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: ν
  - 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:

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

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

Absorption

Light scattering

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:

Molecule concentration: [D]

Beam cross-section = A

Light path length L

Part of the incident energy is absorbed by the molecule

$\Rightarrow I(\omega) / I_0(\omega) < 1$

Beer-Lambert Law

- Absorbance (optical density):

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

- Extinction coefficient:

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

Molecule concentration: [D]

Beam cross-section = A

Light path length L

Part of the incident energy is absorbed by the molecule

$\Rightarrow$ $I(\omega) / I_0(\omega) < 1$

Beer-Lambert Law

- Consider a thin layer of the sample (Thickness = dL)
- When one molecule absorbs one photon at frequency ω, 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:

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

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

$$-\int_{L'=0}^{L} d(\log_{10} I) = -\log_{10}\frac{I}{I_0}$$

$$= \alpha(\omega) = \frac{p(\omega)\,A\,[D]}{\ln 10}\int_{L'=0}^{L} dL'$$

$$= \frac{p(\omega)\,A}{\ln 10}\,[D]\,L = \varepsilon(\omega)\,[D]\,L$$

- 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:
  - $A \rightarrow B$: $-d[A]/dt = +d[B]/dt$
  - $(NH_2)_2CO(aq) + 2\ H_2O(l) \rightarrow 2\ NH_4^+(aq) + CO_3^{2-}(aq)$: $d[NH_4^+] / dt = 2\ d[CO_3^{2-}] / dt = -2\ d[(NH_2)_2CO] / dt$
- Stoichiometrically normalized rate:

$\nu_A A + \nu_B B \rightarrow \nu_C C + \nu_D D$

$$v \equiv \frac{d\,[\mathrm{D}]\,/dt}{\nu_{\mathrm{D}}} = \frac{d\,[\mathrm{C}]\,/dt}{\nu_{\mathrm{C}}} = \frac{d\,[\mathrm{B}]\,/dt}{-\nu_{\mathrm{B}}} = \frac{d\,[\mathrm{A}]\,/dt}{-\nu_{\mathrm{A}}}$$

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

Driving syringes
Mixing chamber
s

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: $\Delta t = \Delta S / v$
      (ΔS = 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 extend 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

Polli et al, Nature 467, 440-443 (2010)

Flash Photolysis (Pump Probe)

Pump pulse

Probe pulse

The sample originally absorbs the pumping radiation but not the probing radiation. After it absorbs the probe pulse, however, it will absorb the probing radiation

Flash Photolysis (Pump Probe)

Pump pulse

Probe pulse

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 absorbs 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

Polli et al, Nature 467, 440-443 (2010)

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: $v \propto [\mathrm{A}]^{\varepsilon_\mathrm{A}} [\mathrm{B}]^{\varepsilon_\mathrm{B}}$ (power law)
  - ε_A, ε_B are not necessarily integers
- Example of more complicated rate law:

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

Rate Laws / Rate Constants

- If the rate law is a power law, for example,
$$v = k\,[\mathrm{A}]^{\varepsilon_\mathrm{A}}\,[\mathrm{B}]^{\varepsilon_\mathrm{B}}$$
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 $\{ \nu_A, \nu_B \}$ 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^{-1}

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 + ... $\rightarrow$ P

  Generally $v = k\,[\mathrm{A}]^{\varepsilon_\mathrm{A}}\,[\mathrm{B}]^{\varepsilon_\mathrm{B}}\,[\mathrm{C}]^{\varepsilon_\mathrm{C}}\ldots$

- Goal: Find k, ε_A, ε_B, and so on.

- To find ε_A, prepare B, C, ... in large excess $\Rightarrow$

  [B], [C], ... can be approximated as constants

  $[B]_0$, $[C]_0$, ... $\Rightarrow$ $v = k\,([\mathrm{B}]^{\varepsilon_\mathrm{B}}\,[\mathrm{C}]^{\varepsilon_\mathrm{C}}\ldots)\,[\mathrm{A}]^{\varepsilon_\mathrm{A}}$

  (pseudo-ε_A-order)

Isolation Method

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

$$\ln v = \ln k'_{\mathrm{A}} + \varepsilon_{\mathrm{A}} \ln [\mathrm{A}]$$

- 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

$$v_0 = k\,[\mathrm{H}]_0^{\varepsilon_\mathrm{H}}\,[\mathrm{G}]_0^{\varepsilon_\mathrm{G}} \Rightarrow \log_{10} v_0 = \log_{10}\left(k\,[H]_0^{\varepsilon_\mathrm{H}}\right) + \varepsilon_\mathrm{G} \log_{10} [G]_0$$

Determine k, ε_H, and ε_G according to the experimental data.

Example

v_0 / M/s		$[G]_0$ / mM			
		1.00	1.54	3.12	4.02
$[H]_0$ / mM	1.34	5.0	7.6	15.5	20.0
	3.00	7.0	11.0	23.0	31.0
	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
$\log_{10}([H]_0)$	-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

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

Example

$$k' \equiv k\,[\mathrm{H}]_0^{\varepsilon_\mathrm{H}}$$

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

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

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

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

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

Example

$$k' \equiv k\,[\mathrm{H}]_0^{\varepsilon_\mathrm{H}}$$

$\log_{10}([H]_0)$ \ $\log_{10}([G]_0)$	-3	-2.81	-2.51	-2.4	$\log_{10}k'$
-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	

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

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

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

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

Example

$$k' \equiv k\,[\mathrm{H}]_0^{\varepsilon_\mathrm{H}}$$

$\log_{10}(v_0)$		$\log_{10}([G]_0)$				
		-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

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

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

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

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

Example

$$k' \equiv k\,[\mathrm{H}]_0^{\varepsilon_\mathrm{H}}$$

$\log_{10}(v_0)$		$\log_{10}([G]_0)$				$\varepsilon_G = 1$
		-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

$\langle x\rangle=-2.46$, $\langle x^2\rangle=6.20$, $\sigma_x^2=0.128$

$\varepsilon_H = 1.0169$, $\log_{10}k = 6.63$

$\langle y\rangle=4.124$, $\langle xy\rangle=-10.023$, $\mathrm{Cov}(xy)=0.1299$

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

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 ]
```

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

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

Integrated Rate Law

- From the differential (instantaneous) rate law we find

$$v = \frac{1}{\nu_{\mathrm{A}}} \left| \frac{d\,[\mathrm{A}]}{dt} \right| = k\,[\mathrm{A}]^{\varepsilon_{\mathrm{A}}}$$

- Often, we may be more concerned about [A](t)

- 1st-order rate law:

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

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

1st-Order Rate Law

- Half-life $t_{1/2}$: time when [A] = $[A]_0$ / 2.
  Let $\nu_A = 1$ and

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

- 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

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

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[•]= $\left\{\left\{a[t] \to a0\, e^{-k\, t\, \nu A}\right\}\right\}$

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[•]= $\left\{\left\{t2 \to \frac{\mathrm{Log}[2]}{k\, \nu A}\right\}\right\}$

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 / μM	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

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

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

- Half-life:

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

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

2nd-Order Reaction

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

$$\left.\begin{array}{ccccc} \mathrm{A} & + & \mathrm{B} & \rightarrow & \mathrm{P} \\ [\mathrm{A}]_0 & & [\mathrm{B}]_0 & & ? \\ -x & & -x & & ? \\ \hline [\mathrm{A}]_0 - x & & [\mathrm{B}]_0 - x & & ? \end{array}\right\} \Rightarrow \frac{dx}{dt} = k\left([\mathrm{A}]_0 - x\right)\left([\mathrm{B}]_0 - x\right)$$

- 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

Time

2nd-Order Reaction

- Case 2, A + B → 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

$$\frac{1}{[\mathrm{A}]} - \frac{1}{[\mathrm{B}]} = \frac{[\mathrm{B}]-[\mathrm{A}]}{[\mathrm{A}]\,[\mathrm{B}]} = \frac{[\mathrm{B}]_0-[\mathrm{A}]_0}{[\mathrm{A}]\,[\mathrm{B}]} \Rightarrow \frac{d\,[A]}{[\mathrm{A}]\,[\mathrm{B}]} = -k\,dt$$

$$\Rightarrow -k\,dt = \frac{1}{[\mathrm{B}]_0-[\mathrm{A}]_0}\left(\frac{d\,[\mathrm{A}]}{[\mathrm{A}]} - \frac{d\,[\mathrm{A}]}{[\mathrm{B}]}\right)$$

$$= \frac{1}{[\mathrm{B}]_0-[\mathrm{A}]_0}\left(\frac{d\,[\mathrm{A}]}{[\mathrm{A}]} - \frac{d\,[\mathrm{B}]}{[\mathrm{B}]}\right)$$

$$\Rightarrow -kt = \frac{1}{[\mathrm{B}]_0-[\mathrm{A}]_0}\left(\ln\frac{[\mathrm{A}]}{[\mathrm{A}]_0} - \ln\frac{[\mathrm{B}]}{[\mathrm{B}]_0}\right)$$

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:

$$\mathrm{E} + \mathrm{S} \underset{k'_a}{\overset{k_a}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_b} \mathrm{E} + \mathrm{P}$$

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

$$\mathrm{E} + \mathrm{S} \rightleftharpoons \mathrm{ES} \rightleftharpoons \mathrm{EP} \longrightarrow \mathrm{E} + \mathrm{P}$$

Henri-Michaelis-Menten

- The above reaction mechanism model under pre-equilibrium approximation ($k'_a >> k_b$)
  - 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 = 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)

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

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

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

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

$$[\mathrm{E}] + [\mathrm{ES}] = [\mathrm{E}]\,(t=0) = [\mathrm{E}]_0$$

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)

$$k_a\,[\mathrm{E}]\,[\mathrm{S}] - (k'_a + k_b)\,[\mathrm{ES}] = 0$$

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

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

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

$$\Rightarrow (K_\mathrm{M} + [\mathrm{S}])\,[\mathrm{ES}] = [\mathrm{E}]_0\,[\mathrm{S}] \Rightarrow [\mathrm{ES}] = \frac{[\mathrm{E}]_0[\mathrm{S}]}{K_\mathrm{M}+[\mathrm{S}]}$$

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

Briggs-Haldane

- Note that, since

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

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

we find that under steady-state approximation

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

Integrated Briggs-Haldane

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

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

$$\frac{[\mathrm{S}] + K_\mathrm{M}}{[\mathrm{S}]}\,d\,[\mathrm{S}] = -k_b\,[\mathrm{E}]_0\,dt$$

$$\boxed{[\mathrm{S}] - [\mathrm{S}]_0 + K_\mathrm{M}\ln\frac{[\mathrm{S}]}{[\mathrm{S}]_0} = -v_\mathrm{max}t}$$

$$[\mathrm{P}] = [\mathrm{S}]_0 - [\mathrm{S}] \Rightarrow \boxed{[\mathrm{P}] + K_\mathrm{M}\ln\frac{[\mathrm{S}]_0}{[\mathrm{S}]_0 - [\mathrm{P}]} = v_\mathrm{max}t}$$

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

- 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[•]= $\left\{\left\{s'[t] \to -\frac{\text{vmax}\, s[t]}{KM + s[t]},\ p'[t] \to \frac{\text{vmax}\, s[t]}{KM + s[t]}\right\}\right\}$

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]
```

Out[•]=

Exercise

- Solve the reversible M&M model:

$$\mathrm{E} + \mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

  - 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