Title

PoGo: Jumping from Peptides to Genomic Loci

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Abstract

Current tools for visualization and integration of proteomics with other omics datasets are inadequate for large-scale studies and capture only basic sequence identity information. We developed PoGo for mapping peptides identified through mass spectrometry to a reference genome to overcome these limitations. PoGo exhibited superior performance over other tools on benchmarking with large-scale human tissue and cancer phosphoproteome datasets. Additionally, extended functionality enables representation of single nucleotide variants, post-translational modifications and quantitative features.

Main Text

Mass spectrometry (MS) and Next-generation sequencing (NGS) technologies have vastly improved our understanding of the cross-talk between genome, transcriptome and proteome, and contribute to a better understanding of the variations between healthy and diseases states. Examples are the identification of new therapeutic target kinases in breast cancer[1] and detection of differentially regulated pathways and functional modules potentially enabling patient stratification in ovarian cancer to inform therapeutic management.[2]

Substantial advances in MS technologies enable more complete identification and quantification of proteomes, making these data more comparable to transcriptomics. Tools like PGx[3] and iPiG[4] to readily visualize proteomics with corresponding RNA-sequencing data on a reference genome are now increasingly indispensable. While iPiG heavily relies on the annotation format used for UCSC genes, PGx uses sample specific protein sequence databases derived from RNA-sequencing experiments and corresponding genomic coordinates. Both tools require reformatting a reference genome annotation in order to enable their mapping.

We developed PoGo to allow direct mapping to reference annotations and improve speed and quality of mapping. PoGo leverages the annotated protein coding sequences (CDS) together with a reference protein sequence database (protein-DB) to map peptides to their genomic loci. Firstly, PoGo maps the genomic coordinates of CDSs onto the protein (Fig. 1),

thereby connecting the protein sequences to the genomic coordinate space. Database search tools enable peptides to be identified from MS using a protein-DB.[5] By using the PoGo-indexed database genomic coordinates of a peptide are retrieved based on the peptide's position within the protein (Fig. 1b and Online Methods). PoGo further takes advantage of distinct attribute columns of the output file formats, such as color, to indicate uniqueness of a peptide across the genome, to show positions of post-translational modifications, to allow quantitative comparison between multiple samples and conditions linking this information to transcripts and genetic loci (Fig. 2 and Online Methods). The main genome browsers, Ensembl[6], UCSC[7], and BioDalliance[8], however, have file size limits for direct upload insufficient for large-scale proteogenomics. Our track-hub generator application, therefore, enables seamless online visualization directly from PoGo output and is crucial for open access proteomics of large datasets.

We first evaluated PoGo's performance on large-scale datasets using the proteogenomic reanalysis of the draft human proteome maps.[9] We used the filtered high stringency level set comprising 233,055 unique peptides across 59 adult and fetal tissues. The mappings were derived from the gene annotation set and protein coding translation sequences for GENCODE (release 20)[9] as GTF and FASTA files. All tools were run with standard parameter settings and evaluated based on speed, memory usage and number of unique and correct mappings. PoGo (94 seconds) was 6.9 and 96.4 times faster than PGx (651 seconds) and iPiG (memory error after 9,064 seconds), respectively, and required 20% less memory compared to PGx (9.7 GB and 11.9 GB respectively). These data show a major improvement of speed and memory usage in addition to application with a readily available reference annotation.

In total 89% of mappings are common between PoGo and PGx. The 10.5% uniquely reported by PGx can be explained by shifting into the correct frame, indicating incorrect assignment. PoGo resulted in 89 completely unique mappings, 72 of these can be attributed to incompletely annotated transcripts (CDS start/end not found). Additionally, 17 unique mappings correspond to alternative splicing, immunoglobulin genes and multiple overlapping mappings in a repeat region. For example, the peptide 'VPEPGCTKVPEPGCTK' (missed cleavage between repeats of 8 amino acids) was mapped by PGx as two consecutive loci in the *SPRR3* gene (Supplementary Fig. 1). PoGo, on the other hand, mapped the sequence 4 times with the repeats overlapping each other (Fig. 1c).

The fast and diverse mapping capabilities of PoGo, as shown above, prompted the current integration of the algorithm into the PRIDE[10] tool suite and soon into the OpenMS framework[9]. This dataset also exemplifies the growing need to handle large numbers of peptides. Therefore, we have generated tissue track-hubs at two different significance thresholds from the draft human proteome maps allowing identification of genes and transcripts unique to single tissues. The scaffolding protein CASS4, for example, was only found in platelets (Supplementary Fig. 2). The genomic region of *RBP3*, only identified in retina, shows full peptide support for all splice junctions (Supplementary Fig. 3).

The large number of single nucleotide variants in individuals can affect the protein sequences and hinder identification of peptides through database searching against a reference genome.[10] Uniquely compared to other tools, PoGo is able to account for up to 2 non-synonymous variants in its mapping (Supplementary Fig. 4). Application with the draft human proteome maps allowing 1 and 2 variants resulted in a 1.5- and 60.8-fold increase in

runtime (Supplementary Fig. 5). Unique mappings to single transcripts and single genes were reduced to 94.9% and 84.1% while the number of peptides belonging to multiple genes increased exponentially by 220.9% and 3,175.2% (Supplementary Fig. 5 and 6). The mapping of additional repeats of the sequence 'VPEPGCTK' following application with mismatches were validated through identified peptides in the sample (Fig. 1c). This highlights the added value to PoGo for mapping peptides to genomic loci with potential single nucleotide variants.

To demonstrate additional PoGo functionalities we chose the phosphoproteome of high-grade serous ovarian cancer with isobaric labelling of 96 tumor samples, identifying 13,646 unique peptides with annotated phosphorylation sites (19,156 phosphopeptides).[2] PoGo mapped 13,617 peptides to 15,944 genomic loci in 66.9 seconds; these could not be mapped by PGx and iPiG. Only a small fraction, 0.2%, of the peptides could not be mapped due to sequence differences of the originating proteins between RefSeq and GENCODE databases. Compared to the other tools PoGo was able to use the annotated post-translational modifications and color code them (Supplementary Table 1) resulting in mappings for 99.8% of the phosphopeptides with their respective localized phosphorylation sites on the reference genome (Supplementary Fig. 7).

PoGo also integrates peptide quantitation with genomic loci through the GCT file format. This allows comparative visualization of multiple samples in the Integrative Genomics Viewer[11] and enables downstream quantitative analysis. The log2-fold changes of phosphopeptides between all 69 ovarian cancer samples and the pooled reference were mapped with PoGo (Supplementary Fig. 7). As an example, *MAPK3* identified with multiple phosphorylated sites in a single peptide and the associated fold changes across samples are shown in Figure 2Figure 2. To our knowledge PoGo is the only tool directly integrating quantitative information for peptides with genomic coordinates.

Our data show that PoGo represents a major advance for peptide-to-genome mapping making it a cornerstone component of proteogenomics workflows. Although the examples used here focus on human tissue and cancer cell lines, PoGo can be applied to any proteomic study for which annotation of coding sequences in GTF format and translated sequences in FASTA format are available. The additional functionalities such as allowing up to two non-synonymous single nucleotide variants, mapping of post-translational modifications and integration of quantitation distinguish it from other tools. Semi-standardized file formats commonly used in genomics for in- and output as well as the scalability for large datasets make PoGo an indispensable component of small and large-scale multi-omics studies. The current integration into the PRIDE tool suite and our track-hub generator application promote open access proteogenomics supporting studies focusing on integration of gene, protein and post-translational modifications expression[12] in the future. PoGo has been developed to cope with the rapid increase of quantitative high-resolution datasets capturing proteomes and global modifications. Integration of orthogonal genomics platforms with these datasets through PoGo will be valuable for large-scale analysis such as personal variation and precision medicine studies.

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Author Contributions

C.N.S conceived and designed the algorithms, implemented the genomic mapping algorithm, performed comparisons with other algorithms and wrote the manuscript; G.P. implemented the protein identification algorithm; A.B. and J.S.C. supervised the work and wrote the manuscript.

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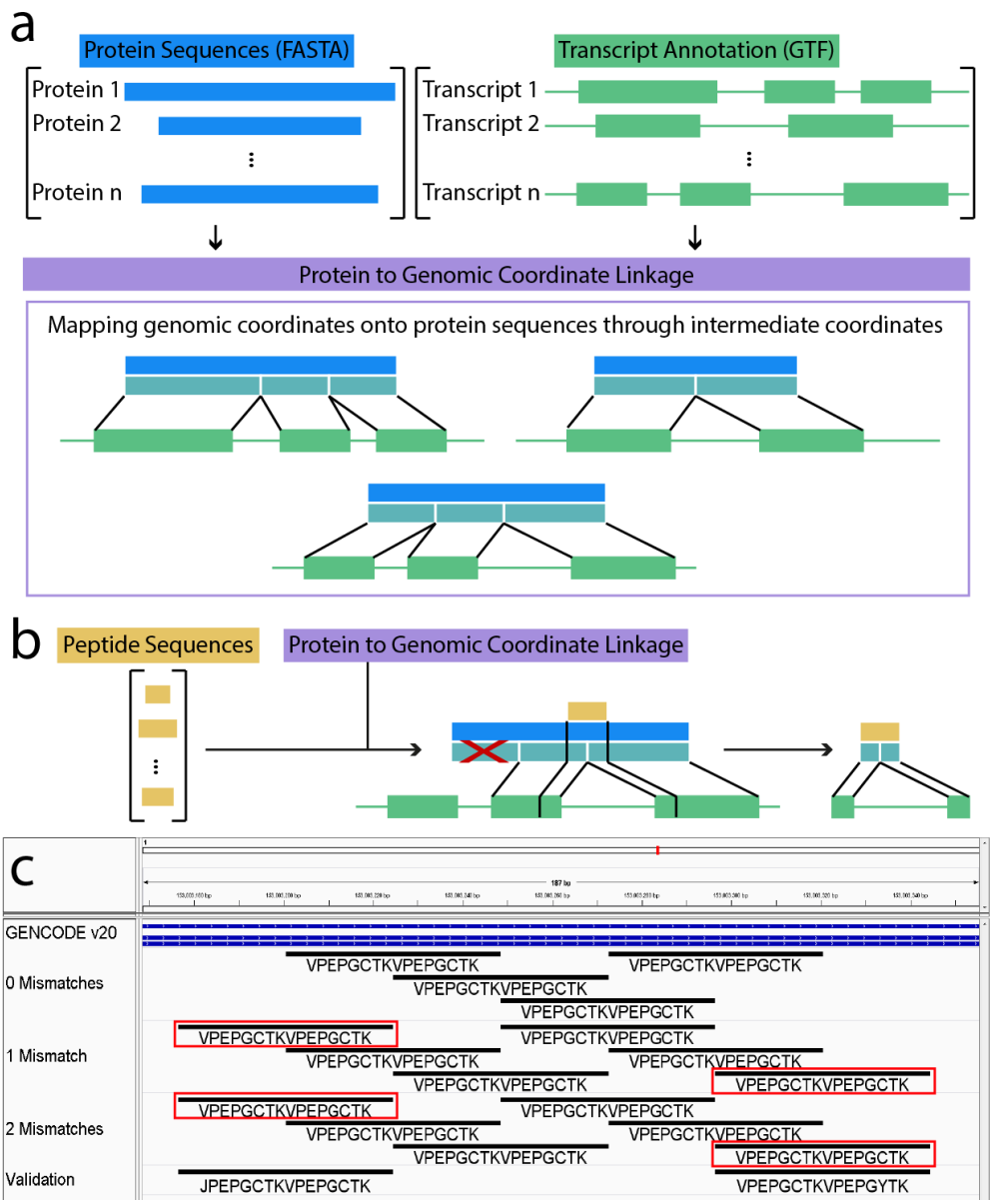
147 **Figures**



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149 **Figure 1** Schema of PoGo algorithm for mapping peptides through proteins to genomic loci.
150 **(a)** Annotated protein coding transcripts in GTF format and respective translated protein
151 sequences in FASTA format are integrated by PoGo through intermediate coordinates
152 (turquoise), representing the exonic structure of the transcript within the protein. **(b)**
153 Peptides, identified through searching mass spectrometry data against the protein sequence
154 database, are mapped against the proteins. The position within the proteins then allow
155 retrieval of overlapping coding exons and enable the calculation of the exact genomic
156 coordinates. **(c)** Example mappings of PoGo for the overlapping repeat peptide
157 'VPEPGCTKVPEPGCTK' in a genome browser (0 Mismatches). Application of PoGo allowing
158 for up to two mismatches results in identification of two additional repeats (1 and 2
159 Mismatches, red boxes) validated through mass spectrometry identified peptides of the
160 exact sequence (Validation).

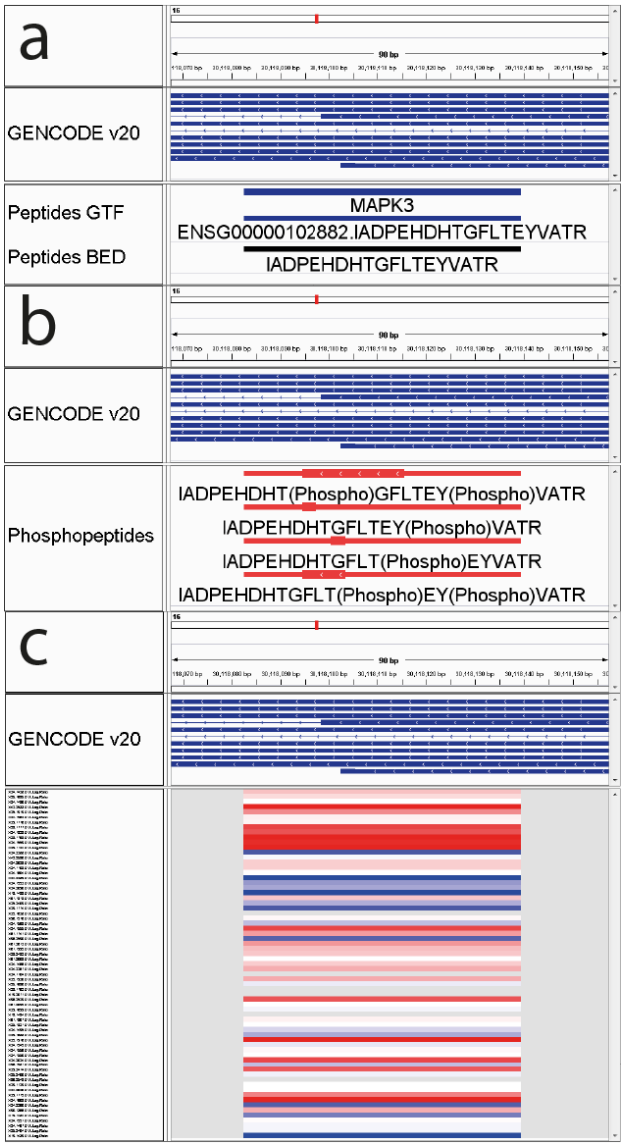

Figure 2 Visualization of different PoGo output formats for the peptide 'IADPEHDHTGFLTEYVATR' within the *MAPK3* gene. Genomic coordinates are shown at the top as x-axis. Gencode (v20) annotations of transcripts are indicated in blue. **(a)** Besides genomic location of the peptide the GTF format holds additional information, such as the gene name and gene identifier, while the BED output visualises uniqueness of the mapping across the genome. Here the black color indicates unique mapping to the gene *MAPK3*. **(b)** Genomic loci of post-translational modifications within a peptide, here phosphorylation identified through brackets in the sequence, are depicted through thick blocks spanning from the first and last modification site. The red color indicated in this output format the presence of phosphorylation. **(c)** Depiction of log2-fold changes mapped for the example peptide to the genomic location across 69 ovarian cancer samples (y-axis). High values are shown in red while blue indicates low log2-ratios.

Online Methods

Software availability. PoGo is implemented in C++. Executable files for Windows and Linux, instructions for running PoGo, and explanations for each output format and their specific visual attributes are available at http://www.sanger.ac.uk/science/tools/pogo. The source code is available through https://github.com/SangerProteomics/PoGo. The track hub generator application, instructions for running it, explanation of visual components of resulting track hubs, and a list of proteogenomic track hubs generated at the Wellcome Trust Sanger Institute are available at http://www.sanger.ac.uk/science/projects/proteogenomichubs. The perl source code is made available through https://github.com/SangerProteomics/TrackHubGenerator.

PoGo algorithm. PoGo is a multi-sample peptide-to-genome mapping tool taking as input tab delimited lists of peptides identified through mass spectrometry (MS) with associated number of peptide-to-spectrum matches (PSMs), quantitative value and sample identifier. PoGo also requires a reference genome annotation in the General Transfer Format (GTF) and translated protein coding sequences in FASTA format as input. The genomic coordinates of annotated coding sequences are mapped onto their respective protein sequences. Peptides identified through MS are then mapped against protein sequences accounting for up to two mismatches. The genomic coordinates for each peptide are calculated based on their position within the proteins. Each mapped peptide is additionally assigned the associated sample identifier as well as the number of PSMs and the quantitative value. Furthermore, post-translational modifications annotated in the peptide sequence are mapped to their respective genomic coordinates and color coded for the type of modification.

Connecting protein sequences with genomic coordinates. PoGo requires protein sequences and gene annotations in FASTA and GTF format, respectively. Protein sequences have to be connected to genes and transcripts through type specific identifiers (IDs). For each protein sequence lines from the GTF file containing the transcript ID and feature-type CDS (coding sequence) are extracted. The order of exons per transcript starts with the first exon in the sequence reflecting the reading direction during translation, regardless of the strand, resulting in a reverse order of genomic coordinates for transcripts on the reverse strand. This way protein sequences and the exons match directionality. The exonic structure is mapped onto the protein sequence through construction of protein exons. Let a transcript T be a set of exons t_1, t_2, … t_n where n is the number of exons and each exon t contains the chromosome identifier, the start and end positions within the chromosome, S_t and E_t respectively, the strand on which the transcript is annotated. The corresponding protein P is defined as a set of protein exons p_1, p_2, … p_n, where each protein exon p contains the start and end positions, s_p and e_p respectively, within the protein sequence so that the protein is mapped onto the transcript as f: $P \rightarrow T$, $p_i \rightarrow t_i$. For each protein in the FASTA file a map of protein exons to genomic exons is generated in PoGo.

To account for frame shifts between genomic exons t_i and t_{i+1} each protein exon p also holds information about the number of base pairs (bp) contributing to the codon of the first (N-term) and last (C-term) amino acid as offsets O={1,2,3}. In general, the N-term offset at the beginning of a protein defined as $O(p_1(\text{N-term}))=3$ resulting in $O(p_n(\text{C-term}))=3$ for complete annotations of coding transcripts. In instances where the annotation is missing a start or end

218 codon the offsets may vary and is identified through the annotated frame. C-term offsets
219 $O(p_i(C\text{-term}))$ for each protein exon p are calculated based on the length of the genomic
220 exon $L(t_i)$ and the offset of the N-term $O(p_i(N\text{-term}))$ so that $O(p_i(C\text{-term}))=X=L(t_i) \bmod 3-$
221 $O(p_i(N\text{-term}))+3$ with the exception $O(p_i(C\text{-term}))=X \bmod 3$ for $X>3$. N-term offsets of
222 following protein exons $O(p_{i+1}(N\text{-term}))$ are calculated so that $O(p_i(C\text{-term}))+O(p_{i+1}(N\text{-term}))$
223 $\bmod 3=0$.

224 **Identifying proteins of origin for input peptides.** To allow fast lookup of proteins containing
225 any given peptide PoGo creates a dictionary of words with length k (k-mer) overlapping by k-
226 1 amino acids from the protein sequences in the FASTA input. Associated with each k-mer is
227 a list of protein entries containing the associated protein with identifiers and the start
228 position of the k-mer in the sequence. The dictionary is designed to consider leucine and
229 isoleucine as equal as they are not distinguishable in MS. Peptides identified through MS are
230 retrieved from the input file and searched against the dictionary. Thereby PoGo allows
231 imperfect matching with up to 2 amino acid substitutions (mismatches m) to also identify
232 proteins with potentially underlying non-synonymous single nucleotide variants. For
233 peptides shorter than $(m + 1) * k$ residues only the first word of length k is used and all
234 combinations with m amino acid substitutions are generated. Each new word is looked up in
235 the dictionary. Peptides longer than $(m + 1) * k$ are split into consecutive k-mers and
236 searched in the dictionary. At most m consecutive k-mers can contain amino acid
237 substitutions leaving one word without any substitutions allowing for perfect matching in
238 the look-up table. The presence of the peptide in each found protein then is validated taking
239 into account the number of mismatches. The gene and transcript identifiers and the
240 respective start position within each protein are retrieved.

241 **Retrieving genomic coordinates for peptides.** Peptides with associated gene and transcript
242 identifiers and the start positions within each protein are used to calculate the genomic
243 coordinates. The length of the peptide sequence A with start position s_A in protein P is used
244 to calculate the end position e_A. To calculate the genomic coordinates for the peptide first
245 the overlapping protein exons p are obtained so that $P(A) = \{x \text{ e } P \mid s_x \leq s_A \leq e_x \text{ v } s_x \leq e_A \leq e_x\}$.
246 Through the mapping of protein exons to genomic exons PoGo can now retrieve the
247 genomic exons for the peptide sequence A through $P(A) \rightarrow T(A)$. The genomic coordinates
248 then are calculated as start $S_A = S_E + dS_A$ and end $E_A = S_E + dE_A$ if the gene is on the forward
249 strand or start $S_A = S_E - dS_A$ and end $E_A = S_E - dE_A$ if on the reverse strand with $dS_A = (s_A - s_P -$
250 $1) * 3 + O(P(N\text{-term}))$ and $dE_A = (e_A - s_P) * 3 + O(P(N\text{-term})) - 1$ denoting the distance of the
251 genomic start and end of the peptide, respectively, from the genomic start position S_E of the
252 genomic exon E.

253 **Mapping post-translational modifications.** Besides mapping peptides, PoGo is also capable
254 of mapping post-translational modifications (PTMs) onto the genome. Post-translational
255 modifications are commonly annotated in the peptide sequence through round brackets
256 containing the PSI (Proteomics Standards Initiative) name of the modification following the
257 modified amino acid. With the position of post-translational modifications in the peptide
258 sequence, start s_{PTM} and end e_{PTM}, the mapping of the underlying peptide to the genome the
259 above equations to calculate the genomic positions are adjusted: $dS_{PTM} = (s_A + s_{PTM} - s_P - 1) *$
260 $3 + O(P(N\text{-term}))$ and $dE_{PTM} = (s_A + e_{PTM}) * 3 + O(P(N\text{-term})) - 1$. Different types of PTMs are
261 mapped separately and color coded in the output while multiple occurrences of the same

262 PTM type, e.g. phosphorylation, within a single peptide are combined into a single mapping
263 using the first and last PTM sites.

264 **Adding quantitative information for multiple samples.** To allow visualization of quantitative
265 information for peptides on a genome, PoGo records this type of information. Peptide and
266 sample pairings may only occur once in the input file uniquely identifying a quantitation
267 value. PoGo stores the tuples of sample identifier, quantitative value and the number of
268 peptide to spectrum matches (PSMs) for each peptide. This information is used in the
269 different output formats to allow comparative analysis.

270 **Generating different output formats.** PoGo generates output in three formats commonly
271 used in genomics. The first and central output format of PoGo is BED. This format stores
272 each mapped peptide as a single line of twelve tab delimited columns. Besides chromosome
273 coordinates, the peptide sequence, strand as well as start and end coordinates of a thick
274 block the start positions and lengths of peptide blocks mapping to genomic exons are
275 included. Additionally, BED files support individual coloring of each feature. PoGo utilizes
276 this in two different forms. Firstly, in the general peptide centric output of PoGo peptides
277 are colored based on their uniqueness within the genome. Peptides unique to a single
278 transcript are colored in red while peptides shared between multiple transcripts of a single
279 gene are shown in black. Peptides mapping to multiple genes are indicated by their grey
280 color. Secondly, PoGo also generates a separate BED file for peptide forms with post-
281 translational modifications. In this instance the thick block element is used to indicate the
282 position of the post-translational modification. Two or more modifications of the same type
283 within a single peptide sequence are collapsed to indicate the range between the first and
284 last modification site. The coloring of the uniqueness per peptide in the genome is
285 substituted to accommodate color coding of post-translational modifications.

286 The second file format supported by PoGo for mapped peptides is the general transfer
287 format (GTF). PoGo redefines some of the feature types to accommodate mapping of
288 peptides. The feature type 'transcript' is used to indicate a mapped peptide while the
289 feature type 'exon' indicates the concrete mapping of the peptide to underlying genomic
290 exons. PoGo additionally stores information such as the gene identifier, name and biotype
291 for the gene as well as the number of peptide-to-spectrum matches (PSMs) and quantitative
292 values for each sample in which the peptide was identified.

293 For comparative or quantitative analysis PoGo generates the output format GCT which can
294 be visualized in the Integrative Genomics Viewer (IGV).[11] This third format is similar to a
295 matrix with rows identifying a peptide with genomic mapping and columns identifying a
296 sample. Each cell holds the quantitative values associated with the peptide and the sample
297 given in the input file.

298 **Human tissue data.** High-resolution MS data from 59 fetal and adult human tissues were
299 used for the validation of PoGo. The raw data of these draft human proteome maps were
300 generated by the Pandey lab[13], the Kuster lab[14], and Cutler lab.[15] All three datasets were
301 combined and reprocessed by Wright et al.[9] The data were retrieved in a tab delimited
302 format combining all results from mzid files available from PRIDE Archive.[10] Identifications
303 were filtered to the highest stringency level described in Wright et al.[9] for identification of
304 novel coding regions (q-value ≤ 0.01 (1% FDR), a PEP of ≤ 0.01, peptide length between 7
305 and 29 residues, full tryptic peptides, a maximum of two missed cleavages).

306 **Phosphoproteomic ovarian cancer data.** We applied PoGo to isobaric labelled
307 phosphoproteome data from an ovarian tumor study comprising 69 samples.[2]
308 Phosphopeptides with associated iTRAQ quantitation were downloaded as tab separated file
309 from https://cptac-data-portal.gorgetown.edu. Lower case characters (s, t and y) in the
310 peptide sequence showing phosphorylation were substituted by upper case characters
311 followed by the PSI name of phosphorylation in brackets.

312 **Protein sequences and gene annotation and PoGo settings.** The annotation of human genes
313 in GTF format and the corresponding protein coding sequence translation as FASTA files
314 were downloaded for GENCODE v20[9] from http://www.gencodegenes.org. Gene and
315 transcript identifiers were set as "ENSG" and "ENST" for genes and transcripts, respectively,
316 followed by 11 digits and the word length for k-mers was set to 5 amino acids. For post-
317 translational modifications 10 biologically relevant types were chosen for easy
318 discriminability of the color code (Supplementary Table 1**Error! Reference source not**
319 **found.**).

320 **Comparison of algorithms for performance evaluation.** For the human tissue and the
321 ovarian cancer phosphoproteome data PoGo's performance was compared against PGx[3]
322 (downloaded from https://github.com/FenyoLab/PGx) and iPiG[4] (downloaded from
323 https://sourceforge.net/projects/ipig/), two standalone tools available to map peptides to
324 their corresponding genomic coordinates. Each dataset was formatted using in-house scripts
325 in R and perl to fit the required input format for each tool. Each program was run using
326 default parameters and the minimum number of required input files. To compare the
327 mappings between the tools, we marked as equal when chromosome name, start and end
328 positions, the exon starts and lengths as well as the peptide sequence were the same.
329 Frameshifts then were identified amongst unique mappings per tool through shifting either
330 start or end position by up to two base pairs and comparing those to the consensus
331 mappings. Remaining unique mappings of the tools then were examined manually by
332 comparing the peptide sequence to the translated sequence of the respective genomic
333 coordinates in the IGV browser.[11]

334 **Generating track hubs.** Track hubs were generated to visualize different aspects of the
335 human proteome maps. The data was filtered to two stringency levels resulting in two sets.
336 The first result set was filtered to a standard significance (q-value of ≤ 0.01 (1% FDR), a PEP
337 of ≤0.05 and a minimum peptide length of 7 residues) while the highest stringency level
338 mentioned in Wright et al.[9] (q-value ≤ 0.01 (1% FDR), a PEP of ≤ 0.01, peptide length
339 between 7 and 29 residues, full tryptic peptides, a maximum of two missed cleavages) was
340 applied to the second set. Additionally, each set was split into subsets for individual tissues,
341 resulting in 60 files per set. PoGo was run with default parameters using the property of
342 passing a comma separated list of input files to be mapped separately. The Track-Hub
343 Generator application then was run using the 60 output files in BED format to generate two
344 track hubs; one for each significance level filter. Folders and files required for track hubs are
345 generated automatically. The script 'fetchChromSizes.sh' and tool 'bedToBigBed' from UCSC
346 (both downloaded from http://hgdownload.cse.ucsc.edu)[16] are used in the Track-Hub
347 Generator to create binary files from the original BED files used for track hubs. The
348 generated track hubs are accessible through ftp and http via
349 http://www.sanger.ac.uk/science/projects/proteogenomichubs.

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