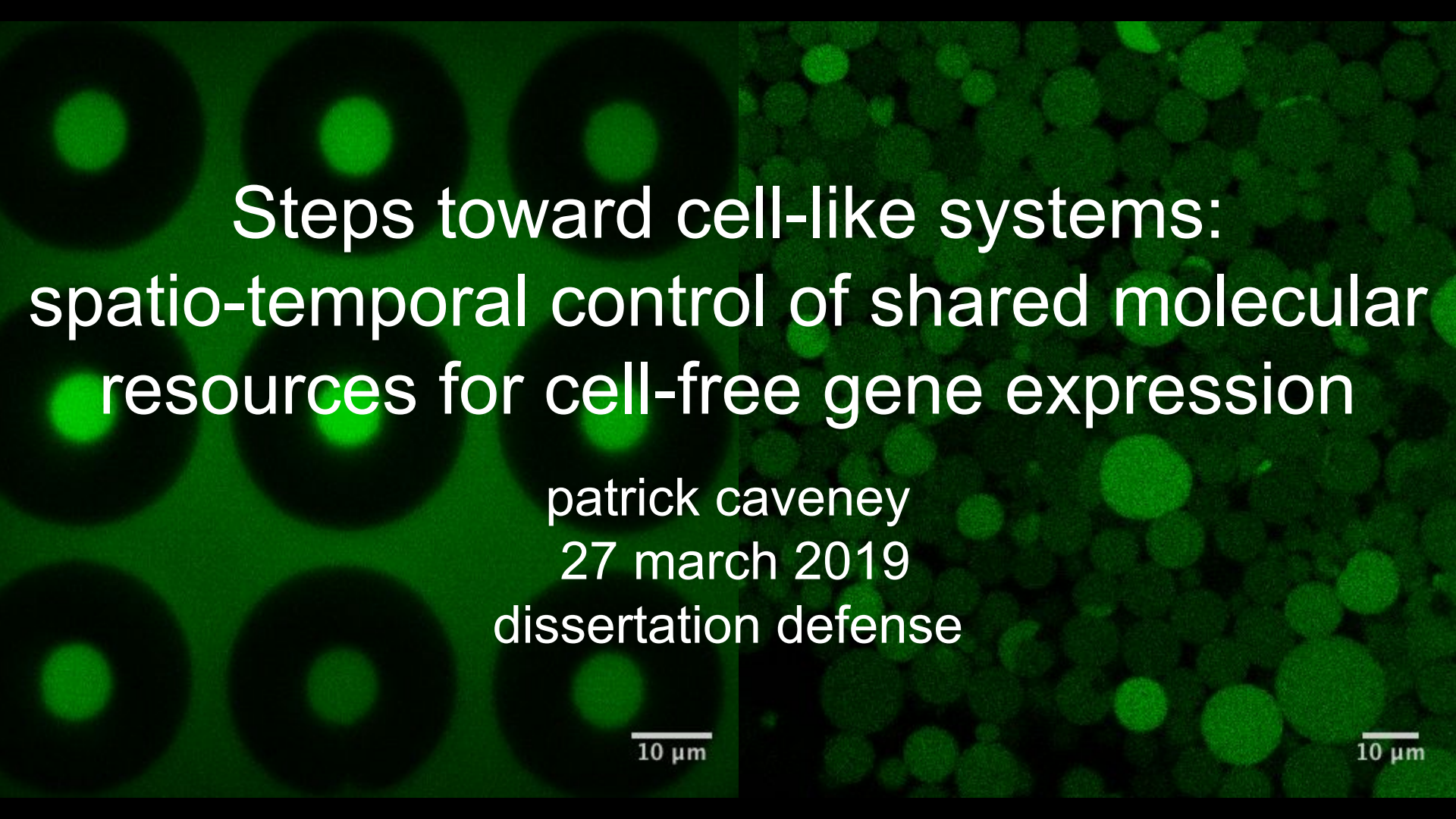


Steps toward cell-like systems: spatio-temporal control of shared molecular resources for cell-free gene expression

patrick caveney

27 march 2019

dissertation defense

Outline

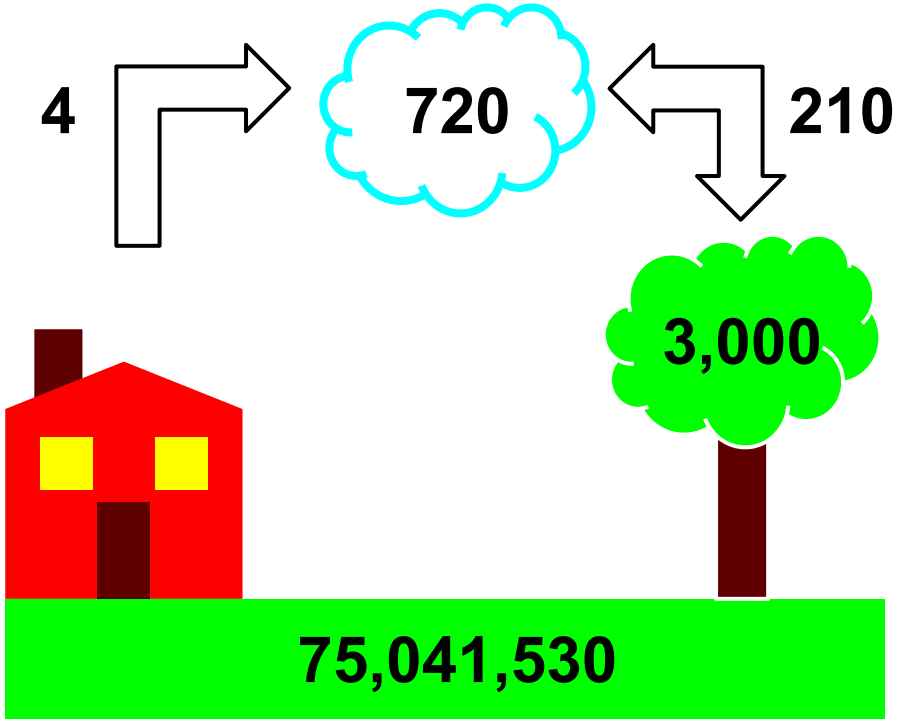
Chapter 1: Introduction

Chapter 2: Resource Sharing Controls Gene Expression Bursting

Chapter 3: Controlling Cell-Free Gene Expression Behavior by Tuning Membrane Transport Properties

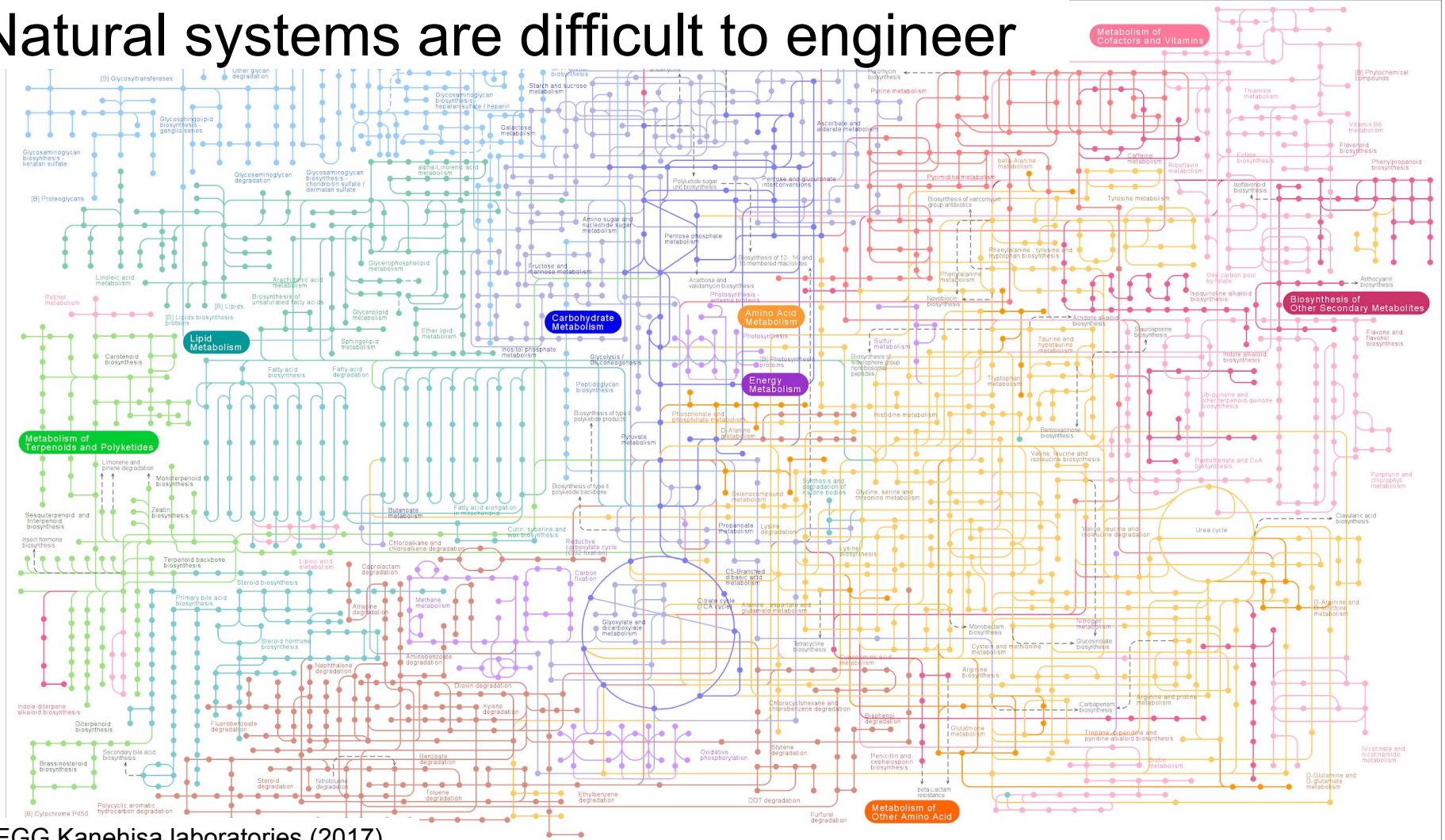
Chapter 4: Conclusion

Biology offers many solutions to energy challenges



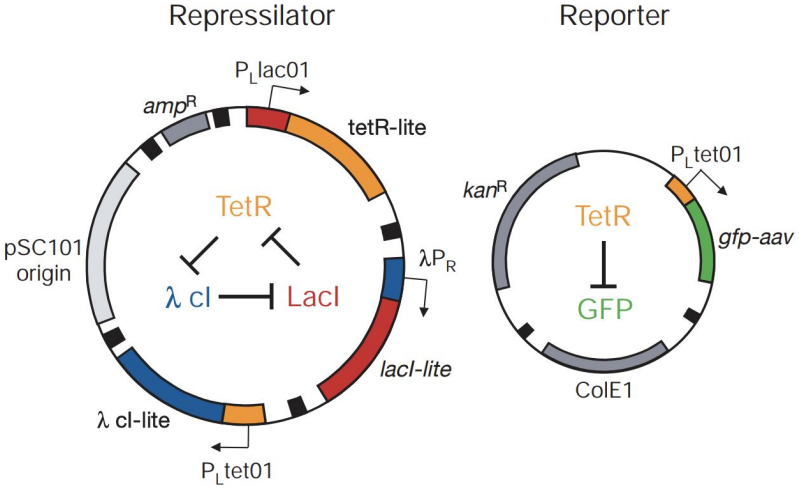
Falkowski, P., Scholes, R. J., Boyle, E. E. A., Canadell, J., Canfield, D., Elser, J., ... & Mackenzie, F. T. (2000)

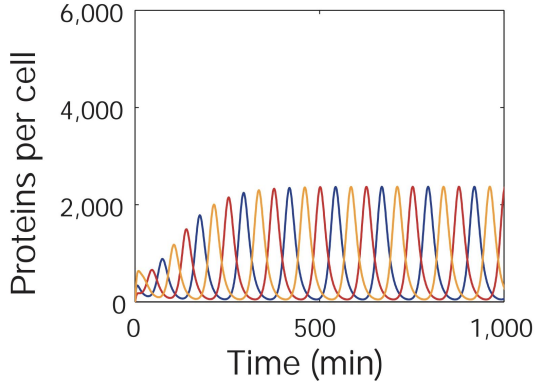
Natural systems are difficult to engineer

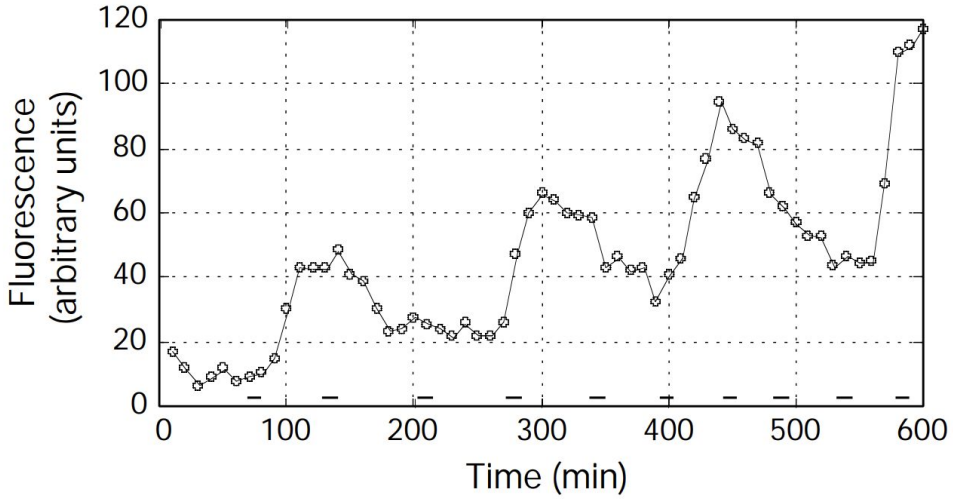


KEGG Kanehisa laboratories (2017)

Biological systems are designed as circuits

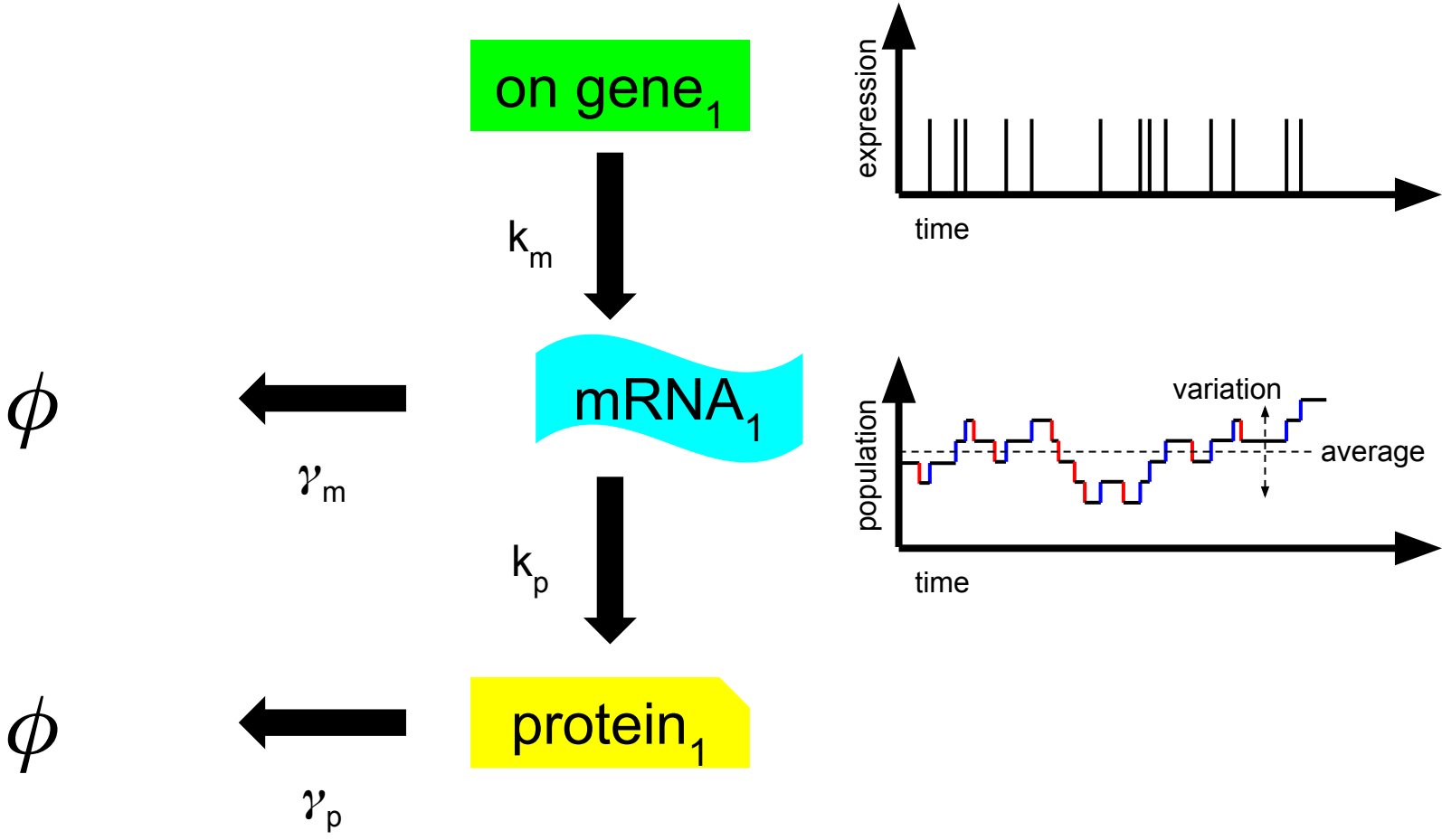




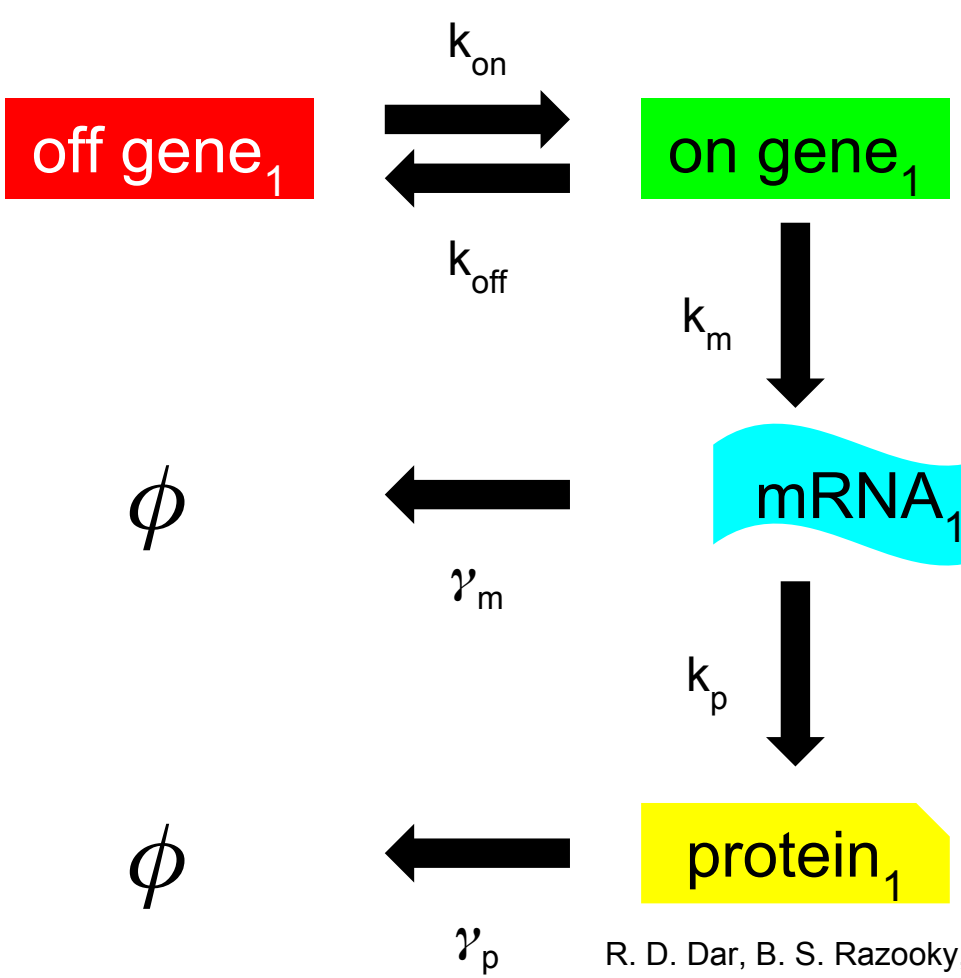


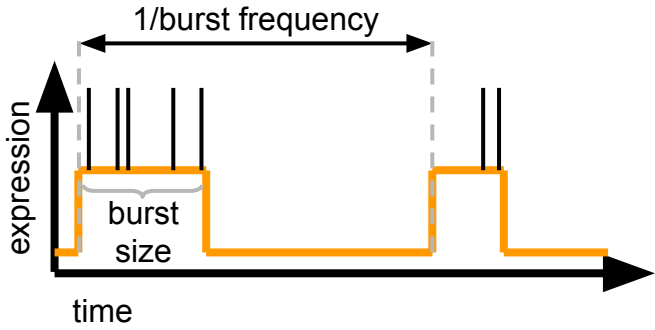
Elowitz, M. B., & Leibler, S. (2000)

Gene expression is a noisy process



Expression bursting further contributes to noise





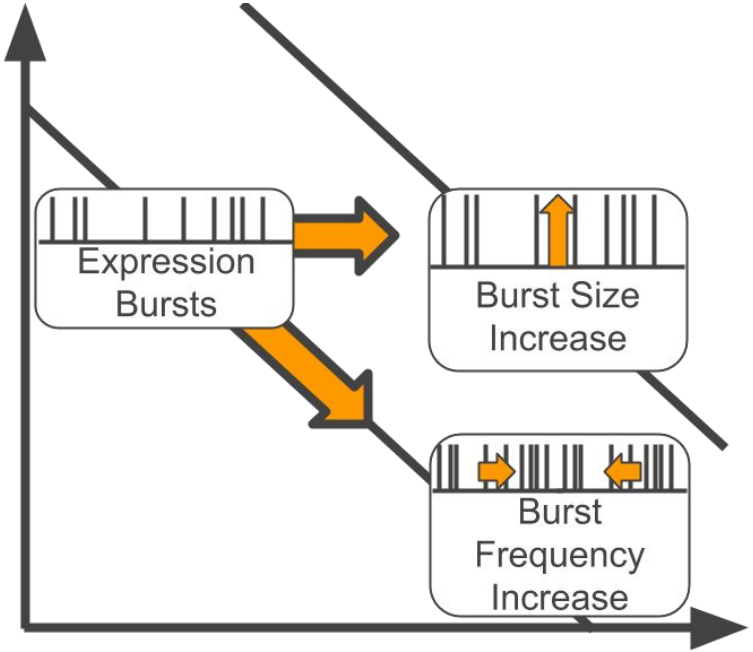
$$< p >= \frac{Bf}{\gamma_p}$$

$$CV^2 \simeq \frac{\gamma_p}{f}$$

R. D. Dar, B. S. Razooky, L. S. Weinberger, C. D. Cox, M. L. Simpson (2015)

Burst size and frequency changes are measurable from protein expression data

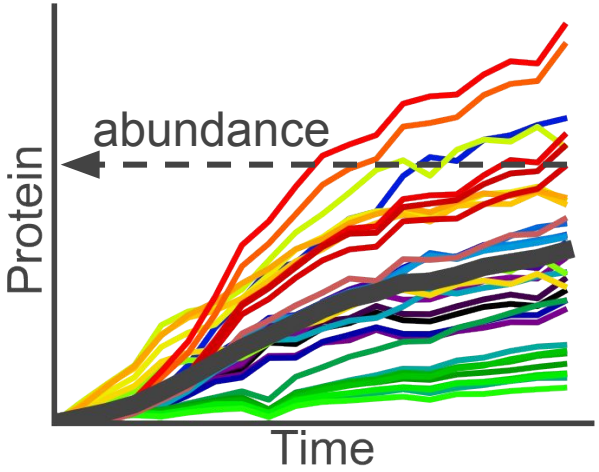
$$CV^2 \simeq \frac{\gamma_p}{f}$$

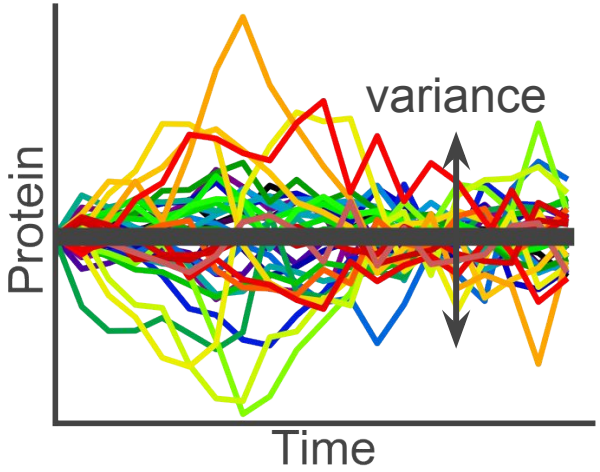


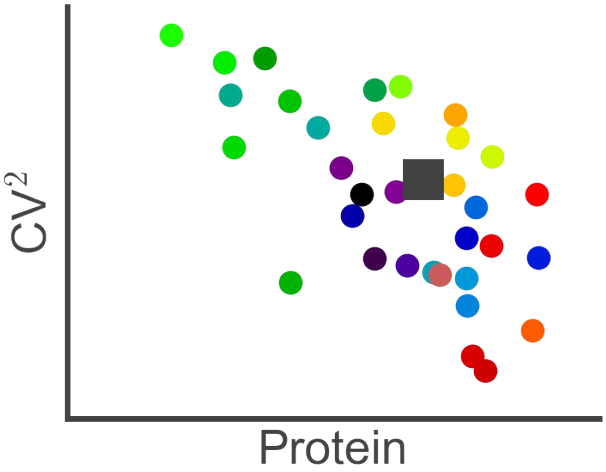
$$< p >= \frac{Bf}{\gamma_p}$$

R. D. Dar, B. S. Razooky, L. S. Weinberger, C. D. Cox, M. L. Simpson (2015)

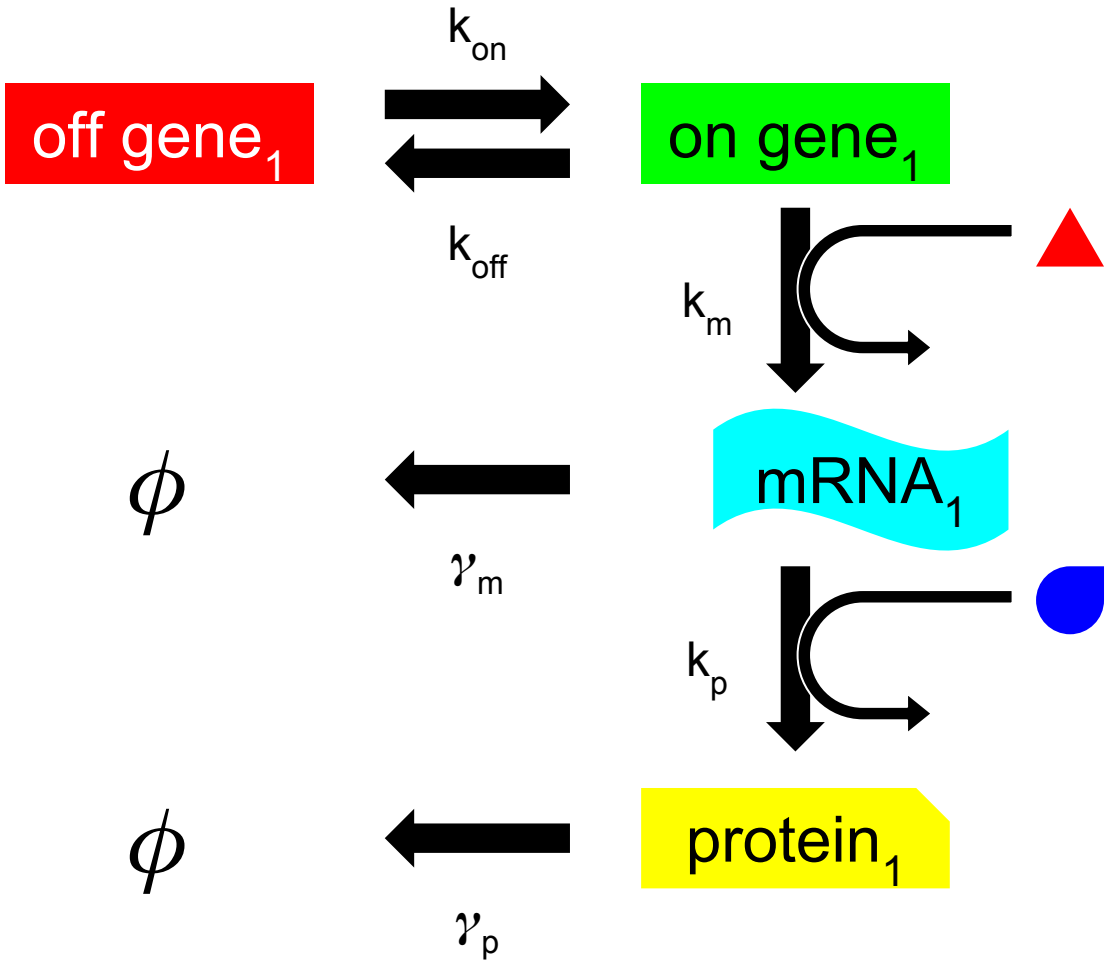
Protein abundance and variance of noise are key measures



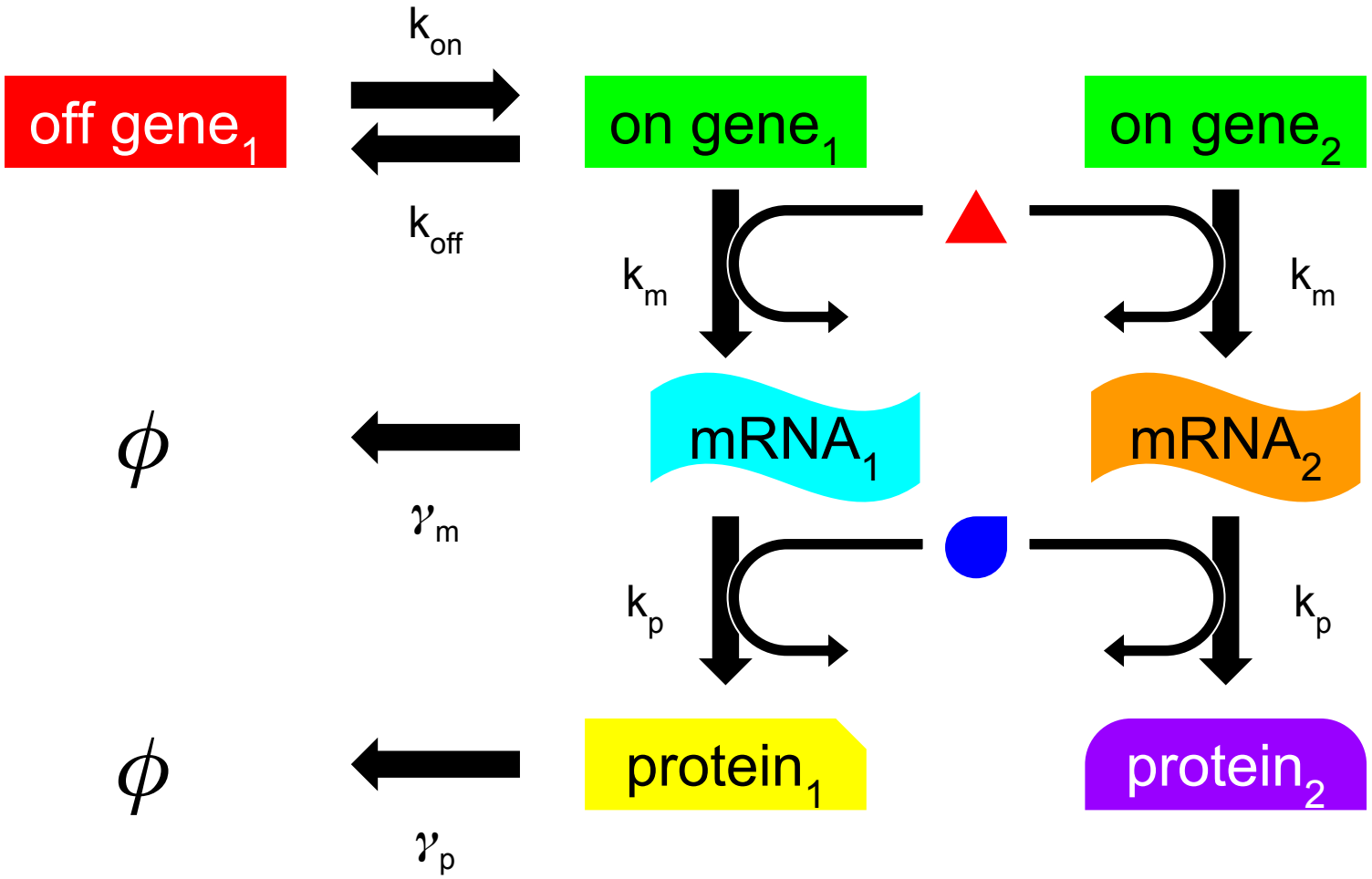




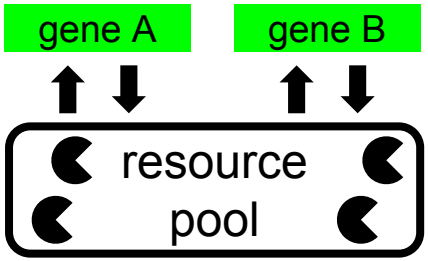
Protein expression requires resources

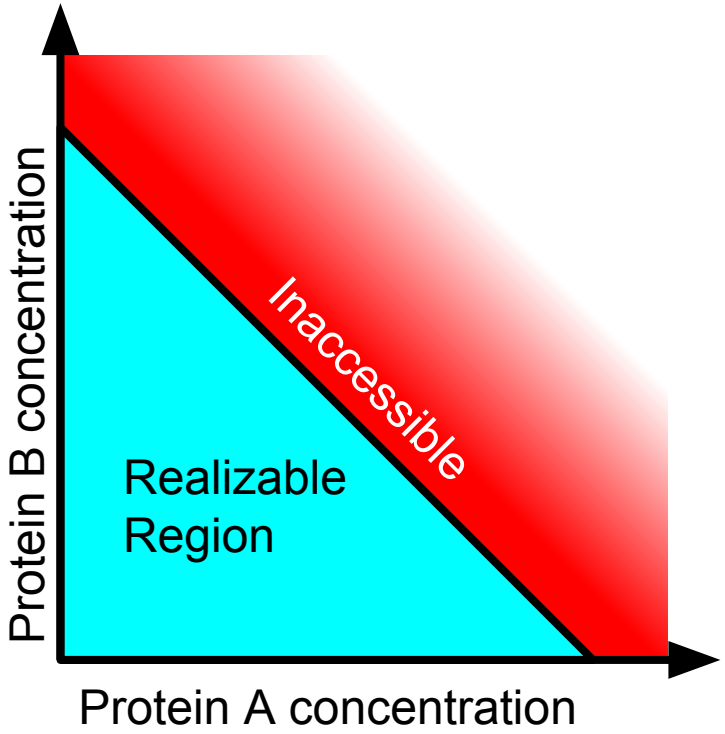


Expression resources are shared between genes



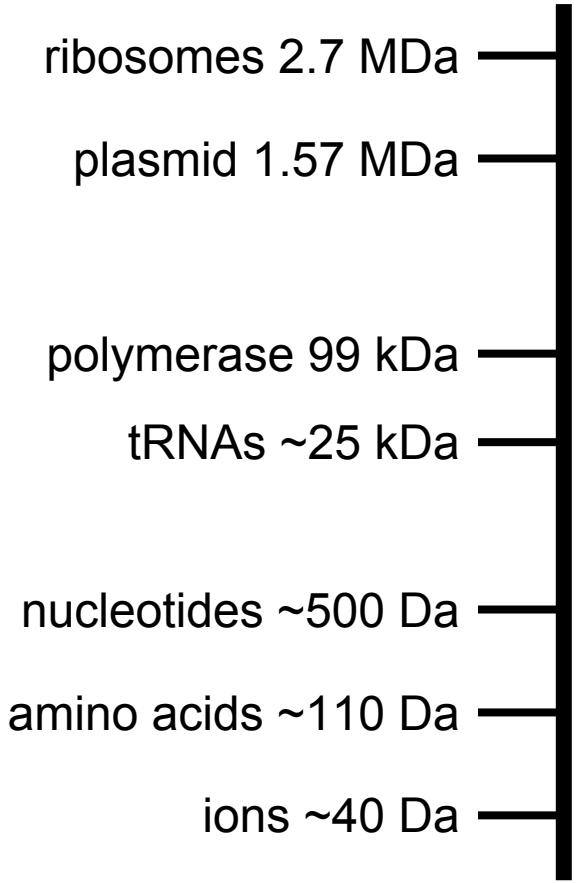
Resource sharing influences protein expression



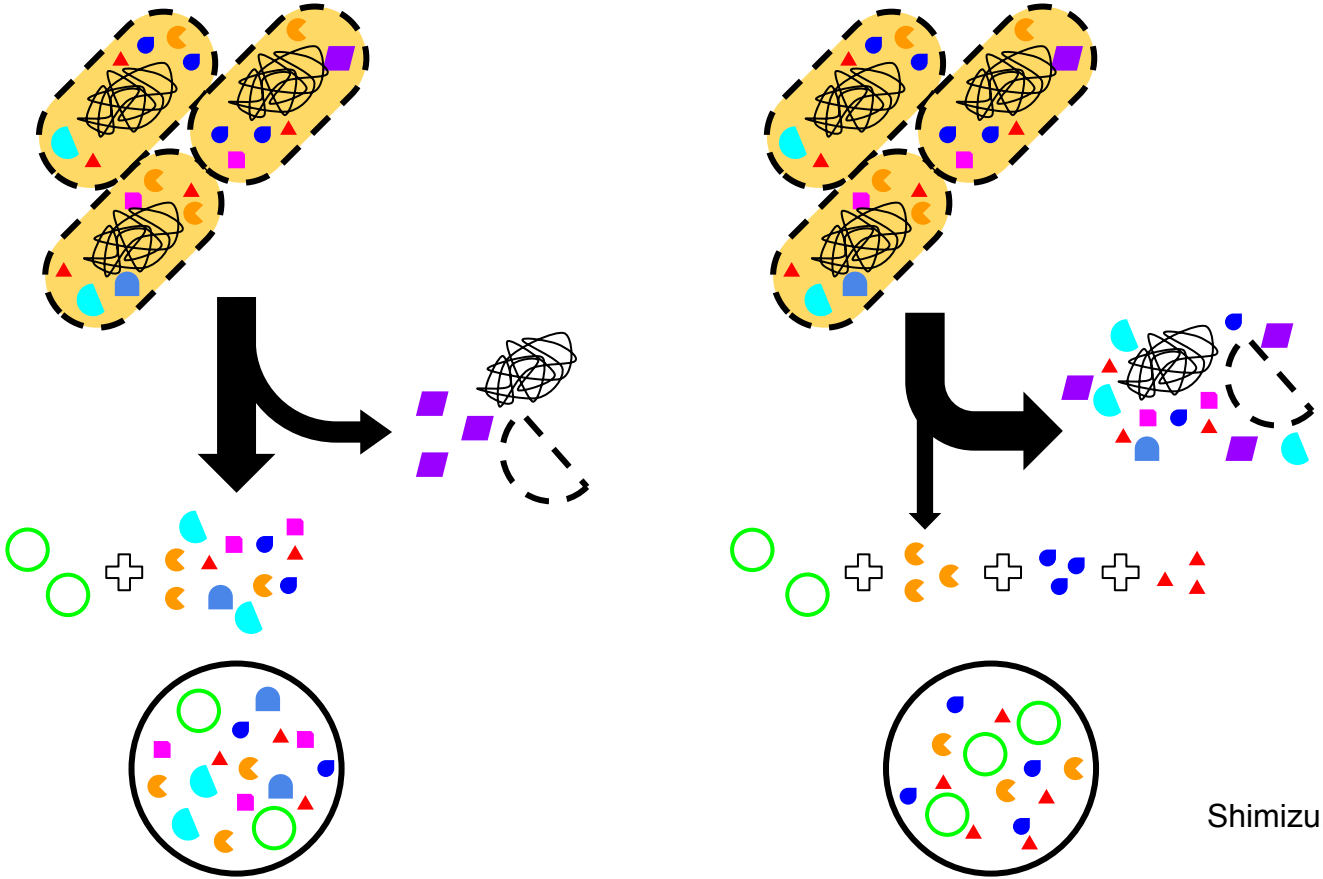


Gyorgy, Andras, José I. Jiménez, John Yazbek, Hsin-Ho Huang, Hattie Chung, Ron Weiss, and Domitilla Del Vecchio (2015)
Gyorgy, Andras, and Domitilla Del Vecchio (2014)

expression resources

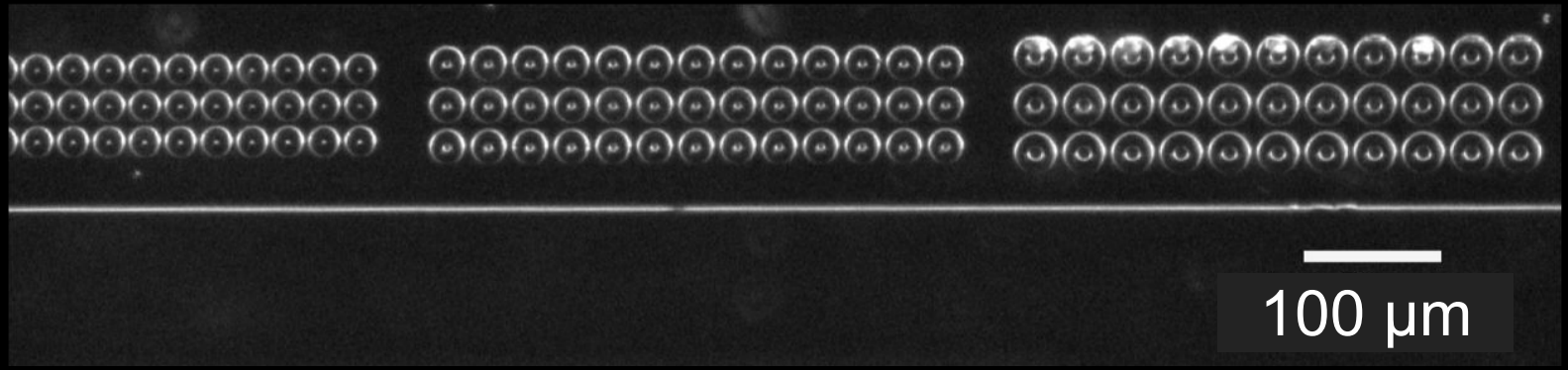


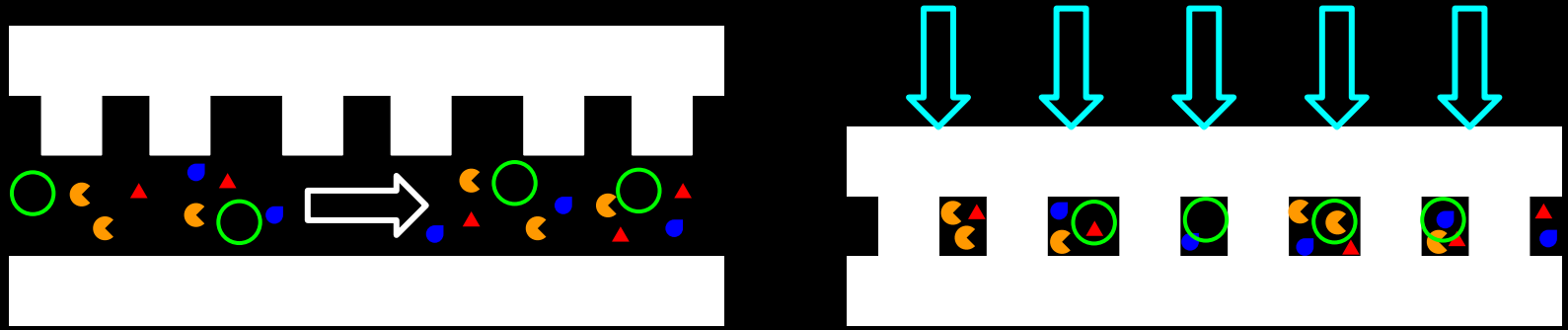
Cell-free protein expression systems allow fine control of the expression environment



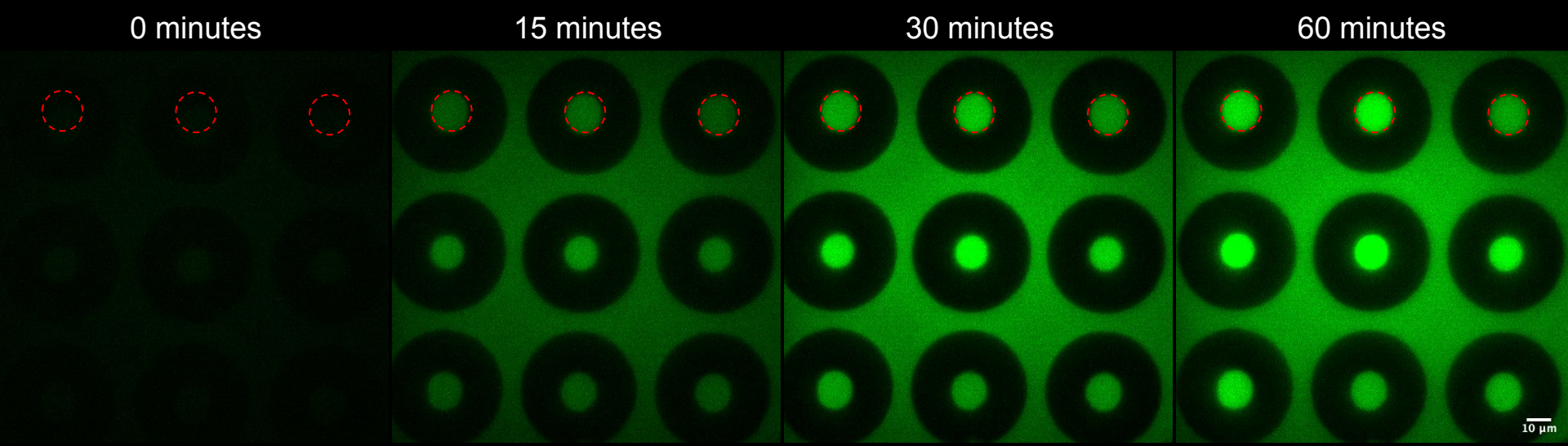
Shimizu, Y., Kanamori, T., & Ueda, T. (2005)

Microfluidic confinement of protein expression to cell-relevant volumes



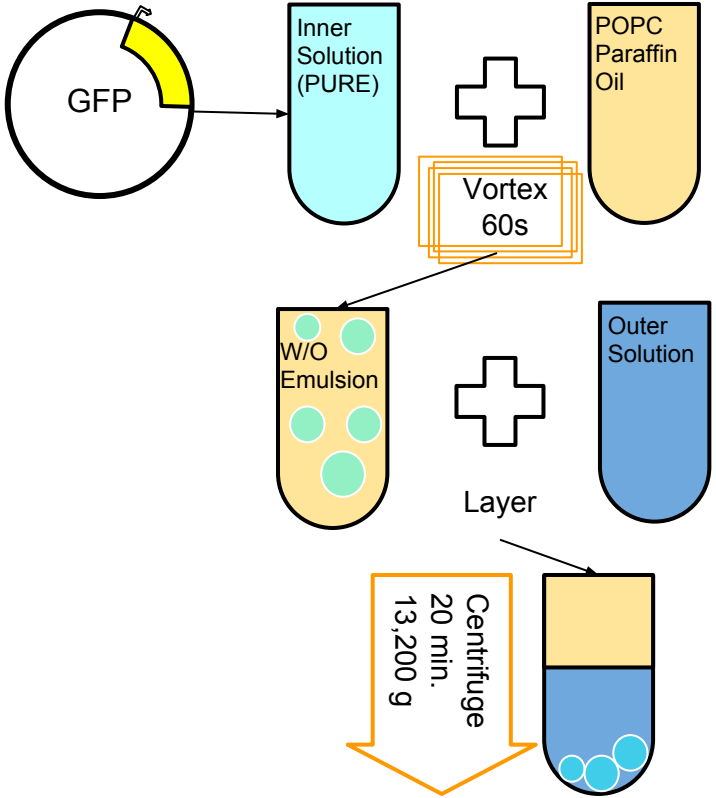


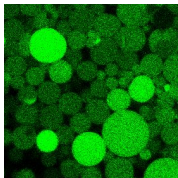
Norred, S. E., Caveney, P. M., Retterer, S. T., Boreyko, J. B., Fowlkes, J. D., Collier, C. P., & Simpson, M. L. (2015)



Norred, S. E., Caveney, P. M., Retterer, S. T., Boreyko, J. B., Fowlkes, J. D., Collier, C. P., & Simpson, M. L. (2015)

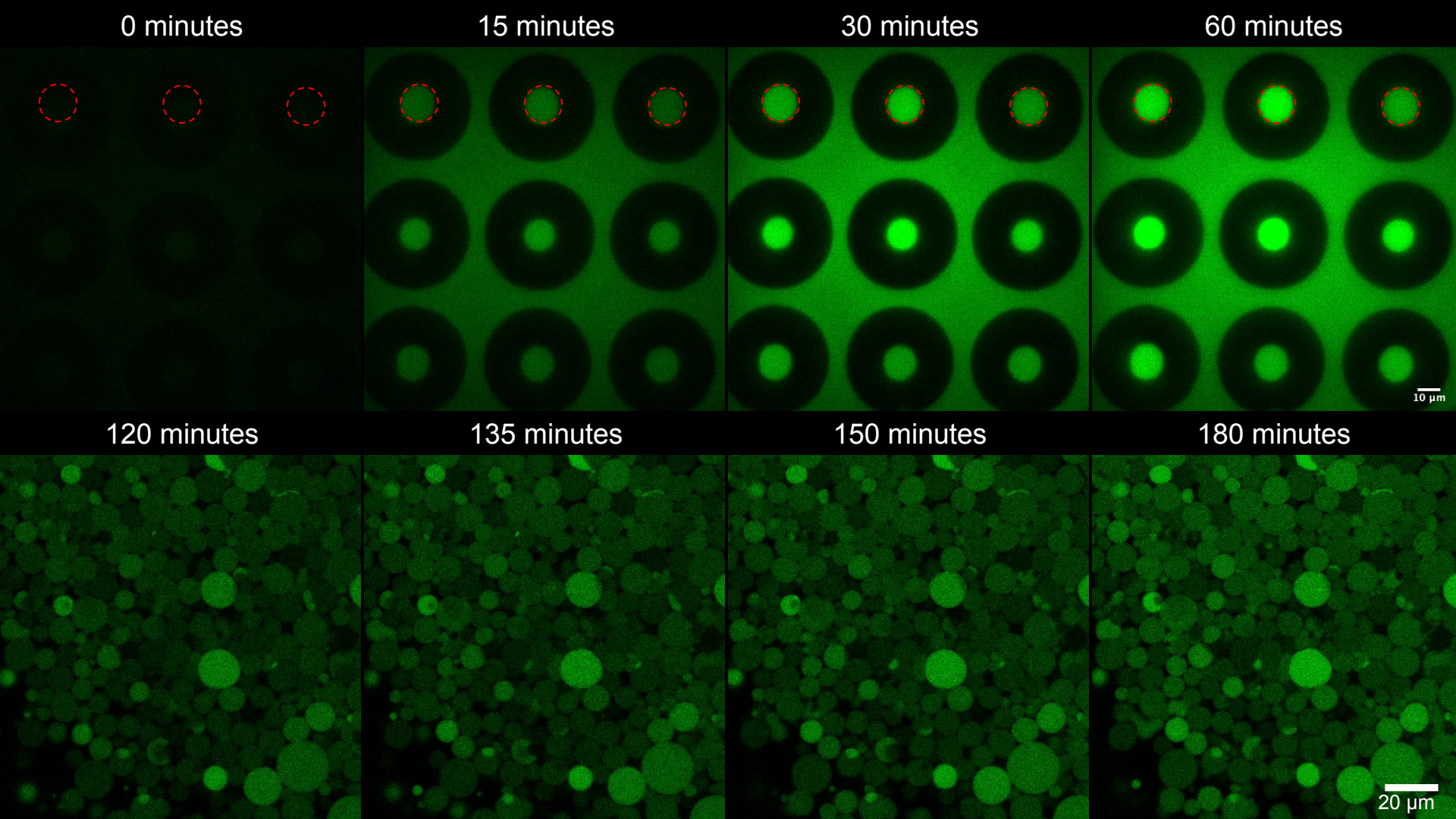
Vesicle confinement of protein expression to cell-relevant volumes





Dr. Brandon Razooky

Nishimura, K., Matsuura, T., Sunami, T., Fujii, S., Nishimura, K., Suzuki, H., & Yomo, T. (2014)


0 minutes
15 minutes
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60 minutes
10 µm
120 minutes
135 minutes
150 minutes
180 minutes
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Purpose

The purpose of this dissertation is to investigate how gene expression bursting and variation is affected by expression resources being shared among genes.

1. How are gene expression characteristics altered by the size of resource pools?
2. Can membrane permeability be tuned to enhance gene expression?
3. How are the dynamics of gene expression affected by resource transport across permeabilized membranes?

Outline

Chapter 1: Introduction

Chapter 2: Resource Sharing Controls Gene Expression Bursting

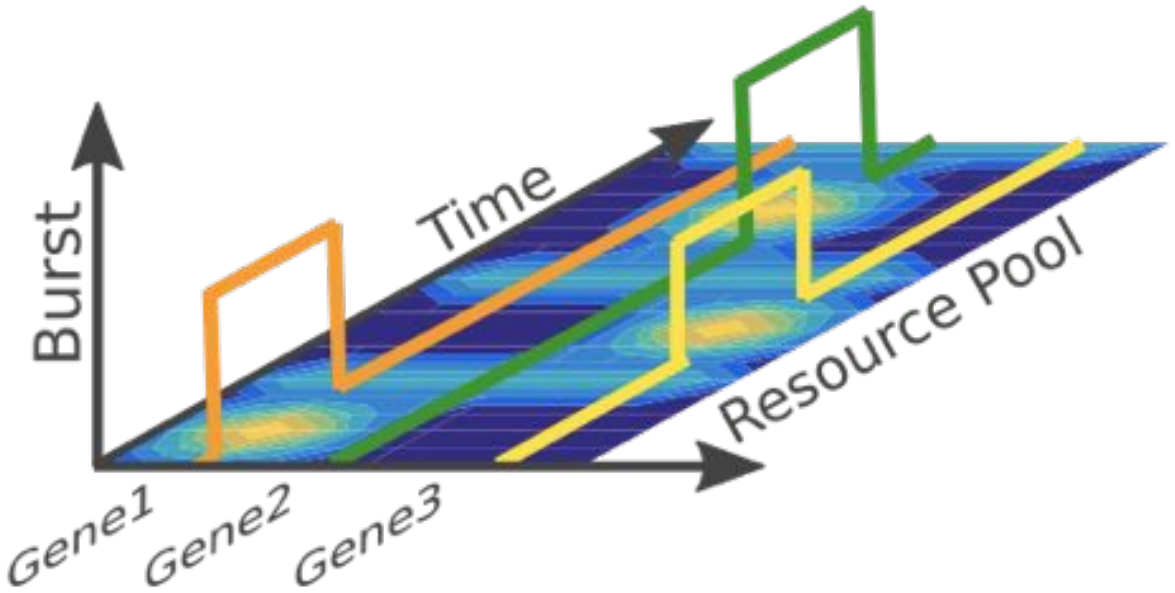
Chapter 3: Controlling Cell-Free Gene Expression Behavior by Tuning Membrane Transport Properties

Chapter 4: Conclusion

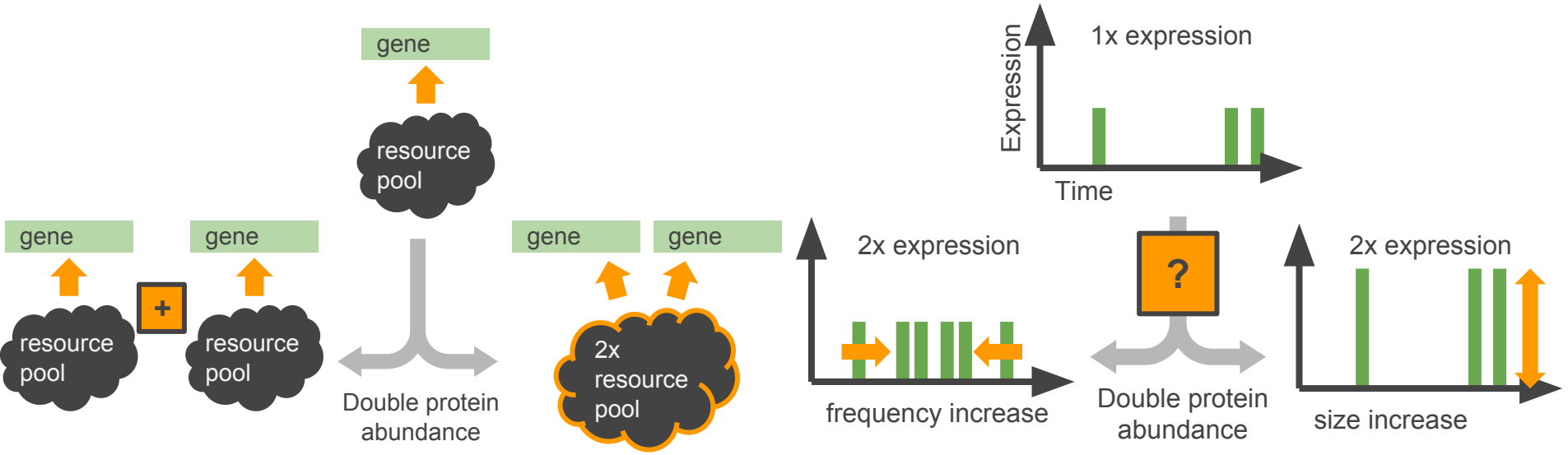
Chapter 2: Resource Sharing Controls Gene Expression Bursting

Caveney, P. M., Norred, S. E., Chin, C. W., Boreyko, J. B., Razooky, B. S., Retterer, S. T., ... & Simpson, M. L. (2016)

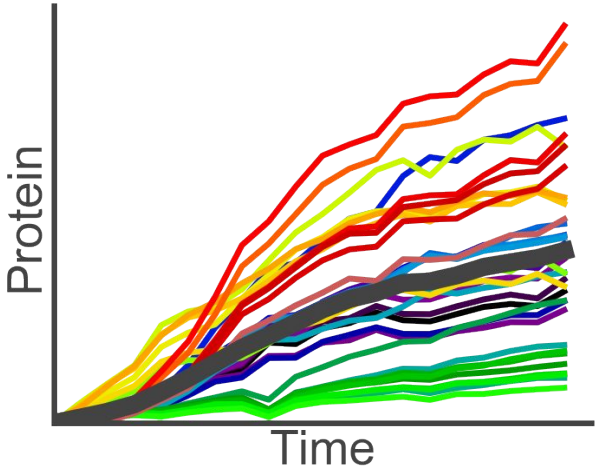
Bursty expression and resource sharing are linked

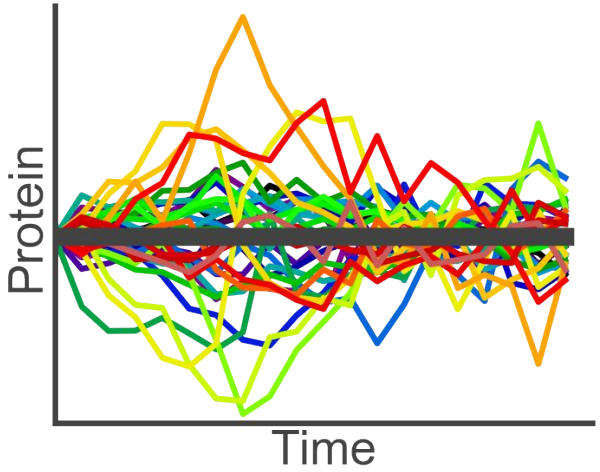


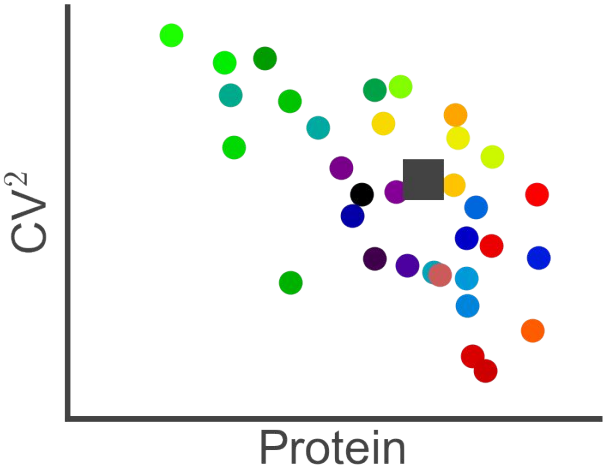
How does resource sharing affect bursty expression?



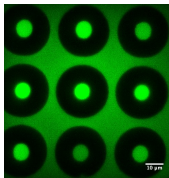
Burst size and frequency changes are measurable from protein expression data



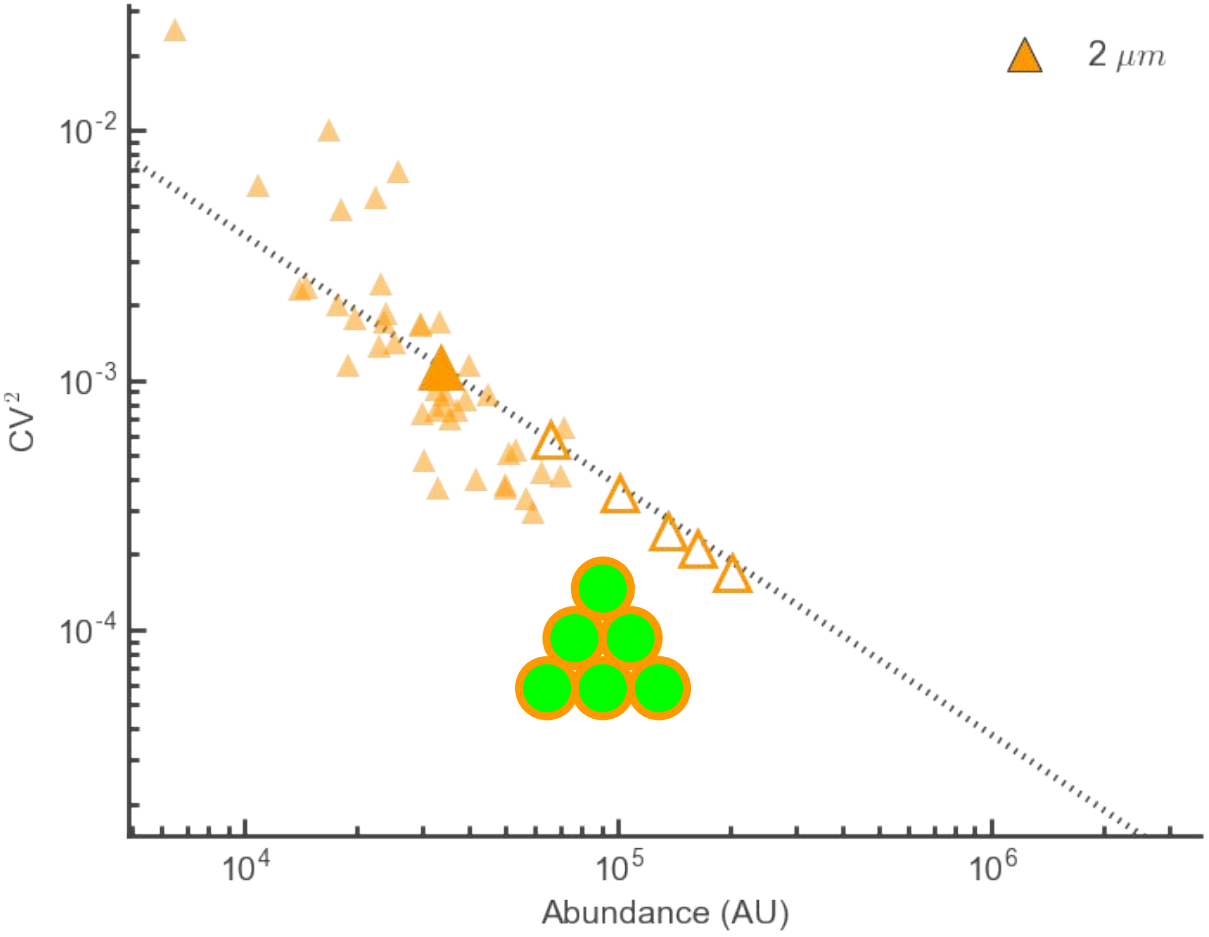


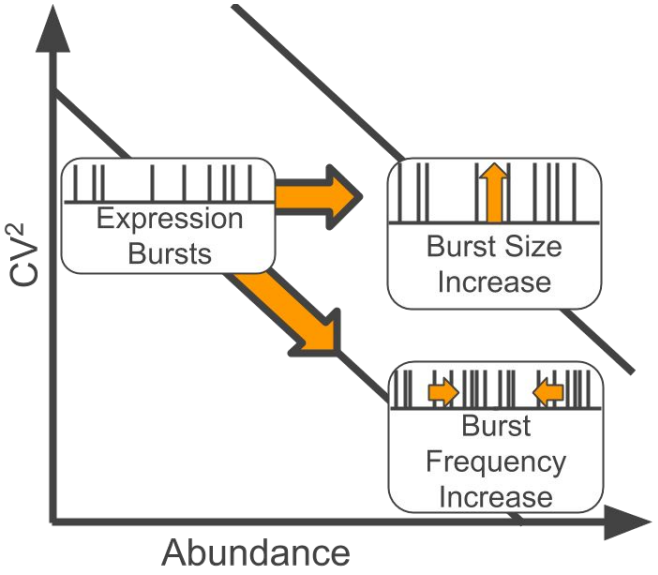


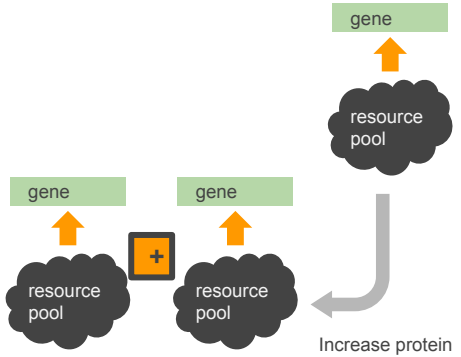
Karig, D. K., Jung, S. Y., Srijanto, B., Collier, C. P., & Simpson, M. L. (2013)
Sun, Z. Z., Hayes, C. A., Shin, J., Caschera, F., Murray, R. M., & Noireaux, V. (2013)

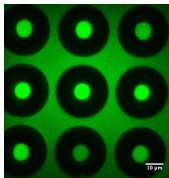


Divided resource pools increase burst frequency

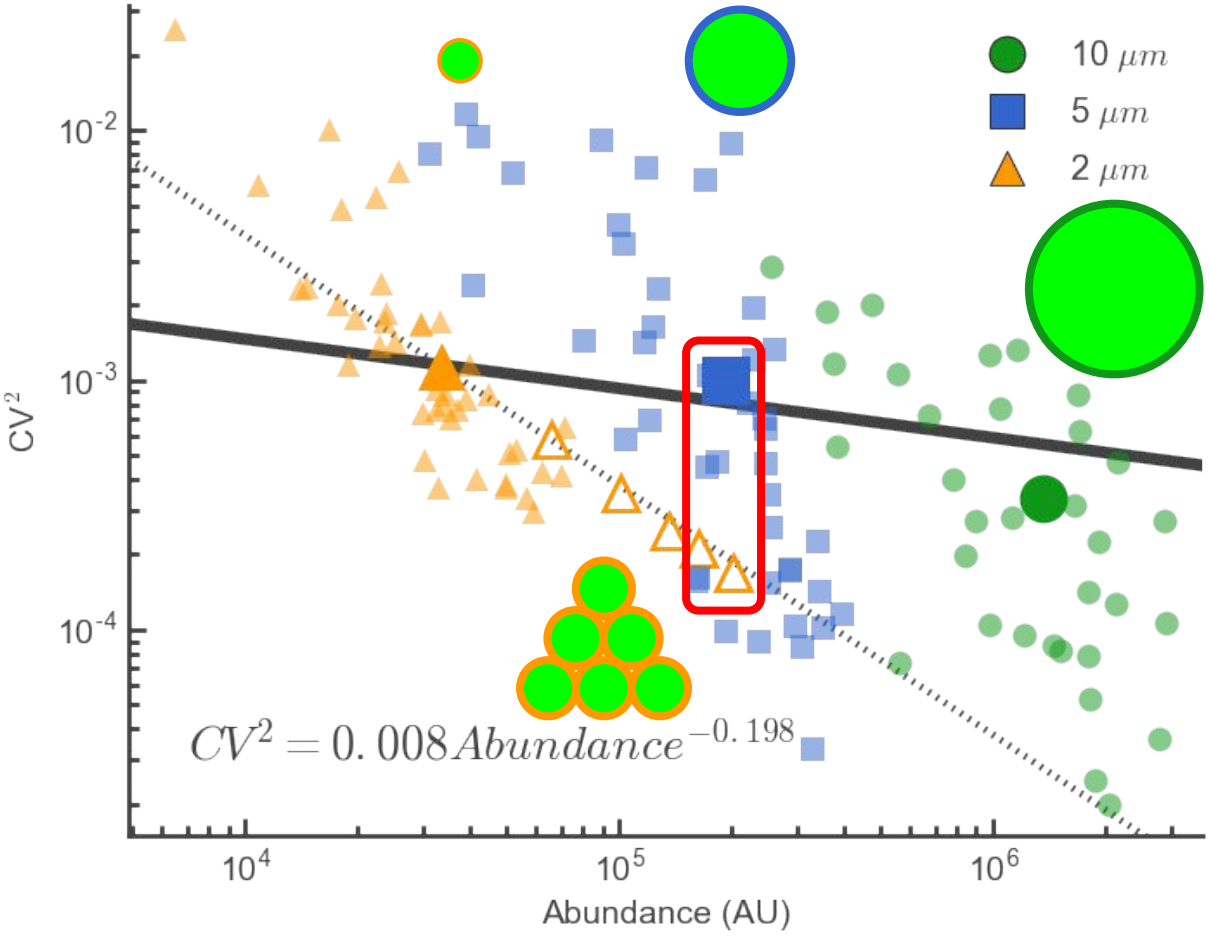


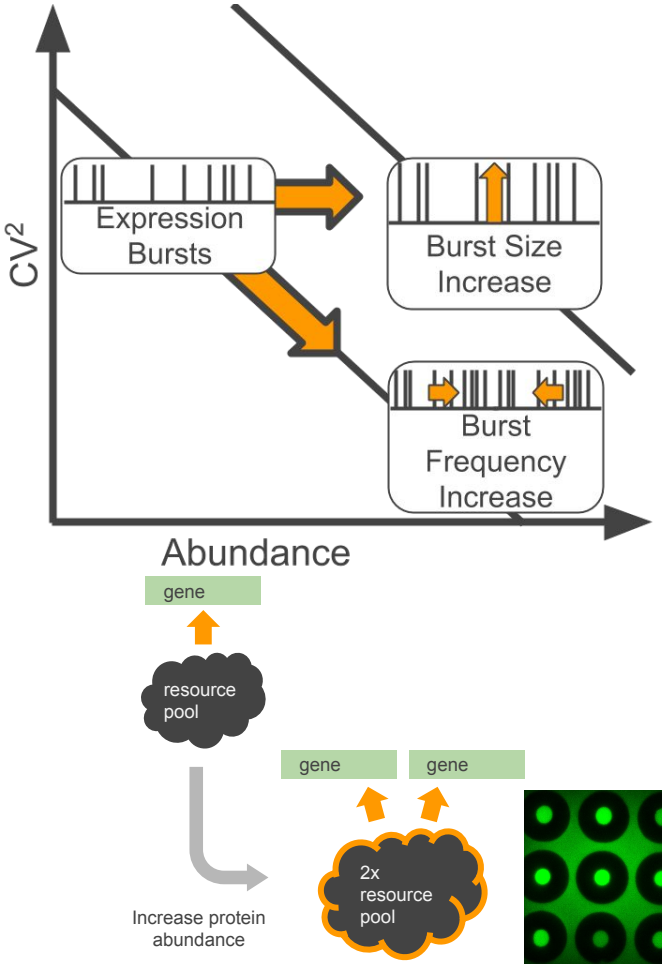




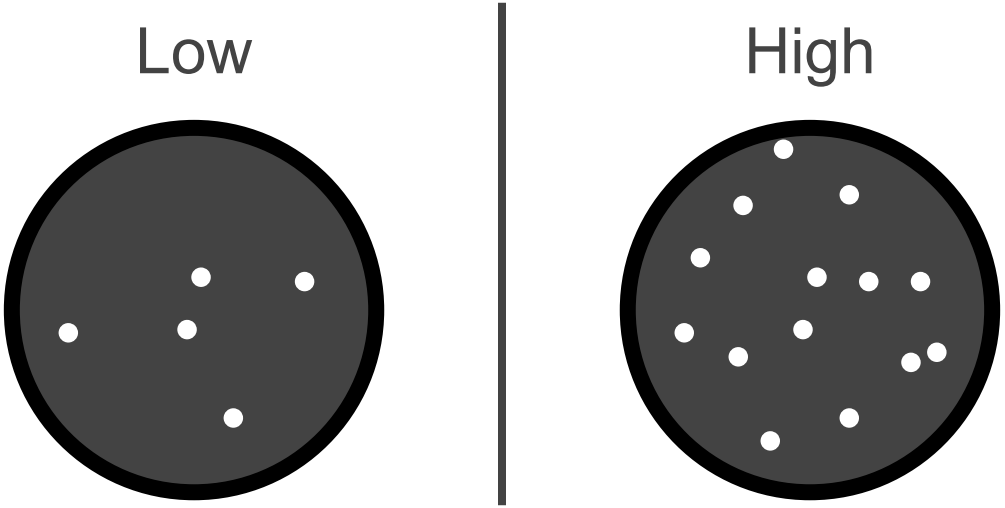


Larger chamber size result in larger burst size

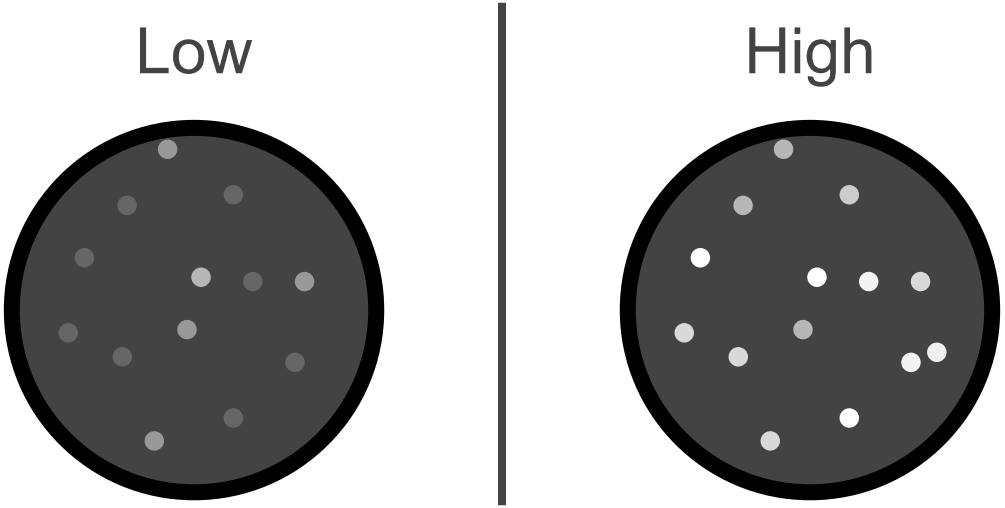




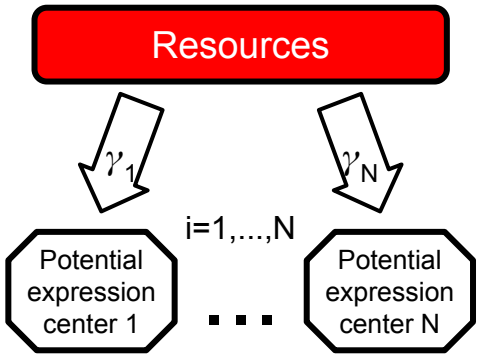
Burst frequency variation of expression centers

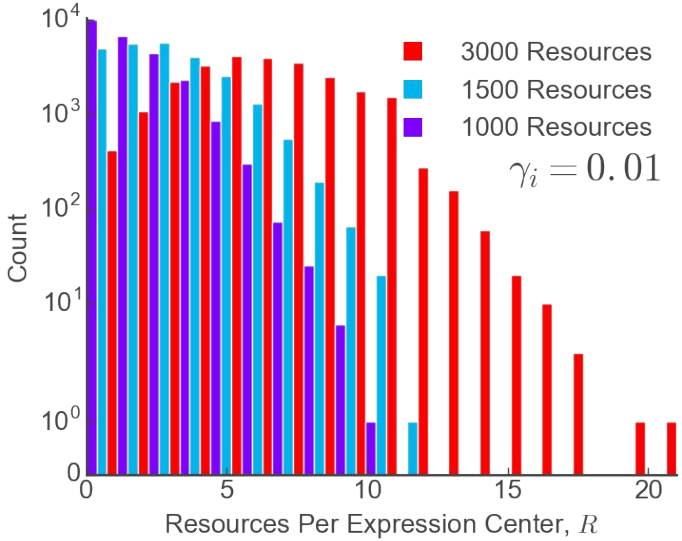


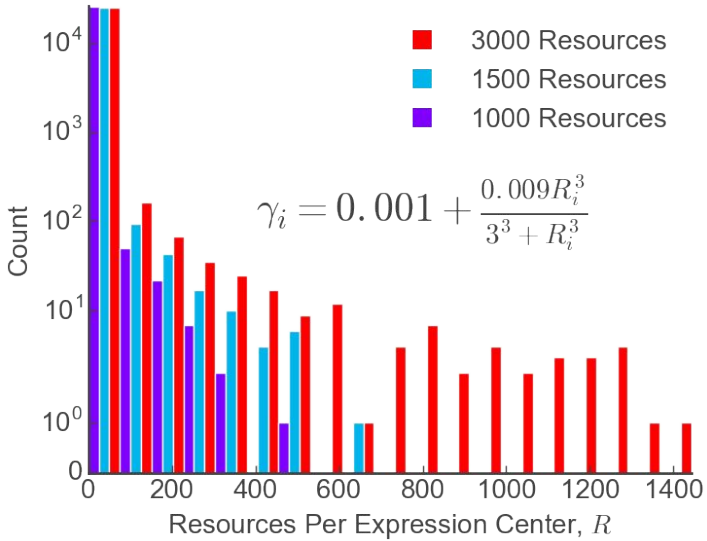
Burst size variation of expression centers



Cooperativity in resource allocation reinforces large bursts instead of creating new bursts



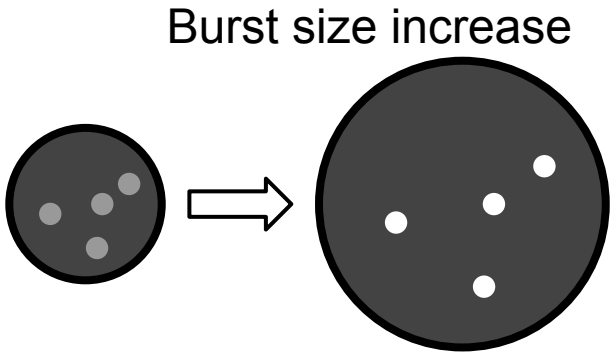


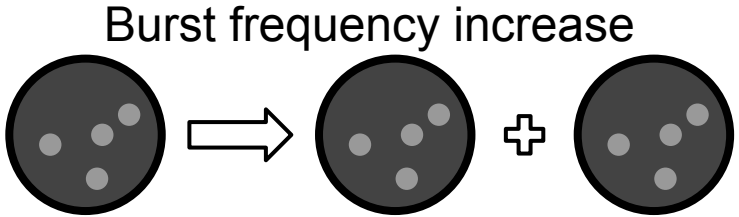


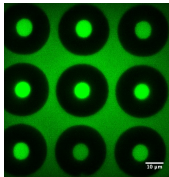
Gaurav Chauhan and Dr. Steven Abel

Summary Chapter 2

- Larger shared resource pools result in larger burst size but relatively constant burst frequency
- Larger divided resource pools resulted in larger burst frequency but constant burst size
- Resources are added to existing bursts instead of nucleating new bursts







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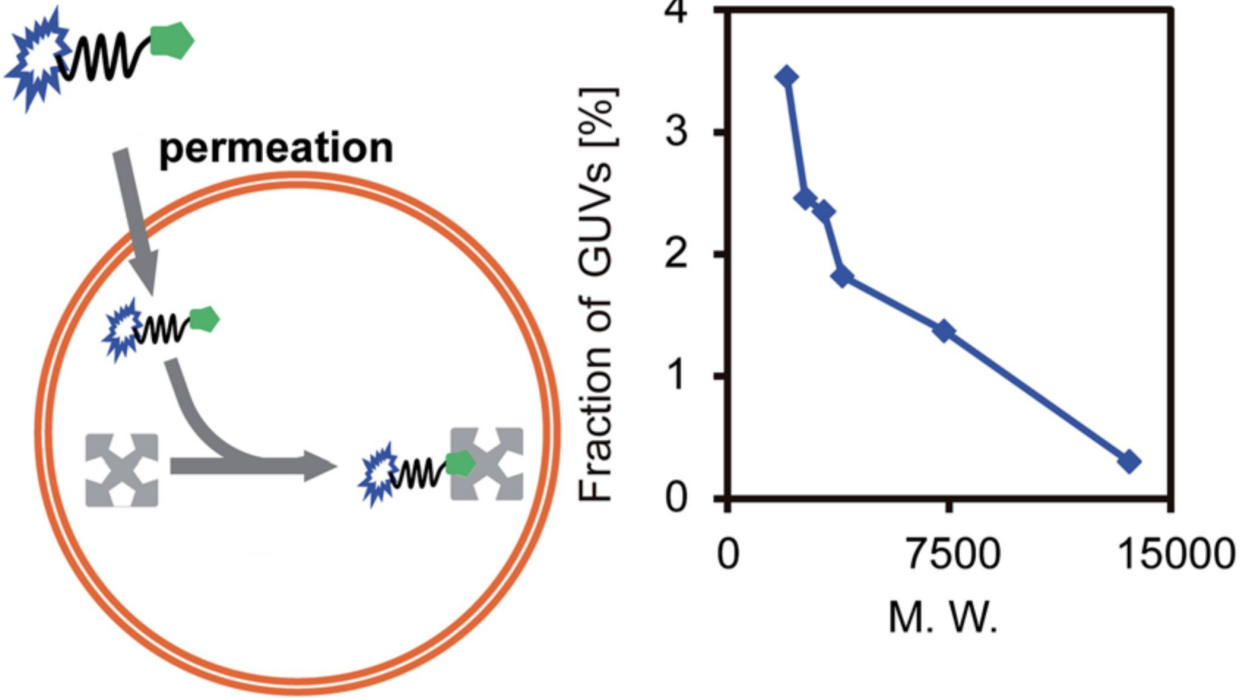
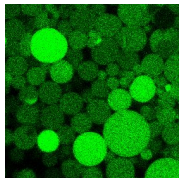
Chapter 3: Controlling Cell-Free Gene Expression Behavior by Tuning Membrane Transport Properties

A small fraction of vesicles are permeable to small, charged expression resources





Nishimura, K., Matsuura, T., Sunami, T., Fujii, S., Nishimura, K., Suzuki, H., & Yomo, T. (2014)

expression resources

ribosomes 2.7 MDa

plasmid 1.57 MDa

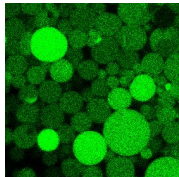
polymerase 99 kDa

tRNAs ~25 kDa

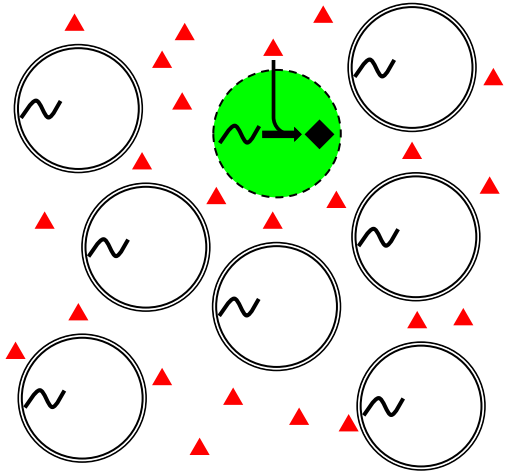
nucleotides ~500 Da

amino acids ~110 Da

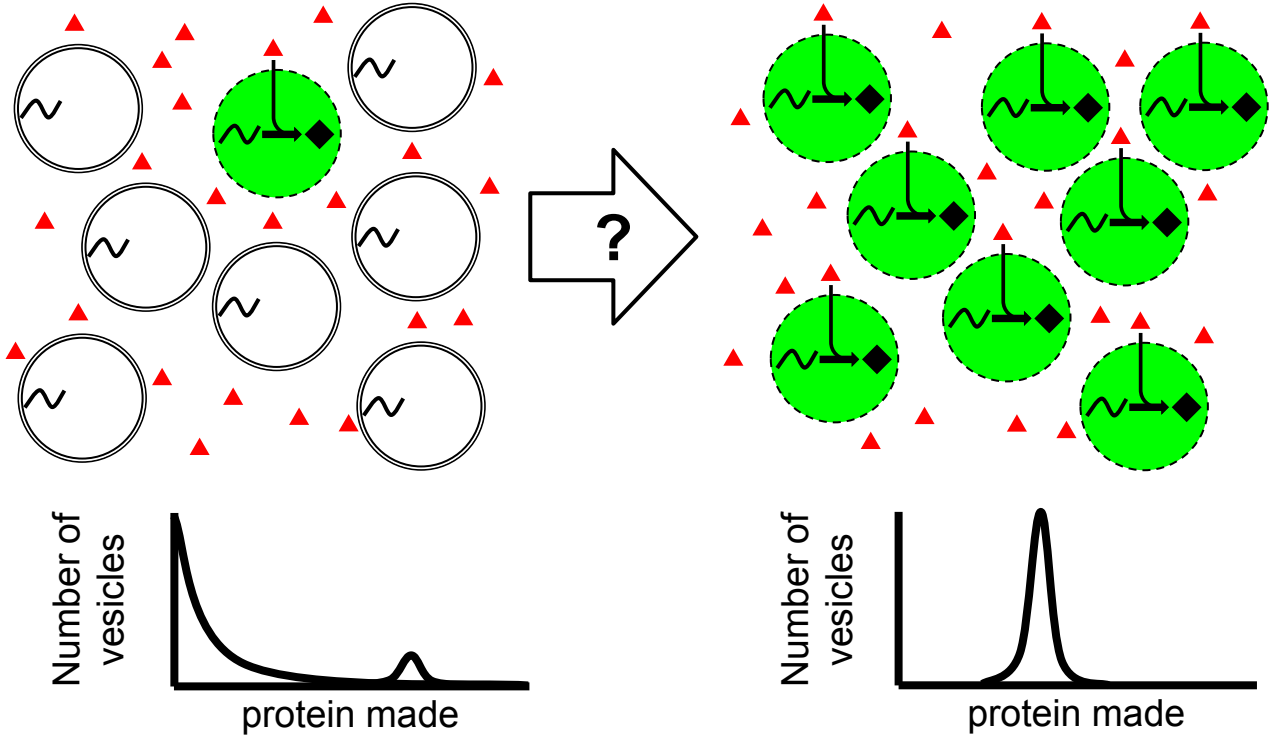
ions ~40 Da



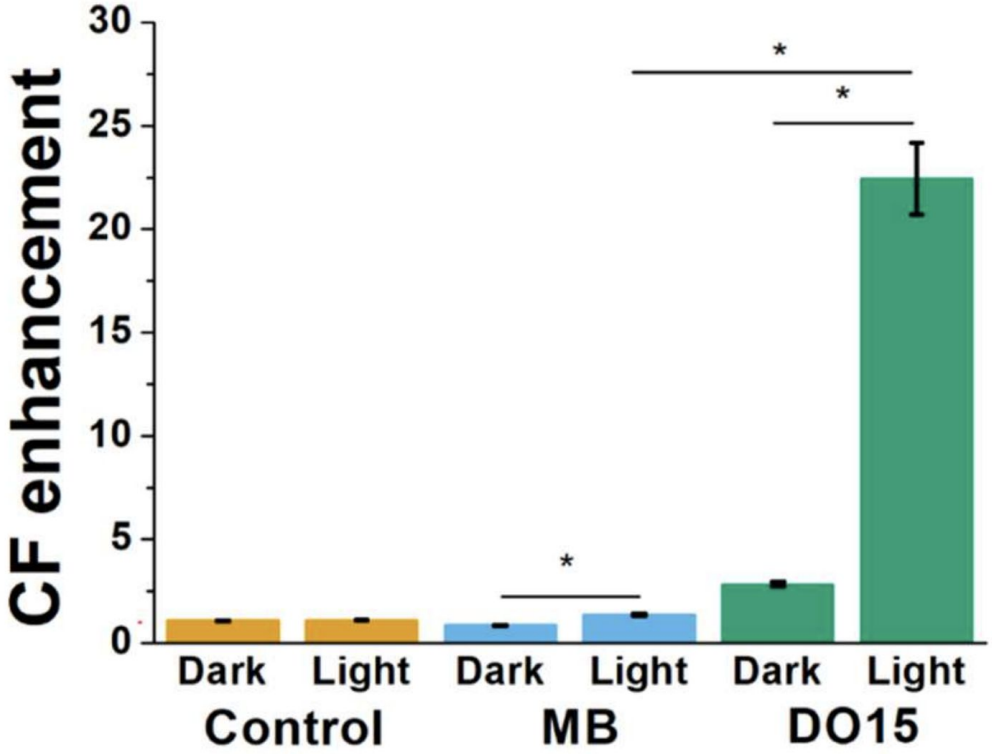
Few vesicles are permeable to expression resources

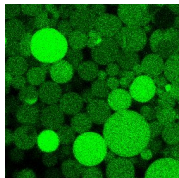


Can most vesicles be made permeable?

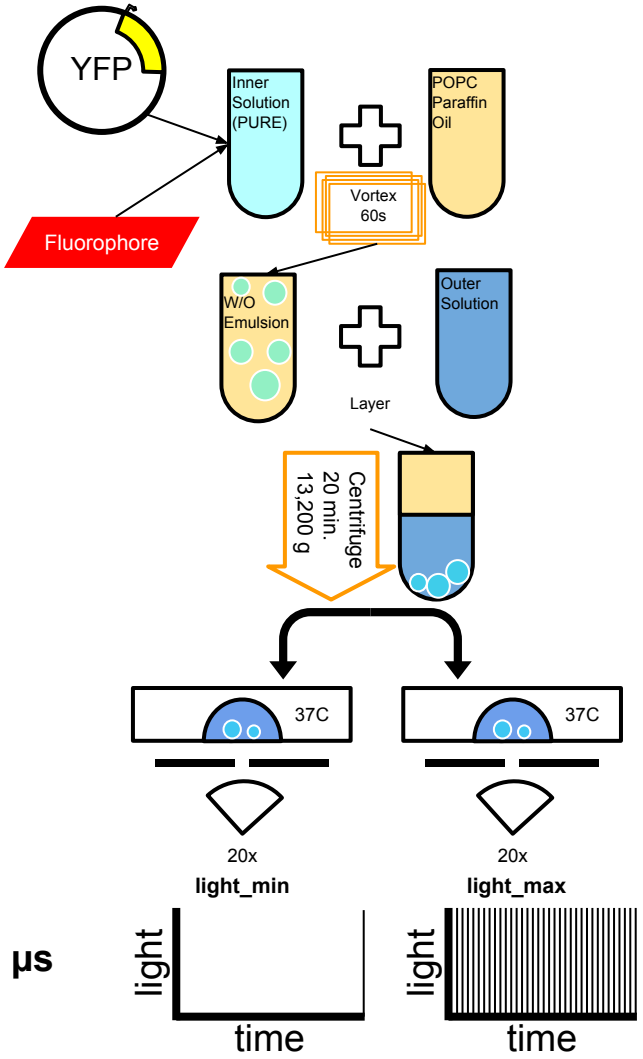


Photosensitized membrane have increased permeability

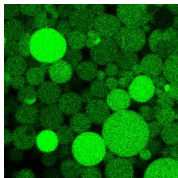




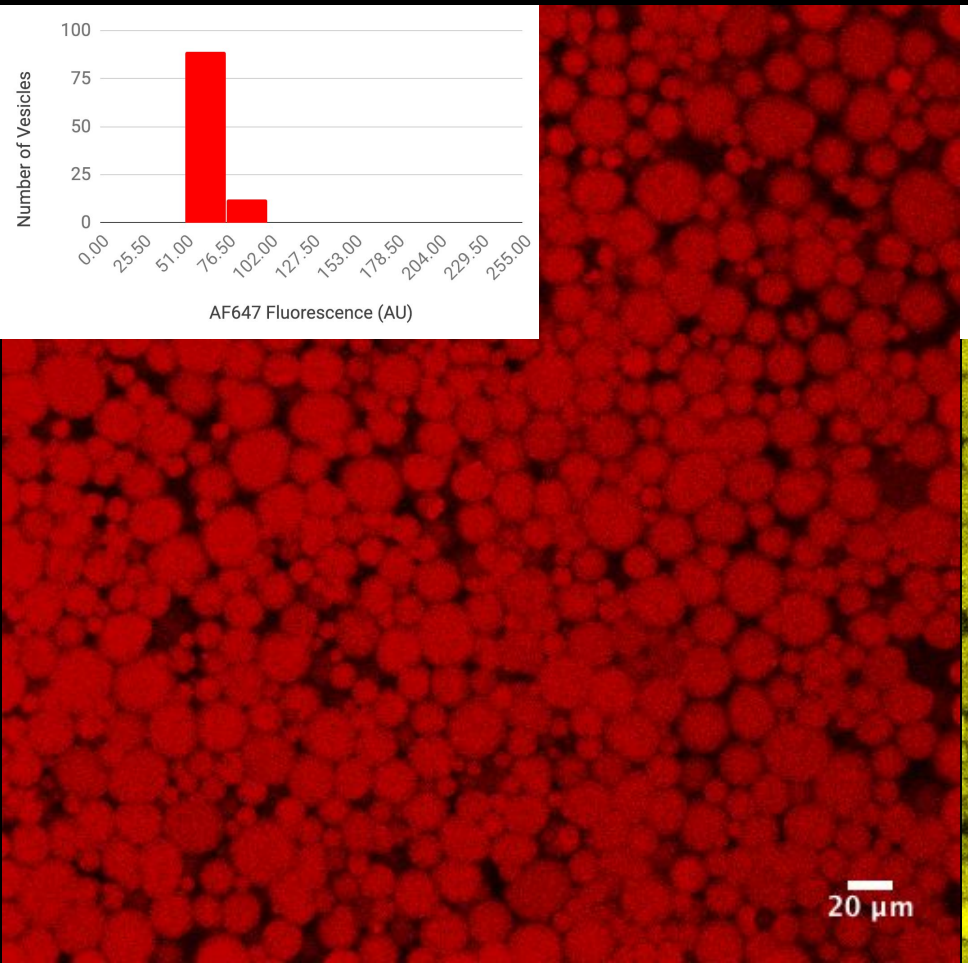
Bacellar, I. O., Oliveira, M. C., Dantas, L. S., Costa, E. B., Junqueira, H. C., Martins, W. K., ... & Miotto, R. (2018)



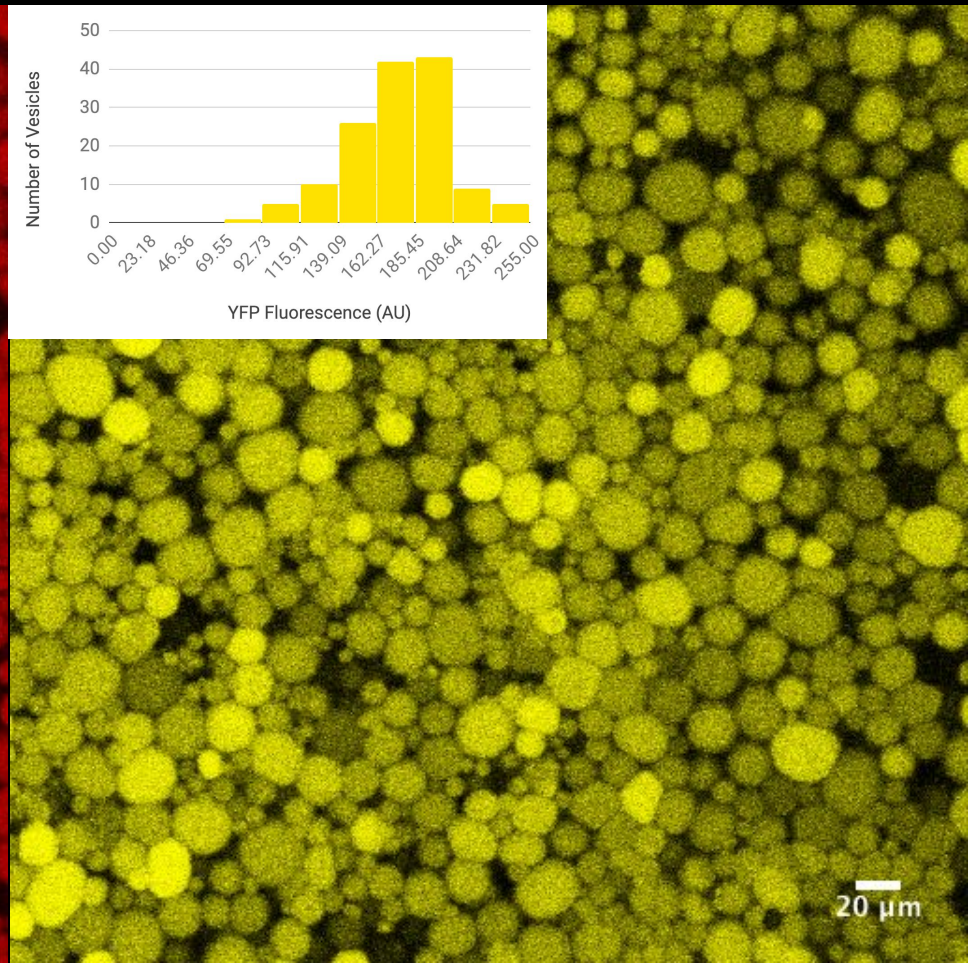
- Zeiss, LSM 710 confocal laser scanning microscope
- Z-stacks of ~23 slices were taken every 3 minutes for 2 hours
- slice was 512x512 pixels
- 405 nm, 6.5 mW; 488 nm, 6.1 mW; and 633 nm, 1.67 mW lasers
- Pixel = **0.81 µm x 0.81 µm**
- Laser dwell time per pixel every three minutes **37.9 µs**

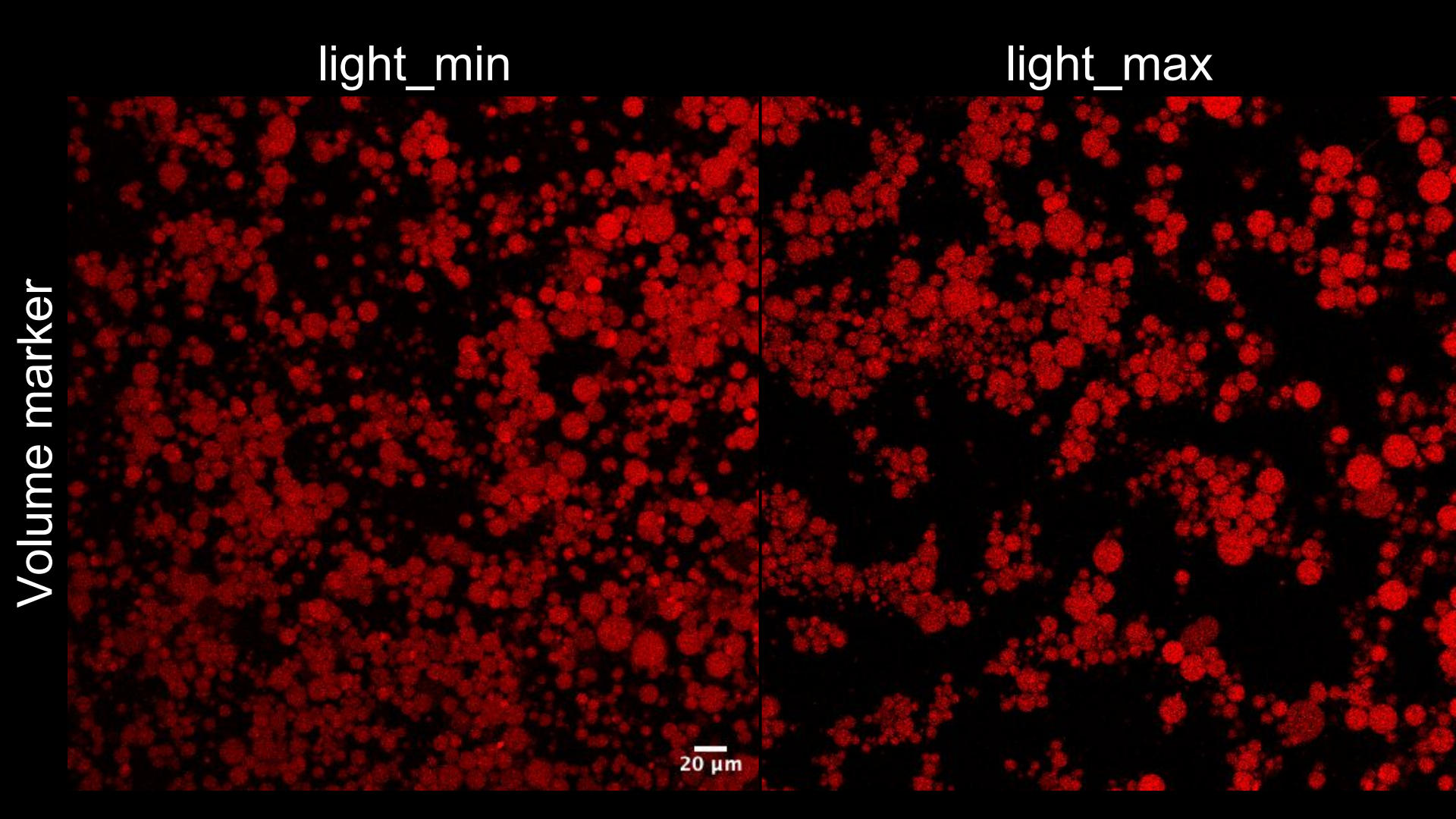


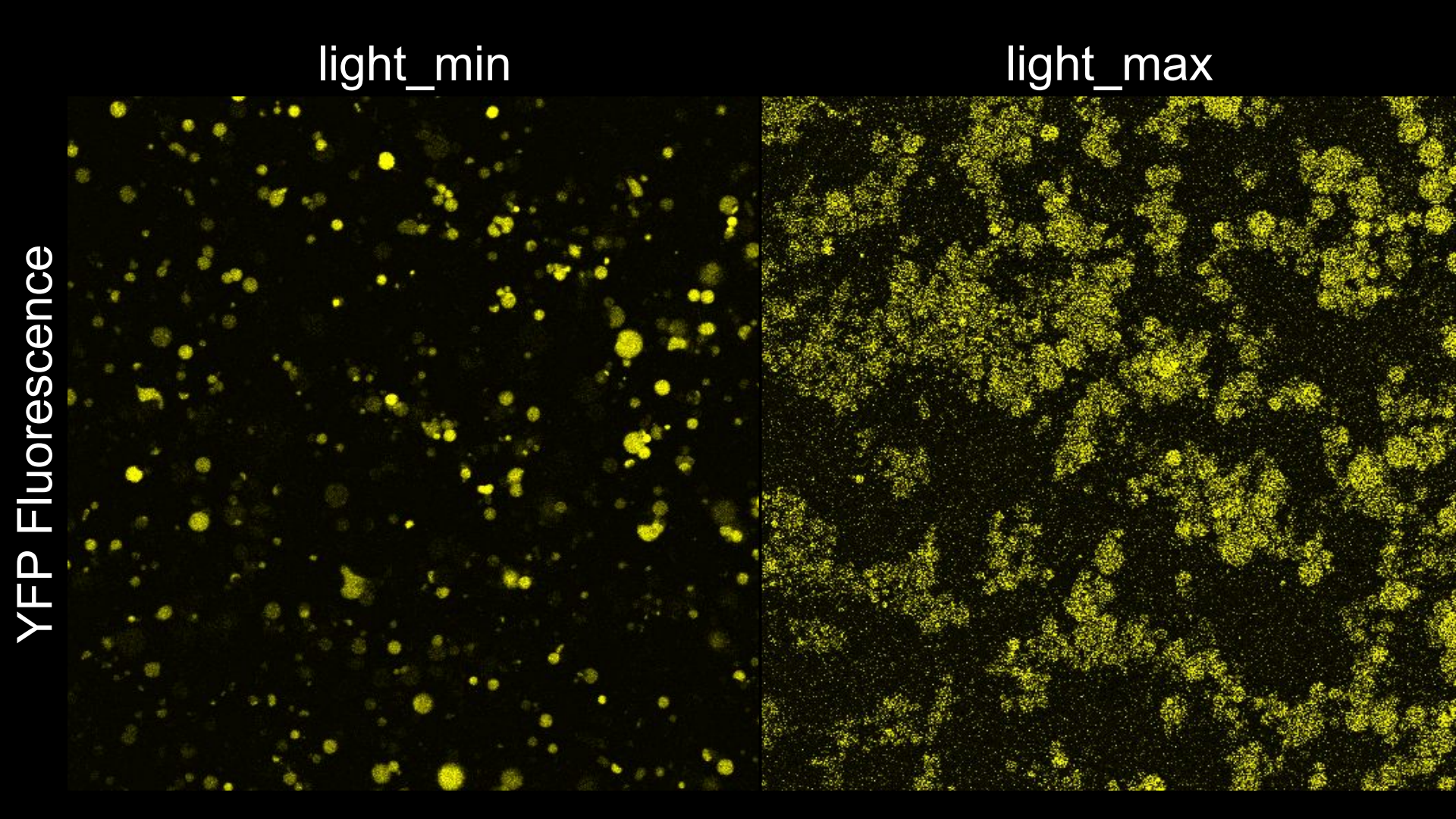
Volume marker



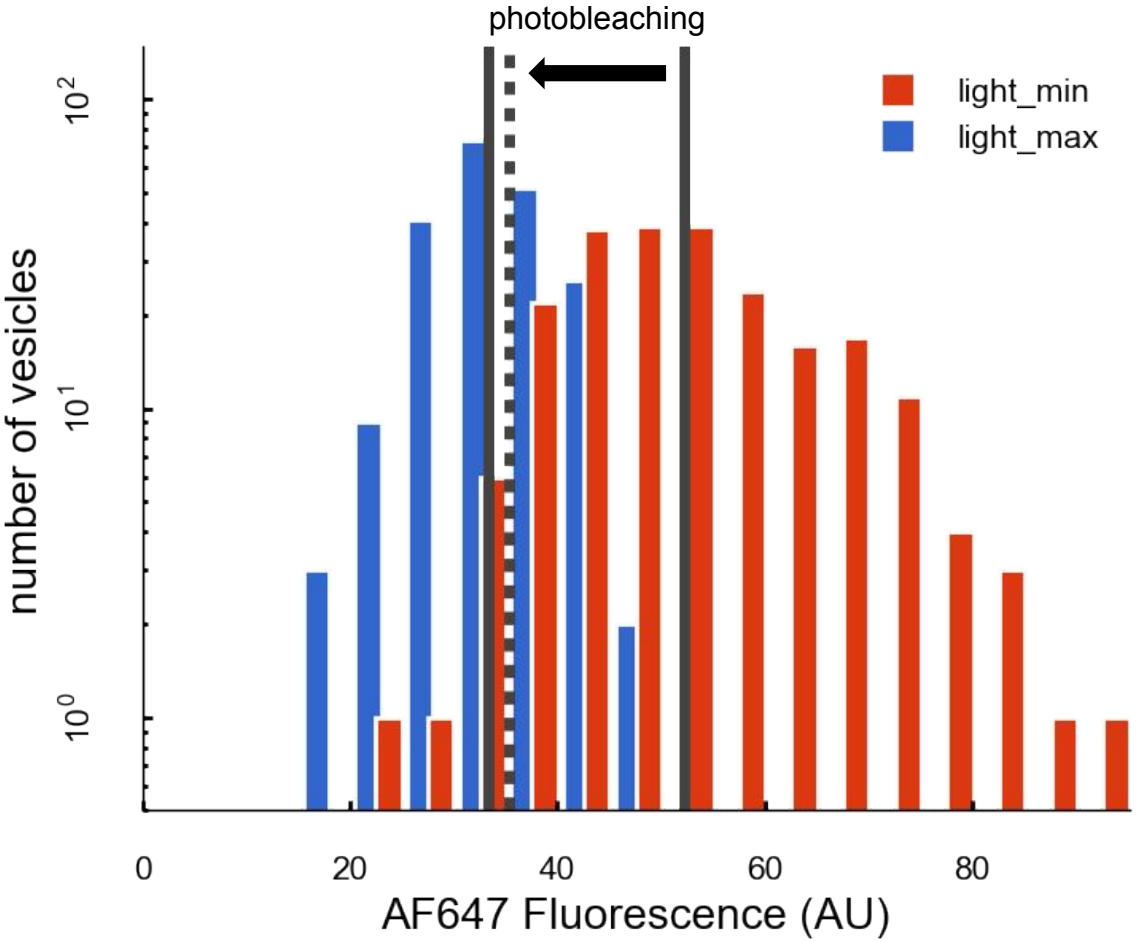
YFP Fluorescence

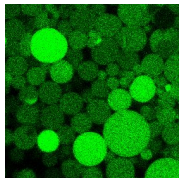



light_min
light_max
Volume marker
20 µm

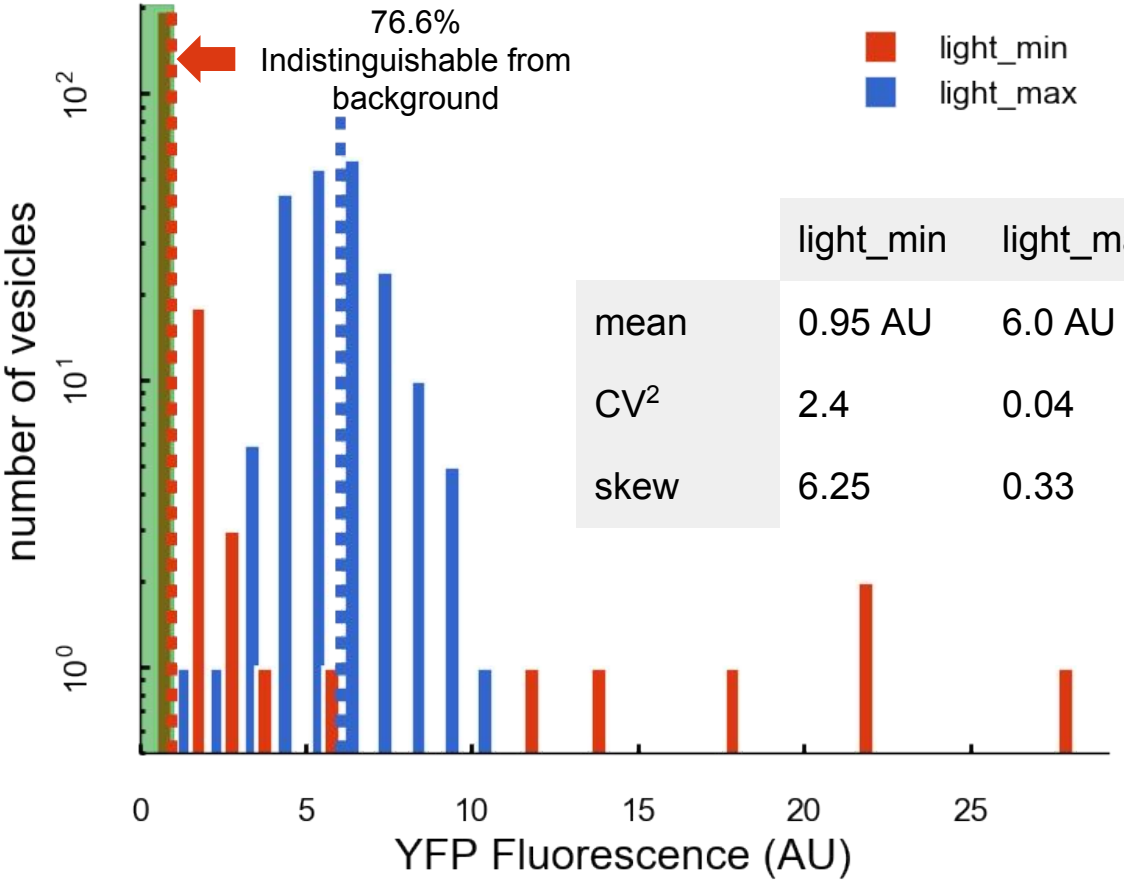

light_min
light_max
YFP Fluorescence

Photobleaching accounts for the decrease in the the AF647 signal

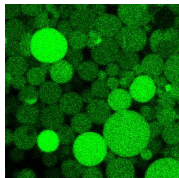




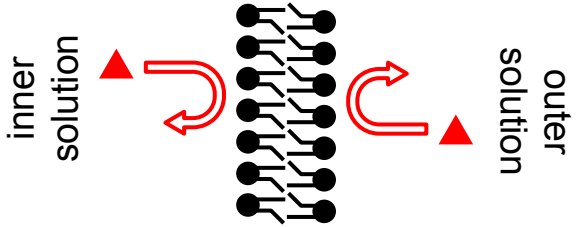
Light_max increases the mean and decreases the variability of protein expression



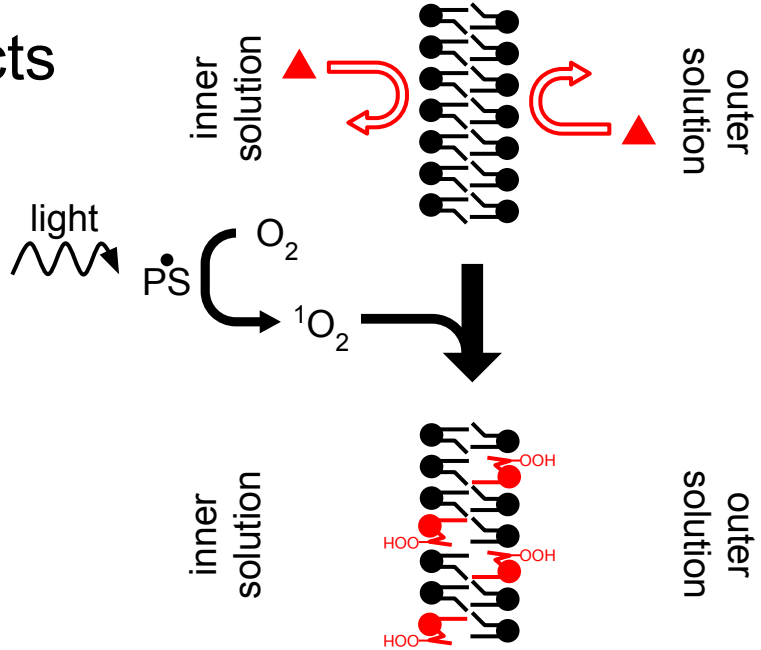
	light_min	light_max
mean	0.95 AU	6.0 AU
CV^2	2.4	0.04
skew	6.25	0.33



Well formed POPC membrane



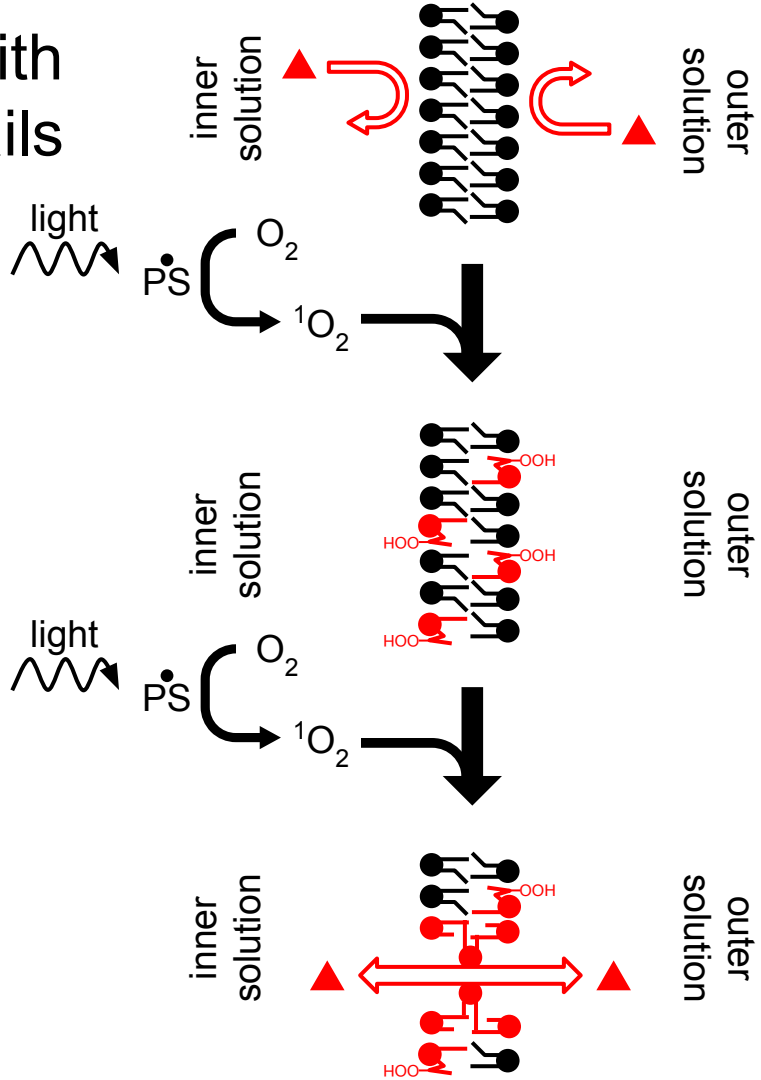
Singlet oxygen reacts with POPC



Bill McClintic and Dr. C. Patrick Collier

Itri, R., Junqueira, H. C., Mertins, O., & Baptista, M. S. (2014)

POPC molecules with aldehyde capped tails lead to pores



Bill McClintic and Dr. C. Patrick Collier

Itri, R., Junqueira, H. C., Mertins, O., & Baptista, M. S. (2014)

expression resources

ribosomes 2.7 MDa

plasmid 1.57 MDa

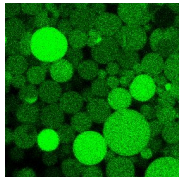
polymerase 99 kDa

tRNAs ~25 kDa

nucleotides ~500 Da

amino acids ~110 Da

ions ~40 Da



expression resources **fluorophores**

ribosomes 2.7 MDa

plasmid 1.57 MDa

polymerase 99 kDa

tRNAs ~25 kDa

AF647-transferrin 80 kDa

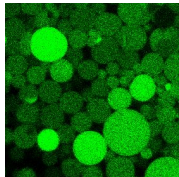
AF633 1.2 kDa

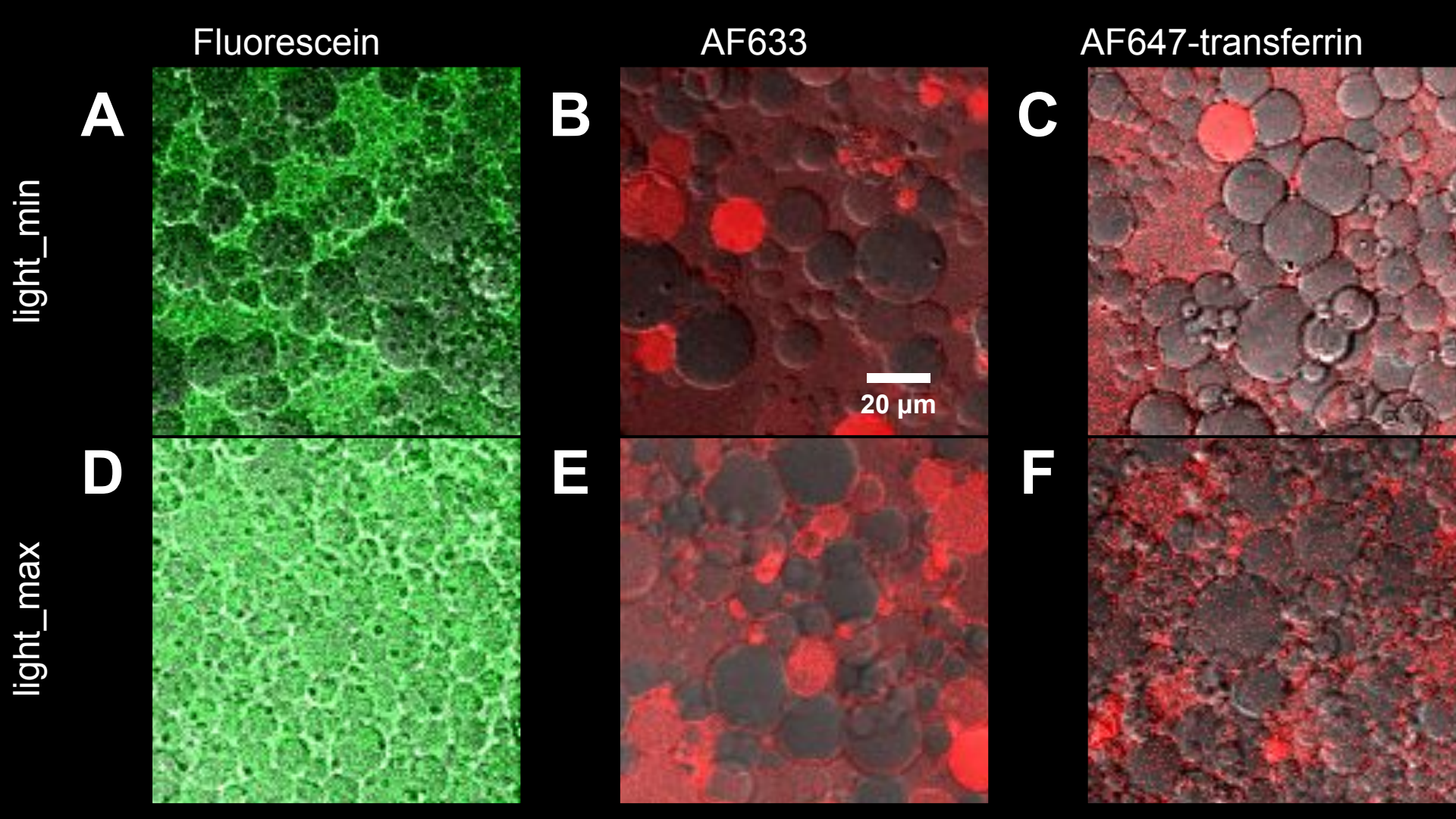
nucleotides ~500 Da

fluorescein 332 Da

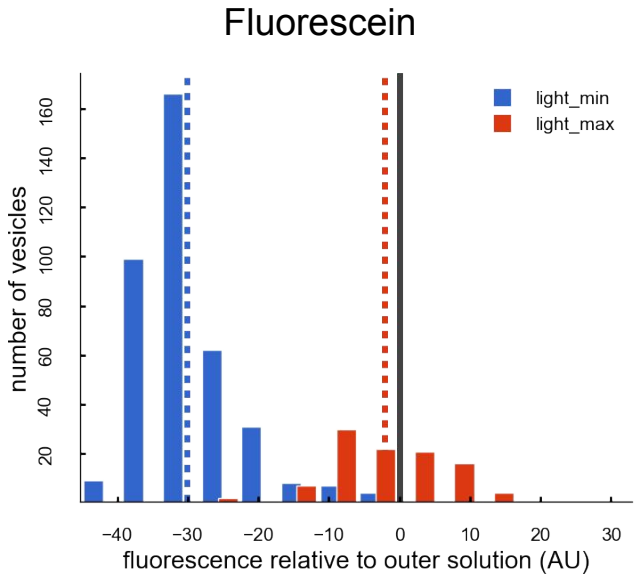
amino acids ~110 Da

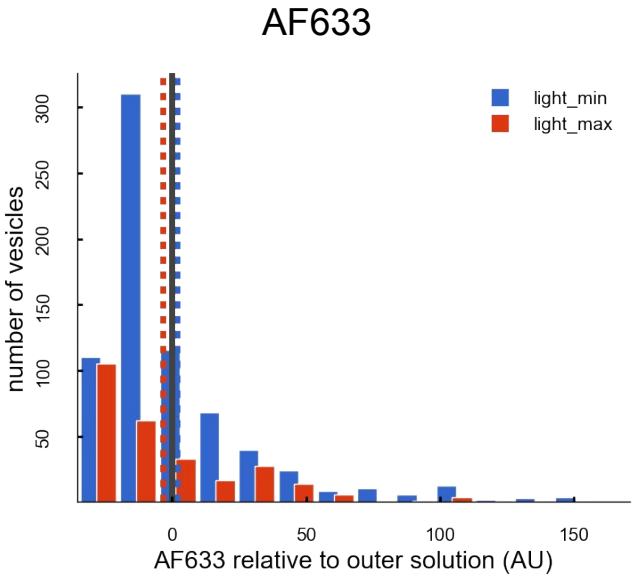
ions ~40 Da

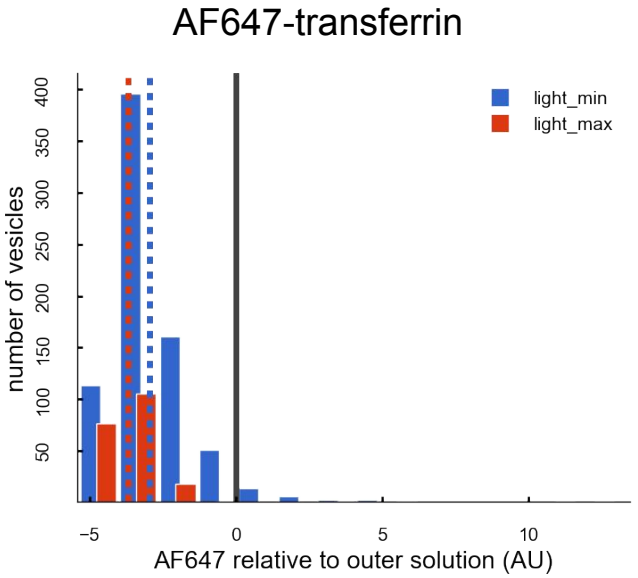


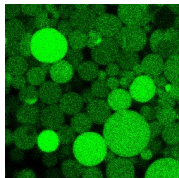

Fluorescein
AF633
AF647-transferrin
A
B
C
D
E
F
light_min
light_max
20 µm

Small molecules cross permeabilized membranes while large molecules do not









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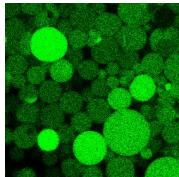
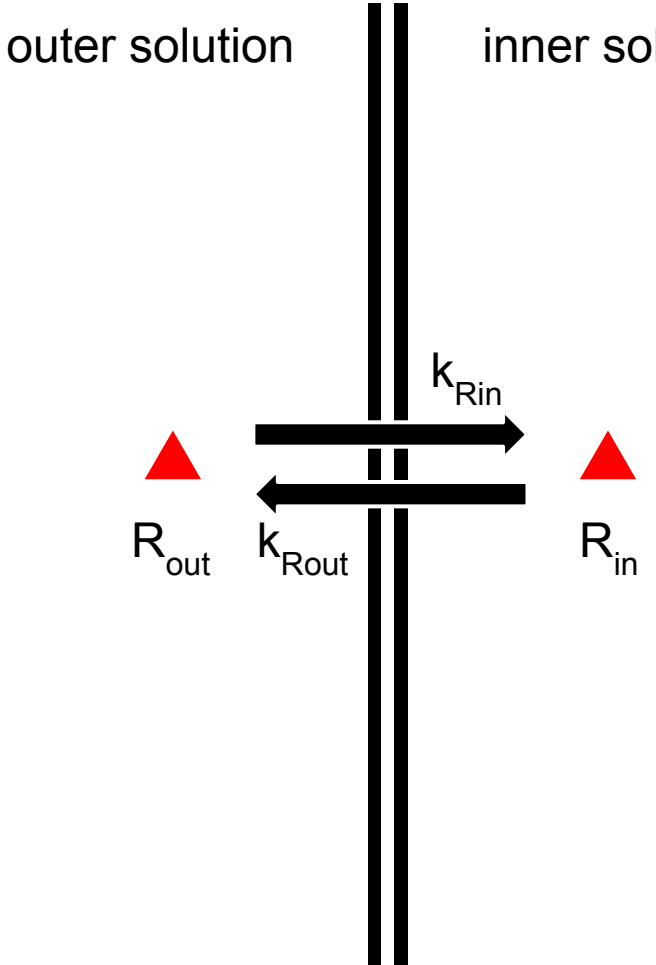
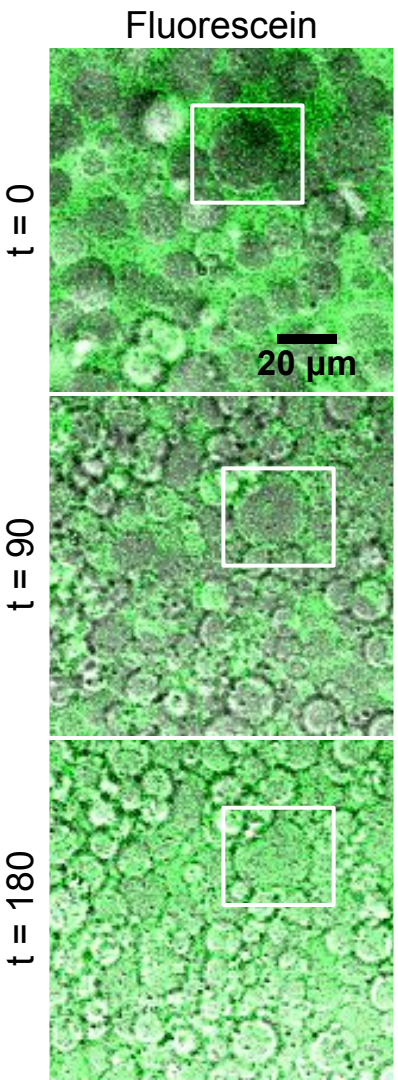


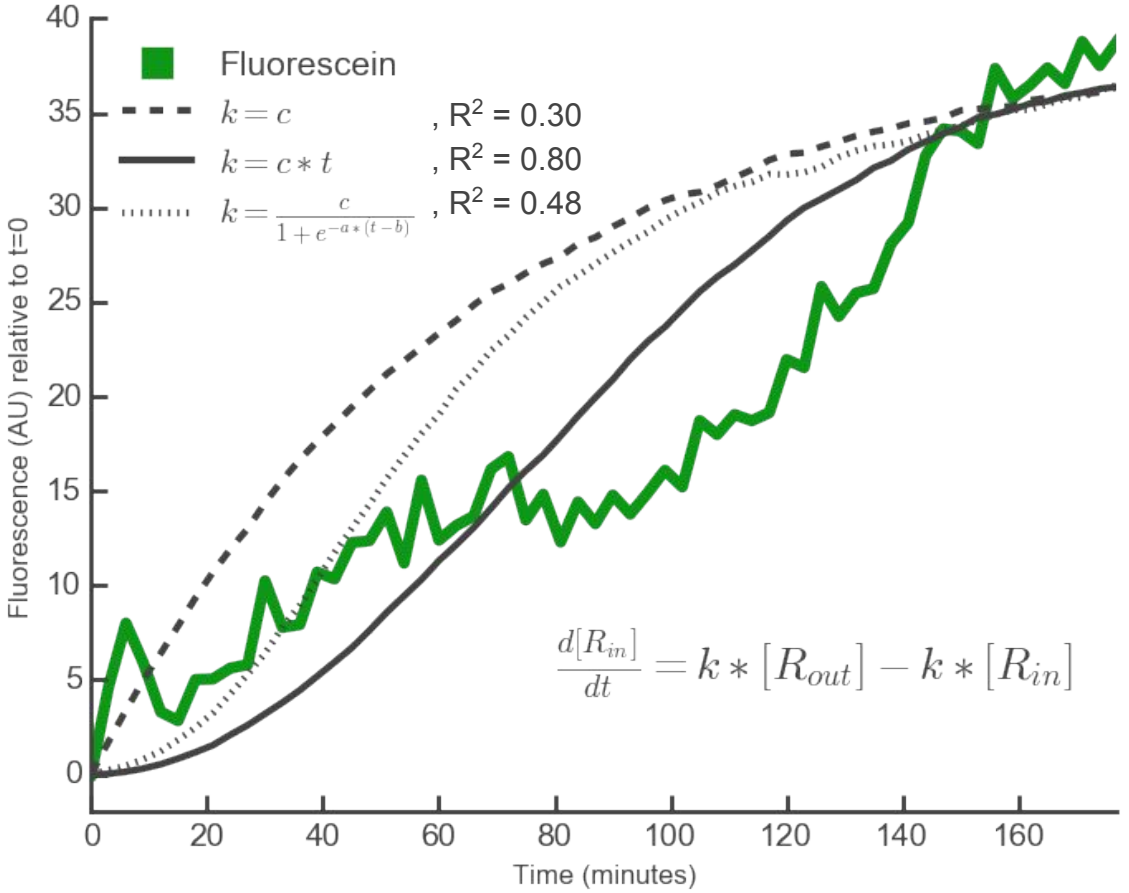
How does membrane permeability change during light_max?

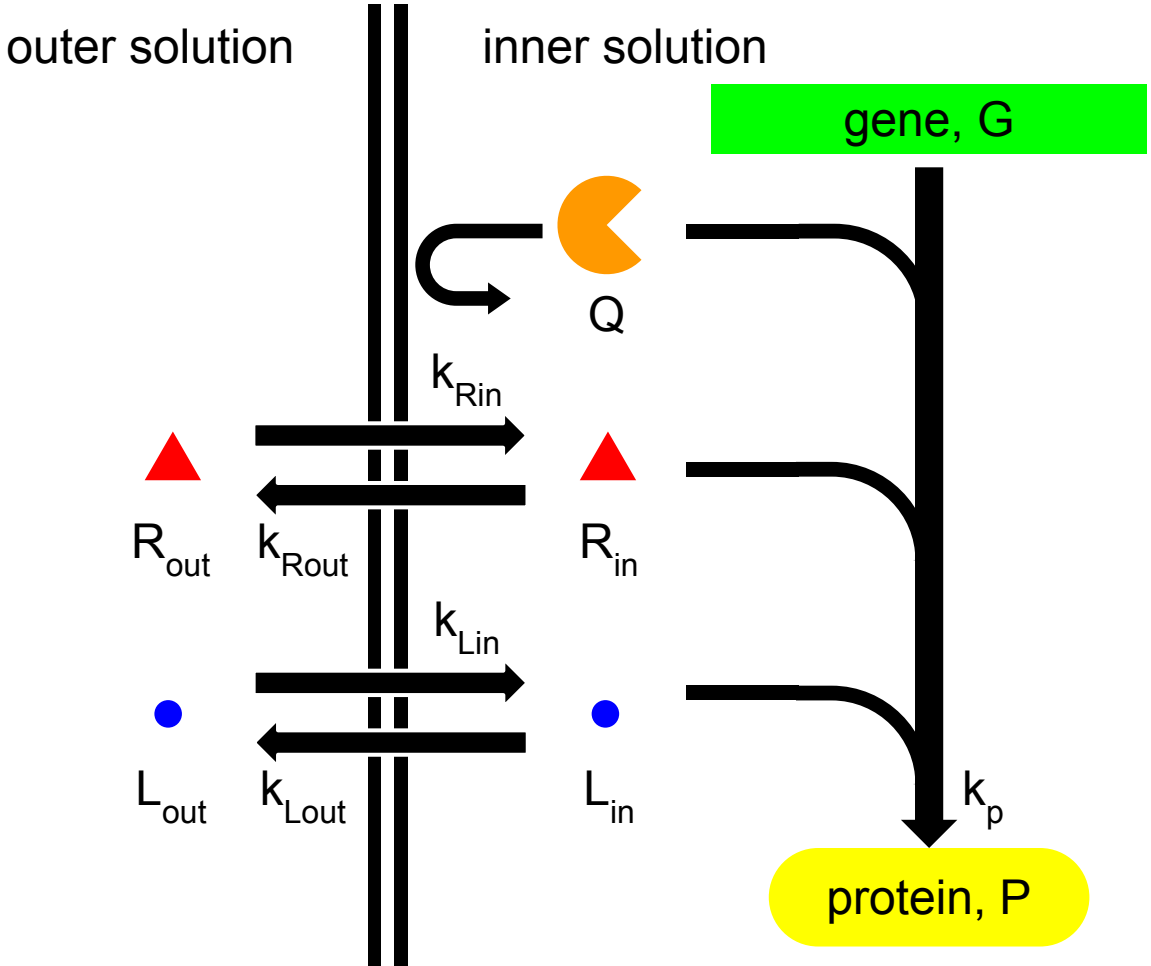


$$\frac{d[R_{in}]}{dt} = k * [R_{out}] - k * [R_{in}]$$

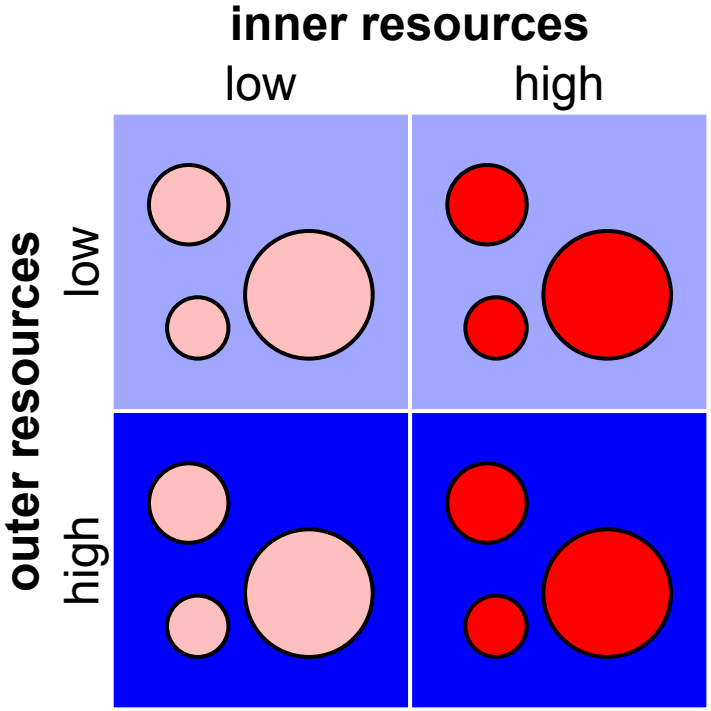
Membrane permeabilization increases linearly with time



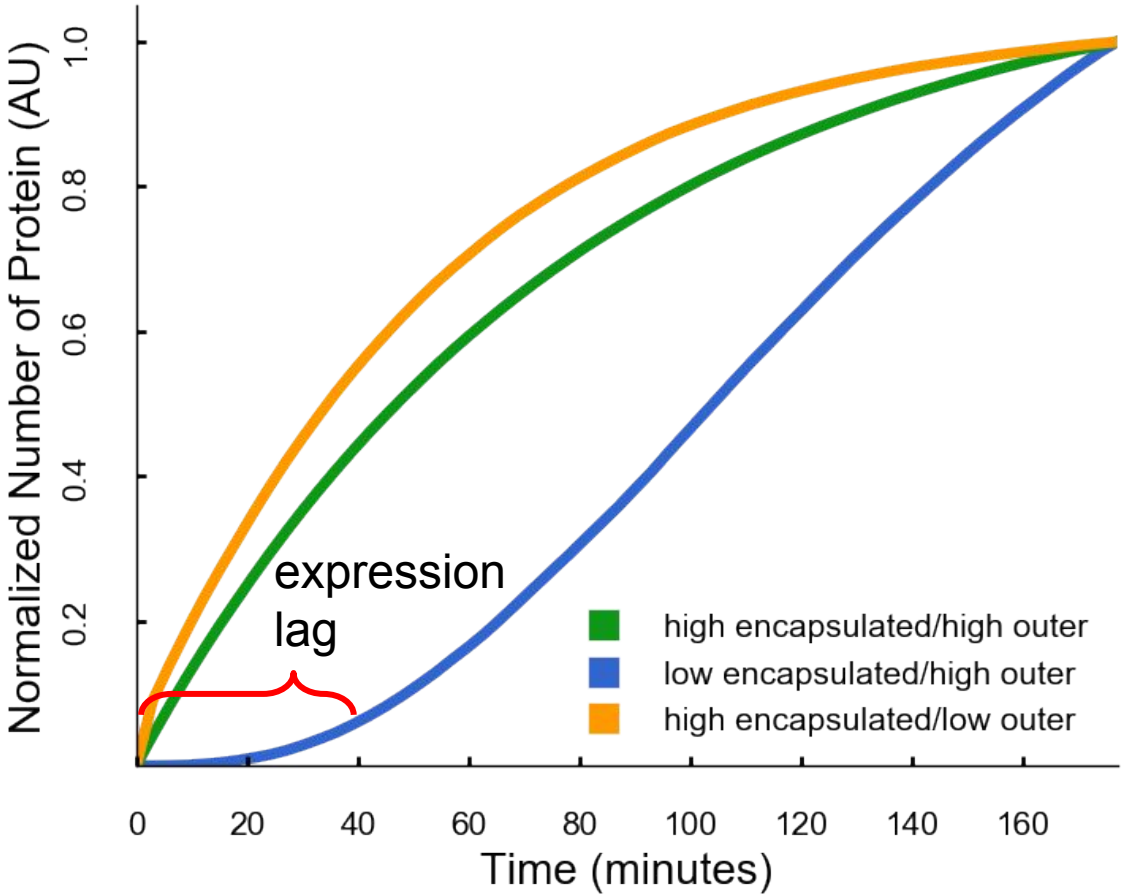



outer solution
inner solution
gene, G
Q
k_{Rin}
R_{out}
k_{Rout}
R_{in}
k_{Lin}
L_{out}
k_{Lout}
L_{in}
k_p
protein, P

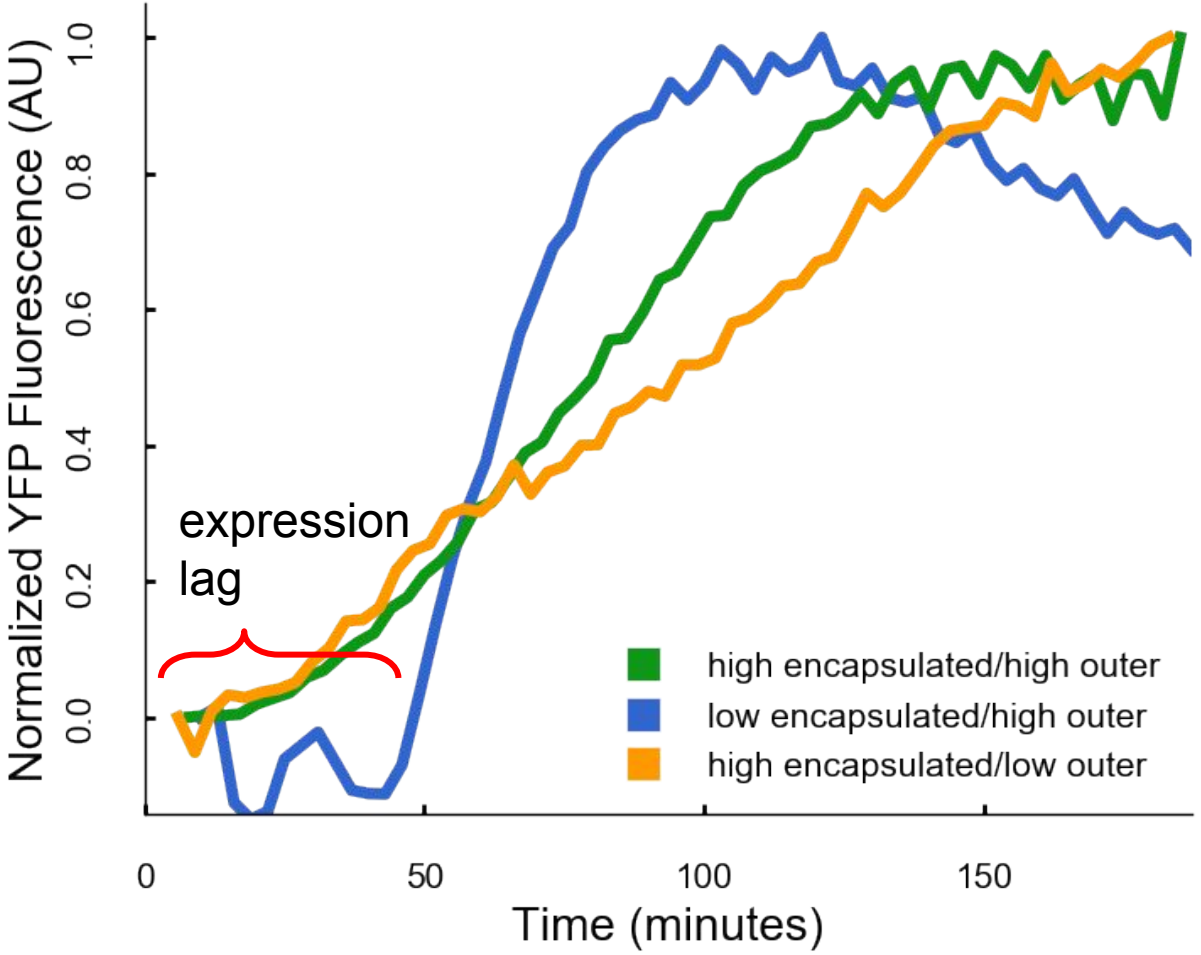
Protein expression dependent upon both encapsulated and outer resources

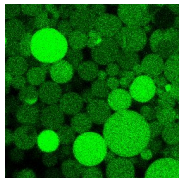


Membrane permeabilization results in a lag in expression

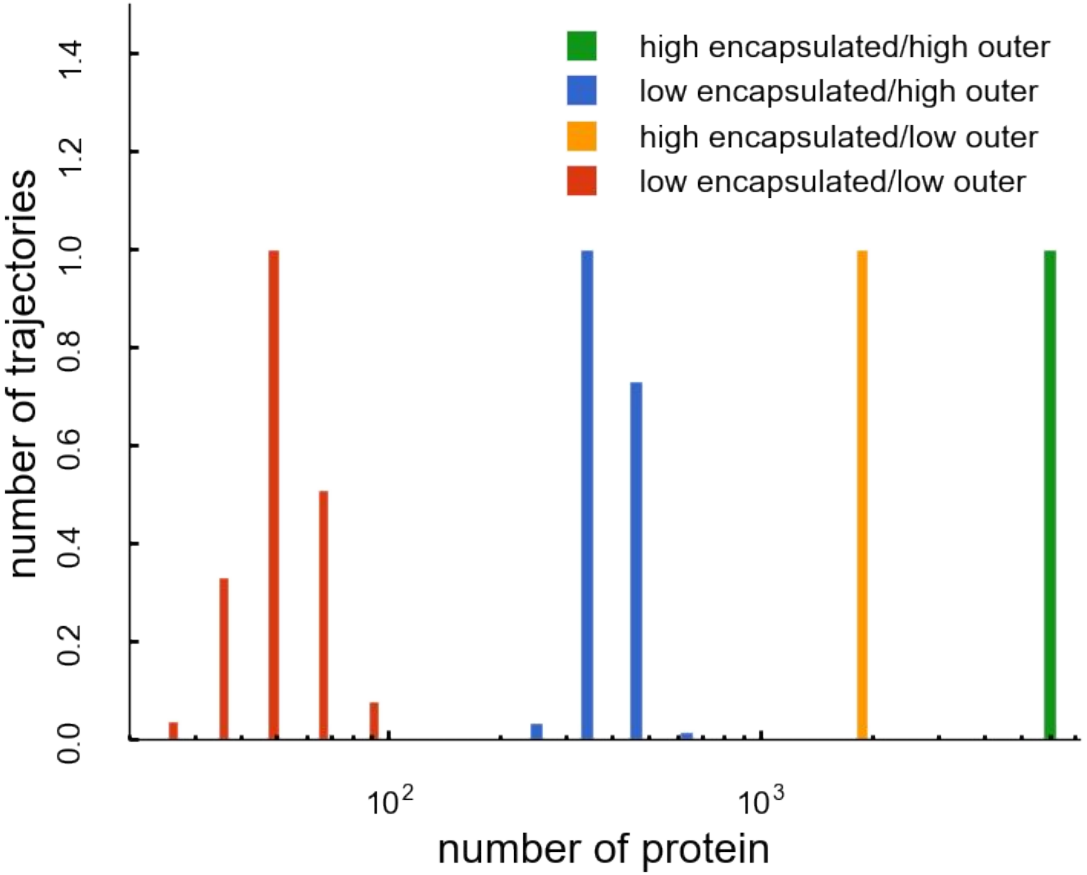


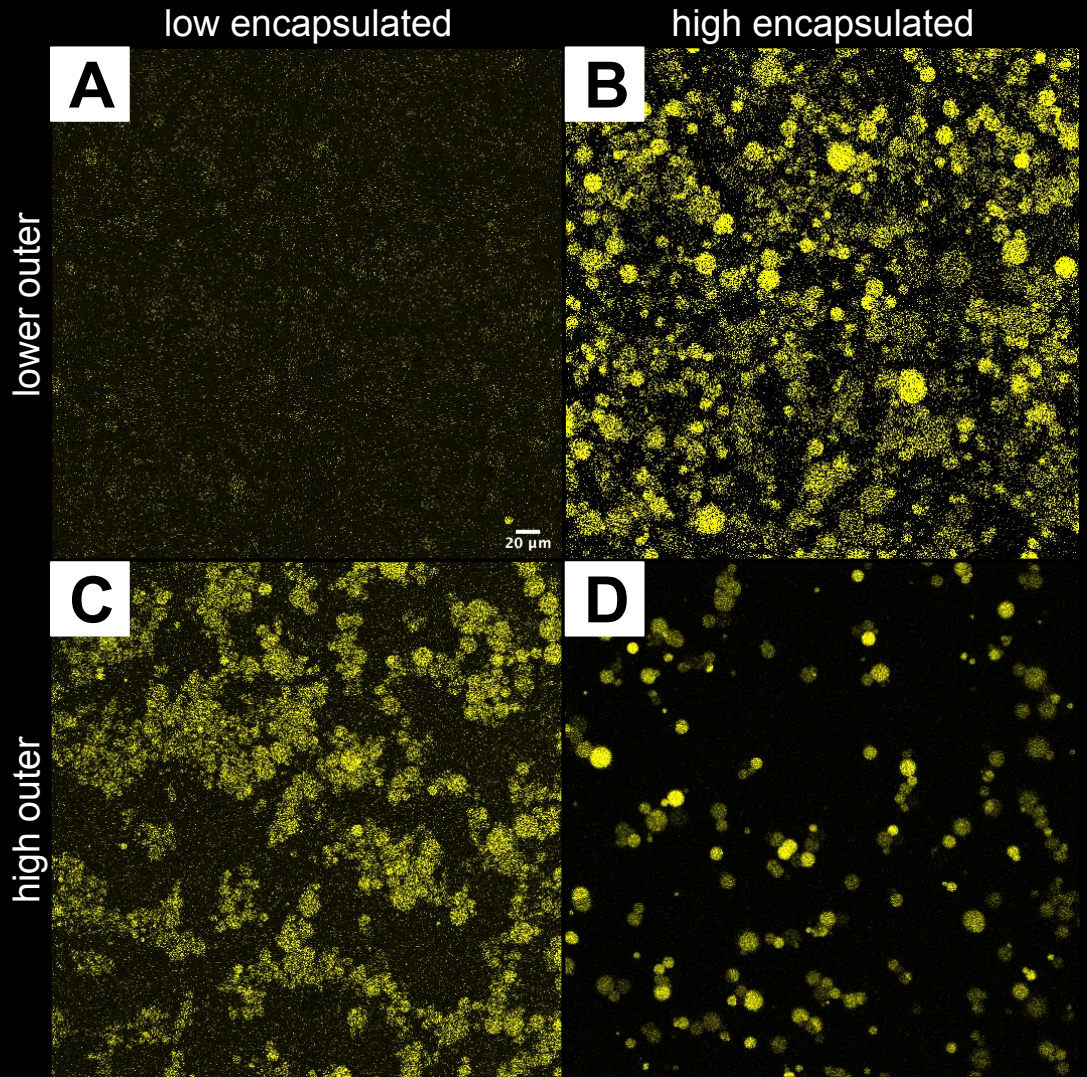
Resource transport delays protein expression



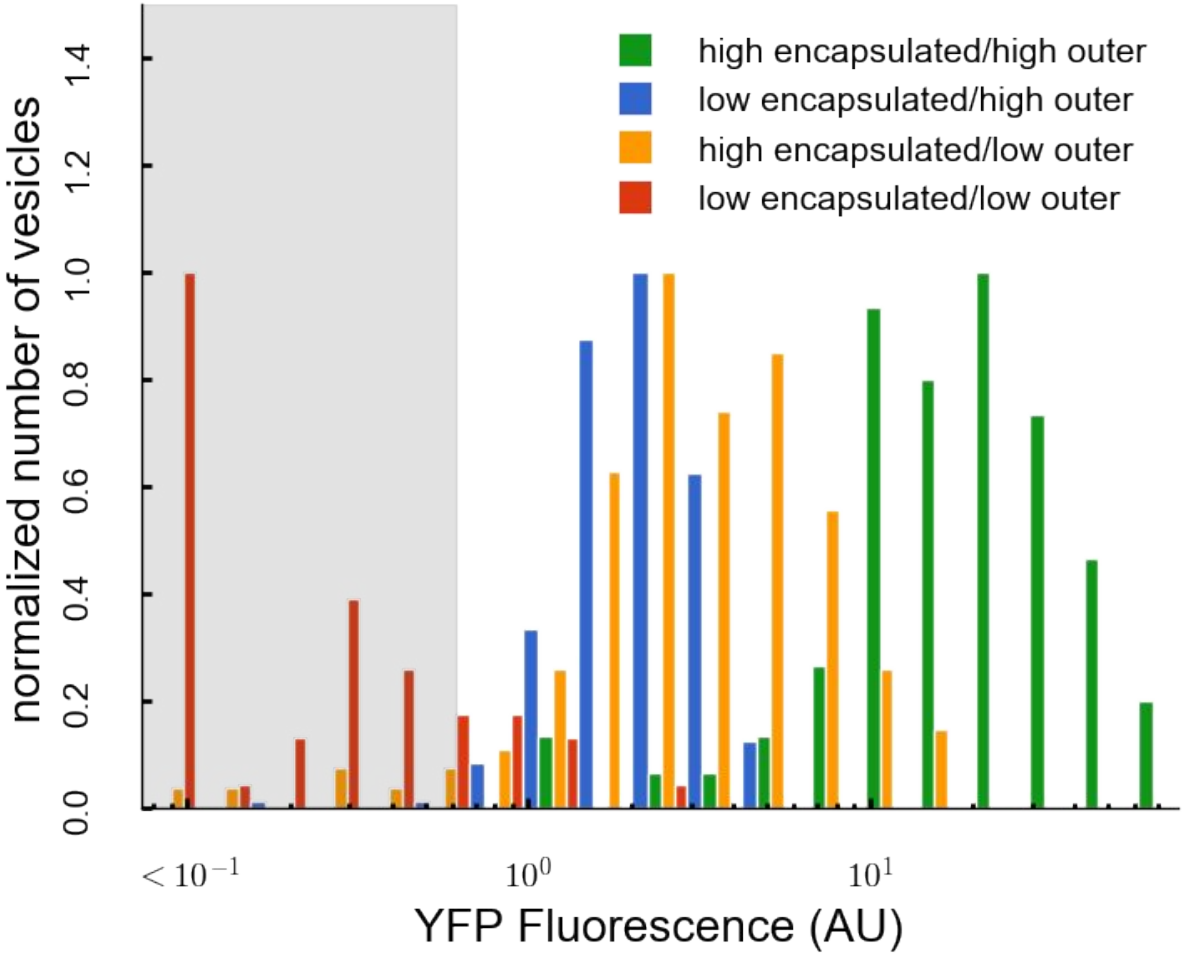


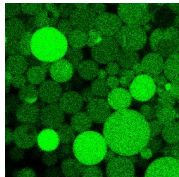
Model predicts greatly reduced mean protein




low encapsulated
high encapsulated
A
B
lower outer
20 µm
C
D
high outer

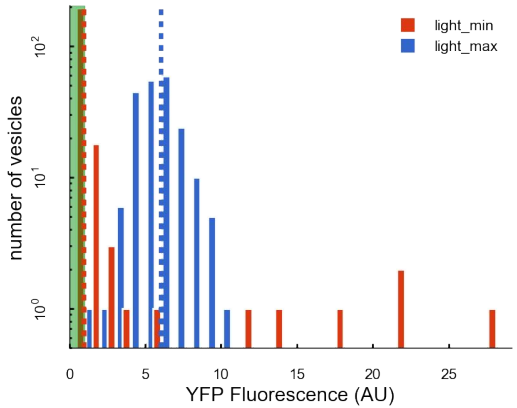
Resource concentrations control mean protein

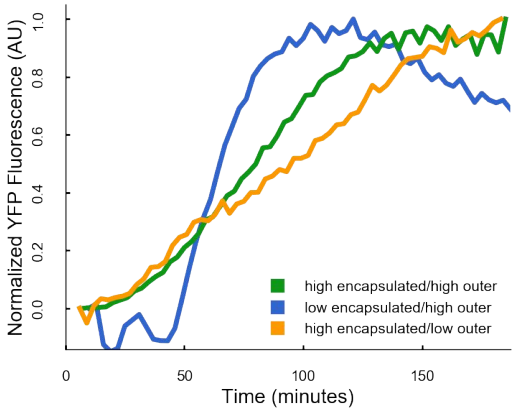


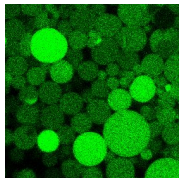


Chapter 3 Summary

1. Small resources cross permeabilized membranes
2. Photo-treatment can increase protein expression in vesicles.
3. Photo-treatment makes protein expression more uniform.
4. Resource transport across the membrane delays protein expression







Outline

Chapter 1: Introduction

Chapter 2: Resource Sharing Controls Gene Expression Bursting

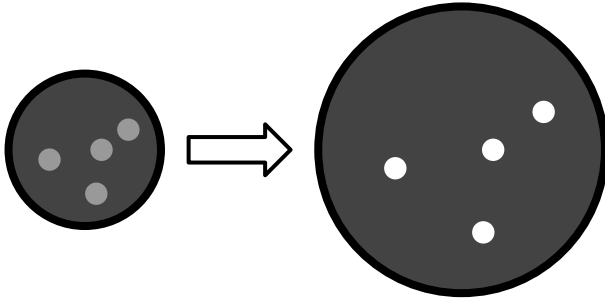
Chapter 3: Controlling Cell-Free Gene Expression Behavior by Tuning Membrane Transport Properties

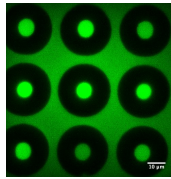
Chapter 4: Conclusion

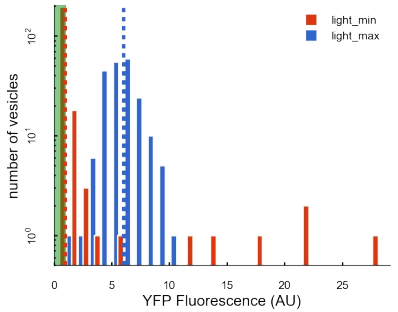
Chapter 4: Conclusion

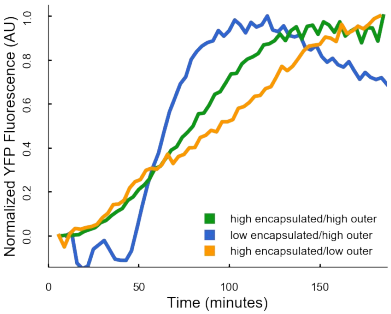
Conclusions

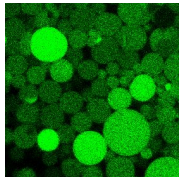
1. Larger resource pools contribute to existing expression centers instead of nucleating new centers
2. Vesicle membranes can be made permeable to small expression resources
3. Permeabilized membranes result in higher average protein expression and more uniform expression
4. Membrane permeabilization delays protein expression











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Rosemary Dabbs
Gaurav Chauhan
Dr. Jonathan Boreyko
Dr. Brandon Razooky
Dr. Jason Fowlkes

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Office of Science

thank you. questions?