Systematic analysis of protein turnover in primary cells

Toby Mathieson\(^1\), Holger Franken\(^1\), Jan Kosinski\(^2\), Nils Kurzawa\(^3\), Nico Zinn\(^1\), Gavain Sweetman\(^1\), Daniel Poeckel\(^1\), Vikram S. Ratnu\(^3\), Maike Schramm\(^3\), Isabelle Becher\(^3\), Michael Steidel\(^1\), Kyung-Min Noh\(^3\), Giovanna Bergamini\(^1\), Martin Beck\(^2\), Marcus Bantscheff\(^1\) & Mikhail M. Savitski\(^3\)

A better understanding of proteostasis in health and disease requires robust methods to determine protein half-lives. Here we improve the precision and accuracy of peptide ion intensity-based quantification, enabling more accurate protein turnover determination in non-dividing cells by dynamic SILAC-based proteomics. This approach allows exact determination of protein half-lives ranging from 10 to >1000 h. We identified 4000–6000 proteins in several non-dividing cell types, corresponding to 9699 unique protein identifications over the entire data set. We observed similar protein half-lives in B-cells, natural killer cells and monocytes, whereas hepatocytes and mouse embryonic neurons show substantial differences. Our data set extends and statistically validates the previous observation that subunits of protein complexes tend to have coherent turnover. Moreover, analysis of different proteasome and nuclear pore complex assemblies suggests that their turnover rate is architecture dependent. These results illustrate that our approach allows investigating protein turnover and its implications in various cell types.
Recent years have seen unprecedented progress in mass spectrometry-based proteomics. This has enabled development of various new methodologies for interrogating the proteome. These include assessment of relative protein expression, detection of protein ligand interactions, monitoring changes in the abundance of post-translational modifications, protein half-life determinations, and many others.

In order to continue improving proteome-wide characterization of protein turnover, a further development of experimental and computational work flows is required. For instance, when using dynamic SILAC (stable isotope labeling by amino acids in cell culture) to measure global protein turnover, precise and accurate peptide ion intensity quantification is needed, since even small deviations in the accuracy of measured fold changes can have a pronounced effect on the half-life measurement. In particular, when measuring protein turnover in non-dividing cells, many proteins will exhibit very slow turnover because the continuous replication of the entire proteome, which occurs in exponentially growing cells, is not required. As primary cells can only be kept in culture for a limited amount of time before adapting to the cell culture conditions or going into senescence, protein turnover determinations have to be based on relatively short-term treatments with stable isotope-encoded amino acids. Consequently, accurate and precise quantification is required in order to allow accurate determination of protein half-lives. We, therefore, developed procedures based on a better utilization of the isotopic distributions of ionized peptides to improve the accuracy and precision of peptide ion intensity-based quantification.

We applied this peptide ion intensity quantification strategy to analyze mass spectrometry data from dynamic SILAC experiments performed in five different, non-dividing cell types: B-cells, monocytes, natural killer (NK) cells, hepatocytes, and mouse embryonic neurons to calculate protein half-lives as previously described. We used this data set to validate and extend the previous observation of coherent subunit turnover of protein complexes, but also observed complex architecture-dependent protein half-life distributions.

To demonstrate the usefulness of our data as a resource, we examined some exemplifying protein complexes in more detail. In agreement with previous literature, we found that histone proteins, aside from some notable exceptions in hepatocytes, have extremely slow turnover. Both, proteasomes and nuclear pore complexes (NPCs), show a clear subcomplex-dependent turnover of their subunits. The extreme longevity of the NPC previously reported in vivo for brain tissue, is not observed for any of the cell types investigated in vitro in this study. These results emphasize that slow NPC turnover is not a general phenomenon occurring in all non-dividing cells, but that specific NPC turnover mechanisms might exist. We conclude that our data set is a useful resource for the scientific community and our method can be broadly applied in the future.

**Results**

**Improvement of peptide ion-based protein quantification.** Protein half-life determination in non-dividing cells requires precise and accurate measurement of protein fold changes. In non-dividing cells the incorporation of heavy isotope labels will be very slow for some proteins, resulting in very-low new-to-old protein ratios because only a very-small portion of the isotope has yet been incorporated. As a consequence, the ratio determination is error prone, particularly at the early time points. Such data might be stringently filtered to select for high-confidence measurements, but at the cost of coverage, specifically affecting long-lived proteins. To achieve accurate protein half-life measurements with good coverage for long-lived proteins in primary cell systems, we investigated and optimized the parameters, which are relevant for determining very reproducible and accurate protein fold changes for the greatest possible number of proteins.

We introduced two innovations into the data analysis workflow. First, we use the exact elemental composition of the identified peptides for calculating the theoretical isotopic envelope (see methods). We established that this method yields a more accurate representation of peptide ion intensities as compared to the previously used averagine model (Supplementary Figure 1) that relies on a scaled version of the average amino acid (Averagine), but does not take into account that the composition of an individual peptide might well differ from the average. Second, we used a measure to detect overlapping isotopic distributions of different peptides (see methods), to improve the quality of quantification (Supplementary Figures 1–3).

Comparison of this quantification strategy with the widely used MaxQuant software shows that our methodology performs significantly better for cases when very-low protein ratios need to be quantified, and more accurately determines the protein fold changes (Supplementary Figure 4). For proteins where the fold changes are less pronounced both softwares perform equally well. This quantification strategy is, thus, well suited for determining protein half-lives of long-lived proteins in non-dividing cells.

**Protein half-life determination.** Five different non-dividing cell types, human B-cells, monocytes, NK cells, hepatocytes, as well as mouse embryonic neurons were each labeled with medium containing 13C615N4 K and 13C615N4 R (heavy SILAC) for four different time intervals ranging between 6 and 72 h (Fig. 1; Supplementary Data 1). Taken together across all five cell types, protein half-lives were determined for a total of 9699 unique protein groups (Supplementary Data 2). The coverage in individual cell types ranged from 4667 protein groups identified in NK cells to 6534 protein groups identified in mouse neurons. To specifically quantify and cross-compare the high-quality subset of the data, we selected protein half-lives where the regression line (the rate constant of the protein degradation) fitted to fold changes at different time points had a Pearson correlation coefficient R2 of >0.85. This criterion was fulfilled by 8804 proteins taken together across the five cell types. The mean half-life was calculated for proteins present in both biological replicates for each cell type. The comparison of protein half-lives between biological replicates (Fig. 2) reveals excellent reproducibility with many proteins showing half-lives of greater than 500 h. The R2 of the log10-transformed half-lives between replicates was 0.94, 0.92, 0.91, 0.93, and 0.93 for B-cells, monocytes, NK cells, hepatocytes, and mouse embryonic neurons, respectively. For 98% of all protein half-lives from the high-quality data set, the replicates differed by less than two-fold. The acquired data set, thus, represents the most extensive, high-quality catalog of protein half-lives in primary cells.

The protein turnover in NK cells was the slowest, with 210 proteins having high quality (R2 of >0.85) half-lives longer than 500 h. The other cell types (monocytes, hepatocytes, B-cells, and mouse embryonic neurons) had, respectively, only 7, 17, 15, and 4 such proteins. Despite having an overall slower turnover rate, the relative log10 transformed half-lives between NK cells and B-cells, as well as NK cells and monocytes were in good agreement: R2 of 0.65 and 0.63, respectively. The same holds true for monocytes and B-cells: R2 of 0.56. In contrast, hepatocytes, which are not of hematopoietic lineage, showed the weakest correlation among the human cells, R2 of 0.36, 0.41, and 0.36
when compared to B-cells, NK cells, and monocytes, respectively. Half-lives determined in the mouse embryonic neurons agreed slightly better with B-cells, NK cells, and monocytes than with hepatocytes ($R^2$ of 0.422, 0.567, 0.413 compared to 0.398). We conclude that a general categorization of the proteome in terms of protein half-lives is to quite some extent preserved over all cell types investigated here.

All protein half-lives determined in this study are summarized in Supplementary Data 2. Amongst the fast turnover proteins, we find members of the Janus family of kinases. In particular Janus kinase 3, which is predominantly expressed in the cells of hematopoietic lineage has a very short half-life between 9 and 11 h (Supplementary Figure 5) in B-cells. Among the longest-lived proteins that were reproducibly observed in more than one cell type, we find the two histone family proteins: HIST1H1C and H2AFY. The average half-life in B-cells is 2242 and 971 h, respectively (Fig. 2b, c). This value goes up to 2741 and 1950 h, respectively, in NK cells. Interestingly, these histone proteins showed a relatively very-fast turnover in hepatocytes with half-lives of 18 and 51 h, respectively. Lamin-B1 has an average half-life in B-cells of 1552 h (Fig. 2d) and in NK cells of 3215 h, the half-life in hepatocytes was faster, 388 h. In mouse embryonic neurons histone HIST1H1B has the slowest turnover, with an average half-life of 1736 h, or 72 days. Our data is in good agreement with a previous study that used radioactive labeling of the long-lived cerebral histone fractions in mice and reported half-lives of 50–100 days.

We also assessed the turnover behavior within the different cellular compartments, (Supplementary Figure 6). Within each compartment a broad range of protein half-lives was observed and there is a large overlap in turnover behavior between all compartments. However, significant differences in general turnover behavior are observed between the different compartments, although the effect size is small, (Supplementary Figure 6). The most prominent and significant trend is the slower turnover of the mitochondrial proteins, which is present in all cell types. Proteins from the Golgi apparatus and nucleus had reproducibly the fastest turnover, while endoplasmic reticulum and cytoplasmic proteins were located in the middle with close to identical turnover distributions.

In summary, we observe a wide spread turnover behavior within the different cell types, with particularly long half-lives observed for histones. We also observe small, but significant and reproducible differences for protein turnover in different cellular compartments.

**Turnover of protein complexes.** Our extensive data set enabled us to assess the turnover of protein complexes on a proteome-wide scale. In an earlier protein turnover study spanning 2500 proteins using $^{15}\text{N}$-pulsed labeling in mice and mass spectrometry, Price et al. observed a coherent turnover behavior of several protein complexes in mouse liver and brain. The complex with the best protein coverage was the 20S proteasome core.
Supplementary Data 3), we observe significant differences in half-lives of the 20S core complex subunits compared to 19S regulatory complex subunits in all cell types, except for mouse embryonic neurons (Fig. 4a), which explains the more coherent turnover behavior in these cells. Interestingly, there is a significant trend for the 20S core complex to be more stable than the 19S regulatory complex in B-cells, monocytes, and NK cells, but a clear and significant opposite behavior is observed for hepatocytes. Clustering of the proteasome subunits according to the similarity between their half-lives across the human cells also leads to distinct separation between the core and regulatory subunits (Fig. 4b).

Next, we looked at the NPC, one of the largest protein complexes in the cell. This complex has been studied in rat brain by Toyama et al., also using an in vivo N labeling strategy and nucleoporins were shown to be particularly long-lived in rat brain tissue. The authors also observed that the half-life of nucleoporins correlates with their allocation in subcomplexes within the NPC. Specifically, while about 40-50% of the inner ring complex members Nup205, Nup155, and Nup93 had not turned over after 6 months, only about 10-20% of the original pool of the Y-complex members Nup160, Nup107, Nup96, and Nup85 remained detectable. To the best of our knowledge, the data set by Toyama et al. thus far accounts for the only investigation in non-dividing cells (see discussion).

In the non-dividing cells in our study, we observe a relatively quick turnover of Nups that is almost one order of magnitude faster as compared to histones (Fig. 2e, f, g; Supplementary Data 4).

Fig. 2 Assessment of protein half-lives in different cell types. a Scatter plots comparing protein half-lives determined in two experiments. On the diagonal the half-lives determined in biological replicate experiments in the same cell type are compared. Red dots indicate nuclear pore complex components. The plots below the diagonal compare the average protein half-lives determined in one cell type against other cell types as indicated. b, c, d Highly reproducible individual peptide fold changes from proteins with very-long half-lives determined from the B-cell experiment. The median protein fold changes are denoted by the slope of the line. The fold changes at the different time points are denoted by the different colors (red, 7 h; gray 11 h; green 24 h, and blue 34 h) with replicate one represented by a solid line and replicate two by a dashed line. The insets on each graph show the heavy and light SILAC intensities plotted on the same scale. b O75367 (Core histone macro-H2A.1, H2AFY) half-life from replicate one/two: 945.9/995.9 h. c P16403 (Histone H1.2, gene HIST1H1C) half-life from replicate one/two: 2168.8/2315.5 h.

b Protein NUP153 (P49790) has the shortest half-life of all the NUPs identified in B-cells with a half-life of 49.8 h in replicate one and 55.1 h in replicate two. g Protein NUP107 (P57740) has a half-life of 91.2 h in replicate one and 104.5 h in replicate two.
Fig. 3 Half-life variability among members of protein complexes is smaller than expected by chance. Distributions of standard deviations (SD) of half-lives from proteins in complexes as annotated in the CORUM database compared to SD of the half-lives of the same proteins shuffled across the different complexes, while preserving the number of proteins in each complex group. Differences in the log$_{10}$ half-lives of true protein complex members vs. the random draws of proteins in a given cell type were assessed by Wilcoxon-rank test (significance levels were encoded as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). Center line in box plots is the median, the bounds of the boxes are the 75 and 25% percentiles i.e., the interquartile range (IQR) and the whiskers correspond to the highest or lowest respective value or if the lowest or highest value is an outlier (greater than 1.5 * IQR from the bounds of the boxes) it is exactly 1.5 * IQR.

**Discussion**

In order to accurately quantify protein turnover in non-dividing cells, we introduced and validated new concepts for peptide ion intensity-based quantification. The methodological improvements are made publicly available as part of the isobarQuant package27 (https://github.com/protcode/isob/archive/1.1.0.zip), which supports all existing approaches for peptide ion intensity-based quantification, such as SILAC28, mTRAQ29, and dimethyl labeling30,31 and allows for adaptation to any type of peptide ion-based quantification strategy. In total we determine half-lives for 9699 proteins taken together from five different non-dividing cell types; B-cells, monocytes, NK cells, hepatocytes, and mouse neurons and make these publicly available as a resource to the scientific community.

In our extensive study of protein turnover in non-dividing cells, we observed very-long half-lives for histone proteins in accordance with previous in vivo work in non-dividing brain cells16 on a relative scale. Similarly, the turnover of proteasomes observed in vivo18, is well in line with our data. For NPCs, Toyama et al. observed an extreme longevity similar to histones in rat brain in vivo16. This is not true for any of the non-dividing cell types investigated in in vitro in this study, but relatively short half-lives of Nups, both on an absolute scale and relative to histones are observed. The technical details and also the biological context of both experiments are very difficult to compare, and either of them have its own benefits. The half-lives measured in non-dividing cells in vitro should yield more accurate values at the cost of losing the endogenous in vivo context, which, of course, is also important. We can nevertheless conclude that the very-slow NPC turnover is not a general phenomenon of all non-dividing cells. This might have important biological implications. Although an NPC surveillance pathway for defective NPC assembly intermediates has been described for yeast32, the turnover of fully assembled NPCs is believed to be extremely rare33 and a corresponding pathway has, to the best of our knowledge, not yet been identified. One might thus speculate that NPC-specific turnover or maintenance pathways might exist that remain to be characterized. It will thus be interesting to look out for NPC turnover in non-dividing cells in the future. One would predict that both should occasionally occur.

For both the NPC and also the proteasome, we observe protein complex architecture-dependent turnover. The 19S regulatory and the 20S core particle have entirely independent assembly pathways34, which would explain the uncoupled turnover, but also indicate that cap and core associate dynamically. An interesting pattern is observed for the beta-type subunits of the 20S core particle that are specifically integrated into immuno-proteasomes34 and show a different turnover in several cell types.
investigated here (Fig. 4a). Since it is difficult to conceive that these subunits dynamically exchange with the assembled 20S core, one might interpret this as two different populations of proteasomes that share most, but not all of their subunits and have different turnover times.

Our observations both for the proteasome and the NPC suggest that there is a trend for shorter half-lives of peripheral subunits. This is exemplified by the 19S proteasome subunits PSMD4 and ADRM1 that are clear outliers. They turn over much quicker than the rest of the 19S subunits and have nearly identical var-

Methods

Primary cell isolation and treatment. Primary human hepatocytes purchased from KaLy-Cell, kept in Williams E medium (ThermoFisher, 22168-020), supplemented with 10% dialyzed FBS and with primary hepatocyte maintenance supplement (ThermoFisher, CM4000), as well as human monococytes, B-cells, and NK cells, isolated from peripheral blood mononuclear cells, derived from buffy coats (German Red Cross, Mannheim) by magnetic-bead based negative selection (STEMCELL Technologies), and resuspended in RPMI-1640 medium (Thermo-Fisher, 21875-034) supplemented with 10% dialyzed FBS, were cultured in the presence of light (L) SILAC amino acids lysine and arginine overnight at 37 °C. The cells were then pulse-labeled with corresponding media containing heavy (H) isotope-labeled amino acids lysine, (13C6, 15N2, Sigma-Aldrich, 608041) and arginine (13C6, SigmaFisher, 88434) for the indicated time periods (Supplementary Data 1), washed, pelleted, and snap-frozen in liquid N2. The cell pellets were lysed in buffer containing 4% SDS and digested with benzonase.

Primary neuron culture. Cortical neuronal cells were isolated from prenatal embryos of CD-1 mouse at embryonic day 15 (E15). To dissociate the cortex tissue, it was finely chopped by scalp followed by digestion in Accutase (ThermoFisher, A1110501) for 12 mins. To prevent clumping due to DNA from dead cells, the tissue was treated with 250 unit/ml of Benzonase (Millipore, 72096-3). Neurons were triturated gently with a fire-polished Pasteur pipette and passed through the 40 μm cell strainer (BD Falcon, 352340) before plating them onto 6-well plate at a density of 1 × 10^6 cells per well. The plates were coated with 0.1 mg/ml of Poly-D-Lysine (Sigma, P0899) and 2.5 μg/ml of laminin (Sigma, 11243217001). Cultures were maintained in Neurobasal medium (ThermoFisher, 21100-044) containing 1% penicillin/streptomycin (ThermoFisher, 15140122), 1% GlutaMAX (ThermoFisher, 35050), and 2% B27 supplement (ThermoFisher, 12587) at 37 °C with 5% carbon dioxide in the incubator. Post-seeding after 1 day in vitro (DIV 1), half of the medium was replaced with fresh pre-warmed Neurobasal medium with all the supplements (above). On DIV 4, the neurons were treated with 1.0 μM of cytosine arabinoside (Tocris, 4520/50). On DIV 5, dynamic SILAC experiments were started by exchanging one-fifth of the medium with a final 10 x excess of heavy lysine (15C6, 15N2, Sigma-Aldrich, 608041) and heavy arginine (15C6, 15N2, ThermoFisher, 88434). The cells were harvested 0, 6, 12, 24, or 36 h after pulse, washed with PBS including protease inhibitors (Sigma-Aldrich, CO-RO Roche), and lysed in 50 mM TRIS-HCl, pH 7.4, supplemented with 4% SDS and benzozene. Lysates were cleared by centrifugation at 20,000 x g at room temperature, followed by protein concentration measurement (BCA assay, ThermoFisher, 23225). A volume of 20 μg protein of each time point were used for MS analysis.

All animal experiments were conducted under veterinarian supervision and rules of the European Molecular Biology Laboratory, following the guidelines of the European Commission, revised directive 2010/63/EU and AVMA Guidelines 2007.
THP-1 SILAC cell mixtures for method evaluation. THP-1 cell cultures (ATCC TIB-202) were established in RPMI-based SILAC media and supplemented with either light or heavy isotope-labeled amino acids (as above). For harvesting, the cells were washed, pelleted, and snap-frozen in liquid N₂. They were lysed with 4% SDS in 50 mM Tris-HEPES pH 7.4 and the DNA was digested with benzonase nuclease (Sigma, E1014-25KU). Three independent dilution series (1:1, 1:9, and 1:49) were created by mixing the 25, 5, and 1% final volume of SILAC-H to SILAC-L medium to a final volume of 50 μL.

The cell line was checked for mycoplasma contamination and authenticated using the Promega kit MycoAlert.

Sample preparation for MS. Cells were washed with PBS and the supernatant was removed completely before cells were lysed in 2% SDS for 3 min at 95 °C in athermolizer (Thermo Fisher Scientific), followed by digestion of DNA with Benzonase at 37 °C for 1.5 h. Lysate was cleared by centrifugation and the protein concentration in the supernatant was determined by BCA assay. Proteins were reduced by DTT and alkylated with iodoacetamide, separated on 4–12% NuPAGE (Invitrogen), and stained with colloidal Coomassie before proceeding to trypsin digestion and mass spectrometric analysis (see below). Gel lanes were cut into three slices covering the entire separation range (~2 cm) and subjected to in-gel digestion. Peptide extracts were additionally fractionated on an Ultimate3000 (Dionex, Sunnyvale, CA) using reversed-phase chromatography at, pH 12, 1 mm Xbridge column (Waters, Milford, MA), as previously described.

LC-MS/MS analysis. Samples were dried in vacuo and resuspended in 0.05 % trifluoroacetic acid (TFA) in water. Of the sample, 50% was injected into an Ultimate3000 nanoLISC (Dionex, Sunnyvale, CA) coupled to a Q-Exactive plus (Thermo Fisher Scientific). Peptides were trapped on a 5 mm × 300 μm C18 column (Pepmap100, 5 μm, 300 Å, Thermo Fisher Scientific) in water with 0.05% TFA at 60 °C. Separation was performed on custom 50 cm × 100 μm (ID) reversed-phase columns (Reprosil) at 55 °C. Gradient elution was performed from 2% acetonitrile to 40% acetonitrile in 61% formic acid and 3.3% DMSO over 2 h. The samples were online injected into Q-Exactive plus mass spectrometers operating with a data-dependent top 10 method. MS spectra were acquired using 70,000 resolution and an ion target of 3 × 10⁶. Higher energy collisional dissociation (HCD) scans were performed with 25% NCE at 17,300 resolution (at m/z 200), and the ion target setting was fixed at 1 × 10⁶. The instruments were operated with Tune 2.3 and Xcalibur 3.0.63

Peptide precursor intensity-based quantification. All acquired, raw data were processed using a modified-version of isobarQuant available from the Github code repository.
information about which can be found in the user manual (see supplementary information). The configuration (.cfg) files were set up for SILAC and processed as described in Pre-Mascot workflow. The .cfg file was set up for SILAC with a custom quantification method for SILAC (silac3). This method contains the mass information for LIGHT (K + 0, R + 0), MEDIUM (K + 13C6, R + 13C6), and HEAVY (K + 13C6 + N2, R + 13C6 + N2) SILAC modifications on lysine and arginine. The quantification configuration was set to use the number of peptides in each group as a single record. This single record is referred to as a SSM (proteinquant file) in Mascot and is used to calculate the protein half-lives.

### Protein inference

Protein groups were inferred from a high-quality subset of the peptide identifications made by Mascot. All of the peptides must pass the 1% (peptide) false discovery rate (FDR) criterion. In addition, at least one peptide of minimum length seven with a Mascot score difference to the next highest scoring non-identical peptide sequence of at least 10 points must be present in the group. Any groups consisting only of peptides present in other groups with higher total scores (Mascot light red peptides) were removed and all groups consisting of peptides fully subsumed by other protein groups were aggregated. Those protein groups sharing a Uniprot gene identifier were merged together (an option in isobarQuant). Peptides passing the FDR cutoff (1%) were termed unique if their peptide sequence was identified in one distinct protein group.

### Protein quantification

Unique peptides quantified in the previous part of the workflow and associated with each protein group were selected and filtered (unless otherwise stated) according to the settings given in the corresponding configuration file (proteinquant.cfg). Mascot score > 15, peptide length ≥ 6, FDR at Mascot score < 1%, maximum least squares fit of both peptides in light/heavy pair ≤ 0.1, and maximum prior ion ratio of both peptides in light/heavy pair ≤ 0.2. The remaining quantification filters in isobarQuant, related to MS2-based quantitation strategies, were not switched on (set to ‘1’). Protein fold changes were calculated using the median of all valid quantified peptide ratios (the fitted intensity of heavy SILAC to light SILAC) linked to the protein group. For protein groups where there was a minimum of one peptide with a positive ratio, any peptides with an undetermined ratio were excluded from the median calculation. The parameters described here were set as the default setting in isobarQuant and have been optimized during this investigation (see Results).

Protein fold changes determined at different time points were extracted from the output of isobarQuant and used to calculate the protein half-lives.

### Comparison between isobarQuant and MaxQuant

In order to compare quantification precision and accuracy to a widely used software, we also analyzed THP-1 data quantified and MaxQuant. Acquired data were processed with MaxQuant (Version1.5.8.0) using the settings generally recommended for SILAC quantification. Searches were performed against the same search database as above using carbamidomethylation of cysteine as a fixed modification and oxidation of methionine and acetyl (protein N terminus) as variable modifications. The mass tolerance for the precursor was 4.5 ppm and 20 ppm for the fragment ions, ‘re-quantity’ option was switched on (a separate analysis was also performed with the ‘re-quantity’ option switched off), in the section ‘group-specific parameters’ a multiplicity of two was selected, with Arg10 and Lys15 chosen as the ‘Heavy label’. A complete list of all MaxQuant settings is available in (Supplementary Data 5).

### Protein half-life determination

Protein half-lives were determined according to a modified-version of the protein decay rate method described by Schwanhäusser et al. Since we were working with non-diving cells, the cell cycle time correction component was removed from the equation to give the formula:

\[
k_{dp} = \frac{\sum_{i=1}^{n} \log(n_{i} + 1) - t_{i}}{\sum_{i=1}^{n} t_{i}^{2}}
\]

where \(k_{dp}\) is the rate constant of the protein decay, \(m\) is the number of time points \(t_{i}\) considered and \(r_{i}\) is the fold-change ratio (heavy / light) of a specific protein at each time point. The half-life of a protein \(T_{1/2}\) is then calculated by

\[
T_{1/2} = \frac{\log 2}{k_{dp}}
\]

For each protein a linear model was fitted to the time course of the logarithmic protein fold changes \[\log_{2}(r_{i} + 1)\] and the coefficient of determination \(R^{2}\) for the
linear regression was recorded (Supplementary Figure 1). The QC value was set to ‘weak’ if it was possible to determine a fold change in at least three out of the four time points, ‘good’ if the protein fold changes at three out of the four time points were based on a minimum of three quantified peptides, and ‘poor’ for the remainder.

### Analysis of half-life variability within protein complexes

All proteins identified in any of the cell types were mapped to complexes using the CORUM database. The complexes were filtered to contain at least five quantified protein members in a given cell type and the standard deviation of the $log_{10}$ transformed half-lives among the complex members was computed. In order to create a reference that indicated half-life variability as expected by chance, random representatives were drawn from the list of proteins associated with the complexes in a given cell type and placed into groups representing the different numbers of true complex members. Thus, in the end, a random group of proteins associated with a complex would have the same number of proteins as the original, true complex group. As previously the standard deviation of the $log_{10}$ transformed half-lives among these proteins within each group was computed. Differences in the $log_{10}$ half-lives of true protein complex members vs. the random draws of proteins in a given cell type were assessed by Wilcoxon-rank test (significance levels were encoded as ***, $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

### Mapping of protein half-lives on protein complex structures

For each cell type, the mean protein half-lives calculated from two replicates were mapped onto protein complex structures as a linear three-color gradient. To avoid distorting the upper half-life values for the linear interpolation were set to the 15th and 85th percentile, respectively. The half-lives outside this percentile range were clipped. The medians and percentiles were calculated as a mean of, respectively, the medians and percentiles of each biological replicate. For coloring the Nup214 complex, which is represented in the structure as a single density segment composed of three subunits, the mean half-life of the three subunits was used. All calculations were performed using half-lives with an R$^2$ of at least 0.25.

### Code availability

The isobarQuant code is freely available from the Github code repository: https://github.com/proteome/isobarchive/1.1.0.zip

### Data Availability

All mass spectrum data (571 raw files) and their corresponding Mascot search files have been uploaded to the Proteome Identifications database (PRIDE) at http://www.ebi.ac.uk/pride/, and are under accession numbers PXD008511 (B-cells), PXD008512 (hepatocytes), PXD008513 (monocytes), PXD008514 (mouse neurons), PXD008515 (NK cells) and PXD008516 (TH1-1 cell). All other data are available from the corresponding authors upon request.

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Author contributions

T.M., M.Ba. and M.M.S. conceived the project; D.P., G.B., M.Ba. and M.M.S. designed the experiments; N.Z., D.P., V.S.R., M.Sc., I.B., M.St. and K.–M.N. conducted and supervised experiments; T.M., G.S. and H.F. developed the software; T.M., H.F., J.K., N.K., M.Be., M.Ba. and M.M.S. performed data analysis; H.F., N.K. and J.K. contributed to the manuscript; and T.M., M.Be., M.Ba. and M.M.S. wrote the manuscript.

Additional information

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