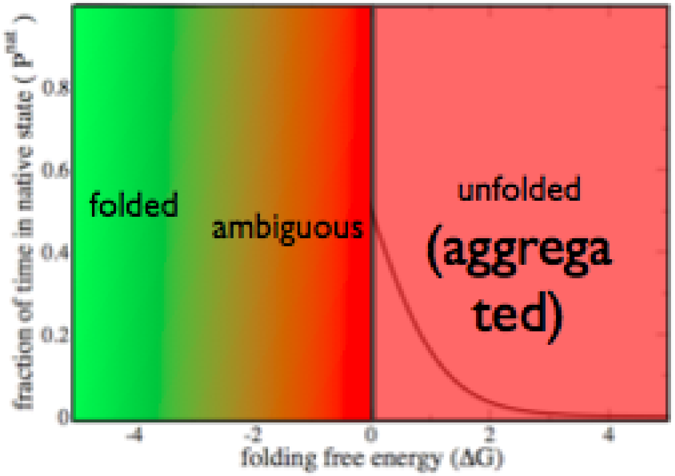


$$P^{nat} = \frac{e^{-G_f/kT}}{e^{-G_f/kT} + e^{-G_u/kT}}$$

$$= \frac{1}{1 + e^{-\Delta G/kT}}$$

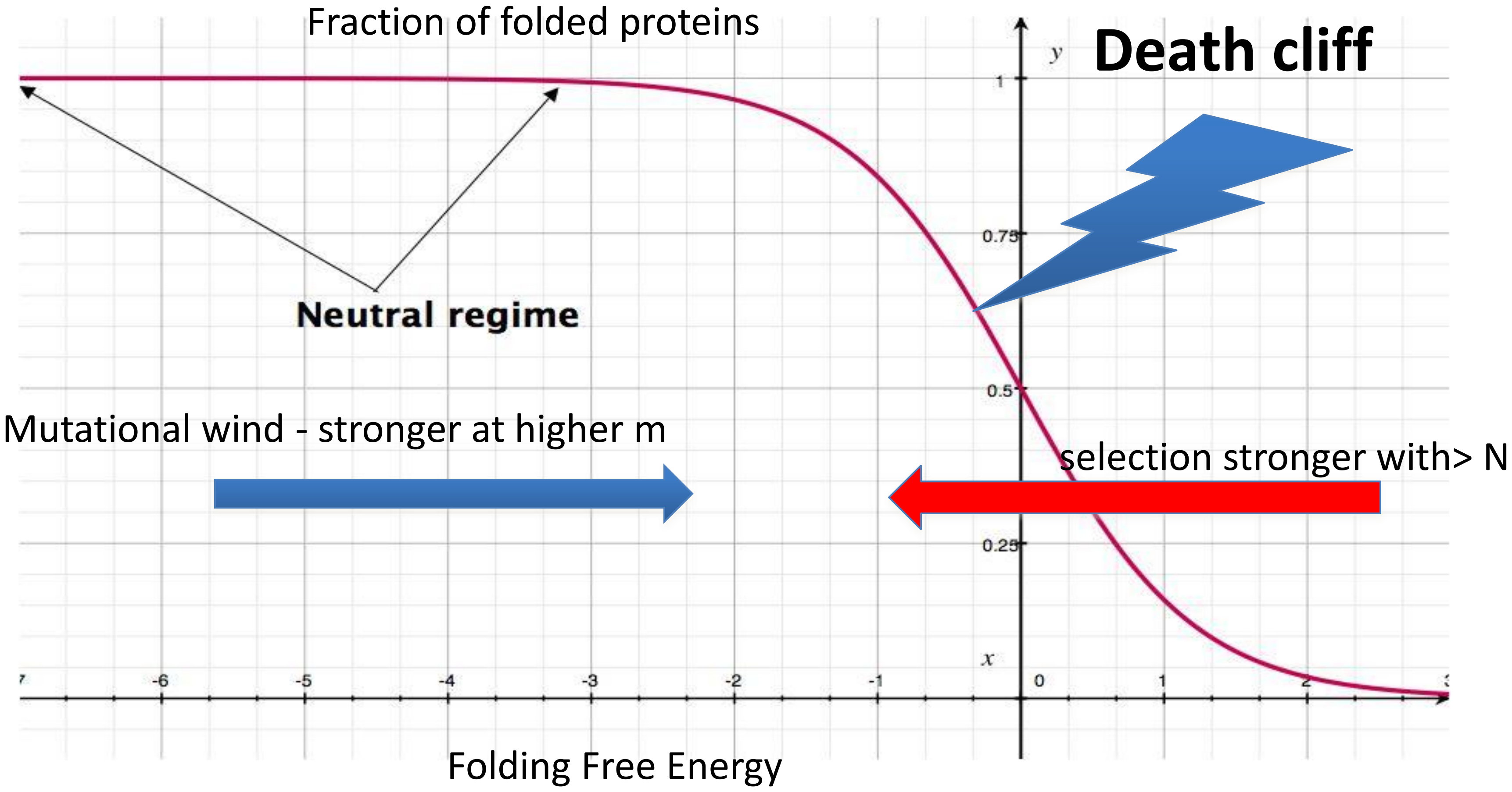
unfolded:
(large entropy, small energy)

folded:
(~ 0 entropy, energy << 0)



ΔG typically ~ -10 to -4 kcal/mole:
"marginally stable"

Evolution of Stability is a Branching (because of cell replication) Random Walk (Biased Diffusional Motion) on the FD Fitness Landscape



Fraction of folded proteins

Death cliff

Neutral regime

Mutational wind - stronger at higher m

selection stronger with> N

Folding Free Energy

Q: How do we know the distribution of step sizes?
A: From experimental ddG statistics!

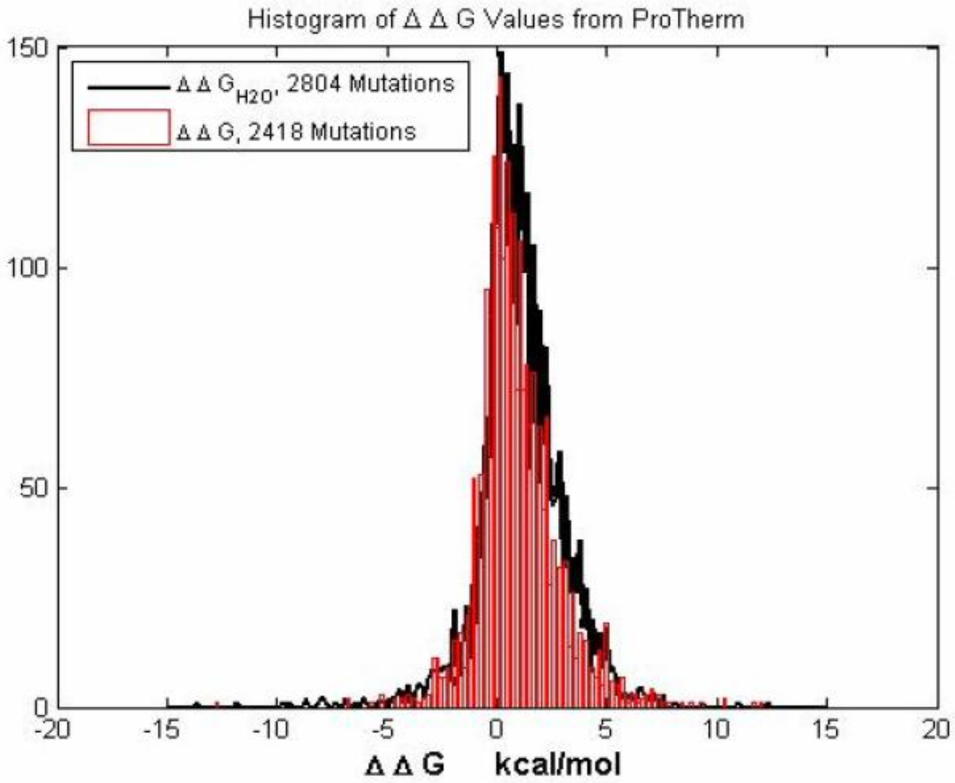
- Asymmetric distribution of ΔΔG; sharper edge on the ΔΔG <0 side, more likely to destabilize protein

•ΔΔG and $ΔΔG_{H2O}$ Shows similar statistics

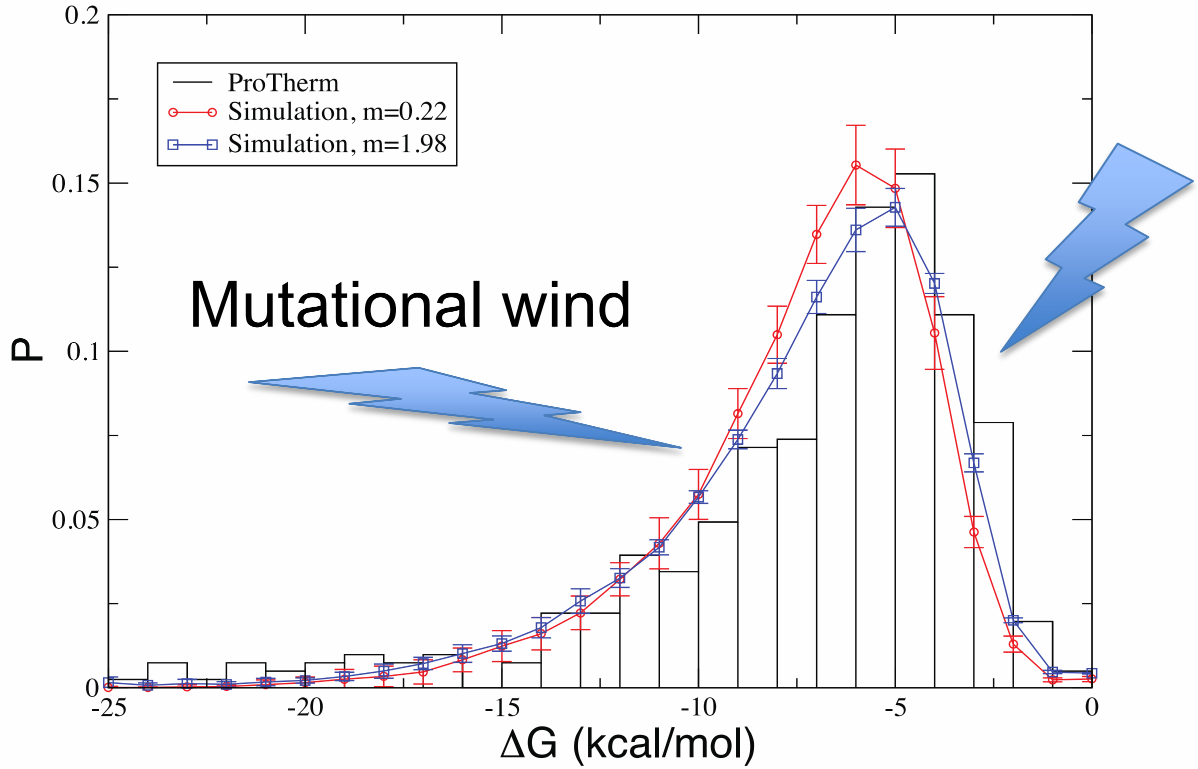
•We could therefore obtain parameters in our random walk model:

•<ddG> =S= 1.1kcal/m

D=<ddg^2> = (3kcal/m)^2



Distribution of protein stabilities : The interplay between protein folding and population genetics



Mutational wind

Cliff hanging

No adjustable parameters

Interplay between mutational wind and ‘’cliff hanging’’:

A.Serohijos, S.Wylie, Peiqiu Chen, K.Zeldovich

Universal Speed Limit on Mutation Rates

~6 Missense mutations Per Proteome Per Replication.

Higher mutation rates ->Population goes extinct

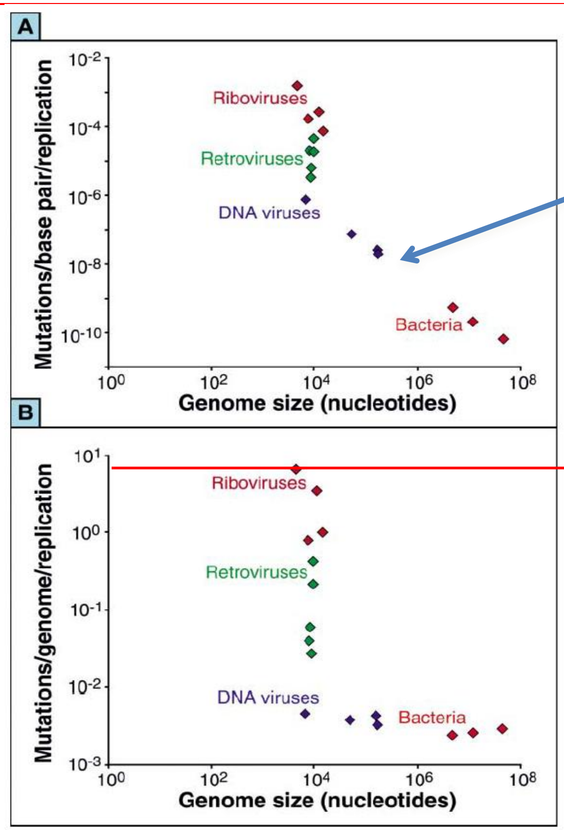
$$\frac{m\mathrm{G}}{b} < \frac{m\mathrm{G}^*}{b} = \frac{2}{\left\{ \frac{s^2}{s^2 + D} + \frac{p^2 \left(s^2 + D \right)}{\left(E_{\min} - E_{\max} \right)^2} \right\}} \approx 6$$

ddG effects of single mutations

# of mutations per genome per replication

K.Zeldovich

RNA world: *At the Edge of the Speed Limit;* **DNA World**: *All organisms are 1000-fold below* (Error correction at work)



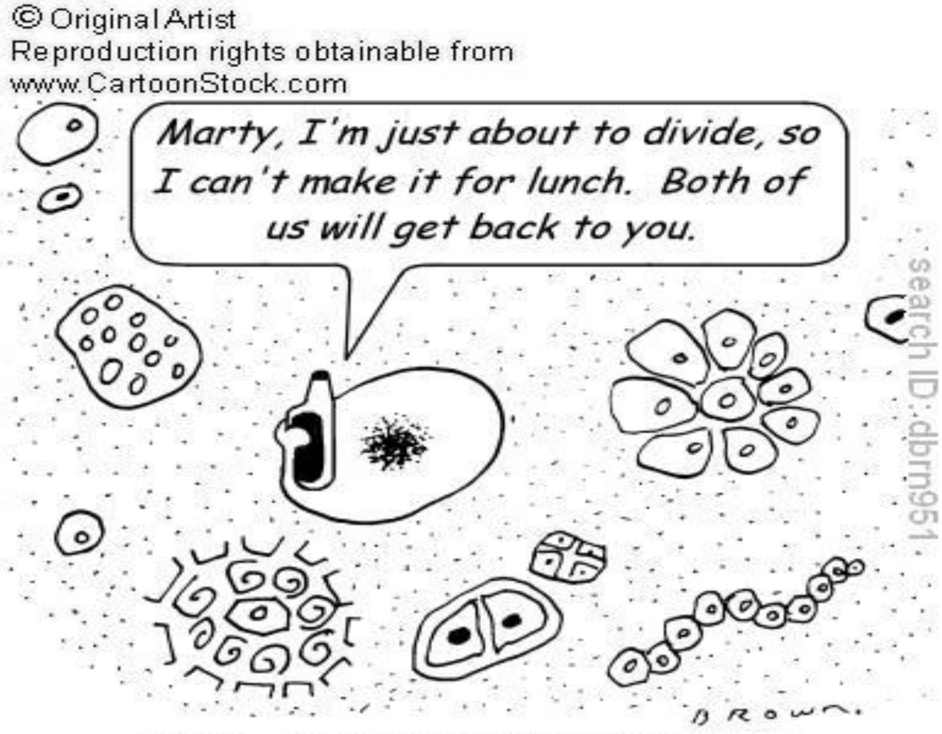
Longer genomes=lower m

$$\frac{2}{\left\{\frac{S^2}{S^2+D}+\frac{\pi^2\left(S^2+D\right)}{\left(\Delta G_{\max}-\Delta G_{\min}\right)^2}\right\}}\approx 6$$

Zeldovich, Chen,ES.PNAS’ 07

Loeb et al, Ann Rev Microbiology, 2005, 58, 183-205

“The dream of every cell is to become two cells.” - F. Jacob -


© Original Artist
Reproduction rights obtainable from
www.CartoonStock.com
Marty, I'm just about to divide, so I can't make it for lunch. Both of us will get back to you.
search ID: dbrn951
BROWN.

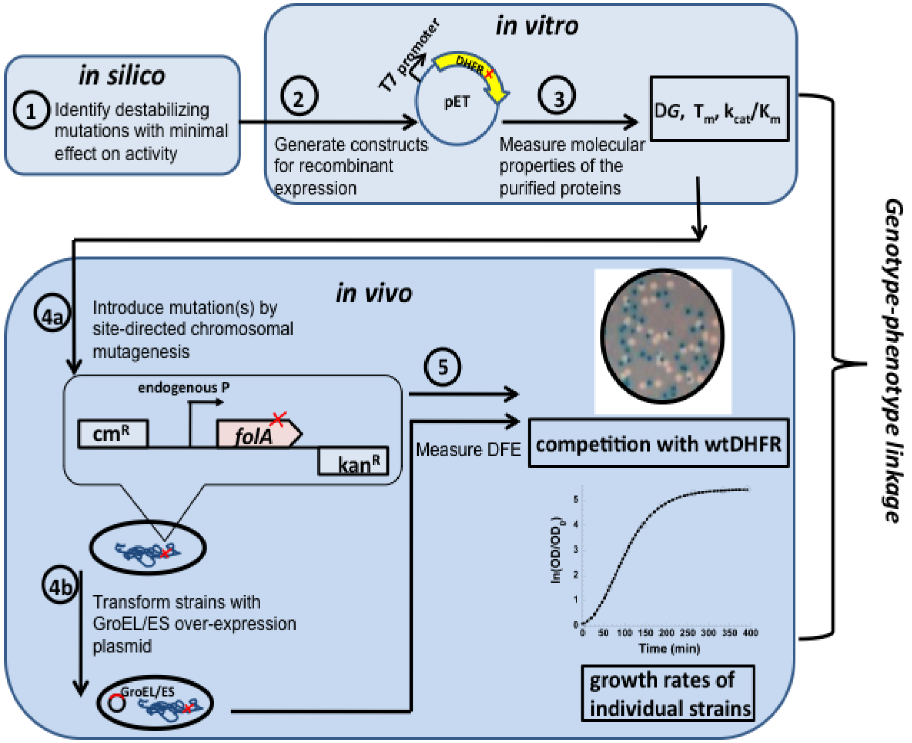
THE CELLULAR PHONE

Cell division rate = fitness
(with caveats –
see below)

How to relate fitness to
Protein Folding? –
That is the question!

Exploring the Genotype-Phenotype relation one mutation at a time:

The experimental setup



Identify destabilizing mutations with minimal effect on activity

Generate constructs for recombinant expression

Measure molecular properties of the purified proteins

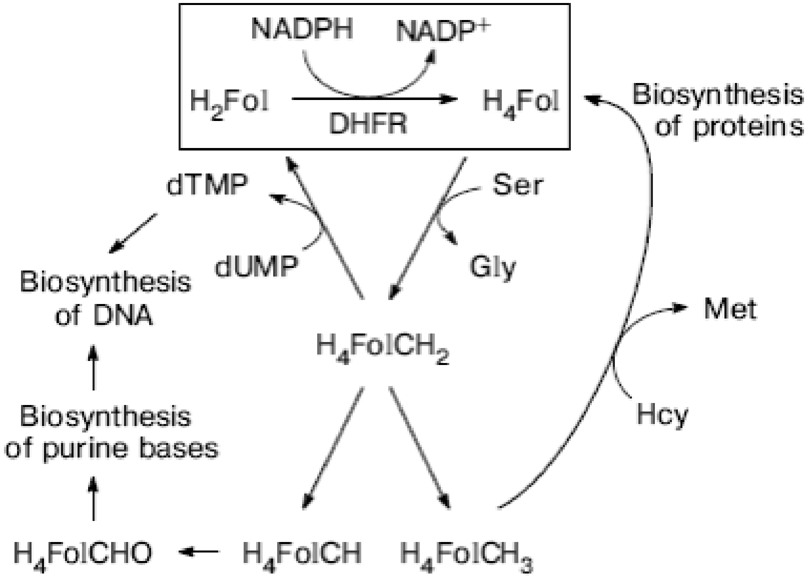
Introduce mutation(s) by site-directed chromosomal mutagenesis

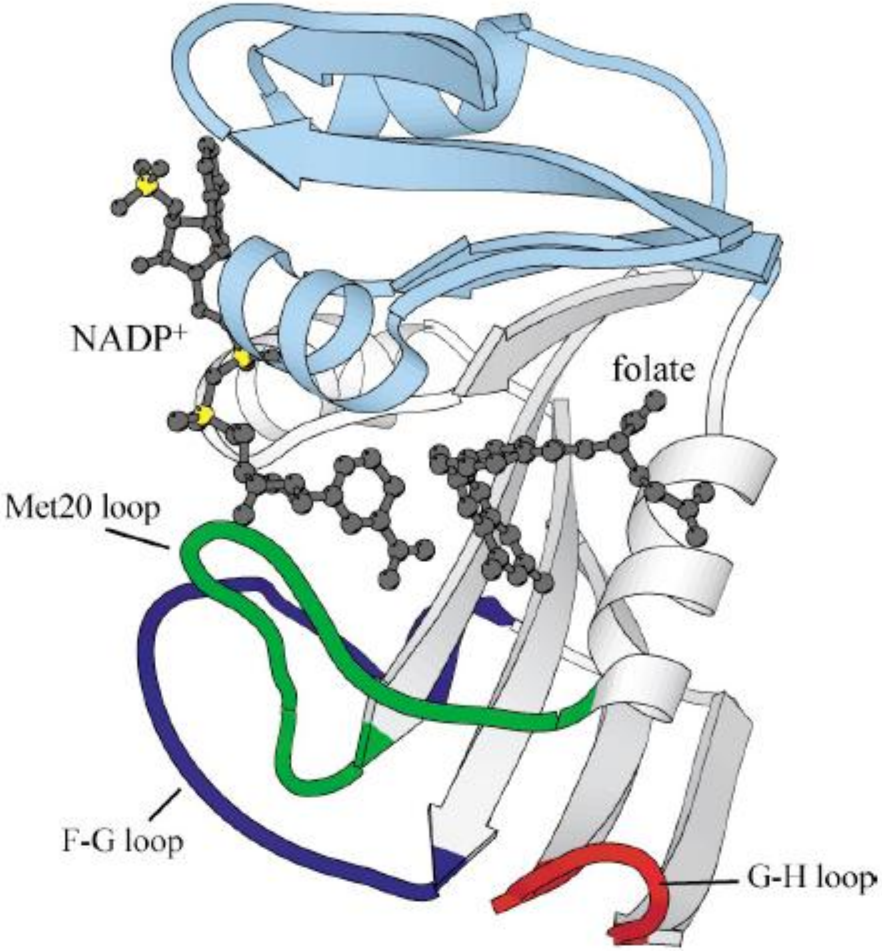
Transform strains with GroEL/ES over-expression plasmid

Measure DFE

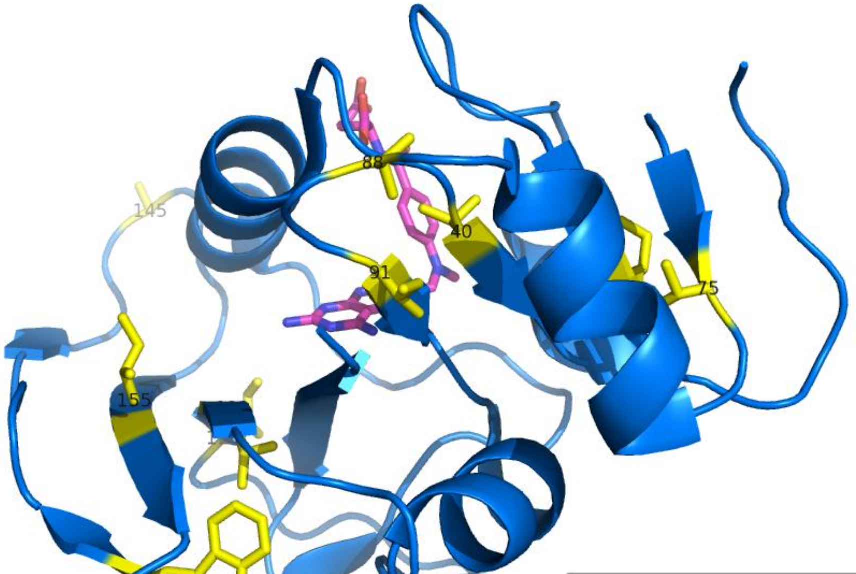
Bottom-up Approach: Introducing mutations of known molecular properties directly on the chromosome.

DHFR Is An Essential Core Metabolism Enzyme

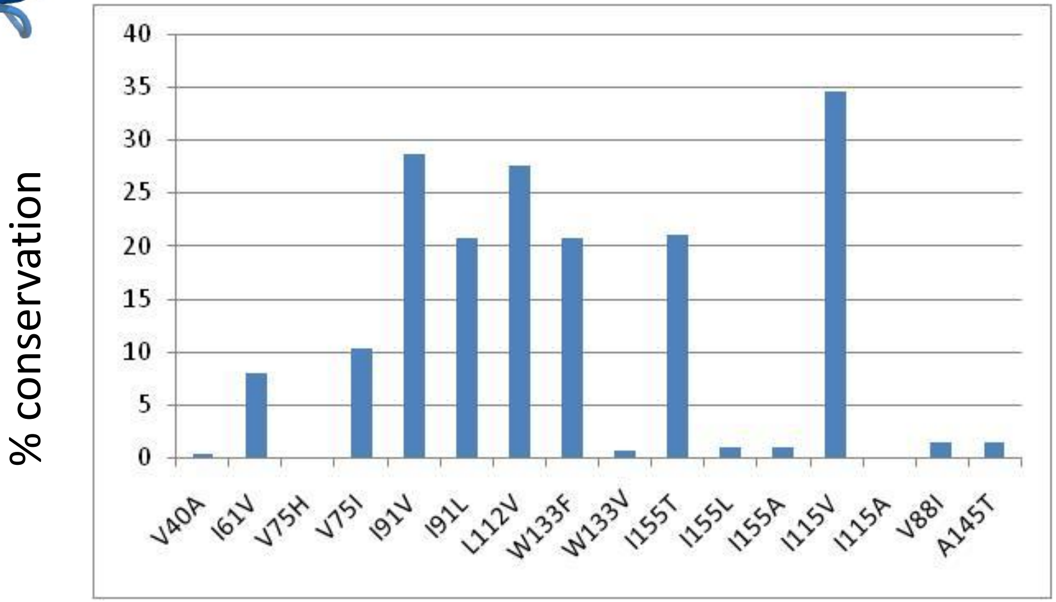




Most mutated residues are deeply buried in the hydrophobic core; half are very conserved evolutionally

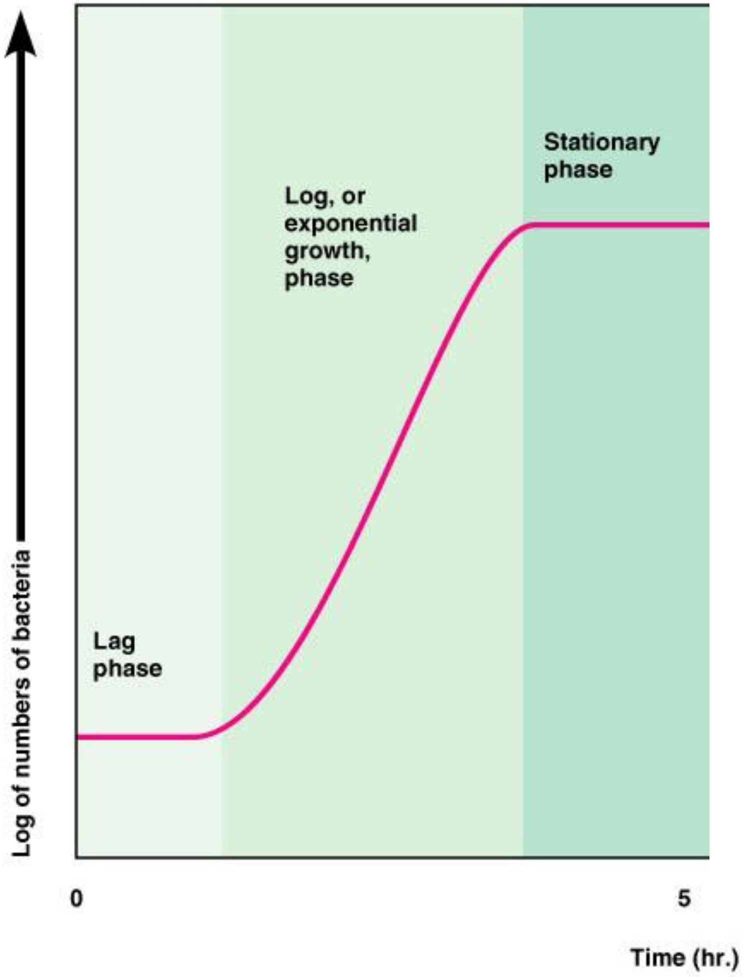


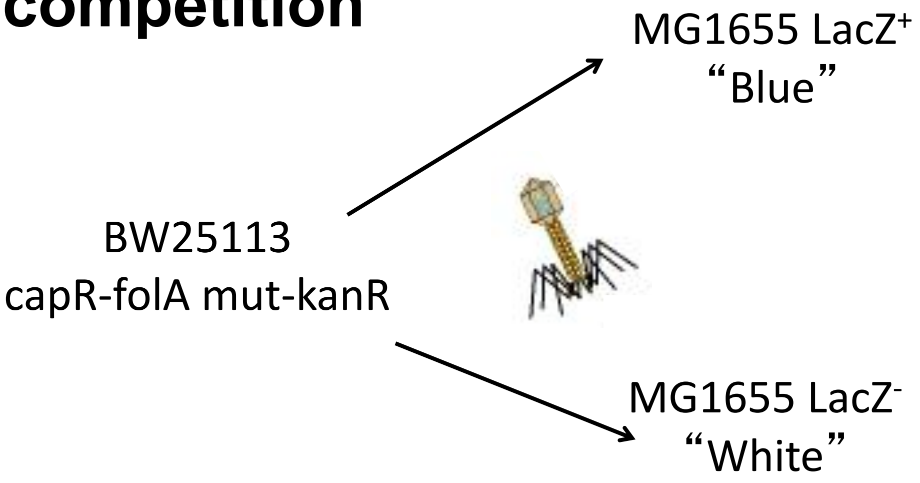
E.*coli*' s DHFR



Mutation	ASA
V40A	0
I61V	0
V75H	0.05
V75I	0.05
I91V	0.07
I91L	0.07
L112V	0
W133F	0.05
W133V	0.05
I155T	0.12
I155L	0.12
I155A	0.12
I115V	0.02
I115A	0.02
V88I	0.34
A145 T	0.89

Bacterial fitness:
Growth rate *vs* competition

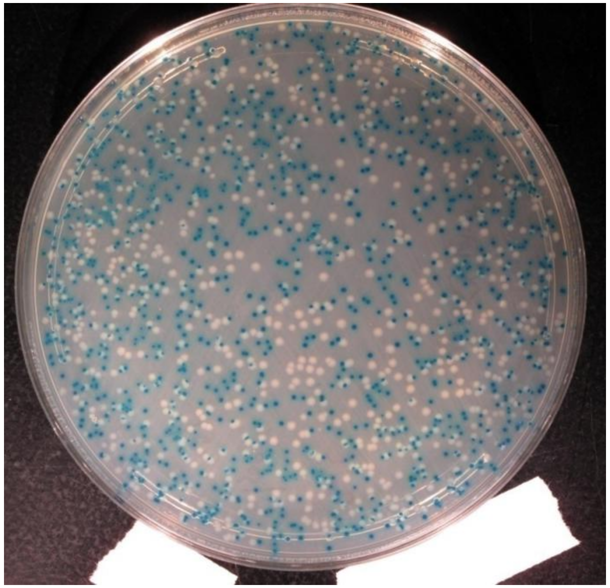




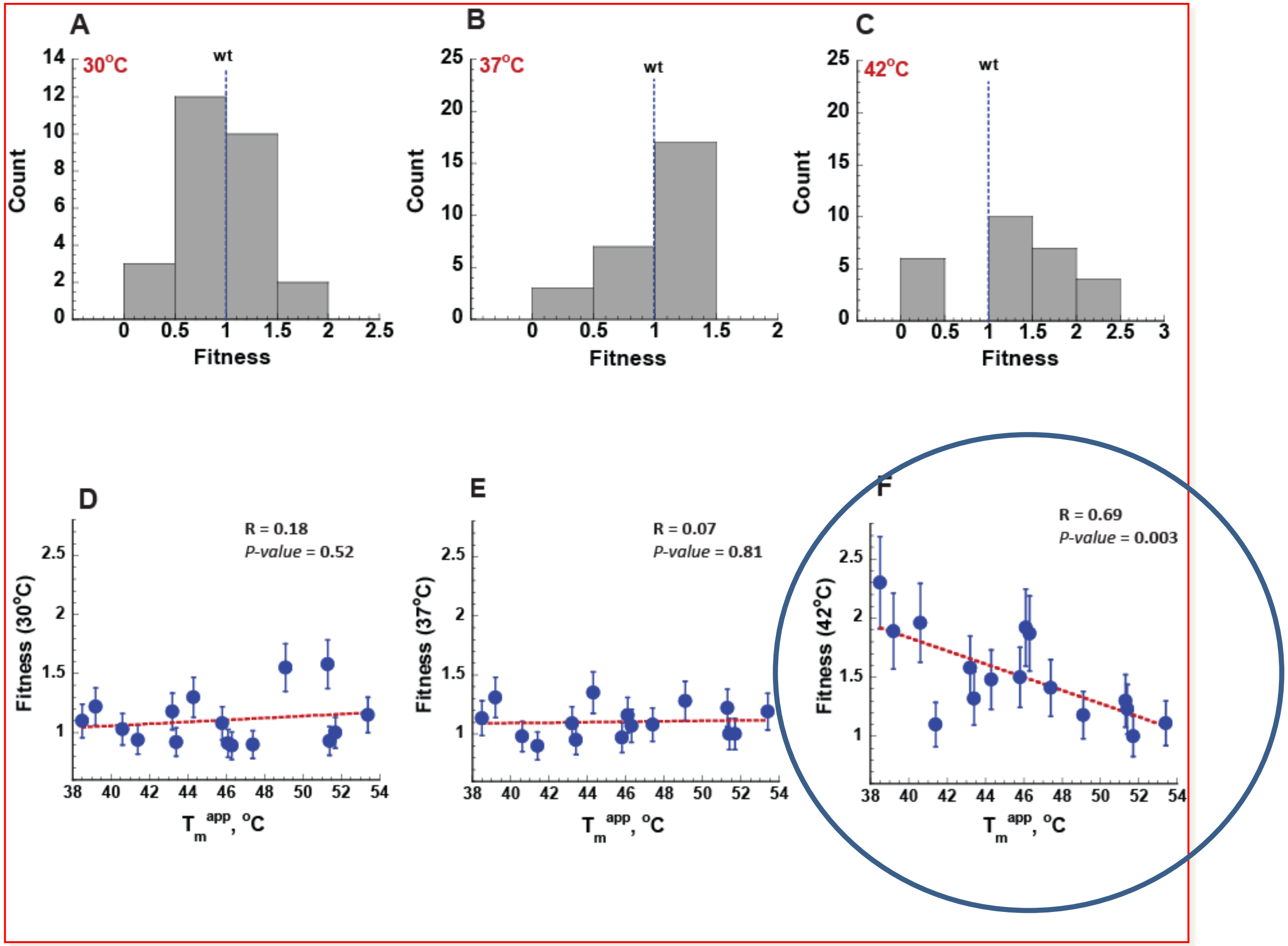
BW25113
capR-folA mut-kanR

MG1655 LacZ^+
“Blue”

MG1655 LacZ^-
“White”

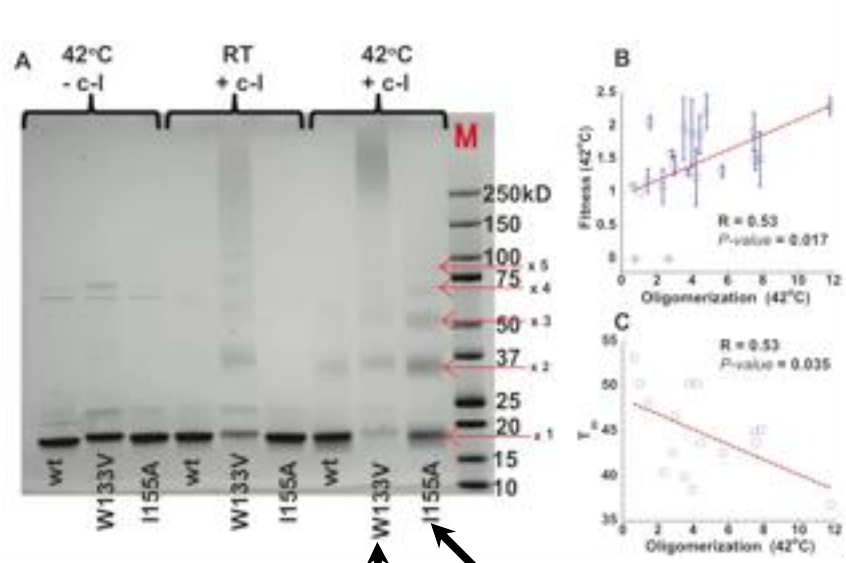


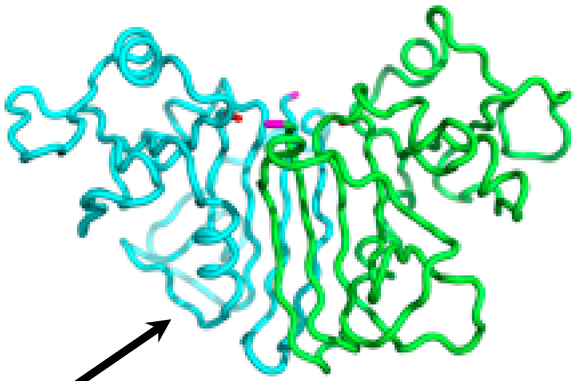
Counter intuitively, for non-lethal mutants fitness is inversely correlated with stability at 42°C!



Why?- see Bershtein, Mu and ES, PNAS, 2012 v.109, pp 4857-62

Soluble oligomerization rescues many mutants from aggregation providing higher fitness to mutant strains

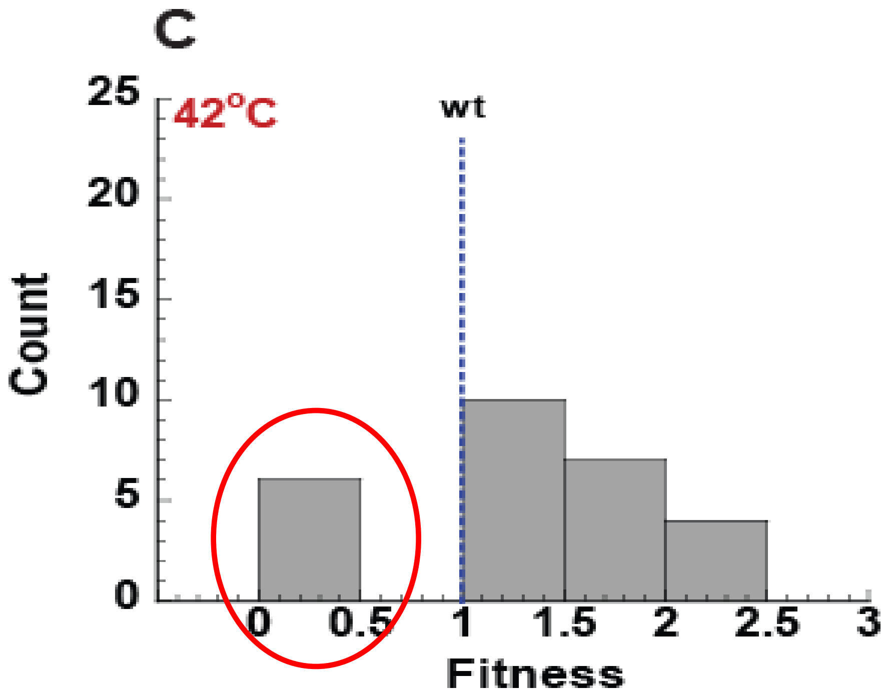




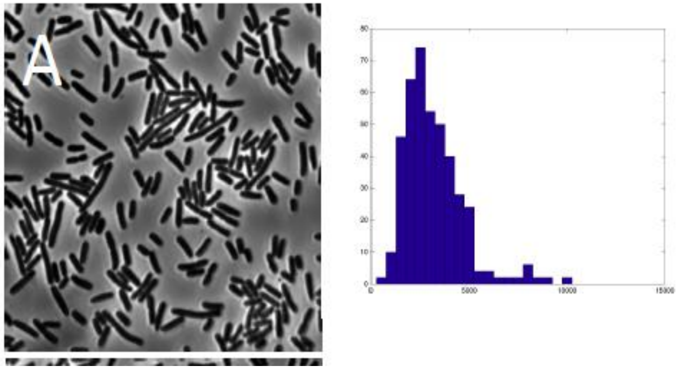
Aggregating mutant

Mutant which oligomerizes

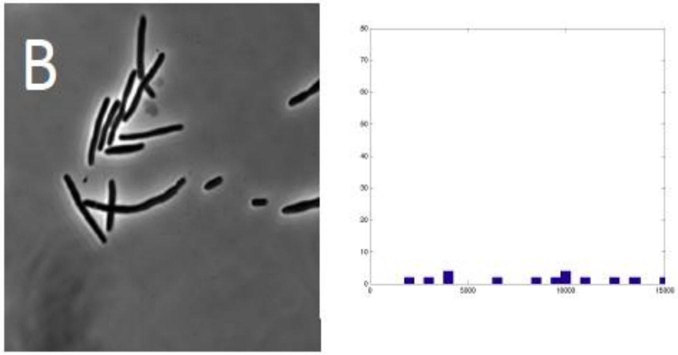
At 42°C 5 out of 27 DHFR mutant strains exhibit slow growth/low fitness



As DHFR activity is diminished the balance between DNA and proteins is shifted: Aggregation prone strains acquire a distinct elongated morphology: a DHFR-deficient phenotype



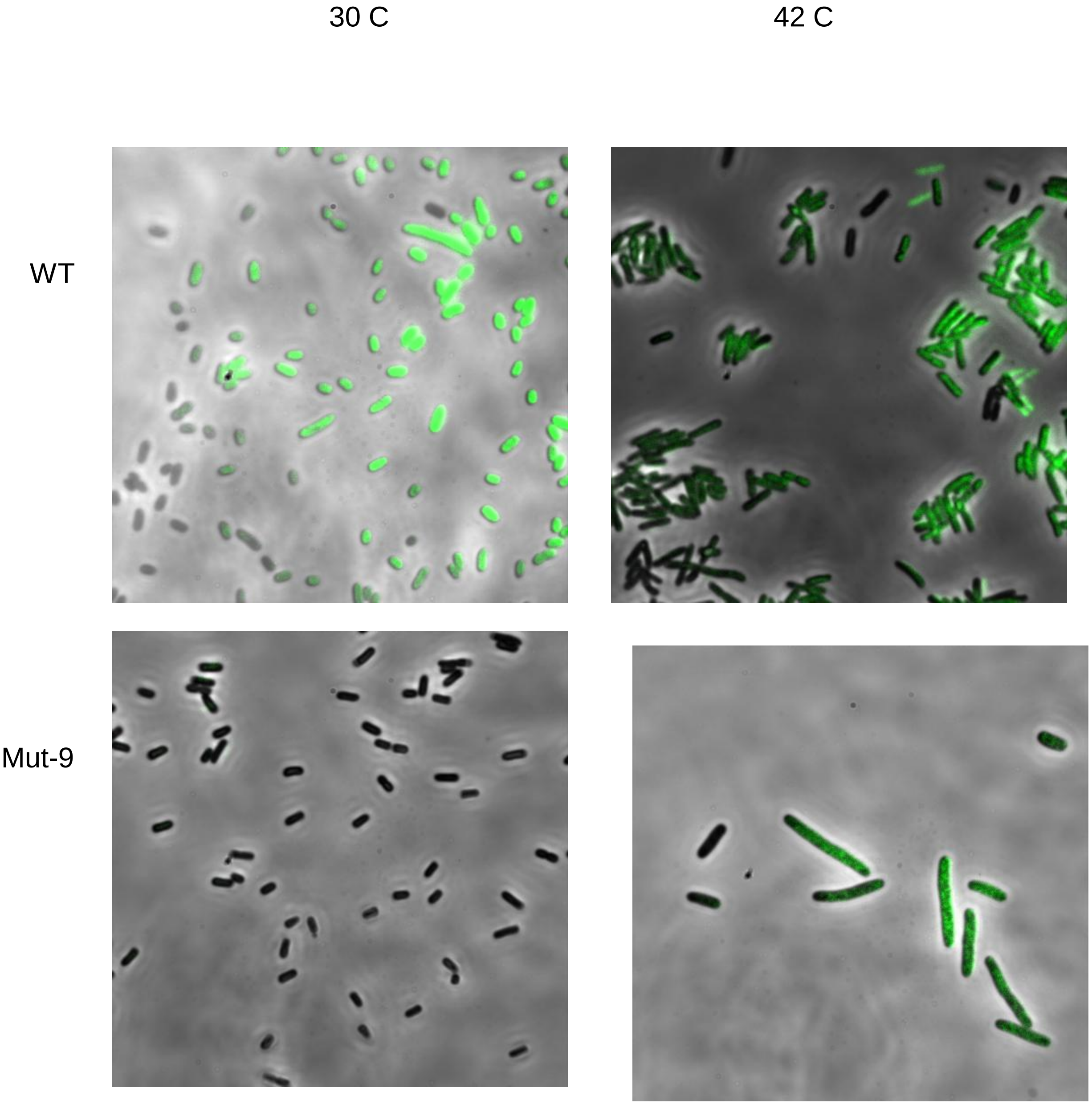
Wt cells



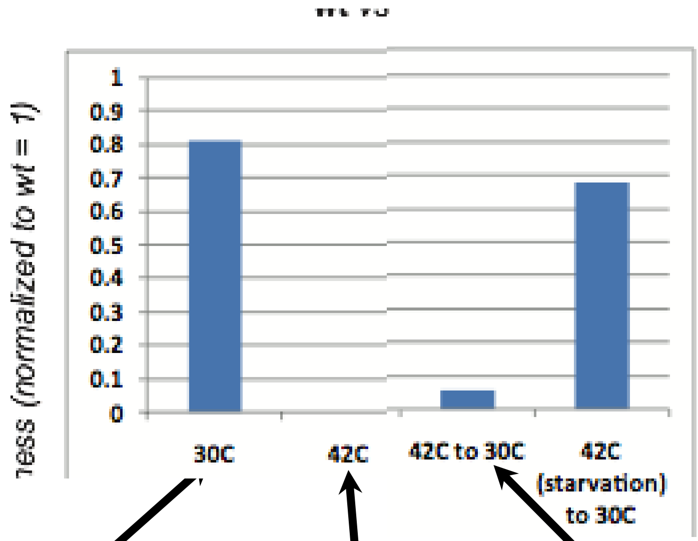
W133V DHFR cells

See also: Kishony et al, Cell, 2010

‘Slow growth mutants of DHFR aggregate in the E.coli cytoplasm: Venus fusion experiment.



Aggregation-prone mutants confer fitness memory on E.coli strains



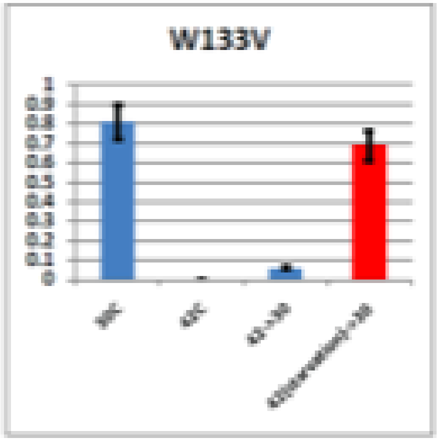
Now:
Before competition at 30C incubate both wt and the mutant at 42 C. Does history of pre-incubation at high T affect fitness at 30C?

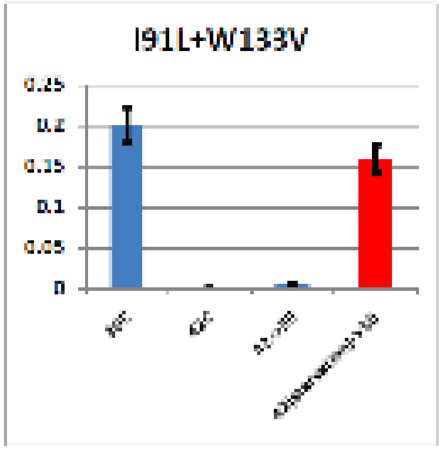
At 30 C the mutant competes well with wt

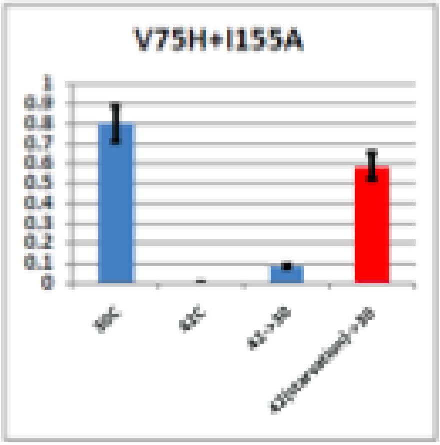
At 42 C the mutant strain does not grow

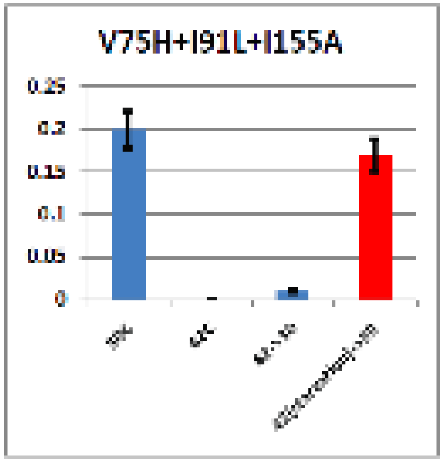
Cells ‘‘memorize’’ past conditions:
after pre-incubation at 42 C fitness at 30 C is lost

Memory effect is due to an active process: Incubation at starvation erases it

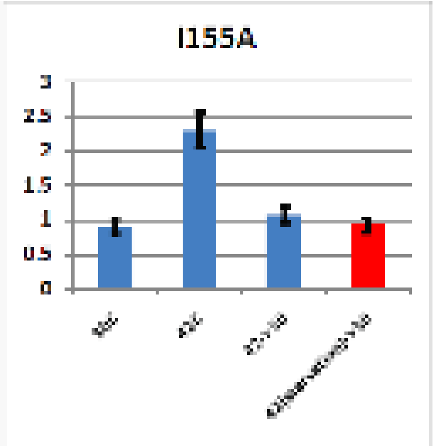




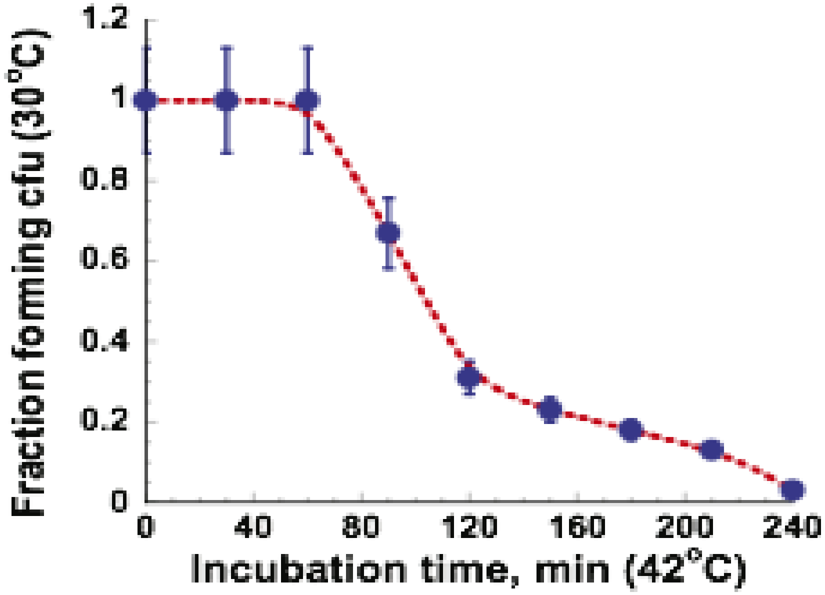




Text



Lag in Fitness Memory : Hint at nucleation of aggregation/amyloid formation of DHFR mutants



Fitness of the mutants strain at 30C depends on how long cells were pre-incubated at 42 C

Q: Why are DHFR aggregates ‘’toxic”?

A: Because aggregates float in the cytoplasm interfering with other cellular processes ‘’non-specifically”.

B: Because aggregates sequester newly made DHFR depleting cells of an essential enzyme

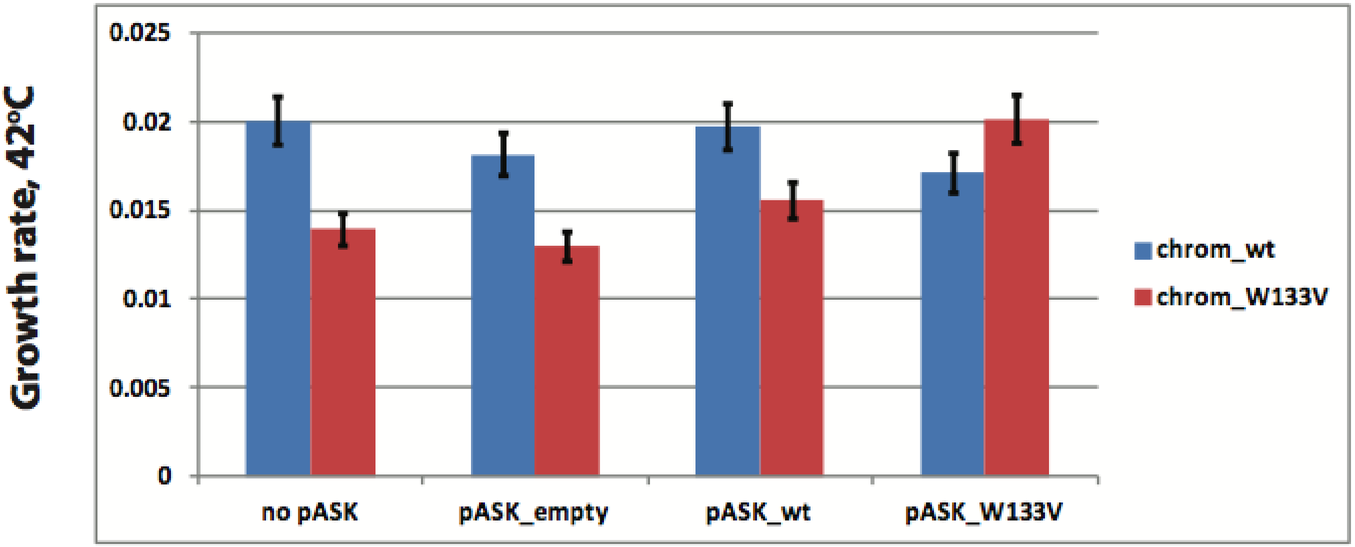
How to answer this question experimentally: ‘’flood” the cells with aggregation-prone DHFR from a plasmid..

If (A) is correct - cells will die.

If (B) is the answer cells will improve fitness.

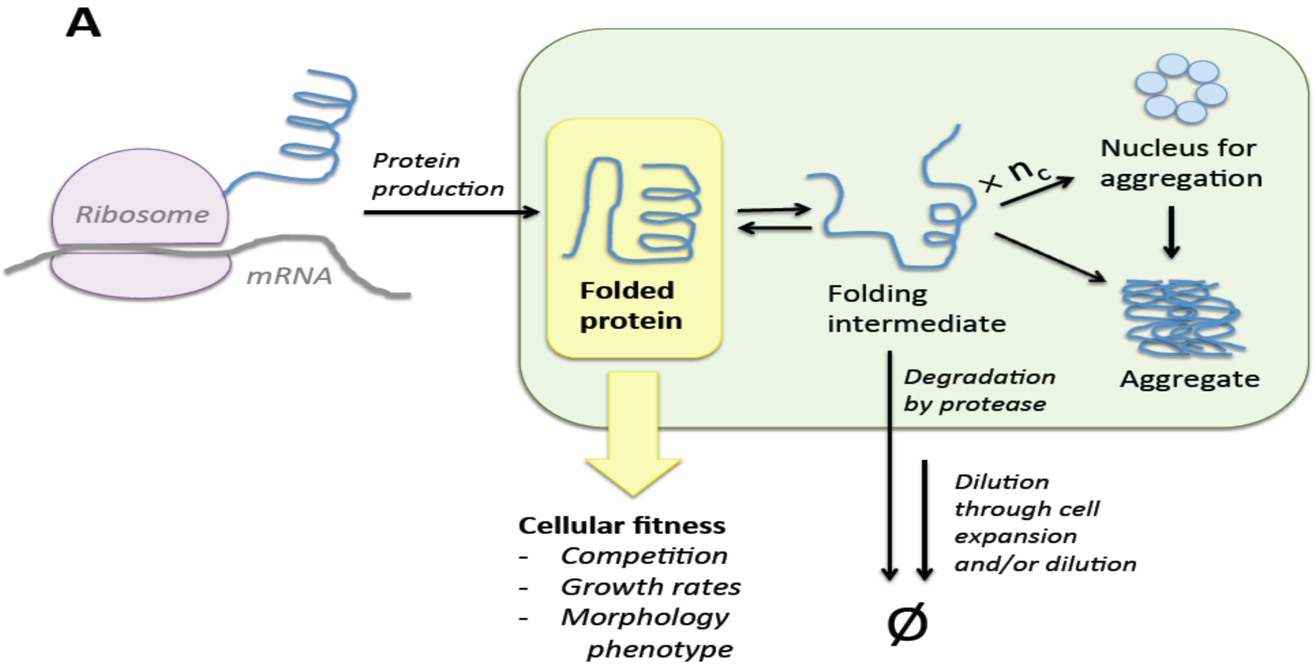
And the answer is…..

B: aggregates sequester newly made DHFR depleting cells of an essential enzyme



Massive overexpression of W133V or I91L+W133V DHFR improves fitness

Theory: a dynamic equilibrium



Physics/Biology Foundations of the model:

1) The cytoplasm is an active medium where concentrations are determined by steady state fluxes rather than Boltzmann equilibrium
2) Aggregates are formed via nucleation, from the Molten-Globule state which competes with cell division.
3) ''Aggregation toxicity'' is due to sequestration of newly folded proteins into aggregates

Equations, shmequations…

$$\frac{dF}{dt} = \mathrm{Pr} - k_u F + k_f U - k_{dil} F$$

$$\frac{dU}{dt} = k_u F - k_f U - n_c k_n U^{n_c} - k_a A U - k_{dil} U$$

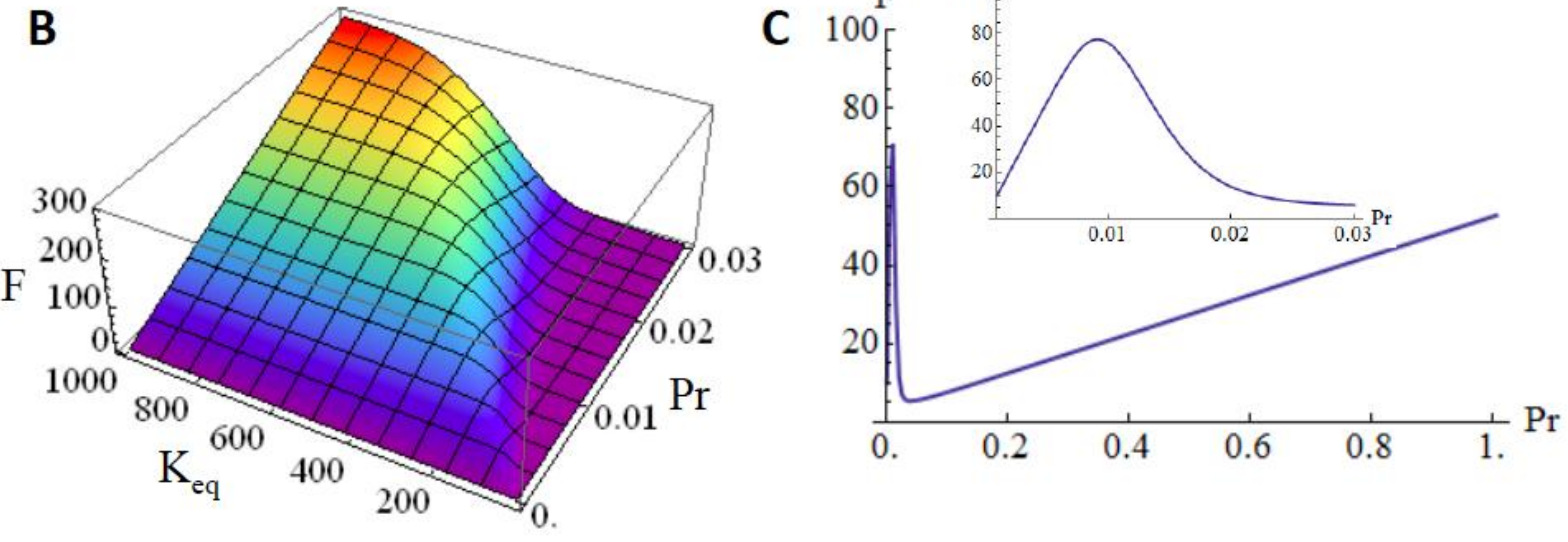
$$\frac{dNuc}{dt} = k_n U^{n_c} - k_a U Nuc - k_{dil} Nuc$$

$$\frac{dA}{dt} = k_a U Nuc - k_{dil} A$$

Jaie Woodard

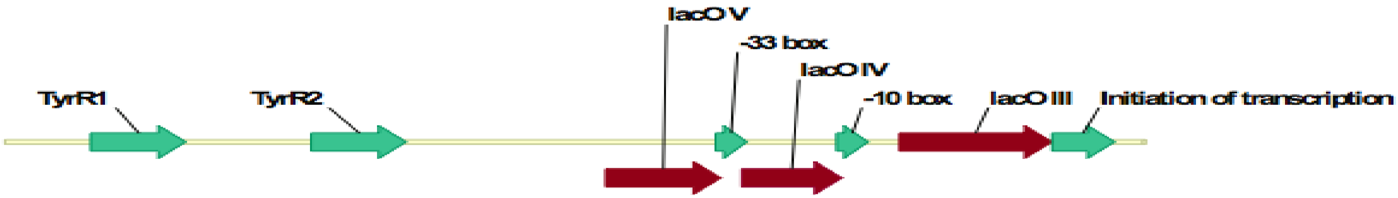
Theory Predicts: Less is more.

Downregulation of the production of an aggregation prone mutant would increase concentration of folded protein and rescue fitness

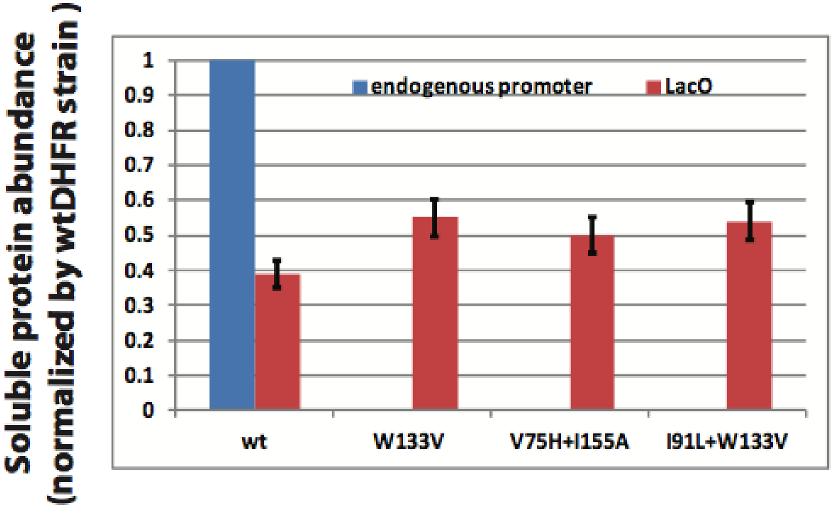


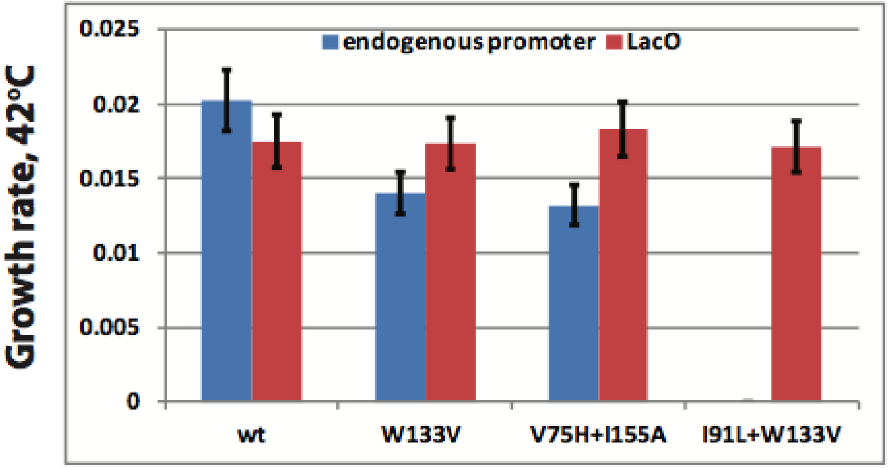
Testing the ‘’less is more” prediction: experiment #1

Downregulation of the DHFR transcription by placing it under an IPTG-controllable promoter indeed restores fitness and folded abundance



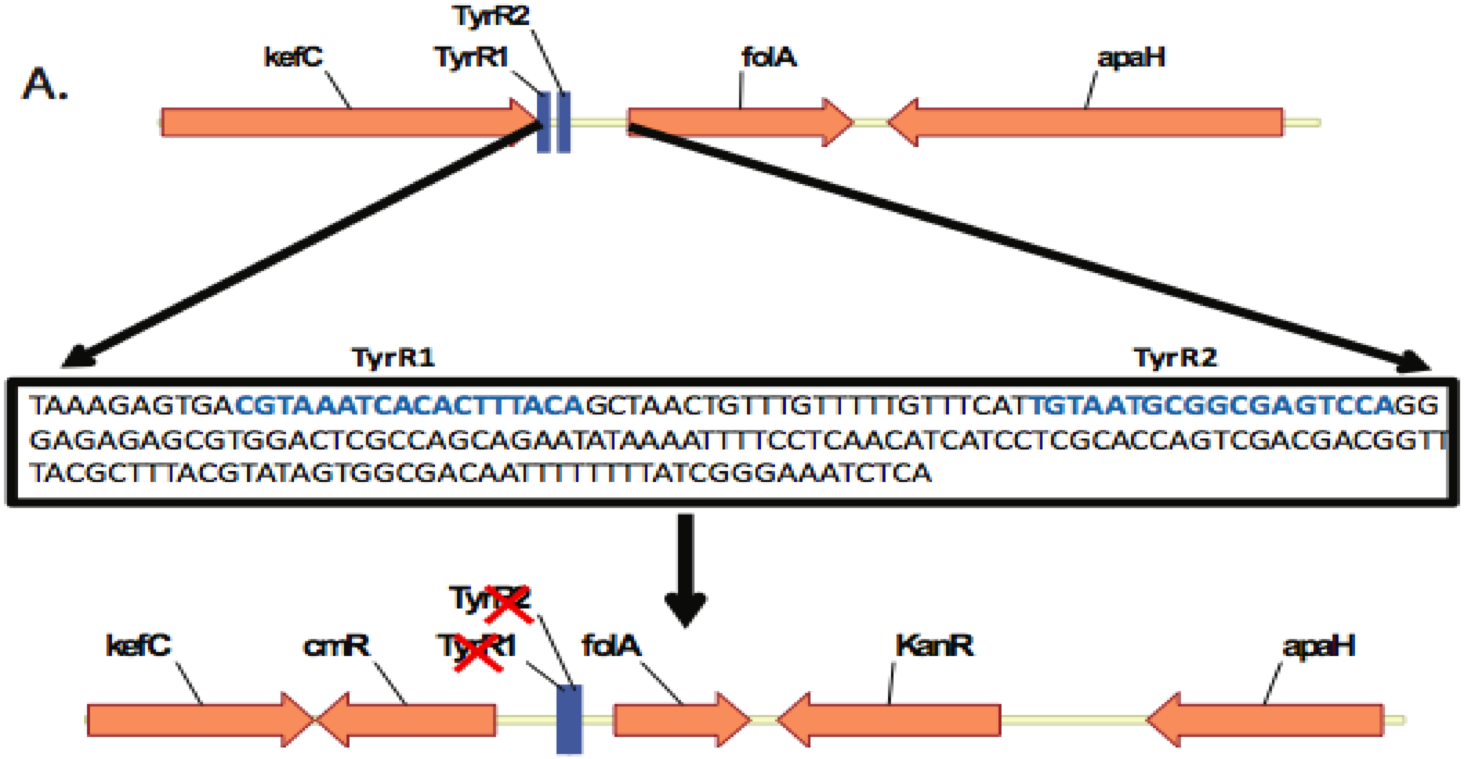
ATCGATTAAAGAGTGACGTAAATCACACTTTACAGCTAACTGTTTGTTTTTGTTTCATTGTAAT
GCGGCGAGTCCAGGGAGAGAGCGTGGACTCGCCAGCAGAATATAAAATTT**AATTGTGAGC**
GGATAACAATTTCGAC**TTGTGAGCGGATAACAATT**ATAGTGGCGAC**AATTGTGAGCGGATA**
ACAATTTCACACAGATCGGGAAATCTCAT





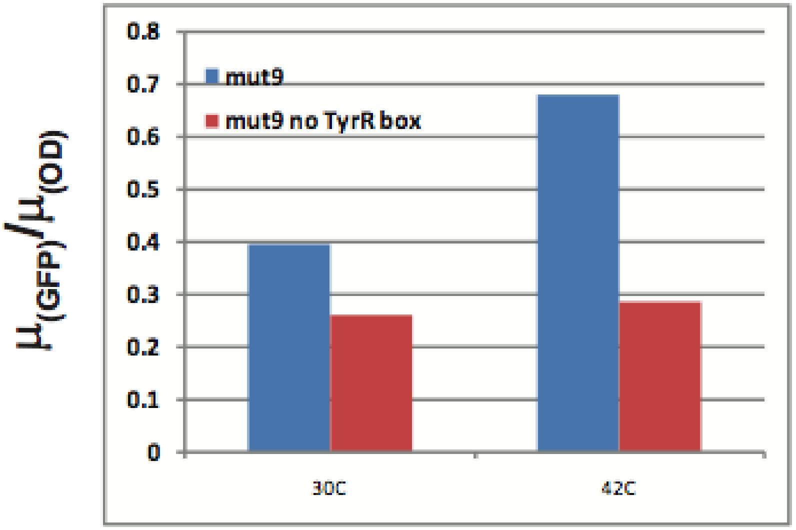
Testing the ‘’less is more” prediction: experiment #2

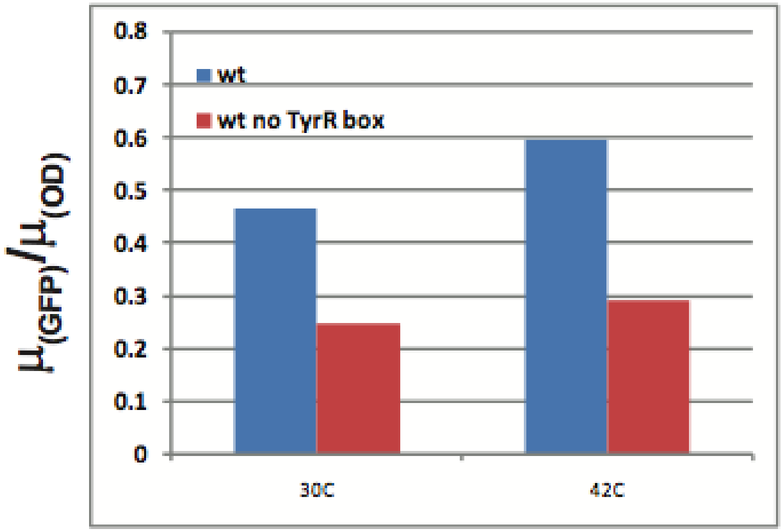
2) **Delete the tyrR box- a genetic element, which controls DHFR expression via a positive feedback loop.**



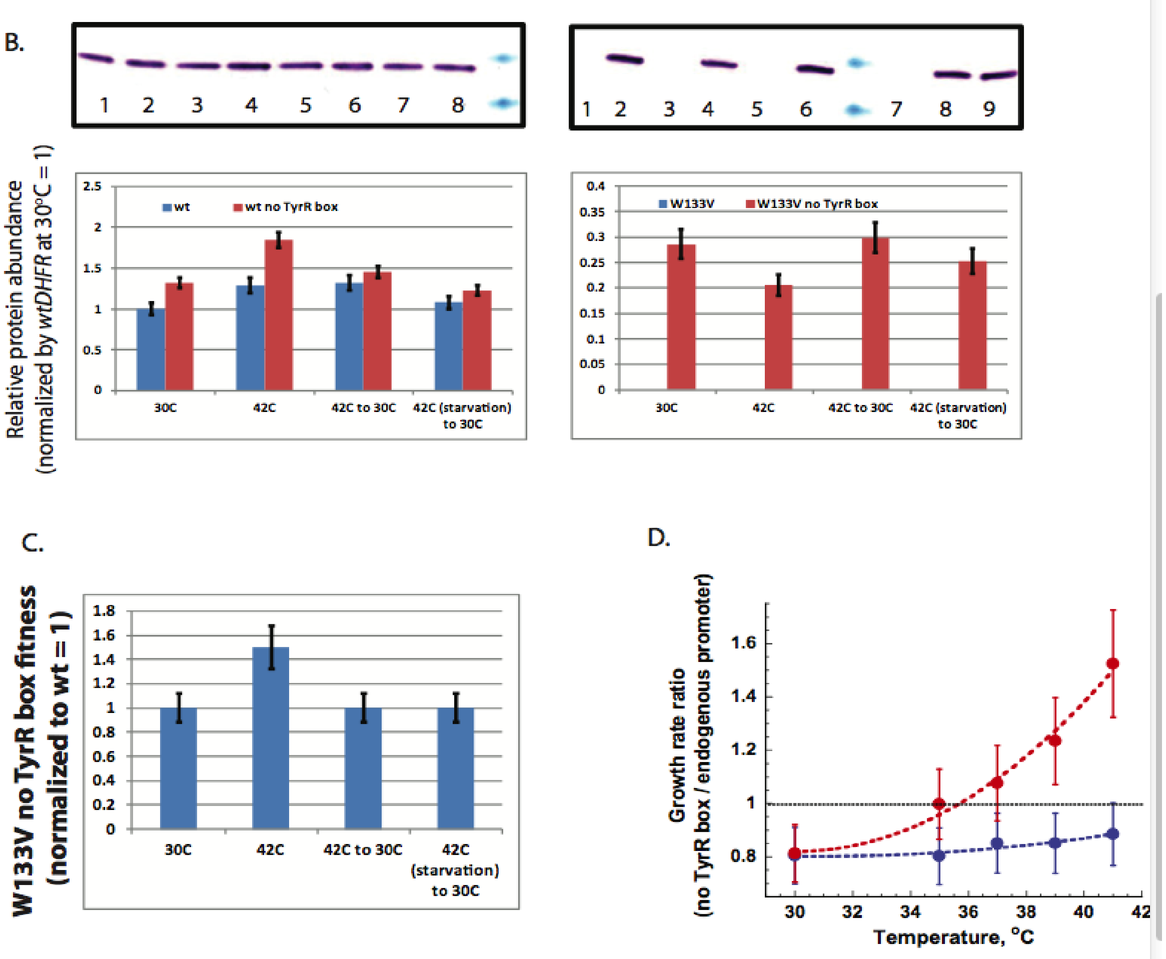
TAAAGAGTGACGTAAATCACACTTTACAGCTAACTGTTTGTTTTTGTTTCATTGTAATGCGGCGAGTCCAGG
GAGAGAGCGTGGACTCGCCAGCAGAATATAAAATTTTCCTCAACATCATCCTCGCACCAGTCGACGACGGTT
TACGCTTTACGTATAGTGGCGACAATTTTTTTTATCGGGAAATCTCA

Real-time qPCR and GFP under DHFR promoter indeed confirm that tyrR controls DHFR expression through positive feedback: deletion of tyrR box decreases production

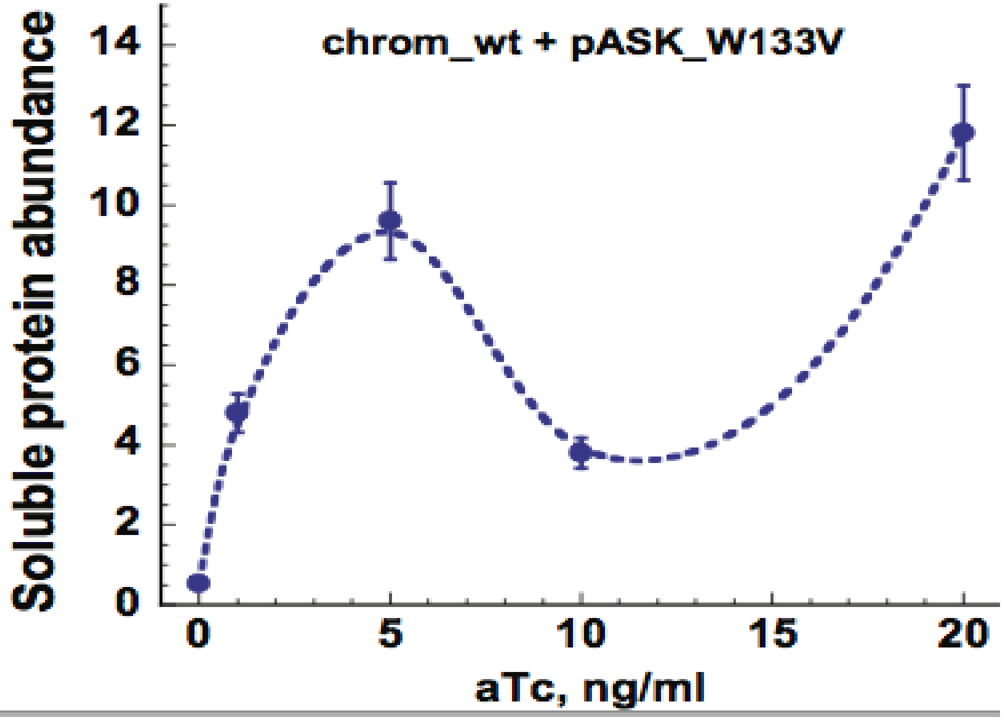


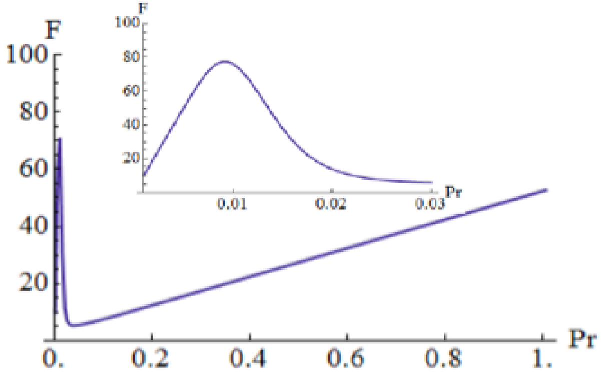


At the same time tyrR deletion hugely increases abundance of folded protein in cytoplasm and rescues fitness:



Finally 3rd experiment: add mutant strains from the aTc controllable plasmid and see how the amount of folded protein depends on production.





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$$$ NIH, HFSF, DARPA

THINK BIG!!!