MHC associated peptide identification from non-pulsed THP-1 differentiated macrophages

Hem R. Gurung, Ph.D.

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Description

THP-1 (DRB1*01:01/15:01) cells were differentiated into macrophages by stimulating with PMA (10 ng/ml) and IFN-gamma (200 activity units/ml). A total of 12 T-175 flasks were used to generate the macrophages from which approximately 0.3 grams cell pellet was obtained. The cell pellet was processed to extract cell membrane bound MHC-peptide complexes. The complexes were then ran through a tandem W632 and L243 affinity columns to purify MHC I and II complexes respectively. The eluted complexes were then acid boiled to release peptides from the MHC grooves. The peptides were then fractionated in RP-HPLC, dried up in speed vaccum, resuspended in 10% sequencing grade acetic acid spiked with iRT peptides, and ran on nanoLCMS (TTOF) system. The raw DDA data were denovo sequenced and peptides were identified using PEAKS software at 1% FDR. Before the cell pellet was processed, cells were tested for HLA-DRB1 expression by flow cytometry.

MHC II expression increases following PMA and interferon gamma stimulation
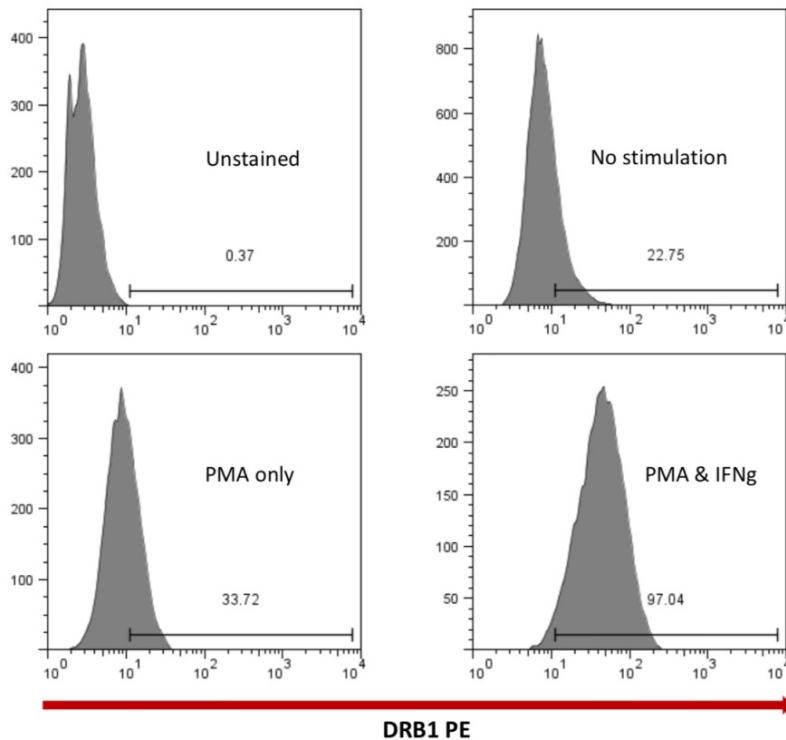
A table of some *MHC I* peptides and including a Biognosys iRT peptide is shown below.

```
## For Class I
library(DT)
setwd("C:/Users/HGURUNG1/Desktop/HG/Projects/Cunningham and Clay/THP-1 mac No pulse 0.3g W632")
data.nopulse.class1 <- read.csv("peptide.csv", header = T)
datatable((data.nopulse.class1[ c(11:14), c(1:7,13:15)]), filter = "top")
```

Show 10 entries   Search:

	Peptide	X.10lgP	Mass	Length	ppm	m.z	RT	Accession	PTM	Proteins
	All	All	All	All	Al	All	A	All	All	All
11	HAYLSKNSL	55.87	1031.5399	9	2.7	516.7786	26.71	Q01518-2|CAP1_HUMAN:Q01518|CAP1_HUMAN		Isoform 2 of Adenylyl cyclase-associated protein 1 OS=Homo sapiens OX=9606 GN=CAP1
12	GTFIIDPGGVIR	54.72	1243.6924	12	1.9	415.5722	49.21	Biognosys|iRT-Kit_peptide_3		
13	VTVPPGPSL	52.16	865.4909	9	0.6	433.753	39.36	P55036-2|PSMD4_HUMAN:P55036|PSMD4_HUMAN:A2A3N6|PIPSL_HUMAN		Isoform Rpn10E of 26S proteasome non-ATPase regulatory subunit 4 OS=Homo sapiens OX=9606 GN=PSMD4
14	LPHVHTVSY	52.08	1051.545	9	2.5	526.7811	28.69	O14773-2|TPP1_HUMAN:O14773|TPP1_HUMAN		Isoform 2 of Tripeptidyl-peptidase 1 OS=Homo sapiens OX=9606 GN=TPP1

Showing 1 to 4 of 4 entries   Previous   1   Next

A total of 857 class I peptides including 11 iRT peptides were detected of which most of them were 9-mers.

```
table.class1 <- table(data.nopulse.class1$Length)
dat.class1 <- data.frame(table.class1)

library(ggplot2)
library(plotly)
ggplot(dat.class1, aes(dat.class1$Var1, dat.class1$Freq, group = 1)) + geom_point() +
  geom_line(color = "red") +
  labs(x = "Peptide length" , y = "Frequency") +
  theme(axis.line = element_line(size = 1),plot.title = element_text(hjust = 0.5)) +
  ggtitle("Frequency of #mer THP-1 Mac MHC I peptides")
```
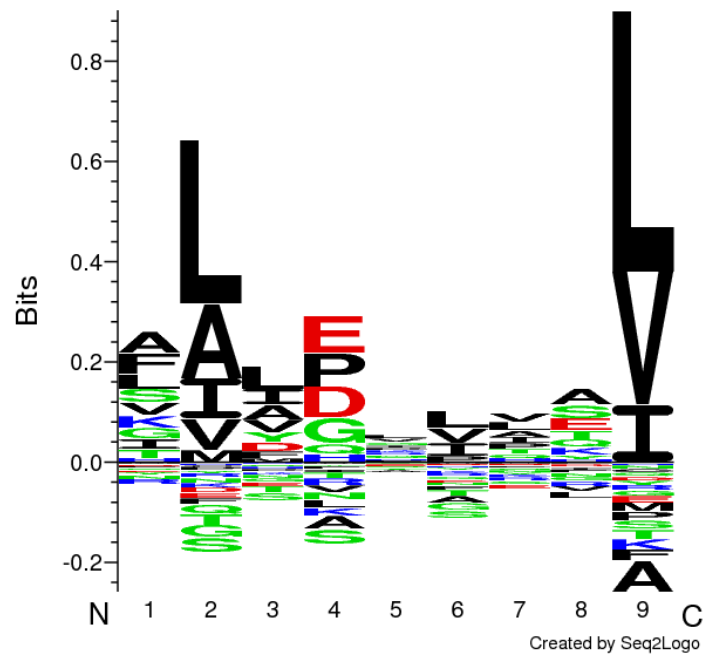
Frequency of #mer THP−1 Mac MHC I peptides

HLA-A02:01 motif

HLA-A0201

THP-1 W632 motif

Created by Seq2Logo

4
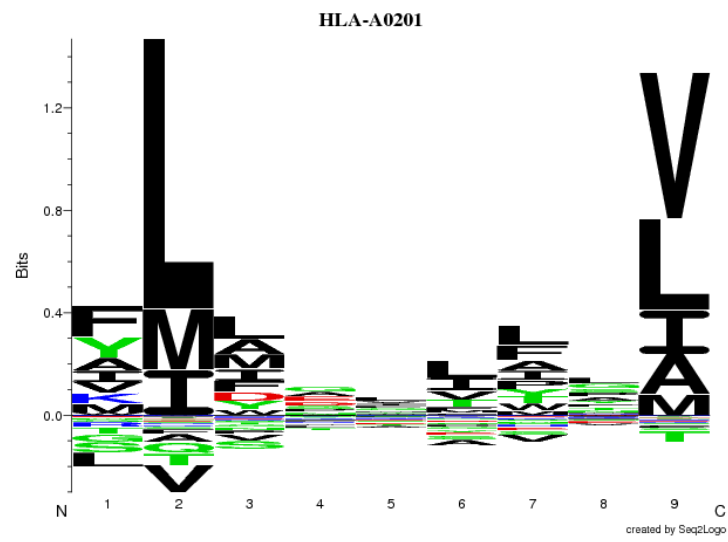
A table of some *MHC II* peptides is shown below.

```
## For class II
library(DT)
setwd("C:/Users/HGURUNG1/Desktop/HG/Projects/Cunningham and Clay/THP-1 mac No pulse 0.3g L243")
data.nopulse.class2 <- read.csv("peptide.csv", header = T)
datatable((data.nopulse.class2[c(10:14) , c(1:7,13:15)]), filter = "top")
```

Show 10 entries   Search: _____

	Peptide	X.10lgP	Mass	Length	ppm	m.z	RT	Accession	PTM	Proteins
	All	All	All	All	All	All	A	All	All	All
10	KTGKLVSLSAQNLVDC(+119.00)	77.18	1793.9015	16	1.6	598.9754	36.54	P25774|CATS_HUMAN	Cysteinylation	Cathepsin S OS=Homo sapiens OX=9606 GN=CTSS PE=1 SV=3
11	ERDALNIETAIKTKGVDE	76.42	2001.0378	18	1.8	668.0211	37.79	P07355|ANXA2_HUMAN:A6NMY6|AXA2L_HUMAN:P07355-2|ANXA2_HUMAN		Annexin A2 OS=Homo sapiens OX=9606 GN=ANXA2 PE=1 SV=2
12	PSSGLGVTKQDLGPVPM(+15.99)	75.01	1697.8658	17	-3.9	849.9369	37.73	P04233-2|HG2A_HUMAN	Oxidation (M)	Isoform 2 of HLA class II histocompatibility antigen gamma chain OS=Homo sapiens OX=9606 GN=CD74
13	Q(-17.03)NLYEKTYLPAVDEKLRDL	74.51	2290.1843	19	2.1	764.4037	54.48	P02655|APOC2_HUMAN	Pyro-glu from Q	Apolipoprotein C-II OS=Homo sapiens OX=9606 GN=APOC2 PE=1 SV=1
14	LPYDYGALEPHINAQ	73.47	1699.8206	15	-7.1	850.9116	46.15	P04179-2|SODM_HUMAN:P04179|SODM_HUMAN		Isoform 2 of Superoxide dismutase [Mn] mitochondrial OS=Homo sapiens OX=9606 GN=SOD2

Showing 1 to 5 of 5 entries   Previous   1   Next

A total of 1404 class II peptides including 11 iRT peptides were detected of which most of them were 15-mers.

```
table.class2 <- table(data.nopulse.class2$Length)
dat.class2 <- data.frame(table.class2)

ggplot(dat.class2, aes(dat.class2$Var1, dat.class2$Freq, group = 1)) + geom_point() +
  geom_line(color = "red") +
  labs(x = "Peptide length" , y = "Frequency") +
  theme(axis.line = element_line(size = 1),plot.title = element_text(hjust = 0.5)) +
  ggtitle("Frequency of #mer THP-1 Mac MHC II peptides")
```
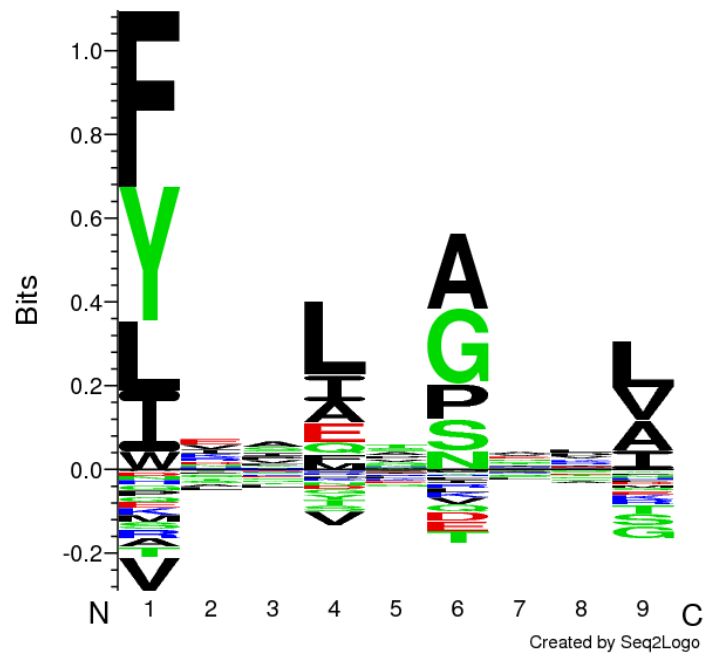
Frequency of #mer THP−1 Mac MHC II peptides
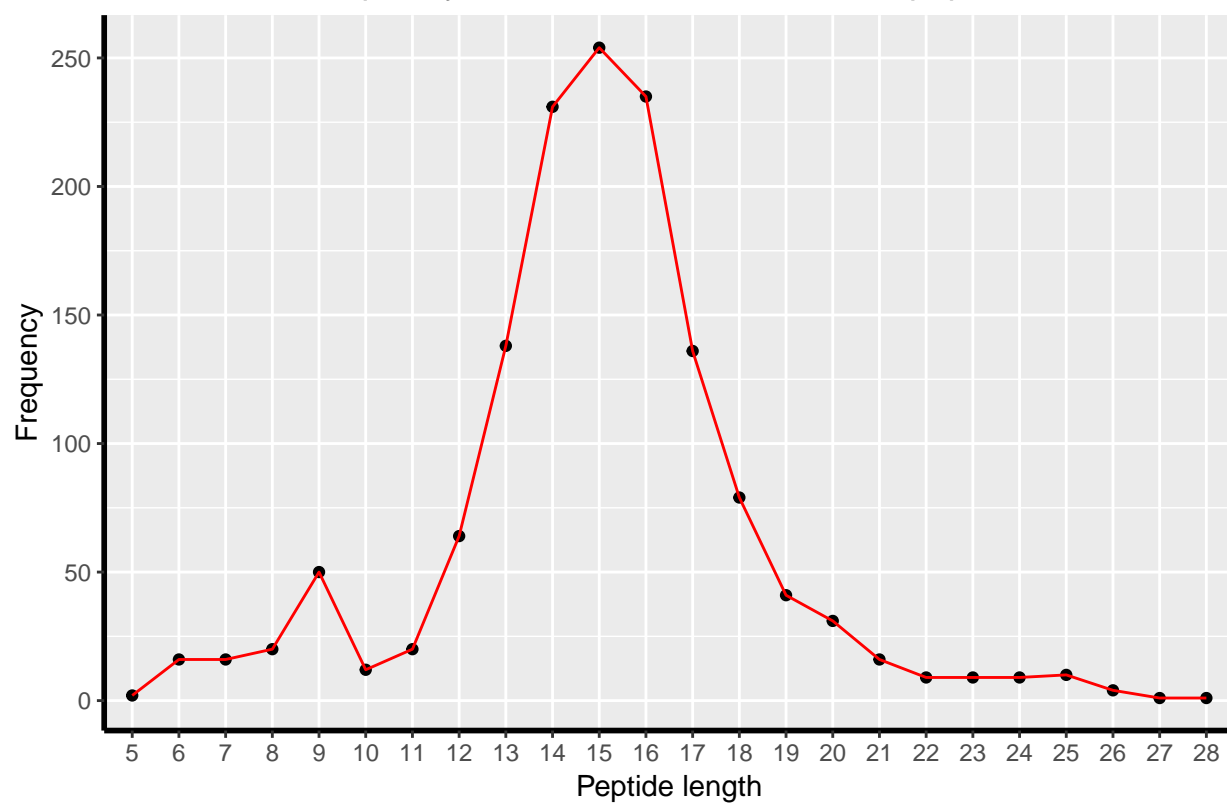
HLA-DRB1*01:01 motif
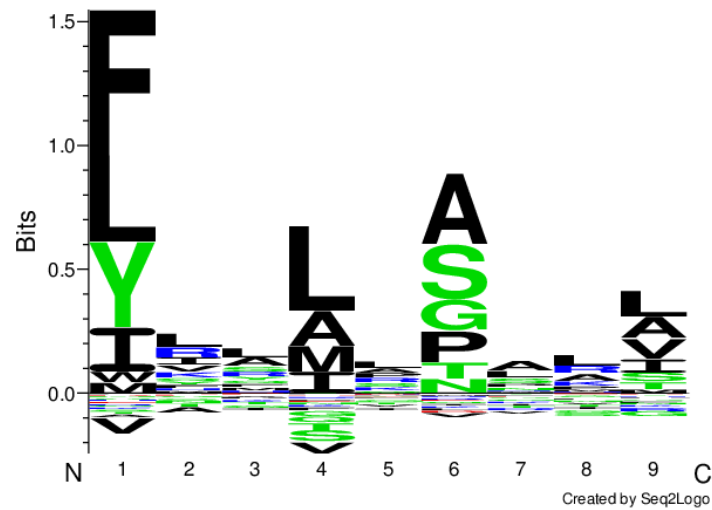
THP-1 L243 motif

Peptide sampling comparing the class I and class II derived peptides looked interesting.

For class I a protein was sampled one time for about 73% of the proteome whereas for class II a protein was sampled one time for only about 37% of the proteome (50% less than class I).

```
setwd("C:/Users/HGURUNG1/Desktop/HG/Projects/Cunningham and Clay/THP-1 mac No pulse 0.3g W632")
library(ggplot2)

class.one <- read.csv("proteins.csv", header = T)

class.one <- class.one[!grepl("Biognosys", class.one$Accession), ]
```

```
par(mfrow= c(1,1))

df1 <- data.frame(table(class.one$X.Peptides))
df1$percent <- (df1$Freq/sum(df1$Freq)) * 100
df1 <- df1[!df1$Var1 == 0, ]
ggplot(df1, aes(Var1, percent)) + geom_bar(stat = "identity") +
  labs(x = "Number of peptides per protein" , y = "Percent total") +
  theme(axis.line = element_line(size = 1),plot.title = element_text(hjust = 0.5)) +
  ggtitle("Sampling frequency of THP-1 Mac MHC I peptides") +
  scale_y_continuous(expand = c(0, 0))
```

Sampling frequency of THP−1 Mac MHC I peptides
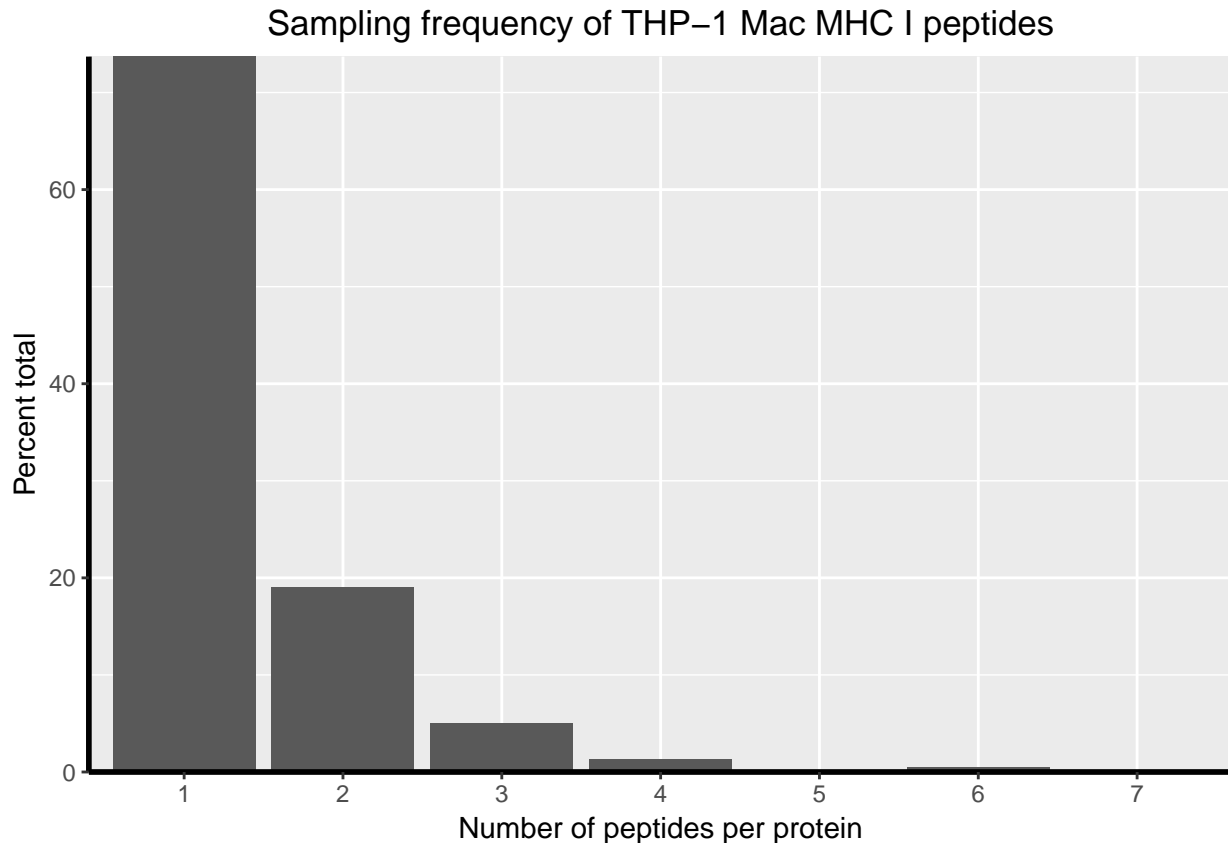

```
setwd("C:/Users/HGURUNG1/Desktop/HG/Projects/Cunningham and Clay/THP-1 mac No pulse 0.3g L243")
class.two <- read.csv("proteins.csv", header = T)

class.two <- class.two[!grepl("Biognosys", class.two$Accession), ]
par(mfrow= c(1,1))

df2 <- data.frame(table(class.two$X.Peptides))
df2$percent <- (df2$Freq/sum(df2$Freq)) * 100
ggplot(df2, aes(Var1, percent)) + geom_bar(stat = "identity")+
  labs(x = "Number of peptides per protein" , y = "Percent total") +
  theme(axis.line = element_line(size = 1),plot.title = element_text(hjust = 0.5)) +
  ggtitle("Sampling frequency of THP-1 Mac MHC II peptides") +
  scale_y_continuous(expand = c(0, 0))
```

Sampling frequency of THP−1 Mac MHC II peptides