Theme Article: Breakthroughs, Challenges, and Opportunities in Biological Data Visualization

ProHap Explorer: Visualizing Haplotypes in Proteogenomic Datasets

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Abstract—In mass spectrometry-based proteomics, experts usually project data onto a single set of reference sequences, overlooking the influence of common haplotypes (combinations of genetic variants inherited together from a parent). We recently introduced ProHap, a tool for generating customized protein haplotype databases. Here, we present ProHap Explorer, a visualization interface designed to investigate the influence of common haplotypes on the human proteome. It enables users to explore haplotypes, their effects on protein sequences, and the identification of non-canonical peptides in public mass spectrometry datasets. The design builds on well-established representations in biological sequence analysis, ensuring familiarity for domain experts while integrating novel interactive elements tailored to proteogenomic data exploration. User interviews with proteomics experts confirmed the tool's utility, highlighting its ability to reveal whether haplotypes affect proteins of interest. By facilitating the intuitive exploration of proteogenomic variation, ProHap Explorer supports research in personalized medicine and the development of targeted therapies.

A key concept in human genetics is haplotypes, which are sets of alleles inherited together from a parent. The increasing availability of population-wide genomic data sets has enabled the mapping of haplotypes across populations, facilitating genotype imputation and genome-wide association studies (GWAS) [1]. While genomics provides valuable insights into the genetic blueprint, it alone is insufficient to capture the complexity of biological systems.

Proteomics, the large-scale study of proteins, offers a deeper understanding of how genes are expressed

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and function within the body. In proteomics, data is usually projected onto a single reference proteome, which disregards the genetic diversity present in human populations. More recently, the field of proteogenomics has emerged, in which proteomic methods are used to detect variants in protein-coding regions [2].

Protein haplotypes, which are unique protein sequences encoded by sets of alleles in linkage disequilibrium (LD), can be discovered through proteomics [3]. Accounting for these protein haplotypes will enable us to more finely chart the human proteome, thereby acknowledging the diversity across populations. This approach not only enhances our understanding of proteins and their function and regulation, but also has the potential to improve the accuracy of biomarker discovery and therapeutic interventions.

Proteogenomic datasets are inherently multimodal, encompassing information concerning genetic variation, mRNA splicing, encoded protein sequences, peptide identifications, and various technical properties and quality metrics. Interpreting these datasets is challenging because experts need to maintain links between these dimensions. For instance, determining which regions of genes encode identified peptides and whether they cover important variant loci or splicing junctions is difficult without a clear visual overview. A robust visualization platform can assist experts in navigating these complex datasets, enabling them to extract relevant information in a meaningful and effective manner.

We have previously developed *ProHap* [4], a tool that creates databases of protein haplotypes from genotype panels of human populations, and allows experts to inspect the presence of these haplotypes in mass spectrometry datasets. Along with *ProHap*, we published a database of protein haplotypes derived from the genotypes of the 1000 Genomes Project [5]. We have used this database to search a publicly available mass spectrometry dataset of healthy human tissues [6], to identify novel variant peptides, and explore their presence across tissues.

In this paper, we present *ProHap Explorer*, a web-based visual interface for proteogenomic data obtained using the *ProHap* workflow. It allows experts to browse the published database of protein haplotypes from the 1000 Genomes Project, and view whether peptides have been identified that cover interesting features in the protein sequences. The availability of such a platform will be instrumental in advancing our understanding of the connection between genetic variation and protein function, ultimately contributing to the field of personalized medicine and the development of targeted therapies.

RELATED WORK

This work is rooted in several research areas, which we divide into two parts: proteogenomics, the field integrating genomic and mass spectrometry-based proteomic research, and visualization of biological sequences. This section reviews the relevant literature and existing tools in each of these domains, highlighting the contributions that have informed the development of *ProHap Explorer*.

Mass Spectrometry-based Proteogenomics

Mass spectrometry-based analysis is the reference platform for sequence-level proteomics. In a so-called bottom-up proteomic experiment, proteins in biological samples are digested using an enzyme (e.g., trypsin) into peptides. These peptides are then separated using liquid chromatography based on their chemical properties before undergoing a tandem mass spectrometry (MS/MS) measurement. The obtained spectra are searched against a database of expected protein sequences using specialized search algorithms, producing a set of peptide-spectrum matches (PSMs) [7]. Rescoring methods are applied to estimate the false discovery rate and posterior error probabilities of PSMs [8].

In proteogenomics, databases of protein sequences are obtained by aligning sites of observed genetic variation with mRNA or cDNA sequences of spliced transcripts, and translating the variants in-silico to obtain putative variant protein sequences [2]. Tools such as *py-pgatk* [9] facilitate this process. Spooner et al. have developed *Haplosaurus* to inspect different haplotypes of specific proteins using a dataset of phased genotypes [10]. Building upon this work, we have introduced *ProHap*, a Python-based pipeline to create databases of protein haplotype sequences from panels of phased genotypes [4].

Biological Sequence Visualization

In the realm of genomic and transcriptomic visualization, several tools have been developed for the exploration and analysis of sequences. Genome browser tools such as the *UCSC Genome Browser*[1] provide robust platforms for visualizing genomic data, offering multiple tracks displaying various features aligned onto a common scale. Similarly, the *Integrative Genomics Viewer* (IGV[2]) is a popular tool that provides a high-performance, interactive platform for visualizing various

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[1] https://genome.ucsc.edu/

[2] https://igv.org/

types of genomic data, including sequence alignments and variant calls [11]. *inPHAP* [12] visualizes haplotypes in genomic datasets using a matrix view with rows representing different haplotypes, and columns representing variant loci. Matrix cells are color-coded based on the allele present in the haplotype.

Ensembl [13] is a widely used resource that provides a comprehensive genome browser, integrating diverse genomic data such as gene annotations, sequence variants, and transcript splicing information. *gnomAD* [14], a key resource for human genetic variation, also offers a similar representation. Additionally, *Vials* is a tool that focuses on the visualization of alternative splicing events and their impact on protein-coding sequences [15].

Resources such as *UniProt*[3] and *neXtProt*[4] play a crucial role in the integration and interpretation of protein data, complementing genomic resources like *Ensembl*. *UniProt* is a generic, high-quality database of protein sequence and functional information, providing detailed annotations on protein structure, function, and interactions across all species. *neXtProt*, although it is now discontinued, extended this by offering a platform specifically designed for human proteins, integrating data from various sources to provide a complete and experimentally-validated representation of the human proteome. *UniProt* features viewer tools highlighting domains, post-translational modifications, annotated sequence features and variant locations. *neXtProt* offered similar visualizations, including a viewer for annotated features and for peptide identifications in mass spectrometry datasets.

DATA MODELING AND DOMAIN GOALS

Despite significant advances in genome and proteome analysis tools, there remains a gap in integrating haplotypes, protein sequence variation, and peptide evidence from mass spectrometry into a single interactive visualization platform. Genome browsers like *Ensembl* and *IGV* provide rich annotations for genomic variants but lack direct integration with proteomic data. Proteogenomic pipelines can generate custom protein databases to detect variant peptides, yet they do not offer interactive exploration of how haplotypes shape the proteome. Proteomic resources such as *UniProt* provide overview of variation in protein sequences, but do not consider haplotypes. Furthermore, even when active, *neXtProt* did not integrate haplotypes into the peptide identification view. *ProHap Explorer* addresses this need by enabling researchers to investigate how common haplotypes alter protein sequences and verify their presence in mass spectrometry datasets within an intuitive, interactive interface.

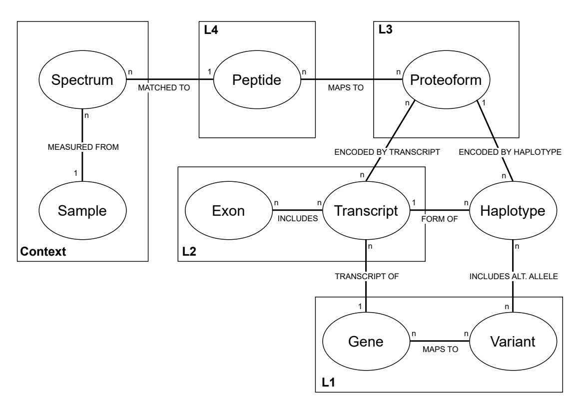

FIGURE 1. Relationships between the elements in proteogenomic datasets created using the ProHap workflow.

We identify four main layers of proteogenomic data (Figure 1), each representing a coordinate system to localize features of interest. The first layer is the gene (L1), defining the relevant region of the chromosome. Positions of features are encoded using their genomic coordinates – the number of base pairs between the start of the chromosome and the feature locus. The second layer is the transcript (L2), which captures the splicing of genes and the location of start/stop codons, denoting the limits of the main open reading frame (mORF). Positions of features within the transcript are determined by the number of nucleotides from the start of the cDNA sequence. The translation of the mORF yields the protein (L3). In our context, a unique protein sequence encoded by a haplotype of a spliced mRNA is referred to as a *proteoform*. Feature positions in the protein are counted as the number of amino acid residues from the first methionine residue at the N-terminal end. Note that features located in the 5' untranslated region of the transcript will have a negative protein-level coordinate. Finally, the fourth dimension is the peptide (L4), representing a part of the protein that has been matched to a spectrum, the features that fall into the peptide range being localized relative to the position of the first amino acid of the peptide.

Peptides matched to mass spectra provide the interface between the theoretical proteome ("what can be encoded by genes") to the experimental data ("what

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[3] https://www.uniprot.org/

[4] https://www.nextprot.org/

has been observed in biological samples"). This context is essential for the expert to assign biological and medical relevance to regions of the proteome. However, inferring the presence of a particular proteoform from a set of peptide identifications, a problem known as protein inference [16], is often impossible to achieve with certainty. A single peptide sequence may be encoded by different genes, and almost always maps to multiple proteoforms encoded by the same gene. While statistical methods have been developed to tackle this problem [17], a visual approach will allow experts to gain an overview of the assignment of peptides to proteoforms.

Working in a multi-disciplinary team with expertise in bioinformatics, proteomics, and genomics, we have identified four key visualization tasks. These were clarified during the iterative development of *ProHap* and the adjacent workflows, and upon discussions with collaborators in meetings and conferences. The tasks can be summarized using the typology introduced by Munzner and Brehmer [18] as follows:

- **T1**: *Explore* unique protein sequences encoded by human genes and the identification rates of matching peptides (e.g., see which genes encode the highest number of variant peptides, and whether any of these are relevant to a particular research project). In particular, it is important to provide tools that allow researchers to contextualize this information. For instance, they need to visually explore how these peptides are distributed across the proteome, identify patterns of variation, and assess their relevance to specific research questions. This is especially valuable when prioritizing genes for further investigation.

- **T2**: *Locate* regions of a protein affected by variation (e.g., once a relevant gene has been identified, see which part of the protein sequence map to variant peptides, and which splicing alternative has the best coverage). Researchers need to be able to intuitively assess the spatial relationships between variants, splicing events, and peptide coverage, facilitating hypothesis generation or experimental planning.

- **T3**: *Locate* weak or ambiguous peptide-spectrum matches for a protein of interest (e.g., once peptides of interest have been identified, review the quality of the peptide-spectrum matches). Although statistical scores can be shown for peptide-spectrum matches, visualization is essential for quickly identifying ambiguous or weak identifications in a list of peptides.

- **T4**: *Browse* identifications of variant peptides across tissues and datasets (e.g., when investigating a particular variant, see in which tissues, or which mass spectrometry datasets these peptides have been identified). In order to understand biological and clinical relevance, researchers need to be able to identify patterns in the expression of variant peptides across tissues and experimental conditions.

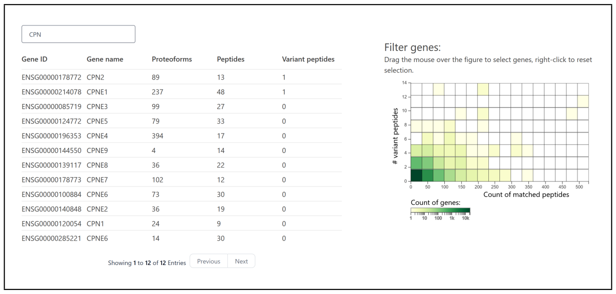
Dataset Presented in ProHap Explorer

We have created a database of protein haplotypes using *ProHap* on the complete dataset of phased genotypes published by the 1000 Genomes Project [5]. Individual variants were thresholded at a 1% minor allele frequency, with no haplotype frequency threshold applied. Variants were aligned with spliced cDNA sequences according to Ensembl v.110 [13] before in-silico translation. Variants mapping to the untranslated regions of transcripts were retained in the haplotypes; however, only the translation of the main open reading frame (mORF) was used in the resulting database of protein haplotype sequences.

We used this sequence database to search a public mass spectrometry proteomic dataset of healthy human tissues [6], downloaded from the PRIDE repository PXD010154. The search and rescoring of peptide-spectrum matches (PSMs) were performed as described by Skiadopoulou et al. [19], specifically, carbamidomethylation of cysteine (Unimod #4) was considered as a fixed modification, and oxidation of methionine (Unimod #35), deamidation of asparagine and glutamine (Unimod #7), and acetylation of the protein N-terminus were considered as variable modifications. The resulting PSMs were filtered at a 1% estimated false discovery rate (FDR). We then used the *ProHap Peptide Annotator* pipeline to annotate the identified peptides with the corresponding transcripts, gene names, and matching alleles of genomic variants. All the data have been stored in a graph database, illustrated in Figure 1, for effective querying.

PROHAP EXPLORER INTERFACE

ProHap Explorer is a web-based visualization tool designed to facilitate the exploration and analysis of



CPN

Gene ID	Gene name	Proteoforms	Peptides	Variant peptides
ENSG00000178772	CPN2	89	13	1
ENSG00000214078	CPNE1	237	48	1
ENSG00000085719	CPNE3	99	27	0
ENSG00000124772	CPNE5	79	33	0
ENSG00000196353	CPNE4	394	17	0
ENSG00000144550	CPNE9	4	14	0
ENSG00000139117	CPNE8	36	22	0
ENSG00000178773	CPNE7	102	12	0
ENSG00000100884	CPNE6	73	30	0
ENSG00000140848	CPNE2	36	19	0
ENSG00000120054	CPN1	24	9	0
ENSG00000285221	CPNE6	14	30	0

Showing **1** to **12** of **12** Entries

Filter genes:

Drag the mouse over the figure to select genes, right-click to reset selection.

FIGURE 2. *Explore View*, searching for gene names containing the “CPN” substring.

proteogenomic data by integrating haplotypic variation with peptide evidence from mass spectrometry datasets. Unlike existing tools that focus solely on genomic sequences or peptide identifications in isolation, ProHap Explorer introduces several novel capabilities, including: (1) linking haplotype-level genetic variation to observed peptide data, (2) an interactive dual-view interface for efficient exploration, (3) proteoform-aware peptide classification, and (4) dataset-specific filtering and highlighting features.

The interface consists of two main views: the *Explore View*, which displays a list of all human genes and allows users to discover which genes are best covered in the mass spectrometry datasets and most affected by variation, and the *Detail View*, which enables users to conduct an in-depth analysis of a gene of interest.

Explore View

The *Explore View* in *ProHap Explorer* (Figure 2) is designed to provide users with a comprehensive overview of human genes and their coverage in mass spectrometry datasets (T1). This view consists of two main components. The first component is a table that lists all human genes along with several key metrics: the number of unique proteoforms they encode, the total number of peptides matched to spectra in the mass spectrometry data, and the number of those peptides that are variant peptides. Users can sort the table by any of these variables, allowing for easy identification of genes with specific characteristics or levels of coverage. Clicking on a gene identifier will open the *Detail View* of this gene, as described below.

In developing the *Explore View*, we considered several alternative visualizations in addition to the data table. One option was to visualize all the genes using a space-filling curve, with color-coded regions representing the number of identified peptides or identified variant peptides. Space-filling curves, such as Hilbert curves, can effectively display large datasets while preserving spatial relationships [20]. However, this approach was ultimately discarded because it can be challenging for users to interpret and navigate, especially when trying to quickly filter genes with multiple variant peptides or a high number of peptides overall. The complexity of space-filling curves can make it difficult to extract specific insights at a glance.

The chosen design of the second component is an interactive 2D histogram. This histogram visualizes the number of peptides matched to spectra on the x-axis and the number of variant peptides of those matched on the y-axis. Each cell in the heatmap is color-coded to represent the number of genes with the respective properties on a logarithmic scale. Users can interact with the histogram by clicking on a specific cell to select it or by making a rectangle selection by dragging the mouse over the heatmap. This selection will filter the genes shown in the table, enabling users to quickly focus on genes that meet specific criteria.

Detail View

Upon selection of a gene in the *Explore View*, the *Detail View* is displayed, which consists of two sections. Each section includes a primary visualization, a data table, and options to download raw data, providing a comprehensive and detailed analysis of the selected gene. The *Detail View* can be active for multiple genes at the same time, with each gene being displayed in a separate tab. The user can freely navigate between the tabs without losing any current filters or selections.

Splicing and Variation Section The first section (Figure 3) represents the layers of genes and transcripts (L1, L2). The primary visualization in this section shows all the different splicing alternatives of the selected gene, with each row representing one transcript. Exons are aligned from the 5’-most to the 3’-most end and shown as rectangles, while introns are displayed as abbreviated lines. The locations of the canonical start and stop codons are highlighted, with the region in between, known as the main open reading frame (mORF), shown in a darker shade to distinguish it from untranslated regions (UTRs).

Vertical lines across all rows represent variant loci, with the variant type (single nucleotide polymorphism (SNP), in-frame insertion or deletion, and frameshift) encoded by different colors. This allows users to quickly identify the types and locations of genetic variants present in the coding regions of the gene (T2). Additionally, regions of exons that encode a sequence of a peptide matched in one of the mass spectrometry datasets are highlighted in dark blue, indicating parts of the protein that have been experimentally validated. Upon selecting a transcript, the user can select whether the horizontal axis should display positions along the chromosome, the spliced transcript, or the protein sequence. All three coordinate systems are based on the Ensembl annotation.

This linear representation, with exons depicted as rectangles and introns as horizontal lines, is the most common way to represent splicing of genes. It is widely used in genome browsers, such as the *UCSC Genome Browser*[5] and *Ensembl* [13], making it a familiar format for experts. This familiarity allows them to interpret the visualization much faster than other designs, enabling us to encode additional information such as variant loci and peptide mappings without overwhelming the user.

The data table in the first section becomes available upon the selection of a transcript. Rows in this table represent the unique non-canonical sequences possibly encoded by haplotypes in this transcript. For each of these proteoforms, the table shows the changes in the cDNA and protein sequence, and the observed frequency of the haplotype in the 1000 Genomes dataset. Amino acid changes that have been observed in an identified peptide are highlighted by a green outline, making it easy to estimate which haplotype have been observed in the analyzed samples.

By clicking on the transcript identifier in the visualization of splicing alternatives, the user will bring up a data table of corresponding proteoforms. The user can then filter the displayed proteoforms by hiding haplotypes that do not encode any matched variant peptides using a checkbox above the table, and by restricting the view to haplotypes that include a variant of interest. The variant can be selected by clicking on the corresponding line element or using a searchable drop-down menu.

The user selects a proteoform by clicking on one of the table rows. Upon selection, the table row expands to reveal the different haplotypes possibly encoding this proteoform sequence, showing associated combinations of variants in the untranslated regions, and associated combinations of synonymous variants (i.e., not encoding an amino acid change) in the protein-coding regions. This iterative filtering approach allows users to gain a deeper understanding of the landscape of proteoforms encoded by the selected gene (T2).

The user may download the protein sequences encoded by all canonical and non-canonical haplotypes of the gene in the FASTA format. Additionally, the details of all the non-canonical haplotypes are available for download as a tab-separated text file, corresponding to the format produced by the *ProHap* pipeline.

Alignment of Identified Peptides After selecting a transcript in the first section, the second section of the *Detail View* is shown, representing the protein and peptides (L3, L4). This section provides a detailed visualization of how peptides align with the selected protein, offering insights into the proteomic data associated with the gene of interest (T3).

The primary visualization in this section (Figure 4) is divided into two parts. The top part represents the canonical proteoform encoded by the selected transcript and becomes available immediately after the transcript selection. This part shows the alignment of peptides with the canonical protein sequence, providing a baseline for comparison. The mirrored bottom part of the visualization shows peptides aligned with the protein haplotype sequence and becomes available after the selection of a non-canonical proteoform in the data table of the first section. This dual representation allows users to compare the canonical and variant protein sequences side by side.

In a similar way to displaying the splicing of genes, it is common to display features within a protein sequence along a linear axis representing the amino-acid position from start to end (L3). This representation is used by *UniProt* and *neXtProt* and is well understood among experts. Color-coding along the axis is used by *inPHAP* [12] to distinguish different alleles. However, the alleles here are distinguished using a separate feature track along the axis, and the color channel is reserved for distinguishing between classes of peptides or a customized highlighting of peptides of interest.

Alternative options, such as space-filling or circular layouts, have their own advantages [20]. Space-filling layouts can show features in greater detail by stretching out the axis and providing more room, but this comes at the cost of rapid interpretability. Circular layouts can easily display relationships between distant elements along the protein; however, these relationships are already provided in the data table of the first section, and a circular layout would distort the display of quantities along the vertical axis. Therefore, we chose to conform to the common practices and provide a linear visualization that is easy to interpret.

By default, each peptide is represented by a rectangle of uniform height, with its position and width encoding the location of the peptide within the protein. Alternatively, users may choose to view the number of spectra matched to peptides at any given position along the protein sequence. In this mode, segments

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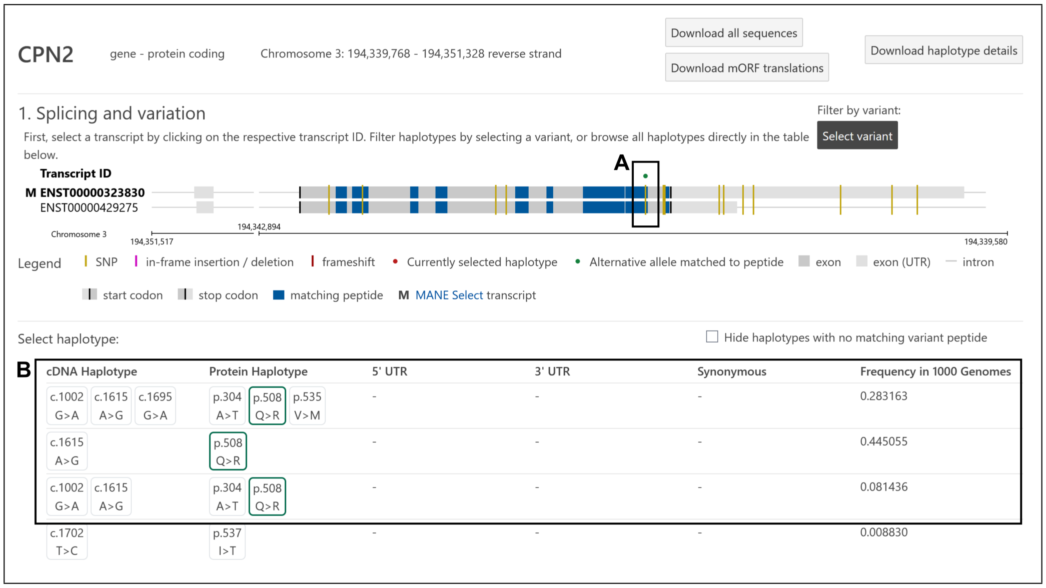
[5] https://genome.ucsc.edu/



First, select a transcript by clicking on the respective transcript ID. Filter haplotypes by selecting a variant, or browse all haplotypes directly in the table below.

cDNA Haplotype	Protein Haplotype	5' UTR	3' UTR	Synonymous	Frequency in 1000 Genomes
c.1002 G>A c.1615 A>G c.1695 G>A	p.304 A>T p.508 Q>R p.535 V>M	-	-	-	0.283163
c.1615 A>G	p.508 Q>R	-	-	-	0.445055
c.1002 G>A c.1615 A>G	p.304 A>T p.508 Q>R	-	-	-	0.081436
c.1702 T>C	p.537 I>T	-	-	-	0.008830

FIGURE 3. First section of the *Detail View*, showing the *CPN2* gene after the selection of a transcript, and before the selection of a haplotype. A: One variant encodes an amino acid substitution that has been observed in a peptide. B: This variant appears in three unique protein haplotype sequences, either in combination with one or two other substitutions, or individually.

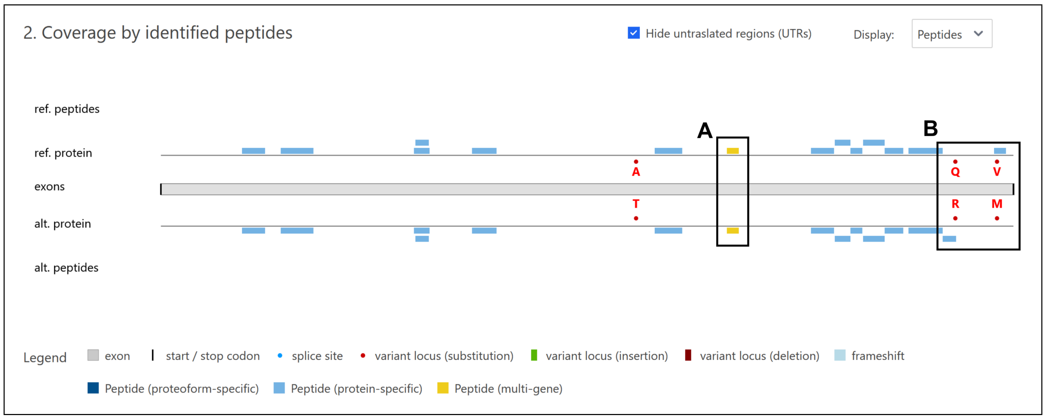


FIGURE 4. Primary visualization in the second section of the *Detail View*, displaying the CPN2 protein with the reference sequence at the top, and a selected non-canonical haplotype at the bottom. A: One canonical peptide matches the protein product of multiple genes. B: Only the alternative allele has been observed in a peptide for the first variant, while only the reference allele has been observed for the second variant.

of peptide sequences are represented as bars, with the height encoding the number of matched spectra and the position and width encoding the location of the segment within the protein. This flexible visualization option helps users to understand the coverage of peptides across the protein sequence.

The color of each peptide rectangle encodes the classification of the peptide based on its ability to distinguish between protein sequences. Multi-gene peptides map to the products of different genes, protein-specific peptides map to multiple sequences that are all products of the same gene, and proteoform-specific peptides map uniquely to a single proteoform sequence [3]. This color-coding allows users to quickly identify peptides that are unique to specific proteoforms or shared among multiple proteins.

The data table in the second section lists all the peptides displayed in the main figure. Each row specifies the peptide sequence, its position within the protein, the classification of the peptide, and the distribution of two quality measures of the peptide-spectrum matches: the posterior error probability (PEP) and the difference between predicted and observed retention time. Additionally, the user can view the raw spectral data in the PRIDE database using the universal spectrum identifier (USI). The last column of the table contains links to PRIDE using the USI of the best, second-best, and median peptide-spectrum match, ordered by the PEP. The user may download the data table in a text format, with each row representing a peptide-spectrum match, with the quality measures and the USI specified.

Users can view the sequence of the peptides by hovering the mouse over the respective elements in the visualization. Additionally, hovering the mouse over the blank spaces displays a corresponding short segment of the protein sequence. Amino acid variants in the protein sequence are highlighted in red. This feature provides immediate access to sequence information, making it easier for users to interpret the alignment and coverage of peptides within the protein.

Details for all the peptide-spectrum matches (PSMs) displayed in the data table are available for download, letting the user make additional queries, or use the data in other workflows.

Highlighting by Tissue, Phenotype, or Proteomic Study

To the left of the two sections of the *Detail View*, a horizontal bar chart displays the number of elements per tissue, phenotype, or proteomic study we reanalyzed. Users select the category to display, as well as whether to display the number of PSMs, unique peptides, or the number of samples where at least one of the displayed peptides was confidently matched. Additionally, each row in the bar chart features a checkbox, allowing users to highlight the relevant category within the elements displayed in the *Protein* section of the *Detail View*. This interactive feature helps users quickly identify and focus on specific tissues, phenotypes, or proteomic studies of interest (T4).

Highlighting elements of interest, as opposed to filtering, ensures that users can see the full context of all peptide identifications, while still emphasizing the selected categories. Although highlighting multiple categories may lead to overplotting in some cases, such as with long proteins that have many identified peptides, it allows users to maintain a comprehensive view of the data. Therefore, this design choice balances the need for detailed inspection with the importance of contextual awareness.

IMPLEMENTATION AND CODE AVAILABILITY

The dataset for *ProHap Explorer* is stored in a graph database implemented in *neo4j*, ensuring efficient querying and management of data entries and relationships between them. A Python-based pipeline is available to transform data from the standard output of *ProHap*, *ProHap Peptide Annotator*, and *Percolator* to the *neo4j* database. The code can be accessed at https://github.com/ProGenNo/ProHap_Graph. The graph database is implemented according to the model illustrated in Figure 1, and is documented in detail in the *GitHub* repository. The server is implemented in Python using the *Flask* library, which queries the *neo4j* database using the *Cypher* language, providing a backend for the application.

The user interface of *ProHap Explorer* was developed using the *Svelte* framework, with *D3.js* employed to render the visualization components. The source code for the web application, along with information about the current deployment, is available at https://github.com/ProGenNo/ProHapExplorer. Temporarily, *ProHap Explorer* is accessible with a limited dataset showing peptide-spectrum matches from the reprocessing of one proteomic study. This temporary deployment provides users with an opportunity to explore the tool and its capabilities while we continue to refine and expand the dataset.

CASE STUDIES

To illustrate the utility of *ProHap Explorer*, we present two case studies demonstrating its application in proteogenomic analysis. These examples highlight how the tool enables researchers to investigate the impact of haplotypes on protein sequences and identify non-canonical peptides in mass spectrometry datasets.

Common Haplotypes of CPN2

In our previous study [4], we identified several variant peptides while searching mass spectrometry datasets against a database of protein haplotype sequences created by *ProHap*. Notably the CPN2 protein commonly carries a substitution of the 508th amino acid from glutamine to arginine (p.508:Q>R), encoded by the genetic variant located at position 194,341,177 on the 3rd chromosome. We have seen the variant peptide mapping to the corresponding location in the CPN2 protein in the majority of the samples processed in our previous research [4].

We have investigated the *CPN2* gene in the *Detail View* of *ProHap Explorer* (Figure 3, Figure 5).

We observed two splicing alternatives present for this gene, both encoding an identical protein sequence as they only differ in the untranslated regions. Moreover, peptides encoded by the alternative allele of the substitution were identified in the underlying dataset (Figure 3A). The p.508:Q>R variant appears in three unique protein haplotypes, either in combination with one or two other amino acid substitutions (p.304:A>T, p.535:V>M), or by itself (Figure 3B). We found that the haplotype encoding only the individual amino acid substitution is more common than the others, appearing in over 44% of the participants of the 1000 Genomes Project.

Upon selecting a haplotype (p.304:A>T, p.508:Q>R, p.535:V>M), we observed that the three variants causing the amino acid substitution are linked with several combinations of variants that do not alter the protein sequence. Most commonly, they are found in combination with one variant in the transcript 3' untranslated region and two synonymous variants in the protein-coding region (Figure 5A). In the second section of the *Detail View*, we inspected the identified peptides encoded by the reference and the alternative haplotype. We noted that no peptides were matched to the reference or alternative allele of the first variant (Figure 5B). Only peptides encoded by the alternative allele of the second variant and the reference allele of the third variant were identified (Figure 5C).

Highlighting the peptides per tissue (Figure 5D) revealed that the variant peptide was uniquely observed in brain tissue from the current underlying datasets, while the peptide mapping to the reference allele at position 535 was uniquely observed in adrenal gland tissue (Figure 5C). This suggests that in the processed samples, only the alternative allele is expressed for the substitution at position 508, and only the reference allele is expressed for the substitution at position 535, consistent with our previous findings. We could not determine whether the reference or the alternative allele is expressed for the substitution at position 304, as this part of the protein was not covered by peptides at all.

New Reference Sequence for CAP1

Similarly to the previous example, we have previously shown that a combination of alleles encodes five amino acid substitutions in a single peptide from the CAP1 protein [4]. This peptide was identified in a dataset of mass spectra from stem cells from a healthy donor. We previously searched these spectra against a personalized database of protein haplotypes created by ProHap using the genotype of the donor, which is publicly available.

Investigating the *CAP1* gene in the *Detail View* of *ProHap Explorer* (Figure 6), we observed that a large number of splicing alternatives have been annotated for this gene. However, all the identified peptides match the alternative marked as *MANE Select*[6], which is the canonical splicing validated by NCBI and Ensembl. Upon selecting the *MANE Select* transcript, we found that a single non-canonical haplotype has been observed for this transcript among all the participants of the 1000 Genomes panel (Figure 6B). This haplotype encodes six amino acid substitutions, all of which have been observed in at least one confidently matched peptide.

In the second section of the *Detail View*, we visualized the number of spectra matched to peptides along the protein sequence. We observed that relatively few spectra support the presence of the peptide spanning the five amino acid substitutions (Figure 6C), while a higher number of spectra support the presence of a downstream peptide, covering the sixth substitution. Upon inspecting the variant peptides in the data table (Figure 7), we noted that the peptide-spectrum matches have low posterior error probability, and the difference between predicted and observed chromatographic retention times is on the lower end (Figure 7C), suggesting these are confident matches. Experts experienced in the interpretation of raw mass spectrometry data may further review these matches by clicking on one of the links in the last column of the table, navigating to the website of the PRIDE database and visualizing the spectrum annotated with names of fragment ions.

This use case demonstrates the evidence that the reference sequence of the CAP1 protein is not expected to be present in any individual, and that we can experimentally verify the presence of the variant peptides encoded by the alternative haplotype. Moreover, five of the six amino acid substitutions can only be observed in peptides if they are considered as a haplotype, highlighting the importance of inspecting haplotypes in proteins in their completeness, rather than each variant individually.

USER FEEDBACK

We conducted user interviews with four domain experts who were not involved in the development of the tool. Participants gave their informed consent prior

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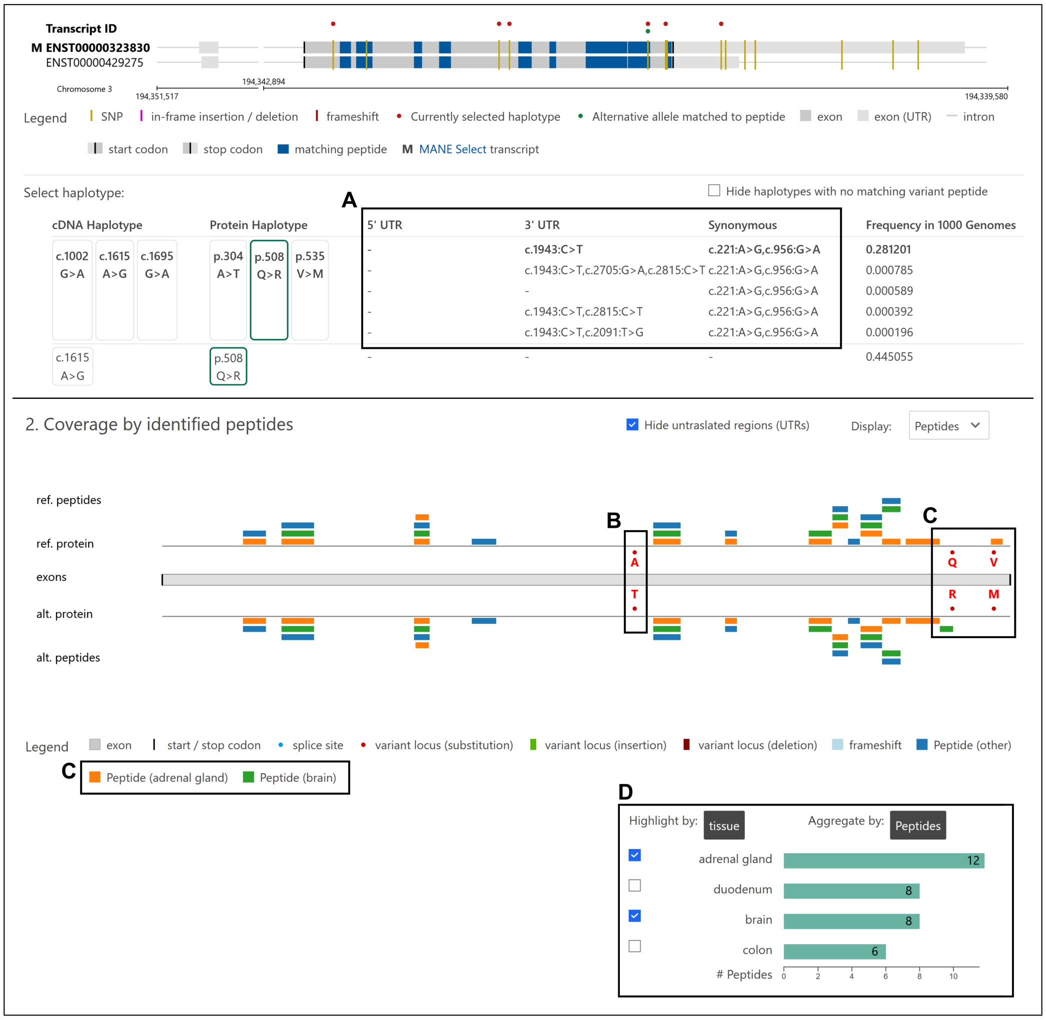
[6] https://www.ncbi.nlm.nih.gov/refseq/MANE/



cDNA Haplotype	Protein Haplotype	5' UTR	3' UTR	Synonymous	Frequency in 1000 Genomes
c.1002 G>A c.1615 A>G c.1695 G>A	p.304 A>T p.508 Q>R p.535 V>M	-	**c.1943:C>T**	**c.221:A>G,c.956:G>A**	**0.281201**
		-	c.1943:C>T,c.2705:G>A,c.2815:C>T	c.221:A>G,c.956:G>A	0.000785
		-	-	c.221:A>G,c.956:G>A	0.000589
		-	c.1943:C>T,c.2815:C>T	c.221:A>G,c.956:G>A	0.000392
		-	c.1943:C>T,c.2091:T>G	c.221:A>G,c.956:G>A	0.000196
c.1615 A>G	p.508 Q>R	-	-	-	0.445055

FIGURE 5. The *Detail View* of the *CPN2* gene after the selection of a transcript and haplotype, and highlighting peptides by tissue in the second section of the view. A: Combinations of variants which do not alter the protein sequence, but are linked with the three variants causing amino acid substitutions in the selected haplotype. B: No reference or alternative peptides have been identified. C: The peptide encoded by the alternative allele of the second variant has been uniquely identified in the brain tissue samples, while the peptide encoded by the reference allele of the third variant has been identified uniquely in the adrenal gland tissue samples. D: The categories highlighted can be selected in the visualization adjacent to the *Detail View*.

to the interview. Two of the participants are post-doc researchers, and two are PhD candidates working in the fields of proteomics and molecular biology. One of the post-docs and one of the PhD students are familiar with bioinformatics, while the remaining two participants mainly work with laboratory methods. This group provided valuable feedback on the usability and functionality of *ProHap Explorer*.

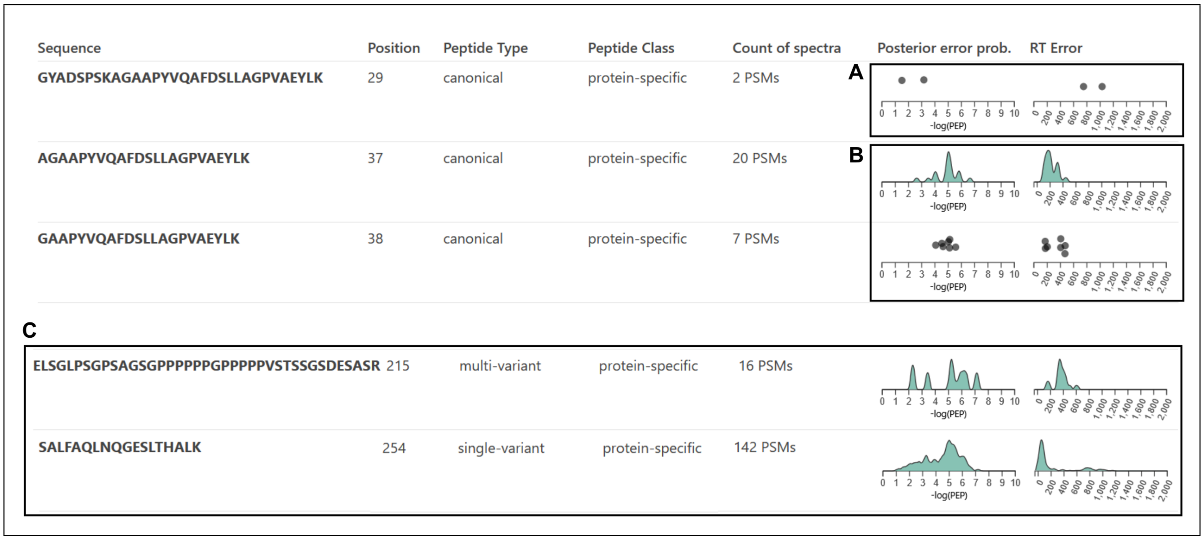
Overall, the participants expressed satisfaction with the clarity of the interface. The linear representations of splicing alternatives and features along the protein sequence were familiar to them, making them easy to interpret. The two participants familiar with bioinformatic tools easily understood the interaction patterns and intuitively selected relevant genes, transcripts, and their haplotypes. They also clearly understood the concept of protein haplotypes and appreciated how this information could enhance their research.

The other participants took more time to familiarize themselves with the interface, as they were more accustomed to static figures. However, they all found it useful to view which parts of proteins had been identified in the mass spectrometry dataset we processed, and mentioned that they would compare this

FIGURE 6. The *Detail View* of the *CAP1* gene. A: A cluster of variants affects one particular region of the gene. B: These variants all form a single haplotype, observed in all participants of the 1000 Genomes panel. C: A peptide encoded by the alternative allele of five variants has been matched to 9 different spectra. Hovering the mouse cursor over the respective element reveals the sequence of the peptide, and the corresponding section of the canonical protein.

with their own data. This will allow them to interrogate whether the lack of peptides in their results might be due to overlooking the influence of genetic variation. Despite the initial learning curve, they recognized the value of the tool in providing a comprehensive view of proteogenomic data.

A few suggested additional features were noted during the interviews. Participants expressed interest in an integration with *UniProt*, which could complement the current reliance on *Ensembl* resources in *ProHap*. Specifically, this integration could enable the annotation of functional domains along the protein sequence, allowing users to quickly identify regions of interest. The detailed descriptions of proteins by *UniProt* and their assumed functions were also noted as valuable additions that could enhance the utility of the tool. Furthermore, participants suggested enabling searches for genes using their respective *UniProt* identifiers. This would reduce reliance on external sources to locate the correct identifiers. One participant mentioned an interest in comparing different genes, although this was not a priority for the others. These suggestions highlight potential areas for future development to further enhance the utility of *ProHap Explorer* for a broader range of users.



Sequence	Position	Peptide Type	Peptide Class	Count of spectra	Posterior error prob.	RT Error
GYADSPSKAGAAPYVQAFDSLLAGPVAEYLK	29	canonical	protein-specific	2 PSMs		
AGAAPYVQAFDSLLAGPVAEYLK	37	canonical	protein-specific	20 PSMs		
GAAPYVQAFDSLLAGPVAEYLK	38	canonical	protein-specific	7 PSMs		
ELSGLPSGPSAGSGPPPPPPGPPPPPVSTSSGSDESASR	215	multi-variant	protein-specific	16 PSMs		
SALFAQLNQGESLTHALK	254	single-variant	protein-specific	142 PSMs		

FIGURE 7. Excerpt from the data table for the second section of the *Detail View* of the CAP1 protein. A: The identification confidence of the first peptide is weak, with only two supporting peptide-spectrum matches (PSMs), one with a high posterior error probability, and both with a high retention time error. B: The second and third peptide are strongly supported by confident PSMs. C: Similarly, the identifications of the two non-canonical peptides of CAP1 are supported by a high number of PSMs, many of which present a low posterior error probability, and a low retention time error.

DISCUSSION AND LIMITATIONS

While *ProHap Explorer* offers a robust and innovative platform for visualizing proteogenomic data, there are some limitations remaining to be addressed to enhance its functionality further. Here, we discuss these limitations and outline potential solutions to ensure the tool remains effective and user-friendly.

The usage of a graph-based backend and effective algorithms for the alignment of features in the visualizations result in a short response time when interacting with *ProHap Explorer*. This ensures an uninterrupted user experience. However, as we add more mass spectrometry data, particularly when thousands of spectra match to each peptide, we anticipate an increase in the latency between user interactions and the rendering of components. If this problem arises, we will need to further optimize the algorithms or pre-compute some of the values that are currently calculated online. These adjustments are feasible and will ensure that the tool remains responsive for large datasets.

Currently, the usage of statistical methods in *ProHap Explorer* is limited to evaluating the confidence of individual peptide matches. Future updates may benefit from integrating protein-level statistics to visually guide users in interpreting the overall reliability of inferred proteoform identifications.

Integration with *UniProt* and the inclusion of extended textual descriptions of proteins will help experts find their proteins of interest more easily. While this feature is not yet implemented, it is an addition that will significantly enhance the usability of *ProHap Explorer*. By providing more context and detailed information about each protein, users will be able to navigate the data more efficiently and make more informed decisions.

In some cases, the visualizations can become cluttered, making it difficult to focus on specific features of interest. A zoom feature would help address this issue by allowing users to hone in on particular areas of the visualization. Furthermore, an optimal approach might involve showing both the overview and the focused view simultaneously, providing context while allowing for detailed exploration. Although this feature is not currently available, the existing modes of interaction and the ability to download raw data enable users to inspect each gene in great detail. This ensures that, despite some visual clutter, users can still access and analyze the information they need. Future updates will aim to incorporate zoom functionality to further improve the user experience.

CONCLUSION

We have presented *ProHap Explorer*, a novel visual exploration tool designed to interrogate the influence of common haplotypes on the human proteome. With the recent introduction of *ProHap*, experts can now create customized sequence databases of protein haplotypes to search mass spectrometry data, or use one of the databases published alongside *ProHap*. However, a resource that allows experts to browse the haplotypes and their influence on protein sequences using public mass spectrometry data was missing. *ProHap Explorer* bridges this gap by providing an interactive visual interface, enabling experts to explore this novel resource effectively.

Our user interviews with participants of varying levels of expertise in proteomics support the utility of *ProHap Explorer*. Participants appreciated the opportunity to investigate whether haplotypes have any consequences on their proteins of interest and whether any non-canonical peptide sequences have been identified in public mass spectrometry datasets. The familiar linear representations and interactive elements facilitated easy navigation and interpretation of the data. Additionally, our interviews highlighted a further need to integrate other resources, such as *UniProt*, to add new layers of information on protein function. This will inform our future steps in the development of *ProHap Explorer*.

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