The Protein Hourglass: First Passage Time Distributions for Protein Thresholds

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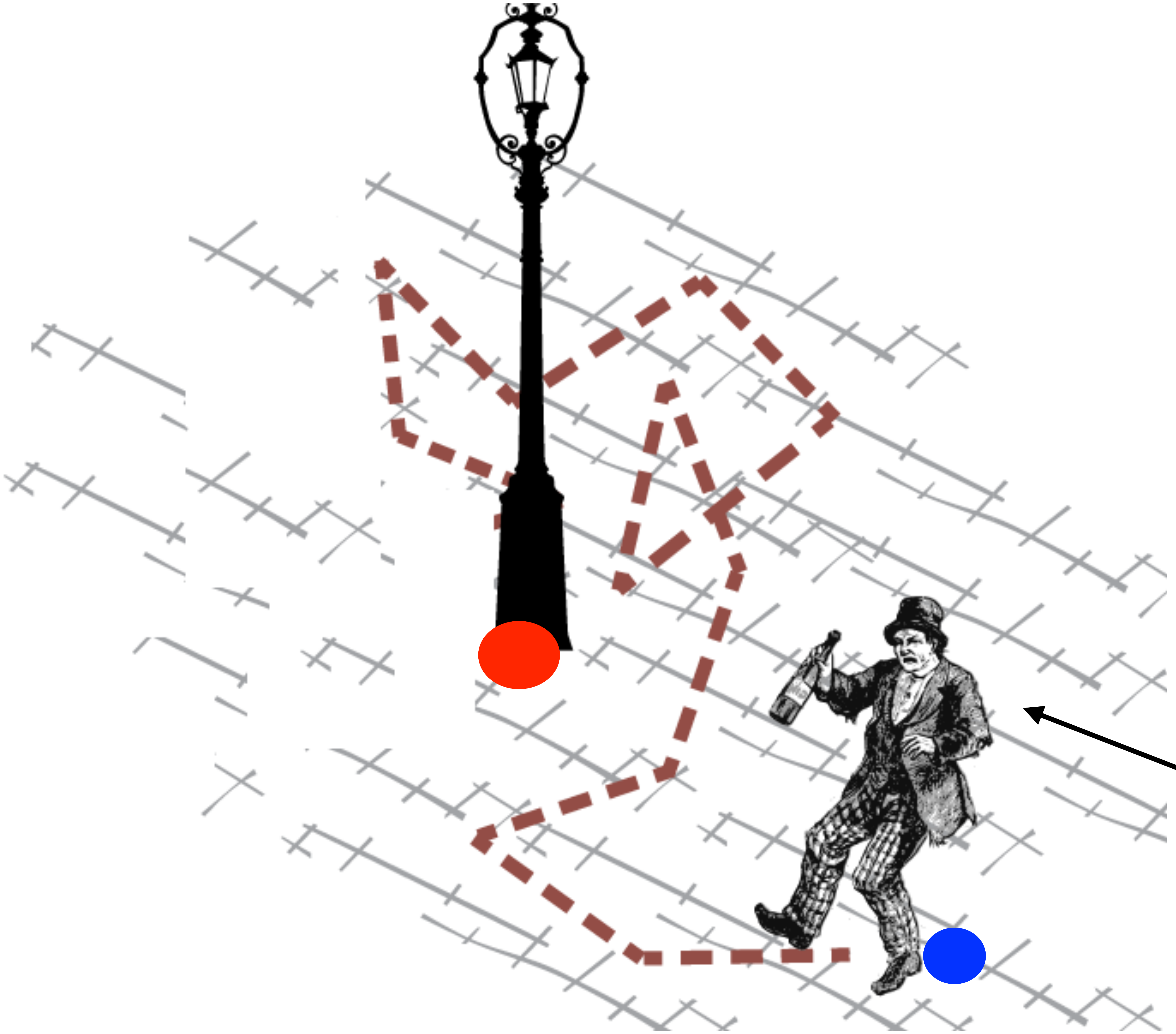
Basic idea of First Passage

Source: shorturl.at/nsxT1

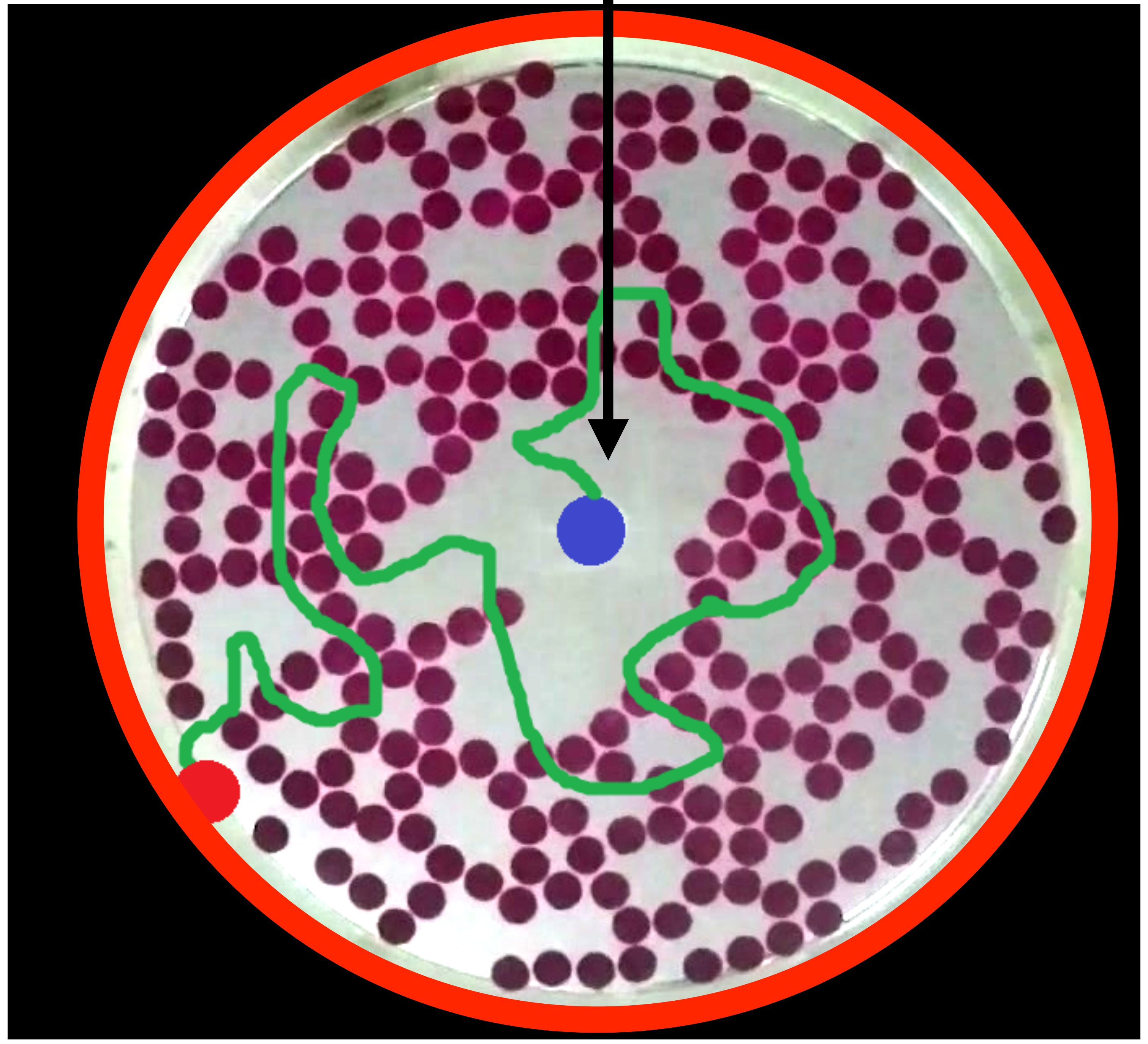


Drunk Person

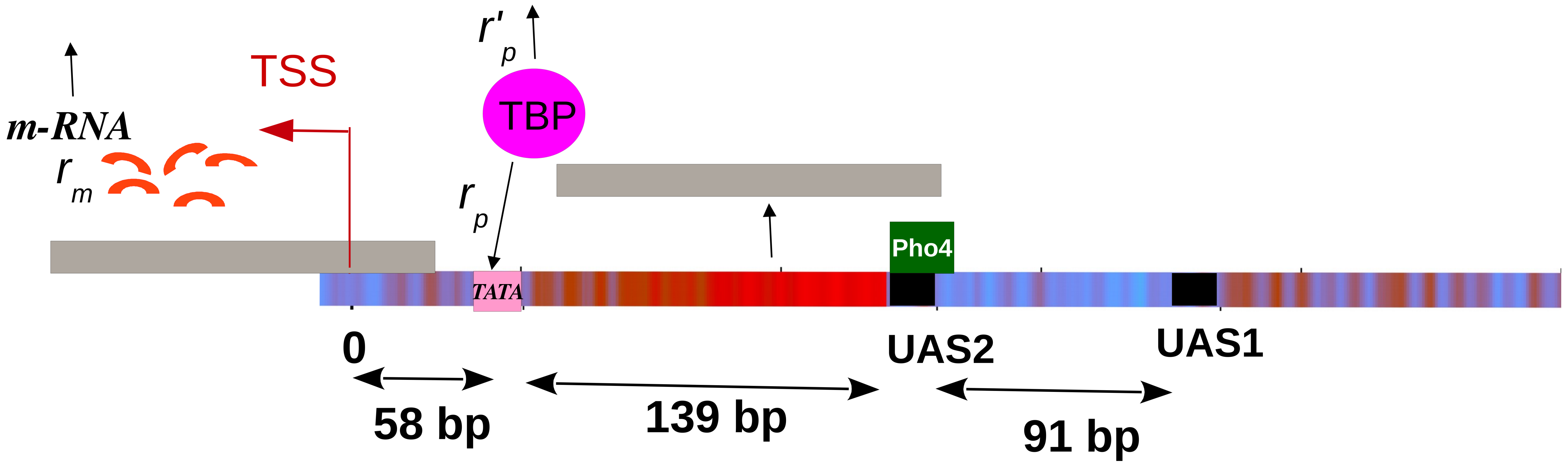
Some works on First Passage from our group

Biswas, Cruz, Parmanada & Das, *Soft Matter* (2020)

Active Camphor boat through passive floating crowders



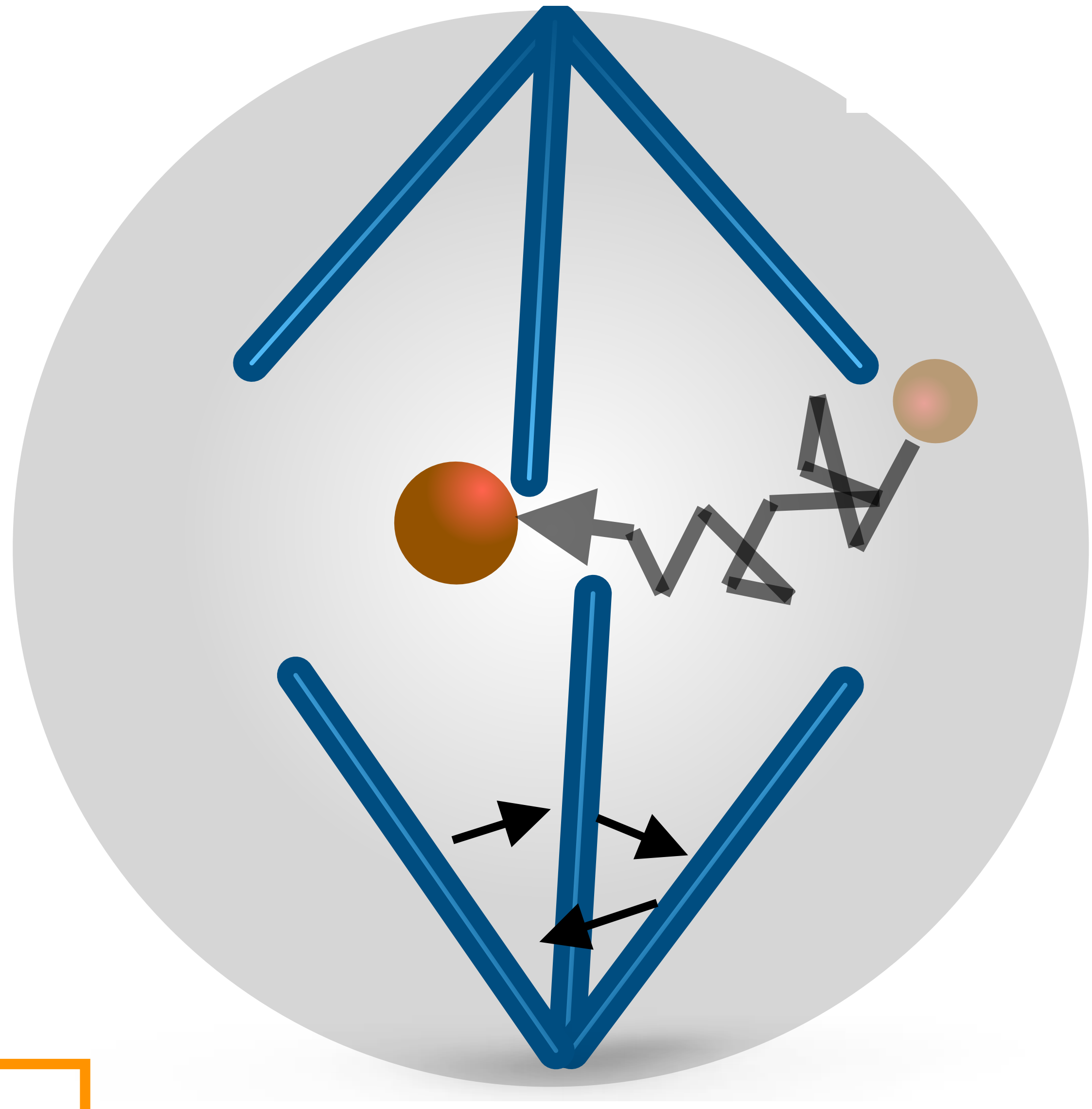
First binding of TBP on TATA box in promoter



Parmar, Das & Padinhateeri, *NAR* (2016)

Kinetochore Capture by multiple microtubules

Nayak, Das & Nandi, *PRR* (2020)



A lamb chased by many lions in confined volume

Nayak, Nandi & Das, *PRE* (2020)

Story of Phage-Lambda infecting E coli

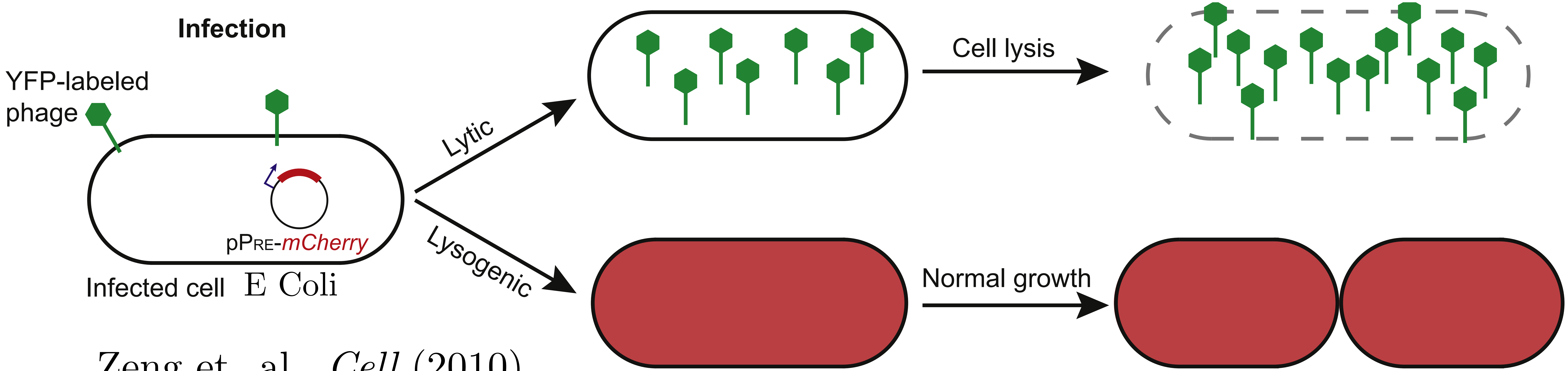
Lysis caused by Holin accumulation

Virus infecting bacteria — Bacteriophages

Virulent (Only lysis, e.g. T4)

Temperate

λ phage: Lysis-Lysogeny switch



Zeng et. al., *Cell* (2010)

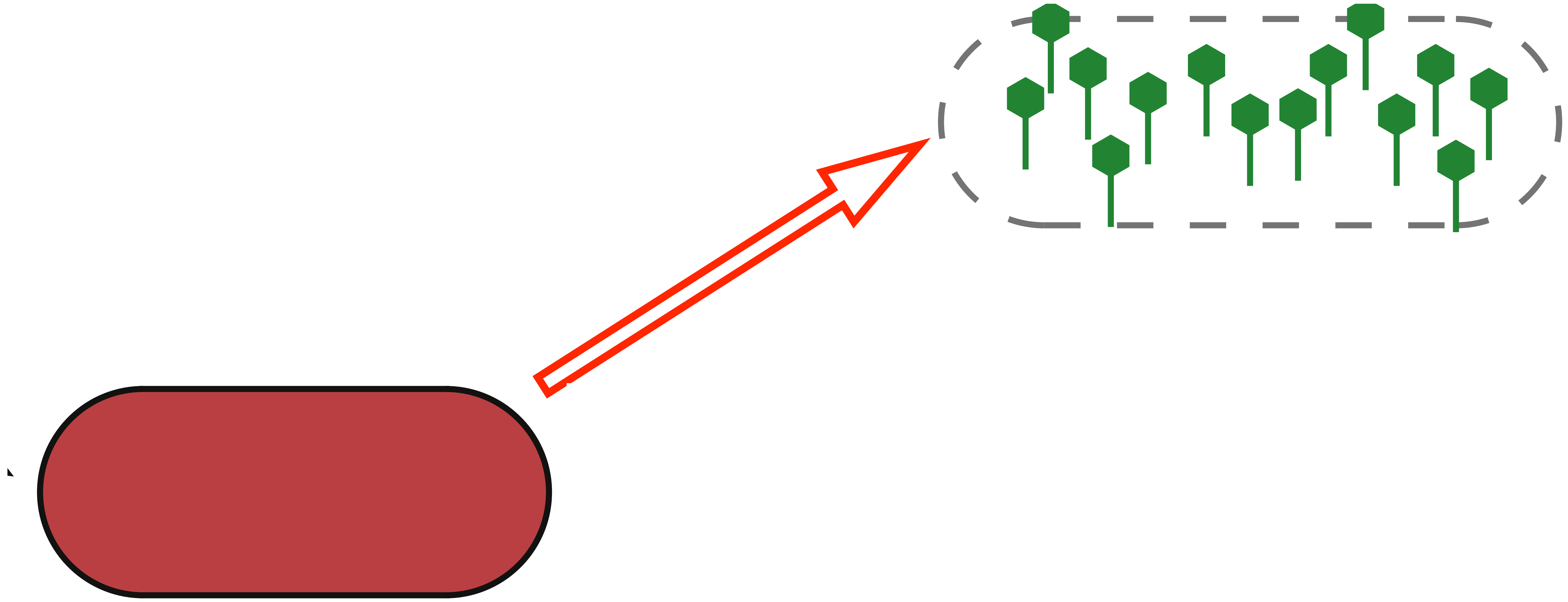
Many controlling factors: M.O.I, E coli cell volume, Stochastic expression of CI, Cro, CII

Virus infecting bacteria — Bacteriophages

Virulent (Only lysis, e.g. T4)

Temperate

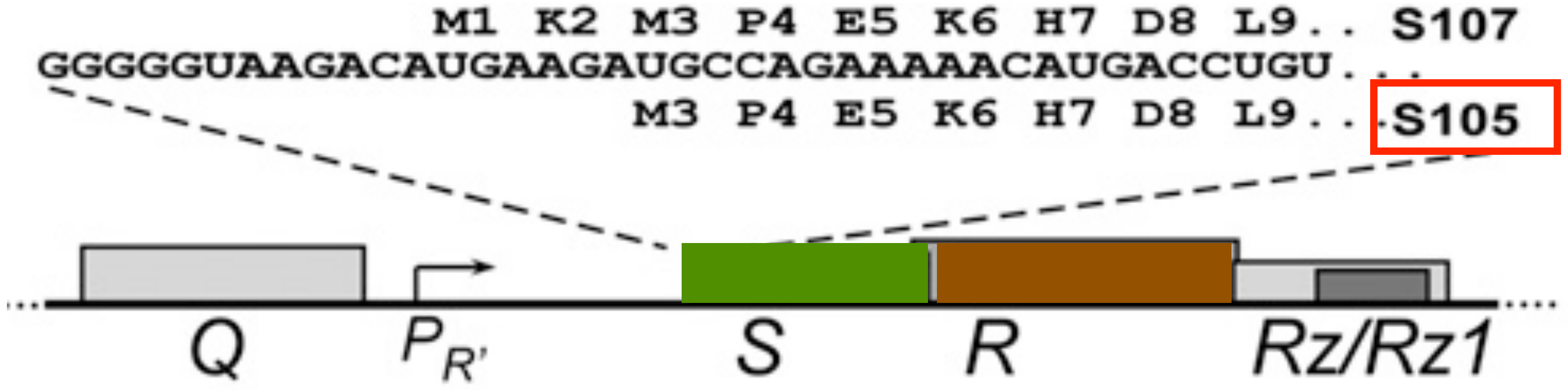
λ phage: Lysis-Lysogeny switch



- Focus on **Lysis – Thermal induction of Lysogenic Bacteria** ($t = 0$ set precisely)
- **What role does Holin proteins play?**

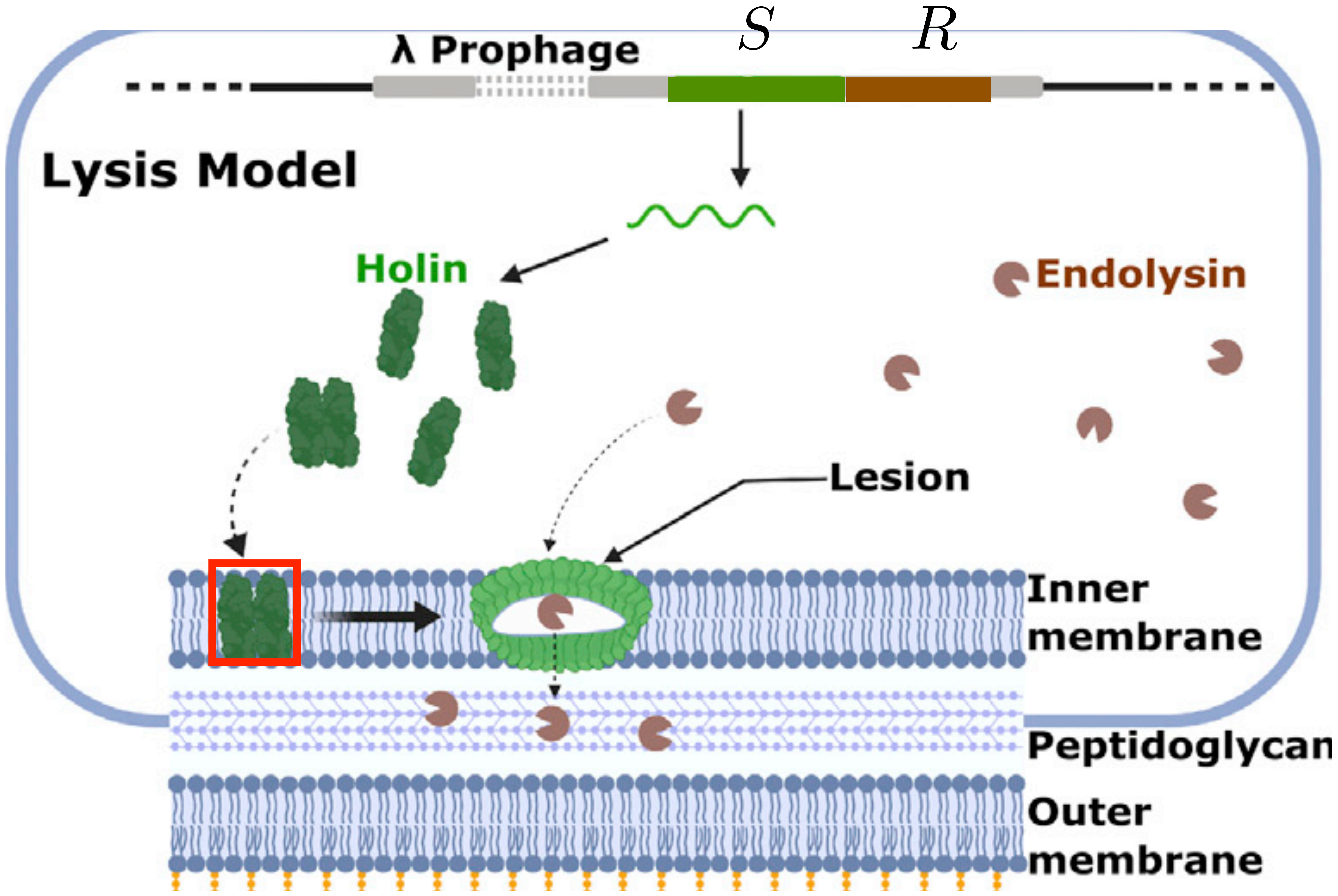
What controls lysis? Holin & Endolysin

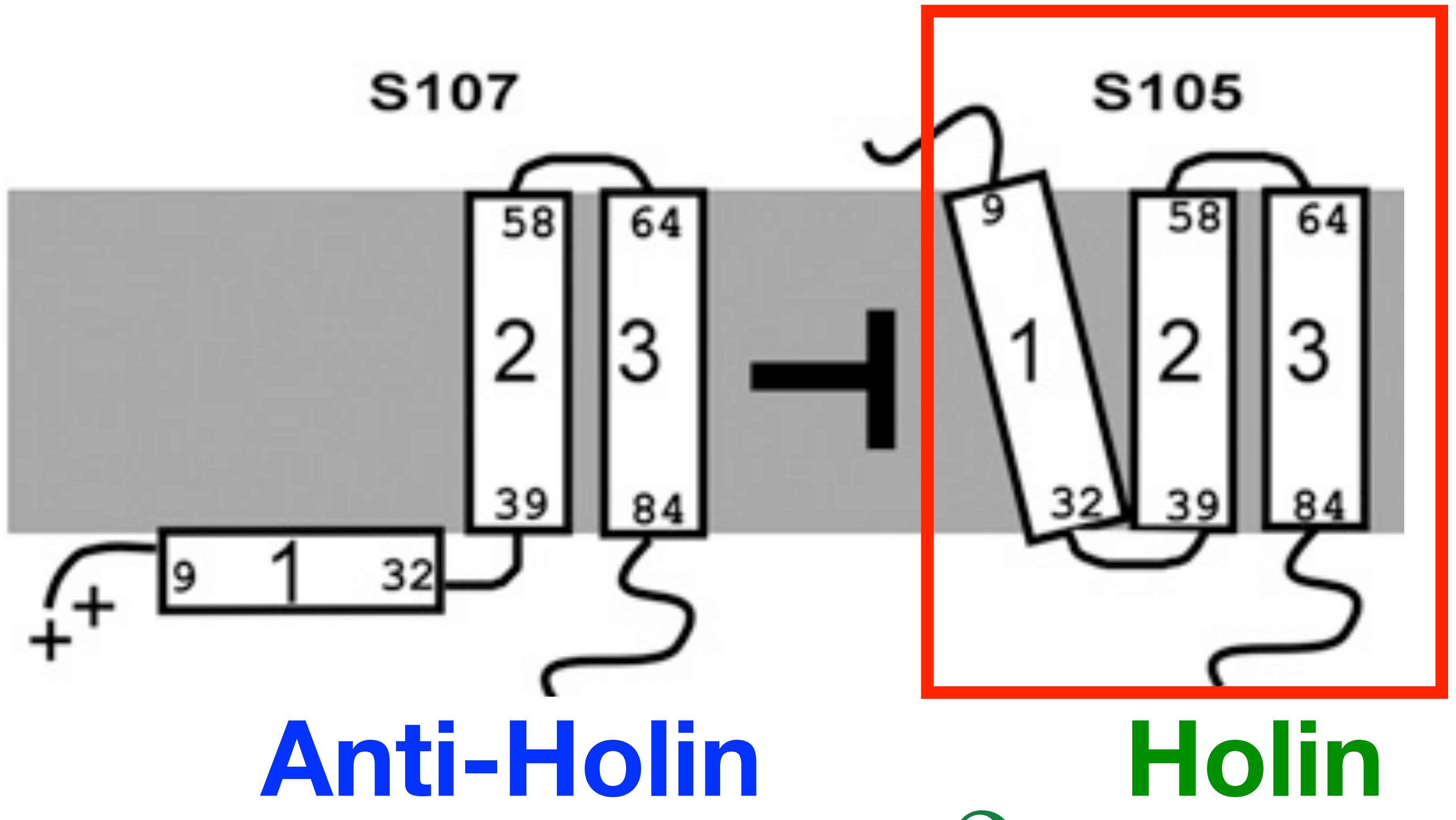
λ-phage genes



White et. al. *PNAS* (2011)

Kannoly et. al., *iScience* (2020)



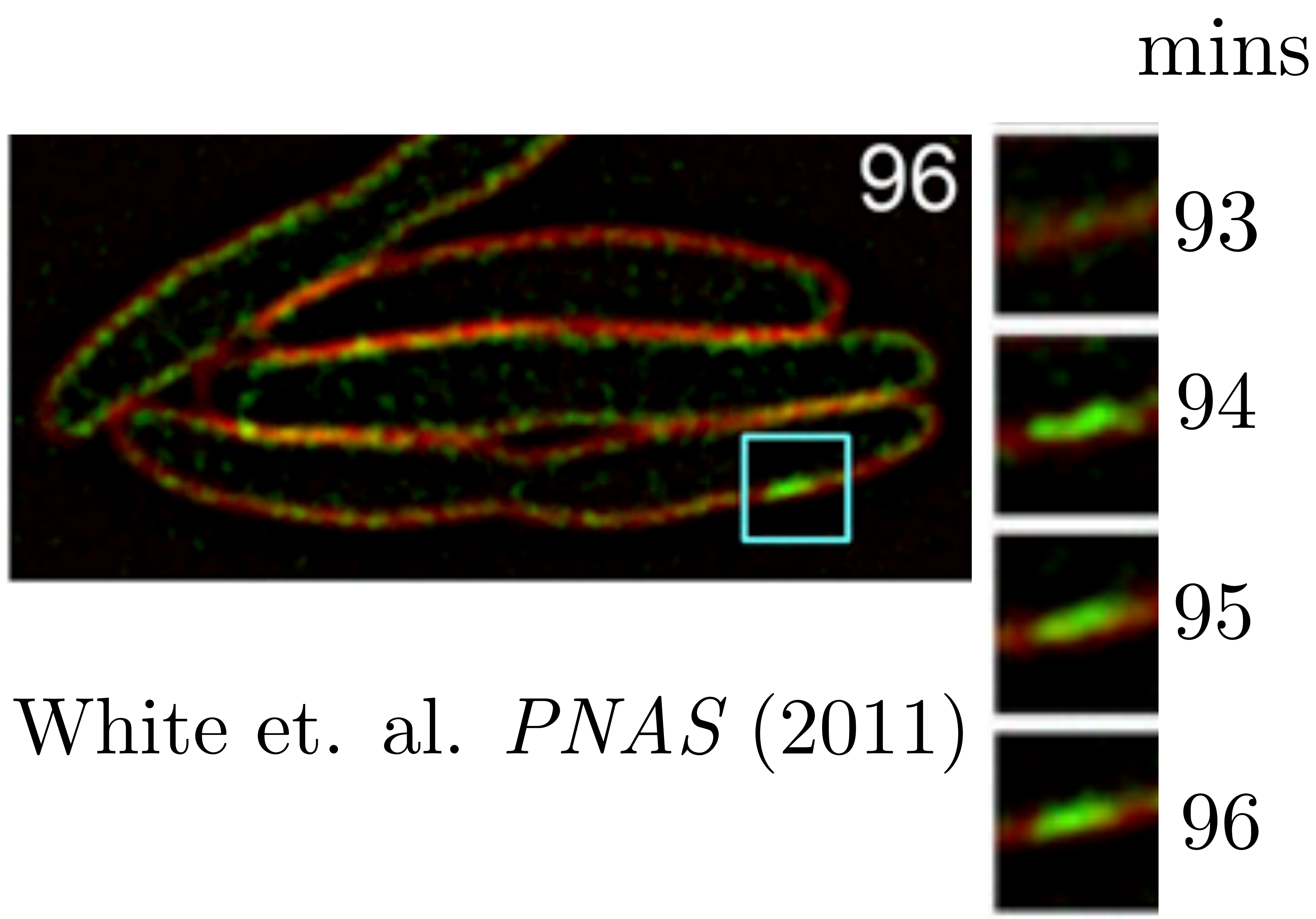


1 : 2

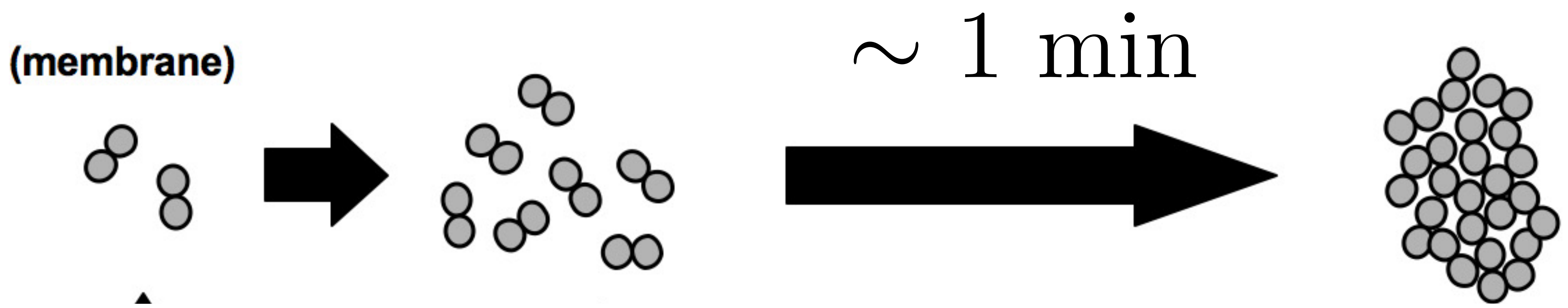
Chang, Nam, Young, *J Bacteriology* (1995)

"Excess" lethal Holin threshold

What is important for Lysis ? — Holin threshold and Time to reach it



White et. al. *PNAS* (2011)



Dennehy & Wang, *BMC Microbiology* (2011)

Critical Raft size — **No**

Critical number on membrane — **YES**

Holin Theshold: $X \approx 1500$

Chang, Nam, Young, *J Bacteriology* (1995)

In WT λ, Lysis time $\approx 65 \pm 3.2$ mins

Dennehy & Wang, *BMC Microbiology* (2011)

$$\langle t_L \rangle = \langle t_{pR'} \rangle + \langle FPT \rangle$$

$$\approx 15 \qquad \approx 50$$

As $n_{\text{Holin}} : 0 \to X$

Holin threshold & First Passage Time

It is very precise ...

HOLINS: The Protein Clocks of Bacteriophage Infections

Wang, Smith & Young, *Annu Rev Microbiol* (2000)

Holin triggering in real time

White et. al. *PNAS* (2011)

It is imprecise too (has stochasticity) ...

Factors influencing lysis time stochasticity in bacteriophage λ

Dennehy & Wang, *BMC Microbiology* (2011)

Stochastic holin expression can account for lysis time variation in the bacteriophage λ

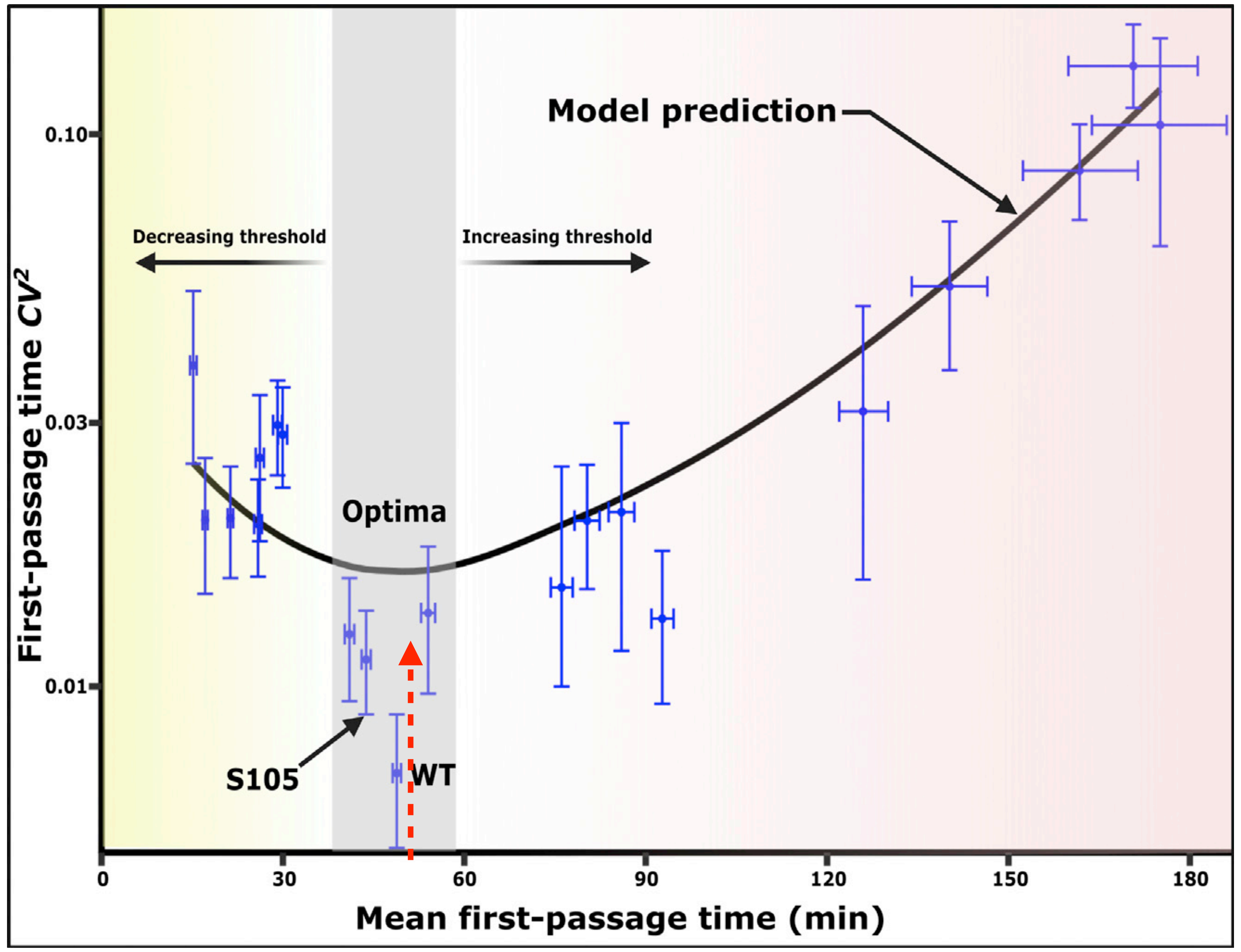
Singh & Dennehy, *J.R. Soc Interface* (2014)

Can one " Control / Engineer " Mean First Passage Time and fluctuations ?

- Bacteria growth rate
- Control late promoter pR' activity
- Holin mutants – alleles, altered amino acid sequences (affect holin number on membrane)

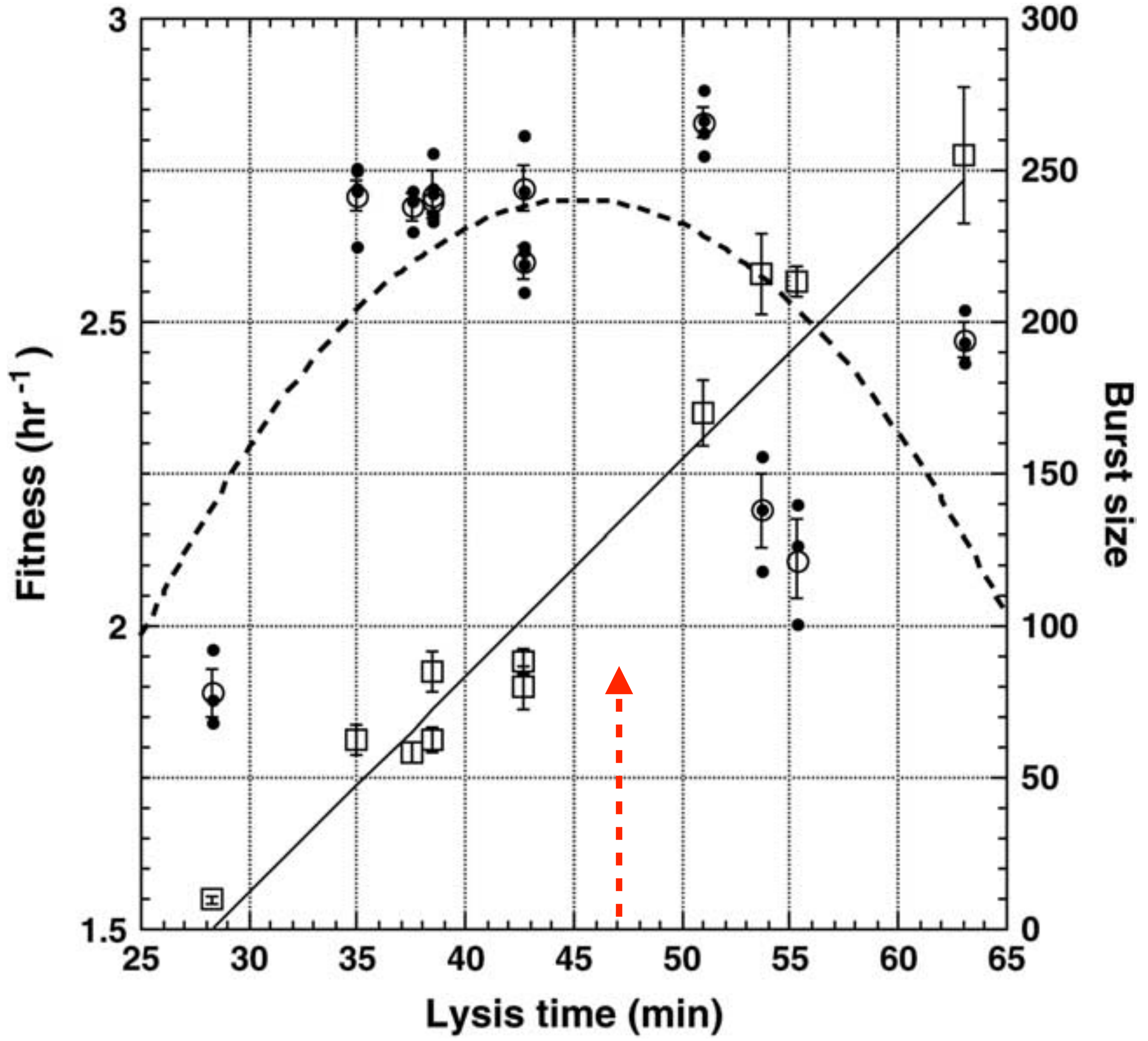
Dennehy & Wang, *BMC Microbiology* (2011)

Kannoly et. al., *iScience* (2020)



Different lysis time ⇒ different CV^2

Wang, *Genetics* (2006)



- Different lysis times ⇒ different fitness

Is lysis time $\langle t_L \rangle$ "Optimal" for WT λ ?

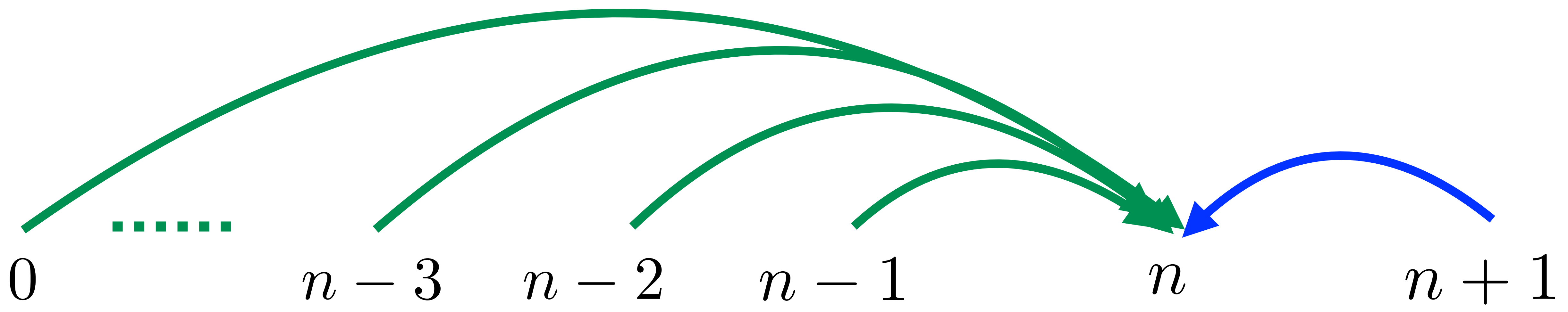
- Gene expression (noisy)– intrinsic

Full Statistics ?

Mathematical formulation of the problem

Calculation of the First Passage distribution

Protein bursts and degradation: Forward Equations



$$\frac{\partial P_n}{\partial t} = \left[\sum_{r=1}^{n} k_{n-r}\frac{b^r}{(b+1)^{r+1}}P_{n-r} - k_n\frac{b}{b+1}P_n\right] + \gamma\left[(n+1)P_{n+1} - nP_n\right]$$

(for $k_n = k =$ constant) Shahrezaei & Swain, *PNAS* (2008)

For $x = n/V$, $$\frac{\partial p(x,t)}{\partial t} = k\int_0^x dx'\left[\nu(x-x') - \delta(x-x')\right]p(x',t) + \gamma\frac{\partial}{\partial x}\left[xp(x,t)\right]$$

with $\nu(x) = (1/b)\exp(-x/b)$

Experiments, *Nature* (2006)

Friedman, Cai, & Sunney Xie, *PRL* (2006)

Steady State: $$p(x, t\to\infty) = \frac{1}{b^a\Gamma(a)}x^{a-1}e^{-x/b}$$ (here $a = k/\gamma$)

Gamma distribution

Moments of the First Passage time to reach the Holin threshold

Ghusinga, Dennehy, Singh, *PNAS* (2017)

Stochastic First Passage time $= min\{t : n(t) \geq X | n(0) = 0)\}$

Solved: Using Forward Master Equation formalism, obtained Mean and Variance for any $k_n \neq$ constant k (with feedback), and $\gamma \neq 0$ (with degradation)

$$CV^2 = \sigma^2 / \langle t \rangle^2$$

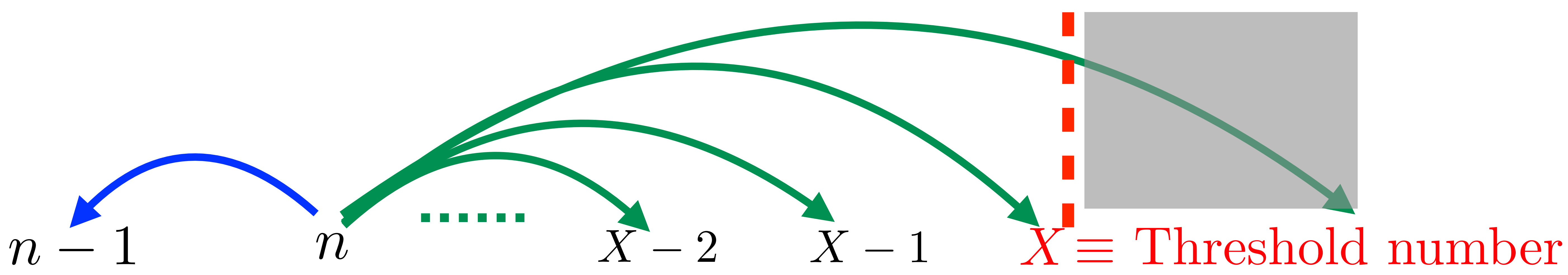
- For Holin ($\gamma \approx 0$), fluctuations (CV^2) minimum for no feedback ($k_n = k$)

- For other proteins which degrade fast ($\gamma > 0$), CV^2 minimised for positive feedback (e.g. $k_n = c_1 + c_2 n$, or Hill form $k_n = k_{max} \frac{r + (cn)^H}{1 + (cn)^H}$)

Full distribution ?

Full First Passage distribution: Backward Formalism

Rijal, Prasad & Das, *PRE* (2020)



Survival probability $S_n(t)$:   Protein number stays below threshold X at time t, starting with n at $t=0$

$$\frac{\partial S_n}{\partial t} = k_n \left[\sum_{r=1}^{X-1-n} \frac{b^r}{(b+1)^{r+1}} S_{n+r} - \frac{b}{b+1} S_n \right] + \gamma \left[n S_{n-1} - n S_n \right]$$

$$S_{n \geq X}(t) = 0 \text{ and } S_{n<X}(0) = 1$$

Compact form: $\quad \frac{\partial}{\partial t} \vec{S}(t) = \mathbf{A} \vec{S}(t)$

First Passage time distribution with $n(0)=0$: $\quad f_{0,X}(t) = -\frac{\partial}{\partial t} S_{0,X}(t)$

Technical details

Laplace Transforms: $\quad \tilde{f}_{0,X}(s) = 1 - s\tilde{S}_{0,X}(s)$ where $\tilde{S}_{0,X}(s) = \sum_{j=1}^{X} (s\mathbf{I} - \mathbf{A})^{-1}_{1j}$

Challenges: (1) To find the matrix elements $(s\mathbf{I} - \mathbf{A})^{-1}_{ij}$ for any X

(2) To find the Laplace Inverse : $f_{0,X}(t) = \mathcal{L}^{-1}[\tilde{f}_{0,X}(s)]$

Our results suitable for Holin ($\gamma = 0$, i.e no degradation)

For equal $k_n = k$ (no feedback):

$$f_{0,X}(t) = \frac{kb^X}{(b+1)^X}\left[\sum_{n=0}^{X-1}\frac{1}{n!}\binom{X-1}{n}\left(\frac{kt}{b+1}\right)^n\right]e^{-\frac{kbt}{b+1}}$$

Note: Exponential tail (not Gaussian)

For unequal k_n (with positive or negative feedback):

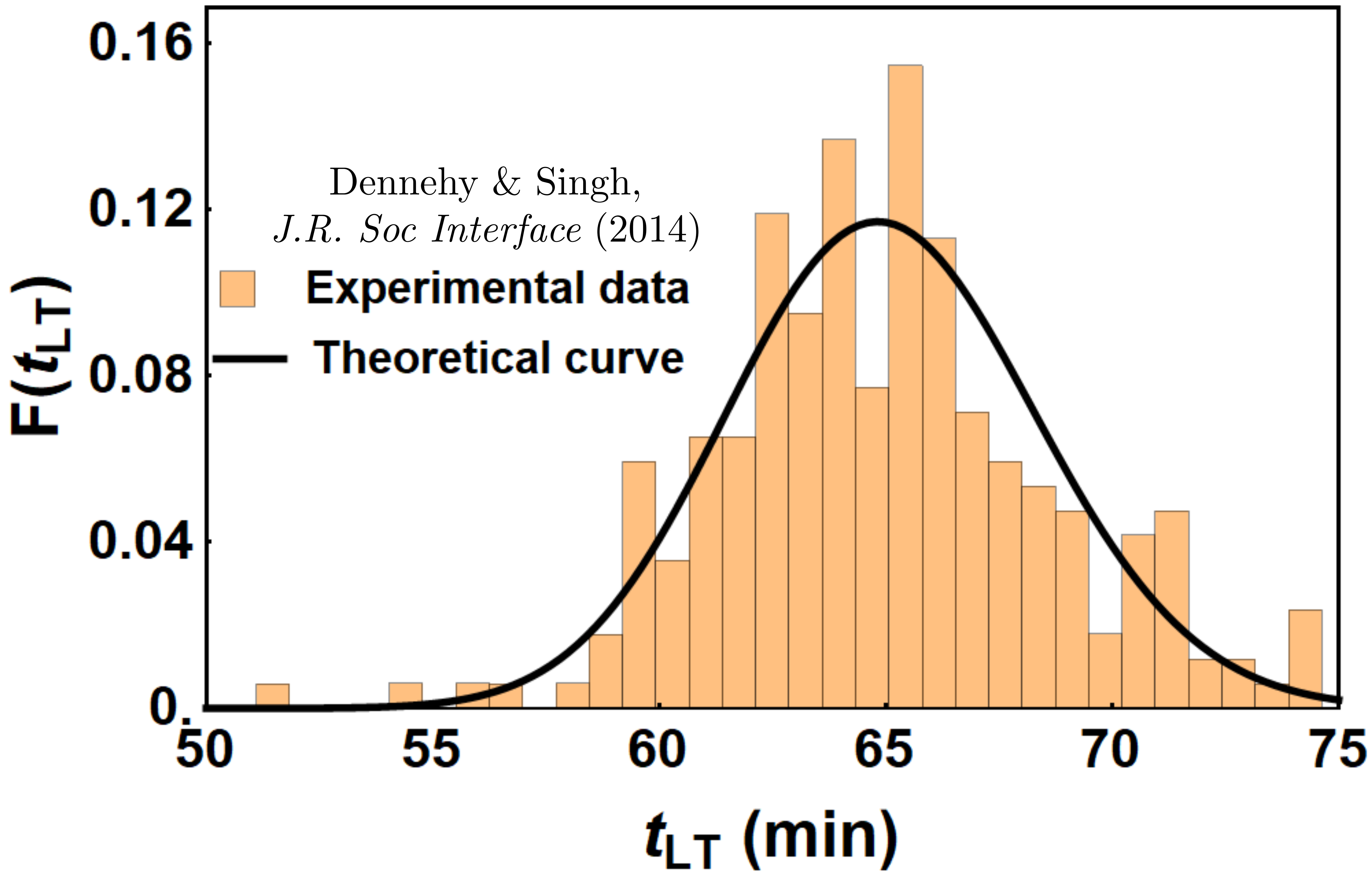
$$f_{0,X}(t) = \frac{bk_0}{(b+1)^2}\Bigg[\left(\frac{k_1}{k_1-k_0}+b\right)e^{-\frac{bk_0t}{b+1}} + \left(\frac{k_1}{k_0-k_1}\right)e^{-\frac{bk_1t}{b+1}} + \sum_{j=3}^{X}\sum_{n=0}^{j-1}\frac{k_n}{(b+1)^{j-2}}\frac{\prod_{q=1}^{j-2}((b+1)k_q - bk_n)}{\prod_{p=0,p\neq n}^{j-1}(k_p-k_n)}e^{-\frac{bk_nt}{b+1}}\Bigg]$$

Comparison with experimental WT Holin, Lysis time distribution

Use Experimental values of: $X = 1500, \quad \langle t_{LT} \rangle \approx 65$ mins & $\sigma_{LT} \approx 3.5$ mins

$$\text{Theory: } \langle t \rangle = \frac{b+X}{bk} \text{ and } CV^2 = \frac{\sigma^2}{\langle t \rangle^2} = \frac{b^2+X+2bX}{(b+X)^2}$$

$$\langle t_{LT} \rangle \approx 15 + \langle t \rangle \qquad \Rightarrow b = 3 \text{ and } k = 10 \text{ min}^{-1}$$

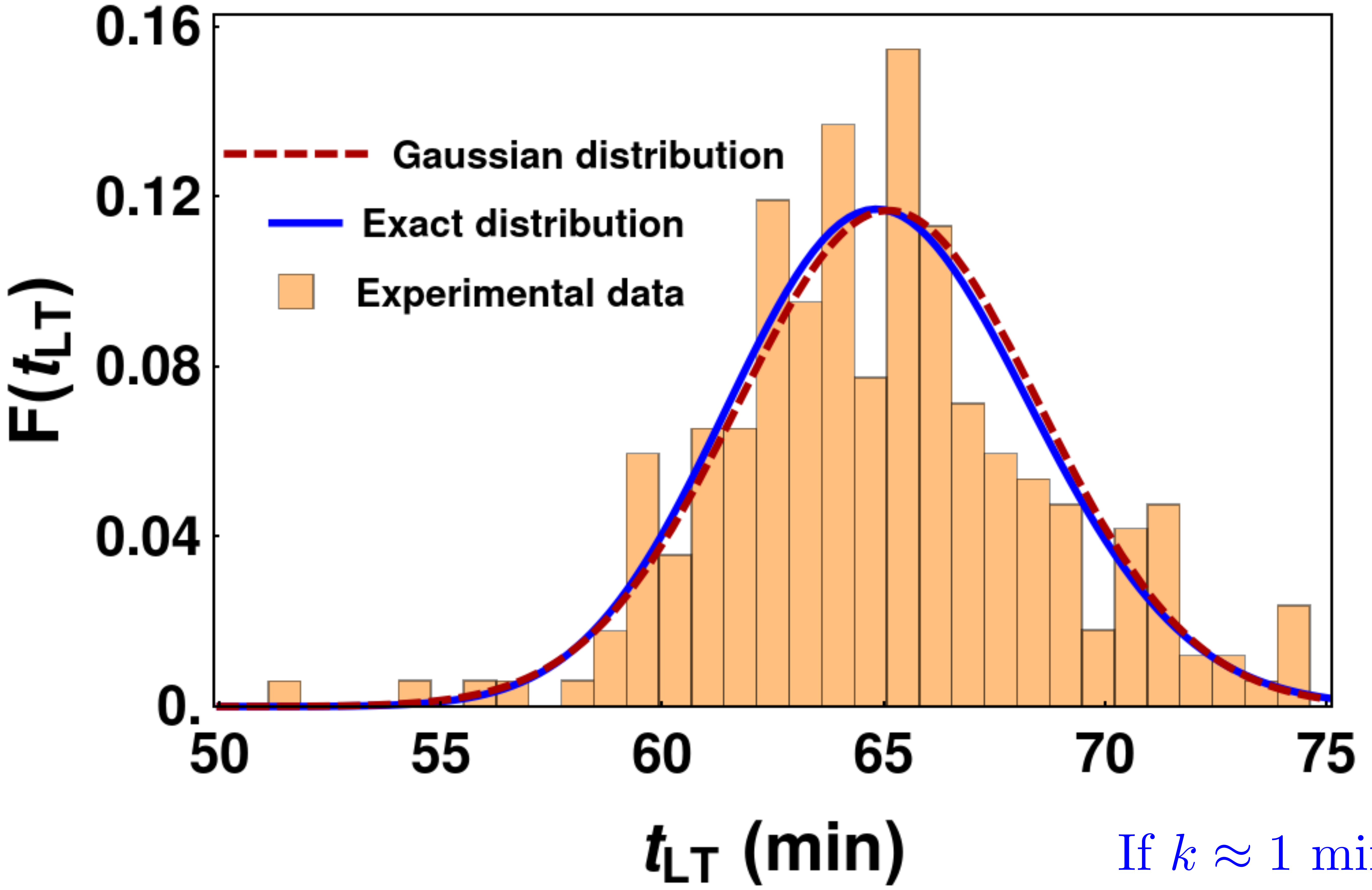


Comparison with experimental (WT lambda) Lysis time distribution

Use Experimental values of: $X = 1500$, $\langle t_{LT} \rangle \approx 65$ mins & $\sigma_{LT} \approx 3.5$ mins

Theory: $\langle t \rangle = \frac{b+X}{bk}$ and $CV^2 = \frac{\sigma^2}{\langle t \rangle^2} = \frac{b^2+X+2bX}{(b+X)^2}$, all cumulants κ_n analyitically

$\langle t_{LT} \rangle \approx 15 + \langle t \rangle$ $\Rightarrow b = 3$ and $k = 10\ \text{min}^{-1}$



$\text{Skewness} = \frac{\kappa_3}{(\kappa_2)^{\frac{3}{2}}}$

Gaussian: 0

Theory: 0.10 , Exp: 0.06

$\text{Kurtosis} = 3 + \frac{\kappa_4}{\kappa_2^2}$

Gaussian: 3

Theory: 3.02 , Exp: 3.54

If $k \approx 1\ \text{min}^{-1}$ and $b \approx 30$, then skewness ≈ 0.30

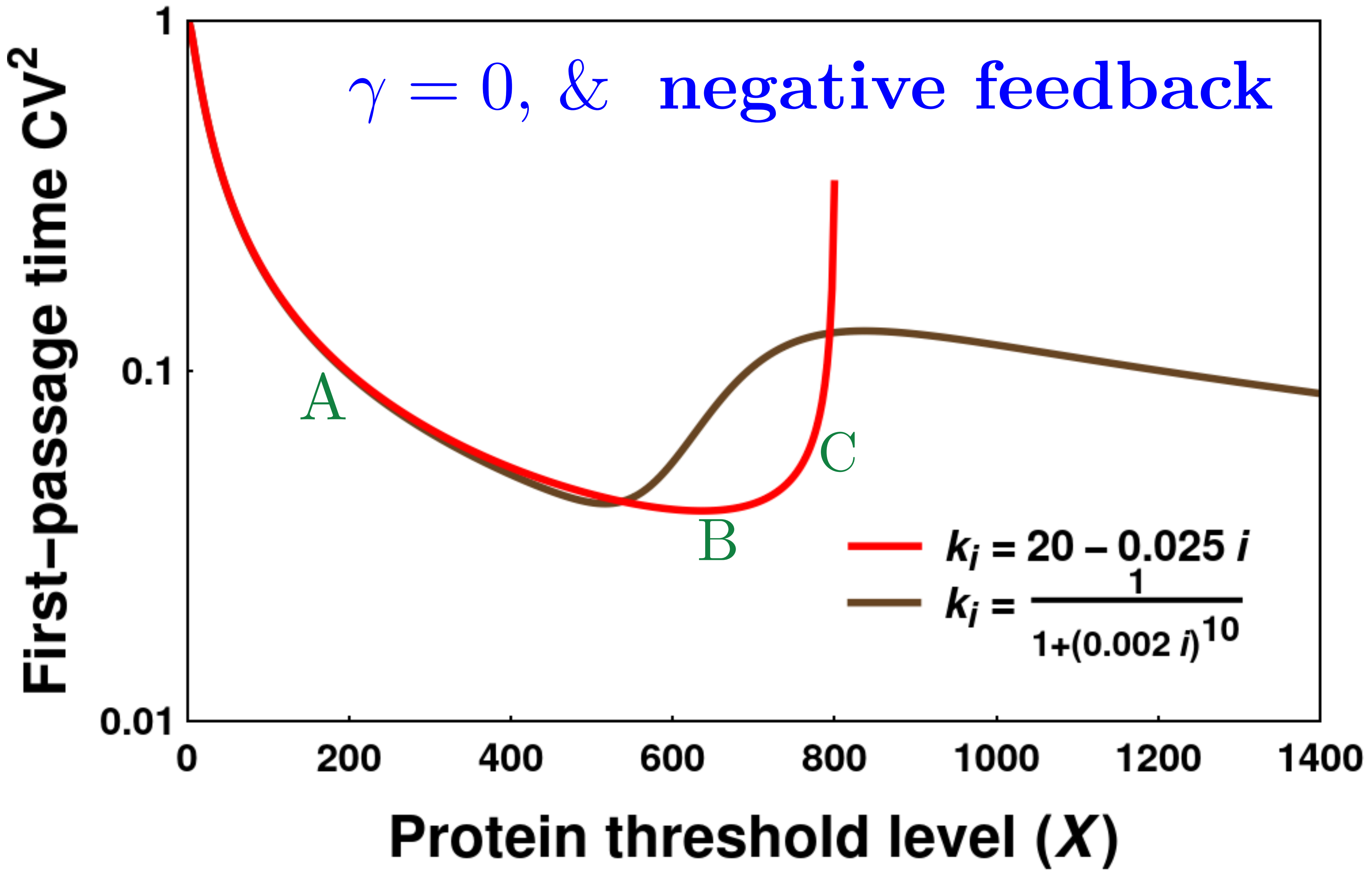
Short lived proteins: with degradation rate $\gamma \neq 0$

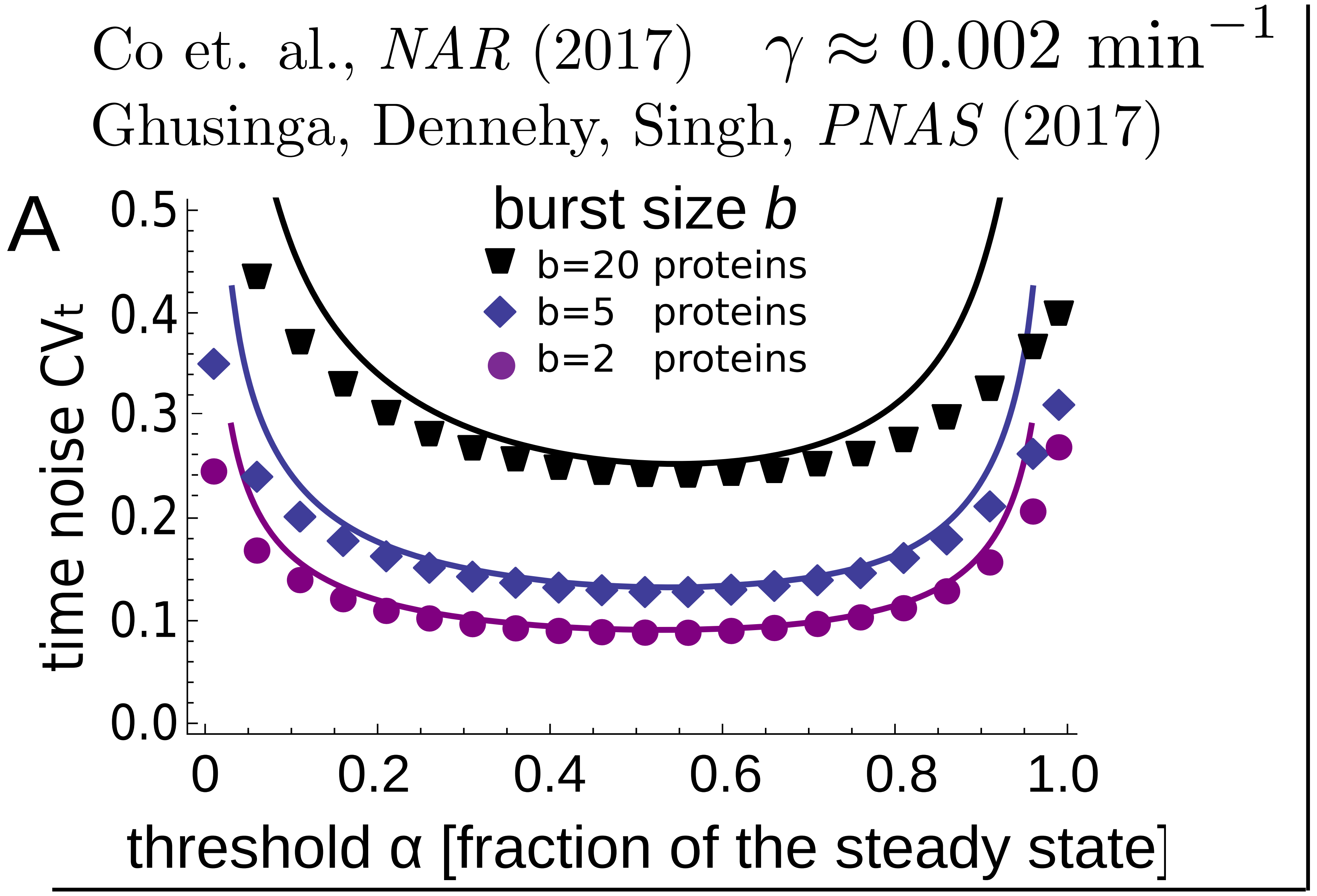
Laplace Transform:

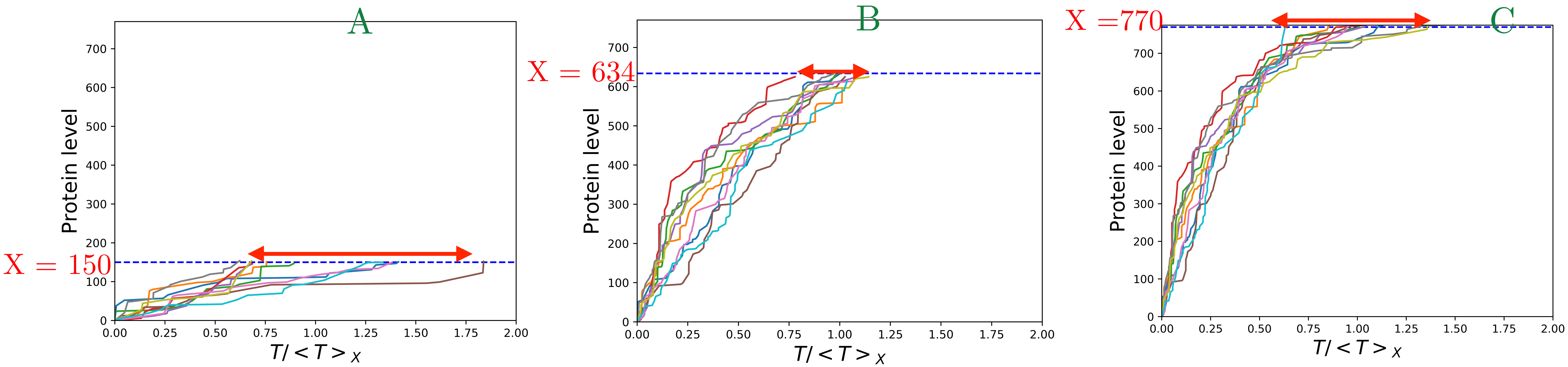
$$\tilde{f}_{0,X}(s) = \frac{b^X k \prod_{l=1}^{X-1} (\gamma l + k + s)}{\prod_{i=0}^{X-1} (\gamma i + s) + b^X (k+s) \prod_{j=1}^{X-1} (\gamma j + k + s) + \sum_{n=1}^{X-1} \left[\binom{X}{n} b^n \prod_{i=0}^{X-1} (\gamma i + k\Theta(i - X + n) + s) \right]}$$

- Finding the poles, for general X, and hence finding $f_{0,X}(t)$ remains difficult.
- We obtained the third moment (not known before), & can predict Skewness.

Noise versus X, non-monotonic behaviour for negative feedback







Concluding comments

1) We obtained the full First Passage time distribution, for long lived proteins (like Holin in Lambda phage).
   [For short lived proteins, we have the exact expression of the Laplace transform of the distribution.]

2) The skewness and departure from Gaussian may be more clearly discernible
   if transcription & translation rates and burst size may be engineered.

3) For long live proteins too, a non-monotonic noise (CV) is possible for “Negative feedback”

4) Results for continuous protein concentration $x = n/V$, have been derived,
   and those are consistent with the results for discrete protein numbers n.

Thank You