tle: “TrpF_kinetics”
thor: “NellySelem”
te: “May 18, 2017”
tput: pdf_document

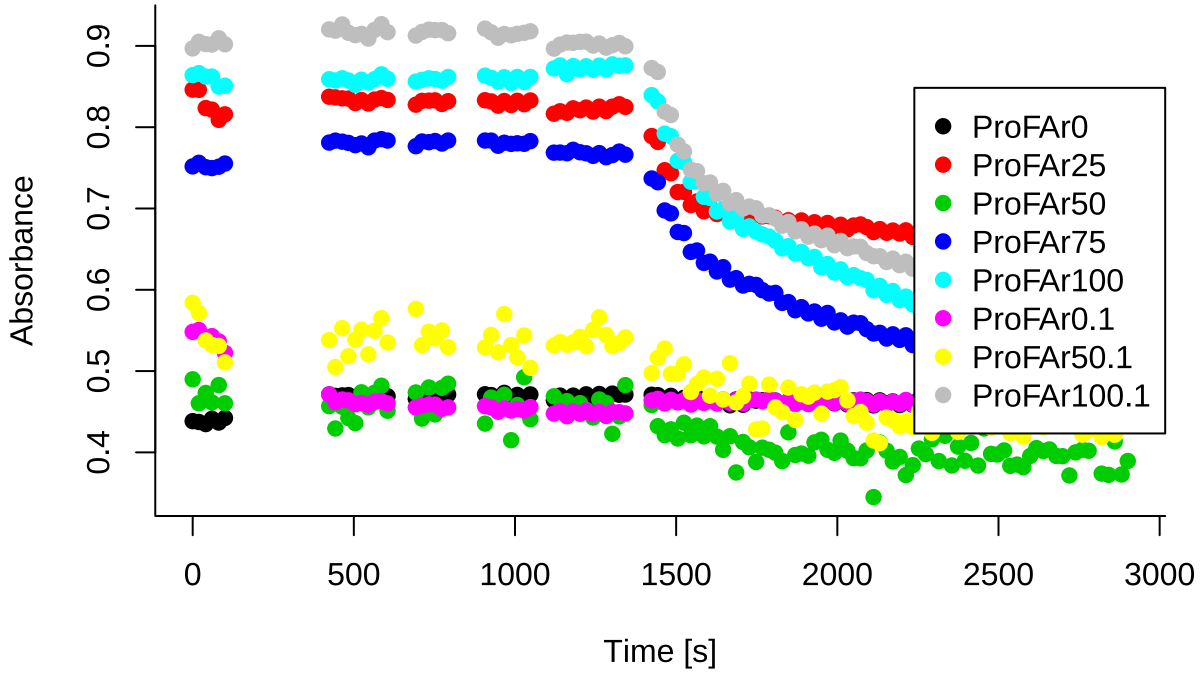
Process data

```
#First I saved file as scv tablar separated
#perl -p -i -e 's/,/\t/g' Pra_scoe1.csv
#An cut it to obtain just data
#tail -n +39 Pra_scoe1.csv | head -n-3 > Pra_scoe1.data
#perl -p -i -e 's/\sNr\.|\s\[\w*\]|\.\s\[.*\]//g' Pra_scoe1.data
#tablePRAPRO <- read.table(s"chapter2/cineticas/Pra_scoe1.data",header=TRUE, sep="\t")
tablePRO <- read.table("cineticas/18082017_PraProfarAbsorbanceCombined.data",header=TRUE, sep="\t")
tablePRA <- read.table("cineticas/18082017_PraProfarFluorCombined.data",header=TRUE, sep="\t")
#tablePRA <- read.table("15082017_praOSCURO.data",header=TRUE, sep="\t")



## visualising full data
tablePRO$Temp<-NULL
tablePRO$Cycle<-NULL

a<-tablePRO$Time[c(TRUE,FALSE)]
b<-tablePRO$Time[c(TRUE,FALSE)]+19.15

tablePRO$Time[c(TRUE, FALSE)] <- a
tablePRO$Time[c(FALSE, TRUE)] <- b
tablePRO$Time
```

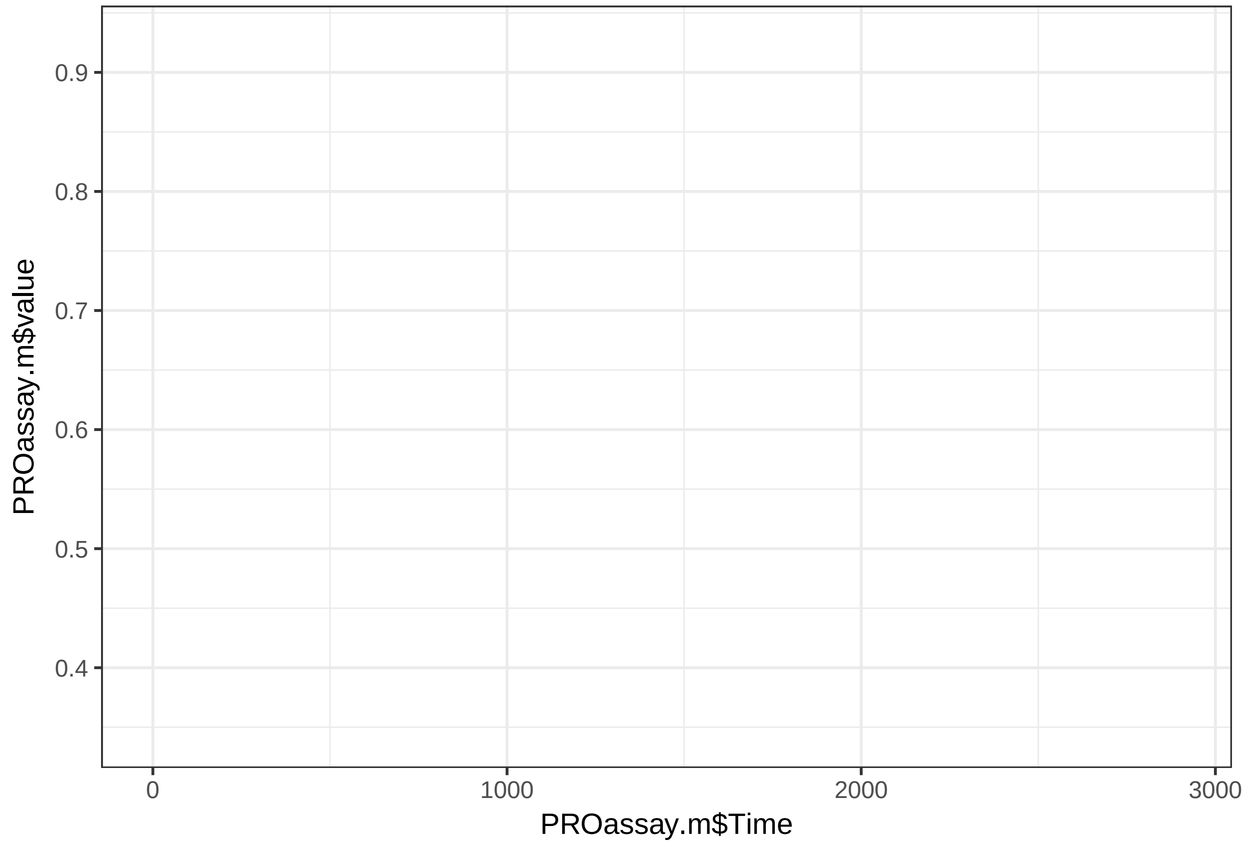
```
##   [1]    0.00   19.15   40.50   59.65   81.00  100.15  423.50  442.65
##   [9]  464.00  483.15  504.50  523.65  545.00  564.15  585.50  604.65
##  [17]  692.40  711.55  732.90  752.05  773.40  792.55  907.00  926.15
##  [25]  947.50  966.65  988.00 1007.15 1028.50 1047.65 1120.70 1139.85
##  [33] 1161.20 1180.35 1201.70 1220.85 1242.20 1261.35      NA      NA
##  [41] 1282.80 1301.95 1323.30 1342.45 1424.00 1443.15 1464.50 1483.65
##  [49] 1505.00 1524.15 1545.50 1564.65 1586.00 1605.15 1626.50 1645.65
##  [57] 1667.00 1686.15 1707.50 1726.65 1748.00 1767.15 1788.50 1807.65
##  [65] 1829.00 1848.15 1869.50 1888.65 1910.00 1929.15 1950.50 1969.65
##  [73] 1991.00 2010.15 2031.40 2050.55 2071.90 2091.05 2112.50 2131.65
##  [81] 2153.00 2172.15 2193.50 2212.65 2234.00 2253.15 2274.50 2293.65
##  [89] 2315.00 2334.15 2355.50 2374.65 2396.00 2415.15 2436.50 2455.65
##  [97] 2477.00 2496.15 2517.50 2536.65 2558.00 2577.15 2598.50 2617.65
## [105] 2639.00 2658.15 2679.50 2698.65 2720.00 2739.15 2760.50 2779.65
## [113] 2801.00 2820.15 2841.50 2860.65 2882.00 2901.15
```

```
tablePRO<-tablePRO[grep("Pro|Time", colnames(tablePRO))]
#tablePRA<-tablePRA[grep("Pra|Time", colnames(tablePRA))]

PROassay.m <- melt(tablePRO,id="Time")

plot(PROassay.m$Time, PROassay.m$value, col=PROassay.m$variable,  xlab="Time [s]",ylab="Absorbance", pch
par(xpd = TRUE)
```

```
legend("right", legend = (unique(PROassay.m$variable)), col = (unique(PROassay.m$variable)),pch=19,bg="
```

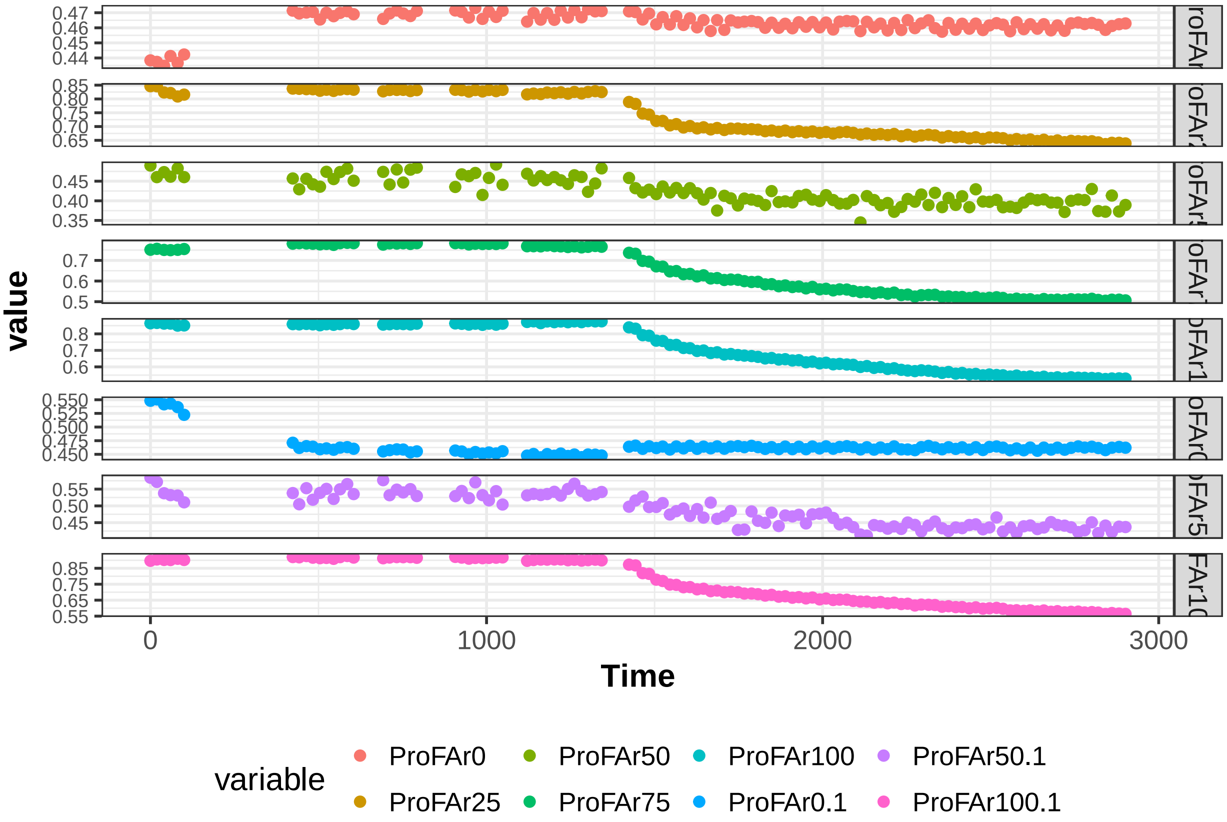


```
ggplot(PROassay.m, aes(x = PROassay.m$Time, y = PROassay.m$value),color="variable") + theme_bw()
```



```
qplot(Time,value, data = PROassay.m,colour=variable)+theme_bw()+ theme(legend.position  ="bottom",legen
```

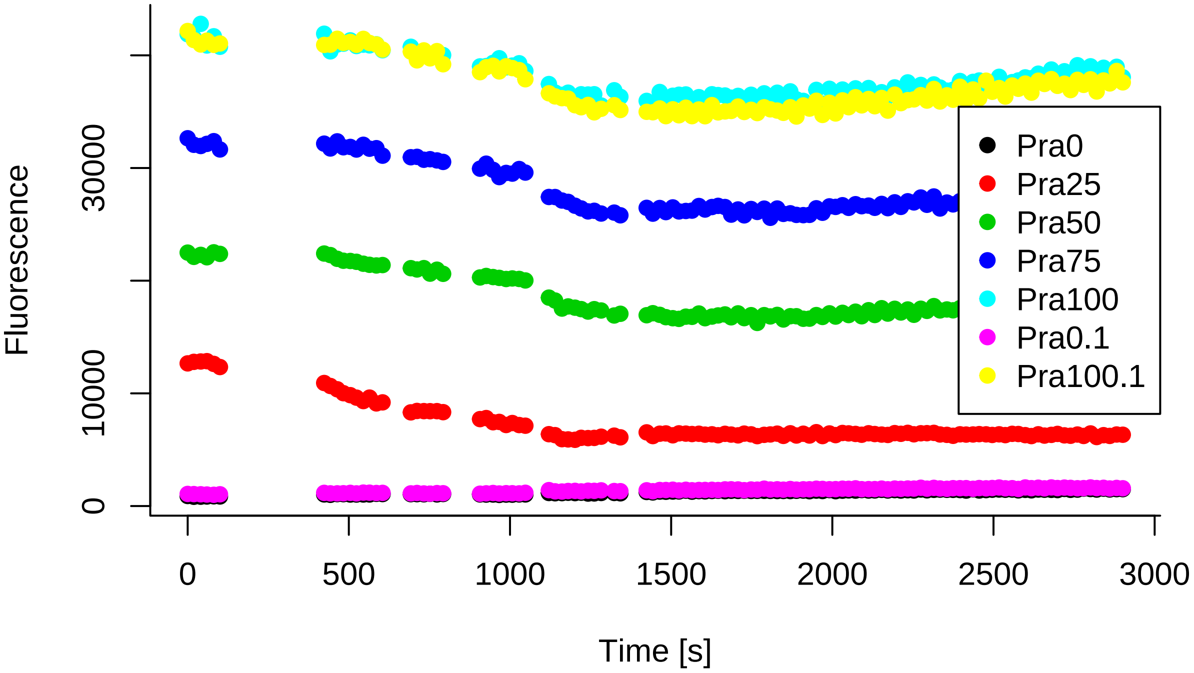
```
## Warning: Removed 16 rows containing missing values (geom_point).
```



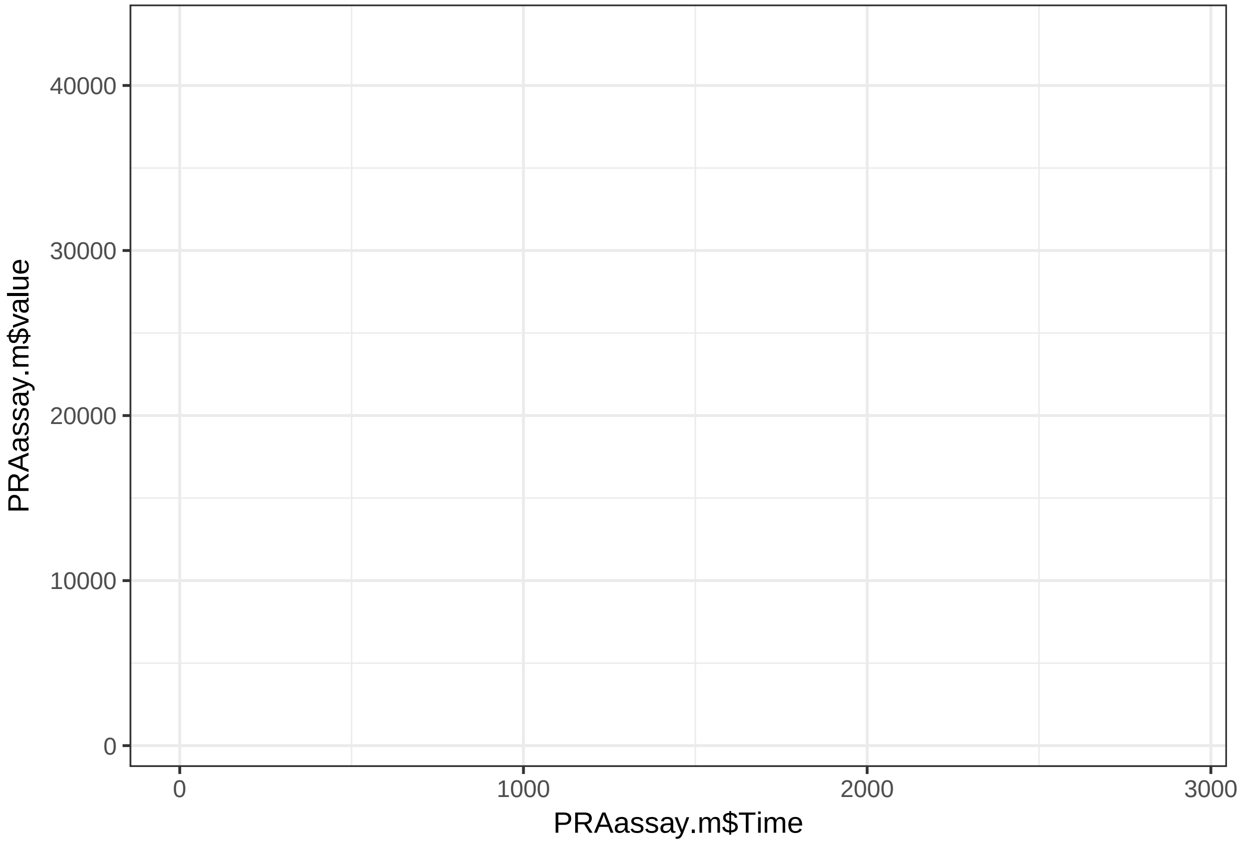
```
## visualising full data
tablePRA$Temp<-NULL
tablePRA$Cycle<-NULL

a<-tablePRA$Time[c(TRUE,FALSE)]
b<-tablePRA$Time[c(TRUE,FALSE)]+19.15

tablePRA$Time[c(TRUE, FALSE)] <- a
tablePRA$Time[c(FALSE, TRUE)] <- b
tablePRA$Time
```

```
##   [1]    0.00   19.15   40.50   59.65   81.00  100.15  423.50  442.65
##   [9]  464.00  483.15  504.50  523.65  545.00  564.15  585.50  604.65
##  [17]  692.30  711.45  732.80  751.95  773.40  792.55  907.00  926.15
##  [25]  947.50  966.65  988.00 1007.15 1028.50 1047.65 1120.70 1139.85
##  [33] 1161.20 1180.35 1201.70 1220.85 1242.20 1261.35 1282.80 1301.95
##  [41] 1323.30 1342.45 1424.00 1443.15 1464.50 1483.65 1505.00 1524.15
##  [49] 1545.50 1564.65 1586.00 1605.15 1626.50 1645.65 1667.00 1686.15
##  [57] 1707.50 1726.65 1748.00 1767.15 1788.50 1807.65 1829.00 1848.15
##  [65] 1869.50 1888.65 1910.00 1929.15 1950.40 1969.55 1991.00 2010.15
##  [73] 2031.50 2050.65 2071.90 2091.05 2112.50 2131.65 2153.00 2172.15
##  [81] 2193.50 2212.65 2234.00 2253.15 2274.50 2293.65 2315.00 2334.15
##  [89] 2355.50 2374.65 2396.00 2415.15 2436.50 2455.65 2477.00 2496.15
##  [97] 2517.50 2536.65 2558.00 2577.15 2598.50 2617.65 2639.00 2658.15
## [105] 2679.50 2698.65 2720.00 2739.15 2760.50 2779.65 2801.00 2820.15
## [113] 2841.50 2860.65 2882.00 2901.15
```

```
tablePRA<-tablePRA[grep("Pra|Time", colnames(tablePRA))]

PRAassay.m <- melt(tablePRA,id="Time")
plot(PRAassay.m$Time, PRAassay.m$value, col=PRAassay.m$variable,  xlab="Time [s]",ylab="Fluorescence", p
par(xpd = TRUE)
legend("right", legend = (unique(PRAassay.m$variable)), col = (unique(PRAassay.m$variable)),pch=19,bg="w
```

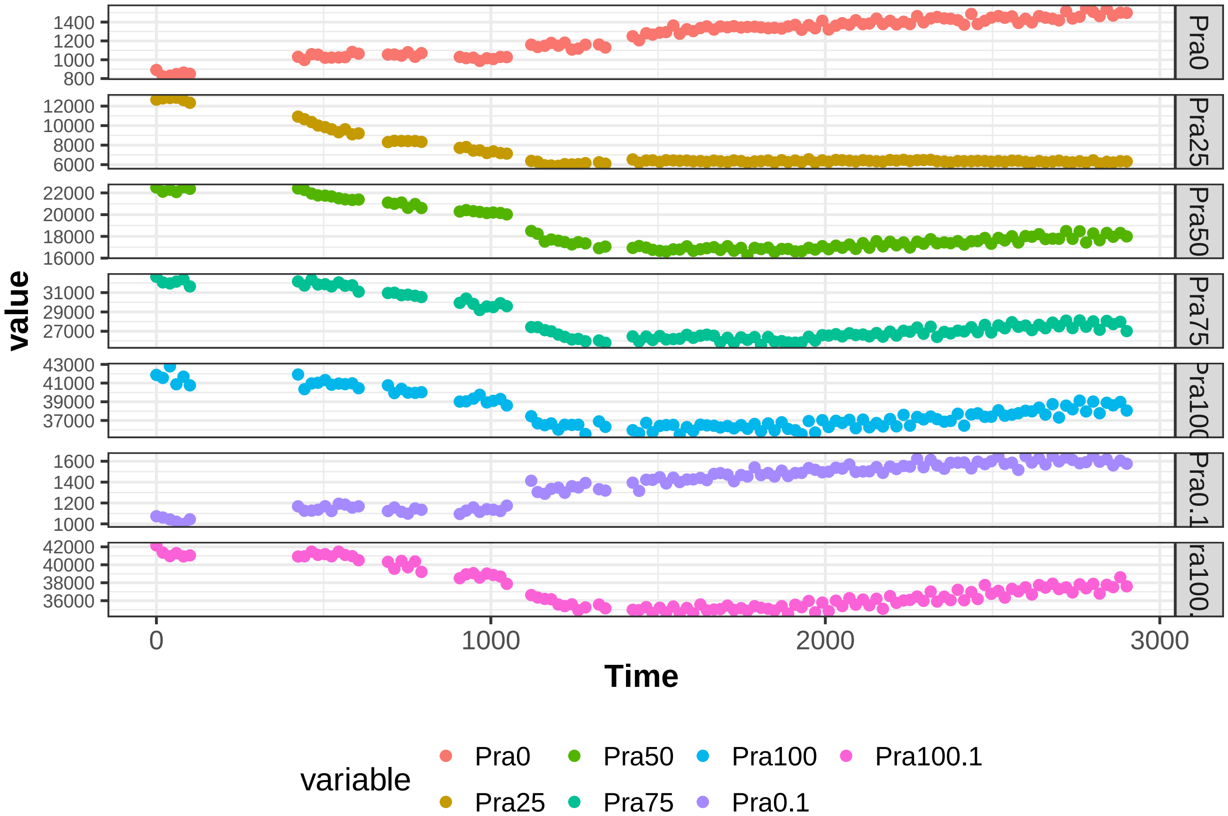


```
ggplot(PRAassay.m, aes(x = PRAassay.m$Time, y = PRAassay.m$value),color="variable") + theme_bw()
```



```
qplot(Time,value, data = PRAassay.m,colour=variable)+theme_bw()+ theme(legend.position  ="bottom",legend
```

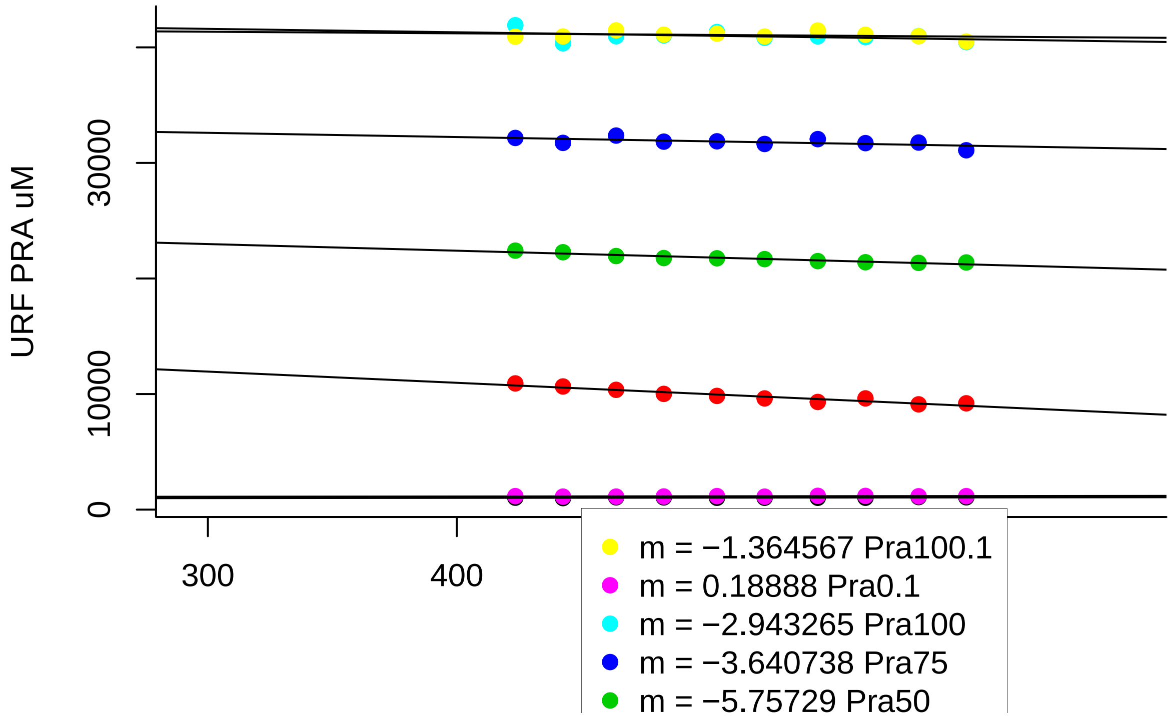
```
## Warning: Removed 7 rows containing missing values (geom_point).
```



```
 ##pendientes (slopes) of linear part of the curve
# time when TrpF is added
cuttime<-344.2
maxtime=620

PRAassay2Time<-tablePRA[which(tablePRA$Time >= cuttime & tablePRA$Time <= maxtime),]

## removi columnas que se salian del margen de medicion
#PRAassay2Time<-PRAassay2Time[, !(colnames(PRAassay2Time) %in% c("C62uM"))]

# vector with slopes for each dataset
V0 <- apply(PRAassay2Time, 2, function(x) coefficients(lm(x ~ PRAassay2Time$Time,na.action=na.omit))[2]

PRAassay2Time.m <- melt(PRAassay2Time,id="Time")
plot(PRAassay2Time.m$Time, PRAassay2Time.m$value, col=PRAassay2Time.m$variable,xlab="Time", ylab="URF P

V1 <- apply(PRAassay2Time[,2:ncol(PRAassay2Time)], 2, function(x) coefficients(lm(x ~ PRAassay2Time$Tim
apply(V1, 2, function(x) {abline(x, col = PRAassay2Time.m$variable)  })
```

```
## NULL
```

```
par(xpd = TRUE)
slopeText2="m ="
slopeText<-paste(slopeText2,rev(round(V0[-1],digits=6)),rev(names(V0[-1])))
legend(450,100, legend = slopeText, col = rev(unique(PRAassay2Time.m$variable)), pch=19,box.lwd=0, bg="
```



```
## get relevant velocities and get correspondent concentrations
## V0 or slopes are on [PRA]uM/s
# slopes stores the slope, with TRUE , FALSE the desired enzyme can be selected, One more than one is t
slopes=(round(V0[-1],digits=6))[c(TRUE)]
concentration<-as.matrix(PRAassay2Time[1,names(slopes)])
#slopes
#concentration
slopes=as.matrix((round(V0[-1],digits=6))[c(TRUE)])
row.names(slopes) <- NULL
slopes<-c(slopes)## plot on r base

slopes
```

```
## [1]  0.196328 -9.695510 -5.757290 -3.640738 -2.943265  0.188880 -1.364567
```

```
# in concentration I stored the inital substrate concentration
row.names(concentration) <- NULL
concentration<-c(concentration)
slopes
```

```
## [1]  0.196328 -9.695510 -5.757290 -3.640738 -2.943265  0.188880 -1.364567
```

```
concentration
```

```
## [1]  1031 10920 22410 32161 41918  1168 40922
```

```
## interpolar michaelis-menden

#https://rpubs.com/RomanL/6752
#https://davetang.org/muse/2013/05/17/fitting-a-michaelis-mentens-curve-using/
#S <-concentration
## from highest to lower concentration
S_ProFAR <-c(0,25,50,75,100,0,50,100)
v<-slopes
```

```
#v<-slopes[-length(slopes)]
#v <-slopes[c(TRUE,FALSE)]
v<--1*v
S_ProFAR
v
mm <- data.frame(S_ProFAR,v)
model.drm <- drm(v ~ S, data = mm, fct = MM.2())
summary(model.drm)
## first value equal km
## second value = vm
Km=coefficients(model.drm)[1]
Vmax=2*coefficients(model.drm)[2]
Enzyme=2.5 #2.5uM
Kcat=Vmax*Enzyme
Km
Vmax
Kcat
mml <- data.frame(S = seq(0, max(mm$S), length.out = 100))
mml$v <- predict(model.drm, newdata = mml)


## plot on r base
plot(mm,log='',xlim=c(0,max(mm$S)), ylim=c(0,max(mm$v)), xlab="Reads", ylab="Transcripts")

##plot on ggplot
ggplot(mm, aes(x = S, y = v)) + theme_bw() + xlab("Concentration [uM]") + ylab("Speed [d[PRA]uM/s]") + g

ggsave("mm.pdf", width = 6, height = 4)
```