

# Studying Chemical Reaction Rates with Wolfram Language

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# Empirical Studies

- Reaction rate (progress with time) measurements:
- The progress of the reactions are expressed as the **change** of the **concentration / pressure** of reactant(s) / product(s) / intermediate(s)
- Measured by monitoring **physical quantities proportional to the concentration or pressure**. For example, absorption of radiation.

# Spectroscopic Methods

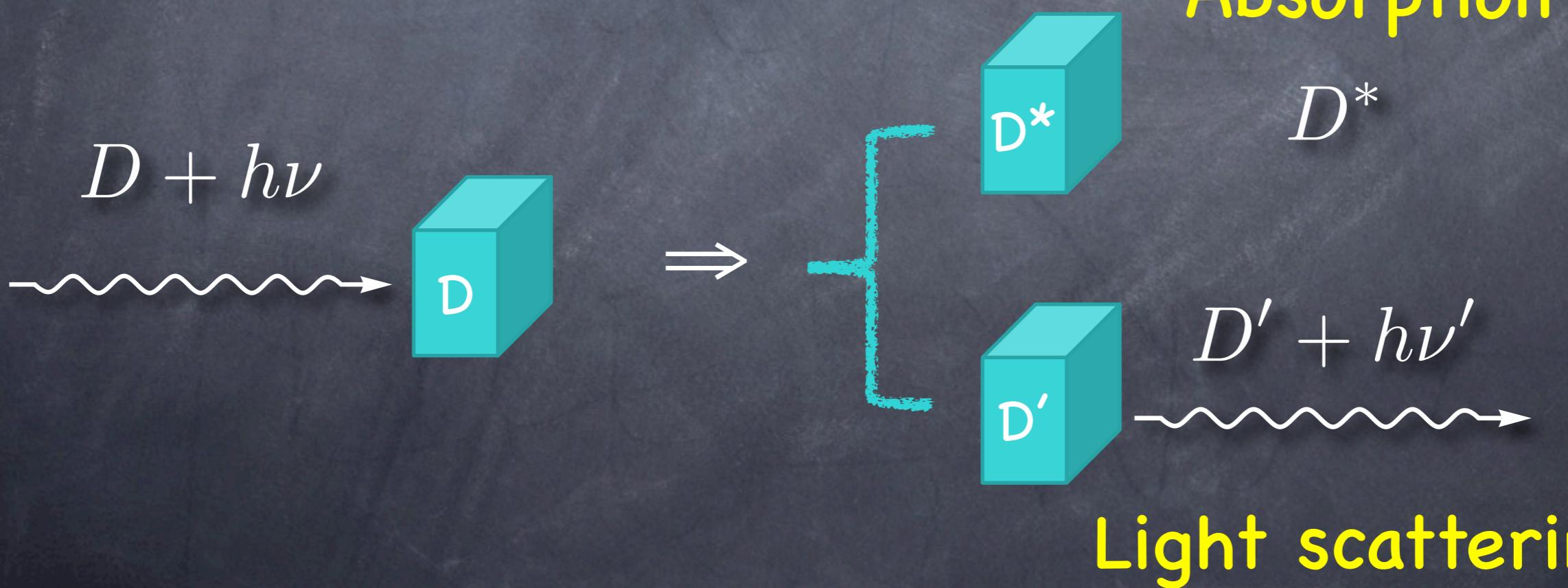
## Photon-matter interactions

- Frequency of light:  $\nu$
- Wavelength:  $\lambda = c/\nu$
- Angular frequency:  $\omega = 2\pi\nu = 2\pi c/\lambda$
- Photon energy:  $E = h\nu = \hbar\omega = hc/\lambda$

Planck's constant:

$$h = 6.626 \times 10^{-34} \text{ J s}$$

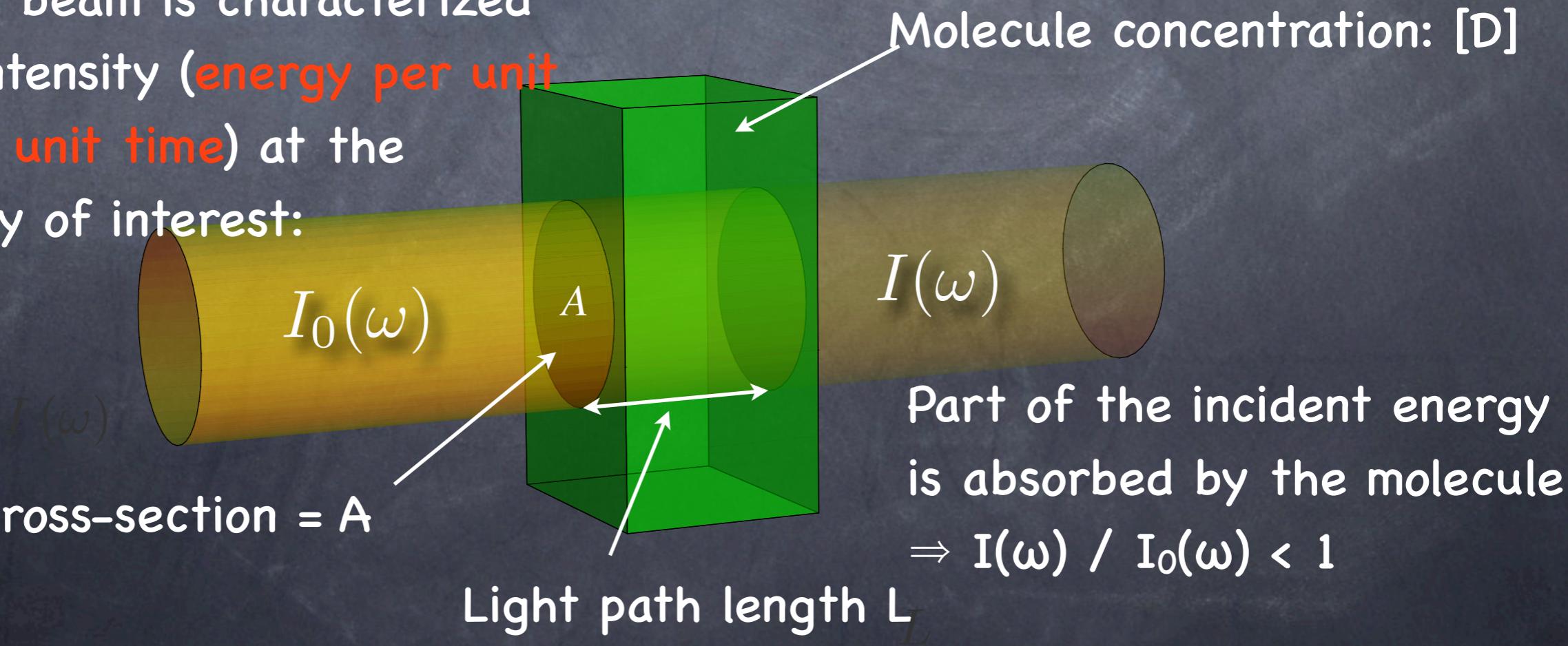
$$\hbar = h / 2\pi = 1.055 \times 10^{-34} \text{ J s}$$



# Absorption Spectroscopy

- Organic / biological molecules:
  - UV-Vis (Ultra-violet and visible)
  - IR (Infrared)
- Fundamental principle of absorption spectroscopy:  
**Beer-Lambert law**

The light beam is characterized by the intensity (energy per unit area per unit time) at the frequency of interest:



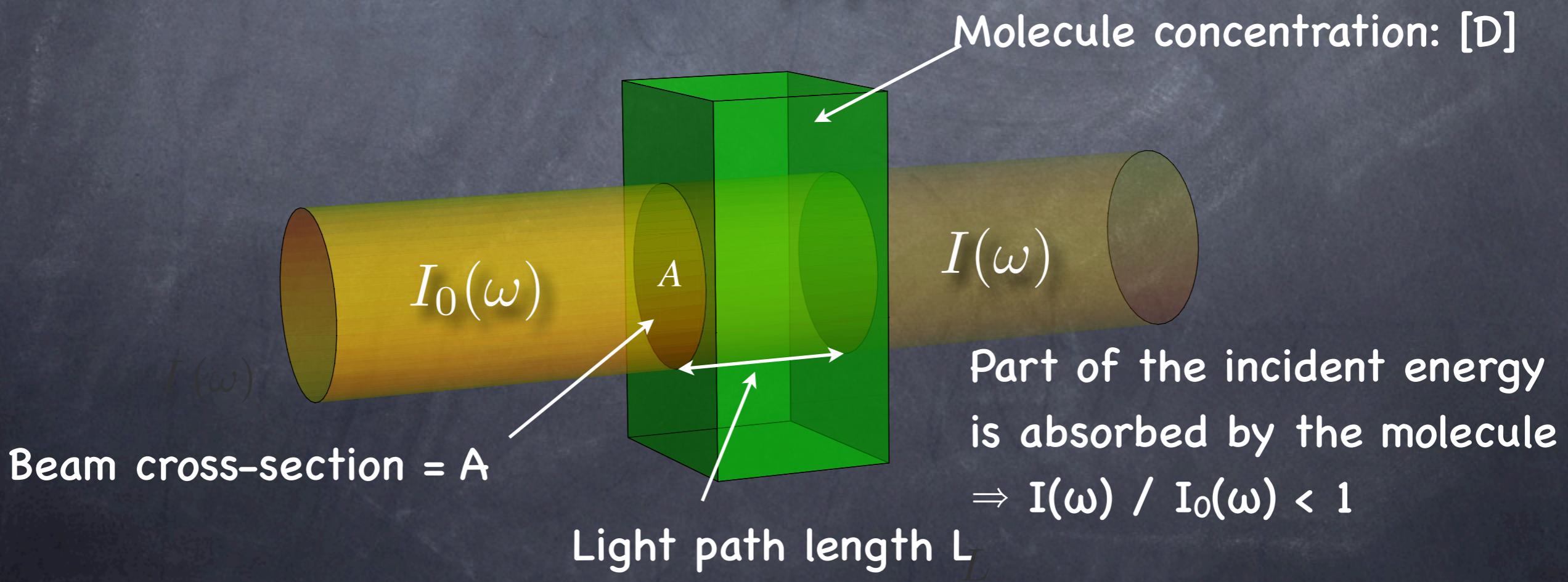
# Beer-Lambert Law

- **Absorbance** (optical density):

$$\alpha(\omega) = - \log_{10} \frac{I(\omega)}{I_0(\omega)}$$

- **Extinction coefficient:**

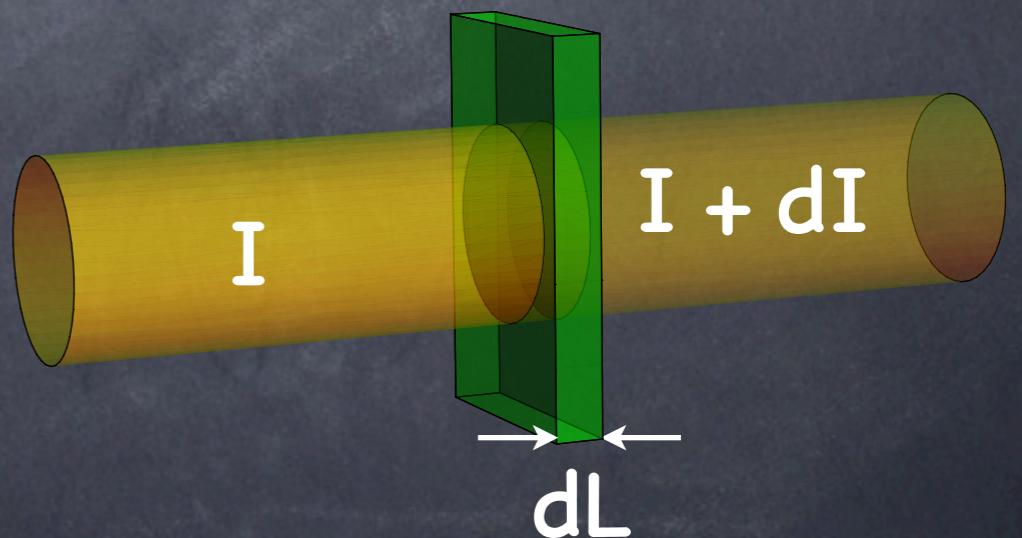
$$\varepsilon(\omega) = \frac{\alpha(\omega)}{[D] L}$$



# Beer-Lambert Law

- Consider a thin layer of the sample (Thickness =  $dL$ )
- When one molecule absorbs one photon at frequency  $\omega$ , the energy of the light beam reduces by  $\hbar\omega$
- Number of incident photon per unit time:  $I(\omega)A/\hbar\omega$
- Number of photons absorbed per unit time:  
 $-A dI/\hbar\omega$ . ( $dI < 0$ )
- The probability per unit time that a photon is absorbed when there is one mole of D in the volume:  $p(\omega) \Rightarrow$

$$-\frac{A dI}{\hbar\omega} = p(\omega) A [D] dL \frac{A I(\omega)}{\hbar\omega}$$



# Beer-Lambert Law

- Linear dependence of the absorbance on molecule concentration and light-path:

$$-\frac{A dI}{\hbar\omega} = \frac{p(\omega) A [D] A I(\omega) dL}{\hbar\omega} \Rightarrow$$
$$\frac{1}{\ln 10} \frac{dI}{I} = d(\log_{10} I) = -\frac{p(\omega) A [D] dL}{\ln 10}$$

$$\begin{aligned} - \int_{L'=0}^L d(\log_{10} I) &= -\log_{10} \frac{I}{I_0} \\ &= \alpha(\omega) = \frac{p(\omega) A [D]}{\ln 10} \int_{L'=0}^L dL' \\ &= \frac{p(\omega) A}{\ln 10} [D] L = \varepsilon(\omega) [D] L \end{aligned}$$

- Exponential dependence of the intensity on absorbance, molecule concentration, and light-path:

$$I(\omega) = I_0(\omega) 10^{-\alpha(\omega)} = I_0(\omega) 10^{-\varepsilon(\omega)[D]L}$$

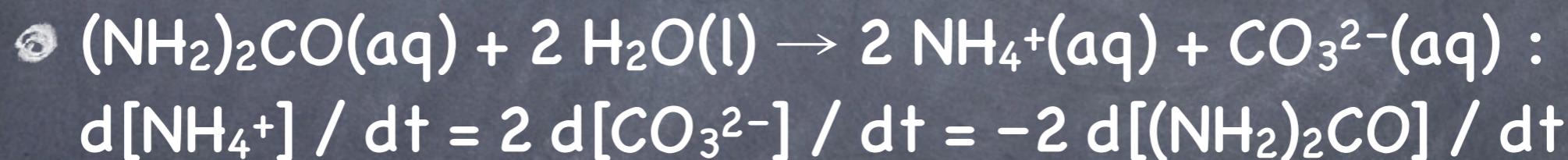
# Definition of Reaction Rate

- Reaction rate: **rate of change** of the **amount** (partial pressure, concentration, ... **not activity**) of certain reactant or product species.
- Rate is always taken as **positive**
  - Production rate(s) of product(s), consumption rate(s) of reactant(s) ...
- Rate of finite process:  $v = |\Delta [J] / \Delta t|$
- Rate of infinitesimal process:  $v = |d [J] / dt|$   
(Differential or instantaneous rate)

# Stoichiometrically Normalized Reaction Rates

- Consider the species based on which the reaction rate is defined, and define a rate which applies to all the species in a reaction

- Examples:**



- Stoichiometrically normalized rate:**

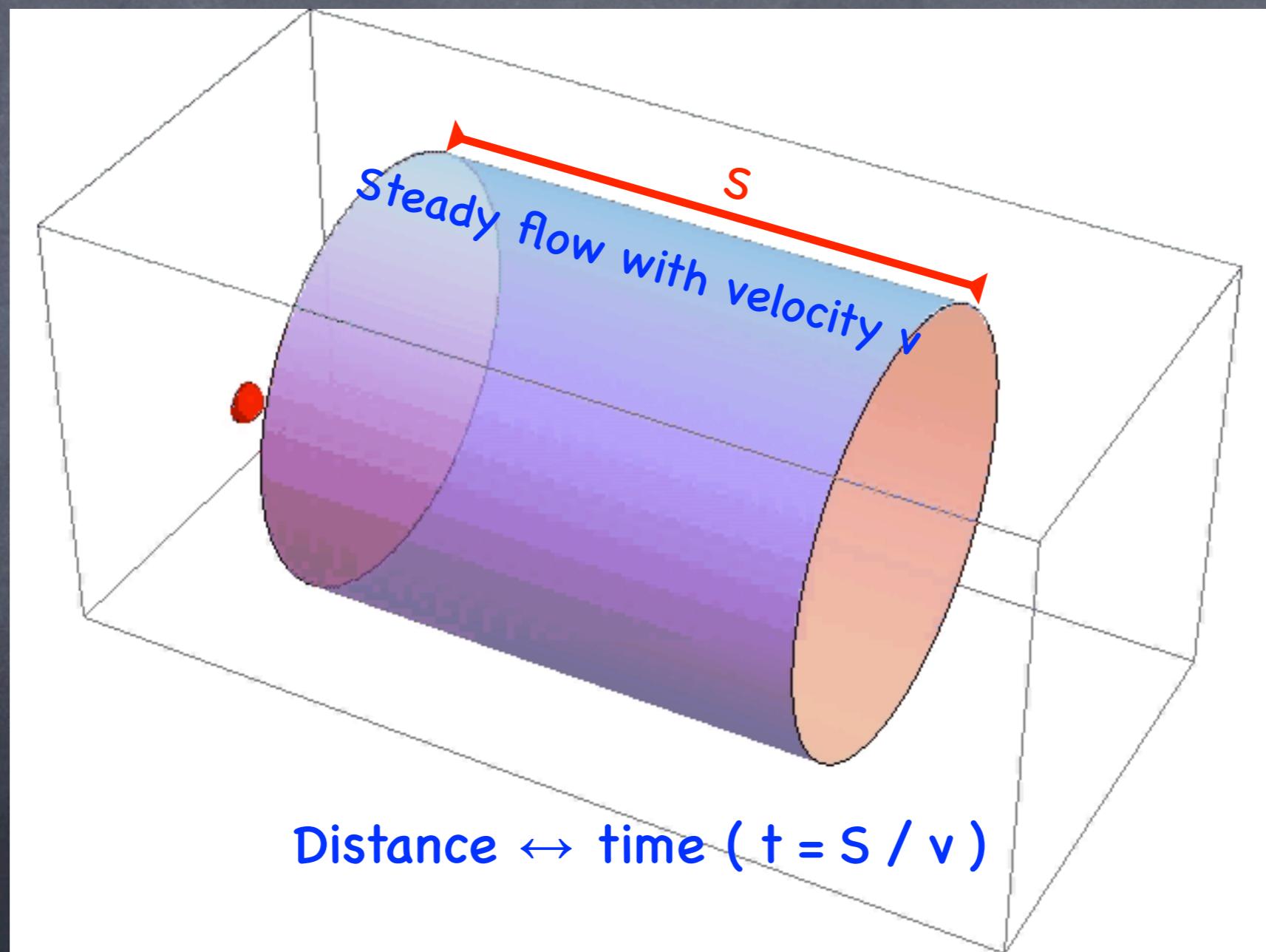


$$v \equiv \frac{d[D]/dt}{v_D} = \frac{d[C]/dt}{v_C} = \frac{d[B]/dt}{-v_B} = \frac{d[A]/dt}{-v_A}$$

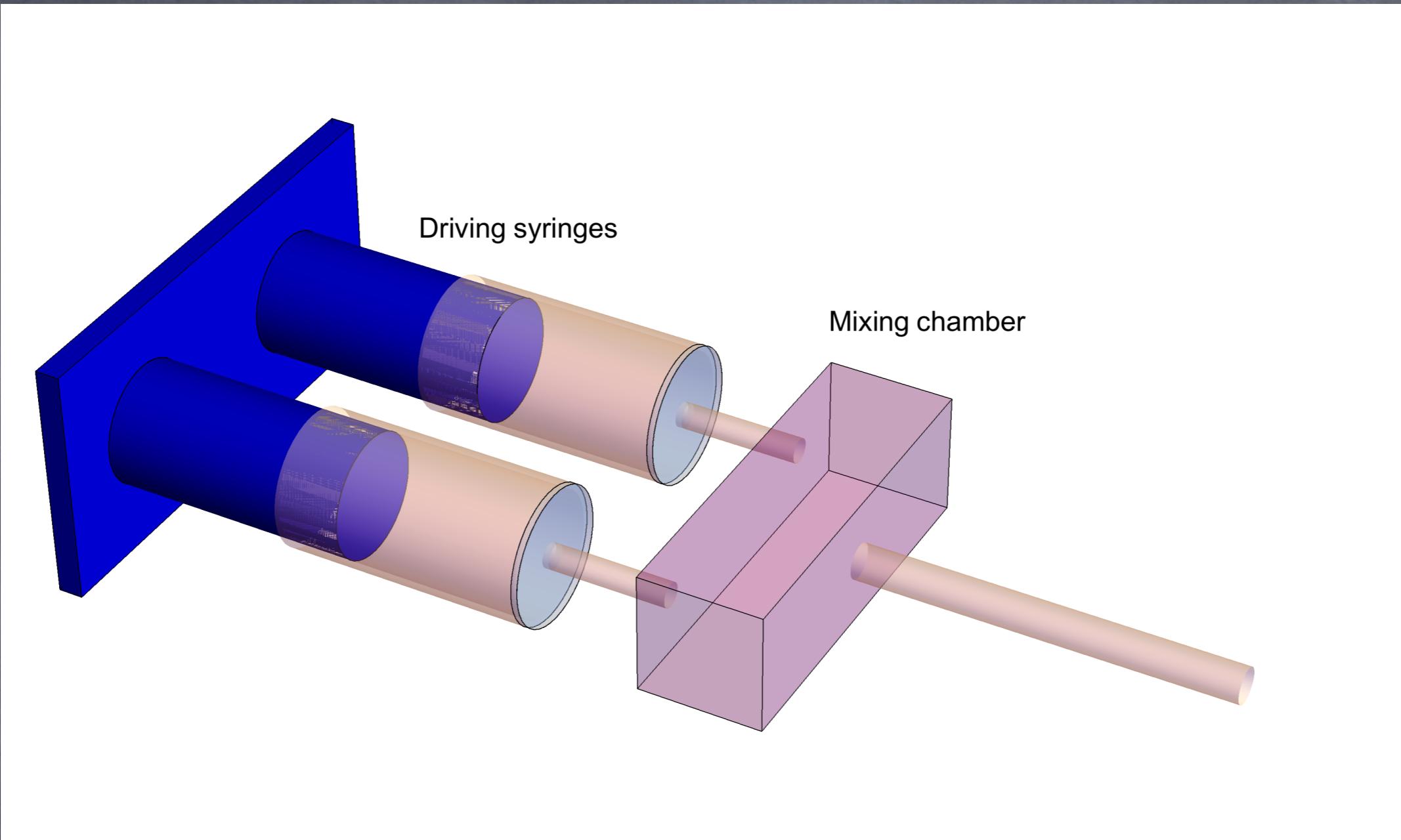
# Measuring Fast Reactions

- ⦿ Real-time measurements with specialized instrumentations
  - ⦿ Flow methods (continuous flow, stopped flow, ...)
  - ⦿ Time-resolved spectroscopic methods
  - ⦿ Relaxation methods  
(ways to apply the above methods)
- ⦿ Batch measurements
  - ⦿ Quenching methods  
(When real-time methods do not have proper dynamic range)

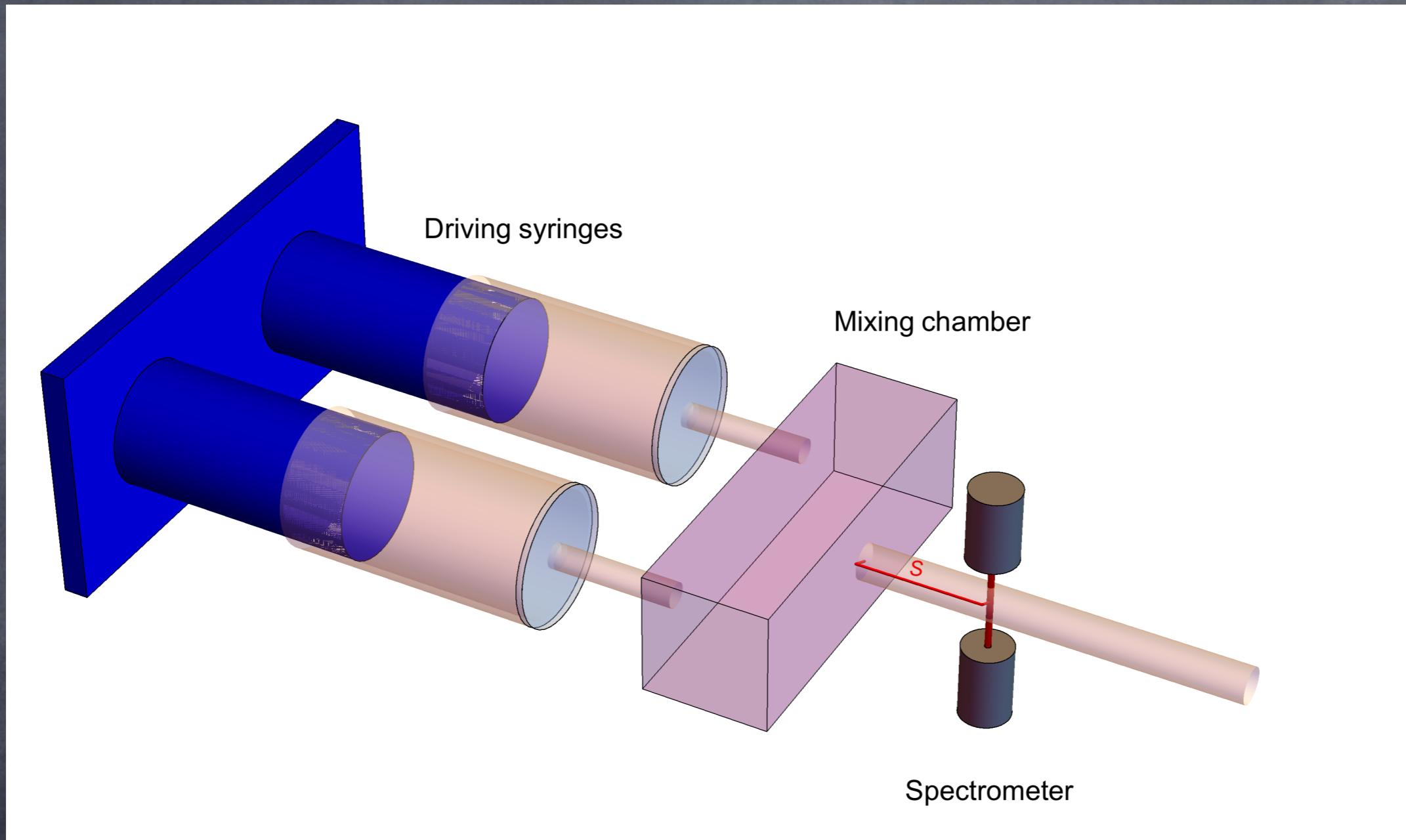
# Flow Method



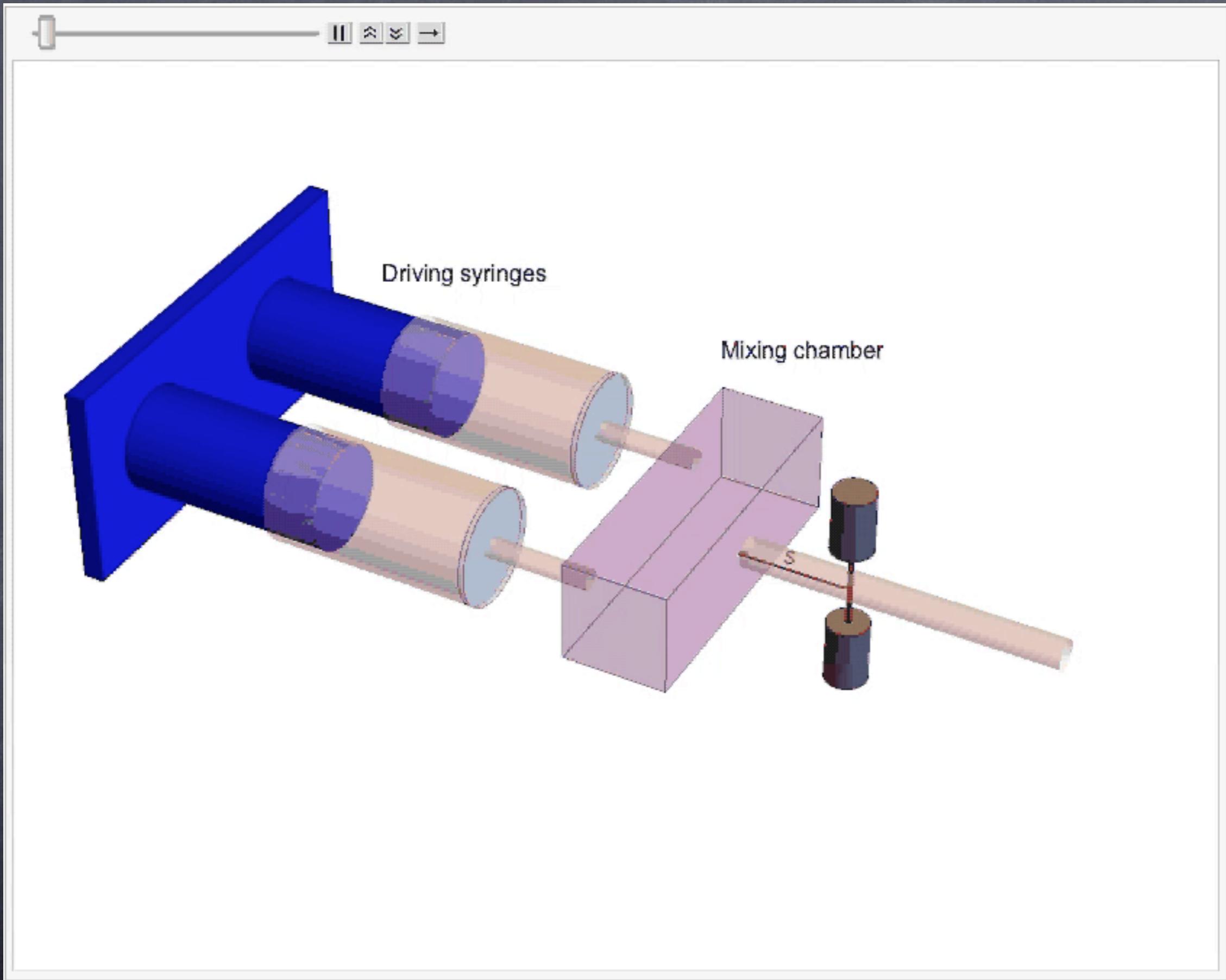
# Continuous-Flow Method



# Continuous-Flow Method



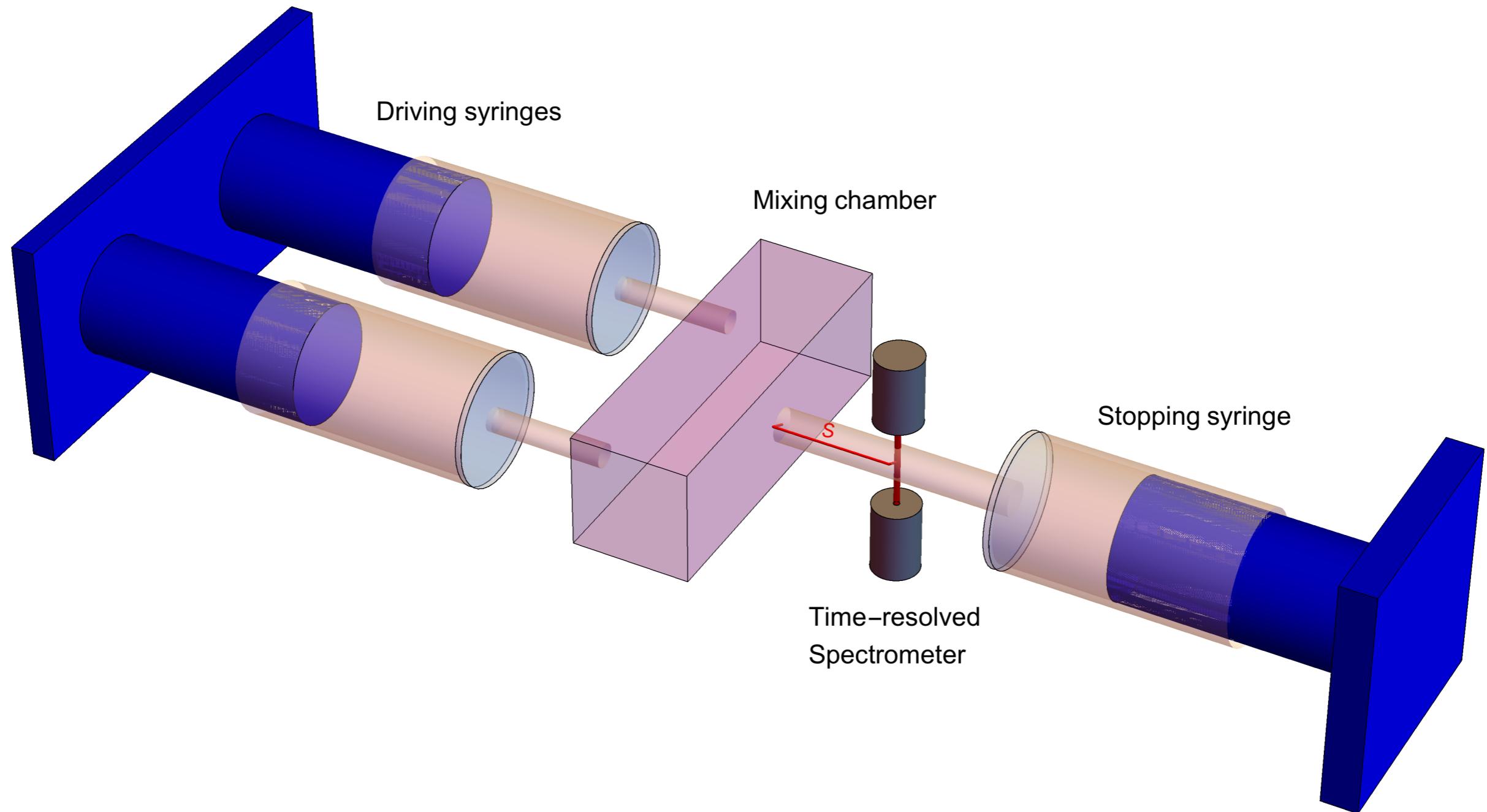
- $t = t_0 + S / v$
- $t_0$ : mixing dead time
- $t$ : time since initiation



# Continuous-Flow Method

- ⦿ Disadvantages:
  - ⦿ Must be liquid-phase reactions
  - ⦿ Require a large amount of reactants
  - ⦿ To achieve higher time-resolution for faster reaction, even more reactants are required
  - ⦿ Time resolution:  $\Delta t = \Delta S / v$   
( $\Delta S$  = spatial resolution)  
Fixed spatial resolution, then  
higher  $v \Rightarrow$  better time resolution

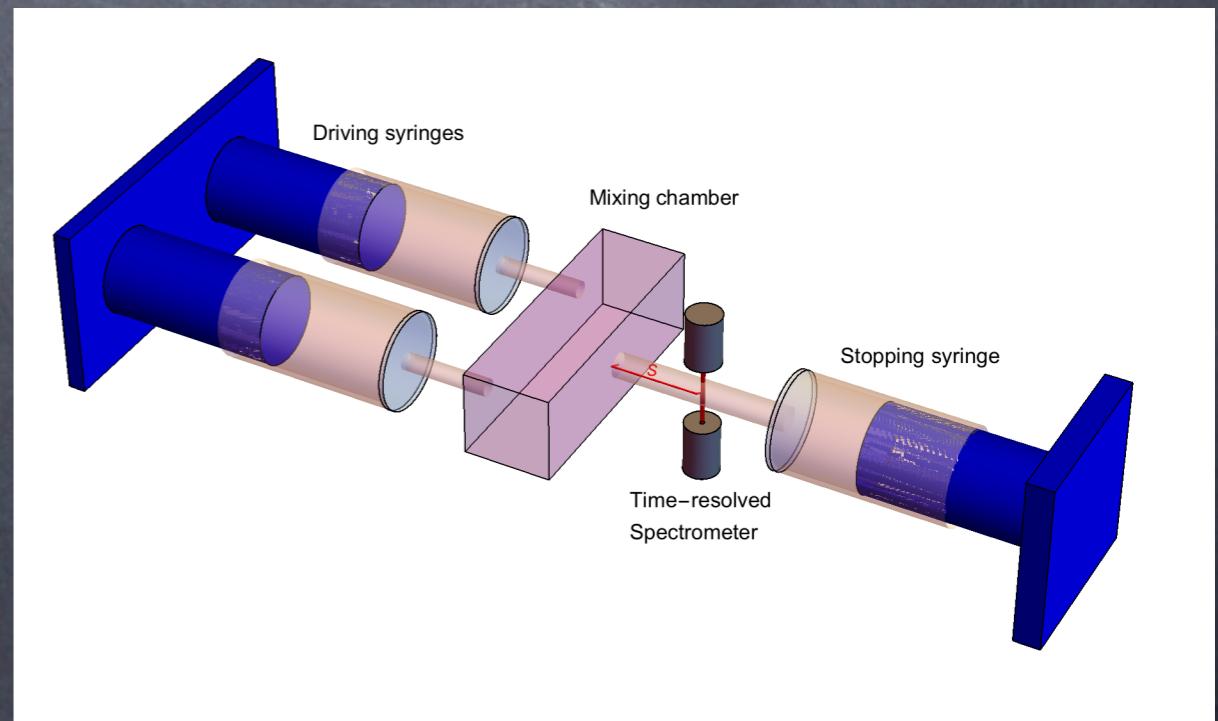
# Stopped-Flow Method



# Stopped-Flow Method

## Procedure:

- Mixing with the flow stopped
- Start the flow
- Stop the flow
- Measurement with time-resolved spectroscopic methods (CD, FTIR, ...)
- **Dead time:**  $t_d = t_0 + S/v$
- **Time-resolution:** depends on the spectroscopic method



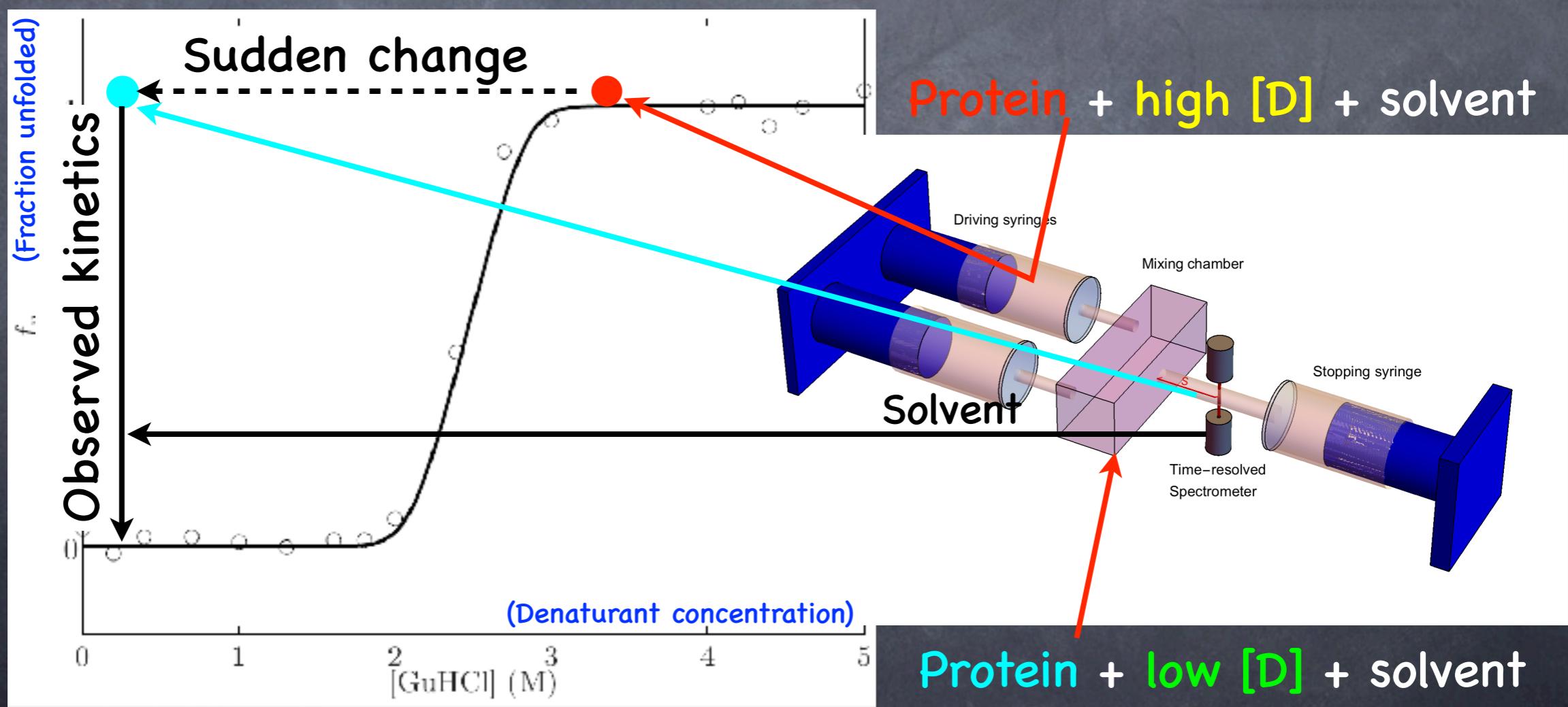
# Relaxation Method

- ⦿ Manfred Eigen, Nobel Prize in Chemistry 1967
- ⦿ If a **parameter** such as temperature, pressure, or denaturant concentration is **switched** to a new value so rapidly that the **chemical system cannot respond during the switching process**, the subsequent **relaxation towards equilibrium** can be monitored.

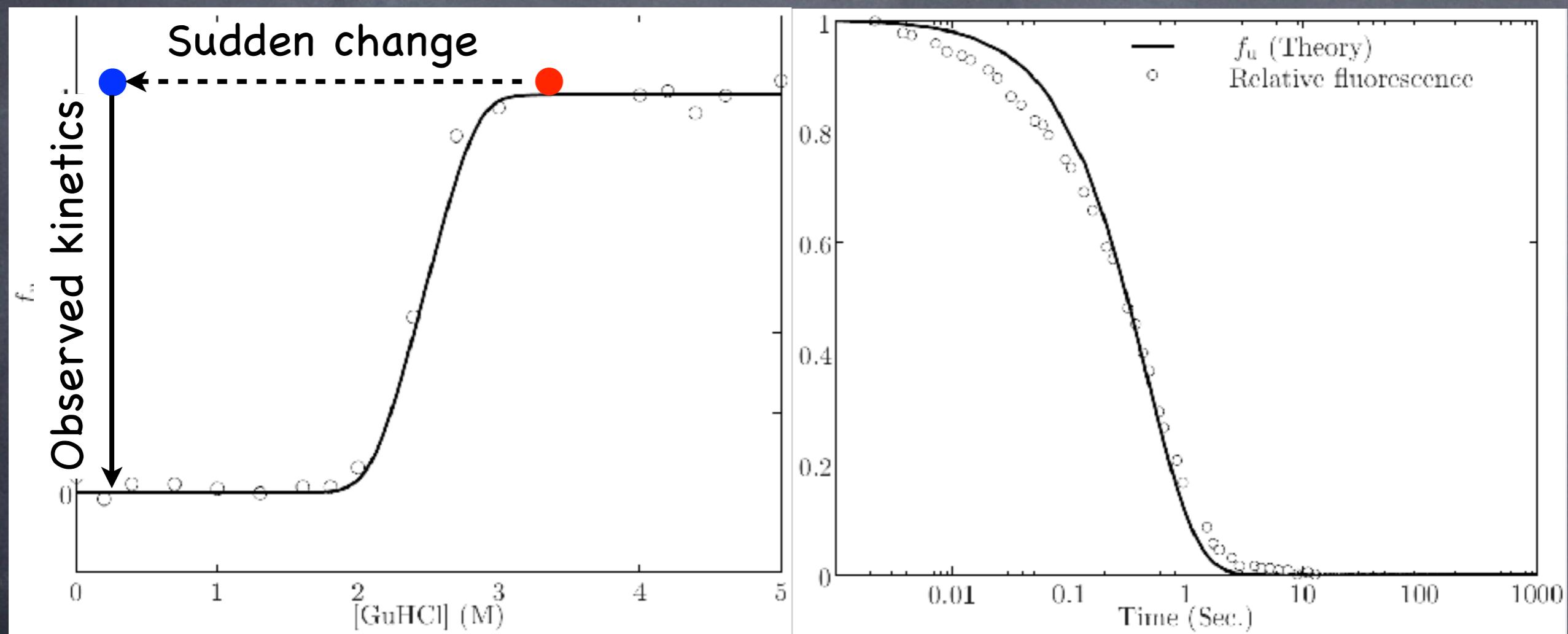


# Protein Folding Kinetics

- Protein unfolding can be induced by denaturant, temperature change, or pH change, etc.
- Protein unfolding/refolding kinetics can be measured by relaxing the unfolding agent



# Refolding Kinetics

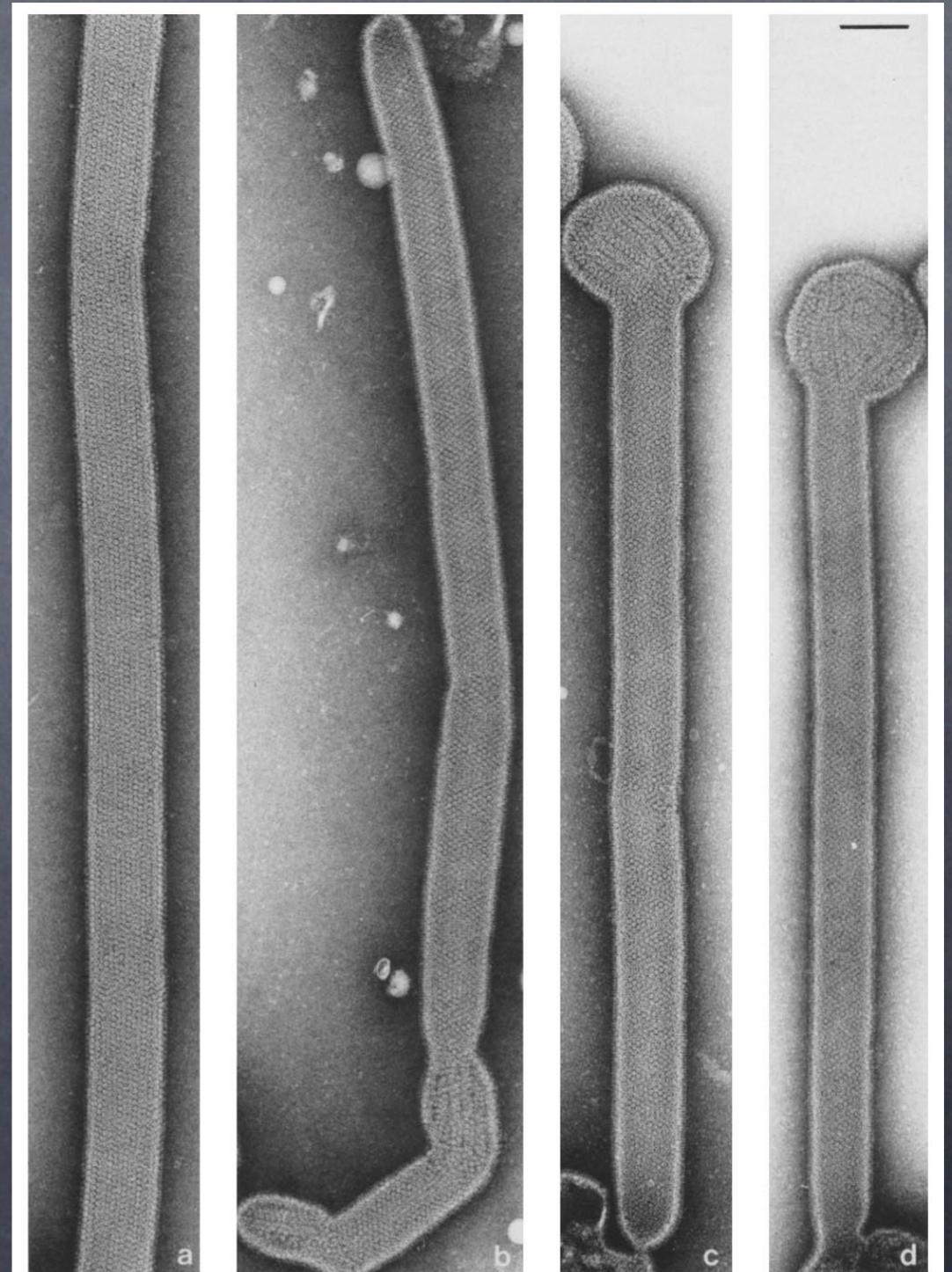


# Quenching Method

- Chemical reaction is stopped some time after it is initiated
- Advantage: composition of the reaction mixture can be studied with slow methods in an off-line manner
- Chemical quench flow: species that can quench the reaction is added into the flow
- Freeze quench: temperature is lowered to an extent that no more reaction will continue

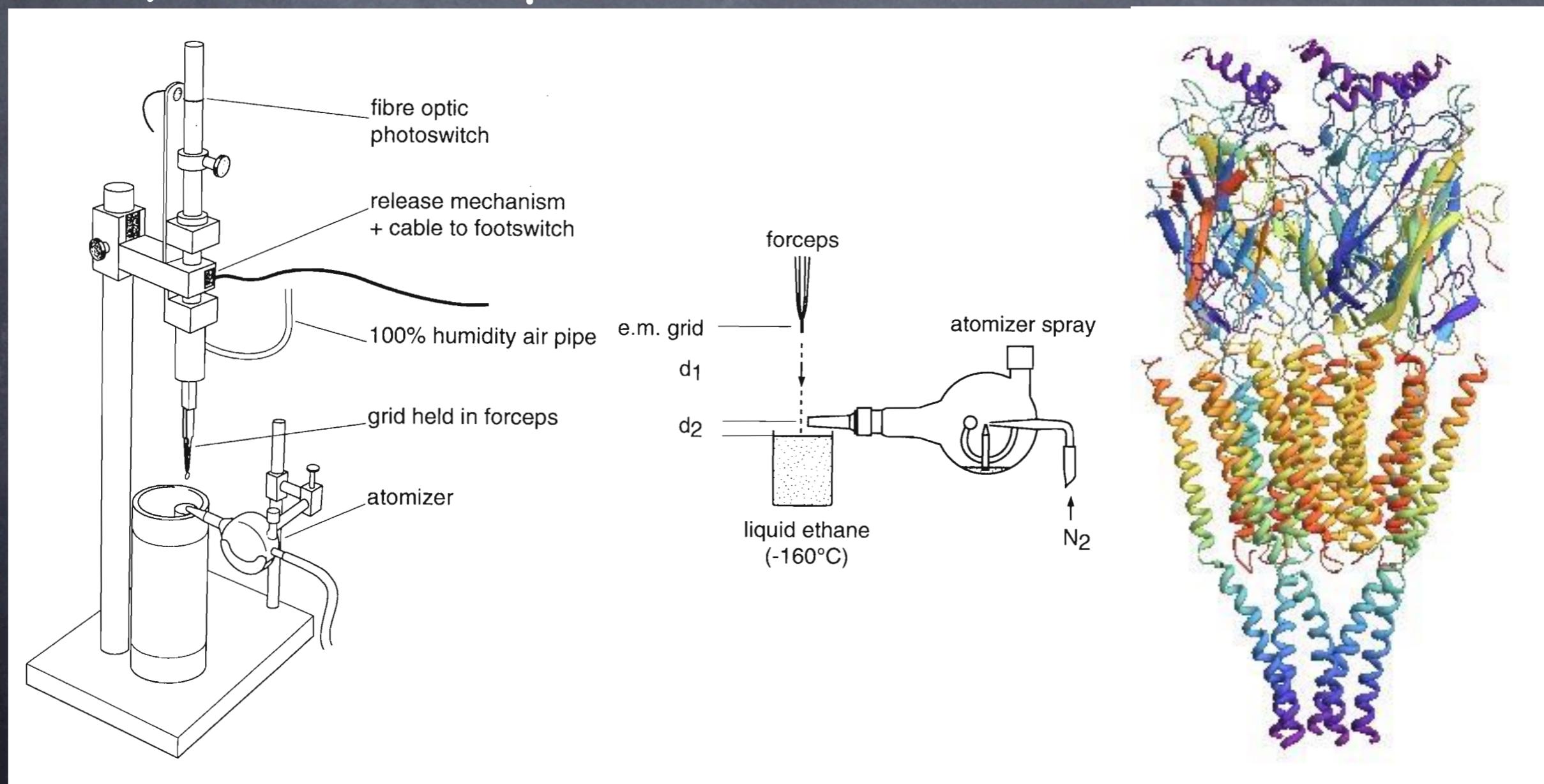
# Freeze quench example: cryo-em

- Nigel Unwin (Cambridge):  
open structure of  
nicotinic acetylcholine  
receptor (nAchR)
- Tube formed by nAchR →



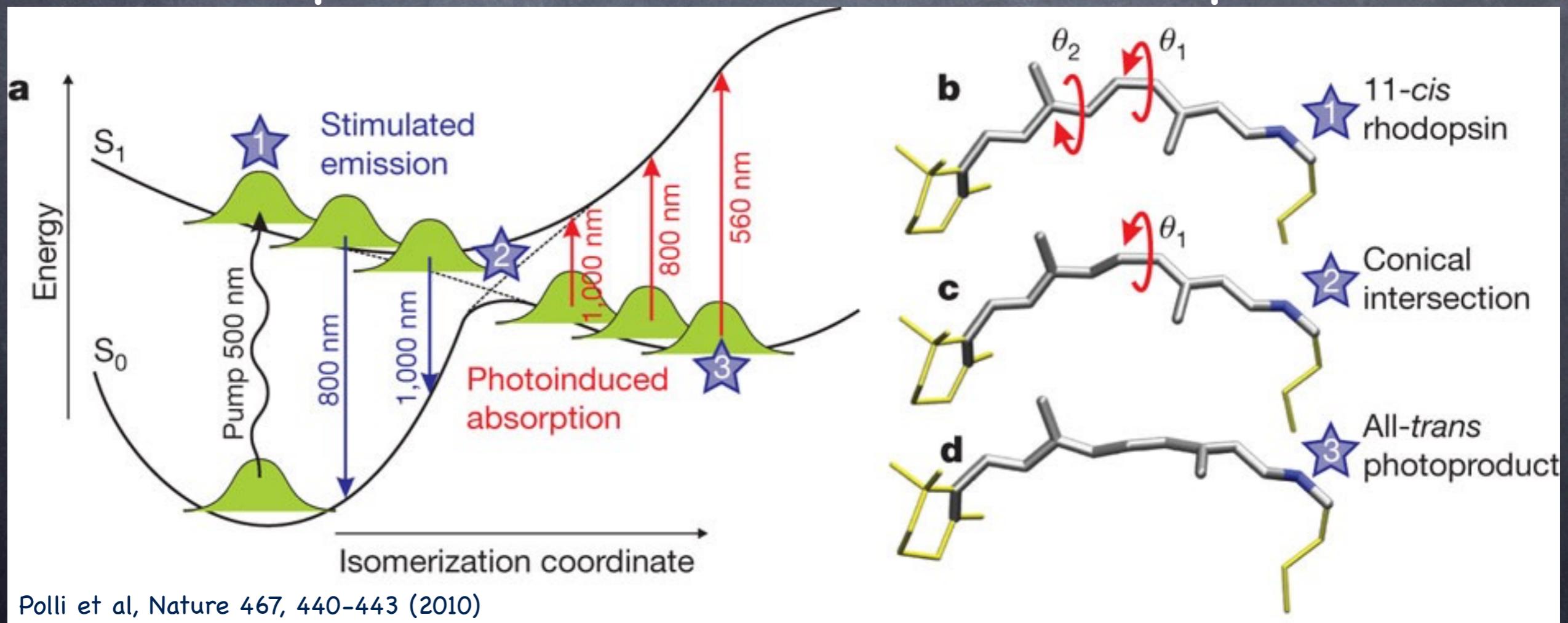
# Free quench example: cryo-em

- Nigel Unwin (Cambridge): open structure of nicotinic acetylcholine receptor (nAChR)

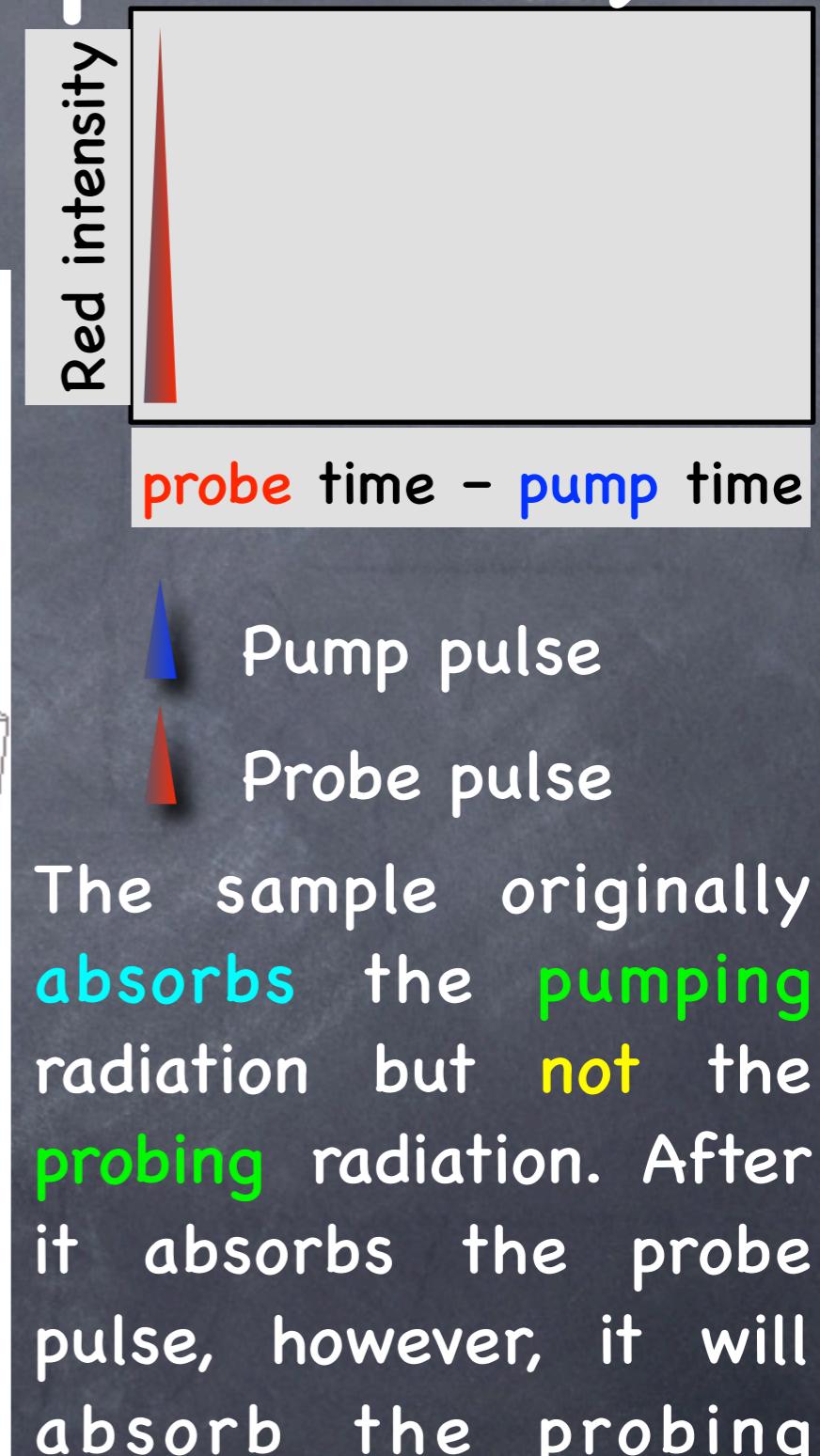
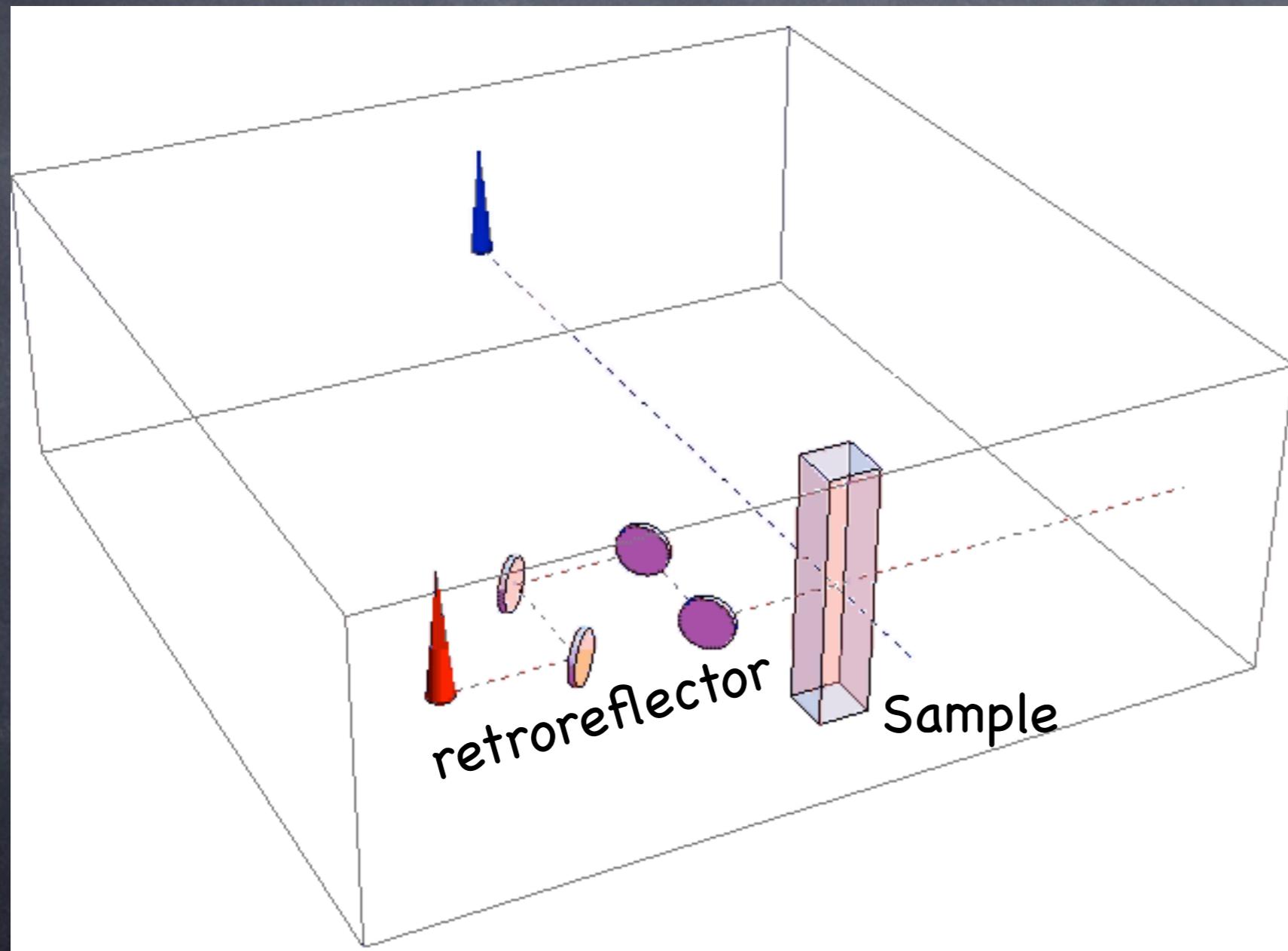


# Flash Photolysis (Pump Probe)

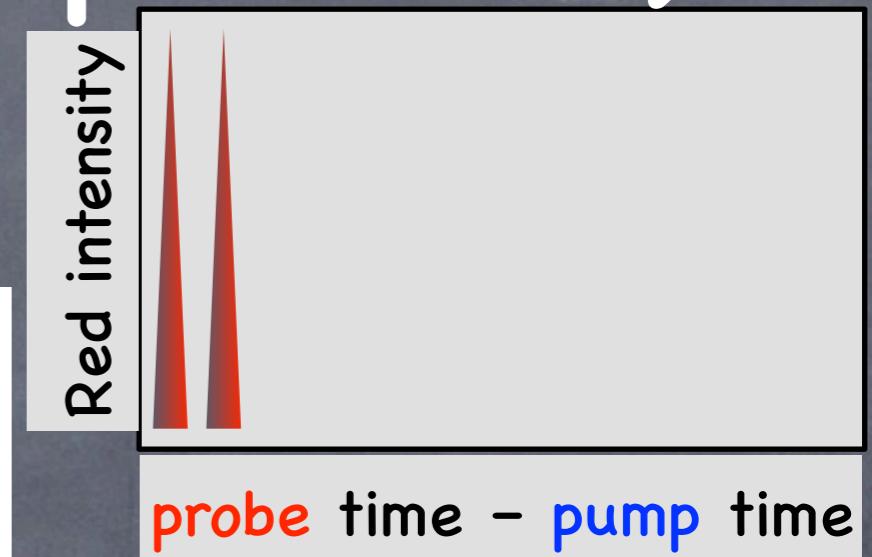
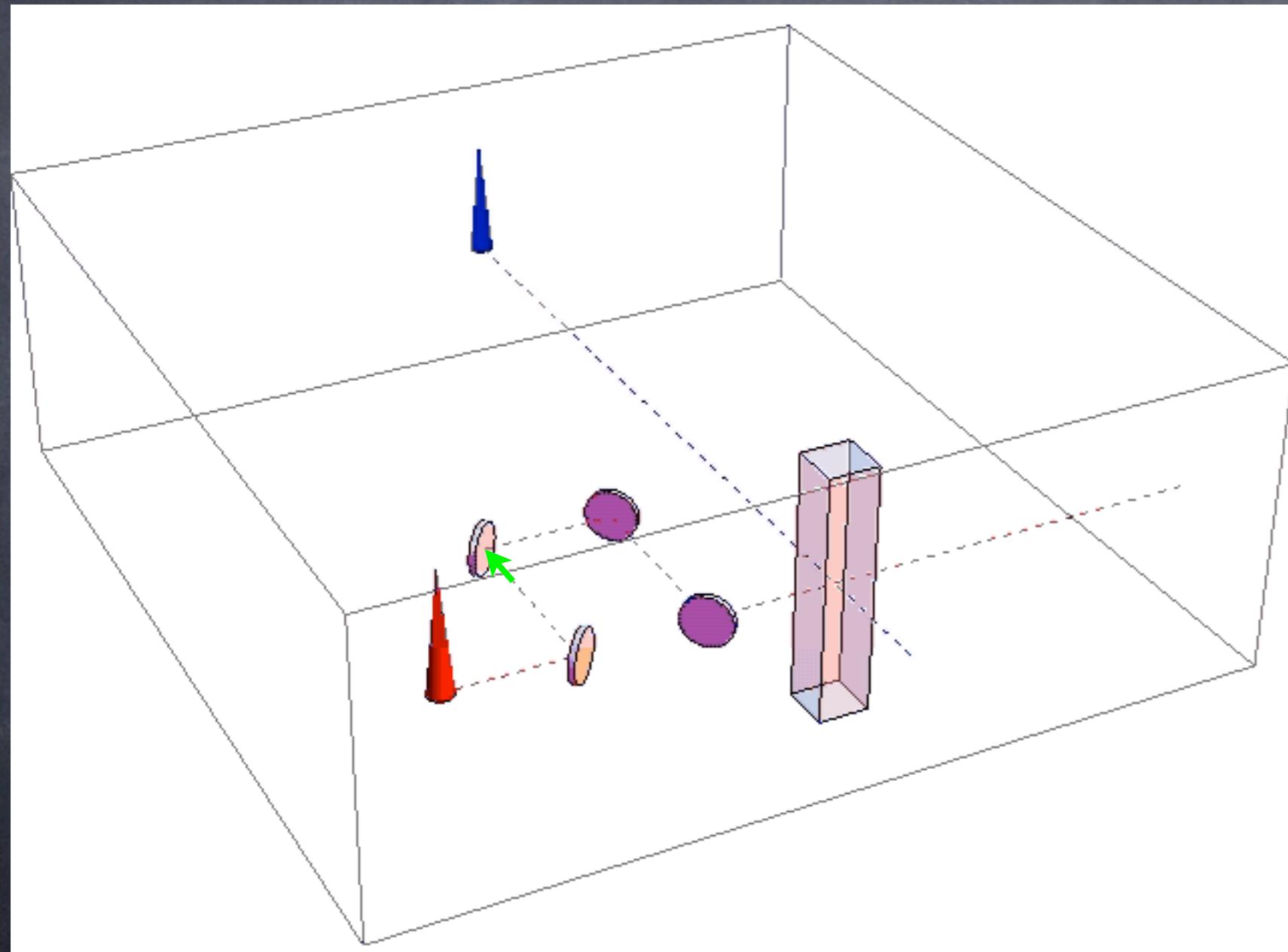
- The absorption spectra of the reactant(s) is different from that of the product(s) in a (photo-induced) reaction
- Example: Photo-isomerization of rhodopsin



# Flash Photolysis (Pump Probe)

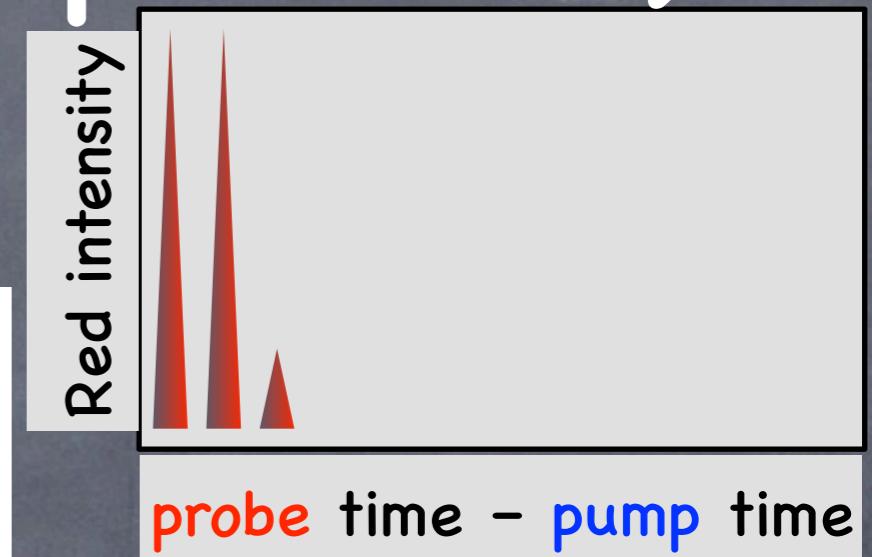
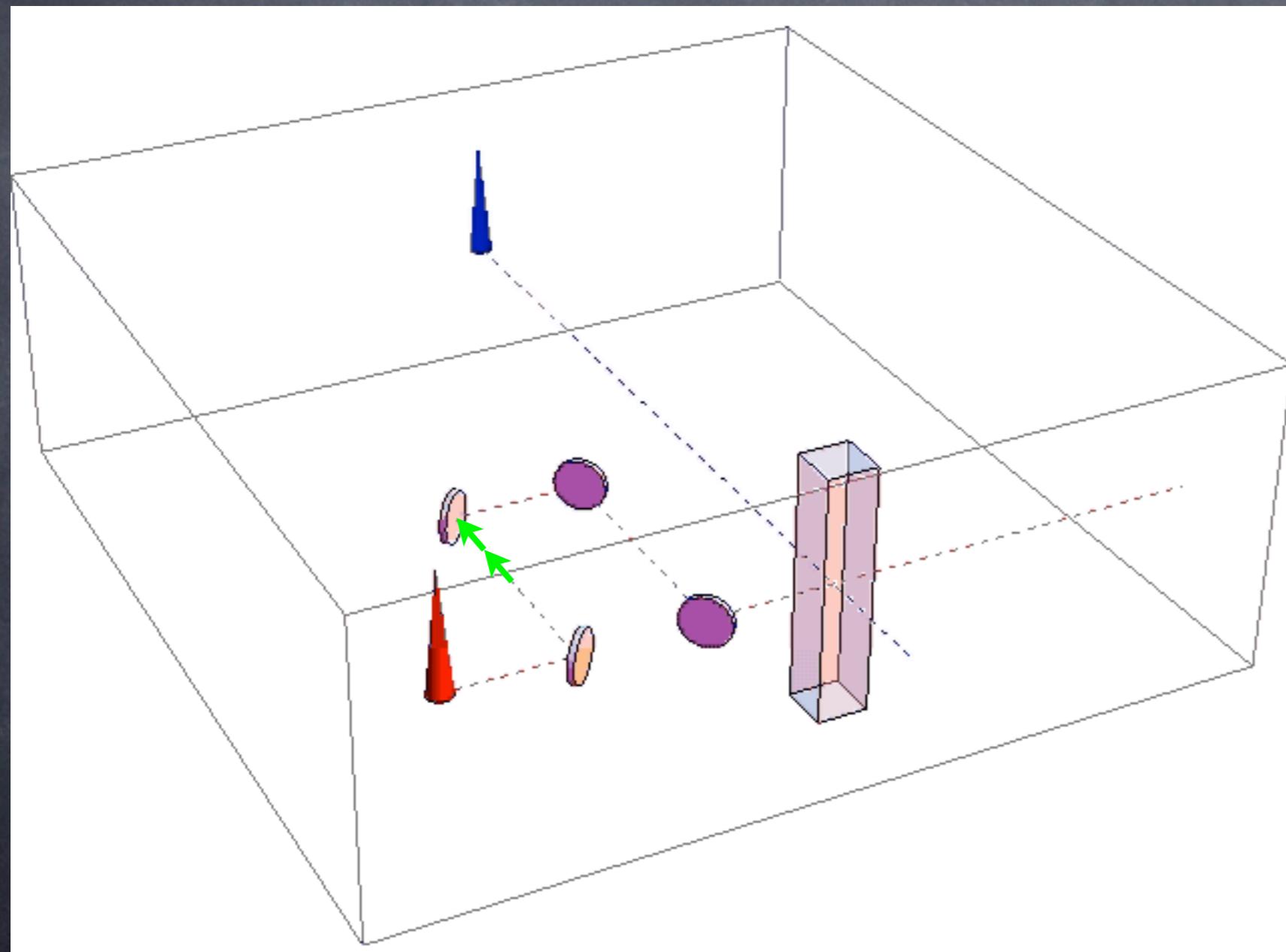


# Flash Photolysis (Pump Probe)



Even if the probe pulse arrives at the same time as the pump pulse, the system may not be able to absorb it, yet.

# Flash Photolysis (Pump Probe)

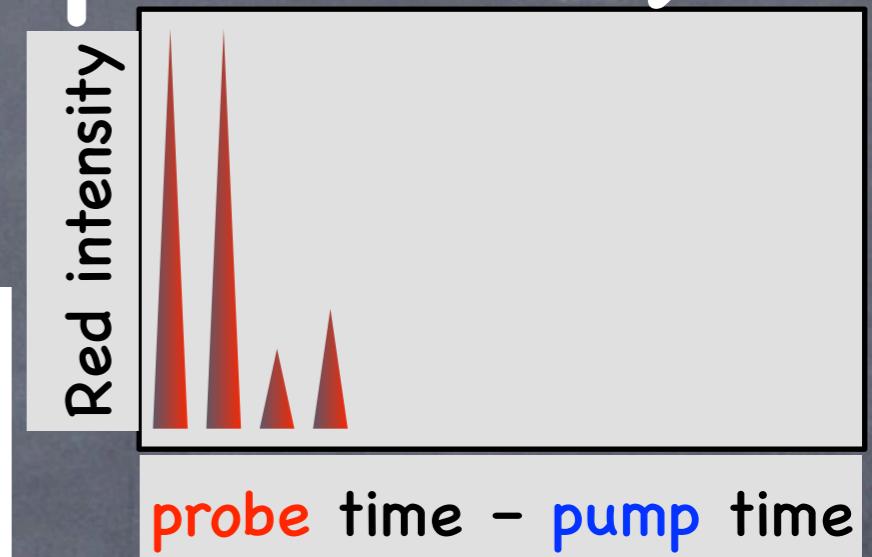
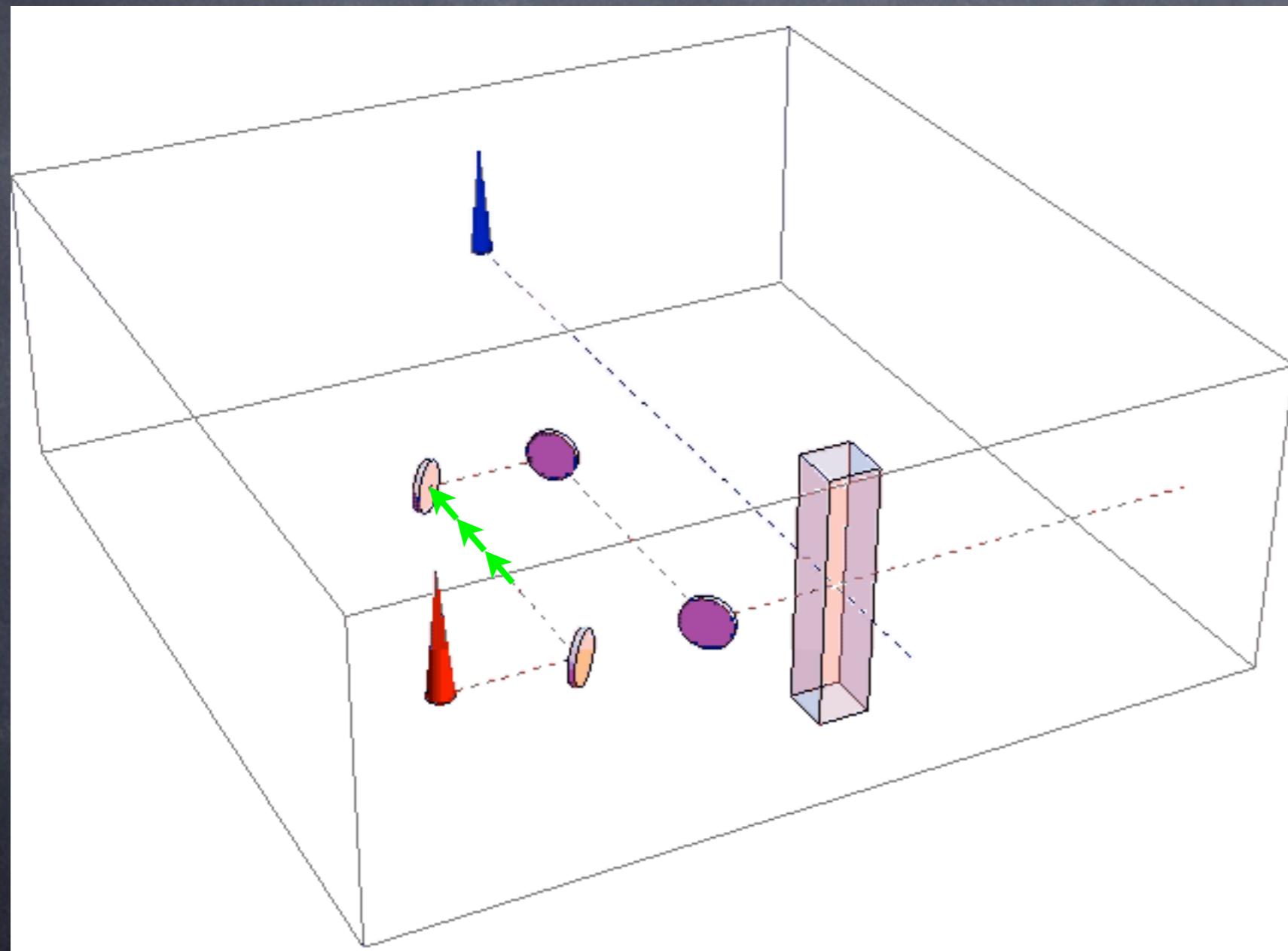


Pump pulse

Probe pulse

If the probe pulse arrives shortly after the arrival of the pump pulse, the system may absorb it quite strongly, resulting in a very weak transmitted probe pulse.

# Flash Photolysis (Pump Probe)

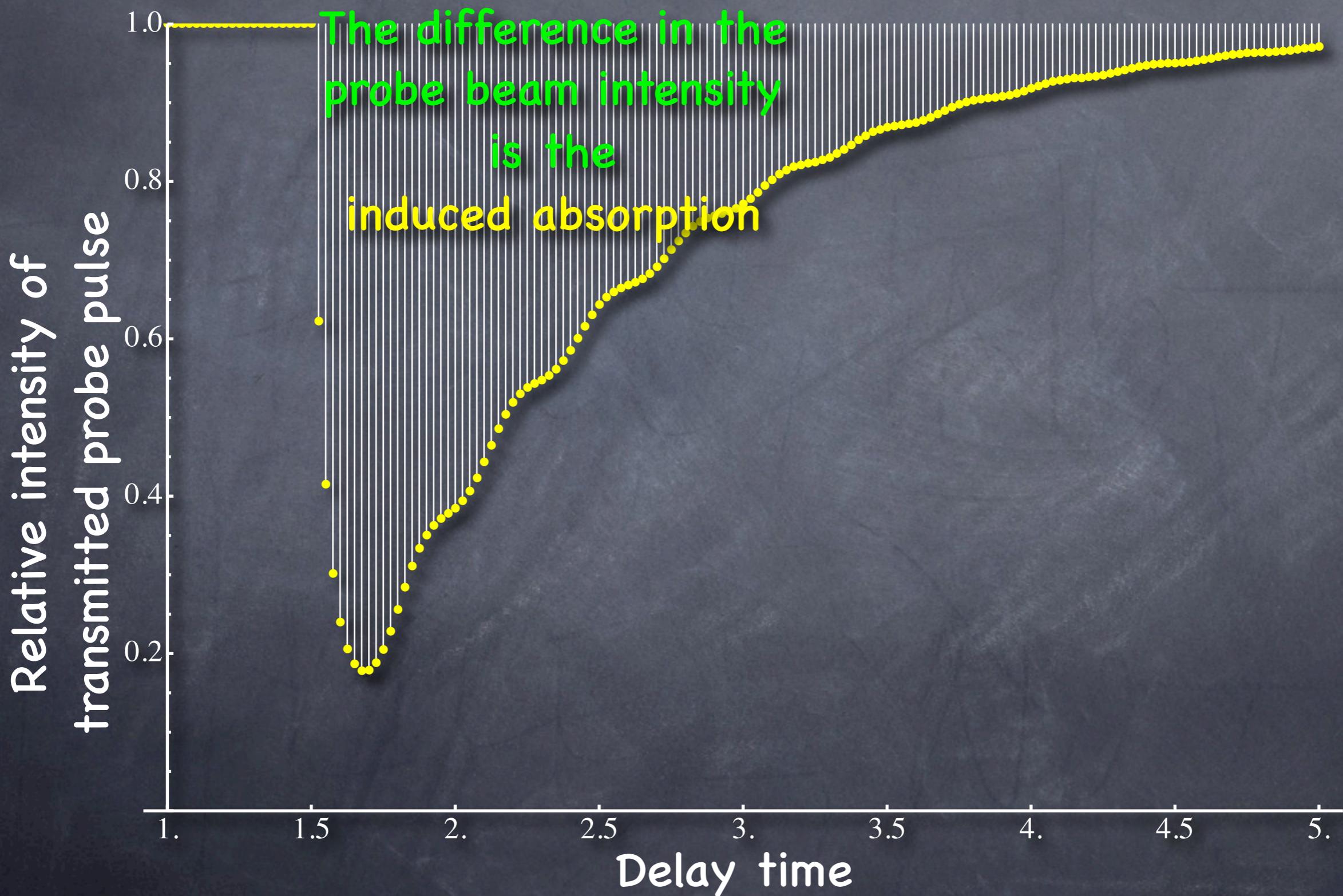


Pump pulse

Probe pulse

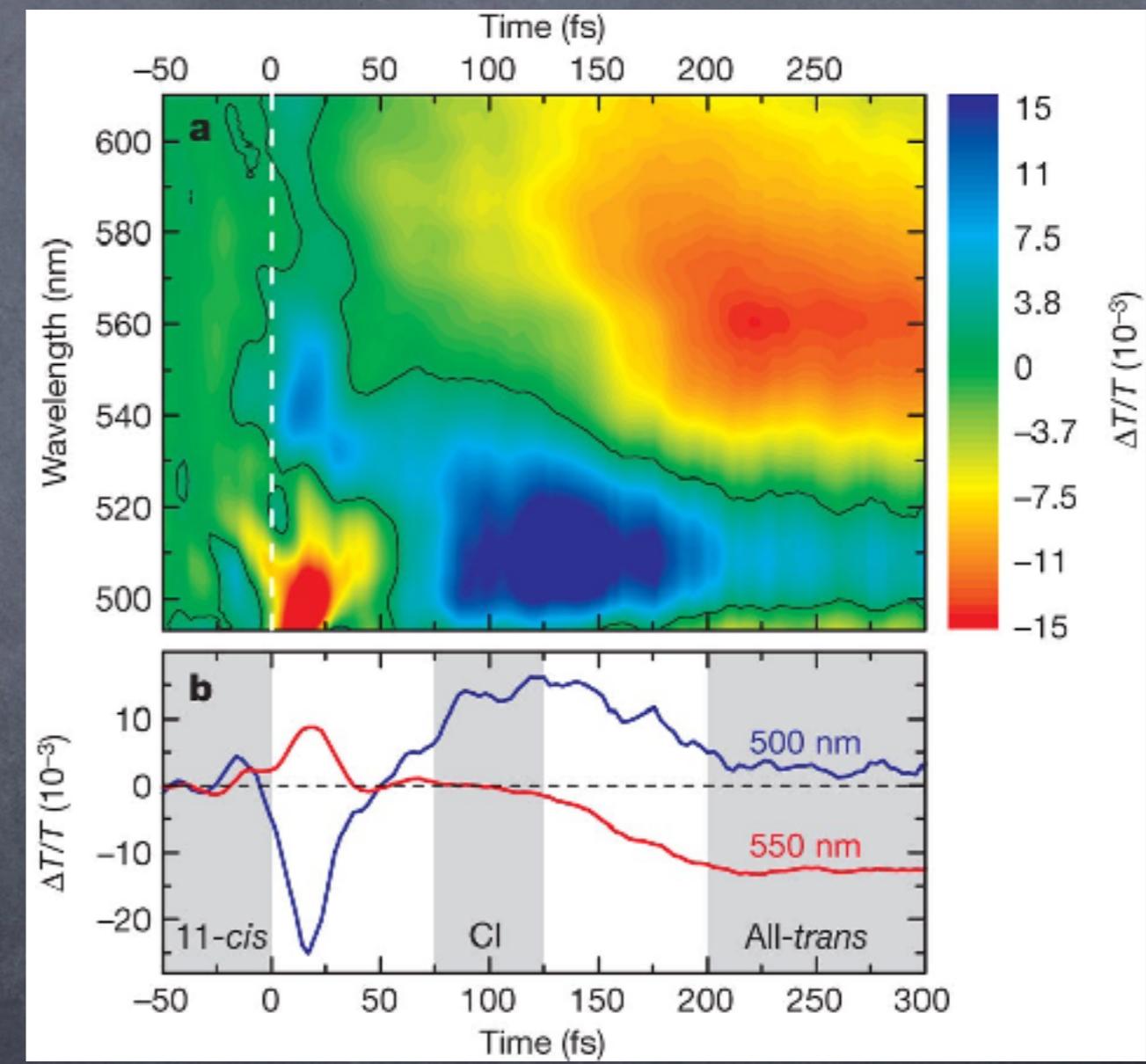
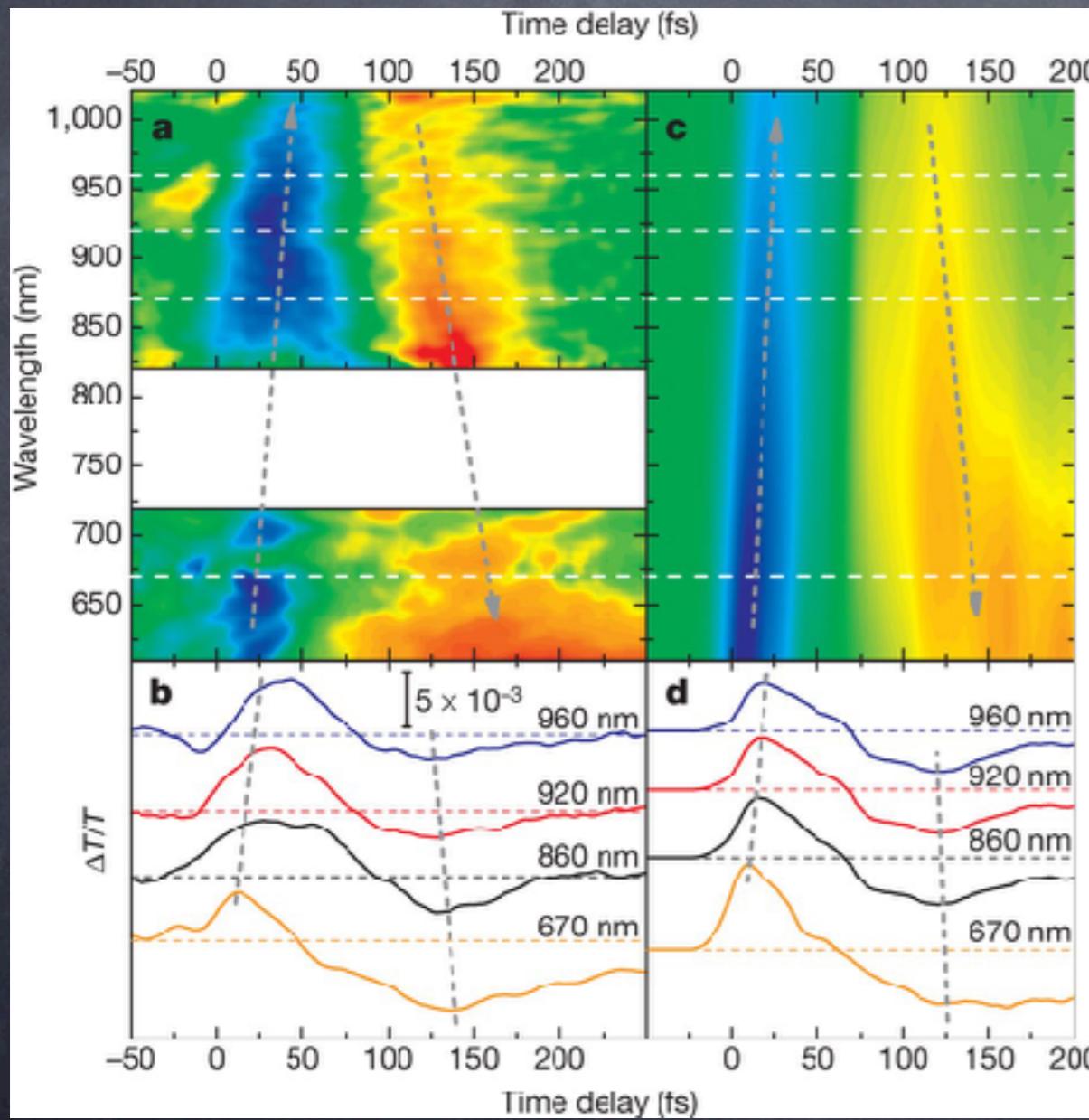
If the probe pulse arrives much later, the 'pumped' molecules would have already relaxed (reacted) so that the probe is not absorbed that much anymore.

# Flash Photolysis (Pump Probe)



# Flash Photolysis (Pump Probe)

Example: Photo-isomerization of rhodopsin



# Empirical Studies of Reaction Kinetics

# Rate Laws / Rate Constants

- The differential rate generally depends on the instantaneous configuration.
- Under given physical condition and configuration, the rate is usually a constant.
- $A + B \rightarrow P$ , typically:  $v \propto [A]^{\varepsilon_A} [B]^{\varepsilon_B}$  (power law)
  - $\varepsilon_A, \varepsilon_B$  are not necessarily integers
- Example of more complicated rate law:

Michaelis-Menten law:  $v = \frac{v_{\max} [S]}{K_m + [S]}$  ← Not a power law

# Rate Laws / Rate Constants

- If the rate law is a power law, for example,

$$v = k [A]^{\varepsilon_A} [B]^{\varepsilon_B}$$

the **order of the reaction** is  $\varepsilon_A + \varepsilon_B$ .

- The constant  $k$  is the **rate constant**.
- There is no definite relation between the stoichiometry coefficients  $\{ v_A, v_B \}$  and the exponents  $\{ \varepsilon_A, \varepsilon_B \}$ .
- The dimension / unit of the rate constant depends on the rate law
- **Example:** first-order reaction  $A \rightarrow B$ , dimension of  $k$ : **time<sup>-1</sup>**

# Determination of Rate Laws

- Rate law = fundamental physical model of the reaction.
- Rate law = expressing the instantaneous rate as a function of time, physical conditions, and system composition.
- Empirical studies: determine **function form** of the **rate law directly from experimental data**.
- Isolation method
- Initial rate method

# Isolation Method

- ⦿ Example:  $A + B + C + \dots \rightarrow P$

Generally  $v = k [A]^{\varepsilon_A} [B]^{\varepsilon_B} [C]^{\varepsilon_C} \dots$

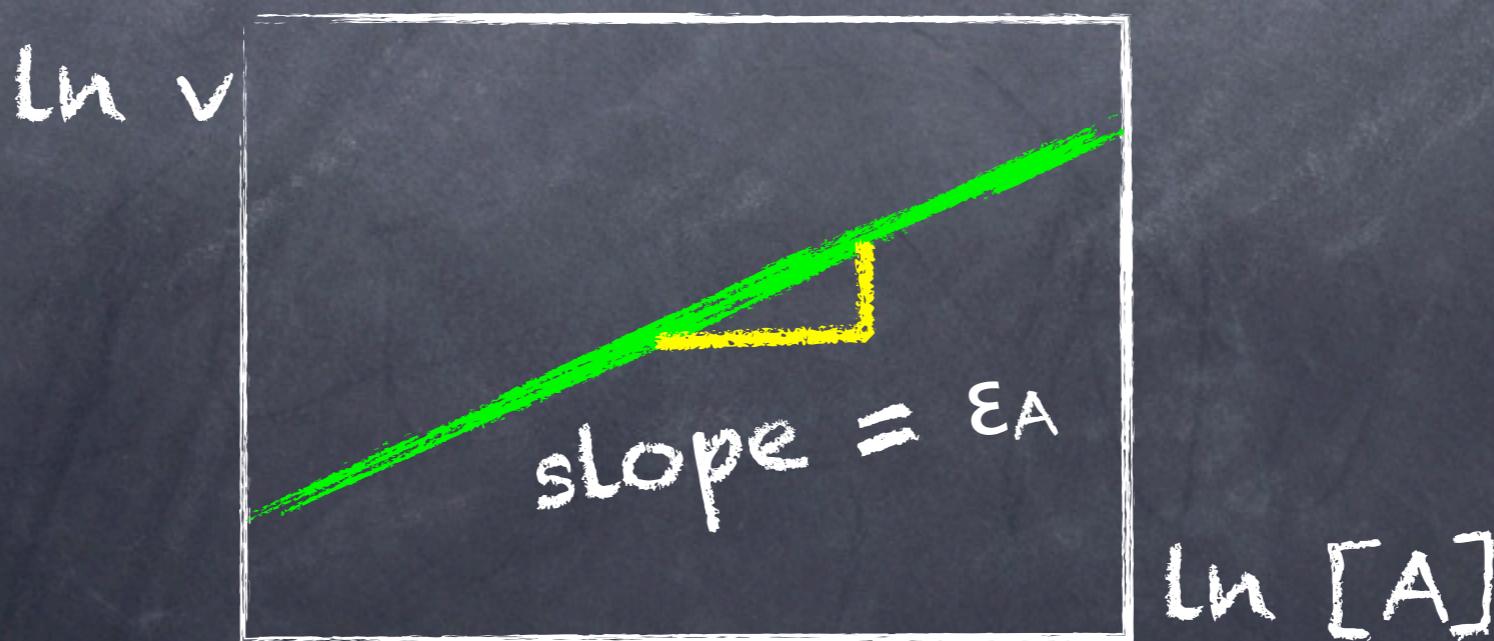
- ⦿ Goal: Find  $k$ ,  $\varepsilon_A$ ,  $\varepsilon_B$ , and so on.
- ⦿ To find  $\varepsilon_A$ , prepare  $B$ ,  $C$ , ... in large excess  $\Rightarrow$   
[B], [C], ... can be approximated as constants  
 $[B]_0, [C]_0, \dots \Rightarrow v = k ([B]^{\varepsilon_B} [C]^{\varepsilon_C} \dots) [A]^{\varepsilon_A}$   
(pseudo- $\varepsilon_A$ -order)

# Isolation Method

- Measure rate and  $[A]$  as functions of time simultaneously.
- Plot the logarithm of rate as function of logarithm of  $[A]$ :

$$\ln v = \ln k'_A + \varepsilon_A \ln [A]$$

- $k'_A$  : effective rate constant



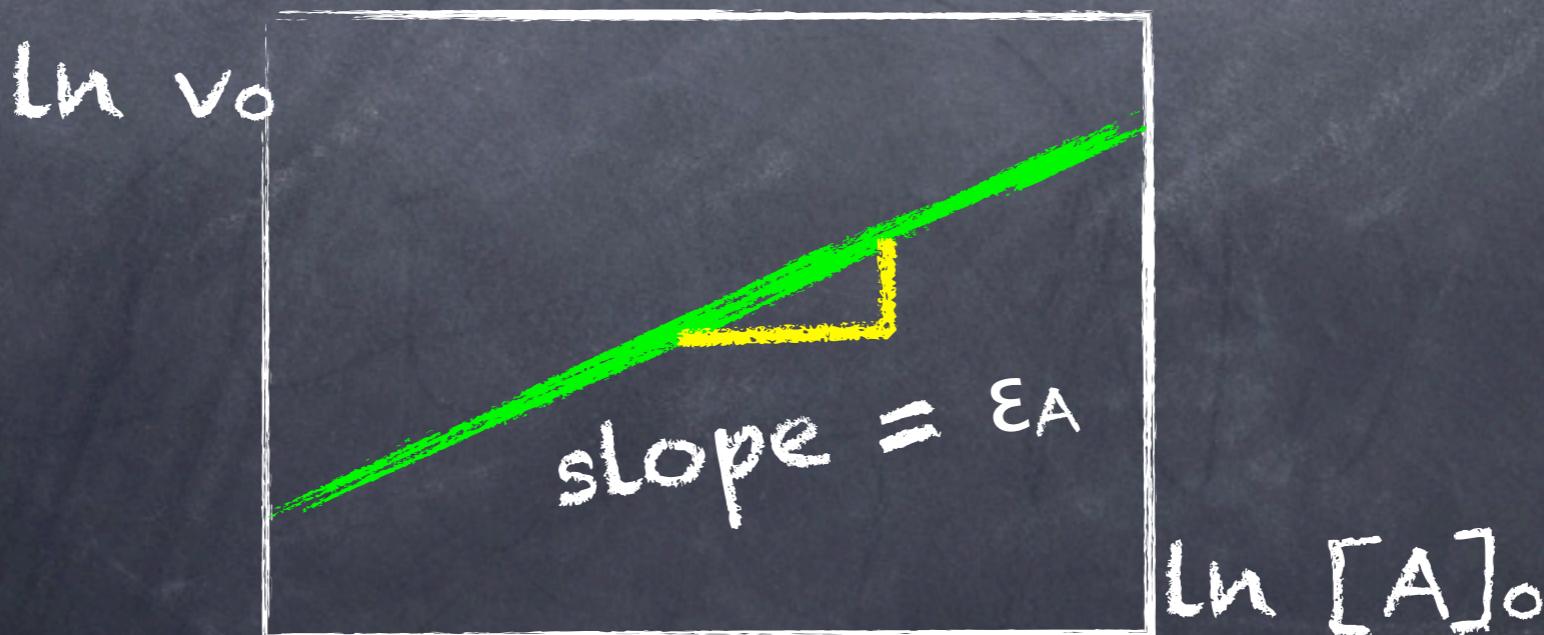
# Initial Rate Method

- ⦿ Isolation method is often combined with the initial rate method.
- ⦿ Different terms in a composite rate law are determined one by one (divide-and-conquer method).
- ⦿ In the isolation method, the “isolation” approximation is worse and worse when more and more A is consumed.

# Initial Rate Method

- If, for different initial concentration of A,  $[A]_0$ , the progress of the reaction is monitored for a short time, even [A] can be treated as a constant. → Initial rate  $v_0$
- Measure  $v_0$  for different  $[A]_0$ , then, like in the isolation method, we have

$$\ln v_0 = \ln k'_A + \varepsilon_A \ln [A]_0$$



# Initial Rate Method

- For power law reaction rate law, linear regression (linear least-square) method can be used to analyze the experimental data.
- Example:** Binding of glucose (G) to the enzyme hexokinase (H). Assume that the rate law is

$$v_0 = k [H]_0^{\varepsilon_H} [G]_0^{\varepsilon_G} \Rightarrow \log_{10} v_0 = \log_{10} (k [H]_0^{\varepsilon_H}) + \varepsilon_G \log_{10} [G]_0$$

Determine  $k$ ,  $\varepsilon_H$ , and  $\varepsilon_G$  according to the experimental data.

# Example

$v_0 / \text{M/s}$	$[G]_0 / \text{mM}$				
	1.00	1.54	3.12	4.02	
$\zeta$	1.34	5.0	7.6	15.5	20.0
$[H]_0 / \text{mM}$	3.00	7.0	11.0	23.0	31.0
$E$	10.0	21.0	34.0	70.0	96.0

# Example

$\log_{10}(v_0)$	$\log_{10}([G]_0)$				
	-3	-2.81	-2.51	-2.4	
$\log_{10}([H]_0)$	-2.87	0.699	0.881	1.19	1.3
	-2.52	0.845	1.04	1.36	1.49
	-2	1.32	1.53	1.85	1.98

# Example

$\log_{10}(v_0)$	$\log_{10}([G]_0)$				
	-3	-2.81	-2.51	-2.4	
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	-2	1.32	1.53	1.85	1.98

$$\langle x \rangle = -2.68, \langle x^2 \rangle = 7.24, \sigma_x^2 = 0.0566$$

# Example

$$k' \equiv k [H]_0^{\varepsilon_H}$$

$\log_{10}(v_0)$	$\log_{10}([G]_0)$					$\log_{10}k'$
	-3	-2.81	-2.51	-2.4		3.72
$\log_{10}([H]_0)$	-2.87	0.699	0.881	1.19	1.3	
	-2.52	0.845	1.04	1.36	1.49	
	-2	1.32	1.53	1.85	1.98	

$$\langle x \rangle = -2.68, \langle x^2 \rangle = 7.24, \sigma_x^2 = 0.0566$$

$$\langle y \rangle = 1.018, \langle xy \rangle = -2.67,$$

$$\text{Cov}(x,y) = 0.0570$$

$$\varepsilon_G = 1.066, \log_{10}k' = 3.72$$

# Example

$$k' \equiv k [H]_0^{\varepsilon_H}$$

$\log_{10}(v_0)$	$\log_{10}([G]_0)$					$\log_{10}k'$
	-3	-2.81	-2.51	-2.4		
$\log_{10}([H]_0)$	-2.87	0.699	0.881	1.19	1.3	3.72
	-2.52	0.845	1.04	1.36	1.49	4.06
	-2	1.32	1.53	1.85	1.98	

$$\langle x \rangle = -2.68, \langle x^2 \rangle = 7.24, \sigma_x^2 = 0.0566$$

$$\langle y \rangle = 1.184, \langle xy \rangle = -3.11,$$

$$\text{Cov}(x,y) = 0.0608$$

$$\varepsilon_G = 1.072, \log_{10}k' = 4.06$$

# Example

$$k' \equiv k [H]_0^{\varepsilon_H}$$

$\log_{10}(v_0)$	$\log_{10}([G]_0)$					$\log_{10}k'$
	-3	-2.81	-2.51	-2.4		
$\log_{10}([H]_0)$	-2.87	0.699	0.881	1.19	1.3	3.72
	-2.52	0.845	1.04	1.36	1.49	4.06
	-2	1.32	1.53	1.85	1.98	4.6

$$\langle x \rangle = -2.68, \langle x^2 \rangle = 7.24, \sigma_x^2 = 0.0566$$

$$\langle y \rangle = 1.670, \langle xy \rangle = -4.41,$$

$$\text{Cov}(x,y) = 0.0619$$

$$\varepsilon_G = 1.0927, \log_{10}k' = 4.60$$

# Example

$$k' \equiv k [H]_0^{\varepsilon_H}$$

$\log_{10}(v_0)$	$\log_{10}([G]_0)$					$\varepsilon_G = 1$
	-3	-2.81	-2.51	-2.4		$\log_{10}k'$
$\log_{10}([H]_0)$	-2.87	0.699	0.881	1.19	1.3	3.72
	-2.52	0.845	1.04	1.36	1.49	4.06
	-2	1.32	1.53	1.85	1.98	4.6

$$\langle x \rangle = -2.46, \quad \varepsilon_H = 1.0169, \quad \log_{10} k = 6.63$$

$$\langle x^2 \rangle = 6.20,$$

$$\sigma_x^2 = 0.128$$

$$\langle y \rangle = 4.124,$$

$$\langle xy \rangle = -10.023,$$

$$\text{Cov}(xy) = 0.1299$$

$$v_0 = k[G]_0[H]_0, \quad k = 4.26 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$$

# Reminder

- ⦿ Due to the finite number of digits and the sensitivity of regression to numbers, everybody may get 'unique' result. Don't mind.

# Example with WL

```
In[•]:= data001 = Import["data001.csv"];  
          |導入
```

```
In[•]:= dgs = GatherBy[data001, First]  
          |按序收集           |第一個
```

```
Out[•]= { {{0.00134, 0.001, 5}, {0.00134, 0.00154, 7.6},  
          {0.00134, 0.00312, 15.5}, {0.00134, 0.00402, 20} },  
          {{0.003, 0.001, 7}, {0.003, 0.00154, 11}, {0.003, 0.00312, 23}, {0.003, 0.00402, 31} },  
          {{0.01, 0.001, 21}, {0.01, 0.00154, 34}, {0.01, 0.00312, 70}, {0.01, 0.00402, 96}} }
```

```
In[•]:= Map[{Log10[#[[1, 1]]], LinearModelFit[Log10[#[[;; , 2 ;; 3]]], lg, lg][0]} &,  
          |映射   |常用對數           |線性模型配適           |常用對數  
          dgs]
```

```
Out[•]= { {-2.8729, 3.69244}, {-2.52288, 4.03258}, {-2., 4.55552} }
```

```
In[•]:= LinearModelFit[%, lh, lh]  
          |線性模型配適
```

```
Out[•]= FittedModel[ 6.53324 + 0.989646 lh ]
```

$$\log_{10} v_0 = 6.53324 + \log_{10} [\text{H}]_0 + \log_{10} [\text{G}]_0$$

$$v_0 = 3.41 \times 10^6 [\text{H}]_0 [\text{G}]_0$$

# Integrated Rate Law

- From the differential (instantaneous) rate law we find

$$v = \frac{1}{\nu_A} \left| \frac{d[A]}{dt} \right| = k [A]^{\varepsilon_A}$$

- Often, we may be more concerned about  $[A](t)$
- 1st-order rate law:

$$v = -\frac{1}{\nu_A} \frac{d[A]}{dt} = k [A] \Rightarrow \boxed{-k\nu_A dt} = \frac{d[A]}{[A]} = \boxed{d \ln[A]}$$

$$\int_{[A]_0}^{[A]} d \ln [A]' = -k\nu_A \int_0^t d\tau \Rightarrow \ln \frac{[A]}{[A]_0} = -k\nu_A t$$

# 1st-Order Rate Law

- ⦿ **Half-life  $t_{1/2}$**  : time when  $[A] = [A]_0 / 2$ .

Let  $v_A = 1$  and

$$\ln \frac{[A]_0/2}{[A]_0} = -k v_A t_{1/2} \Rightarrow t_{1/2} = \frac{\ln 2}{k}$$

- ⦿ For a 1st-order reaction, half-life is independent of  $[A]_0$ .
- ⦿ **Lifetime (time constant)**:  $\tau = 1/k$
- ⦿ When  $t = \tau$ ,  $[A] = [A]_0 e^{-1}$ .
- ⦿ **Integrated rate law**:  $[A] = [A]_0 e^{-kt} = [A]_0 e^{-t/\tau}$

# Meaning of Lifetime

- At time  $t = 0$ , in one liter of the reaction mixture, there are  $[A]_0$  moles or  $N_A \times [A]_0$  molecules inside.
- At time  $t$ , there are  $N_A \times [A]$  molecules in the reaction mixture, where  $[A] = [A]_0 e^{-kt}$ .
- During time  $t$  and  $t + dt$ , the number of A molecules which react and disappear is  $k \times N_A \times [A] \times dt$ . The time that these molecules remain as A is  $t$ .
- The average time that each molecule A remain as A, that is, their lifetime as A, is

$$\tau = \frac{\int_{t=0}^{\infty} k N_A [A]_0 e^{-kt} t dt}{N_A [A]_0} = \frac{1}{k} \int_{x=0}^{\infty} x e^{-x} dx = \frac{1}{k}$$

# Integrated Rate Law w/ WL

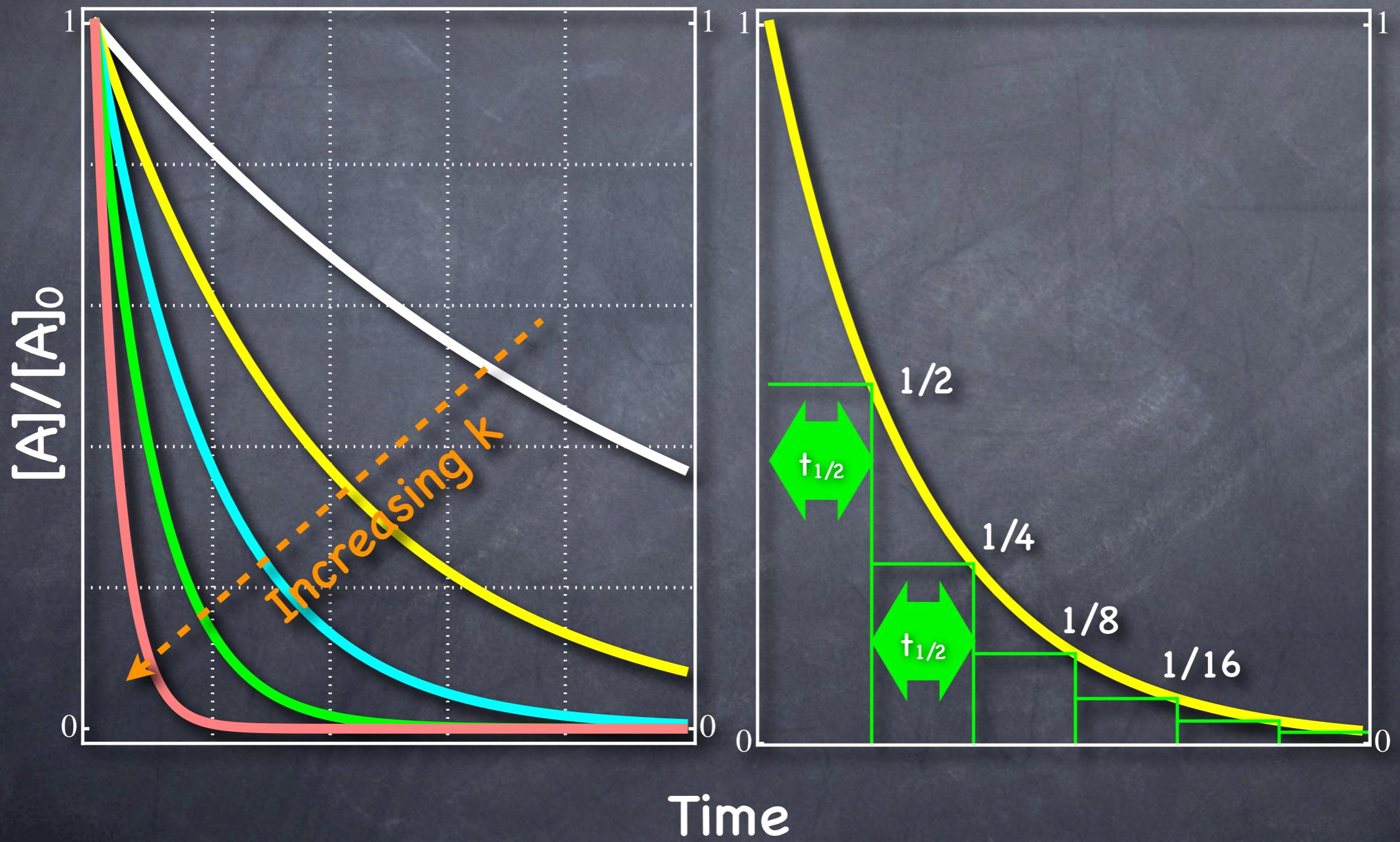
```
In[•]:= asol = Block[{k, a0, νA, deq, $Assumptions = k > 0 && a0 > 0 && νA > 0},  
  SetAttributes[{k, a0, νA}, Constant];  
  deq = -D[a[t], t] / νA == k a[t];  
  DSolve[deq && a[0] == a0, a[t], t]  
 ]
```

```
Out[•]= { {a[t] → a0 e-k t νA} }
```

```
In[•]:= Block[{k, a0, νA, kim, $Assumptions = k > 0 && a0 > 0 && νA > 0},  
  SetAttributes[{k, a0, νA}, Constant];  
  kim[t_] = a[t] /. asol[[1]];  
  Solve[kim[t2] == a0 / 2, t2, Reals]  
 ]
```

```
Out[•]= { {t2 →  $\frac{\text{Log}[2]}{k \nu A}$ } }
```

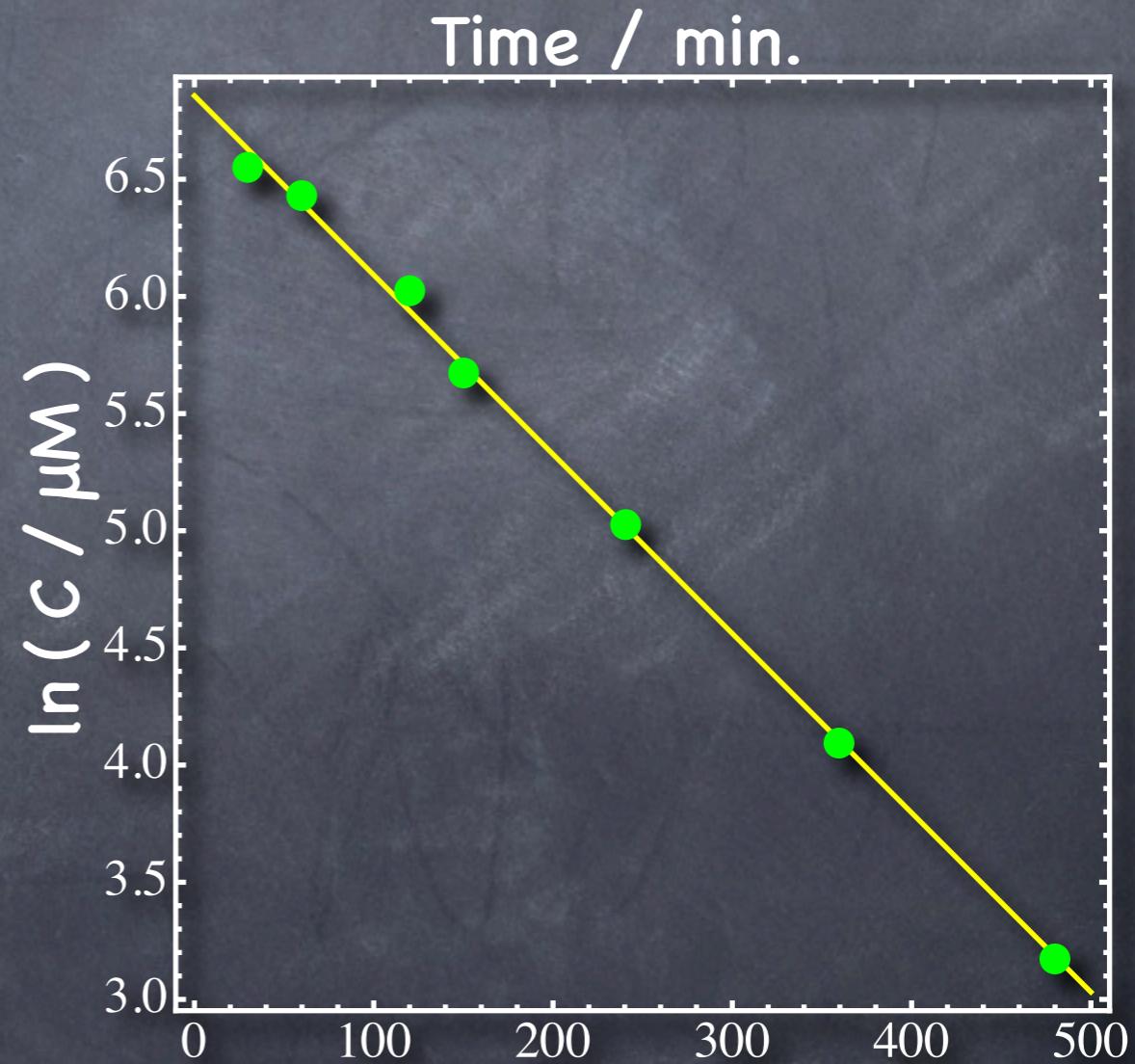
# 1st-Order Rate Law



# 1st-Order Rate Law

- If  $\ln [A]$  is plotted against  $t$ , the result should be a straight line for 1st-order reaction. If it is not a straight line, the reaction may not be a 1st-order reaction.

$t$ / min.	$C$ / $\mu\text{M}$	$\ln C$
30	699	6.55
60	622	6.43
120	413	6.02
150	292	5.68
240	152	5.02
360	60	4.09
480	24	3.18



# Exercise

- Use the file data002.csv, which corresponds to the raw data in the previous slide, and fit  $\ln[A]$  or  $\log_{10}[A]$  to time  $t$ . Plot the result (data and model) in log-linear plot like that in the previous slide, or in linear-linear plot if you like to practice.

# 2nd-Order Reaction

- Case 1, A → P:  $d[A]/dt = -k[A]^2$

$$-\frac{d[A]}{[A]^2} = d\left(\frac{1}{[A]}\right) = k dt$$

$$\Rightarrow \frac{1}{[A]} = \frac{1}{[A]_0} + kt$$

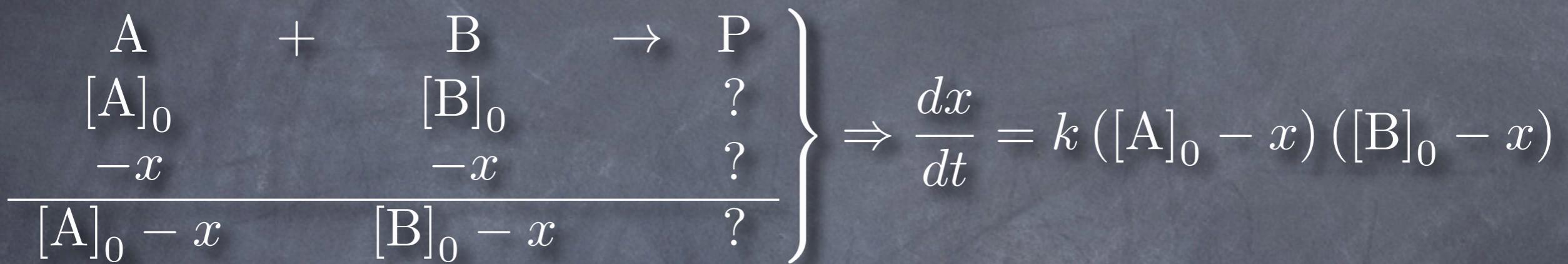
- Half-life:

$$[A]_0 = [A](1 + kt [A]_0) \Rightarrow [A] = \frac{[A]_0}{1 + kt [A]_0}$$

$$\frac{2}{[A]_0} = \frac{1}{[A]_0} + kt_{1/2} \Rightarrow kt_{1/2} = \frac{1}{[A]_0} \Rightarrow t_{1/2} = \frac{1}{k [A]_0}$$

# 2nd-Order Reaction

- Case 2,  $A + B \rightarrow P$ :  $d[A]/dt = d[B]/dt = -k[A][B]$

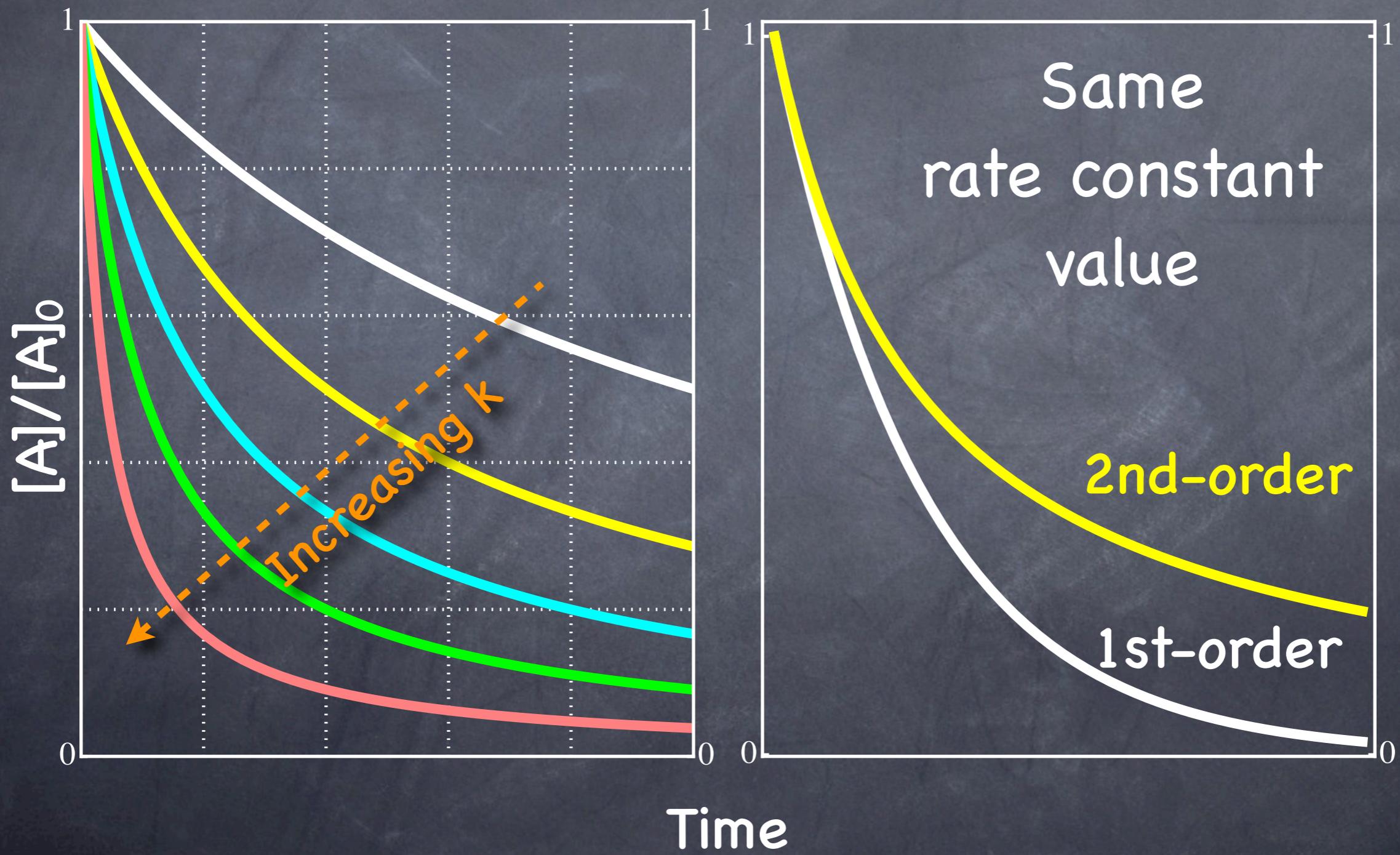


- If  $[A]_0 = [B]_0$ ,  $X = [A]_0 - x$  and  $dX/dt = -kX^2$ .  
The function form of the result is the same as in case 1.

# Exercise

- Solve Case 1 of 2nd-order kinetics above with Wolfram Language. Find the half-life to confirm the given result.

# 2nd-Order Reaction



# 2nd-Order Reaction

- Case 2,  $A + B \rightarrow P$ :  $d[A]/dt = d[B]/dt = -k[A][B]$
- If  $[A]_0 \neq [B]_0$ ,  $[B] - [A] = [B]_0 - x - [A]_0 + x = [B]_0 - [A]_0$ , so

$$\begin{aligned}\frac{1}{[A]} - \frac{1}{[B]} &= \frac{[B] - [A]}{[A][B]} = \frac{[B]_0 - [A]_0}{[A][B]} \Rightarrow \frac{d[A]}{[A][B]} = -k dt \\ \Rightarrow -k dt &= \frac{1}{[B]_0 - [A]_0} \left( \frac{d[A]}{[A]} - \frac{d[A]}{[B]} \right) \\ &= \frac{1}{[B]_0 - [A]_0} \left( \frac{d[B]}{[A]} - \frac{d[B]}{[B]} \right) \\ \Rightarrow -kt &= \frac{1}{[B]_0 - [A]_0} \left( \ln \frac{[A]}{[A]_0} - \ln \frac{[B]}{[B]_0} \right)\end{aligned}$$

# Enzyme Kinetics

# Enzymes and Biology

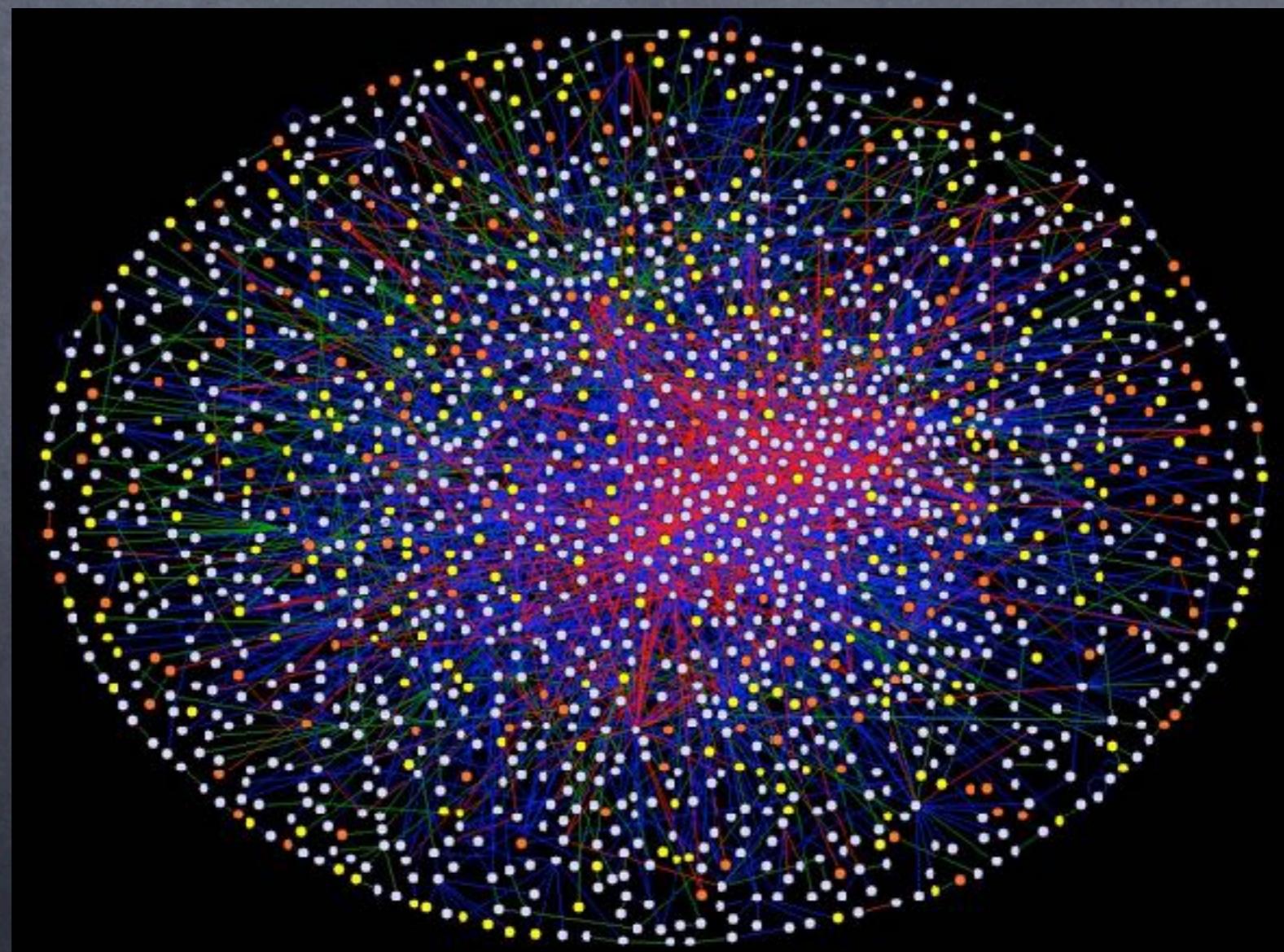
- For millennia people had watched planets wander through the nighttime sky. They named them, gave them symbols, and charted their complicated comings and goings. ...

J. E. Ferrell Jr quoted Stas Shvartsman, J. Biology 2009, 8 (1) 2.



# Enzymes and Biology

- ⦿ Where there is an enzyme, there is a reaction.

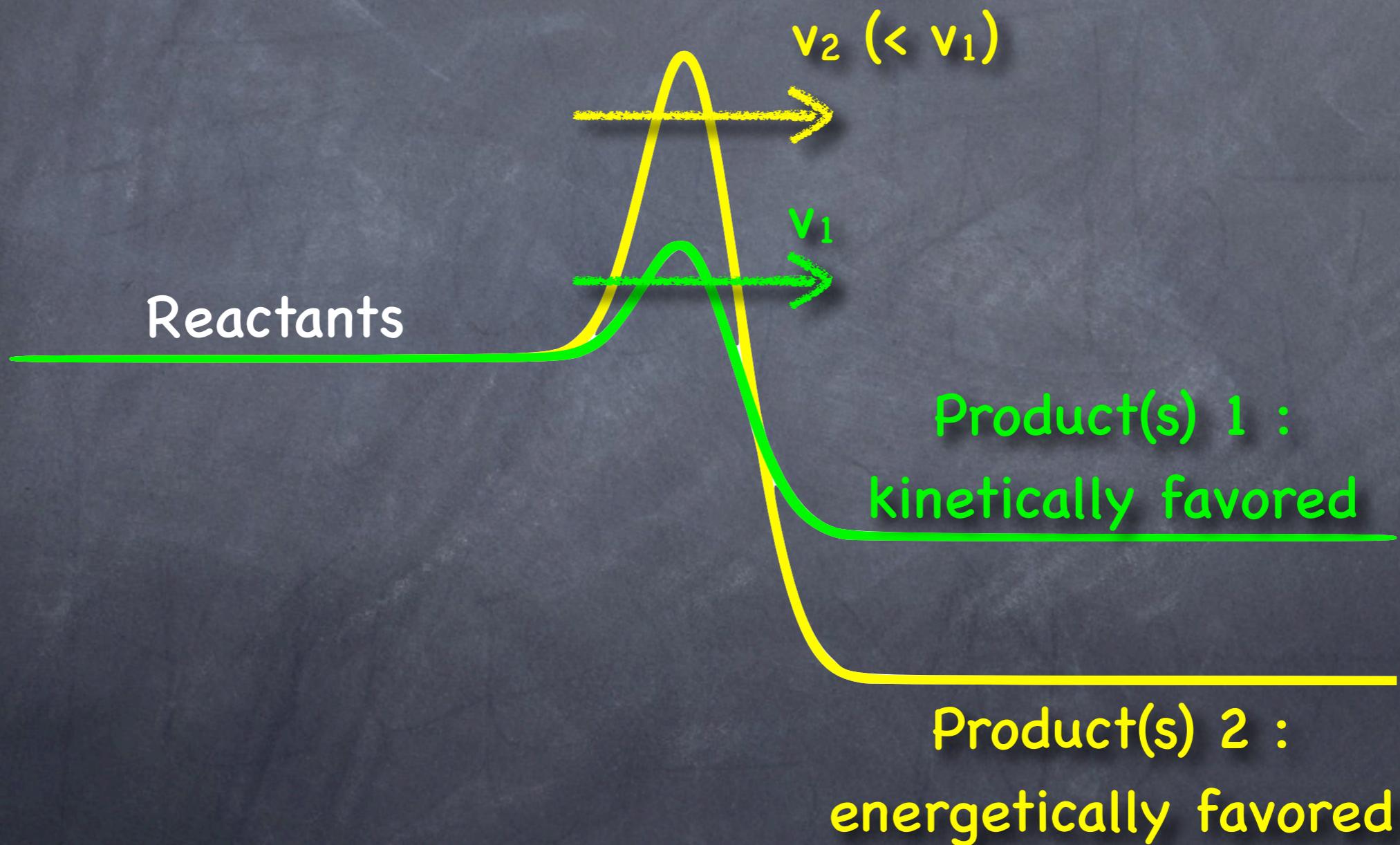


# Kinetic-Controlled Rxns

- ⦿ Kinetic control:
- ⦿ Competing reactions: Same substrates, different products
- ⦿ Instantaneously, ratio between amounts of products of different reactions is proportional to the ratio between the rates of different reactions (instead of determined by equilibrium constants)
- ⦿ Biological systems make a lot of uses of enzyme systems to regulate the rate constants of reactions and to achieve kinetic control of complex reactions

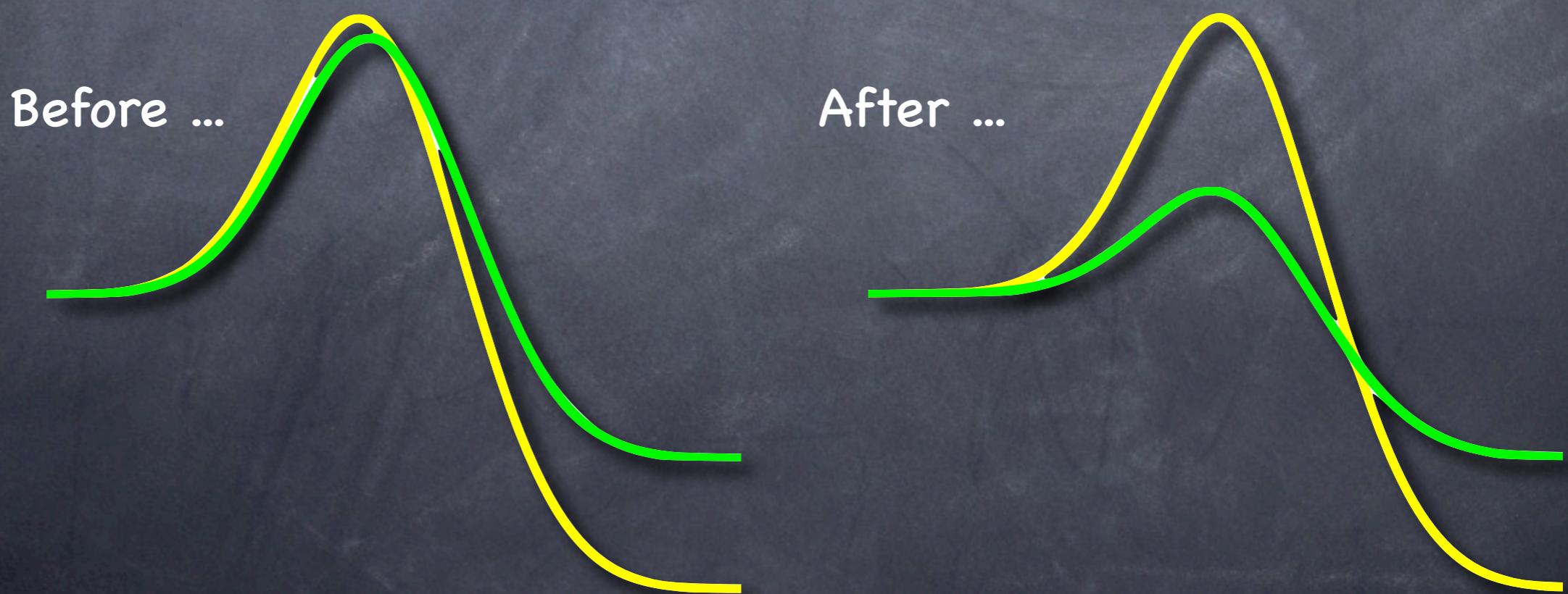
# Kinetic-Controlled Rxn

Example:



# Enzymes

- ⦿ Homogeneous (?) biological catalysts
- ⦿ Lower the activation energies of reaction pathways (how?) ⇒ Kinetically influence the relative abundance of products, without being able to influence the equilibrium properties



# Enzymes

- ⦿ Special biopolymers that contain **active sites** which are in charge of **binding** the substrates (reactants) and **processing** them into products
- ⦿ The **active sites** return to their original states when products are released

# Enzymes Kinetics

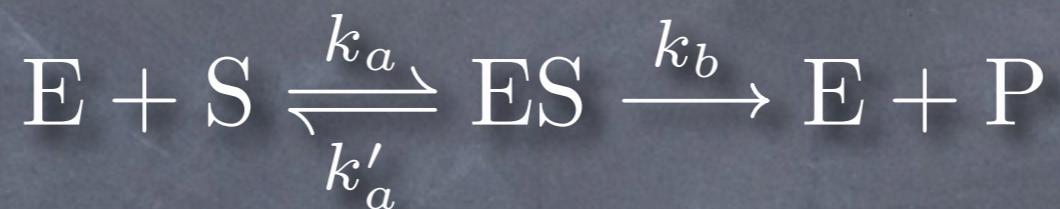
- ⦿ Enzyme kinetics = kinetics of reactions catalyzed by enzymes
- ⦿ Reaction rate depends on both substrates (reactants, products, cofactors, effectors, ...) and enzyme concentrations
- ⦿ Usually studied by initial rate measurement with very low enzyme concentration
- ⦿ Products often play no role

# Michaelis-Menten Mechanism

- A **simple** model that describes many **single-site** reactions
- **Properties to be expected:**
  - Given  $[S]_0$  (substrate), the initial production rate is proportional to  $[E]_0$  (enzyme)
  - Given  $[E]_0$  and low  $[S]_0$ , the initial production rate is proportional to  $[S]_0$
  - Given  $[E]_0$  and high  $[S]_0$ , the initial production rate reaches a maximum  $v_{max}$

# Michaelis-Menten Mechanism

- Reaction mechanism model considered:



- Only **one** enzyme-substrate complex presents as **intermediate**. For otherwise we may have to consider an alternative model such as:



# Henri-Michaelis-Menten

- The above reaction mechanism model under **pre-equilibrium** approximation ( $k'_a \gg k_b$ )
- The RDS is the breaking down of the enzyme-substrate complex ES into E + P
- Enzyme, substrate and the enzyme-substrate complex are at **pre-equilibrium**  $\Rightarrow$   
 $[E][S]/[ES] = K = \text{dissociation constant of ES}$
- Total concentration of enzyme is  $[E]_0 = [E] + [ES]$

$$\frac{[S]}{K} = \frac{[ES]}{[E]} \Rightarrow \frac{[S]}{K + [S]} = \frac{[ES]}{[E] + [ES]} = \frac{[ES]}{[E]_0}$$
$$\Rightarrow \frac{d[P]}{dt} = k_b [ES] = \frac{k_b [E]_0 [S]}{K + [S]}$$

# Henri-Michaelis-Menten

- If  $[S]$  is extremely high, almost all of the enzyme will be in bound form,  $[ES] = [E]_0$ . Ideally, the **enzyme turnover rate**  $k_b[ES]$  will approach its maximum  $v_{max} = k_b[E]_0$ .
- **Turnover rate:** the rate at which the enzyme resumes its free form by releasing the product.



Mike Ehrmann / Getty Images

<http://nbcsports.msnbc.com/id/46506624/ns/sports-nba/>

# Briggs-Haldane

- The above reaction mechanism model under **steady-state** approximation (pre-equilibrium or non-reversible conditions may not be achieved)

$$\frac{d [E]}{dt} = -k_a [E] [S] + k'_a [ES] + k_b [ES]$$

$$\frac{d [S]}{dt} = -k_a [E] [S] + k'_a [ES]$$

$$\frac{d [ES]}{dt} = k_a [E] [S] - k'_a [ES] - k_b [ES]$$

$$\frac{d [P]}{dt} = k_b [ES]$$

$$[E] + [ES] = [E] (t = 0) = [E]_0$$

**Nonlinear differential  
equation set normally  
cannot be solved**

# Briggs-Haldane

- Or we apply the steady-state approximation to let  $d[ES]/dt = 0$  (ES is the only intermediate)

$$k_a [E] [S] - (k'_a + k_b) [ES] = 0$$

$$K_M \equiv \frac{k'_a + k_b}{k_a} \Rightarrow [ES] = k_a [E] [S] / (k'_a + k_b)$$

$$[ES] = \frac{k_a [E]_0 [S]}{k'_a + k_b} - \frac{k_a [ES] [S]}{k'_a + k_b} = \frac{[E]_0 [S]}{K_M} - \frac{[ES] [S]}{K_M}$$

$$\Rightarrow \left(1 + \frac{[S]}{K_M}\right) [ES] = \frac{[E]_0 [S]}{K_M}$$

$$\Rightarrow (K_M + [S]) [ES] = [E]_0 [S] \Rightarrow [ES] = \frac{[E]_0 [S]}{K_M + [S]}$$

$$\Rightarrow v = \frac{d[P]}{dt} = \frac{k_b [E]_0 [S]}{K_M + [S]} = \frac{v_{\max} [S]}{K_M + [S]}$$

# Briggs-Haldane

⦿ Note that, since

$$\frac{d[S]}{dt} = -k_a [E] [S] + k'_a [ES]$$

$$\frac{d[ES]}{dt} = k_a [E] [S] - k'_a [ES] - k_b [ES] = 0$$

we find that under steady-state approximation

$$\frac{d[S]}{dt} = -k_b [ES] = -\frac{d[P]}{dt}$$

# Integrated Briggs-Haldane

- To compare with the experiments, we often need the integrated rate law

$$\frac{d[S]}{dt} = -k_b [ES] = -k_b \frac{[E]_0 [S]}{\frac{k'_a + k_b}{k_a} + [S]}$$

$$\frac{[S] + K_M}{[S]} d[S] = -k_b [E]_0 dt$$

$$[S] - [S]_0 + K_M \ln \frac{[S]}{[S]_0} = -v_{\max} t$$

$$[P] = [S]_0 - [S] \Rightarrow [P] + K_M \ln \frac{[S]_0}{[S]_0 - [P]} = v_{\max} t$$

# M&M w/ WL

```
In[•]:= sssol = Block[{s, e, es, p, k1, k2, k3, e0, s0, deqs, ss, cons, seq,
  $Assumptions = e0 > 0 && s0 > 0 && k1 > 0 && k2 > 0 && k3 > 0},
  SetAttributes[{k1, k2, k3, e0, s0}, Constant];
  ss = -k1 e[t] s[t] + k2 es[t] + k3 es[t] == 0;
  cons = e[t] + es[t] == e0 && s[t] + es[t] + p[t] == s0;
  deqs = dsdt == -k1 e[t] s[t] + k2 es[t] && dpdt == k3 es[t];
  seq = Eliminate[ss && cons && deqs, {e[t], es[t], p[t]}] // Simplify;
  Solve[seq, {dsdt, dpdt}] /. Rule → Equal
]
```

$$Out[•]= \left\{ \left\{ dsdt == -\frac{e0 k1 k3 s[t]}{k2 + k3 + k1 s[t]}, dpdt == \frac{e0 k1 k3 s[t]}{k2 + k3 + k1 s[t]} \right\} \right\}$$

# M&M w/ WL

- 4 Replace the rate constants and  $[E]_0$  with Michaelis constant and maximum turnover rate

```
In[•]:= Block[{vmax, KM, mme},  
  mme = Eliminate[Union[sssol[[1]], {vmax == e0 k3, KM == (k2 + k3) / k1}],  
  {k1, k2, k3}];  
  Solve[mme, {dsdt, dpdt}] /. {dsdt → D[s[t], t], dpdt → D[p[t], t]}  
 ]
```

$$Out[•]= \left\{ \left\{ s'[t] \rightarrow -\frac{v_{max} s[t]}{K_M + s[t]}, p'[t] \rightarrow \frac{v_{max} s[t]}{K_M + s[t]} \right\} \right\}$$

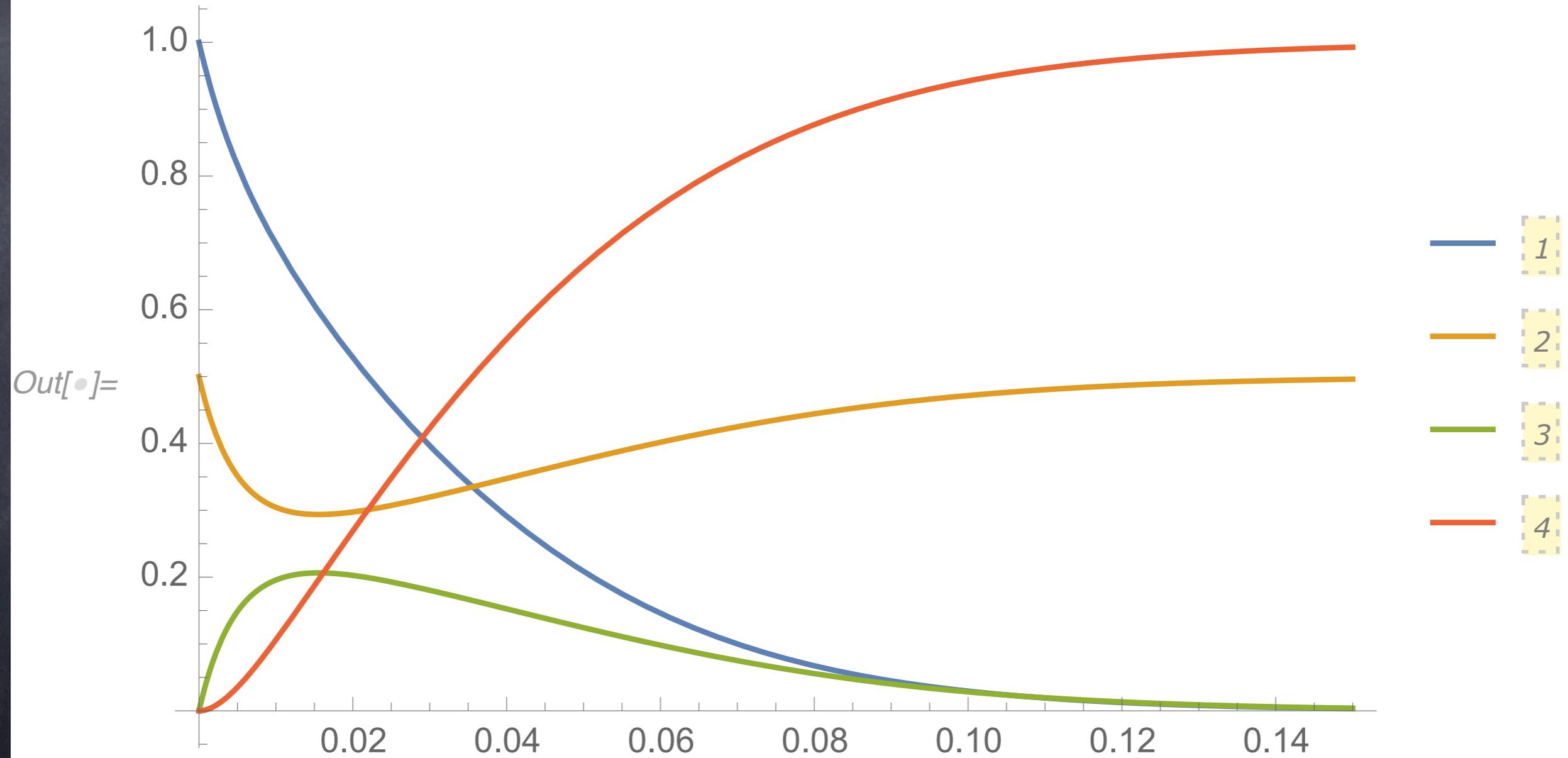
# Visualize the Kinetics

- Totally numerical solution of the M&M model:

```
In[•]:= nnsol = Block[{s, e, es, p, k1 = 100, k2 = 5, k3 = 80, e0 = 0.5, s0 = 1,
  tmax = 0.15, deqs, inc},
  SetAttributes[{k1, k2, k3, e0, s0}, Constant];
  deqs = D[s[t], t] == -k1 e[t] s[t] + k2 es[t] &&
  D[e[t], t] == -k1 e[t] s[t] + k2 es[t] + k3 es[t] &&
  D[es[t], t] == k1 e[t] s[t] - k2 es[t] - k3 es[t] &&
  D[p[t], t] == k3 es[t];
  inc = s[0] == s0 && e[0] == e0 && es[0] == 0 && p[0] == 0;
  NDSolve[deqs && inc, {s[t], e[t], es[t], p[t]}, {t, 0, tmax}]
];
```

# Visualize the Kinetics

```
In[•]:= Plot[Evaluate[{s[t], e[t], es[t], p[t]} /. nnsol],  
{t, 0, 0.15}, PlotLegends → Automatic]
```



# Exercise

- Solve the reversible M&M model:



- Solve numerically and plot result
- Apply steady-state approximation and solve analytically

# Advanced Techniques

- ⦿ For large reaction networks, it is important to
  - ⦿ be able to **solve the problem with well-organized procedure** – don't write the similar programs again and again
  - ⦿ be able to **book-keep information of large systems** so that various computations can be done on the same system easily

# Advanced Techniques

- ⦿ Computation procedure reuse:
- ⦿ Write reusable functions and notebooks
- ⦿ System information reuse:
  - ⦿ Store all useful information in JSON
  - ⦿ Effectively, object-oriented programming in Wolfram Language

# JSON example

- Record the information of the reactions in a JSON file/string.
- Read the JSON and construct the equations you need.
- Use the WL notebook AdvancedRxn.nb and the JSON file assoctest.json to play around.