RStudio - 03; Enzymology data

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Contents

1 Enzymology data

Our student colleague and friend "Freddie" is running an enzymology experiment and is asking our help to present the result and update it later if necessary.

He first wants to plot the data with the Michaelis-Menten method (Michaelis and Menten 1913) and deduct values for the parameters of the equation.

Michaelis and Menten showed that the rate of an enzyme-catalyzed reaction is proportional to the concentration of the enzyme–substrate complex predicted by the Michaelis–Menten equation. (Michaelis et al. 2011)

The Michaelis-Menten equation can be written as a function of S with constant parameters to be evaluated:

$$f(S, (K, V_m)) = \frac{V_m S}{K_m + S}$$

where S is the value for the substrate also denoted $[S]$ in some equations, V_m is the asymptote `Vmax` and K_m is the value half-way between 0 and the asymptote.

2 Data

Freddie is not very good with electronic records and has written down the data on a piece of paper that contains the results if his experiements:

Table 1: Freddie's data:

S	v
0	0.0
1	11.1
2	25.4
5	44.8
8	54.5
12	58.2
30	72.0
50	60.1

How can we help Freddie to write his report?

3 Method

We will create a "dynamic" report for Freddie that can be updated with new data if necessary.

3.1 Create an RMarkdown document

If we are continuing from the previous section we already have created an Rstudio project file *e.g.* `Project_1` potentially stored in a directory on the desktop.

You can create a new project or use this existing project `Project_1`. The project will contain the files that we want to use or save.

You can start a new `R Markdown` file with the following menu cascade:

File > New File > R R Markdown

In the selection window that opens provide a name for your document, the default is "untitled" but it could be call *e.g.* `Freddie1.Rmd` where `.Rmd` is the standard `R` markdown filename extension.

The document will come pre-filled with sample text and data that we will need to erase.

3.1.1 Top section

But first, note that the title you gave to your file is found at the very top of the document and will be used as the document title at the top of the final report. In turn this information is found within a structure bonded by 2 lines with 3 dashes:

For example my document had this default top section:

```
---
title: "TEST"
author: "Jean-Yves Sgro"
date: "May 2, 2017"
output:
  html_document:
    toc: yes
  pdf_document:
    toc: yes
```

This is an important section in a special language called `YAML` that provides a space to place special options.

For our immediate purpose this is sufficient for our goal and we can explore and tweak these options later.

3.1.2 Body text

The rest of the document is written in `R` markdown and we can immediately get a glimpse of its structure organization.

The sample data is a built-in dataset about cars from the 1970's in tabular form, used here as a quick example.

We can recognise immediately the code that will be executed by `R` as marked by `{r}` and usually referred to as `code chuncks`.

The first `chunck` is a set-up parameter:

Here `include=FALSE` means that the code will be executed but the code itself will be not be shown in the final output.

The rest of the file is a simple plot and a simple table output.

3.1.3 Report Output

To obtain an output from this page:

- click the `Knit HTML` button at the top of the `Scripts` quadrant.
- a new windowm, in HTML format will appear containing the report.

This is how we create dynamic documents with `R` code executed on the fly and the "narrative" story telling.

3.1.4 Making a "Freddie" report

We can now erase what we don't need, that is everything except the first `{r}` chunk that we can keep.

We can then prepare the document for writing a nice report for Freddie. As stated before the `Title` of the report will be the title provided in the `title:` section in the `YAML` description at the top.

To start we can set-up the report with section names that we can fill later. For example:

```
---
title: "Report for Freddie"
author: "your name"
date: "May 5, 2017"
output: html_document
---

# Introduction

In this report we present ....

# Data source and method

Enzyme was from **Promega** etc.

# Raw data
```

```
The data was obtained from an experiement performed by.... on this date:...

# Data exploration

We can explore the data with a simple first plot describing the reaction....

# Model building

This enzymatic data follows a Michaelis-Menten plot
and we can use a statistical method to create a model
and calculate constant parameters Km and Vmax.

# Final plot based on data and model

This plot depicts...

# Conclusion

In conclusion we can report that...
```

This "raw" template can be filled as we go but for now it would be rendered as follows by the `Knit HTML` button. Note that the YAML section is not shown in the final output.

> Introduction
>
> In this report we present ....
>
> Data source and method
>
> Enzyme was from **Promega** etc.
>
> Raw data
>
> The data was obtained from an experiement performed by.... on this date:...
>
> Data exploration
>
> We can explore the data with a simple first plot describing the reaction....
>
> Model building
>
> This enzymatic data follows a Michaelis-Menten plot and we can use a statistical method to create a model and calculate constant parameters Km and Vmax.
>
> Final plot based on data and model
>
> This plot depicts...
>
> Conclusion
>
> In conclusion we can report that...

4 Fill-in the report

Now that you have a rough template to fill-in, we can explore **how** we can fill it (!) with `R` code and descritions.

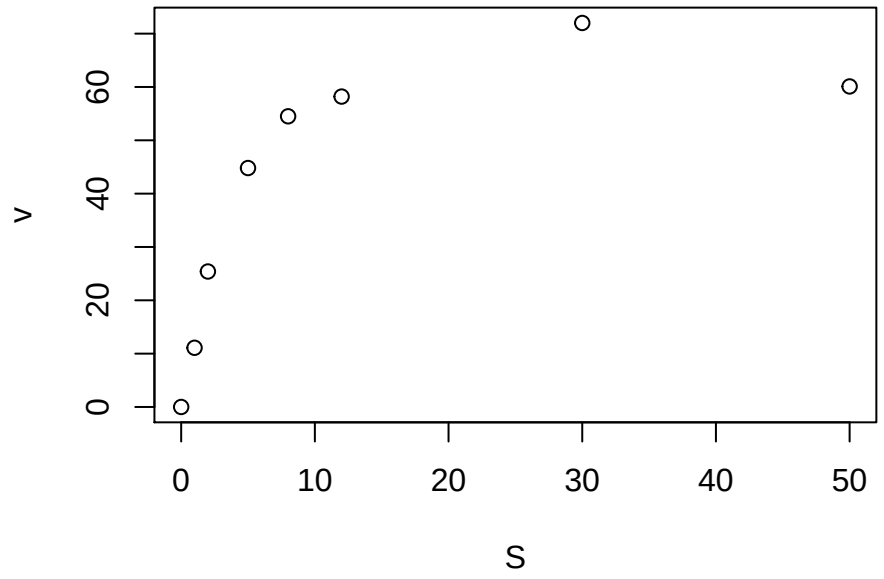
4.1 Import and explore data

First we need to import the data within `R` and since there are only a few numbers we can first type the data into vectors. We can call `S` the substrate concentration and `v` the observed "velocity" result.

```
# Data can be entered as vectors S and v
S <- c(0,1,2,5,8,12,30,50)
v <- c(0,11.1,25.4,44.8,54.5,58.2,72.0,60.1)
```

We can then create a simple plot and see if the data "makes sense" in the plot:

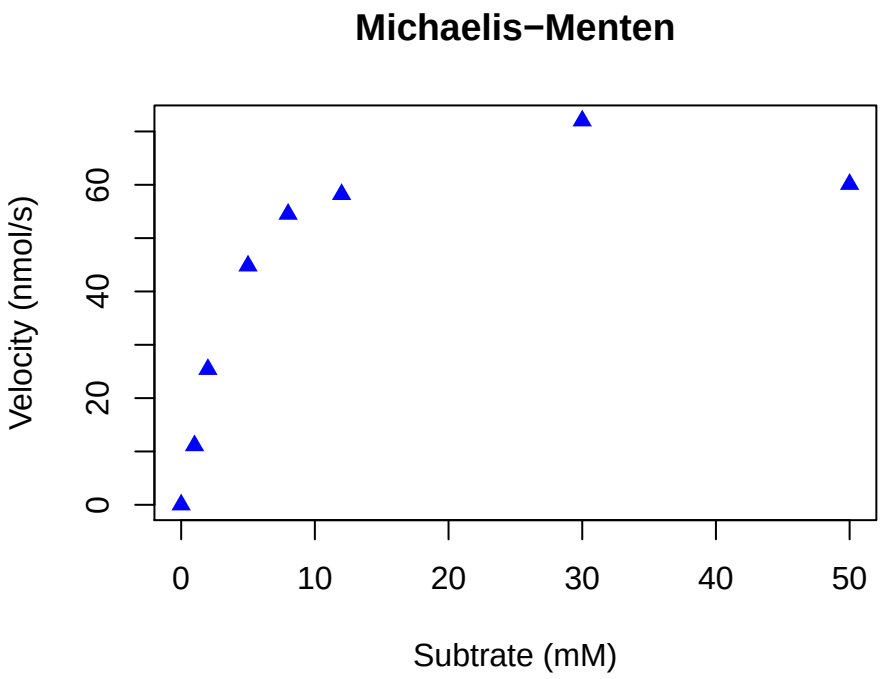
```
plot (S,v)
```



What do you think?

We can make the plot beter by adding a plot title, axis labels, change the default circles to filled triangles and make them blue:

```
plot (S,v,
      xlab="Subtrate (mM)",
      ylab="Velocity (nmol/s)",
      main="Michaelis-Menten",
      pch=17, col="blue")
```



Does it look better? You can change the color to `red` or another color if you wish to try.

Does it look OK?

We can make the data into a `data frame` for `S` and `v` so that we don't have to specify both vectors each time.

We can create the dataframe with the command:

```
kinData <- data.frame(S,v)
```

4.2 Theoretical equation

It would be nice to plot a theoretical curve in the midst of the data, and also find a way to evaluate K and V_m (`Vmax`) in the process.

The theoretical curve can be defined into an `R` object to contain the Michaelis-Menten curve, for example we can call it `MMcurve`.

```
# "velocity = Vmax times S divided by (Km plus S)", stored in MMcurve
MMcurve<-formula(v ~ Vmax*S/(Km+S))
```

Briefly: The `~` operator [...] is interpreted as a specification that the response `v` is modelled by a linear predictor `Vmax*S/(Km+S)`.

For more details on the `formula()` function:

```
?formula
```

From our enzymology classes we need to remember that:

V_m is the maximum value, the asymoptote of the curve.

The value of K_m is equal to S when V_m is half-way ($\frac{1}{2}V_m$)

By observing the plot we can make a "guess" as to what the values should be approximately, and then use an R method to solve the equation based on this rough data.

By observing the plot we can deduct that:

$V_m \approx 50$

$K_m = S$ at $(\frac{1}{2}V_m)$ *i.e.* $K_m \approx 2$

4.3 Nonlinear least square

Nonlinear least square is a mathematical method that can help solve the Michaelis-Menten equation.

Any basic R installation contains the `stats` (statistics) package and the `nls()` function is a built-in Nonlinear least square solver.

Now that we have an estimate of the parameters to initialize the calculation, we can ask R to help compute an answer for the values of the constants V_m and K_m (see estimation above.)

```
bestfit <- nls(MMcurve, kinData, start=list(Vmax=50,Km=2))
# print the results
bestfit
```

```
Nonlinear regression model
  model: v ~ Vmax * S/(Km + S)
   data: kinData
  Vmax     Km
73.261  3.437
 residual sum-of-squares: 156.4

Number of iterations to convergence: 7
Achieved convergence tolerance: 5.971e-06
```

Therefore we have established that:

$V_m = 73.26$

$K_m = 3.44$

> **Important note**: We are learning here to make "dynamic" document and therefore ***all*** the numbers have to be calculated by R when the document is updated.
>
> Therefore here is the "under the hood" code to write the results so that **no manual data entry** will be required!
>
> First, we could see the results of `bestfit` by simply having it print to the screen and in the document.
>
> Now we need to be able to "grab" the values presented for `Vmax` and `Km` and for now we'll dispense of the "pretty" nomenclature of V_m and K_m to simply concentrate on what's important.
>
> These two parameters can be extracted from bestfit with the `coef()` function, a generic function to extract model coeficients from objects returned by modeling functions, in our case it was `nls()`. The function can be written as either `coef()` or `coefficients()`. Hence:
>
> ```
> coef(bestfit)
> ```
>
> ```
>     Vmax        Km
> 73.261388  3.437164
> ```
>
> We can use subsetting methods to "grab" them independently:

```r
# Vmax is the first:
coef(bestfit)[1]
```

```
   Vmax
73.26139
```

```r
# We can also round and show only 2 significant digits:
round(coef(bestfit)[1], digits = 2)
```

```
Vmax
73.26
```

```r
#Km is the second:
coef(bestfit)[2]
```

```
     Km
3.437164
```

```r
# rounded:
round(coef(bestfit)[2], digits = 2)
```

```
 Km
3.44
```

To write the "narrative" and use these values within the text we make use of the fact that `R` can compute code within a `code` text if marked properly as we have seen in a previous section.

Since `R` can extract and round-off these numbers we can indeed include them in the text as such:

which is processed as:

Vm = 73.26 and **Km = 3.44**

5 Theoretical curve

We can use the `bestfit` calculation to now calculate and plot the theoretical curve.

First, we create a set of theoretical S concentration values from `0` to `50` in step increments of `0.1` (intervals). We can then use these substrate concentrations to calculate ***predicted velocities*** that will make the theoretical curve line

```r
# Concentration ranges for S:
SconcRange <- seq(0,50,0.1)
```

Now we can use the `stats` package `predict()` function, *"a generic function for predictions from the results of various model fitting functions."*

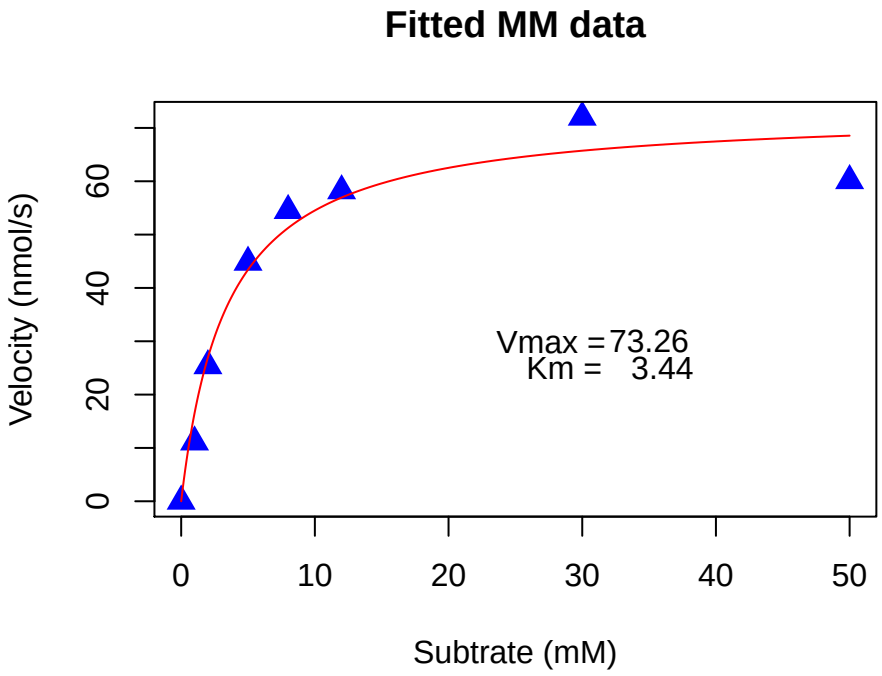
```r
# Then, calculate the predicted velocities using the predict() function:
theorLine <- predict(bestfit,list(S=SconcRange))
```

We can now redo the plot with all the data, theoretical line and constant values:

```r
# Now plot the data, the best fit line, and put the best fit coefficients in the plot
plot (kinData,
      xlab="Subtrate (mM)",
      ylab="Velocity (nmol/s)",
      title(main="Fitted MM data"),
      pch=17, col="blue", cex=1.5)
```

```r
# Now add the theoretical line with the lines() function:
lines(SconcRange,theorLine,col="red")

# Add text with the values:
text(28,30, "Vmax = ")
text(35,30,round(coef(bestfit)[1],2))
text(29,25, "Km = ")
text(36,25,round(coef(bestfit)[2],2))
```



6 Final Freddie report

We now have all the elements to write a final report for Freddie...

When you press `Knit HTML` the `HTML` file is saved in one single file (containing all figures as well) and you can hand it to Freddie.

It is possible to export at `PDF` and MSWord`in addition to`HTML‘ but that might require the installation of at least one more package.

7 R Session.

It is customary to add information about the `R` version and the packages that were loaded at the time of the calculation. This is accomplished with the simple command `session()` that will display all this information. This is extremely useful to record the `R` version etc.

```r
sessionInfo()
```

```
R version 3.3.3 (2017-03-06)
Platform: x86_64-apple-darwin13.4.0 (64-bit)
Running under: OS X El Capitan 10.11.6

locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] knitr_1.15.1

loaded via a namespace (and not attached):
 [1] backports_1.0.5 magrittr_1.5    rprojroot_1.2   tools_3.3.3
 [5] htmltools_0.3.5 yaml_2.1.14     Rcpp_0.12.10    stringi_1.1.5
 [9] rmarkdown_1.4   highr_0.6       stringr_1.2.0   digest_0.6.12
[13] evaluate_0.10
```

8 Acknowledgments

The data, ploting method and nls calculation were from the script online at: http://rforbiochemists.blogspot.com/2015/05/plotting-and-fitting-enzymology-data.html

Other web examples for MM plots:

Fitting a Michaelis-Menten model and drawing the results in R

Fitting a Michaelis-Menten curve using R

REFRENCES:

Michaelis, L., and M.L. Menten. 1913. "Die Kinetik Der Invertinwirkung." *Biochemische Zeitschrift* 49: 333–69.

Michaelis, L., M. L. Menten, K. A. Johnson, R. S. Goody, L. Michaelis, and M. L. Menten. 2011. "The original Michaelis constant: translation of the 1913 Michaelis-Menten paper." *Biochemistry* 50 (39): 8264–9.