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Minimal Information About an Immuno-Peptidomics Experiment (MIAIPE)

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Minimal information about an immuno-peptidomics experiment (MIAIPE) is an initiative of the members of the Human Immuno-Peptidome Project (HIPP), an international program organized by the Human Proteome Organization (HUPO). The aim of the MIAIPE guidelines is to deliver technical guidelines representing the minimal information required to sufficiently support the evaluation and interpretation of immunopeptidomics experiments. The MIAIPE document has been designed to report essential information about sample preparation, mass spectrometric measurement, and associated mass spectrometry (MS)-related bioinformatics aspects that are unique to immunopeptidomics and may not be covered by the general proteomics MIAPE (minimal information about a proteomics experiment) guidelines.

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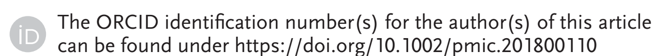
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1. Introduction

The immunopeptidome is the ensemble of peptides presented by major histocompatibility complex (MHC) molecules to T cells and it plays a key role in mediating specific elimination of diseased or abnormal cells by the immune system. MHC class I molecules are ubiquitously expressed on almost all cells and they present their peptide ligands to $CD8^+$ T cells, while MHC class II molecules are mainly expressed on immune cells that function as professional antigen presenting cells that present peptides to $CD4^+$ T cells. Highly specialized cellular machineries process intracellular and extracellular proteins into short peptide fragments, of which some are further loaded on the MHC molecules.[1] The presented peptides are mostly derived from the degradation of the normal cellular proteins, yet during pathologies, some may also be derived from disease-related antigens, such as tumor-associated antigens and pathogens. Therefore, the comprehensive knowledge of the MHC bound peptide (pMHC) repertoire accessible to patrolling T cells is an excellent basis for designing innovative therapeutics against cancer, autoimmunity, and pathogenic infections. Currently, nanoscale liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)-based immunopeptidomics is the prime methodology to comprehensively interrogate the repertoire of pMHC presented in vivo.[2] Given the rising number of research laboratories performing large-scale immunopeptidomics and the growing interest in detecting clinically relevant peptides by LC-MS/MS, comprehensive data of naturally presented MHC class I and class II peptidomes from thousands of donors and MHC allotypes will be acquired in the coming years.[3] We foresee that, as is happening already in other proteomics-based workflows, extensive reporting of experimental procedures and deposition of data in dedicated repositories will facilitate the reuse of the data and consequently will enhance the general value of the scientific work.

In 2015, the Human Immuno-Peptidome Project (HIPP) was created as a new initiative under the umbrella of the Human Proteome Organization (HUPO; https://hupo.org/Human-Immuno-Peptidome-Project/) to further accelerate research toward comprehensive and reproducible analysis of immunopeptidomes. HUPO-HIPP has the long-term goal of increasing the impact of immunopeptidomics in biomedical research by enhancing the accessibility of immunopeptidomics data, as well as experimental and computational techniques, to the whole scientific community. During the first international workshop of the HUPO-HIPP initiative, leaders in the immunopeptidomics field acknowledged the importance of drafting the minimal information about an immuno-peptidomics experiment (MIAIPE) guidelines as a community effort to ensure transparency and reproducibility in published data.[4] To this end, three working groups comprised of leading experts in different aspects of immunopeptidomics have drafted the guidelines, which describe key information about: 1) sample preparation and the biochemical isolation of pMHC from cells or tissues, 2) LC-MS/MS parameters, and 3) informatics and statistics. Importantly, the main principle of the guidelines is not to enforce any particular experimental design but rather to ensure that published MS-based immunopeptidomics studies provide essential information to the readers to 1) explain the experimental data, 2) assess the reliability of the results and avoid misinterpretation, and 3) support the consistent reuse of the data in the future.

The analysis of immunopeptidomes using LC-MS/MS technologies is a relatively complex process. Caron et al. have reviewed the three main data acquisition concepts in MS that have been applied in immunopeptidomics,[2] commonly used in other proteomics MS workflows. These include: 1) data-dependent analysis (DDA) which is the widely used strategy for so-called "discovery experiments" where the researcher is interested in profiling the repertoire of pMHC from a given sample[5,6]; 2) selected reaction monitoring (SRM), also referred to as multiple reaction monitoring (MRM), targeted analyses whereby ion signatures from both the peptide precursor and its fragmentation product ions are monitored, and the area under the curve or peak height can be used for quantification purposes; this method ensures a higher degree of selectivity than a DDA experiment, but at the expense of the experimental depth and throughput[7]; and 3) data independent analysis (DIA) strategies, where all peptides within a defined mass-to-charge (m/z) window are subjected to fragmentation and recording of their combined MS/MS spectra; the precursor isolation window sequentially marches up the full m/z range and this analysis is repeated again and again during chromatographic elution.[8] This less mature technique promises excellent performance for both discovery and quantitative analyses, especially with regard to reproducibility and more accurate label-free quantification.[9]

Most of immunopeptidomics studies are discovery oriented; therefore, the main focus of the current MIAIPE guidelines is on reporting and interpreting DDA MS data. Furthermore, as the immunopeptidomics MS approach and the supportive bioinformatics tools are those commonly used in general proteomics techniques, several parts of the guidelines below overlap with the existing general MIAPE guidelines,[10–13] developed under the umbrella of the HUPO Proteomics Standards Initiative, and the Paris Guidelines on reporting and deposition of proteomics datasets (http://www.mcponline.org/site/misc/ParisReport_Final.xhtml). For the sake of completeness and clarity, we have included overlapping content and elaborate further on the immunopeptidomics specific aspects.

2. Preparation of pMHC Samples

The first methods used for the isolation of pMHC have been described by the labs of Sette for class II[14] and Rammensee for class I.[15] Since these pioneering studies, pMHC have been isolated biochemically from a wide range of biological samples for fundamental research,[16] as well as for translational and clinical research.[17] Three accepted methods for the isolation of MHC class I or class II peptides have emerged in the last decades: 1) mild acid elution, where the cell surface MHC class I but not class II complexes are denatured at approximately pH 3.3, therefore releasing the MHC class I peptides while leaving the cells intact[18]; 2) immunoaffinity purification of the endogenous pMHC from cells, tissues, and body fluids using MHC-specific monoclonal antibodies bound to solid support[19]; and 3) immunoaffinity purification of transfected recombinant soluble or membrane-anchored MHC molecules using anti-MHC

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antibodies or antibodies against affinity tags.[20] Stable isotope labeling of the cellular proteins has been used in order to follow their synthesis, degradation dynamics, and the MHC presentation of their derived peptides and to assess the degree to which pMHC is derived from defective ribosome products[21] and has been applied for the identification of pathogen-derived pMHC.[22] A comprehensive description of such advanced experimental approaches is required, and in this section, we cover aspects related to the extraction of pMHC from the biological samples and their preparation and storage prior to the LC-MS/MS characterization.

2.1. Sample Preparation

2.1.1. Sample Metadata

Indicate the sample name or identifier, specifically, host (use NCBI taxonomy terms or identifiers), tissue of origin, and cell type (include catalogue number, if commercially available). Indicate the MHC allotypes according to the MHC Restriction Ontology (MRO) terminology/identifiers available, for example, via the Ontology Lookup Service (https://www.ebi.ac.uk/ols/ontologies/mro), for example, HLA- A*02:01. Indicate the method used for typing MHC alleles (e.g., PCR-based and exome-capture sequencing data). In case of transgenic modified cells, describe the vector and the recombinant MHC molecule. Indicate if nonhost antigens have been externally supplied or presumably expressed in the samples. Indicate if and how chemical or metabolic labeling was applied, if the sample has been fractionated and how, and if exogenous standards have been added to the sample. Indicate amount of starting material per sample (e.g., number of cells, amount of tissue, volume of plasma, and cultured cell growth medium) and indicate patient consent if applicable. The number of technical and biological replicates should be indicated for each experiment.

2.1.2. Reagents and Equipment

Indicate buffer compositions, concentration, pH, and temperature (e.g., detergents, salts, protease inhibitors). Indicate detailed information and suppliers of plasticware and chromatography materials (e.g., ultrafiltration units, C18 tips, and columns).

2.1.3. . Cell Culture Conditions

Indicate the media and serum used for the cell culture. Indicate if specific additives were added, including antibiotics used for the selection of transfected cells. Indicate if stable isotope labeled amino acids were used, their concentration, supplier, and isotopes used for their labeling.

2.1.4. Mild Acid Elution Method

Describe washing of cells prior to the acid elution. Indicate the exact buffer composition, volume, concentration, pH and temperature, and duration at which one performed the acid elution per sample. Describe how the peptides-containing samples were cleared (e.g., filtration, centrifugal force, temperature, and duration).

2.1.5. Antibody Coupling to Resin for IP

Indicate the name of antibody used for the affinity purification and, in case the antibodies are described for the first time, add information about isotype and origin and the MHC allele specificity. Provide information on the type of coupling resin (manufacturer information) and coupling chemistry/biochemistry employed. Indicate the ratio of antibody to resin, buffer composition, temperature, and duration of coupling and quenching reactions. Indicate the cartridge or column device used for the cross linking procedures.

2.1.6. IP Method

Describe the storage and preparation of cells, tissues, or body fluids prior to the IP. If MHC complexes have been isolated from tissues, include disruption conditions (e.g., mechanical or enzymatic), temperature and duration, and if any washing steps were involved. If isolated from body fluids, include the addition of anticoagulation factors and protease inhibitors, centrifugations, etc.

Indicate the lysis buffer volume used per sample, incubation time and temperature, agitation or physico-mechanical process. Describe how the lysates were cleared (e.g., filtration, centrifugal force, temperature, and duration). Describe the solutions used for conditioning and washing the resin prior to the IP. Indicate the format of the IP device (type of column or tube), how cell lysates were introduced into the IP device and the duration of the IP process. Indicate the ratio of lysate per IP resin, if agitation was used during incubation or if peristaltic flow or gravity forces were used to maintain the flow. Describe composition, volume, and administration of all washes and elution steps.

2.1.7. Extraction and Separation of the pMHC

Describe the cleanup approach taken to separate the peptides from cell debris and other contaminating proteins. If molecular weight cutoff (MWCO) filters or C18 cartridges were used, indicate the manufacturer and catalog name. Include conditioning and washing steps, amount of the sample loaded, centrifugation force, temperature and duration, and details about the buffers and volume used to elute the peptides.

If peptide samples were fractionated, enriched for modification (e.g., phosphopeptides) or chemically labeled for quantitative analyses, describe the procedures. Provide information on the column dimensions, stationary phase, buffers, column temperature, flow rate, and number of fractions collected and combined.

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2.1.8. Concentration and Storage of Samples

Indicate if eluted peptide solutions were reduced in volume by vacuum centrifugation or lyophilization, and the conditions of storage.

2.1.9. Preparation of Sample Prior to LC-MS/MS

Describe the solvents used for resuspending the peptides prior to MS and the volume and percentage of injected sample. Specify the number of LC-MS/MS runs per sample (injection replicates).

3. LC-MS/MS-Based Detection of Immunopeptidomes

The Rammensee lab reported the first application of mass spectrometry and Edman degradation for the identifications of pMHC,[23] while in 1992, Hunt et al.[24] published the first LC-MS/MS approach for the identification of eluted pMHC. Their basic setup consisted of a C18 reversed phase liquid chromatography (RP-LC) system coupled online via electrospray ionization (ESI) to a MS/MS. This general framework became the standard method for identifying pMHC. An increasing number of reports have highlighted the influence of the applied fragmentation methodology (e.g., CID, HCD, and EThcD) on the depth and features of the identified immunopeptidome. In fact, MS/MS spectrum generation and acquisition is even more important in immunopeptidomics as compared to other sample types due to the very diverse physical chemical properties of these endogenous peptides.[25] Thanks to tremendous technological development in the MS/MS field over the last decade, large-scale MHC-class I and MHC-class II immunopeptidome analyses have taken off in recent years,[6,26,27] thus drastically increasing the availability of immunopeptidome datasets.

3.1. LC-MS/MS Analysis—Compatible with the General MS MIAPE Guidelines[28]

3.1.1. LC-MS/MS Metadata

Indicate the name of the manufacturer and model of the mass spectrometer and chromatography system. Indicate the acquisition method (DDA, DIA, SRM) and fragmentation method (CID, HCD, ETD, EThcD).

3.1.2. Trapping Column

If applicable, indicate the name of the manufacturer and model of the trapping column and if packed in-house. Describe the stationary phase including the name of manufacturer for in-house packed trapping columns. Indicate the length and inner diameter of the trapping column.

3.1.3. Analytical Column

Indicate the name of the manufacturer and model of the analytical column and if packed in-house. Describe the stationary phase including the name of manufacturer for in-house packed analytical columns. Indicate the length and inner diameter of the analytical column. For in-house packed columns, indicate the frit or Arched-Tip used for packing.[29]

3.1.4. Mobile Phases and Gradient

Indicate the composition of the mobile phases, the total run time, flow rate, and temperature of the LC. Describe the gradient in terms of time and percentage of mobile phases.

3.1.5. Ion Source Type

Indicate the type of ion source (e.g., ESI, MALDI). List any modifications made to the standard specification. If the interface is entirely custom-built, describe it or provide a reference if available. Indicate the manufacturing company and model name for the ESI emitter; list any modifications made to the standard specification. If the emitter is entirely custom-built, describe it briefly or provide a reference if available. If the ion source is MALDI, indicate the laser energy absorbing matrix in which the sample is embedded (e.g., alpha-cyano-4-hydroxycinnamic acid).

3.1.6. MS Acquisition Approach

Describe the instrument's parameter settings and the acquisition method. Importantly, a sizeable number of pMHC only occur as singly charged species[26]; therefore, indicate the charge state selection. Furthermore, indicate the mass ranges, the automatic gain control (AGC) ion target values and maximal allowed injection times for full MS scan and MS/MS, the isolation window, and resolution. Specify the acquisition sequence, for instance a top-five method with a cycle made of one full MS scan followed by a precursor selection of the five most intense ions for fragmentation applying a dynamic exclusion window of 30 s. In the case of SRM experiments, specify all transitions and detection windows.

4. Informatics and pMHC Identification

Large-scale immunopeptidomics experiments create increasing amounts of data, which makes it impractical or even impossible to present all potential results within a typical manuscript. For example, post-translationally modified pMHC (e.g., phosphorylations,[30] symmetric vs asymmetric demethylation,[31] O-GlcNAc vs O-GalNAc[32]) may be directly identified by enabling variable modifications in the database searches. Therefore, all raw and processed data should be submitted to a public proteomic or immunopeptidomic repository (e.g., the resources included in the ProteomeXchange

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Consortium [http://www.proteomexchange.org/], such as PRIDE, MassIVE, jPOST, and iProX repositories,[33] or the recently created SysteMHC Atlas immunopeptidomic data repository [https://systemhcatlas.org/][34]) to enable better collaborations among researchers and data reuse by third parties, to advance the field more efficiently. As discussed above, immunopeptidomics experiments are carried out by applying different methods, using a variety of instrument types, and employing different computational analysis tools. Therefore, accurate reporting of both experimental and computational parameters is essential to improve interpretation, accessibility, and reproducibility of immunopeptidomics data.

4.1. Informatics—Compatible with the MIAPE for Quantitative MS[11] and MIAPE for MS Informatics[10] Guidelines

4.1.1. Informatics Metadata

Indicate if the identification of pMHC has been performed using a sequence based or a de novo approach. Indicate if a quantification analysis was performed. If applicable, indicate the method used for assigning peptides to their corresponding MHC allotypes, for example, using mono-allelic cell lines, clustering tools (including version number), and affinity prediction algorithms (including name and version number), and if the list of reported pMHC has been filtered accordingly.

4.1.2. Raw Data Processing and Database Searches

Indicate all the specific parameters used in the identification analysis. Follow the specific MIAPE guidelines related to the different techniques (including DDA, SRM, spectral library searches).[10,11] Indicate the version name and number of the tool used for generating "peak list" files. Include parameters used in the creation of this peak list (e.g., whether smoothing was applied, any signal-to-noise criteria, whether charge states were calculated or peaks de-isotoped).

Indicate the name and version number of the search engine tool used. In case of multiple tools, indicate the order in which they were used and how the results were combined. Include the parameters used for the database search (e.g., precursor-ion mass tolerance, fragment-ion mass tolerance, as well as dynamic and static modifications allowed). If possible, provide the configuration file for the search tool.

Indicate the name and version of the reference sequence database including the number of entries within the database. In case of custom compiled databases, submission of the respective .fasta files of the target and decoy databases to a public repository is encouraged.

Indicate retention time and precursor mass, charge and mass error observed, and identification score(s). Indicate the decoy strategy applied to estimate false discovery rate (FDR), and if the FDR has been applied at the peptide spectrum match or at the peptide level. Indicate the thresholds and values specific to judging certainty of identifications and any related additional statistical analyses. If possible, indicate the number of peptide-spectrum matches, score difference to the second best fit, any associated statistical information obtained for the conducted searches (e.g., posterior error probability [PEP][35] and q values of the peptides), and the number of observed fragment ions. If possible, both target and decoy peptide sequences should be deposited.

4.1.3. Identification and Mapping of pMHC to Proteins

For each identified pMHC, provide the accession number of the parent protein(s) and position within the protein. Indicate whether the peptide mappings to the proteins included in the search database are unique/unambiguous or not. In case of ambiguous mappings, all potential proteins (protein groups) mapped should be reported. As identical peptide sequences can be included in multiple unique protein sequences due to biological variation such as single amino acid variants, alternative splice forms, and homologs, all potential source proteins should be reported.

4.1.4. Post-translationally Modified pMHC

For each post-translationally modified pMHC, indicate the modification name and site in the peptide sequence and the number of modifications. When ambiguity with regard to the modification site cannot be resolved, cite whether specific software has been used or not. If specific software has been used, the name, version, and all corresponding scores should be indicated. The ambiguity must be explicitly shown.

If possible, provide evidence for assigning a specific modification or variant peptide sequence over another in case of isobaric modifications or different isobaric amino acid combinations (e.g., acetylation vs trimethylation, phosphorylation vs sulfation, symmetric vs asymmetric dimethylation, O-GlcNAc vs O-GalNAc etc.).

4.1.5. Quantification of pMHC

Indicate all the specific parameters used in the quantification analysis. Follow the specific MIAPE guidelines related to the different quantification techniques (including labeled, e.g., SILAC and iTRAQ, label-free, or absolute quantification).[11]

Indicate the signal extraction method and for each peptide the MS intensity per measured sample (mean and standard deviation) or per LC run. Specify how MS intensities were normalized.

Indicate any data manipulation such as related to systematic error effects, interference from overlapping precursor ions, incomplete isotope labeling, correction for pipetting error, imputation of missing values, rejection of outliers, and the categorical exclusion of data by thresholds.

4.1.6. Data Availability

Data should be made openly available to the scientific community. It is encouraged that data is submitted to one public

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repository, following the requirements established by the ProteomeXchange Consortium.[33] Data submissions must then include at least: the metadata information described in Section 2.1.1, raw data (MS data coming from the mass spectrometer), and processed results, including the output of the analysis (ideally in an open standard format if possible, e.g., mzIdentML, mzTab). In addition, it is encouraged that peak list files are always included (in a suitable open format, e.g., mzXML, mzML, mgf, etc.), together with any other data type that can help to improve experimental reproducibility.

If there are selected peptides of key interest (e.g., virus-derived), the annotated MS/MS spectra of the key peptides should be provided as supplemental material accompanying the manuscript. If this is not possible, ensure that the submission to a public repository enables this functionality (the so-called "complete" submissions, which use PSI standard formats).

4.1.7. Software Availability

If possible provide any "home-made" tailored scripts (e.g., in a source code repository such as GitHub, Bitbucket, among others), that were used for data manipulation, between the different analysis steps.

5. Conclusion

The main objective of the MIAIPE guidelines is to harmonize the way immunopeptidomics studies are reported in scientific manuscripts and deposited in public repositories. This community effort wishes to facilitate transparent experimental reporting, data sharing, and distribution and reuse of datasets and by that to advance best practice in this emerging field. The broadness of the required MIAIPE description matches to similar guidelines from other disciplines where such guidelines are more mature and therefore well implemented and acknowledged.[13,36] We anticipate that these guidelines will evolve in parallel with the field. We envision that widespread compliance with the MIAIPE guidelines will standardize the reported content of MS-related immunopeptidomics studies and will increase the general value of the conducted research for the advantage of the growing immunopeptidomics scientific community.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

antigen processing and presentation, immunopeptidomics, major histocompatibility complex

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