

Gel-based activity assays for PRMT2

Methylation assays were performed at 23 °C in 20 mM Tris-HCl pH 8, 100 mM NaCl, 1 mM EDTA, 0.5 mM DTT. PRMT2 at varying concentrations was incubated for 3 to 5 hours with 5 μM RSF1 and 0.05 mM S-adenosyl-L-[Me-14C]methionine (14C - SAM) at 50 mCi.mmol⁻¹ (PerkinElmer). The reaction was stopped by addition of SDS loading buffer 4X and analyzed on 15% SDS-PAGE gel. After electrophoresis, the gel was stained with Coomassie Brilliant Blue, dried on a Whatman 3MM paper sheet and exposed for 48 hours against a BAS-MS 2025 imaging plate (Fujifilm). The amount of radioactivity incorporated by the substrate was quantified by phosphorimager analysis (Typhoon, Molecular Dynamic). The amount of arginine methylation was deduced from the radioactive signal using spots of 14C - SAM as a reference.

```
**IC50 calculation **
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```
Data fit with R
```

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Script:
```

```
library(base)
```

```
library(stats)
```

```
library(nlme)
```

```
library(Hmisc)
```

```
#Lit 2 jeux de données comportant colonnes abcisse et ordonnée chacun.  
Fusionne tout en matrice et trace le graphique.
```

```
mat1 <- matrix(scan("C:/Vincent/PRMT2/Activité/tab1.txt", n = 3*7, skip =  
1), 7, 3, byrow = TRUE)
```

```
mat2 <- matrix(scan("C:/Vincent/PRMT2/Activité/tab2.txt", n = 3*7, skip =  
1), 7, 3, byrow = TRUE)
```

```
matT <- cbind(mat1,mat2)
```

```
matT
```

```
matx <- matrix(c(log10(matT[,1]),log10(matT[,4])), ncol=2)
```

```
maty <- matrix(c(matT[,2],matT[,5]), ncol=2)
```

```
up1 <- matT[,2]+matT[,3]
```

```
down1 <- matT[,2]-matT[,3]
```

```
up2 <- matT[,5]+matT[,6]
```

```
down2 <- matT[,5]-matT[,6]
```

```
up <- cbind(up1,up2)

down <- cbind(down1,down2)

matplot(matx, maty, type="p", pch = 21:22, xlab=expression(paste(
"log[compound,", n,"M]" )), ylab="Activity, %", cex=1.5,
cex.axis=1.5, cex.lab=1.5, lwd=2 ,xlim= c(0,5.5), ylim= c(0,130))

errbar(matx, maty, up, down, add=T, pch=46, cap=.015)

#fit et affichage du fit

df1 <- data.frame(X1=matT[,1], Y1=matT[,2])

df2 <- data.frame(X2=matT[,4], Y2=matT[,5])

fitt1 <- nls(Y1 ~ Total/(1+(X1/IC50)^Hill),df1,
start=list(Hill=1,Total=100,IC50=10000))

fitt2 <- nls(Y2 ~ Total/(1+(X2/IC50)^Hill),df2,
start=list(Hill=1,Total=100,IC50=10000))

summary(fitt1)

summary(fitt2)

tab<-data.frame(coef(fitt1),coef(fitt2))

tab

xfit <- seq(0, 5.5, 0.05)

x<-10^xfit

y1<- tab[2,1]/(1+(x/tab[3,1])^tab[1,1])

y2<- tab[2,2]/(1+(x/tab[3,2])^tab[1,2])

lines(xfit,y1, lwd = 3)

lines(xfit,y2,lty = "dotted",col="red", lwd = 3)
```

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Last update: **2023/11/01 20:15**

