**Quantitative Life Sciences: from Infectious Diseases to Ecosystems**

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# Pharmacokinetics/-dynamics & Stoichiometry
Exercise sheet

## Objectives

- to understand the drug distribution in the body and how to model it quantitatively with ordinary differential equations (ODE)
- to understand how the drug effect can be described in terms of drug concentrations
- to simulate HIV entry of pseudotyped HIV virus and understand how it can be quantified
- **to quantify the interactions between biological organisms and chemical substances**

## Available scripts

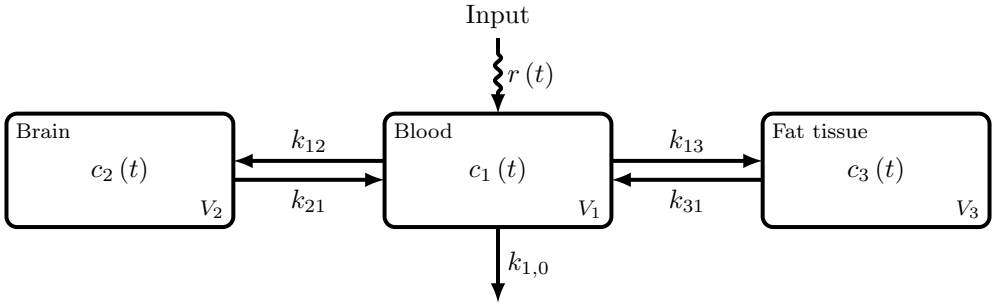
Files `PK_scripts.R`, `PD_scripts.R` and `Stoichiometry_scripts.R` contain different functions that will be used in this hands-on session. You can load them by calling

```
source("<path_to_the_folder>/PK_scripts.R")
source("<path_to_the_folder>/PD_scripts.R")
source("<path_to_the_folder>/Stoichiometry_scripts.R")
```

Alternatively, you can open then files and run each function separately. You may use the templates from `PK_PD_Stoichiometry-Templates.R` file.

## Problem 1: Pharmacokinetics in anesthesia

In order to sufficiently describe the pharmacokinetics of intravenous anesthetics the following three compartment model has been applied for various agents




After intravenous administration of the anesthetic into the blood compartment a rapid distribution to the central nervous system starts (brain compartment). The initial rapid equilibrating phase is followed by slower distribution (fat tissue compartment) before finally the drug elimination from the blood begins.

The blood ($CL$), brain ($CL_2$) and fat tissue ($CL_3$) clearances are defined as follows

$$\begin{aligned} CL &= k_{10} \cdot V_1, \\ CL_2 &= k_{12} \cdot V_1 = k_{21} \cdot V_2, \\ CL_3 &= k_{13} \cdot V_1 = k_{31} \cdot V_3. \end{aligned}$$

The pharmacokinetics is described by the system of ODEs

$$\begin{aligned} V_1 \cdot \frac{\mathrm{d}c_1(t)}{\mathrm{d}t} &= r(t) + CL_2 \cdot c_2(t) + CL_3 \cdot c_3(t) - (CL + CL_2 + CL_3) \cdot c_1(t), \\ V_2 \cdot \frac{\mathrm{d}c_2(t)}{\mathrm{d}t} &= CL_2 \cdot c_1(t) - CL_2 \cdot c_2(t), \\ V_3 \cdot \frac{\mathrm{d}c_3(t)}{\mathrm{d}t} &= CL_3 \cdot c_1(t) - CL_3 \cdot c_3(t), \end{aligned}$$

where $r(t)$ is dosing regime (delivered anesthetic per time unit).

Or

$$\begin{aligned} \frac{\mathrm{d}a_1(t)}{\mathrm{d}t} &= r_a(t) + k_{21} \cdot a_2(t) + k_{31} \cdot a_3(t) - (k_{10} + k_{12} + k_{13}) \cdot a_1(t), \\ \frac{\mathrm{d}a_2(t)}{\mathrm{d}t} &= k_{12} \cdot a_1(t) - k_{21} \cdot a_2(t), \\ \frac{\mathrm{d}a_3(t)}{\mathrm{d}t} &= k_{13} \cdot a_1(t) - k_{31} \cdot a_3(t), \end{aligned}$$

aa

$$a_1 = V_1 \cdot c_1$$

The model parameters depend on the anesthetic and may also vary between the patients. Propofol is an intravenous anesthetics whose PK have been studied with the above model and the corresponding parameters have been estimated in various studies. The Schnider model for propofol uses the estimates from Table 1.

| Parameter | Estimated parameter value for **propofol** |
|---|---|
| $V_1$ [L] | 4.27 |
| $V_2$ | $18.9 - 0.391 \cdot (\text{Age} - 52)$ |
| $V_3$ | 238 |
| $CL$ [L/min] | $1.89 + 0.0456 \cdot (\text{Weight} - 77) - 0.0681 \cdot (\text{LBM} - 59) + 0.0264 \cdot (\text{Height} - 177)$ |
| $CL_2$ | $1.29 - 0.024 \cdot (\text{Age} - 52)$ |
| $CL_3$ | 0.836 |

Table 1: Schnider model for propofol.

LBM is the lean body mass, calculated from the gender, weight (in kilograms) and height (in centimeters) as

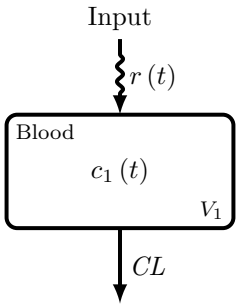
$$\text{LBM}_M = 1.1 \cdot \text{Weight} - 128 \cdot \left(\frac{\text{Weight}}{\text{Height}}\right)^2 \quad \text{for males, and}$$

$$\text{LBM}_F = 1.07 \cdot \text{Weight} - 148 \cdot \left(\frac{\text{Weight}}{\text{Height}}\right)^2 \quad \text{for females.}$$

1. **Model parameters:** Using Table 1 calculate the parameters for your imaginary patient (be reasonable!) and save them for later.

   `Hint:` Functions `LBM` and `Schnider_model` from `PK_scripts.R` calculate the Schnider model parameters for propofol from gender, age (in years), height (in centimeters) and weight (in kilograms).

2. **Single compartment simplification:** Assume that the anesthetic only distribute in the blood compartment, namely $CL_2 = CL_3 = 0$. In other words, suppose that the simplified one compartment model




sufficiently describes the PK profile of propofol. The corresponding ODE is

$$V_1 \cdot \frac{\mathrm{d}c_1(t)}{\mathrm{d}t} = r(t) - CL \cdot c_1(t). \tag{1}$$

(a) *Single IV bolus:* For the anesthesia induction a single IV injection containing $D_0$ of anesthetic is given to your patient. Therefore, $r(t) \equiv 0$ (no infusion) and $c_1(0) = \frac{D_0}{V_1}$. Suppose that the initial dose $D_0$ to induce anesthesia equals

$$D_0 = 6 \cdot V_1.$$

The drug concentration in the blood compartment follows

$$c_1(t) = \frac{D_0}{V_1} \exp\left(-\frac{CL}{V_1} \cdot t\right).$$

Calculate the initial dose $D_0$ for your patient and plot the drug concentrations over the first 240 min for propofol.

Hint: Function `exp` from R is vectorized, i.e., to calculate exponent over a range of values a single function call does its job.

```
times <- c(0, 1, 2)
exp(times)

## [1] 1.000000 2.718282 7.389056
```

(b) *Continuous IV infusion:* In order to induce and maintain anesthesia the anesthetic is delivered with IV infusion at the constant rate $r(t) \equiv r$. The drug concentration in the blood compartment follows

$$c_1(t) = \frac{r}{CL} \cdot \left(1 - \exp\left(-\frac{CL}{V_1} \cdot t\right)\right)$$

assuming that upon starting the infusion the drug was absent ($c_1(0) = 0$). The infusion rate for your patient equals $r = 6 \cdot CL$.

(i) Plot the concentrations for the first 240 min after the infusion start.

(ii) What is the maximum concentration? Is it ever reached?

(c) *IV infusion with loading dose:* Assume that in addition to the continuous IV infusion with rate $r = 6 \cdot CL$ at time of the infusion initiation additional IV bolus dose $D_0 = \frac{V_1 \cdot r}{CL}$ is injected. This IV bolus is called the *loading dose*.

(i) What are the the infusion rate, the loading dose and initial concentration for you patient?

(ii) Load the R-package `deSolve` with

```
require(deSolve)
```

Use the function `c1_loading` from `PK_scripts.R` to obtain propofol concentrations for the first 240 min and plot them.

Hint: In addition to the continuous IV infusion with the loading dose the function also allows to stop the infusion after time `T_stop`. For this task leave the argument `T_stop` unspecified (default). ***With minor step-wise modifications this function can also be used for the subsequent questions, so it is worth playing with it a bit. We will upgrade it step-by-step, hence you do not need to understand every detail at this point.*** What do you get if you set `r=0`? What if you choose `D_0=0`?

(iii) What are the benefits of such loading dose?

(iv) Stop the infusion after 90 min by setting `T_stop = 90`. Save the results into variable `c1_stop` and plot the concentrations for the first 240 min. Take a look at the attribute `time_to_event` by

```
time_to <- attr(c1_stop, "time_to_event")
```

Can you interpret the time `time_to` by observing the concentrations in the blood after the infusion has been stopped?

3. **Three compartment model with continuous IV infusion:** Assume that the anesthetic is delivered to the blood compartment with the IV infusion at the constant rate $r(t) \equiv r = 6 \cdot CL$ (same as in 2.) and that the concentrations prior start are 0 in all three compartments.

(a) *Steady-state concentrations:* Calculate the steady-state concentrations (i.e. stable concentrations which do not change with time).

Hint: What should be the values of derivatives $\frac{\mathrm{d}c_1(t)}{\mathrm{d}t}$, $\frac{\mathrm{d}c_2(t)}{\mathrm{d}t}$ and $\frac{\mathrm{d}c_3(t)}{\mathrm{d}t}$ for steady-state concentrations $c_1 \equiv c_1(t)$, $c_2 \equiv c_2(t)$ and $c_3 \equiv c_3(t)$?

(b) *Concentration dynamics:* Solve the ODEs numerically for first 240 min and 5 s time step and plot the concentrations in all three compartments.

Hint: Use the template below. You might also find the implementation of the `c1_loading` helpful. What do you need to change?

To combine all three plots you can set `mfrow=c(3,1)` in the `plot` function.

```
c3_infusion <- function(T_max, dt, r,
                        V_1, CL, V_2, CL_2, V_3, CL_3) {

  # model parameters:
  params <- c("V_1" = V_1,
              "CL" = ???,
              ???)

  # initial concentrations (at time = 0)
  c_init <- c("c_1" = ???, # blood compartment
              "c_2" = ???, # brain compartment
              "c_3" = ???) # fat tissue compartment

  # time grid
  times <- seq(from=???, to=???, by=???)

  # instantaneous concentrations change
  # i.e. the derivatives describing the ODE system
  dc <- function(time, conc, params) {

    # infusion rate - constant
    rate <- params["r"]

    # dc_1/dt
    dc_1 <- 1/params["V_1"]*(rate+params["CL_2"]*conc["c_2"]+???)

    # dc_2/dt
    dc_2 <- ???
```

```
    # dc_3/dt
    ???

    derivatives <- c("c_1" = dc_1, # dc_1/dt
                     "c_2" = ???, # dc_2/dt
                     "c_3" = ???) # dc_3/dt

    return(list(derivatives))
  }

  # numerical solution
  c_out <- ode(y = ???, # initial values in each compartment
               times = ???, # time grid
               func = ???, #ODEs
               parms = ???) # model parameters

  return(c_out)

}
```

(c) *Context sensitive half-life:* The infusion is stopped after 90 min. Assume that the patient wakes up when the concentration is halved.

(i) How long does it take before the concentration in the central compartment is halved after having stopped the infusion (*context sensitive half-life*)?
Hint: Combine the functions `c1_loading` and `c3_infusion` which you implemented in (3.b). You can use the provided template specific for this question.

(ii) Compare the context sensitive half-life of propofol from this model with the time from the task (2.c.iii). Why are they different?

(iii*) Does the context half-life depend on the infusion duration?
Hint: You can either repeat the previous step for a short duration `T_stop` = 15 minutes and for a long duration of 180 minutes or (if you feel comfortable with repeating the steps over the whole range of infusion durations), you can repeat it over a range of infusion durations `T_stop`, for instance $15 - 180$ minutes in 15 minutes steps.

# Problem 2: Pharmacodynamics

1. **Sigmoid $E_{max}$ response model:** Suppose that the drug receptor $R$ with $\gamma$ binding sites binds to the drug $D$ with the following reaction kinetics

$$\gamma \cdot D + R \underset{k_{\text{on}}}{\overset{k_{\text{off}}}{\rightleftharpoons}} D_\gamma R.$$

Under the assumption of total receptor concentration conservation, the total receptor concentration $[R_T]$ equals the sum of free receptor conectration $[R]$ and drug-receptor complex (bound receptor) concentration $[D_\gamma R]$, namely $[R_T] = [R] + [D_\gamma R]$ at all times.
In the equilibrium, the bound receptor concentration $[D_\gamma R]_{eq}$ is (derived from the law of mass action and assuming that the binding has negligible effect on the drug concentration, i.e., $[D] \approx [D]_{eq}$)

$$[D_\gamma R]_{eq} = \frac{1}{K_D^\gamma} [D]^\gamma [R]_{eq},$$

where $K_D^\gamma := \frac{k_{\text{off}}}{k_{\text{on}}}$ is a binding-reaction constant.
The equibilibrium receptor occupancy hence equals

$$RO := \frac{[D_\gamma R]_{eq}}{[R_T]} = \frac{[D_\gamma R]_{eq}}{[R]_{eq} + [D_\gamma R]_{eq}} = \frac{\frac{1}{K_D^\gamma} [D]^\gamma [R]_{eq}}{[R]_{eq} + \frac{1}{K_D^\gamma} [D]^\gamma [R]_{eq}} = \frac{[D]^\gamma}{K_D^\gamma + [D]^\gamma}.$$

Sigmoid $E_{max}$ response model assumes that the effect of drug on the body is proportional to the equilibrium receptor occupancy, namely

$$E = f(RO) = \alpha \cdot RO.$$

(a) *Sigmoid $E_{max}$ curve:* One possible model is given by

$$E(c) = E_0 + \frac{E_{max} \cdot c^{\gamma}}{EC_{50}^{\gamma} + c^{\gamma}}.$$

Interpret its variables and parameters with the help of the derivation above. Can you link its parameters with $K_D$ and receptor occupancy $RO$? Implement the sigmoid $E_{max}$ function in R as a function `E` with parameters `conc`, `E0`, `Emax`, `EC50` and `gamma`.
Hint: What is the receptor occupancy at $[D] = K_D$?

```
E <- function(conc, E0, Emax, EC50, gamma) {
  tot_eff <- ???
  return(tot_eff)
}
```

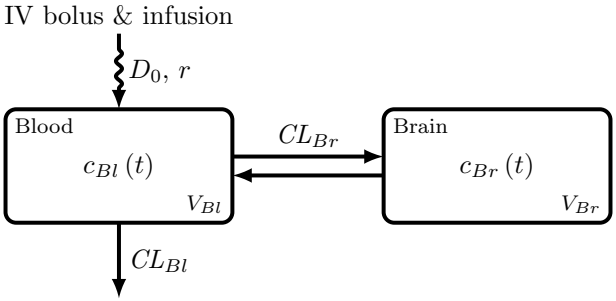
(b) *Minimum effective concentration and potency:* Assume that the drugs A, B and C all have maximum treatment effect $E_{max} = 90$, baseline effect $E_0 = 10$ and $EC_{50} = 0.3$. Drug A has Hill coefficient $\gamma_A = 1$, drug B $\gamma_B = 4$ and drug C $\gamma_C = \frac{1}{4}$. Plot their effects for different drug concentrations ranging from 0 to 1.5.
Hint: To include multiple lines in the same plot you can use function `lines`.

```
plot(???, ylim = c(???,???))
lines(???)
```

(i) Suppose that the minimum therapeutic effect is 20 (for instance to kill tumor cells faster than they are growing) and that the concentrations above 0.25 are too toxic for the patient. Which drug would you choose?

(ii) Which drug would you prefer if the therapeutic concentration window was between 0.45 and 1.2?

2. **Direct response and biophase distribution models:** Assume the following simplified model for an IV anesthetic: The anesthetic is delivered intravenously with a single dose $D_0$ and maintained




with IV infusion at the constant rate $r$ for the first 75 minutes. First, it is distributed in blood from where it is then transported to the brain compartment where its target cells are present. Finally, it is returned to the blood compartment from where it is eliminated. The effect of the anesthetic is measured with percentage change in EEG (electroencephalogram), which measures the brain activity during sedation/anesthesia.

(a) *PK model:* The PK of the anesthetic is already implemented in the function `conc_Blood_Brain` and has the following parameters:

`T_max:` the last time at which the concentrations are calculated

`dt:` time steps at which the concentrations are approximated

`V_Bl:` volume of distribution of blood compartment

`V_Br:` volume of distribution of brain compartment

`CL_Bl:` clearance of the elimination from blood

`CL_Br`: clearance between the blood and the brain compartment

`D_0`: bolus dose

`r`: infusion rate

`T_stop`: infusion duration

Plot drug concentrations in both compartments over the first 120 minutes for parameters $V_{Bl} = 5$ L, $V_{Br} = 1.2$ L, $CL_{Bl} = 0.1$ L/min and $CL_{Br} = 0.3$ L/min. Bolus dose is $D_0 = 10$ mg and the infusion rate is $r = 0.15$ mg/min.
Hint: Set `mfrow = c(2,1)` in the `plot` function.

(b) *Direct response model:* The anesthetic's effect on EEG is described with the sigmoid $E_{max}$ model with $E_{max} = 100$, $EC_{50} = 1$ and $\gamma = 2$. The baseline change in EEG is 0.

(i) Plot the EEG change over time after a single IV bolus of anesthetic and continuous IV infusion.
Hint: Where does the effect take place, i.e. which is the effect compartment? Use the function `E` which you implemented in (1.c).

(ii) When do you expect the highest effect?
Hint: Function `which.max`.

(iii) Plot the obtained time-depending effects against the drug concentration in brain at the same time points. Do you see the expected sigmoid shape?

(c) *Biophase distribution model:* Usually the drug concentrations cannot be measured in the effective compartment. In addition, often the effective compartment is not known (as well as its PK parameters). Based on the above example we will illustrate how neglecting the effective compartment affects the results.
Suppose that the effect of the anesthetic is described with the same sigmoid $E_{max}$ model parameters, but since the drug concentrations in brain cannot be measured, you predict the effect based on the concentrations in blood.

(i) Plot the EEG percentages against time. When do you expect the anesthetic to be the most effective based on the drug concentrations in blood?

(ii*) Plot the "real" EEG effect that the drug causes (the effect predicted in (2.b.i)) against the observable blood drug concentrations. Does it look like a sigmoid curve?

## Bonus-Problem 3: Stoichiometry of HIV entry

The production of pseudotyped viruses with mixed envelope (Env) trimers expressed on their surface enables the quantification of the HIV entry and neutralization. Each HIV trimer consists of three envelope proteins and the pseudotyped viruses are produced by transfecting cells with a mixture of plasmids encoding wild-type and mutant Env proteins. Therefore, the generated virions express four types of trimers: wild-type Env homotrimers, mutant Env homotrimers, and Env heterotrimers with one or two mutant Env proteins.

The stoichiometry of HIV entry $T$ is the number of trimer-receptor interactions which the virus needs to infect target cell. Let $f_M$ denote the fraction of mutated Env proteins forming the virion. Since one mutated envelope protein is thought to be enough to render a trimer nonfunctional the probability that the trimer is functional therefore equals

$$p = (1 - f_M) \cdot (1 - f_M) \cdot (1 - f_M) = (1 - f_M)^3 .$$

The fact that $p \neq 1$ enables us to estimate $T$ from the infectivity of the pseudotyped viruses compared to the infectivity of the wild-type virus (i.e. *relative infectivity*).

1. **Virion:** Assume that each Env protein forming a trimer is chosen at random from an (endless) sample of mixed Env proteins. Given a mixture of Env proteins a virion can be then constructed as follows. To build the first trimer 3 proteins are randomly drawn from the set of Env proteins - a mutant Env protein is drawn with probability $f_M$ and the wild-type protein with the probability $1 - f_M$. This is repeated until the total number of trimers is sampled.
Construct a virion with $s$ trimers from a mixture of Env proteins with fraction $f_M$ of mutated Env proteins and calculate its number of functional trimers by performing the following steps.

(a) *Trimer and its functionality:* Function `sample_trimer(f_M)` from `Stoichiometry_scripts.R` builds a trimer from Env pool with fraction of mutated Env proteins `f_M`.

(i) Construct one trimer with `f_M=0.5` and one with `f_M=0`.

(ii) Write a function `is_functional(trimer)` which returns `TRUE` when the `trimer` is functional and `FALSE` otherwise. Are your two trimers functional?
Hint: Can you use the R function `all`?

```
trimer1 <- ???
trimer2 <- ???

is_functional <- function(trimer) {

  ???

}

is_functional(trimer1)
???
```

(b) *Number of functional trimers:* Function `sample_virion(s, f_M)` from `Stoichiometry_scripts.R` constructs a virion with `s` trimers from Env pool with fraction of mutated Env proteins `f_M`.

(i) Test the function for $s = 4$ and $f_M = 0.5$.

(ii) Write a function `g_trimers(virion)` which calculates how many trimers of the `virion` are functional. How many functional trimers does your simulated virion have? Assume that the one single functional trimer is sufficient for HIV to infect a cell ($T = 1$). Is your virion able to infect the cell? Repeat this one more time by sampling another virion with the same parameters. Does the number of functional trimers change?
Hint: Functions `lapply` and `is_functional`.

```
g_trimers <- function(virion) {

  # Which trimers are functional?

  # How many functional trimers are there?
}
```

2. **Virus population and its infectivity:** Assume that each virion in the pseudotyped virus generation of $N = 10^4$ virions with fraction $f_M = 0.103$ of mutant Env proteins has exactly $s = 13$ trimers on the surface. How does the infectivity of the population depend on the stoichiometry of HIV entry $T$?

(a) *Virus population:* Using the function `sample_virion` sample a virus population `virions` of $10^4$ virions. What is the distribution of the number of functional trimers in your viral population?
Hint: Use function `replicate` to generate a viral population. For each virion calculate the number of functional trimers (if you are using `lapply`, do not forget to `unlist` the output) and plot the distribution using `hist` with the option `freq = FALSE`. Compare the result with the theoretical distribution as demonstrated below:

```
s <- ???
f_M <- ???
virions <- replicate(n = ???, # nr. of repetitions
                     expr = ???, # function call to be repeated n-times
                     simplify = FALSE)
# Distribution of number of functional trimers
nr_func_trimers <- unlist(lapply(X = ???, FUN = ???))
## histogram
h <- hist(???,
          col="lightgray",
          border = "white",
          freq=FALSE,
```

```
          main="Distribution of the number of functional trimers",
          xaxt="n",
          xlab="Number of functional trimers",
          ylab="Probability",
          breaks=seq(0, s+1))
axis(side=1, at=h$mids[1:s], labels=h$breaks[2:(s+1)])
## theoretical distribution
p <- (1-f_M)^3
pdf_binom <- dbinom(x=seq(1, s), size=s, prob=p)
lines(h$mids[1:s], pdf_binom, lty="dotted")
points(h$mids[1:s], pdf_binom, pch=19, cex = 1.2)
```

(b) *Infectivity:* Infectivity of viral population is defined as the proportion of the virions which are able to infect cells and depends on the stoichiometry of HIV entry.

(i) Write a function `infectivity(virions, TT)` which calculates the infectivity given that the required number of functional trimers on the virion surface is `TT` (stoichiometry of HIV entry).
Hint: Functions `lapply` and `g_trimer`.

```
infectivity <- function(virions, TT) {

  # Number of functional trimers for each virion (see (a))
  nr_func_trimers <- unlist(lapply(X = ???, FUN = ???))

  # Which virions have enough functional trimers?
  inf_virions <- which(nr_func_trimers ???)

  # Proportion of "infectious" virions
  prop_inf <- ???

  return(prop_inf)
}
```

(ii) For your simulated viral population `virions` calculate the infectivity for each `TT` $\in \{1, 2, \ldots, 14\}$ and plot the results. Is the infectivity increasing or decreasing with $T$?
Hint: Functions `sapply` and `infectivity`.

(iii*) How would the same plot look like for the wild-type virus population?

3. **Trimer number distribution:** In question (2.) we assumed that all the virions from the population have the same number of trimers on the surface. However, this is not the case and hence the infectivity of the population also depends on the trimer number distribution.

(a) *Empirical trimer distribution:* Import file `trimer_numbers.csv` to R, which contains the trimer numbers for different virus strains and from different assays (column `"mean_eta"`). Plot the sample of trimer numbers using `hist`. What is the `mean` number of trimers expressed on a single virion?
Hint: You can import `.csv` files with `read.csv` function. You may want to check how the imported data look like.

4. **HIV entry stoichiometry $T$ estimate:** Since the relative infectivity depends on the HIV entry stoichiometry $T$, the latter can be estimated from the observed relative infectivites from different virus strains and fractions $f_M$, and by taking the trimer number distribution into account.

(a) *Experimental relative infectivity (RI):* File `RI_data.csv` contains the experimental data collected to estimate $T$. Import the dataset and plot the relative infectivities (RI) against fractions of mutant plasmids $f_M$. Did you expect such shape? Do you expect the same relative infectivity curves for all virus strains? For the interpretation of this part please see [**?**].

(b) *HIV entry stoichiometry estimate for a single viral strain:* Column `"virus"` indicates the virus strain. For each strain the experiment was performed for two different *Env* mutations (column `"mutation"`), namely R508S/R511S and V513E. Finally, the infectivity of wild type *Env*s (`wt` in column `"env"`) were compared to the $\Delta$V1V2 *Env*s with deletions of the gp120 variable loops 1 and 2 (`V1V2` in column `"env"`).

(i) Choose one viral strain among P3N, Ac10 and Cap88, and one of the R508S/R511S and V513E mutations. Prepare two separate datasets for your virus - one containing the relative infectivity data for wild-type *Env* and one for *Env* with V1V2 deletion. Plot the relative infectivity data against $f_M$ for both datasets and compare them. Which type of *Env* do you expect to have higher entry stoichiometry?

(ii) Estimate the HIV entry stoichiometry for wild-type *Env* virus with function `estimate_T(data, trimer_number_sample)` from `Stoichiometry_scripts.R`. `data` is the dataset containing the relative infectivities RI and the corresponding $f_M$ for your virus. `trimer_number_sample` is the corresponding sample of trimer numbers.
`Hint:` Dataset `trimer_numbers.csv` from (3.a).

(iii*) Estimate the HIV entry stoichiometry for the same virus with $\Delta$V1V2 *Env*. What could you conclude from the two estimated stoichiometries?