Optimization of methods for the analysis of class I MHC peptides by mass spectrometry

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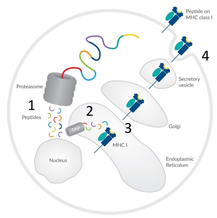
1. Objective

The major histocompatibility complex (MHC) is a region of highly polymorphic genes encoding for glycoproteins (MHC molecules) that form part of the cell-mediated branch of the acquired immune system. In the cytosol, cellular self and foreign (non-self) proteins are constantly being degraded; it is the peptides generated that are presented, non-covalently bound to MHC molecules, on the surface of cells for inspection by T-lymphocytes. CD8+ cytotoxic T lymphocytes (CTL) recognize peptides presented by MHC Class I, and CD4+ helper T lymphocytes recognize peptides presented by MHC Class II. The recognition of peptide presented by MHC Class I results in the destruction of the presenting cell by the CTL.

Characterizing the factors associated with T cell recognition of peptide/MHC complexes is an attractive proposition for anyone interested in generating tools for targeted cell destruction. In the field of oncology the obvious application then is the targeted destruction of cancerous cells. To enable the molecular level characterization of peptides associated with molecules of the major histocompatibility complex requires a targeted protein complex enrichment, an unbiased peptide elution and finally a peptide analysis method. Most frequently immunoprecipitation is used to isolate the target complex followed by peptide elution performed under conditions minimizing protein contamination and finally peptide analysis is accomplished by mass spectrometry.

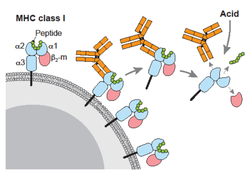
Here we present a case study of our recent work optimizing and performing a workflow for the analysis of peptides associated with Class I MHC molecules. The goal of the assay optimization was to minimize the amount of antibody required for the assay, to minimize the amount of biological material needed from which the complex is isolated and to achieve the optimum sensitivity towards the hitherto unknown target peptides.

2. MHC Peptide Presentation

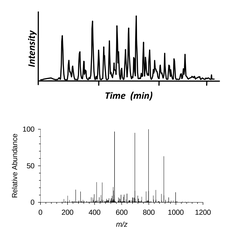


The processing of an antigen within a cell is prerequisite for antigen recognition by T lymphocytes via their T cell receptors (TCRs). Processing describes the sequence of events that takes an intra- or extra-cellular protein in it native state and leads to presentation of proteolytically derived fragments on the cell surface bound to an MHC molecule. The cartoon shows 1) Protein Processing, 2) Peptide transport into the endoplasmic reticulum (ER), 3) Peptide assembly with class I molecules and 4) Peptide presentation on the cell surface.

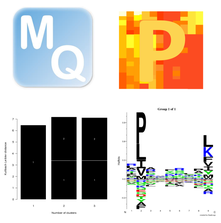
3. Immunopeptidome Analysis Workflow



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 Collision Induced Dissociation (CID) or Electron-Transfer/higher-energy collision Dissociation (EThcD) fragmentation..



MaxQuant[1] is used for peptide identification and quantitation. Processed data can be further interrogated using bioinformatics tools such at NetMHC[2] or GibbsCluster[3]..

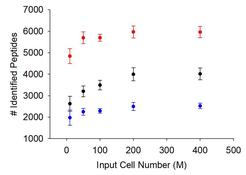
1. Cox J. *et al.*, Nat. Biotech. 2008, 26, 1367-722.
2. http://www.cbs.dtu.dk/services/NetMHC/ Andreatta M. *et al.*, Bioinformatics 2016, 32, 511-7
3. http://www.cbs.dtu.dk/services/GibbsCluster/ Andreatta M. *et al., Bioinformatics* (2012) doi: 10.1093/bioinformatics/bts621

4. Immunopeptidome Profiling

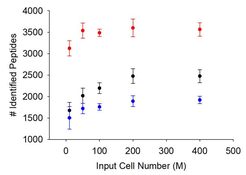
In an effort to better understand the HLA-I peptide yield and to improve the practical application of this assay we varied the input amount.

Using our standard workflow we enriched Class I MHC peptides from 2 human cell lines and 1 mouse cell line, **HCT116**, **Colo205** and **CT26** respectively. Peptide enrichments were performed in technical triplicate. Samples were analyzed in singlet.

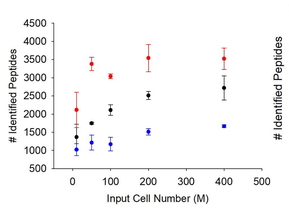
Enriched peptides from 10M, 50M, 100M, 200M and 400M cells were analyzed using 2hr LC-MS/MS.



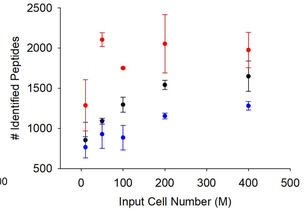
The figure above contains data for all the unique peptides identified in each sample.



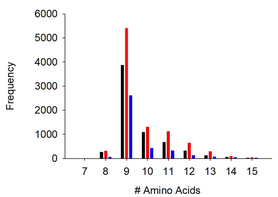
The figure above contains data for the 9mer unique peptides identified in each sample.



The figure above contains data for all the unique peptides identified in each sample. Match Between Runs turned off.



The figure above contains data for the 9mer unique peptides identified in each sample. Match Between Runs turned off.



	HCT116	Colo205	CT26
Bin	*Frequency*	*Frequency*	*Frequency*
7	0	4	0
8	260	302	51
9	3871	5406	2611
10	1081	1298	422
11	671	1119	316
12	319	637	123
13	125	278	60
14	51	83	38
15	17	34	14
More	0	0	0

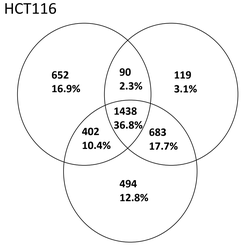
The histogram and table above show the length distribution for all the identified peptides in the combined data for each cell type.

5.0 Reproducibility 1

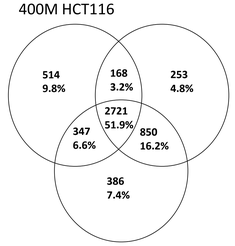
We considered the detection of unique peptides in each replicate. The data in the Venn diagrams below are from the 10M and 400M HCT116 samples.

The Venn diagrams below contain the data for all the identified unique peptides.

10M HCT116



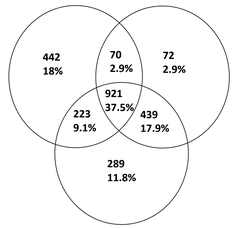
400M HCT116



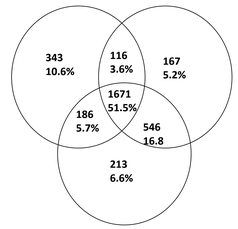
5.1 Reproducibility 1

The Venn diagrams below contain the data for the 9mer identified unique peptides.

10M HCT116



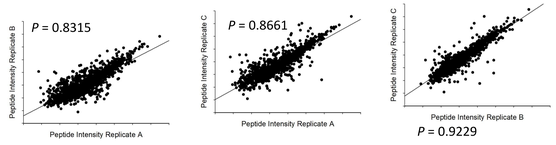
400M HCT116



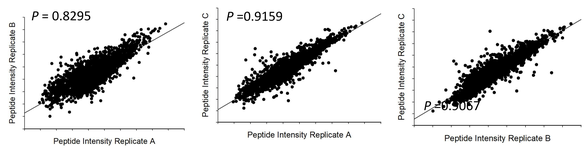
6. Reproducibility 2

The common peptides from the Venn diagrams in section 5 were used to assess the reproducibility of the described method.

10M HCT 116 common peptidome reproducibility



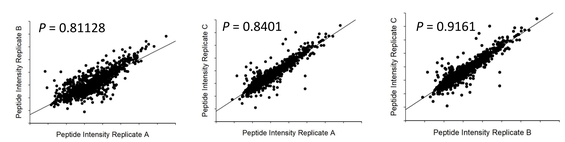
400M HCT 116 common peptidome reproducibility



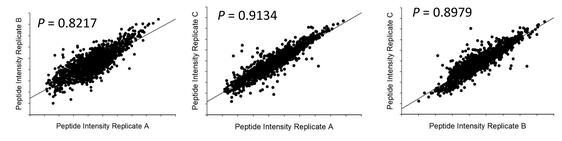
7. Reproducibility 3

The common 9mer peptides from the Venn diagrams in section 5 were used to assess the reproducibility of the method.

10M HCT 116 9mer common peptidome reproducibility



400M HCT 116 9mer common peptidome reproducibility

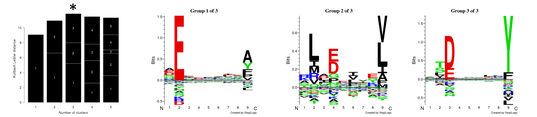


8. Reproducibility 4

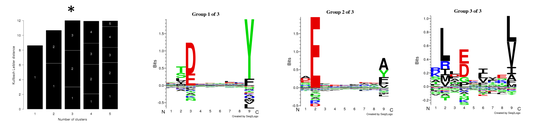
Motif Analysis was perform analysis on the 9mer population from the combined 10M and 400M HCT116 input experiments. Motif analysis was performed with the GibbsCluster tool[3]. Default parameters for MHC Class I ligands of the same length were used.

The sequence motifs identified in the cluster with largest Kullbach Leibler distance (marked with an asterisk on the bar chart) are below.

The figures below are from the 10M HCT 116 9mer motif analysis.



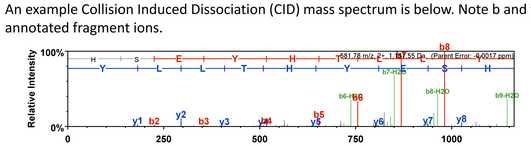
The figures below are from the 400M HCT 116 9mer motif analysis.



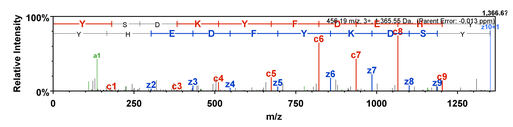
9. CID EThcD IDK?

Peptides are sequenced based on their fragmentation spectra.

An example Collision Induced Dissociation (CID) mass spectrum is below. Note b and y annotated fragment ions.



An example Electron Transfer/higher energy collision Dissociation (EThcD) mass spectrum is below. Note c and z annotated fragment ions.



We evaluated three methods of analyzing immunopeptidomes 1) CID 2) EThcD and 3) a combined CID+ EthcD method . Results from a 2hr LC-MS/MS method using each of three approaches is below.

	CID	EThcD	EThcD + CID
No. of Unique Peptides	3207	2685	3555

The Venn diagrams below contain the data for the unique peptides identified using each of the fragmentation modes in the evaluation.