

Week 48

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```
try:
    import fysisk_biokemi
    print("Already installed")
except ImportError:
    %pip install -q "fysisk_biokemi[colab] @ git+https://github.com/au-mbg/fysisk-biokemi.git"
```

Qualitative description of Michaelis-Menten kinetics

```
import fysisk_biokemi.widgets as fbw
from fysisk_biokemi.widgets.utils import enable_custom_widget_colab,
disable_custom_widget_colab

import plotly
from IPython.display import display, HTML
enable_custom_widget_colab()
fbw.michaelis_menten_demo()
```

Use the widget created by the cell above to answer the following the questions.

(a)

Setting $V_{\max} = 400$ and $[E]_{\text{tot}} = 0.0005$, change K_M from 0.5 to 5 to 10 to 50. Describe the change in curve appearance and explain it using the Michaelis-Menten (MM) equation.

(b)

What is the biological meaning of K_M ?

(c)

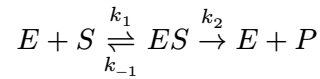
Now set $[E]_{\text{tot}} = 0.0005$, and $K_M = 5$, change $V_{\max}$ from 40 to 100 to 900. Do you observe any changes? Explain your answer using the widget and the MM equation

(d)

Now set $K_M = 5$ and $V_{\max} = 100$, change $[E]_{\text{tot}}$ from 0.0005 to 0.05. Do you observe any change in curve appearance, in kcat or in kcat/KM? Explain your answer

The Michaelis Menten equation

Consider a reaction catalyzed by an enzyme obeying the Michaelis-Menten kinetics model.



$$V_0 = V_{\max} \frac{[S]}{[S] + K_M}$$

(a) Enzyme saturation

Explain what is meant by enzyme saturation.

(b)

Calculate the level of enzyme saturation, f_{ES} , at substrate concentrations of

- $[S] = 0.1 \cdot K_M$
 - $[S] = 0.5 \cdot K_M$
 - $[S] = 1 \cdot K_M$
 - $[S] = 10 \cdot K_M$
 - $[S] = 100 \cdot K_M$
-

The Michaelis-Menten constant K_M is defined as:

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

(c) Calculate K_M

In a specific reaction, the following rate constants were determined:

- $k_1 = 7 \cdot 10^7 \cdot \text{M}^{-1} \cdot \text{s}^{-1}$,
- $k_{-1} = 8 \cdot 10^5 \text{ s}^{-1}$
- $k_2 = 10^3 \text{ s}^{-1}$

Calculate the value of K_M . Does K_M approximate the dissociation constant for the ES complex in this case?

(d) Calculate k_2

Assume that a solution of an enzyme at a concentration of $1 \cdot 10^{-7} \text{ M}$ with a substrate concentration of $[S] = 100 \cdot K_M$ has $V_0 = 10^{-4} \text{ M} \cdot \text{min}^{-1}$

Given this information and with your answers to (b) and (c) in mind, calculate the constant k_2 . What is this constant also called?

(e) Lineweaver-Burk plot

The values of $V_{\max}$ and K_M have historically been determined from a Lineweaver-Burk plot.

How does the x- and y-intercepts in a Lineweaver-Burk plot relate to $V_{\max}$ and K_M ?

Enzyme inhibitors (I)

```
import matplotlib.pyplot as plt
import pandas as pd
from scipy.optimize import curve_fit
pd.set_option('display.max_rows', 6)
```

An enzyme obeying the Michaelis-Menten kinetics model was tested for substrate conversion in the absence and presence of an inhibitor, called inhibitor1 at a concentration of $[I] = 2.5 \cdot 10^{-3}$ M. The data set is contained in the file enzyme-inhib-i.xlsx. Using this data a researcher wanted to determine the type of inhibition.

Start by loading the dataset

```
from fysisk_biokemi.widgets import DataUploader
from IPython.display import display
uploader = DataUploader()
uploader.display()
```

Run the next cell **after** uploading the file

```
df = uploader.get_dataframe()
display(df)
```

(a) Convert units

Convert the concentrations of substrate and the initial velocities to units given in M and $\text{M} \cdot \text{s}^{-1}$, respectively.

```
df['[S]_ (M)'] = ...
df['V0_no_inhib_ (M/s)'] = ...
... # This one you'll have to do all on your own.
display(df)
```

(b) Plot

Plot the initial velocities of both experiments as a function of substrate concentration in one plot. Estimate k_{cat} and V_{max} in the presence and absence of inhibitor from the plot.

```
fig, ax = plt.subplots(figsize=(6, 4))

... # Your code for plotting here
... # and here.

ax.set_xlabel('[S] (M)')
ax.set_ylabel('$V_0$ (M/s)')
ax.legend()
```

The researcher wanted to determine V_{max} and K_M values for both experiments in order to correctly conclude on the type of inhibitor.

(c)

Determine V_{max} and K_M by fitting.

Start writing the function to fit with

```
def ...  
    ...
```

And use the `curve_fit`-function to find the parameters

```
popt, _ = ...  
vmax, km = popt  
  
# With inhibitor  
popt, _ = ...  
vmax_inhib, km_inhib = popt  
  
# Printing  
print('Without inhibitor:')  
print(f'\t V_max = {vmax:.2e}')  
print(f'\t Km = {km:.2e}')  
print('With inhibitor:')  
print(f'\t V_max = {vmax_inhib:.2e}')  
print(f'\t Km = {km_inhib:.2e}')
```

(d)

What type of inhibitor is inhibitor1?

(e)

Calculate K_i .

Enzyme inhibitors (II)

```
import matplotlib.pyplot as plt  
import pandas as pd  
from scipy.optimize import curve_fit  
pd.set_option('display.max_rows', 6)
```

Two enzyme inhibitors were identified as part of a drug discovery program. To characterize the mechanism of action, the reaction kinetics were analysed for the enzyme alone and in the presence of 5 μM of each of the inhibitors as a function of substrate concentration resulting in the data in the file `enzyme-inhib-ii.xlsx`

Load the dataset

```
from fysisk_biokemi.widgets import DataUploader  
from IPython.display import display  
uploader = DataUploader()  
uploader.display()
```

Run the next cell **after** uploading the file

```
df = uploader.get_dataframe()
display(df)
```

(a) Convert & plot

Convert the measurements to SI units and plot the reaction rate against time for all three reactions.

Start by converting the units

```
... # Your code to convert units here.
```

And then plot

```
fig, ax = plt.subplots()

col_names = ['enz_(M/s)', 'inhibitor2_(M/s)', 'inhibitor3_(M/s)']

for col_name in col_names:
    ax.plot(..., ..., 'o', label=col_name) # Replace ... with your code here.

ax.legend()
ax.set_xlabel('Substrate concentration [M]', fontsize=14)
ax.set_ylabel('$V_0$', fontsize=14)
plt.show()
```

Based on the appearance of these plots:

- Can you determine the type of inhibition?
- Alternatively, can you exclude some mechanisms?

(b) Intuit parameters

Based on your answer to in (a) what do you expect for the parameters K_M and $V_{\max}$?

- Which dataset would have the highest $V_{\max}$, which the lowest?
- What about K_M ?

(c) Fit

As always, we need the function we're fitting with

```
def michaelis_menten(S, Vmax, Km):
    return (Vmax * S) / (S + Km)
```

Given we're fitting on multiple datasets we will make our lives a bit easier by making a little function

```
def fit_parameters(df, col_name):
    popt, _ = curve_fit(..., ..., ...) # Replace ... with your code.
    vmax, km = popt
    # Printing
    print(f'{col_name}:')
```

```
print(f'\t V_max = {vmax:.2e}')
print(f'\t Km = {km:.2e}')
```

```
for col_name in col_names:
    fit_parameters(df, col_name)
```

(d) Change

In the fits above you will likely see that all the fitted parameters change between the different data sets – some by a little and some by a lot. How big does a change have to be to be considered significant?

(e) K_i

Determine the K_i for each of the two inhibitors.

A snake dependent enzyme

When (or *if* depending on your outlook in life) you are bit by a cobra, the snake protein CVF interacts with the human protein FB to form a proteolytically active enzyme in the blood called CVF:FB. CVF:FB enzyme is able to cleave another human protein known as C5 into C5b and C5a (C5a is the protein you visually inspected in your first PyMOL session). With C5 as a substrate, CVF:FB obeys the Michaelis-Menten kinetics model, whereas CVF alone does not show any catalytic activity towards C5.

Now consider two different CVF proteins, CVF1 and CVF2 from two different cobra species. Upon characterization it was found that CVF1:FB has a $K_M = 0.036 \mu\text{M}$ and a $k_{cat} = 0.43 \text{ min}^{-1}$, whereas CVF2:FB has a $K_M = 14 \mu\text{M}$ and a $k_{cat} = 0.73 \text{ min}^{-1}$. The concentration of C5 in blood is $0.37 \mu\text{M}$.

(a)

Convert the given K_M values and the C5 concentration to SI-units given in M

```
# CVF1
K_m_cvf1 = ...
k_cat_cvf1 = ...

# CVF2
...
...

# Concentration
C_c5 = ...
```

(b)

Assuming that $[\text{CVF1:FB}] = [\text{CVF2:FB}] = 0.1 \cdot 10^{-6} \text{ M}$, calculate $V_{\max}$ of both enzymes.

```
V_max_cfv1 = ...
...
```

```
print(f"{V_max_cfv1 = :.3e}")
print(f"{V_max_cfv2 = :.3e}")
```

(c)

Calculate the fraction of enzyme:substrate complexes (CVFx:FB:C5) in blood.

```
theta_cfv1 = ...
theta_cfv2 = ...

print(f"{theta_cfv1 = :.3f}")
print(f"{theta_cfv2 = :.3f}")
```

(d)

Calculate the initial velocities of C5 cleavage in blood by the two enzymes.

```
v0_cfv1 = ...
v0_cfv2 = ...

print(f"{v0_cfv1 = :.3e}")
print(f"{v0_cfv2 = :.3e}")
```

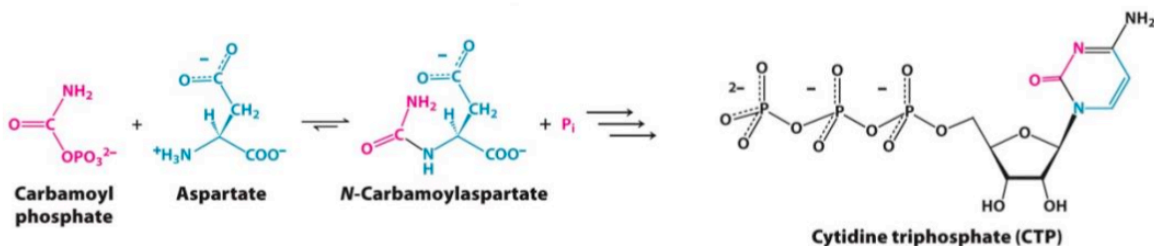
(f)

Which of the two enzymes, CVF1:FB or CVF2:FB, cleaves the most C5 molecules in blood?

Enzymatic behaviour of the enzyme ATCase

```
import matplotlib.pyplot as plt
```

The enzyme aspartate transcarbamoylase (ATCase) catalyzes the first reaction in the biosynthesis of pyrimidines such as CTP as shown in the reaction below:



ATCase does not obey the Michaelis-Menten kinetics model but instead shows the behaviour recorded in the enzyme-behav-atcase.csv dataset.

The dataset consists of three columns; the aspartate concentration in mM and the rate of formation of N-carbamoylaspartate with and without the presence of CTP.

Load the dataset with the widget below

```

from fysisisk_biokemi.widgets import DataUploader
from IPython.display import display
uploader = DataUploader()
uploader.display()

```

Run the next cell **after** uploading the file

```

df = uploader.get_dataframe()
display(df)

```

(a) Plot the dataset

```

fig, ax = plt.subplots()

... # Put some code here
... # And here

ax.set_xlabel('[Aspartate] (mM)')
ax.set_ylabel('Rate of formation')
ax.set_yticklabels([]);

```

(b) Kinetic profile

Describe the kinetic profile of ATCase and explain what it tells us about the way ATCase works. (You may find inspiration in the material previously covered on protein-ligand interactions)

(c) Quarternary structure

What does the figure tell us about the quaternary structure of ATCase?

(d) DNA synthesis

Which other pyrimidine based trinucleotide besides CTP is used to synthesize DNA?

(e) Effect of CTP

Qualitatively describe the effect of CTP on the rate of N-carbamoylaspartate formation

(f) Physiological advantage

In fact many enzymes are regulated by certain end products in a fashion similar to the CTP effect on ATCase. Can you explain why this might be a physiological advantage?