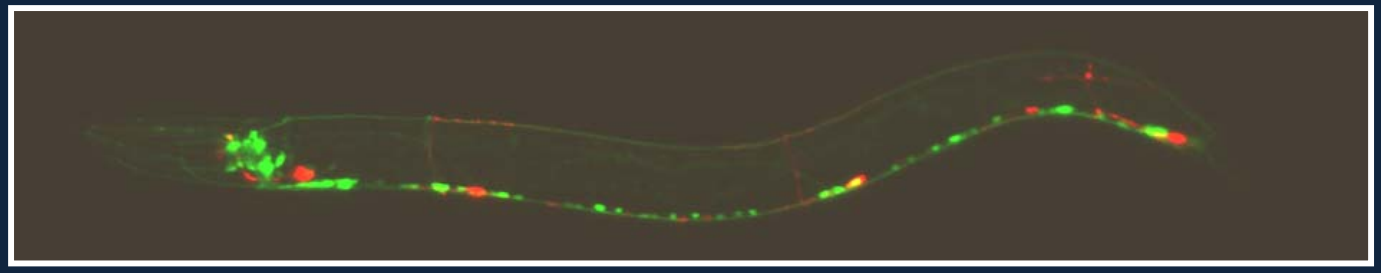
Reaction/Diffusion Dynamics in the *C. Elegans* Axon



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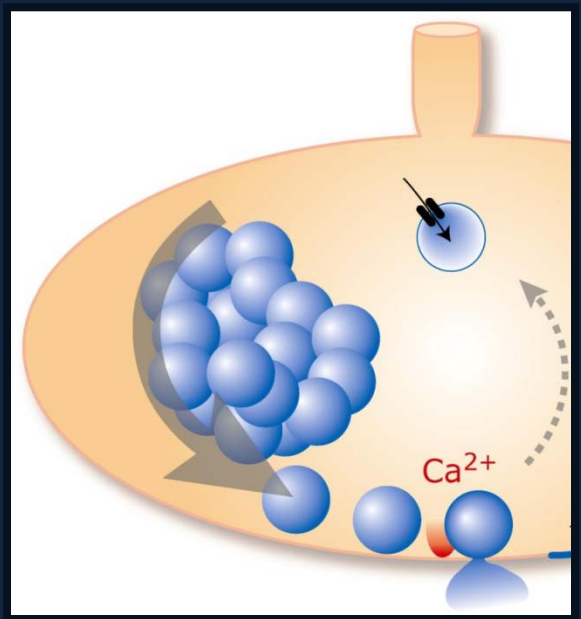
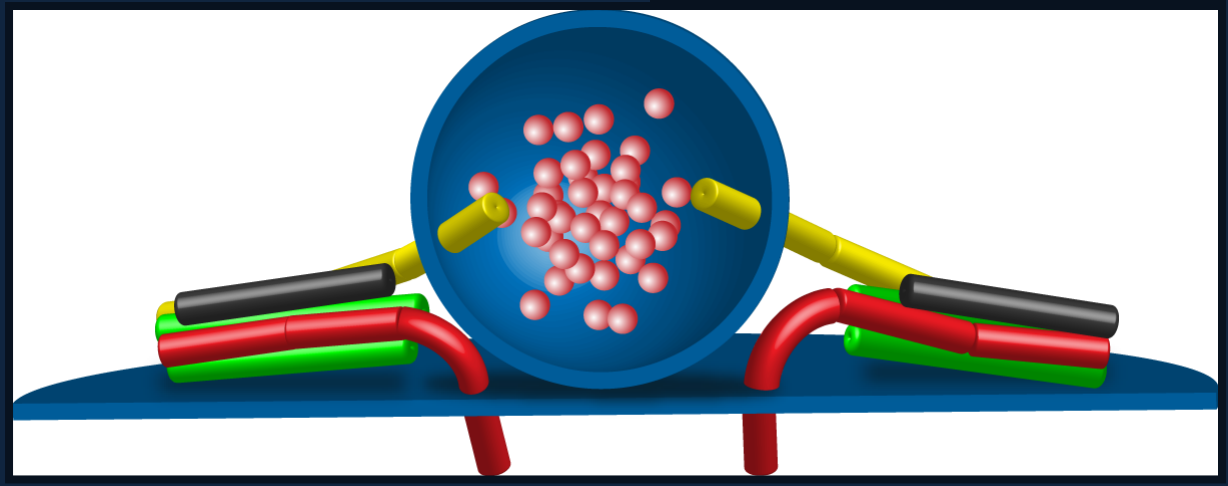
Synaptic Transmission

- goal: to understand the molecular mechanisms involved in the function and plasticity of synaptic transmission
- focus on role of presynaptic proteins that control the fusion of synaptic vesicles
- what recruits these proteins to the synapse and what makes them stay there?

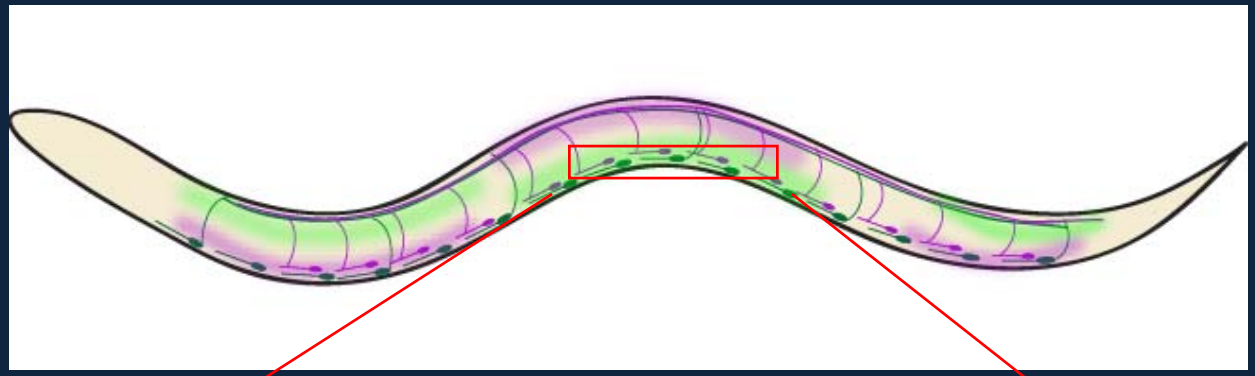
Critical Synaptic Vesicle Proteins

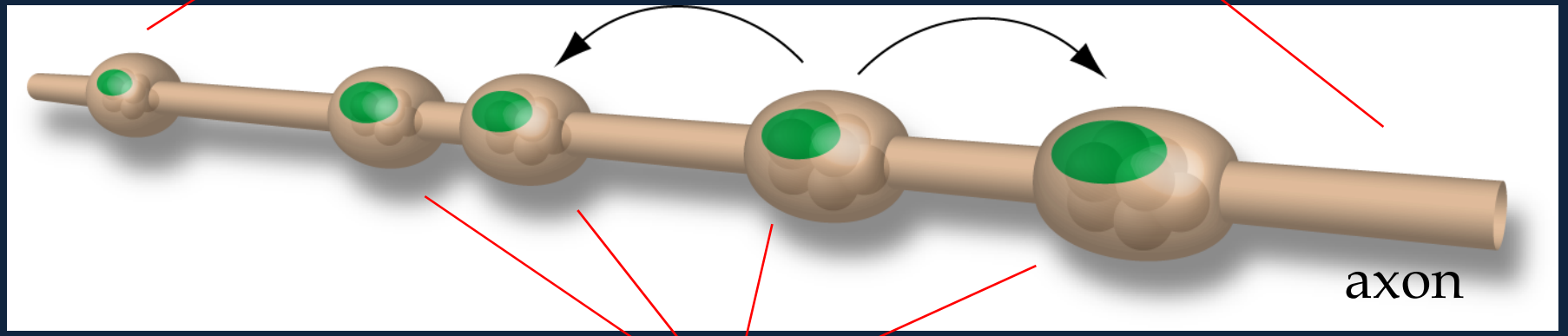
- Major players:
  - SNAREs:
    - synaptobrevin-2
    - syntaxin-1
    - SNAP-25
  - synaptotagmin-1
  - complexin





C. Elegans Nervous System

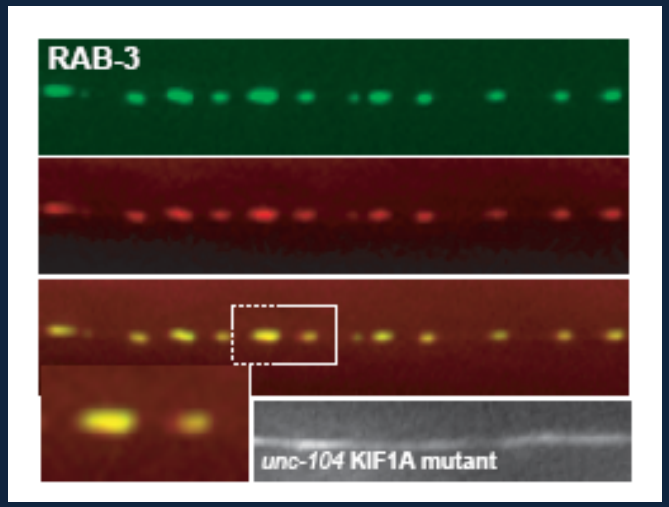


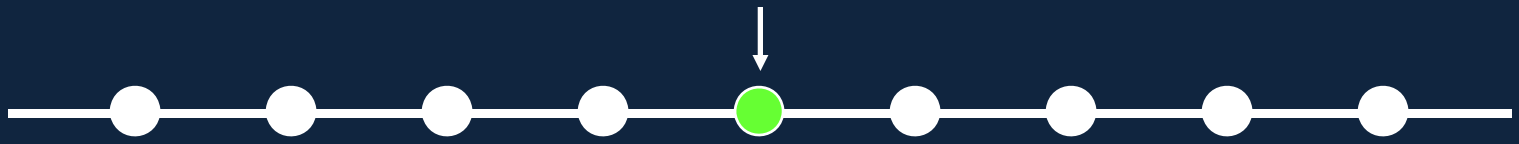


Presynaptic terminals

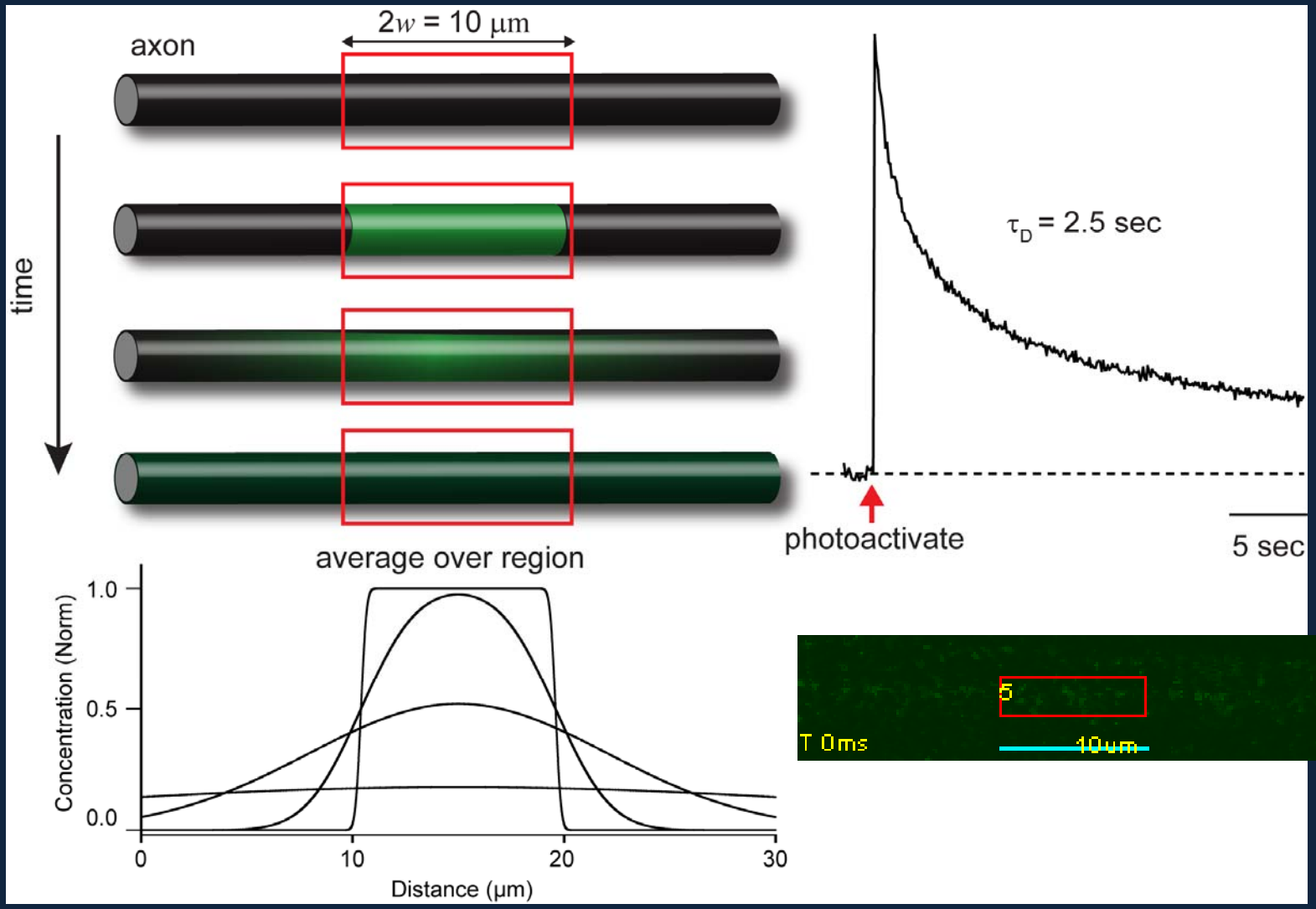
Experimental Set-up

- simple geometry of axon
- photoactivable GFP (pGFP)
  - soluble
  - fused to protein of choice
- quantify retention/spread
- mutations

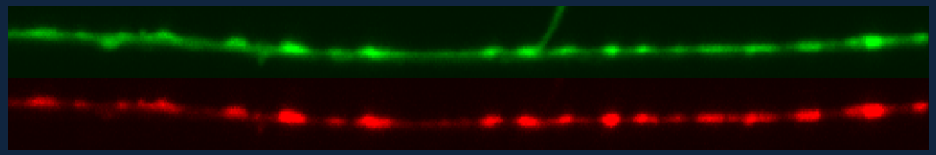


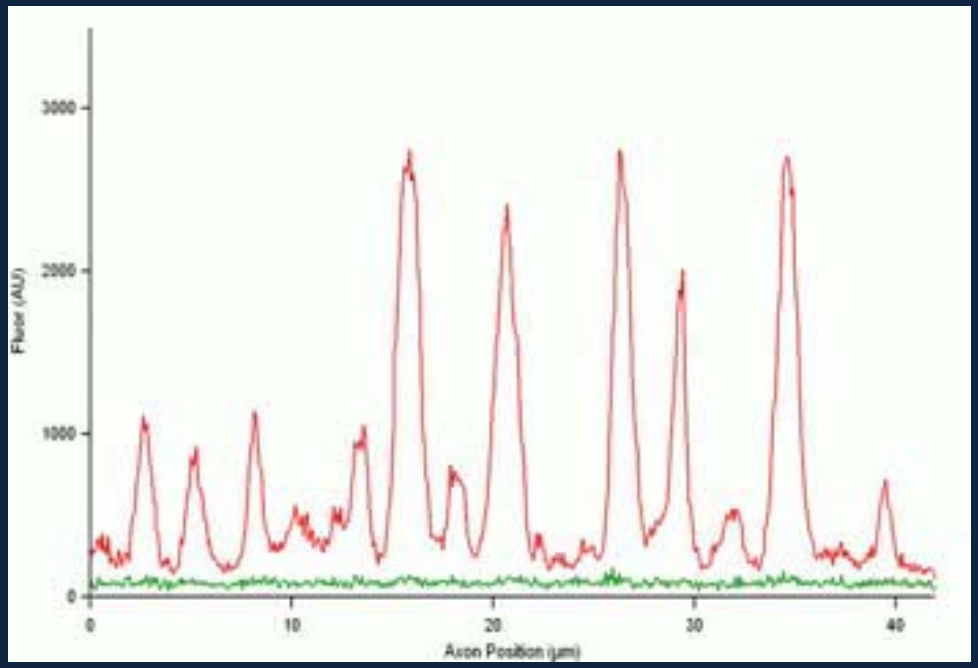


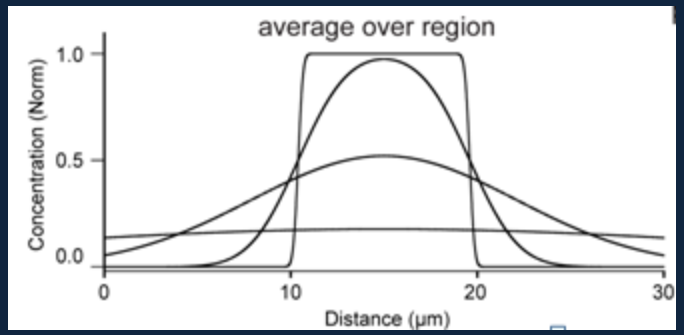
Quantifying Mobility Using pGFP

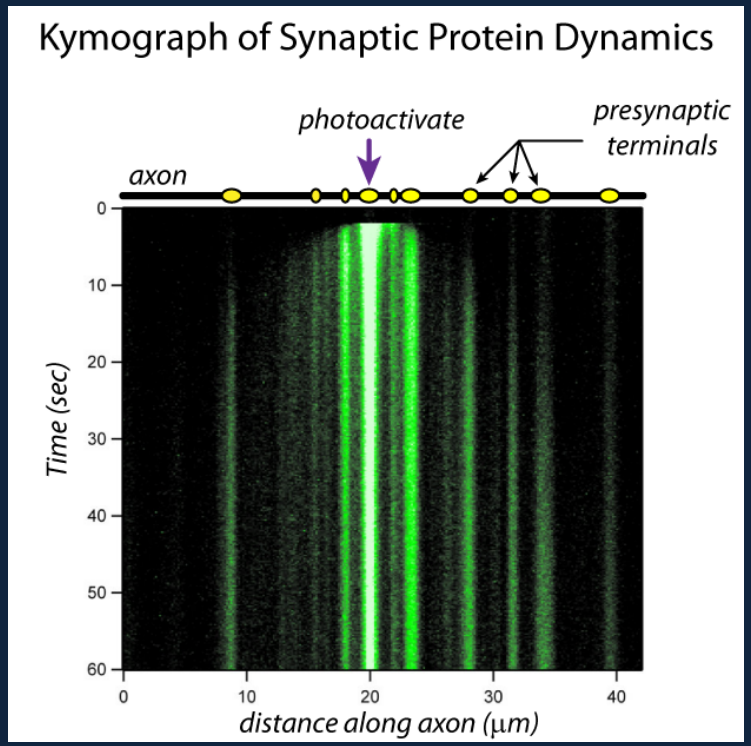


Quantifying Mobility Using pGFP

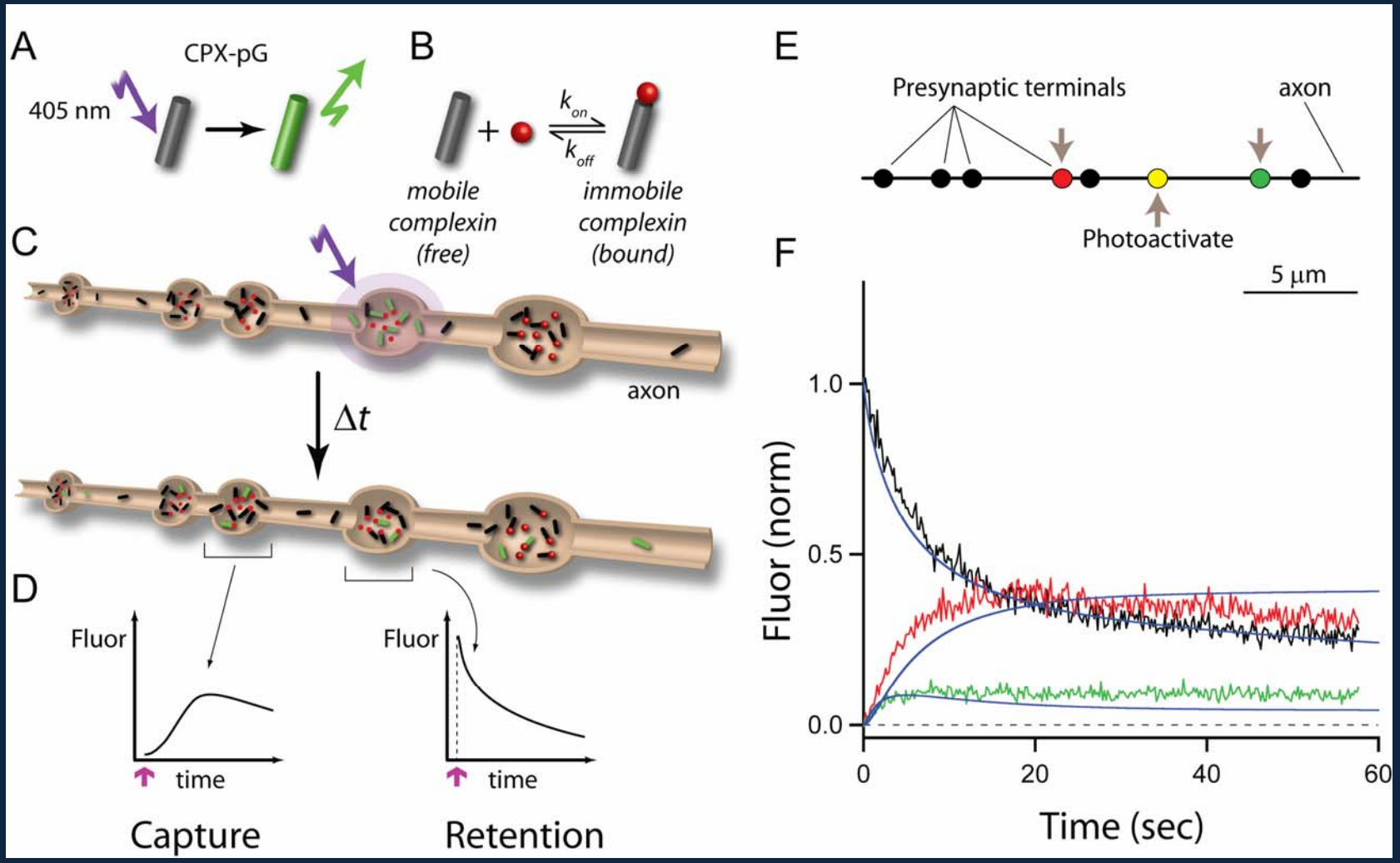






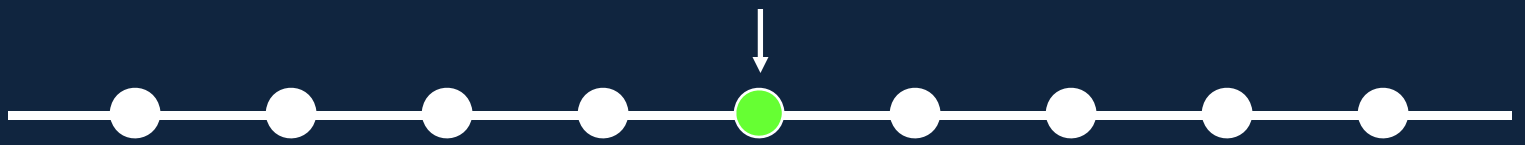


Example: complexin



Modeling

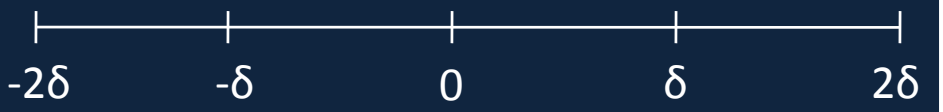
- two extremes: diffusion versus reaction
- focus on synapse where GFP is activated



Diffusion: microscopic

- random migration of molecules arising from motion due to thermal energy
- average position: particles go nowhere
- measure spread as root-mean-square displacement
  - spreading increases as the square-root of time

- Diffusion equation



Position of *i*th particle after *n*th step:

$$x_i(n) = x_i(n-1) \pm \delta$$

$$= x^2(n-1) + \delta^2$$

$$D = \delta^2 / 2\tau$$

$$x(t) = (2Dt)^{1/2}$$

Diffusion: macroscopic

- Fick's First Law: flux goes from regions of high concentration to low
  - J is net flux

$$J = -D\frac{\partial C}{\partial x}$$

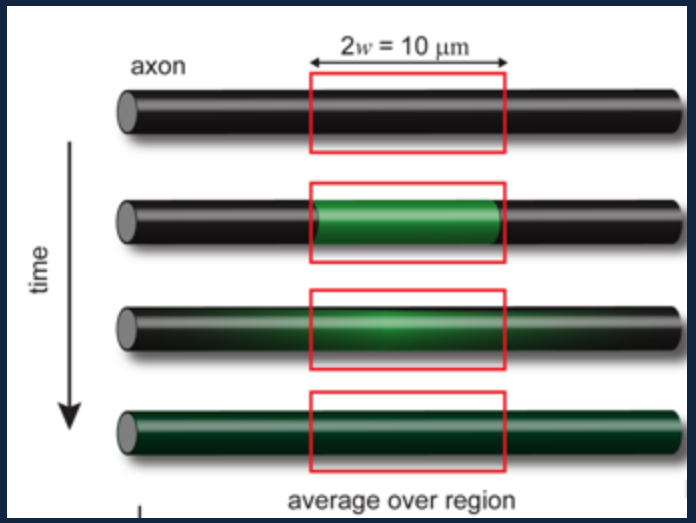
- Fick's Second Law: predicts how diffusion causes the concentration field to change with time
  - particles neither created or destroyed
  - 1 dimensional approximation for free diffusion
  - infinite thin cable

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$$

Diffusion: time course of decay

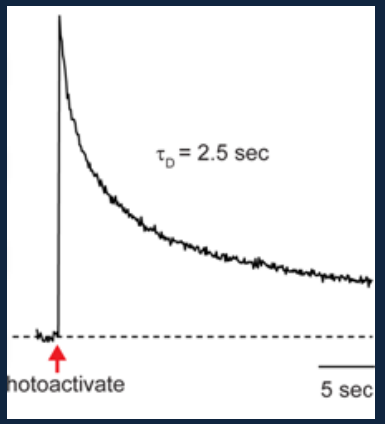
- To solve for average of C in our box:

$$C(x,t) = \frac{C_o}{2}\left[\operatorname{erf}\left(\frac{w+x}{\sqrt{4Dt}}\right) + \operatorname{erf}\left(\frac{w-x}{\sqrt{4Dt}}\right)\right]$$



$$\bar{C}(t) = \frac{1}{2w}\int_{-w}^{w} C(x,t)dx = C_0\left[\operatorname{erf}\sqrt{\frac{\tau_D}{t}} + \sqrt{\frac{t}{\pi\tau_D}}\left(e^{-\tau_D/t} - 1\right)\right]$$

- Where $\tau_D = \frac{w^2}{D}$, the characteristic diffusion time
  - equal to about half of decay



Reaction

C = molecule

F = what molecule is binding to

B = bound

$$C + F \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} B$$

$$B_T = B + F$$

$$\frac{dB}{dt} = k_{on}\, C B_T - \left(k_{off} + k_{on}\, C\right) B$$

$$B(t) = B_\infty - \left(B_\infty - B_o\right) e^{-t/\tau}$$

where

$$B_\infty = \frac{B_T}{1 + K_D/C_o}$$

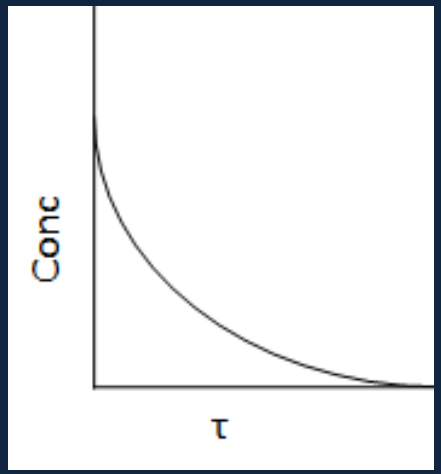
$$\tau = \frac{1}{k_{off} + k_{on} C_o}$$

So diffusion or reaction?

- similar decay curves?

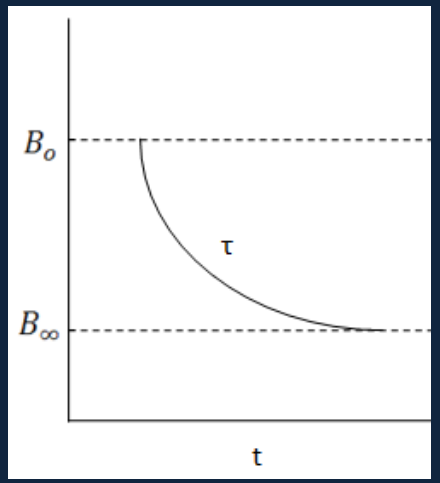
Diffusion:

$$\tau_D = \frac{w^2}{D}$$



Reaction:

$$\tau = \frac{1}{k_{off} + k_{on}C_o}$$



- turns out to be somewhere inbetween
  - width of window (w) matters

- next step: simulate curves to separate diffusion and reactions

Summary

- goal: to understand the molecular mechanisms involved in the function and plasticity of synaptic transmission
- method: use pGFP fused to protein of choice (e.g. complexin) to study binding affinities under various conditions
- use modeling techniques to differentiate between diffusion and reaction components

Why is this helpful?

- Allows us to quantitatively measure biochemical reactions *in vivo*

- How do synapses fight against diffusion to maintain stability?