Progress on Identifying and Characterizing the Human Proteome: 2018-2019 Metrics from the HUPO Human Proteome Project

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PERSPECTIVE

Progress on Identifying and Characterizing the Human Proteome: 2018-2019 Metrics from the HUPO Human Proteome Project

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ABSTRACT: The Human Proteome Project (HPP) annually reports on progress made throughout the field in credibly identifying and characterizing the complete human protein parts list and making proteomics an integral part of multi-omics studies in medicine and the life sciences. NeXtProt release 2019-01-11 contains 17 694 proteins with strong protein-level evidence (PE1), compliant with HPP Guidelines for Interpretation of MS Data v2.1; these represent 89% of all 19 823 neXtProt predicted coding genes (all PE1, 2, 3, 4 proteins), up from 17 470 one year earlier. Conversely, the number of neXtProt PE2, 3, 4 proteins, termed the “missing proteins” (MPs), has been reduced from 2949 to 2129 since 2016 through efforts throughout the community, including the chromosome-centric HPP. PeptideAtlas is the source of uniformly re-analyzed raw mass spectrometry data

for neXtProt; PeptideAtlas added 495 canonical proteins between 2018 and 2019, especially from studies designed to detect hard-to-identify proteins. Meanwhile, the Human Protein Atlas has released version 18.1 with immunohistochemical evidence of expression of 17 000 proteins and survival plots as part of the Pathology Atlas. Many investigators apply multiplexed SRM-targeted proteomics for quantitation of organ-specific popular proteins in studies of various human diseases. The 19 teams of the Biology and Disease-driven B/D-HPP published a total of 160 publications in 2018, bringing proteomics to a broad array of biomedical research.

KEYWORDS: neXtProt Protein Existence metrics, missing proteins (MPs), unannotated protein existence 1 (uPE1), neXt-MP50 and CP50 Challenges, Human Proteome Project (HPP) Guidelines 3.0, Chromosomal-HPP (C-HPP), Biology & Disease-HPP (B/D-HPP), PeptideAtlas, Human Protein Atlas

Progress on the Human Proteome Parts List: neXtProt and PeptideAtlas

Since its launch in September 2010, the HUPO Human Proteome Project (HPP) has provided a productive framework for international communication, collaboration, quality assurance, guideline generation, data sharing and re-analysis, and acceleration of progress in building and utilizing proteomic knowledge globally. The HPP has 50 collaborating research teams organized by chromosome (1-22, X, Y) and mitochondria (C-HPP), biological processes and disease categories (B/D-HPP), and resource pillars for antibody-based protein localization, mass spectrometry, knowledgebases, and pathology.

Remarkable progress has been documented on the chromosome-centric human proteome parts list, as officially curated by neXtProt[1-7]. Table 1 shows the increase from 13,975 PE1 proteins in 2012 to 17,694 in release 2019-01. This PE1 total now represents 89% of the PE1, 2, 3, 4 proteins predicted to be coded in the latest version of the human genome (GRCh38). Conversely, the number of yet-to-be detected proteins, termed missing proteins, are those whose evidence is limited to transcript expression (PE2),

homology from other species (PE3), or gene models (PE4) (MPs), which have declined from 5511 in 2012 to 2129 in 2019. Splice variants, sequence variants, and some post-translational modifications (PTMs) are tabulated for each protein entry[8]. We acknowledge that neXtProt continues to carry a category PE5, which represents dubious or uncertain genes, including many pseudogenes. However, the HPP in 2013 removed PE5 from the denominator of all predicted proteins to be found[3] and excluded these from the MP-50 Challenge[7].

As shown at the bottom of Table 1, there has been a corresponding increase in the number of proteins in the human build of PeptideAtlas. PeptideAtlas provides an essential HPP function by uniformly processing raw MS data/metadata registered in ProteomeXchange through its Trans-Proteomic Pipeline (TPP)[9-10] and applying the communally-agreed HPP Guidelines 2.1 for Interpretation of Mass Spectrometry Data[11]. The number of proteins in PeptideAtlas that are assessed as canonical has increased from 12 509 to 16 293, despite the implementation of more stringent guidelines, which accounts for the observed dip between 2014 and 2016. We present the main sources of the increment of 495 canonical proteins from 2018 to 2019 in Figure 3, below.

Table 1. neXtProt Protein Evidence Levels from 2012 to 2019: Progress in Identifying PE1 Proteins[a] and PE2,3,4 Missing Proteins[b]

PE Level	2012-02	2013-09	2014-10	2016-01	2017-01	2018-01	2019-01
1: Evidence at protein level	13 975	15 646	16 491	16 518	17 008	17 470	17 694
2: Evidence at transcript level	5205	3570	2647	2290	1939	1660	1548
3: Inferred from homology	218	187	214	565	563	452	510
4: Predicted	88	87	87	94	77	74	71
5: Uncertain or dubious	*622*	*638*	*616*	*588*	*572*	*574*	*576*
Human PeptideAtlas canonical proteins	12 509	13 377	14 928	14 569	15 173	15 798	16 293

[a] PE1/PE1+2+3+4 = 17 694/19 823 = 89.3%; [b] PE 2+3+4 = 2129 "missing proteins" as of 2019-01.

In Figure 1 we account for the types of experimental evidence upon which neXtProt has classified the human proteome. Of all 17 694 PE1 proteins, 16 600 are now based on validated mass spectrometry results (Figure 1, green). Of these, 98% represent canonical proteins in the human PeptideAtlas build. In addition, 1094 PE1 proteins (yellow) were identified based upon protein-protein interactions (388), PTMs or proteolytic processing (158), disease mutations (123), Edman sequencing (90), 3D structure (50), Ab-based data (46), or other biochemical studies (239). The 2129 PE2,3,4 MPs are

comprised of 1688 with no MS data (red) plus 441 with insufficient or unconfirmed MS data (orange) that were not compliant with HPP Guidelines 2.1; the full list can be retrieved by query NXQ_00204.

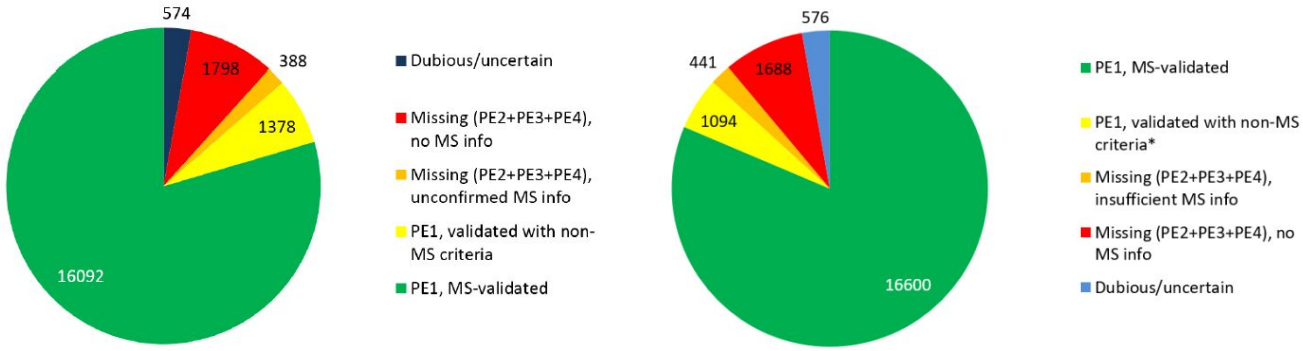

Fig. 1. Pie charts showing distribution of human proteins based on the type of protein evidence data. Shown here are the numbers of PE1 proteins (green plus yellow), PE2,3,4 MPs, and PE5 entries as of neXtProt releases 2018−01 and 2019-01.

The year-to-year changes in neXtProt HPP metrics are quite complex to track, due to such factors as revisions of total gene entries in UniProt/SwissProt, reassessment of evidence level in neXtProt upon review of additional data, and policy changes about

potential inclusion of claimed products of small open reading frames (smORFs), long non-coding RNAs, or immunoglobulin genes. The flow chart illustrating all PE level transitions from neXtProt release 2018-01 to release 2019-01 is presented in Figure 2 (right), along with the previously published transition from 2017 to 2018 for comparison (left).[7]

During 2017, 431 MPs were promoted to PE1 and 44 new SwissProt proteins were added as PE1s, while 3 PE1s were demoted to MPs and 10 PE1s were deleted. During 2018, 213 MPs and 5 PE5 entries were promoted to PE1 along with 78 new proteins, while 65 PE1s were demoted and 7 PE1s deleted. This results in a net gain of 224 PE1 proteins and a net reduction of 57 PE2, 3, 4 MPs in the HPP neXtProt baseline for 2019. Note especially that the number of MPs was inflated by 40 new entries in 2017 and 116 in 2018. Uncertainties in the reference human genome produce a significant undertow which impacts the global efforts to reduce the number of the MPs.

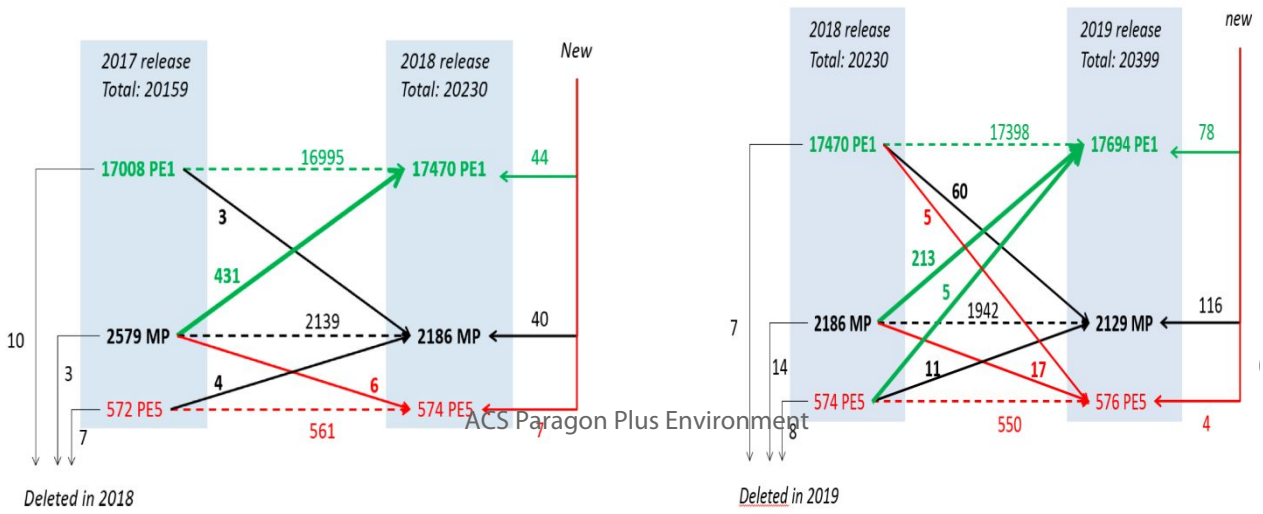

Fig 2. These flowcharts depict the changes in neXtProt PE1−5 categories from release 2017−01 to 2018−01 (left) and from 2018-01 to 2019-01 (right).

Table 2 presents a detailed chromosome-by-chromosome accounting of the status of the MPs search, elaborated from neXtProt 2019-01 [https://www.nextprot.org/about/protein-existence and ftp://ftp.nextprot.org/pub/current_release/Chr_reports/]. Also tabulated are the numbers of functionally unannotated PE1 proteins (uPE1) of the human proteome[12]. Together the PE2,3,4 MPs (NXQ_00204) and the uPE1 proteins (NXQ_00022) constitute a large part of what has been termed the "Dark Proteome" (DP)[13].

Chr	PE1	PE2	PE3	PE4	PE5	PE1-4 (total)	PE1/ PE1-4	PE2,3,4 2016/2019	uPE1 Proteins
1	1796	150	66	8	49	2020	1796/2020=88.9%	309/224	138
2	1178	71	18	1	17	1268	1178/1268=92.9%	134/90	90
3	969	76	19	3	15	1067	969/1067= 90.8%	141/98	59
4	678	46	21	2	21	747	678/747=90.8%	95/69	50
5	796	55	9	3	13	863	796/863=92.2%	122/67	52
6	983	79	12	6	30	1080	983/1080=91.0%	136/97	54
7	808	89	41	5	42	943	808/943=85.7%	137/135	50
8	602	45	14	2	39	663	602/663=90.8%	95/61	36
9	671	71	21	12	35	775	671/775=86.6%	129/104	58
10	665	60	7	1	17	733	665/733=90.7%	115/68	57
11	1014	184	95	1	39	1294	1014/1294=78.3%	319/280	72
12	932	64	14	0	23	1010	932/1010=92.3%	119/78	52
13	297	20	4	1	12	332	297/332=89.5%	43/25	27
14	613	40	56	4	14	713	613/713=90.0%	93/100	34
15	513	43	17	1	30	574	513/574=89.4%	73/61	40
16	745	58	10	2	24	815	745/815=87.7%	99/70	47
17	1049	82	13	3	22	1147	1049/1147=91.5%	148/98	65
18	250	13	1	2	10	266	250/266=94.0%	24/16	10
19	1271	104	25	2	32	1402	1271/1402=90.7%	261/131	77
20	488	43	3	5	12	539	488/539=90.5%	82/51	44
21	186	28	18	1	23	233	186/233=79.8%	49/47	9
22	432	39	5	1	21	477	432/477=90.6%	64/45	30
X	720	76	18	5	29	819	720/819=87.9%	145/99	102
Y	26	11	3	0	7	40	26/40=65%	16/14	1
Mito	15	0	0	0	0	15	15/15=100%	0/0	
Unk	2	2	0	0	0	4	2/4=50%	2/2	
ALL	**17 694**	**1548**	**510**	**71**	**576**	**19 823**	**17 694/19 823=89.3%**	**2949/2129**	**1254**
Sums	17 699	1549	510	71	576	19 829	17 699/19 829=89.3%	2950/2130	1254

NOTE: There are discrepancies between the true total numbers of proteins in each PE category and the Sums because six proteins derive from two genes on two different chromosomes, and thus appear twice under the per-chromosome table values.

Table 2. Chromosome-by-Chromosome Status of Predicted Proteins in neXtProt 2019-01

Progress from the Chromosome-centric C-HPP

The C-HPP initiated a Next-MP50 challenge at the Sun Moon Lake Workshop following the Taipei HUPO Congress in 2016. The aim was to galvanize the 24 chromosome teams to each find 50 missing proteins as a step toward completing the human proteome parts list. As shown in Table 2, that 50 MP target has been reached for Chromosomes 19, 1, 5, and 17. Chromosomes 2, 3, 10, and X have 43 to 47 net reduction of MPs. The Chr 17 team has published detailed analyses of the paths to reduce PE2,3,4 and increase PE1[14-15]. As depicted for the whole proteome in Figure 2 flowcharts, the dynamics of promoting, demoting, gaining, and deleting protein entries in neXtProt is quite complex. More detailed chromosome-by-chromosome analyses are planned for the coming year.

C-HPP investigators generated 10 articles for the 6th annual HPP Special Issue of *J Proteome Research* (2018) that reported evidence for detection and characterization of a total of 108 MPs[16]: 13 from cerebrospinal fluid[17], 1 from mesenchymal stem cells[18], 5 from olfactory epithelium[19], 1 from HeLa cells[20], 3 from mitochondria[21], 14 with the use of multiple proteases for digestion[22], 2 using LysargiNase[11] which mirrors trypsin specificity[23], 6 from embryonic stem cells[24], 30 membrane proteins[25], and 9 from other

unusual/rare/stressed tissues[26]. In addition, evidence for detection of 107 MPs was put forward from re-analyses by MassIVE[27], many of which are included in the 2019-01 PeptideAtlas build.

The limited numbers of MPs found each year by the C-HPP teams and the global proteomics community reflect the increasing difficulty in devising and executing deep discovery of MPs in the human proteome. More sensitive methods (including enrichment techniques) and targeted analyses of understudied tissues and cells are needed. Examples include organ-specific endothelium, hard connective tissues with cartilaginous structures and cortical *versus* trabecular *versus* membranous bones[28], dental tissues like dentin and odontoblasts[29], and brain regions. In embryonic tissues, limited bursts of transcription factor expression and specific hydrolases likely account for many differentiating aspects of each cell and tissue in the body. Identifying such temporally or spatially rare proteins will require dedicated searches at precise developmental windows—an ethical and practical challenge. Likewise, tissue responses to bacterial, viral, and parasitic infections, or after injury may harbor MPs key for regeneration or repair

of these specific tissues. Integration with the B/D-HPP teams in this task is highly desirable.

Meanwhile, the C-HPP initiated the neXt-CP50 challenge for the functional characterization of the dark proteome, that is, human proteins with no known or inferred function. These proteins often have antibody-based tissue distribution and intracellular localization in Human Protein Atlas, mass spectrometry-identified peptides in PeptideAtlas, and other information. At the March 2018 launch of the neXt-CP50 challenge there were 1937 PE1,2,3,4 proteins with no known function, of which 1260 were uPE1 proteins[30] (1254 in Table 2 as of 2019-01).

An interesting example of functional annotation and unusual cell types is MALT1 in B and T cells. MALT1 deficiency causes a rare immunodeficiency with ~50% reduced NFkB activation. The function of this mutant protease was uncovered by TAILS N-terminal positional proteomics. A highly selective molecular connector therapeutic compound was able to graft together two domains of the mutant MALT1 to restore molecular stability in the patient's B and T cells, rescue NFkB activation, and provide MALT1 cleavage activity in B and T cell antigen receptor activation[31]. Meanwhile, a computational approach to

predict functions of uPE1 proteins using I-TASSER and COFACTOR was introduced by the Chromosome 17 team[32] and subjected to blinded analyses of newly annotated proteins in CAFA3 and in neXtProt-2019[33].

Newly Identified Canonical Proteins in Peptide Atlas

PeptideAtlas[34-35] reprocessed and incorporated data from 120 new human sample datasets between the 2018-01 and 2019-01 builds. Not all newly deposited and released MS datasets are automatically captured and incorporated into PeptideAtlas because, with each additional dataset, the stringency applied to all data must be increased to maintain a constant false discovery rate (FDR). Thus, only datasets that are sufficiently novel are subjected to TPP reanalysis and are incorporated. These additional MS data resulted in an increase in the number of PeptideAtlas proteins for which there are at least two uniquely-mapping, non-nested 9+ amino acid peptides by 495 to a 2019-01 count of 16 293 (Table 1). Figure 3 depicts the contributions of the top 10 datasets to this increase in the number of proteins, accounting for 292 (59%) of the 495. The three datasets contributing the most canonical proteins (a total of 161) were PXD009737[36],

PXD010630[37], and PXD009840[24], all resulting from the 6th HPP special issue in this journal (2018). It is impressive that major progress has been made with membrane proteins. The PeptideAtlas reanalysis of data from the use of multiple proteases by Sun *et al*[36] yielded 73 new canonical proteins, far more than the 14 new PE1 proteins validated by synthetic peptides and proposed in their original article. Note the important distinction between new PE1 proteins and new canonical proteins in PeptideAtlas (which may already be PE1 via other means). Neither neXtProt nor PeptideAtlas requires confirmatory synthetic peptide MS results for their annual re-analyses of community MS data for the HPP. Also, it is notable that the raw data for PXD009840 were accessed at jPOST[38] along with one other new canonical protein from a second jPOST dataset. No MS datasets from iProX[39] have been included in the current PeptideAtlas or neXtProt releases, but there will be in the next release (see B/D-HPP, below). Also, PeptideAtlas does not yet incorporate SRM results.

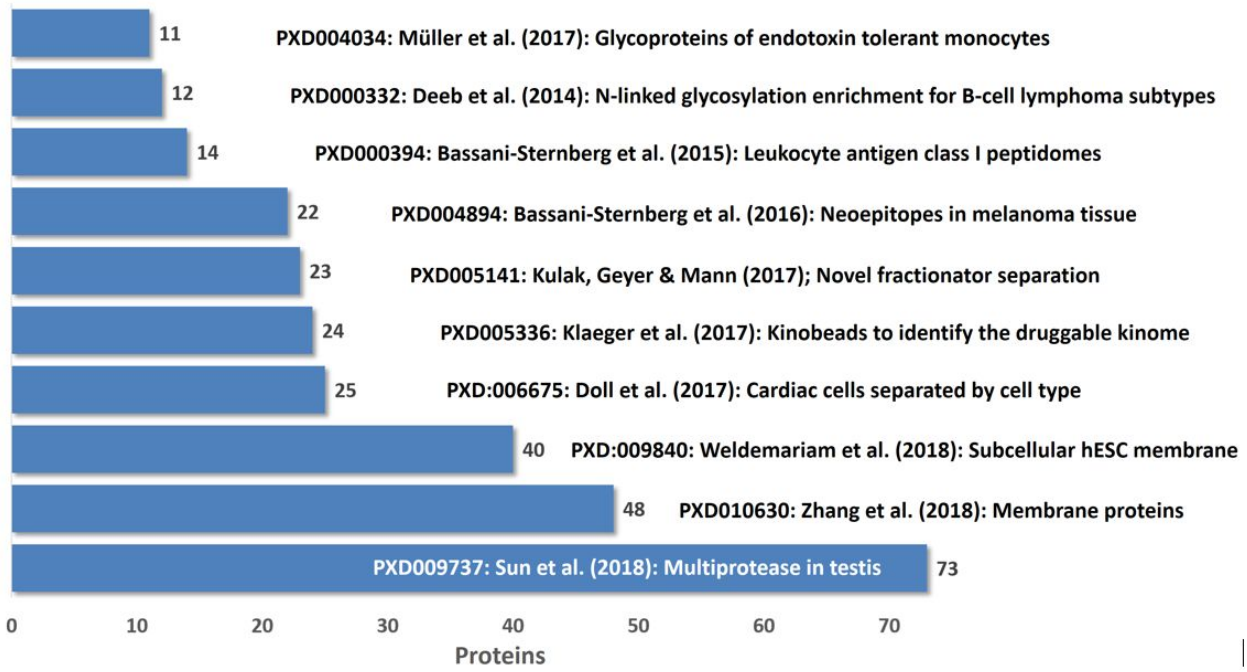

Figure 3. Top 10 newly-analyzed deposited ProteomeXchange datasets contributing to the increased number of canonical proteins found in PeptideAtlas between 2018 and 2019 builds. Each dataset is labeled with the number of new canonical proteins, ProteomeXchange identifier (PXD), reference citation[22, 25, 40-47], and methods highlighted.

From the point of view of PeptideAtlas, the progress in pursuit of a complete human proteome is depicted in Figure 4, using data from Table 1 since 2016, when the HPP Guidelines 2.0 had been applied. In green on the left are the PeptideAtlas canonical proteins, progressing to 16 293 in 2019. These proteins have at least two uniquely-mapping, non-nested, peptides of 9 or more amino acids in PeptideAtlas. In yellow in the

middle are the proteins classified as PE1 in neXtProt based on evidence from other techniques (see Figure 1 and associated text); this number has been steadily decreasing over the past four years. In red on the right are the PE2,3,4 MPs. The total has been increasing at a small rate as understanding and annotation of the human proteome improves each year. A simple extrapolation of increases in green and decreases in yellow and red (based on 2016 to 2019 data) yields a convergence of all three on the year 2026. Clearly, additional strategies need to be developed to accelerate progress to reach near-complete human proteome coverage at a stringency required by the HPP Mass Spectrometry Data Interpretation Guidelines 3.0 (see below).

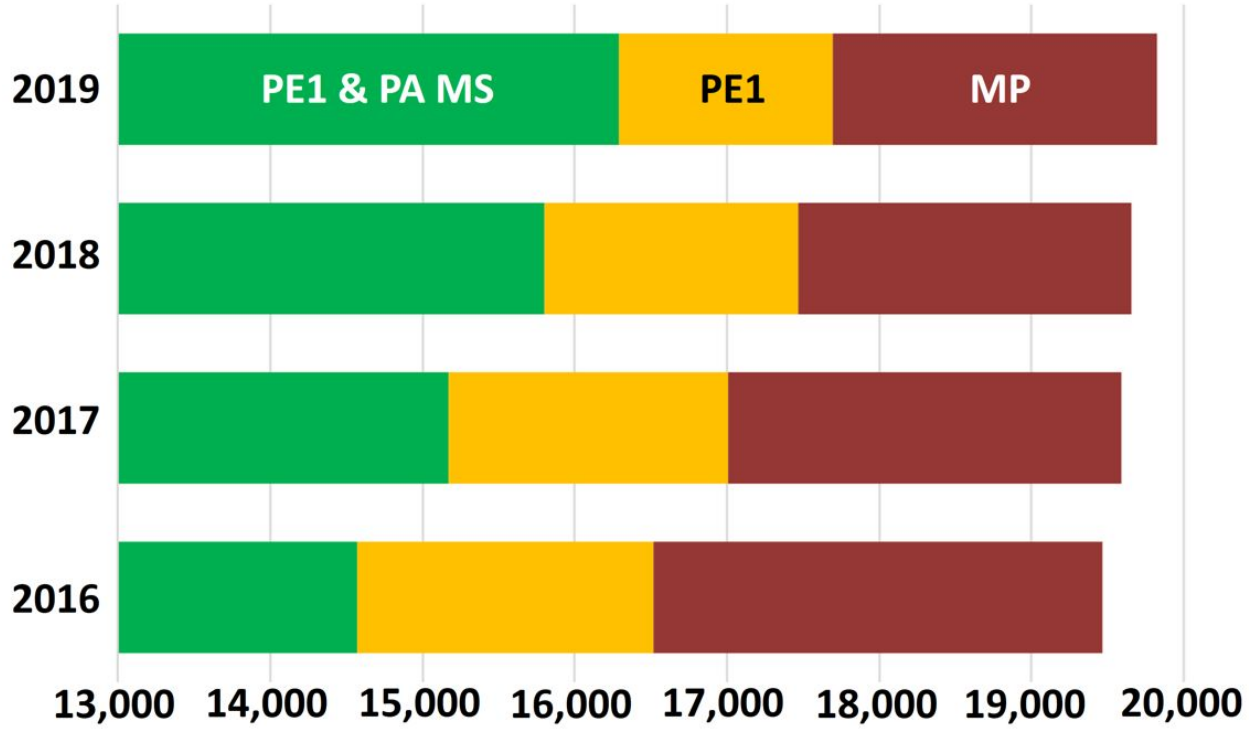

Figure 4. Summary of the evolution since 2016 of the proteome categories of PE1 proteins based on PeptideAtlas (PA) MS evidence (green), PE1 proteins based on non-MS evidence (yellow), and PE2,3,4 MPs (red). Progress has been remarkably linear since 2016.

HPP Mass Spectrometry Data Interpretation Guidelines 3.0

At a full-day workshop prior to the 21st Saint-Malo C-HPP Workshop in May 2019, the HPP leadership gathered for an extended discussion of 25 open questions from the HPP Knowledgebase Resource Pillar and other HPP teams relating to updates of the HPP

Mass Spectrometry Data Interpretation Guidelines 2.1[11] and implementation of the workflow by which the HPP confirms the translation of potential coding genes. Each of these questions was debated, potential solutions listed, and consensus decisions achieved by the participants. The result will be an update of the Guidelines from version 2.1 to version 3.0, with a manuscript[48] describing the set of questions, potential solutions, proposed consensus decisions, and the logic behind those proposals. For the checklist required for submitted manuscripts, numbered items will be re-factored into logical subgroups and authors will be required to provide page numbers on the checklist so reviewers can see where specific guidelines are addressed in the manuscripts. The term "extraordinary detection claims," which appears to have caused some confusion, will be replaced with "new PE1 protein detection claims." The revised guidelines will refine how peptide nesting is defined and specify how sequence-identical protein entries should be handled.

Two new guidelines will be added: (1) the provision of Universal Spectrum Identifiers (USI; http://psidev.info/USI), a feature developed by the HUPO Proteomics Standards Initiative (PSI) that enables the unique identification of a particular spectrum being held

up as evidence for a new PE1 protein detection claim across proteomics repositories, suitable for searching; and (2) a guideline for handling HPP datasets that use data-independent acquisition (DIA) workflows including SWATH-MS[49]. These guideline changes are expected to take effect for contributions to the 2020 JPR HPP Special Issue.

At the workshop, several changes to the overall pipeline for tracking detections of MPs were considered. The current pipeline begins with deposition to ProteomeXchange, reprocessing of datasets by PeptideAtlas, and final mapping and incorporation by neXtProt. It was agreed that there would be no substantial change to the basic set of guidelines for calling a protein successfully identified by mass spectrometry. However, the meaning of the terms *non-nested* and *uniquely-mapping* has been interpreted and implemented in slightly different ways among the different components of the pipeline. A consensus interpretation was clarified and will be documented.

For proteins that come close to meeting the guidelines, but do not meet them due to their extreme sequence composition (*e.g.,* very short or very hydrophobic), it was decided that complex exception rules are not warranted. Rather, a panel of researchers including neXtProt curators will be established to review evidence for special cases and classify

proteins as PE1 if the available evidence is compelling but falls short of the Guidelines 3.0 due to valid physiochemical reasons. No proteins would be declared too difficult to detect yet, although there was substantial discussion about the extreme difficulties of detecting olfactory receptors and other categories of membrane-bound proteins, let alone those proteins predicted to come from purported genes lacking measurable transcripts. Discussion sections of previous JPR HPP Special Issue Metrics papers have addressed these challenges[7]. Finally, a plan was initiated for incorporating the dataset reprocessing results of MassIVE-KB ProteinExplorer[27, 50], including BioPlex data (while excluding bait) through the PeptideAtlas TPP to feed into the 2020 neXtProt HPP release. Refinements to the overall HPP pipeline for tracking high stringency identification of MPs should facilitate confident completion of the attainable protein parts list over the next several years.

ProteomeXchange (PX)

The ProteomeXchange Consortium[51] was founded a decade ago by the European Bioinformatics Institute (EBI) and HUPO based on PRIDE and PeptideAtlas. As of 23

May 2019, ProteomeXchange (PX) contained 8134 publicly-released data submissions, of which 3395 are human datasets. PRIDE[52-53] (EMBL-EBI, Cambridge, UK) and PeptideAtlas (ISB, Seattle, WA, USA) are the founding members, joined now by MassIVE (UCSD, San Diego, CA, USA), jPOST (various institutions, Japan), iProX (Phoenix National Center for Protein Sciences, Beijing, China), and Panorama Public[54] (targeted proteomic datasets from Skyline, University of Washington, Seattle, USA). Each assigns ProteomeXchange identifiers (PXD) to its datasets. The PRIDE (PRoteomics IDEntification) database had 12 585 projects [https://www.ebi.ac.uk/pride/archive/]. Peptide Atlas has 1547 human data sets (120 new in 2018) with 16 303 canonical PE1-4 proteins and 2949 uncertain or redundant protein entries (www.peptideatlas.org/hupo/c-hpp/). MassIVE-KB [Mass Spectrometry Interactive Virtual Environment; https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp] comprised 9286 public datasets, with 20 116 proteins, 11M peptide variants, and 505 modifications. iProX [www.iprox.org] contained 553 projects (247 public), and 112 608 data files (14 404 added in the past year). The large dataset for the 2019 early-stage hepatocellular carcinoma paper from Jiang *et al*[55] will be included in 2020 HPP updates; this dataset was uploaded

via ProteomeXchange to PRIDE as well as to iProX. JPOSTdb [https://globe.jpostdb.org/] held 66 human, 25 mouse and 12 bacterial datasets.

Highlights of Progress from the B/D-HPP

The Biology and Disease-driven Human Proteome Project (B/D-HPP) currently encompasses the efforts of 19 independent initiatives (https://www.hupo.org/B/D-HPP). The main aims are to elucidate the molecular basis of human biology, to uncover protein drivers of human disease, and to promote the development of novel proteomics-based tools to improve the clinical management of patients. Collaborative research is conducted in close interaction with specific biomedical and clinical communities. Overall, the research work done in 2018 by B/D-HPP teams resulted in 160 published papers and participation in 82 international congresses, among which 40% were organized by scientific and clinical societies focused on specific topics, including cardiovascular, cancer, infectious diseases, gastroenterology, and nutrition. Also, there were 28 educational activities, including the first Summer School in Immunopeptidomics (https://hupo.org/B/D-HPP/).

Web tools for computational bibliometric analyses provide researchers prioritized lists of proteins in relation to particular organ systems[56] or disease categories[57], and metabolites or chemicals[58]. In the annual survey of B/D teams, 13 responded that they are using (11) or planning to use (2) such popular proteins methodology and databases in their research.

B/D-HPP teams have made substantial contributions to the elucidation of molecular mechanisms driving human disorders, moving toward "precision medicine." For example, pathological conformations of TDP-43, a nucleic acid binding protein that regulates splicing and expression of CFTR, can distinguish four pathological subtypes of frontotemporal lobar degeneration[59]. The human arterial proteome has been extensively analyzed for proteins associated with early atherosclerosis; a subset of plasma proteins emerged as efficient predictors of angiographically-defined coronary disease[60]. Biomarker identification has been pursued in urine of diabetic patients[61], peptides from mucins, fibrinogen, and collagen fragments in ~1000 cancer patients[62], and co-regulated groups of circulating proteins correlating to past, current and future disease states in 5500 individuals in Iceland[63]. Studies from the Beijing Proteome Research Center addressed

human HBV-associated HCC[64] and a mouse model of metabolic syndrome and fatty liver[65]. A major analysis of patients with early-stage hepatocellular cancers delineated three subtypes, notably S-III with high expression of sterol-O-acyl transferase (SOAT1), which is well-suited for targeted therapy, as demonstrated in xenograft models[55].

PTMs represent a functional regulatory level that is central to understanding progression of diseases and to define novel therapeutic targets. Cross-talk across different PTMs in the context of cardiovascular physiopathology has been addressed[66]. A total of 1655 proteins with 3324 oxidized cysteine sites were identified in a mouse cardiac hypertrophy model[67]. Changes in protein phosphorylation patterns have been associated with HCC[68] and ovarian tumors[69]. Protein glycosylation profiles have been explored in plasma from 300 cancer patients combining N-glycosite enrichment and SWATH; some glycoproteins, notably those related to blood platelets, are common changes across several cancers, while others are highly cancer-type specific[70]. Finally, Murray *et al*. have demonstrated how dynamic changes in protein acetylation participate in the herpes virus human cytomegalovirus replication and in the host cell defense[71].

Many efforts have been devoted to development and standardization of new proteomics-based applications. MALDI-TOF profiling has been used to discriminate different infant milk formula in pediatric clinical settings[72]. Piazza *et al* have defined protein-metabolite interaction networks and identified 1700 interactions and 7000 interaction sites, revealing principles of chemical communication, mechanisms of enzyme promiscuity, and estimates of metabolite binding at proteome-wide scale[73]. Reference proteomes have been reported for clinical studies of cerebrospinal fluid[74] and the NCI-7 cell line panel[75]. Gut microbiota have been investigated increasingly as a complex community that influences many aspects of human physiology and disease; the platform iMetaLab (http://imetalab.ca) facilitates functional studies of microbiota[76]. A key need in proteomic workflows is format and data analysis standardization; an example is the Minimal Information About an Immuno-Peptidomics Experiment (MIAIPE) guidelines[77]. Finally, an interlaboratory study to assess the performance of glycoproteomics software for automated intact N- and O-glycopeptide identification from high resolution MS/MS spectral data is ongoing.

The functional annotation of the human proteome requires a collaborative effort. Fertilization of interactions across C-HPP and B/D-HPP teams[21, 78] to share resources and knowledge will help decipher the code of life and set the basis for future molecular precision medicine.

Highlights from the Human Protein Atlas

During 2018, the Human Protein Atlas (HPA)[79] released its version 18.1 that summarizes data from 26 000 antibodies targeting proteins from 17 000 human protein-coding genes. There is continuously growing global interest with 150 000 web visitors per month. The latest update included interactive survival scatter plots as part of the Pathology Atlas[80]. The Cell Atlas[81] is being recognized in other international projects, such as the Human Cell Atlas[82], as well as deep learning and citizen science projects[83]. HPA continues to enhance its work on antibody validation[84] and on annotation of protein expression in currently under-explored tissues[85]. Fredolini *et al* have applied a magnetic bead-assisted workflow and immunoprecipitation-MS/MS to assess antibody selectivity

for enrichment and detection of 120 proteins in human EDTA-plasma; most of the antibodies co-enriched other proteins besides the intended target, due to sequence homology[86].

During 2019, the HPA plans to release three new components focusing on blood, the brain, and metabolism. In addition, HPA is combining mRNA expression data obtained from human tissues that are currently derived from different types of sequencing data; the harmonized RNA expression data will be a valuable reference resource for neXtProt Protein Evidence curation. A quantitative paired analysis of the proteome and transcriptome abundance for 29 healthy human tissues identified 13,640 proteins, but only 37 without prior protein level evidence. Hundreds of proteins were not detected even when the corresponding mRNAs were highly expressed, particularly in testis (PXD010154)[87]. These findings, and many others, will be captured for standardized reanalysis with the PeptideAtlas TransProteomicPipeline to be included in PeptideAtlas 2020-01 and incorporated into neXtProt release 2020-01.

CONCLUSIONS

The Human Proteome Project continues to provide a quality-assured framework for capturing sustained progress on the protein parts list and the characterization of the features and functions of human proteins. PeptideAtlas identified 495 new canonical proteins during the past year, including many neXtProt PE1 proteins that previously were classified based on non-MS data. NeXtProt had a net increase of 224 PE1 proteins. As of the baseline for the 2019 studies (neXtProt release 2019-01), there were still 2129 "Missing Proteins" (PE2,3,4) awaiting the application of advanced techniques and analysis of under-studied specimen types. Since 2016 there has been a surprisingly linear decrease in the number of missing proteins, which is likely to continue, though a hard core of perhaps 1000 predicted proteins may be so low in abundance, so unusual in the sites or conditions of expression, or so unsuited to detection that they will be "out of reach." We suspected that we were approaching the limit 2-3 years ago, but the pace of progress has not diminished. Over the next few years we will clarify that question while

enhancing our knowledge of the proteins and stimulating the broader and broader use of proteomics in biomedical research.

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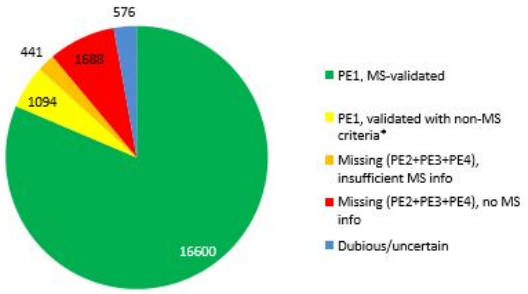
TOC graphic



Human Proteome Protein Existence Metrics (neXtProt Release 2019-01-11)