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The Human Proteoform Atlas: a FAIR community resource for experimentally derived proteoforms

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ABSTRACT

The Human Proteoform Atlas (HPfA) is a web-based repository of experimentally verified human proteoforms on-line at http://human-proteoform-atlas.org and is a direct descendant of the Consortium of Top-Down Proteomics' (CTDP) Proteoform Atlas. Proteoforms are the specific forms of protein molecules expressed by our cells and include the unique combination of post-translational modifications (PTMs), alternative splicing and other sources of variation deriving from a specific gene. The HPfA uses a FAIR system to assign persistent identifiers to proteoforms which allows for redundancy calling and tracking from prior and future studies in the growing community of proteoform biology and measurement. The HPfA is organized around open ontologies and enables flexible classification of proteoforms. To achieve this, a public registry of experimentally verified proteoforms was also created. Submission of new proteoforms can be processed through email *via* nrtdphelp@northwestern.edu, and future iterations of these proteoform atlases will help to organize and assign function to proteoforms, their PTMs and their complexes in the years ahead.

INTRODUCTION

Genomic data types probe human biology at the gene and transcript level, yet proteins are the direct source of much activity within our bodies and cells. Current, data on the expression of proteins is not complete as expressed proteins are often highly modified in ways which affect their structure and function. A single human gene usually expresses as a family of related forms of a protein. The term proteoforms has been introduced to refer to the specific molecular species of an expressed and translated gene including the precise combination of sequence variants, alternative splicing events, and post-translational modifications (PTMs) (Figure 1) (1). Proteoforms represent the main determinants of cellular phenotypes in protein-level biology yet remain largely understudied. According to UniProt, the total number of 'canonical' (i.e. the representative protein in UniProt for a given gene) human proteins across the entire proteome, is currently 20 386 (2). The number of proteoforms is larger and difficult to estimate because of phenomena such as mRNA splicing, PTMs, coding single-nucleotide polymorphism (cSNPs), and similar events (3).

Understanding expressed proteoforms as the molecular phenotype is fundamental. One illustrative example is the case of the KRAS protein, which belongs to the RAS gene family. KRAS is alternatively spliced at the fourth exon, yielding the KRAS4A and KRAS4B isoforms (4). These genes encode for small GTPases which play important roles in cell growth and proliferation via the MAPK and PI3K pathways. The RAS gene family is also among the most frequently mutated in cancer, for example at residues G12, G13 or Q61 in KRAS4. Combined with oncogenic mutations, KRAS undergoes post-translational modification, including farnesylation and carboxymethylation of the C-terminal C185, which is critical for plasma membrane association. Given these sources of variation, there are thousands of possible theoretical proteoforms for the KRAS protein, with <40 mapped thus far in cancer cell lines and tumors (5).

The Protein Ontology (PRO) (6), another major protein knowledgebase, provides proteoform-level permanent identifiers, but only after a proteoform is published in the peer-reviewed literature and has been manually curated. This process leads to high quality annotations but, consequently, lags behind the experimental forefront. Therefore, at present, experimentally verified proteoform related information is mostly locked away from researchers by the lack of a findable, accessible, interoperable and reusable (FAIR) data infrastructure. As highlighted above for the KRAS protein, only those proteoforms representing different protein isoforms coming from alternatively spliced

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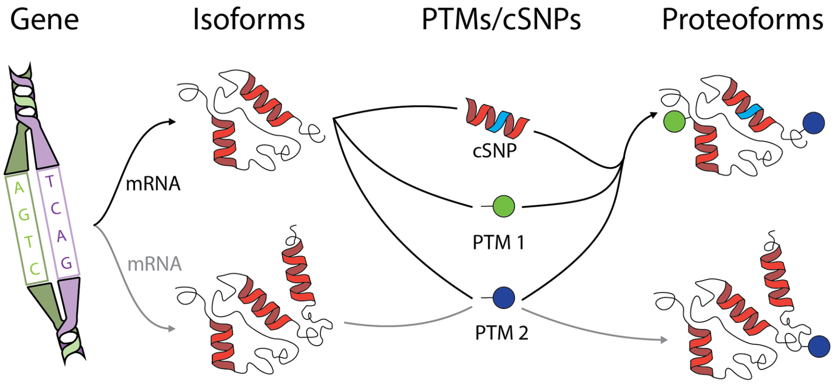


Figure 1. Scheme explaining the concept of a proteoform. A single gene (depicted at left) can be processed to generate different isoforms *via* alternative splicing of mRNA, which in combination with site-specific post-translational modifications (PTMs) and/or coding single-nucleotide polymorphism (cSNPs) that might be present in the population, generates different proteoforms (at right).

transcripts have their own unique protein identifiers in UniProt. For other types of proteoforms (proteins containing PTMs and protein variants) there is a need to normalize a widely accepted representation and to assign accession numbers, enabling interoperability between protein resources. Naturally, this limitation propagates to other bioinformatics resources that rely on UniProt as the way to represent proteins. Therefore, there is a need to enable a 'proteoform-centric view' for proteins, by creating resources and infrastructure for the handling, analysis, and validation of proteoforms. To overcome the shortage of dedicated biological cyberinfrastructure on human proteoforms we have established a Human Proteoform Atlas (HPfA) to serve as a registry for experimentally verified proteoforms and build upon the general Proteoform Atlas hosted by the Consortium of Top-Down Proteomics' (CTDP) (http://atlas.topdownproteomics.org/).

HUMAN PROTEOFORM ATLAS CONTENTS AND FEATURES

We have established the HPfA, using FAIR principles, to durably store and identify proteoforms as they are experimentally verified from *Homo sapiens* (UniProt taxon identifier: 9606). The atlas, on-line at http://human-proteoform-atlas.org, is organized around existing open ontologies. It is currently structured to include Anatomy, Blood, Cancer, Cariology, Cell Lines, Cells, Immunology, and Neurologic Atlases, but new atlases can be instantiated as needed.

At present, the Human Proteoform Atlas contains 37 071 unique experimentally verified proteoforms, each of which has been assigned a durable proteoform identifier. These proteoforms come from 30 datasets in 27 peer-reviewed publications (Supplementary Table S1) (7–38), published by 15 unique laboratories over the last seven years (2014 to 2021), and represent 3055 protein isoforms derived from 2465 unique genes (∼13% of the protein-coding human genome). The proteoforms contain 369 separate types of modifications including phosphorylation (∼13% of the total PTMs observed), acetylation (∼43%), disulfides (∼2%), methylation (∼29%), Oxidation (∼2%), Dehydro (∼2%) palmitoylation (<1%), farnesylation (<1%), geranylation (<1%), nitrosylation (<1%), sulfinic acid (<1%) and ADP-ribosylation (<1%).

The Human Proteoform Atlas is built around existing open and community-supported ontologies. Terms from these ontologies are used to classify proteoform entries. Currently, there are 68 ontologic terms used from seven ontologies (Figure 2A), including 11 different tissue types (Figure 2B), and 12 separate ontology terms associated with disease (Figure 2C).

The Human Proteoform Atlas is designed to be open, scalable, and adopted as part of future community-driven activities. It creates the infrastructure needed to maximize dissemination of human proteoforms to the biomedical community while creating a single resource which leverages knowledge of human proteoforms. This repository links to protein-centric databases such as UniProt and has data visualization and integration tools.

User interface

Proteoforms and datasets contained in the Human Proteoform Atlas are broken down into eight current sub-atlases, displaying both dataset and proteoform counts and a description of that Atlas. Atlases are organized around existing open ontologies and can be broken down further by ontology terms or dataset.

A user visiting the site first encounters a view of the eight sub-atlases, with dataset and proteoform counts. If the user is interested in the proteoforms associated with a particular cell type, for example, an eosinophil cell, they may select the Cell atlas which displays a breakdown of all ontological terms from the Cell ontology (CL) in the atlas. Selecting the eosinophil cell ontology term (CL:0000771), proceeds to an overview, including a description of the ontology term, links to EBI (39) and PURL (40), as well as a list of proteoforms associated with eosinophil cells, including protein associations, and modifications. These proteoforms can be further queried using the provided search tool. Proteoforms may be selected to give a detailed description, including biological and chemical proteoform accession numbers, associated proteins, ProForma sequence (41) and

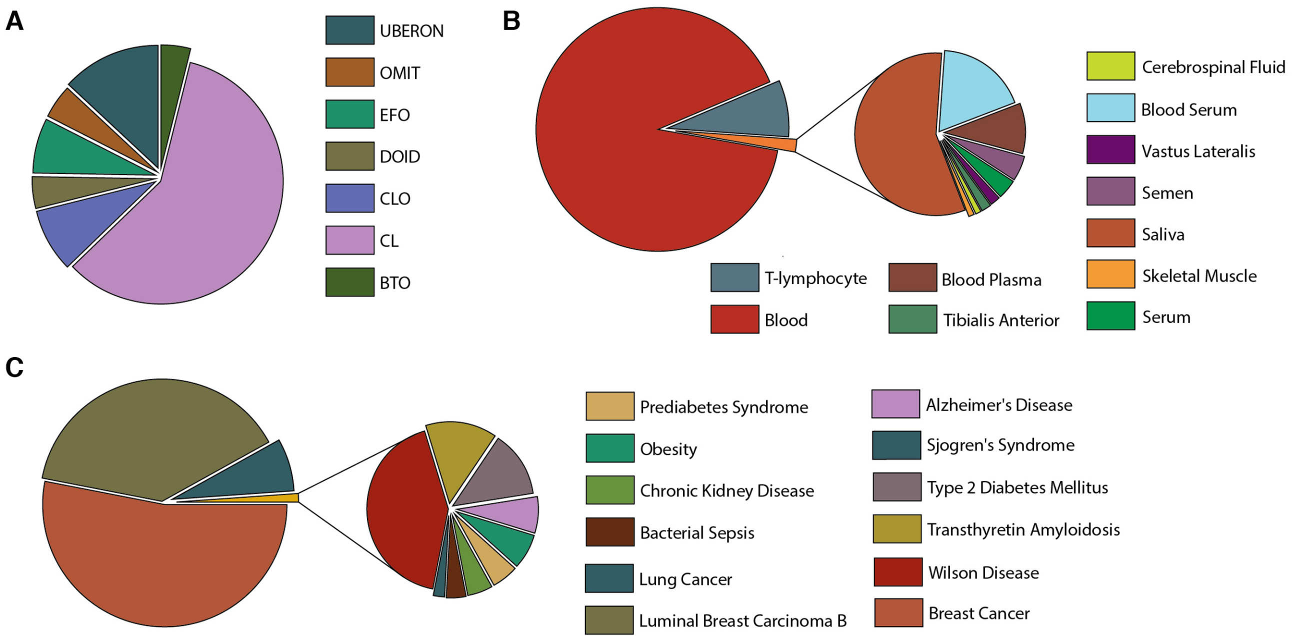
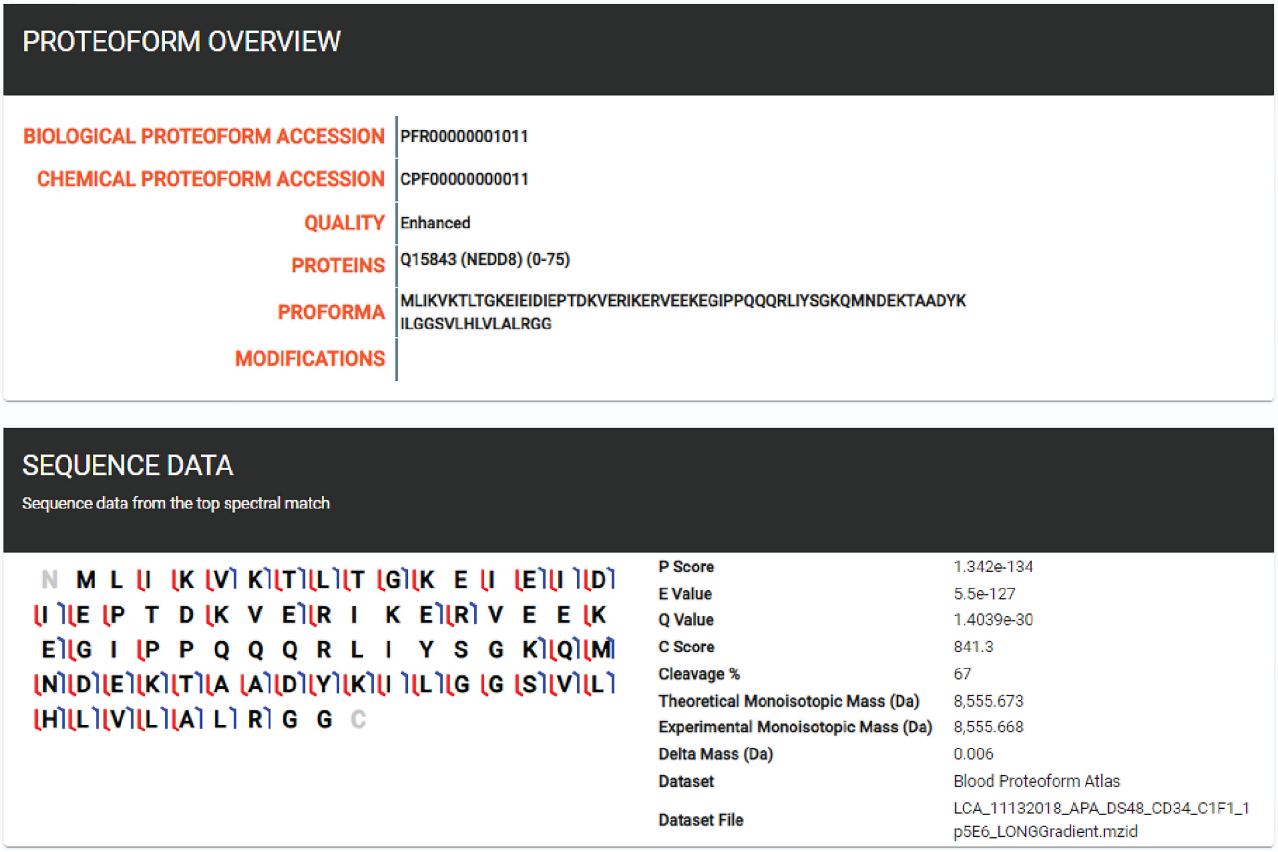


Figure 2. Proteoform breakdown in the HPfA by ontology in panel (**A**), tissue in (**B**) and human disease in (**C**). Ontology codes are: Uber-anatomy ontology (UBERON), Ontology for MIRNA Target (OMIT), Experimental Factor Ontology (EFO), Human Disease Ontology (DOID), Cell Line Ontology (CLO), Cell Ontology (CL) and BRENDA Tissue Ontology (BTO).



PROTEOFORM OVERVIEW

BIOLOGICAL PROTEOFORM ACCESSION	PFR00000001011
CHEMICAL PROTEOFORM ACCESSION	CPF00000000011
QUALITY	Enhanced
PROTEINS	Q15843 (NEDD8) (0-75)
PROFORMA	MLIKVKTLTGKEIEIDIEPTDKVERIKERVEEKEGIPPQQQRLIYSGKQMNDEKTAADYK ILGGSVLHLVLALRGG
MODIFICATIONS	

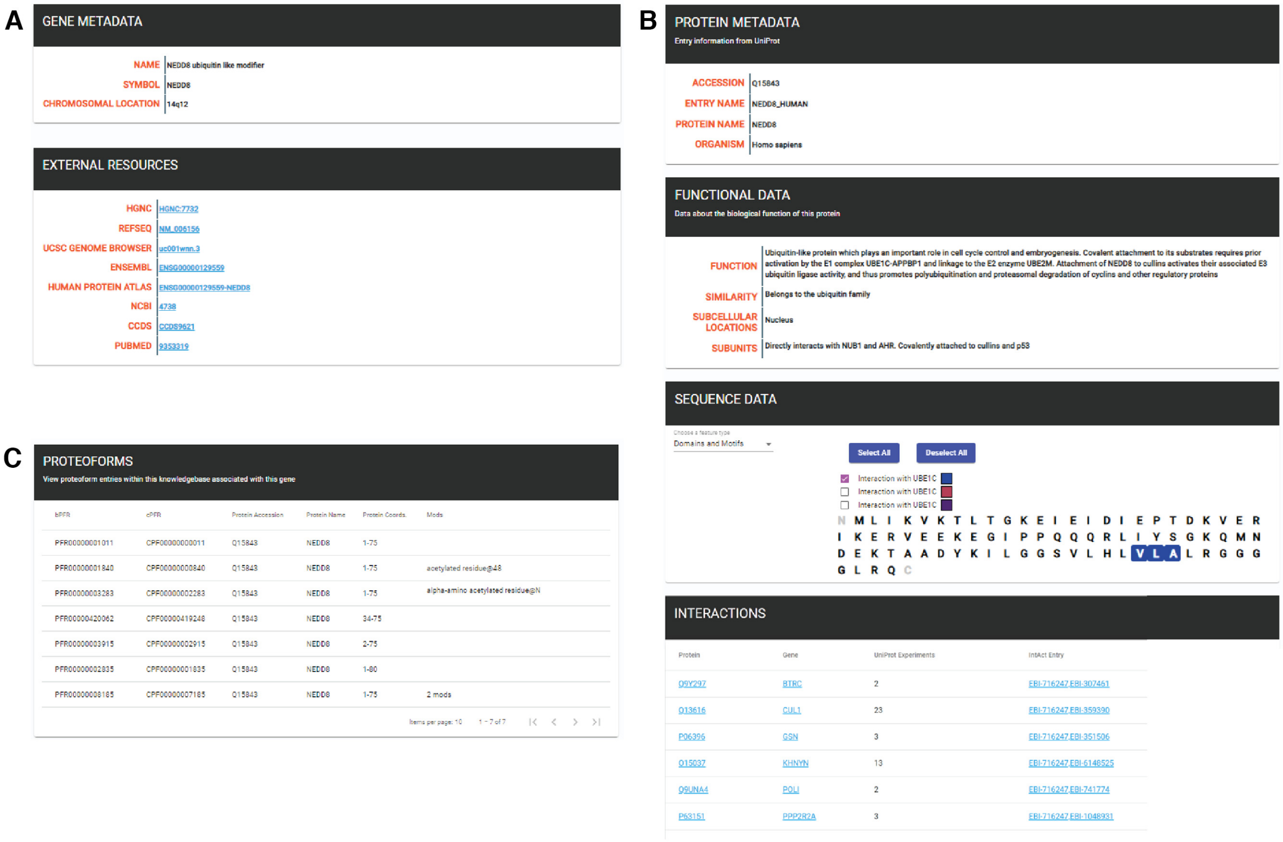
SEQUENCE DATA

Sequence data from the top spectral match

N M L I K V K T L T G K E I E I D
I E P T D K V E R I K E R V E E K
E G I P P Q Q Q R L I Y S G K Q M
N D E K T A A D Y K I L G G S V L
H L V L A L R G G C

P Score	1.342e-134
E Value	5.5e-127
Q Value	1.4039e-30
C Score	841.3
Cleavage %	67
Theoretical Monoisotopic Mass (Da)	8,555.673
Experimental Monoisotopic Mass (Da)	8,555.668
Delta Mass (Da)	0.006
Dataset	Blood Proteoform Atlas
Dataset File	LCA_11132018_APA_DS48_CD34_C1F1_1 p5E6_LONGGradient.mzid

Figure 3. Screenshots of the Human Proteoform Atlas. A proteoform page for PFR00000001011, an unmodified proteoform of the protein NEDD8.



GENE METADATA

NAME	NEDD8 ubiquitin like modifier
SYMBOL	NEDD8
CHROMOSOMAL LOCATION	14q12

EXTERNAL RESOURCES

HGNC	HGNC:7732
REFSEQ	NM_006156
UCSC GENOME BROWSER	uc001wnn.3
ENSEMBL	ENSG00000129559
HUMAN PROTEIN ATLAS	ENSG00000129559-NEDD8
NCBI	4738
CCDS	CCDS9621
PUBMED	9353319

PROTEOFORMS

View proteoform entries within this knowledgebase associated with this gene

bPFR	cPFR	Protein Accession	Protein Name	Protein Coords.	Mods
PFR00000001011	CPF00000000011	Q15843	NEDD8	1-75	
PFR00000001840	CPF00000000840	Q15843	NEDD8	1-75	acetylated residue@48
PFR00000003283	CPF00000002283	Q15843	NEDD8	1-75	alpha-amino acetylated residue@N
PFR00000420062	CPF00000419248	Q15843	NEDD8	34-75	
PFR00000003915	CPF00000002915	Q15843	NEDD8	2-75	
PFR00000002835	CPF00000001835	Q15843	NEDD8	1-80	
PFR00000008185	CPF00000007185	Q15843	NEDD8	1-75	2 mods

Items per page: 10 1 – 7 of 7 |< < > >|

PROTEIN METADATA

Entry information from UniProt

ACCESSION	Q15843
ENTRY NAME	NEDD8_HUMAN
PROTEIN NAME	NEDD8
ORGANISM	Homo sapiens

FUNCTIONAL DATA

Data about the biological function of this protein

FUNCTION	Ubiquitin-like protein which plays an important role in cell cycle control and embryogenesis. Covalent attachment to its substrates requires prior activation by the E1 complex UBE1C-APPBP1 and linkage to the E2 enzyme UBE2M. Attachment of NEDD8 to cullins activates their associated E3 ubiquitin ligase activity, and thus promotes polyubiquitination and proteasomal degradation of cyclins and other regulatory proteins
SIMILARITY	Belongs to the ubiquitin family
SUBCELLULAR LOCATIONS	Nucleus
SUBUNITS	Directly interacts with NUB1 and AHR. Covalently attached to cullins and p53

SEQUENCE DATA

Choose a feature type
Domains and Motifs ▾

Select All Deselect All

☑ Interaction with UBE1C 🟦
☐ Interaction with UBE1C 🟥
☐ Interaction with UBE1C 🟪

N M L I K V K T L T G K E I E I D I E P T D K V E R
I K E R V E E K E G I P P Q Q Q R L I Y S G K Q M N
D E K T A A D Y K I L G G S V L H L V L A L R G G G
G L R Q C

INTERACTIONS

Protein	Gene	UniProt Experiments	IntAct Entry
Q9Y297	BTRC	2	EBI-716247,EBI-307461
Q13616	CUL1	23	EBI-716247,EBI-359390
P06396	GSN	3	EBI-716247,EBI-351506
O15037	KHNYN	13	EBI-716247,EBI-6148525
Q9UNA4	POLI	2	EBI-716247,EBI-741774
P63151	PPP2R2A	3	EBI-716247,EBI-1048931

Figure 4. Three screenshots from the Human Proteoform Atlas. (**A**) A gene entry summary page for NEDD8. (**B**) The protein summary page for NEDD8 including sequence, function and interaction data from UniProt. (**C**) A display of seven proteoforms associated with the NEDD8 gene.

post-translational modifications (Figure 3). If the proteoform is from an enhanced dataset (see below), a graphical fragment map is populated and displayed, including various proteoform scores, experimental/observed masses, and dataset information.

If a user is interested in a specific gene or protein, these may be selected from the gene and protein tabs. Selecting a gene will display a summary page, including the full gene name, its symbol, chromosomal location, and various links to external resources, including but not limited to; HUGO Gene Nomenclature Committee (HGNC) (42), NCBI Reference Sequence Database (REFSEQ) (43), ENSEMBL (44), NCBI, and PubMed (Figure 4A). Selecting a protein displays a protein summary page that includes protein metadata obtained from UniProt (Figure 4B). If available, information about protein functionality, sequence data, and published interactions are also displayed, with the sequence data section displaying different features, including domains and motifs, known mutagenesis information, UniProt PTMs and their locations, and secondary structural information.

A user may also select a specific dataset. Selecting the datasets tab displays a current list of submitted datasets (Figure 5A). When selected, a summary page for a dataset is displayed, featuring gene, protein/proteoform counts, any published manuscripts associated with the dataset (including PubMed links) and whether a dataset is enhanced (Figure 5B).

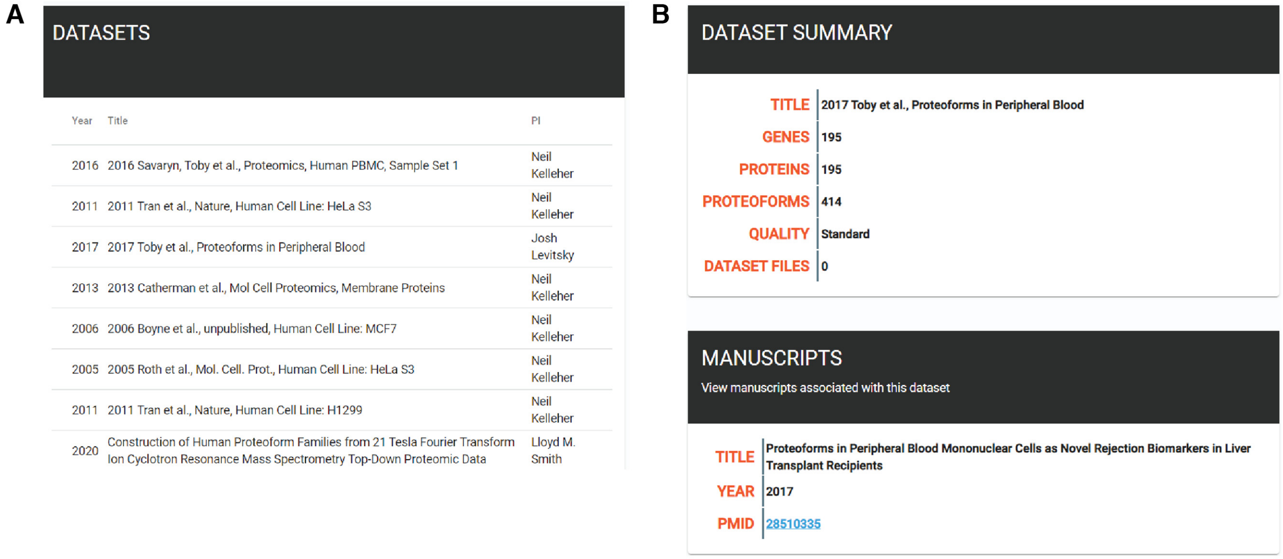
DEVELOPMENT AND IMPLEMENTATION

We developed two seperate Application Programmable Interfaces (APIs); The Proteoform Repository and the Human Proteoform Atlas (Figure 6). The former, allows the verification and registration of identified proteoforms, *via* their ProForma (41) or sequence and PTMs, and is administered by the Consortium for Top-Down Proteomics (CTDP). The HPfA API is used to generate the UI for the Human Proteoform Atlas.

The Proteoform Repository API

Proteoform (GET) requests are sent to The Proteoform Repository, and a biological and chemical proteoform record number (PFR) returned along with a validated ProForma string. In a collaboration with UniProt, regular reports of added proteoforms are included in the PTM / Processing section of associated protein entries (under Proteomic databases: TopDownProteomics). The Proteoform Repository acts as a standalone API that can be utilized outside of the Human Proteoform Atlas.

The central databases for the Proteoform Repository were created using a MariaDB v10.3 database server hosted at Northwestern University. Web services were created using Microsoft's ASP.NET 5.0 framework using C# v9.0. Documentation of the web API was created using OpenAPI 3.0 and Swagger. All APIs support only ProForma version



DATASETS

Year	Title	PI
2016	2016 Savaryn, Toby et al., Proteomics, Human PBMC, Sample Set 1	Neil Kelleher
2011	2011 Tran et al., Nature, Human Cell Line: HeLa S3	Neil Kelleher
2017	2017 Toby et al., Proteoforms in Peripheral Blood	Josh Levitsky
2013	2013 Catherman et al., Mol Cell Proteomics, Membrane Proteins	Neil Kelleher
2006	2006 Boyne et al., unpublished, Human Cell Line: MCF7	Neil Kelleher
2005	2005 Roth et al., Mol. Cell. Prot., Human Cell Line: HeLa S3	Neil Kelleher
2011	2011 Tran et al., Nature, Human Cell Line: H1299	Neil Kelleher
2020	Construction of Human Proteoform Families from 21 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Top-Down Proteomic Data	Lloyd M. Smith

DATASET SUMMARY

TITLE	2017 Toby et al., Proteoforms in Peripheral Blood
GENES	195
PROTEINS	195
PROTEOFORMS	414
QUALITY	Standard
DATASET FILES	0

MANUSCRIPTS

View manuscripts associated with this dataset

TITLE	Proteoforms in Peripheral Blood Mononuclear Cells as Novel Rejection Biomarkers in Liver Transplant Recipients
YEAR	2017
PMID	28510335

Figure 5. Screenshots from the Human Proteoform Atlas. (**A**) A list of contributed datasets. (**B**) A dataset summary page.

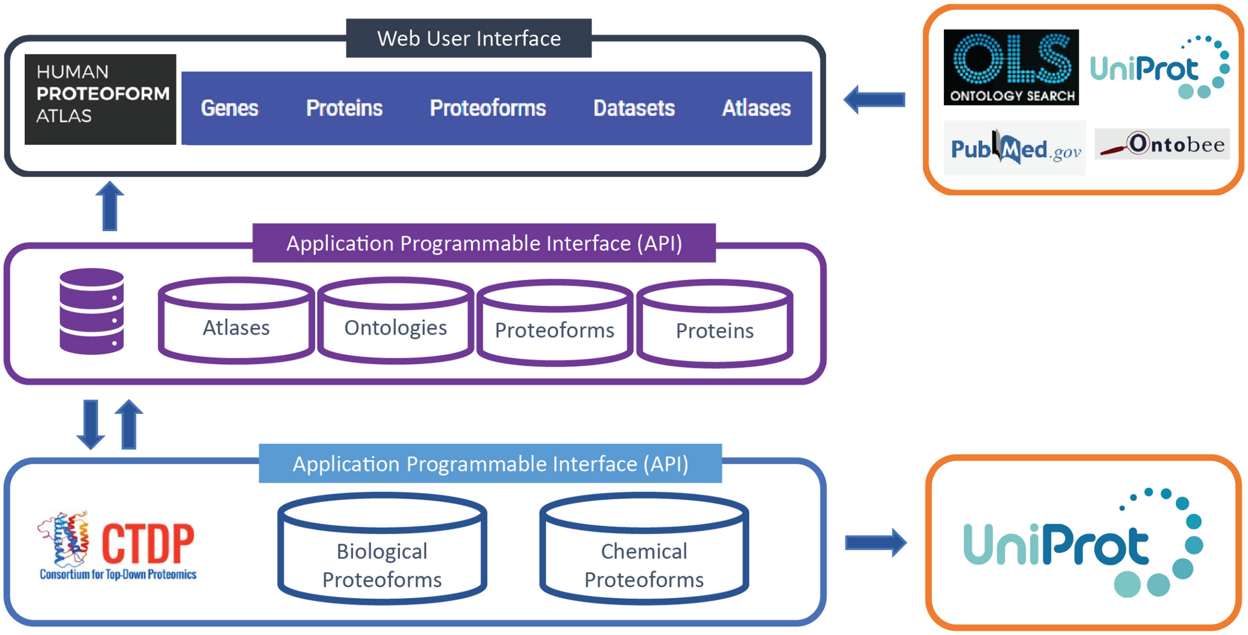


Figure 6. Technical overview of the HPfA. The arrows indicate the flow of data between the Human Proteoform Atlas API and User Interface, the Proteoform Repository API and other external databases.

2.0 and are not backward compatible with version 1.0. The PFRs generated are guaranteed to be immutable, persistent and unique by consistently processing the ProForma strings received. Each string is validated, translated to use a standard set of modifications, hashed and compared against a database of previously seen ProForma hashes (using a .NET process-level concurrency lock to prevent race conditions).

The Human Proteoform Atlas

The Human Proteoform Atlas (HPfA) consists of a web frontend and database backend established on a cloud platform (Microsoft Azure). The graphical user interface was implemented in Angular 10 using the Angular Material component library. The backend is comprised of a .NET 5 REST API, Entity Framework Core 5 for database access, and a Microsoft SQL Server database. The Human Proteoform Atlas API makes requests to The Proteoform Repository API during datasets submission. Proteoforms are validated and PFRs obtained and incorporated into the SQL database.

External database interactions

The Human Proteoform Atlas UI and backend API both interact with external APIs. The UI utilizes calls to the EBI UniProt API for protein entry information on Protein

Overview pages, the HUGO Gene Nomenclature Committee API (42) for gene information, and to the EMBL-EBI Ontology API (39) for ontology data. During dataset submission the Human Proteoform Atlas backend REST API makes calls to the EMBL-EBI Ontology API for ontology term validation.

Data collection and integration

Custom .NET 5 scripts were created to facilitate the internal loading of existing public top-down proteomic data, hand-curated from the literature. In the interest of facilitating data exchange, we have used community-generated data standards including mzIdentML (45) and ProForma for the submission of datasets. Submitted proteoforms do not require full PTM localization (modifications may have a range), corresponding to a level 3 proteoform (or less) as described in Smith *et al.* (46). Users wanting to submit their own datasets can submit by emailing nrtdp-help@northwestern.edu. Similar to the Proteomics Identification Database (PRIDE) (47), two types of dataset submission are available: standard and enhanced. The standard submission requires at least one target Atlas, a dataset name, any associated ontology terms, at least one associated publication (increasing confidence in the reported proteoforms), and a list of proteoforms as either ProForma or TopPIC strings (48). Optionally, external links to data repositories (such as PRIDE) can also be included, further increasing confidence in reported proteoforms. Enhanced submissions require mzIdentML files (which can be converted from TDReports generated in TDPortal and Thermo Fisher's ProSightPD). Each mzIdentML file may be given its own ontology terms, allowing the grouping of dataset files with different tissues/cell types into one dataset. Enhanced datasets contain spectral information, including fragment scores and coverage, allowing the creation of fragment maps.

FUTURE DIRECTIONS

Given the difficulty of housing all proteoform information from all biological domains in one central location, an open-source framework which creates a freestanding proteoform knowledgebase with the minimum functionality needed to store and display proteoforms would be desirable. This will allow other research communities to establish their own domain-specific proteoform knowledgebases. Associated with the Human Proteoform Project (49–51), proposed by the Consortium for Top-Down Proteomics, we plan to package and adapt an open-source codebase to build robust and scalable proteoform atlases of high-utility.

Currently, dataset submission is coordinated *via* email and can be cumbersome. Therefore, we plan to work with the community to create a web and/or desktop submission client, allowing ontology validation, client-side proteoform validation and ORCID integration. In addition, the National Resource for Translational and Developmental Proteomics intends to modify their tool, ProSight Lite, to take advantage of the Chemical Proteoform API such that it will be able to easily upload individual user-identified chemical proteoforms.

DATA AVAILABILITY

The Human Proteoform Atlas is a collaborative repository for human proteoforms (http://human-proteoform-atlas.org), with a publicly available API (http://api.human-proteoform-atlas.org). The Proteoform Repository is a publicly available web API that supports ProForma v2.0 submissions (http://www.proteoform.org/api). An open-source codebase is also available for the conversion of tdReports to mzIdentML files (https://github.com/NRTDP/tdReport-to-mzIdentML), and an open-source ProForma parser is available as part of the Consortium for Top-Down Proteomics' open-source software solution (https://github.com/topdownproteomics/sdk).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Conflict of interest statement. M.T.R., R.T.F. and N.L.K. contribute to the development of commercial proteomics software.

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