Mass spectrometry as a tool for MHC Class I and II neoantigen discovery

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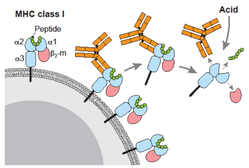
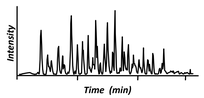
MS Bioworks
Protein Mass Spectrometry Services

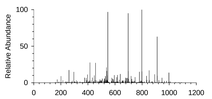
Cayman CHEMICAL

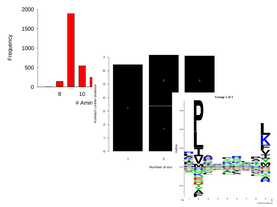
Class I

1. Workflow



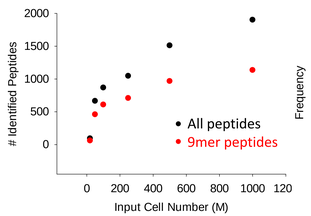


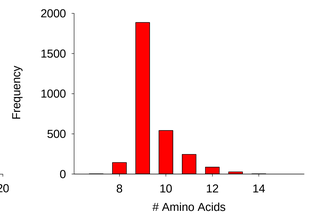




- Cells are lysed and resulting preparations are subjected to immunoaffinity capture. Following multiple washes peptides are eluted from the MHC cleft with acid.
- Purified peptides are analyzed by LC-MS/MS using nano-scale chromatography combined with a Fusion Lumos mass spectrometer using electron-transfer/higher-energy collision dissociation (EThcD) fragmentation. MaxQuant[2] is used for peptide identification and quantitation.
- Processed data can be further interrogated using bioinformatics tools. For example NetMHC[3,4] or GibbsCluster[5] allow the elucidation of binding strengths and motifs.

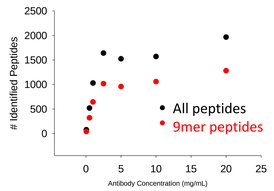
4. Pulldown Optimization I





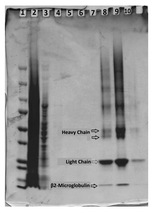
- To better understand the Class I peptide yield and to improve practical application of this assay we varied the cell count while holding the antibody concentration constant (20mg/mL). The histogram shows the distribution on peptide lengths in the combined data. The scatter plot includes data for all the observed peptides and exclusively the 9 mers.

5. Pulldown Optimization II

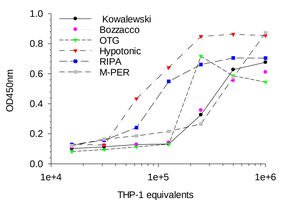


- Here we considered the antibody concentration and its impact on the Class I peptide yield. The input was 500M cells. Reducing the high antibody concentration associated used has the potential to reduce costs and again improve the practical application of this assay. The scatter plot includes data for all the observed peptides and exclusively the 9 mers.

2. Quality Control

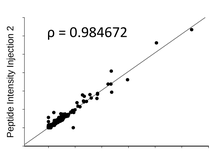


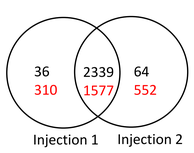
1-MW Markers,
2-Lysate,
3-Flow Thru,
4-7-Washes,
8-Eluate,
9-3kDa MWCORetentate
10-3kDa MWCO Filtrate



- SDS-PAGE of material from all steps of the peptide isolation protocol can be used to assess the pulldown specificity and extraction efficiency.
- A custom ELISA that detects the combination of heavy chain and beta 2 microglobulin light chain allows us to identify lysis buffer formulations that are optimal for solubilization and recovery of intact Class I. It also allow us to assess the success of immunoaffinity capture of Class I from the cell lysates.

3. LC-MS/MS

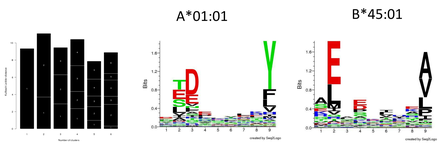




Unique peptides MBR
Unique peptides

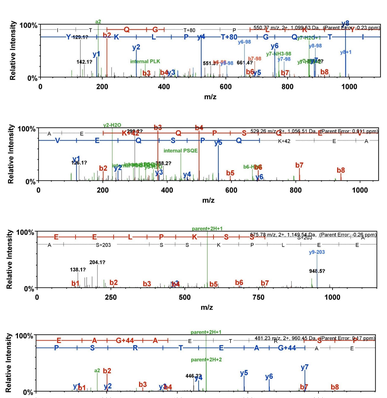
- A single sample (Peptide enrichment from 1Billion cell input) was analyzed twice by LC-MS/MS. The data were processed with and without the MaxQuant Match Between Runs (MBR) feature enabled. The Pearson correlation was calculated with the MBR data.

6. Motif Analysis



- Putative Class I peptides can be processed to assess binding strength and to extract motif information. These data can be used to confirm or predict HLA allele information. Here data specific to HCT116 cells are presented.

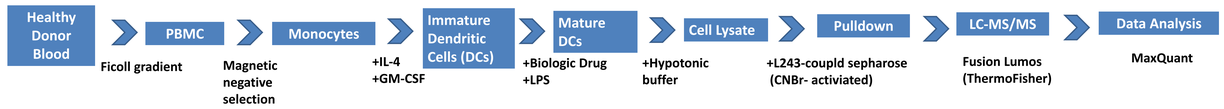
7. Neoantigen Identification



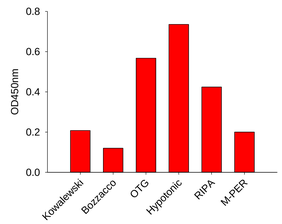
- Phosphorylation (T1463) Nuclear receptor corepressor 2 NCOR2
- Acetyl Lysine (K970) Histone acetyltransferase p300 EP300
- O-Linked Glycosylation (S1019) Nuclear pore complex protein Nup153
- AA Substitutions (G282T) ATP-dependent RNA helicase DDX24

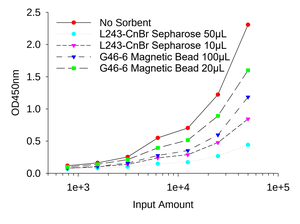
Class II

1. Workflow



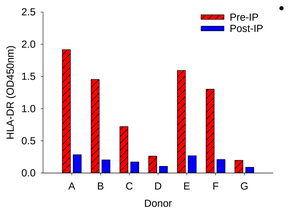
2. Pulldown Optimization





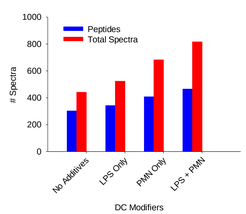
- Using an ELISA that detects the intact Class II heterodimer, we optimized lysis buffer formulations, IP antibodies, and sorbents to maximize Class II immunoaffinity capture.

3. Quality Control



- The Class II ELISA is used to assess the quality of the target cell preparation and immunoaffinity capture prior to LC-MS/MS analysis. In this case, the seven dendritic cell samples had widely divergent levels of Class II expression, but all Class II was captured by the immunoaffinity sorbent. Thus, we expect to recover far more peptides from Donor A than Donor D.

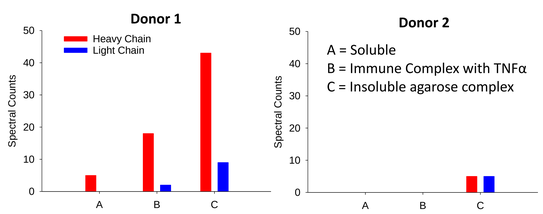
4. LPS Stimulation



- Human dendritic cells are adept at ingesting apoptotic whole cells, immune complexes, and insoluble aggregates, and then processing those materials for presentation on Class II.
- We fed apoptotic human neutrophils to dendritic cells in the presence or absence of LPS to assess the processing and presentation of neutrophil-specific peptides.

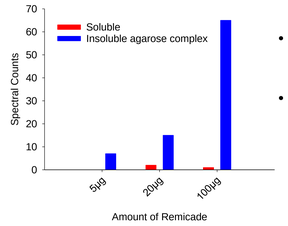
5. Biologic Treatment

- We used the commercial biologic Remicade to assess treatment conditions with samples from two donors.



- Dendritic cells prefer immune complexes or insoluble aggregates over soluble proteins for ingestion, processing, and display on Class II.
- The ability to present peptides derived from Remicade is donor-dependent.

6. Biologic Input Level



- Soluble protein antigen (Remicade) is taken up and processed far less efficiently than aggregated antigen.
- Increasing the concentration of soluble protein antigen does little to improve the outcome.

7. Peptide Identification

- The sequence coverage map for the heavy chain of the target protein in the Donor 1 samples is below. Identified peptides are highlighted in yellow.

Remicade_HC (100%), 49,515.8 Da
Remicade_HC
19 exclusive unique peptides, 34 exclusive unique spectra, 43 total spectra, 54/450 amino acids (12% coverage)

EVKLEESGGG LVQPGGSMKL SCVASGFIFS NHWMNWVRQS PEKGLEWVAE
IRSKSINSAT HYAESVKGRF TISRDDSKSA VYLQMTDLRT EDTGVYYCSR
NYYGSTYDYW GQGTTLTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT
YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

- The details of the heavy chain peptides identified in the Donor 1 samples are below. Note the highest number of peptides were identified in the AA402 – AA 421 region of the sequence.

Sequence	# Matching Spectra	Start AA	Stop AA
(Q)SPEKGLEWVAEIRSKSINSA(T)	1	40	59
(E)KGLEWVAEIRSKSINS(A)	1	43	58
(K)GLEWVAEIRSKSIN(S)	2	44	57
(K)GLEWVAEIRSKSINS(A)	1	44	58
(K)SAVYLQmTDLRTED(T)	1	79	92
(L)DSDGSFFLYSKLTVDK(S)	2	402	417
(L)DSDGSFFLYSKLTVDKS(R)	2	402	418
(L)DSDGSFFLYSKLTVDKSR(W)	3	402	419
(D)SDGSFFLYSKLTVDK(S)	2	403	417
(D)SDGSFFLYSKLTVDKS(R)	3	403	418
(D)SDGSFFLYSKLTVDKSR(W)	8	403	419
(D)SDGSFFLYSKLTVDKSRW(Q)	1	403	420
(D)SDGSFFLYSKLTVDKSRWQ(Q)	1	403	421
(S)DGSFFLYSKLTVDK(S)	2	404	417
(S)DGSFFLYSKLTVDKS(R)	1	404	418
(S)DGSFFLYSKLTVDKSR(W)	4	404	419
(D)GSFFLYSKLTVDK(S)	2	405	417
(D)GSFFLYSKLTVDKS(R)	2	405	418
(D)GSFFLYSKLTVDKSR(W)	4	405	419

8. Innovator vs. Biosimilar

- Peptides from Remicade and the biosimilar Remsima are processed and presented by Class II in a similar manner.
- The Remsima heavy chain coverage map is presented below. Peptide density around the 402-421 position was as Remicade.

Remicade_HC (100%), 49,515.8 Da
Remicade_HC
19 exclusive unique peptides, 31 exclusive unique spectra, 33 total spectra, 60/450 amino acids (13% coverage)

EVKLEESGGG LVQPGGSMKL SCVASGFIFS NHWMNWVRQS PEKGLEWVAE
IRSKSINSAT HYAESVKGRF TISRDDSKSA VYLQMTDLRT EDTGVYYCSR
NYYGSTYDYW GQGTTLTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK
DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT
YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP
KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK