

Benchmarking accuracy and precision of intensity-based absolute quantification of protein abundances in *Saccharomyces cerevisiae*:
Supplementary material

BJ Sanchez, PJ Lahtvee, K Campbell, S Kasvandik, R Yu, I Domenzain, A Zelezniak and J Nielsen

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1 Summary

Here we will go through the typical way of deducing protein abundances [fmol/ μ g protein] from SILAC/iBAQ data, and compare it to rescaling values to a fix total protein abundance based on MS intensities, to assess the usefulness of the external standard curve and iBAQ data. The main observation that comes from this is that as MS measurements are so variable, it's impossible to find a unique external standard (ES) curve, hence normalizing to a fixed total protein abundance is as good as using the "optimal" fit from the ES curve. We can then use the MS intensity directly and bypass the ES curve, the iBAQ values and the internal standard.

2 Additional experimental details

2.1 Cell lysis and sample preparation for LC/MS/MS

Cell pellets were suspended in lysis buffer consisting of 6 M guanidine HCl, 100 mM Tris-HCl pH 8.0, 20 mM dithiothreitol (DTT), heated at 95°C for 10 min and sonicated with Bioruptor (Diagenode, Denville, NJ, USA) sonication (15 min, "High" setting). Samples were further homogenized by FastPrep24 (MP Biomedicals, Santa Ana, CA, USA) bead beating device 2x at 4 m/s for 30 s with cooling between cycles. After removal of beads, the samples were precleared with centrifugation at 17,000 g for 10 min at 4°C. Aliquots of samples were precipitated overnight with 10% trichloroacetic acid (TCA) at 4°C and used for protein concentration measurement with the Micro BCA™ Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). For absolute quantification of the internal standard (IS), 1.1 μ g of Proteomics Dynamic Range Standard Set (UPS2, Sigma Aldrich) (ES) was mixed with 6 μ g of heavy-labelled yeast grown in previously described minimal medium supplemented with $^{15}\text{N}_2^{13}\text{C}_6$ -labelled lysine (Lys8) (Cambridge Isotope Laboratories, Tewksbury, MA, USA). The biological replicate samples were spiked in 1:1 ratio with the IS. Next, samples were precipitated with 10% TCA and suspended in 7:2 M urea:thiourea, 100 mM ammonium bicarbonate (ABC) buffer. After reduction with 5 mM dithiothreitol and alkylation with 10 mM chloroacetamide, samples were digested for 4 h at room temperature with 1:50 (enzyme to protein) Achromobacter lyticus Lys-C (Wako Pure Chemical Industries, Osaka, Japan). Solutions were diluted 5x with 100 mM ABC and further digested overnight at room temperature. Peptides were desalted using in-house made C18 (3M Empore, Maplewood, MO, USA) tips and reconstituted in 0.5% trifluoroacetic acid (TFA).

2.2 Nano-LC/MS/MS analysis

Peptide samples were injected to an Ultimate 3000 RSLC nano system (Dionex, Sunnyvale, CA, USA) using a 0.3 \times 5 mm trap-column (5 μ m C18 particles, Dionex) and an in-house packed (3 μ m C18 100 Å, Dr Maisch, Ammerbuch, Germany) analytical 50 cm \times 75 μ m emitter-column (New Objective, Woburn, MA, USA). Peptides were eluted at 250 nL/min with an 8-40% (4 h) A to B gradient (buffer A: 0.1% (v/v) formic acid; buffer B: 80% (v/v) acetonitrile + 0.1% (v/v) formic acid) to a quadrupole-orbitrap Q Exactive Plus (Thermo Fisher Scientific) MS/MS via a nano-electrospray source (positive mode, spray voltage of 2.5 kV). The MS was operated with a top-5 or top-10 data-dependent acquisition strategy. Briefly, one 350-1,400 m/z MS scan at a resolution setting of $R = 70,000$ was followed by higher-energy collisional dissociation fragmentation (normalized collision energy of 26) of the 5 or 10 most intense ions (z : +2 to +6) at $R = 17,500$. MS and MS/MS ion target values were 3,000,000 and 50,000 ions with 50 and 100 ms injection times, respectively. Dynamic exclusion was limited to 70 s.

2.3 Mass-spectrometric raw data identification and quantification

Raw data were identified and quantified with the MaxQuant 1.4.0.8 software package (Tyanova, Temu, and Cox 2016). For samples spiked with the heavy IS, the labelling state (*multiplicity*) was set to 2, and Lys8 was defined as the heavy label. Methionine oxidation, asparagine/glutamine deamidation and protein N-terminal

acetylation were set as variable modifications, and cysteine carbamidomethylation was defined as a fixed modification. Search was performed against the UniProt (www.uniprot.org) *S. cerevisiae* S288C reference proteome database (version from July 2016), with this reference genome being selected over CEN.PK due to better annotation. This was performed using the LysC/P digestion rule. Only protein identifications with a minimum of 1 peptide of 7 amino acids long were accepted, and transfer of peptide identifications between runs was enabled. Signal integration (re-quantification) of missing label channels was enabled. Protein quantification was reported when at least one peptide had been quantified (i.e. the protein minimum ratio count was set to 1). Peptide-spectrum match, peptide and protein false discovery rate (FDR) were kept below 1% using a target-decoy approach. All other parameters were default.

For absolute quantification of the heavy IS with the ES, the iBAQ feature (Schwanhäusser et al. 2011) of MaxQuant was enabled. Briefly, this function normalizes protein intensities by the number of theoretically observable peptides and enables rough intra-sample estimation of protein abundance. The UPS2 proteins \log_{10} iBAQ intensities are then plotted against \log_{10} quantities of the UPS2 proteins. This regression is then used to derive absolute quantities of all other proteins in the IS.

3 Loading and pre-processing data

3.1 iBAQ data

ES: The commercial kit brings 10.6 ug of protein, however only 1.1 ug is injected in the MS.

In the iBAQ data (IS+ES) there are 6 samples: measured in 3 batches (`batch1`, `batch2` & `batch3`) and each of them processed with 2 different MS methods `top5` & `top10`. We will have then 6 different ES curves:

- `top5_batch1`
- `top5_batch2`
- `top5_batch3`
- `top10_batch1`
- `top10_batch2`
- `top10_batch3`

In each sample of the iBAQ data there are:

- 6 ug of IS: yeast samples, all marked; i.e. will appear in the heavy fraction (H)
- 1.1 ug of ES: universal protein standard (UPS2) unmarked; i.e. will appear in the light fraction (L)

3.2 SILAC data

There are 18 different samples:

- 3 biological replicates (`R1`, `R2` & `R3`)
- each measured on 3 different batches (`batch1`, `batch2` & `batch3`)
- each estimated with a different MS method (`top5` & `top10`)

Each injected sample consisted of:

- 6 ug of IS (detected in the H fraction)
- 6 ug of actual sample (detected in the L fraction)

3.3 Other data

Number of theoretical peptides: We can obtain the number of theoretical peptides for each of the proteins if we remove the UPS2 sequences and label from the MaxQuant search, which leads the software to report only the iBAQ intensities of the proteins. We then divide the total raw intensity with the total iBAQ intensity to get the desired number, and merge this information with the ES, IS and sample data.

Peptide data: Will be used later for misc checks at the peptide level.

Ribosomal proteins: We used a list of ribosomal genes based on previous work (Jenner et al. 2012).

4 Methods evaluated

Method 1: iBAQ

Method 1 uses the computed iBAQ abundances available in the MaxQuant (Tyanova, Temu, and Cox 2016) output file, which are inferred using an ES curve of the UPS2 proteins (in the L fraction) (Schwanhäusser et al. 2011). As the data comes in fmol/sample, the only steps missing are to convert all values to fmol/ μ g protein (by dividing by the sample mass), and then use the values from the IS (H fraction) together with the normalized L/H ratios in the SILAC data for getting absolute abundances in each sample of the SILAC data (fmol/ μ g protein), by doing:

```
abundance(sample) = (L/H)ratio * abundance(IS)
```

Method 2: Rescaling iBAQ values

As iBAQ values don't add up always to the total injected protein mass (Figure S1), we should assess the benefits of rescaling all of these values to add up to the injected amounts:

```
abundance = (iBAQ abundance)*(injected amount)/(sum of all iBAQ abundances*MW values)
```

Method 3: TPA

If we are rescaling the iBAQ values in method 2, we can alternatively consider skipping iBAQ values and ES curves entirely, and assume that all MS intensities summed up together (mass-wise) are proportional to the injected amount in ug. This is known as the total protein approach (TPA) (Wiśniewski and Rakus 2014):

```
abundance = (MS intensity)*(injected amount)/(sum of all MS intensities*MW values)
```

This can be performed separately for the ES, the IS and the samples.

Method 4: Normalized TPA

We will also try out to first normalize all MS intensitiy values by the corresponding number of theoretical peptides, to later rescale the data as before (Wiśniewski et al. 2012):

```
abundance = (normalized MS intensity)*(injected amount)/(sum of all normalized MS intensities*MW values)
```

Note that for methods 3 and 4 we have essentially created a linear model:

```
abundance = m*intensity, where m = 1/(sum of intensities*MWs)
```

```
log(abundance) = log(intensity) + log(m) -> linear model with a = 1 and b = log(m) in the log space.
```

5 Method comparison

5.1 Protein totals

First, let's take a look at the total detected protein amount in each of the 6 samples of IS, for all methods:

```
## [1] "Method 1: iBAQ - 3497 proteins detected - 16.9% variation"
## [1] "Method 2: iBAQ rescaled - 3497 proteins detected - 0% variation"
## [1] "Method 3: TPA - 3534 proteins detected - 0% variation"
## [1] "Method 4: TPA normalized - 2923 proteins detected - 0% variation"
```

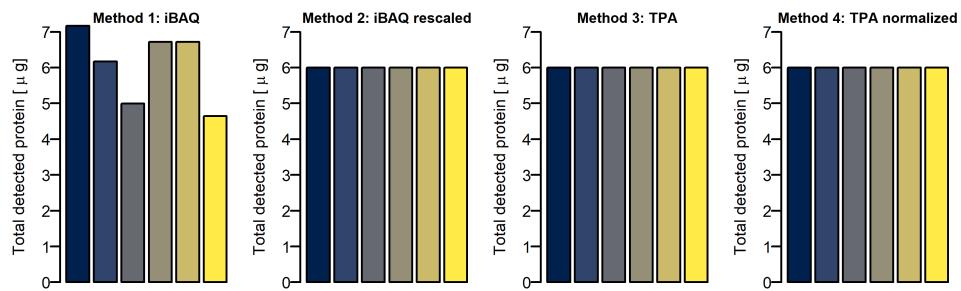


Figure S1: Total detected protein amounts in all 6 IS, according to all methods.

Let's also take a look at the total detected protein amount of each of the 18 samples, colored by the original ES curve used for the calibration (Figure S1):

```
## [1] "Method 1: iBAQ - 1587 proteins detected - 15.4% variation"
## [1] "Method 2: iBAQ rescaled - 1587 proteins detected - 0% variation"
## [1] "Method 3: TPA - 1726 proteins detected - 0% variation"
## [1] "Method 4: TPA normalized - 1488 proteins detected - 0% variation"
```

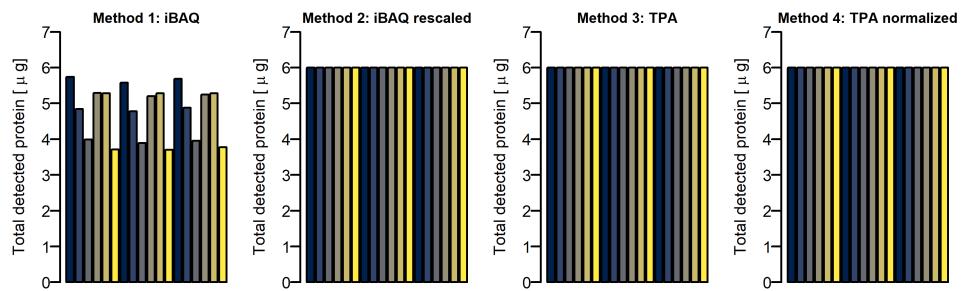


Figure S2: Total detected protein amounts in all 18 samples, according to all methods.

Both in figures S1 and S2, the total detected protein varies considerably among samples calculated with method 1 (a coefficient of variation of ~16%), and as expected the variation goes to zero if we rescale (methods 2, 3 and 4). Note that for method 1, the total amount of protein detected in the samples is lower than the amount detected in the internal standard, due to more proteins detected in the internal standard (as the latter is a mix of different conditions and not just one sample).

Note as well that the coverage of method 4 is lower than methods 1, 2 and 3. This appears as a limitation of method 4, but it is actually a limitation of the MaxQuant software, which does not provide as an output the number of theoretical peptides for each protein, which led us to have to infer them as described in section ???. In fact methods 1 and 2 also employ the number of theoretical peptides, so this would not be a limitation if choosing other software. In any case, the coverage decrease is overall low for the proteins later analyzed: When analyzing accuracy (section 5.2) we only lost one value when comparing UPS2 values (167 → 166), no values when comparing ribosome stoichiometry in the IS (312 → 312) and we gained 19 values when comparing ribosome stoichiometry in the samples (731 → 750). On the other hand, When analyzing precision (section 5.3.1), we lost 4.5% of values (14,858 → 14,182) in the IS data, but gained 6.3% of values (21,320 → 22,657) in the sample data.

5.2 Evaluating accuracy

Let's now compare accuracy. First, we compare the ES values predicted by each method to the actual UPS2 values:

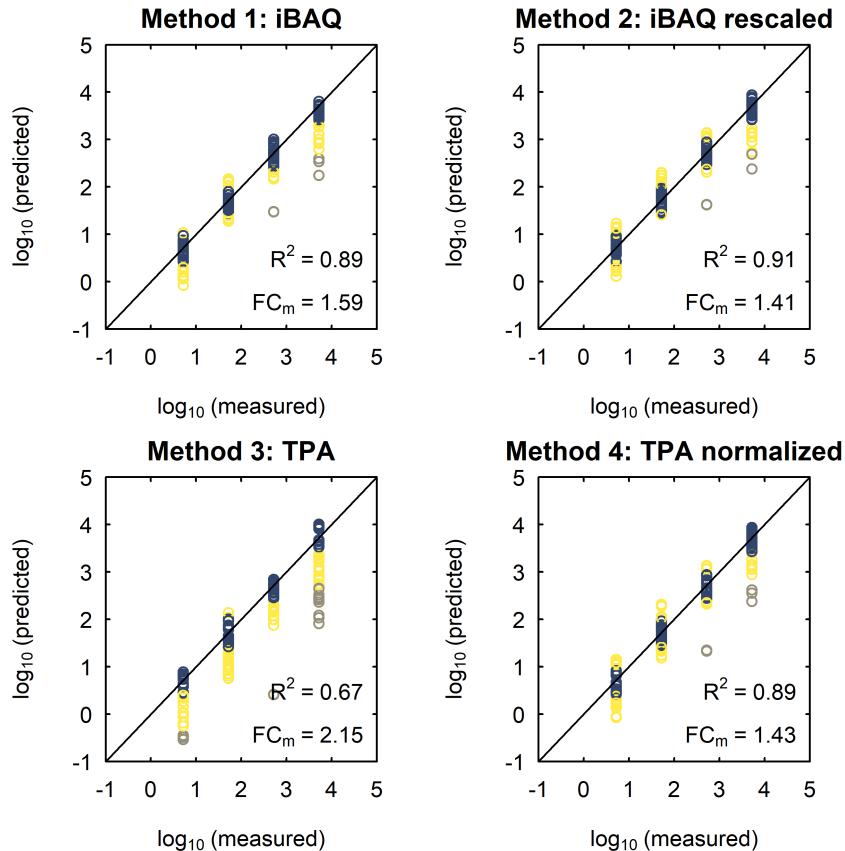


Figure S3: Comparison of predicted Vs real abundance values [fmol/µg protein] from UPS2, according to all methods. Blue is a FC lower than 2, yellow between 2 and 10, and gray over 10.

We see that predictions from methods 1, 2 and 4 are similar; by using ES curves (methods 1-2) we don't

gain much prediction power than if we simply rescale the normalized data (method 4). However, method 3 performs significantly worse.

Now, let's see how are the predictions of ribosomal subunit stoichiometry in the 6 measurements of the internal standard, as they should all be the same, being that a) they all belong to the same sample, and b) all ribosomal proteins are expressed in a 1:1 ratio. For this, first we need to create dataframes with only ribosomal proteins, and then plot for each method the corresponding data:

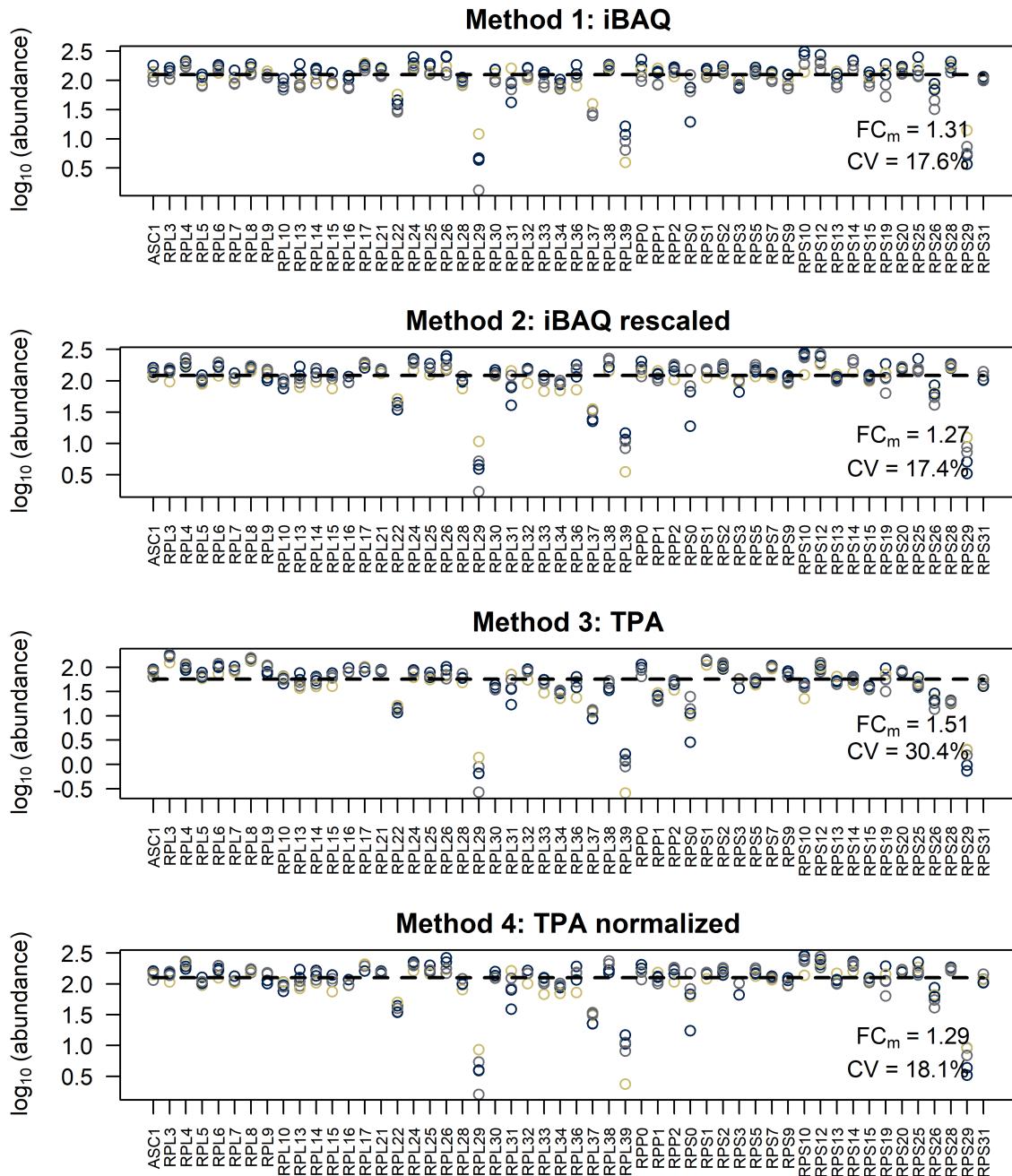


Figure S4: Predicted ribosomal subunit abundances [fmol/µg protein] in the internal standard, by each method. Colors correspond to different technical replicates. The median value is displayed with a segmented line, and both the median fold change to that line and the coefficient of variation for all data are displayed.

These distributions are not very different between them (with exception of method 3), as we can see in the cumulative distributions (Figure S5).

```

## [1] "UPS2 error - number of FC compared = 167"
## [1] "UPS2 error - number of FC compared = 167"
## [1] "UPS2 error - number of FC compared = 167"
## [1] "UPS2 error - number of FC compared = 166"
## [1] "UPS2 error of iBAQ = iBAQrescaled: p-val = 0.0130003208923511"
## [1] "UPS2 error of iBAQ = TPA: p-val = 6.30399116685609e-07"
## [1] "UPS2 error of iBAQ = TPAnorm: p-val = 0.161194686406416"
## [1] "UPS2 error of iBAQrescaled = TPA: p-val = 1.77198722361993e-09"
## [1] "UPS2 error of iBAQrescaled = TPAnorm: p-val = 0.746934008834625"
## [1] "UPS2 error of TPA = TPAnorm: p-val = 4.42783796072987e-07"

## [1] "Ribosomal error - number of FC compared = 312"
## [1] "Ribosomal error - number of FC compared = 312"
## [1] "Ribosomal error - number of FC compared = 312"
## [1] "Ribosomal error - number of FC compared = 312"
## [1] "Ribosomal error of iBAQ = iBAQrescaled: p-val = 0.193148716047241"
## [1] "Ribosomal error of iBAQ = TPA: p-val = 3.02259165252394e-07"
## [1] "Ribosomal error of iBAQ = TPAnorm: p-val = 0.314460419659206"
## [1] "Ribosomal error of iBAQrescaled = TPA: p-val = 1.01396668839016e-10"
## [1] "Ribosomal error of iBAQrescaled = TPAnorm: p-val = 0.863645796682843"
## [1] "Ribosomal error of TPA = TPAnorm: p-val = 1.47339151990167e-09"

```

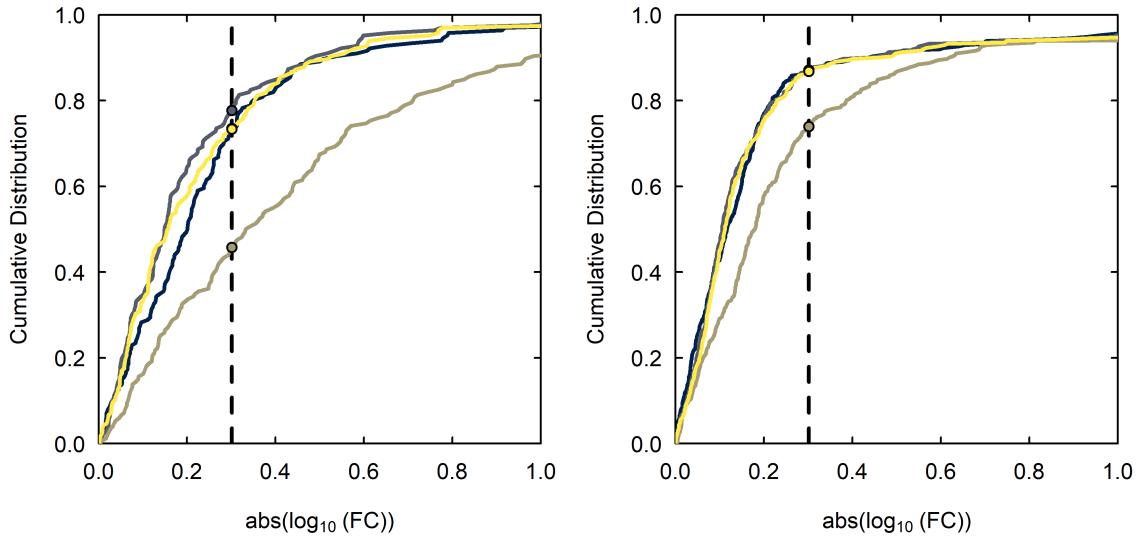


Figure S5: Cumulative distributions of absolute fold changes for both accuracy evaluation metrics: differences of predicted Vs experimental values of UPS2 (left) and differences to median value in ribosomal measurements of IS (right). A fold change of 2 is indicated with a vertical segmented line. Colors represent the methods: 1) iBAQ (blue), 2) iBAQ rescaled (gray), 3) TPA (brown) and 4) TPA normalized (yellow).

We can confirm this for the 18 samples:

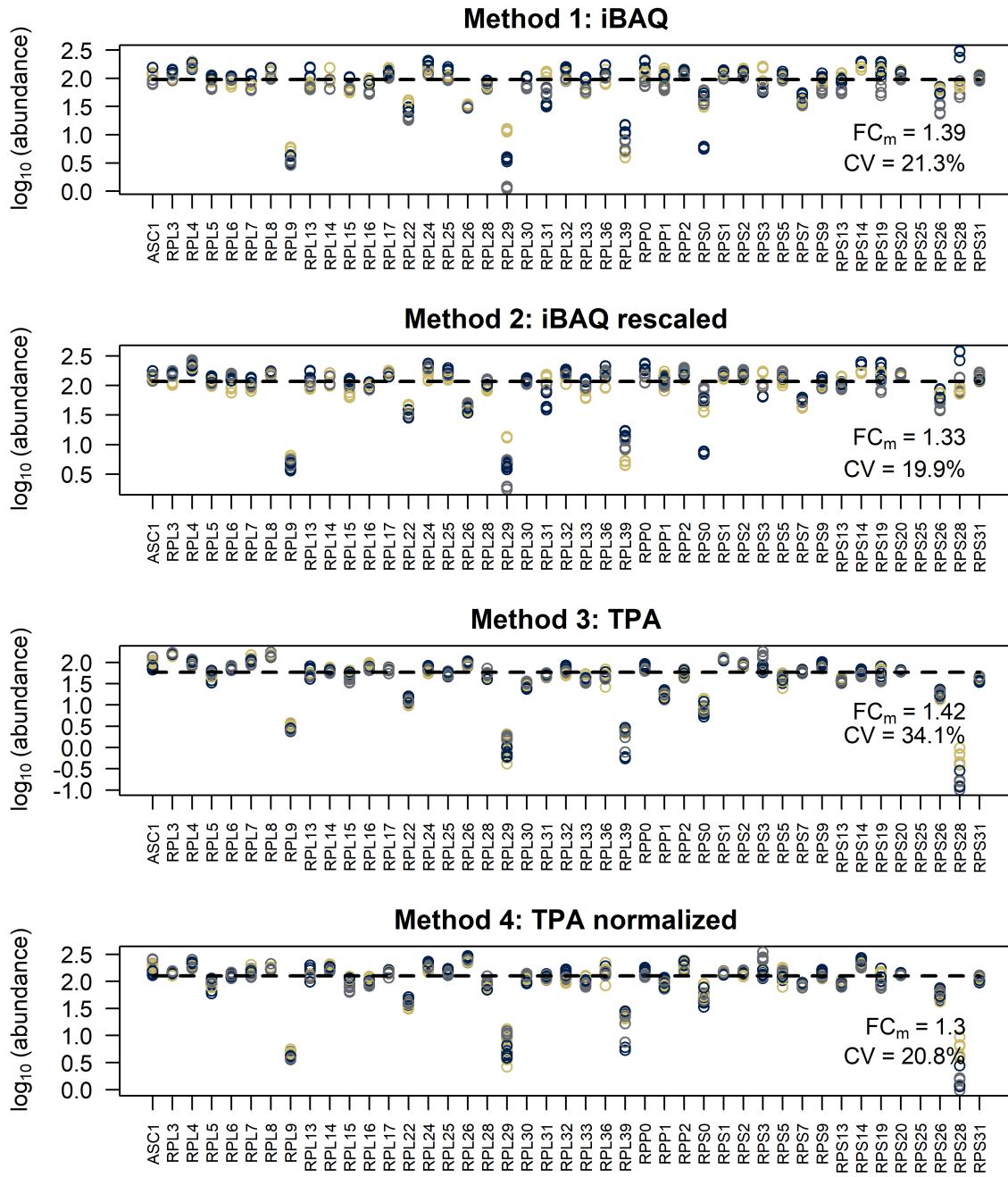


Figure S6: Predicted ribosomal subunit abundances [fmol/ μ g protein] in the samples, by each method. Colors correspond to different technical replicates. The median value is displayed with a segmented line, and both the median fold change to that line and the coefficient of variation for all data are displayed.

```

## [1] "Ribosomal error - number of FC compared = 731"
## [1] "Ribosomal error - number of FC compared = 731"
## [1] "Ribosomal error - number of FC compared = 750"
## [1] "Ribosomal error - number of FC compared = 750"
## [1] "Ribosomal error of iBAQ = iBAQrescaled: p-val = 0.00245409015321552"
## [1] "Ribosomal error of iBAQ = TPA: p-val = 0.000993737106838144"
## [1] "Ribosomal error of iBAQ = TPAnorm: p-val = 0.00187816598008894"

```

```

## [1] "Ribosomal error of iBAQrescaled = TPA: p-val = 5.58856405152852e-06"
## [1] "Ribosomal error of iBAQrescaled = TPAnorm: p-val = 0.00742541104603345"
## [1] "Ribosomal error of TPA = TPAnorm: p-val = 5.1796594568887e-05"

```

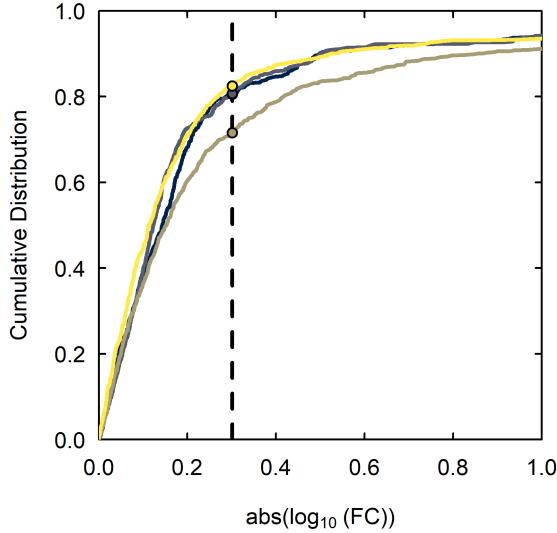


Figure S7: Cumulative distributions of absolute fold changes for differences to median value in ribosomal measurements of samples (right). A fold change of 2 is indicated with a vertical segmented line. Colors represent the methods: 1) iBAQ (blue), 2) iBAQ rescaled (gray), 3) TPA (brown) and 4) TPA normalized (yellow).

So in conclusion, methods 1, 2 and 4 perform similarly well in terms of accuracy, and method 3 performs significantly worse.

5.3 Evaluating precision

We now display the variability of the abundance between biological replicates and between batches, for all methods. For that we first define a function that gives all possible combinations between replicates, and we then plot the data with scatter plots and PCAs:

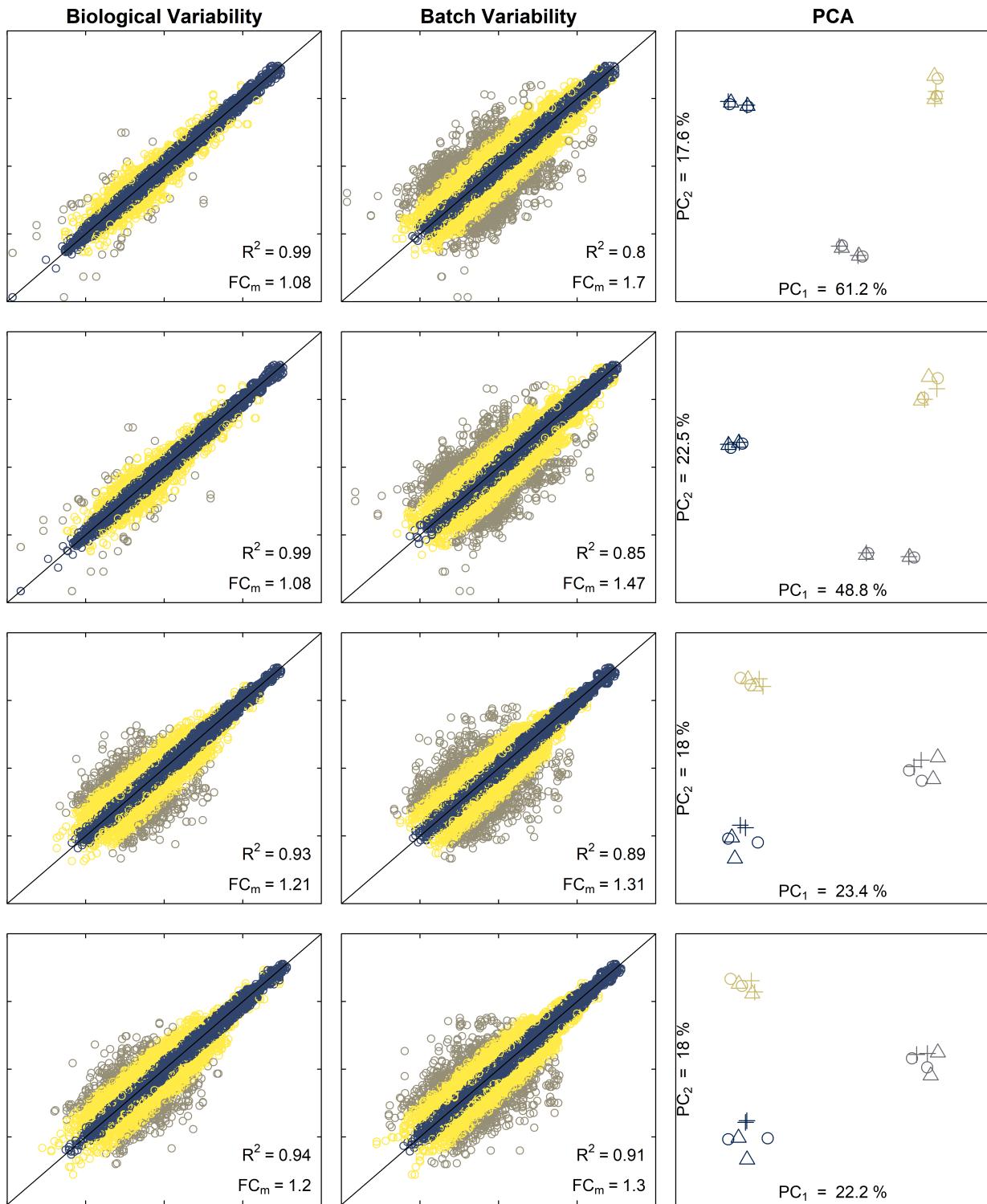


Figure S8: Comparison of data variability from 1) iBAQ (1st row), 2) rescaling iBAQ (2nd row), 3) TPA (3rd row) and 4) normalized TPA (4th row). In the variability plots (left and middle columns, $\log_{10}(\text{abundance})$ [fmol/ μg protein]) both in the x-axis and y-axis), 2 abundance values for a given protein are plotted if they belong to the same biological replicate or batch, respectively. Blue is a FC lower than 2, yellow between 2 and 10, and gray over 10. In the PCA plots (right column), colors refer to MS batches and shapes to biological replicates.

We see a lower median fold change between batches + a better separation of the “batch clusters” in the PCA when we use method 3 or 4 (PC1+PC2 represents less variability). This means that by using rescaled MS data (methods 3 or 4) we achieve lower variability between batches than with methods 1 and 2. In turn, methods 3 and 4 have a higher median fold change between biological replicates, but within acceptable margins. Overall, variability between replicates is the lowest for method 4, if we look at the breakdown of proteins by method:

Table S1: Protein breakdown by method and type of replicate. For each protein, the median fold change is considered.

	Method 1	Method 2	Method 3	Method 4
Variability between biological replicates:				
FC < 2	98.6%	98.6%	91.2%	92.4%
2 <= FC <= 10	1.2%	1.2%	8.3%	7.2%
10 < FC	0.2%	0.2%	0.5%	0.4%
Variability between batches:				
FC < 2	57.2%	67.6%	79.8%	82.3%
2 <= FC <= 10	40.6%	30.6%	19.1%	16.7%
10 < FC	2.2%	1.8%	1.1%	1%
Variability between all replicates:				
FC < 2	76.1%	82.6%	84.5%	86.6%
2 <= FC <= 10	22.7%	16.5%	14.8%	12.9%
10 < FC	1.2%	0.9%	0.7%	0.5%

5.3.1 Evaluating inter-batch precision

Let’s look further into the reduction of batch variability, by looking at the cumulative distribution of batch variability for each method. We see that predictions for methods 3 and 4 are less variable, both in the IS data:

```
## [1] "Inter-batch precision - number of FC compared = 14858"
## [1] "Inter-batch precision - number of FC compared = 14858"
## [1] "Inter-batch precision - number of FC compared = 14908"
## [1] "Inter-batch precision - number of FC compared = 14182"
## [1] "Inter-batch precision of iBAQ = iBAQrescaled: p-val = 0"
## [1] "Inter-batch precision of iBAQ = TPA: p-val = 0"
## [1] "Inter-batch precision of iBAQ = TPAnorm: p-val = 0"
## [1] "Inter-batch precision of iBAQrescaled = TPA: p-val = 0"
## [1] "Inter-batch precision of iBAQrescaled = TPAnorm: p-val = 0"
## [1] "Inter-batch precision of TPA = TPAnorm: p-val = 0.4644560752872"
```

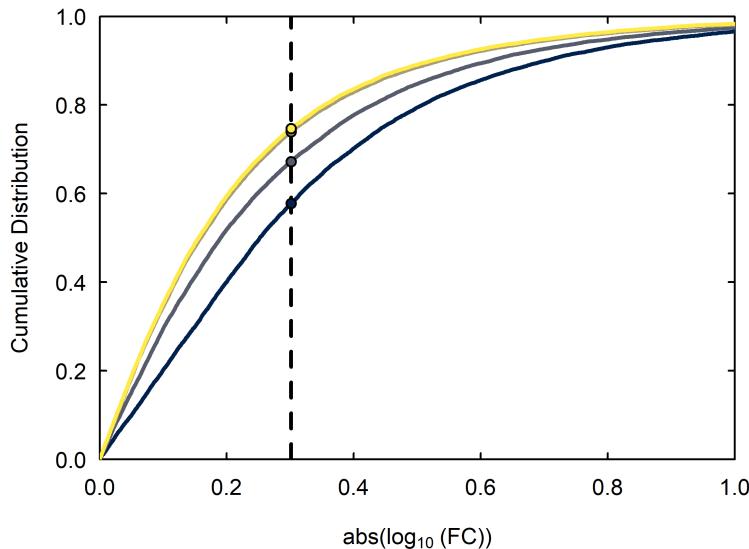


Figure S9: Fold change cumulative distributions of batch variability within the IS measurements for all methods. A fold change of 2 is indicated with a vertical segmented line. Colors represent the methods: 1) iBAQ (blue), 2) iBAQ rescaled (gray), 3) TPA (brown) and 4) TPA normalized (yellow).

And also in the sample data:

```
## [1] "Inter-batch precision - number of FC compared = 21320"
## [1] "Inter-batch precision - number of FC compared = 21320"
## [1] "Inter-batch precision - number of FC compared = 23699"
## [1] "Inter-batch precision - number of FC compared = 22695"
## [1] "Inter-batch precision of iBAQ = iBAQrescaled: p-val = 0"
## [1] "Inter-batch precision of iBAQ = TPA: p-val = 0"
## [1] "Inter-batch precision of iBAQ = TPAnorm: p-val = 0"
## [1] "Inter-batch precision of iBAQrescaled = TPA: p-val = 0"
## [1] "Inter-batch precision of iBAQrescaled = TPAnorm: p-val = 0"
## [1] "Inter-batch precision of TPA = TPAnorm: p-val = 0.0616669688864653"
```

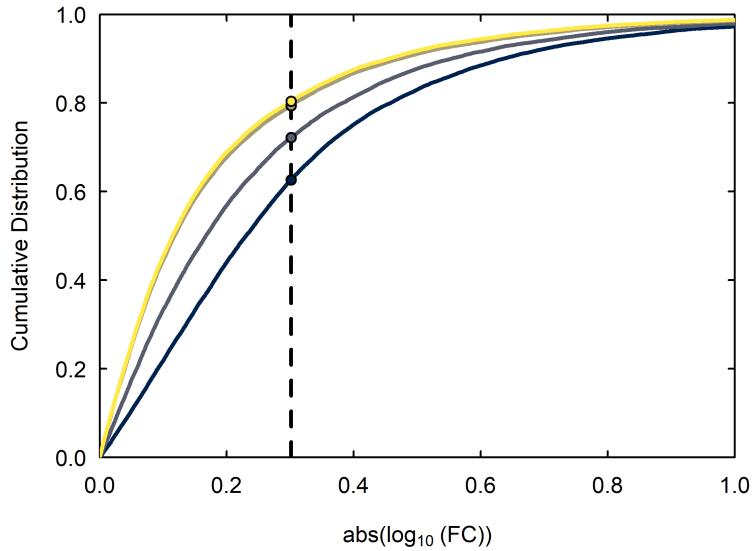


Figure S10: Fold change cumulative distributions of batch variability within samples for all methods. A fold change of 2 is indicated with a vertical segmented line. Colors represent the methods: 1) iBAQ (blue), 2) iBAQ rescaled (gray), 3) TPA (brown) and 4) TPA normalized (yellow).

We can also look at batch variability by plotting each FC to the corresponding abundance, together with a “UPS2 window” for method 1, that shows the abundance levels that are detected by the UPS2:

```
## [1] "Method 1: iBAQ -> 736 proteins below UPS2 detection range"
## [1] "Method 2: iBAQ rescaled -> 820 proteins below UPS2 detection range"
## [1] "Method 3: TPA -> 600 proteins below UPS2 detection range"
## [1] "Method 4: TPA normalized -> 825 proteins below UPS2 detection range"
```

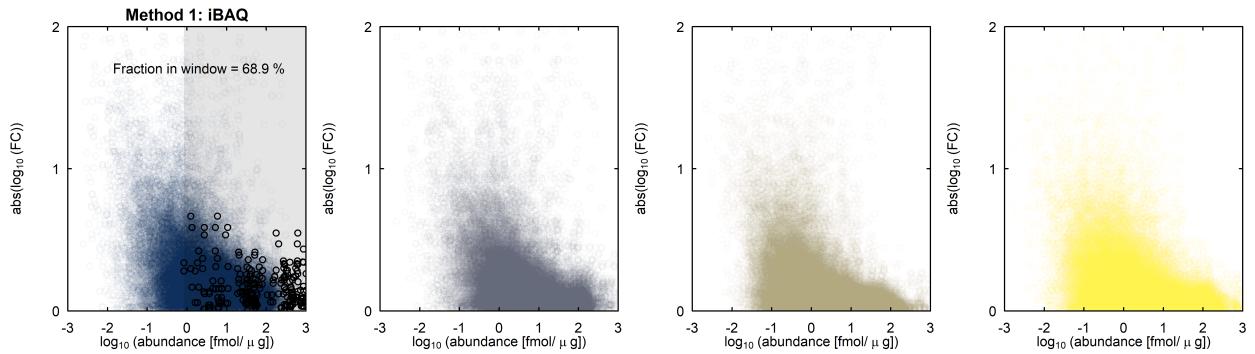


Figure S11: Fold change Vs abundances in samples for all methods. The detection window of UPS2 and the UPS2 datapoints are highlighted in gray and black, respectively, for method 1.

We see that all datasets look somewhat similar in shape, so instead let's look at trends with the help of smooth splines:

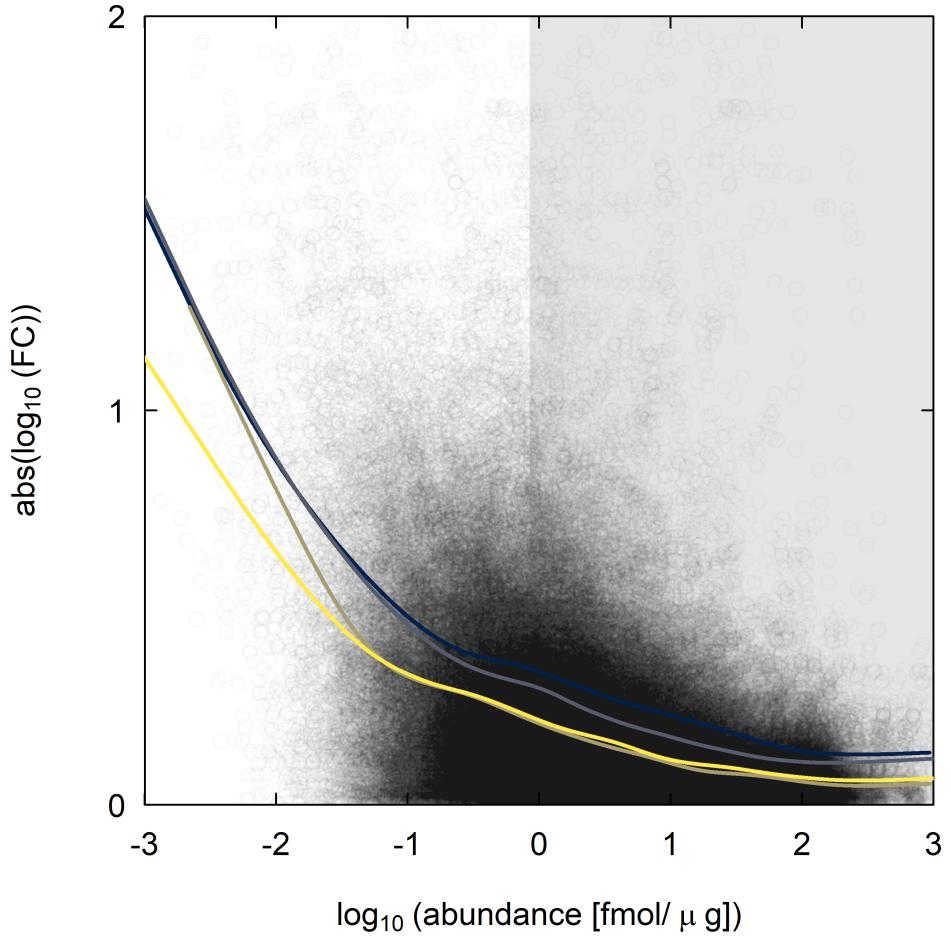


Figure S12: Fold change Vs abundances in samples for all methods + smoothing splines. Colors represent the methods: 1) iBAQ (blue), 2) iBAQ rescaled (gray), 3) TPA (brown) and 4) TPA normalized (yellow). The detection window of UPS2 is highlighted in gray.

We see that method 4 has overall less variability than methods 1 and 2, both for lowly and highly abundant proteins. It also performs better than method 3 at low abundances. So in conclusion, method 4 is the best method from all 4 assessed, as it is as accurate as methods 1 and 2, but significantly more precise.

5.4 Additional comparisons

5.4.1 ES curves

Let's take a look at the ES real abundance values Vs the normalized intensity data from method 4, and compare the “linear model” mentioned in section ?? (method 4) to a linear fit to the data (method 1):

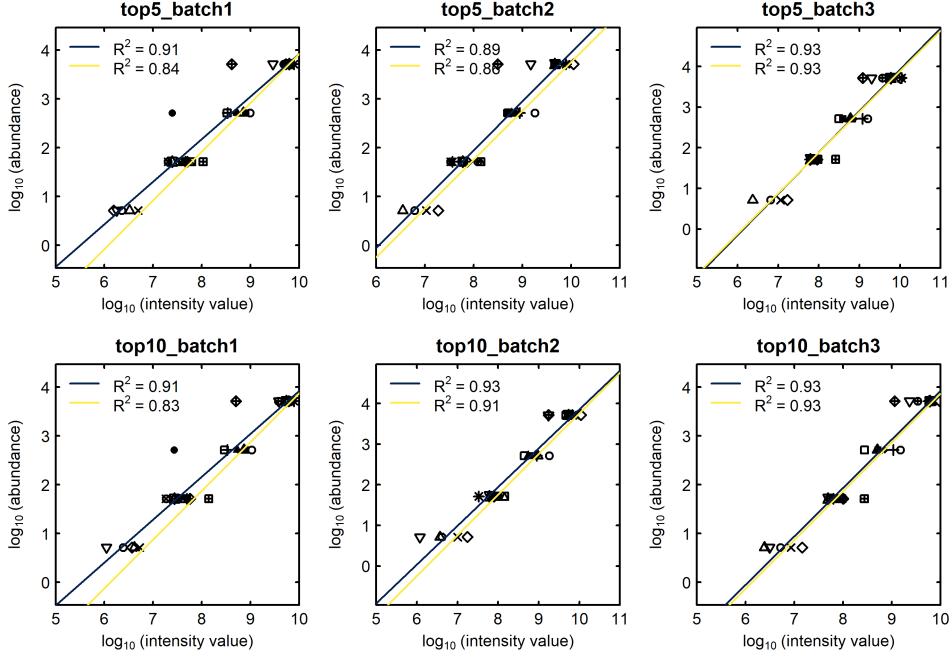


Figure S13: $\log_{10}(\text{abundance} [\text{fmol}/\mu\text{g protein}])$ Vs $\log_{10}(\text{normalized MS intensity})$ of the 30/48 UPS2 proteins that were detected and measured by the MS, together with 2 linear models used later for converting the data: method 1, iBAQ (blue); and method 4, rescaling the normalized intensities (yellow). Within each of the 4 orders of magnitude, each symbol corresponds to a different protein.

The blue fits give us the transformation from light (L) MS intensity of the UPS2 proteins to abundance ($\text{fmol}/\mu\text{g protein}$) with iBAQ. But they don't look the best (considering they are in \log_{10} space), many other curves (as the yellow ones) can almost equally well fit that data. Let's see everything in the same plot:

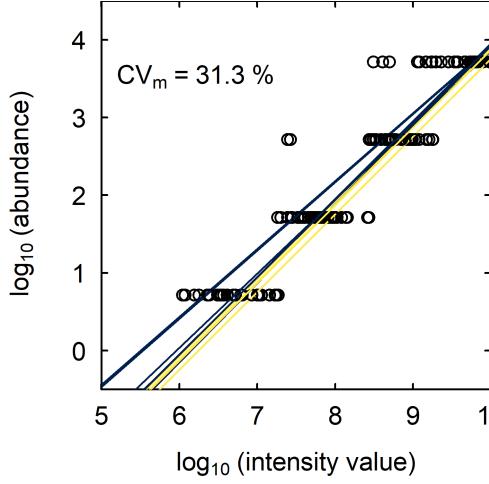


Figure S14: $\log_{10}(\text{abundance} [\text{fmol}/\mu\text{g protein}])$ Vs $\log_{10}(\text{normalized MS intensity})$ of the 30/48 UPS2 proteins that were detected and measured by the MS, together with 2 linear models used later for converting the data: method 1, iBAQ (blue); and method 4, rescaling the normalized intensities (yellow). Average coefficient of variation (CV_m) within each protein is shown in the upper left corner.

In conclusion, we can skip entirely the UPS2 data and iBAQ values, and instead assume that the normalized

MS intensities should always add up to a given protein amount. With this, we can reproduce very closely the ES curves, and achieve more consistent results across replicates.

5.4.2 Peptide performance

We will look into some metrics at the peptide level. First we will look at differences between the number of peptides detected and assigned to each protein (known as the razor unique peptides):

```
## [1] "Bio mean peptide difference: 4.83"
## [1] "Tech mean peptide difference: 4.83"
## [1] "All mean peptide difference: 4.83"
```

Table S2: Peptide breakdown by type of replicate. For each protein, the mean difference of peptides with the union of peptides detected under all conditions was calculated.

Peptides	
Differences between biological replicates:	
Mean Difference <= 2	27.6%
2 < Mean Difference < 5	32.7%
5 <= Mean Difference	39.7%
Differences between batches:	
Mean Difference <= 2	25.9%
2 < Mean Difference < 5	34.1%
5 <= Mean Difference	40%
Differences between all replicates:	
Mean Difference <= 2	23.8%
2 < Mean Difference < 5	37.3%
5 <= Mean Difference	38.9%

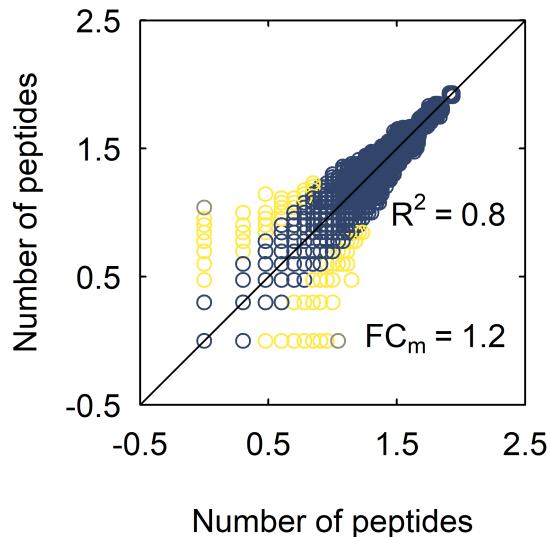


Figure S15: Variability of number of peptides detected by protein.

We will also look at some other basic metrics at the peptide level in the samples, so as to compare bio and tech replicates, including:

1. Charge state variability

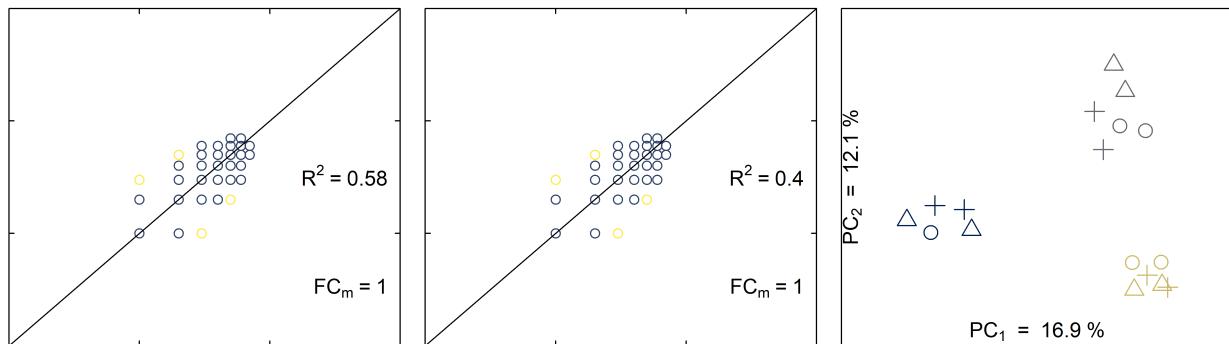


Figure S16: Peptide charge state variability. In the log/log variability plots (left and middle), 2 peptide charges are plotted if they belong to the same biological replicate or batch, respectively. Blue is a FC lower than 2, yellow between 2 and 10, and gray over 10. In the PCA plots (right column), colors refer to MS batches and shapes to biological replicates.

2. Retention time variability

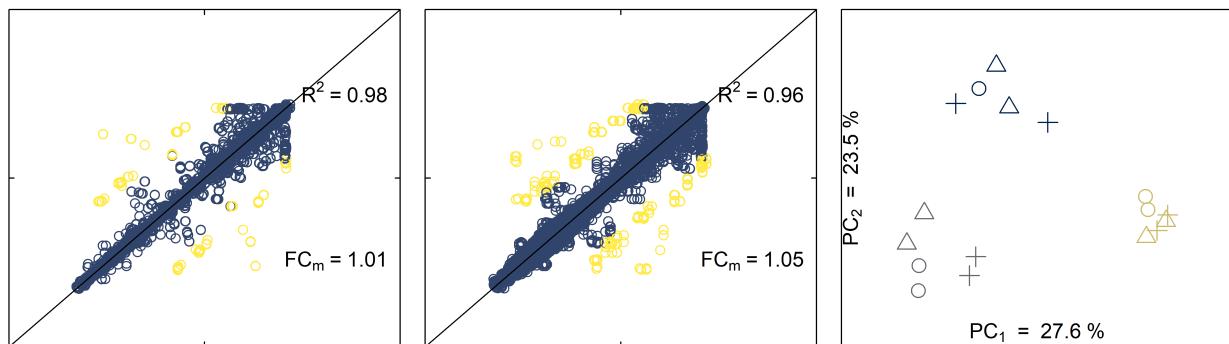


Figure S17: Peptide retention time variability. In the log/log variability plots (left and middle), 2 peptide retention times are plotted if they belong to the same biological replicate or batch, respectively. Blue is a FC lower than 2, yellow between 2 and 10, and gray over 10. In the PCA plots (right column), colors refer to MS batches and shapes to biological replicates.

3. Ion intensity variability

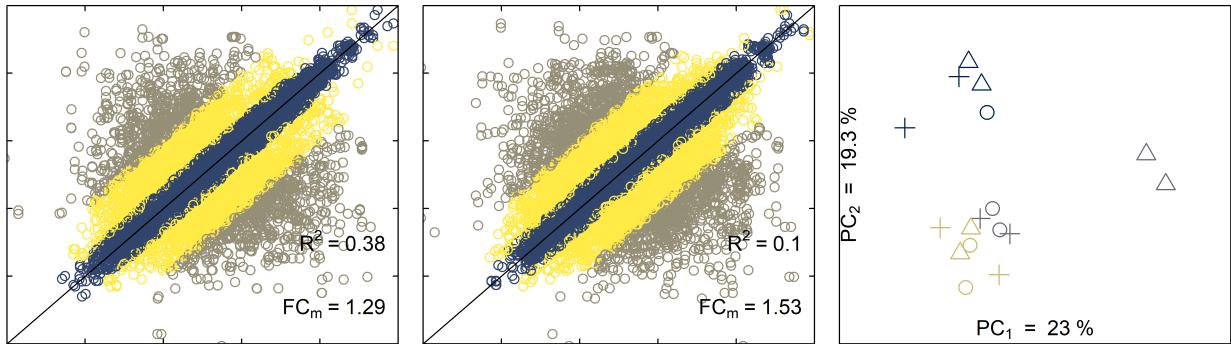


Figure S18: Peptide ion intensity variability. In the log/log variability plots (left and middle), 2 peptide intensity are plotted if they belong to the same biological replicate or batch, respectively. Blue is a FC lower than 2, yellow between 2 and 10, and gray over 10. In the PCA plots (right column), colors refer to MS batches and shapes to biological replicates.

5.4.3 Predictions between methods

Plotting the data between methods shows us that predictions are not very different:

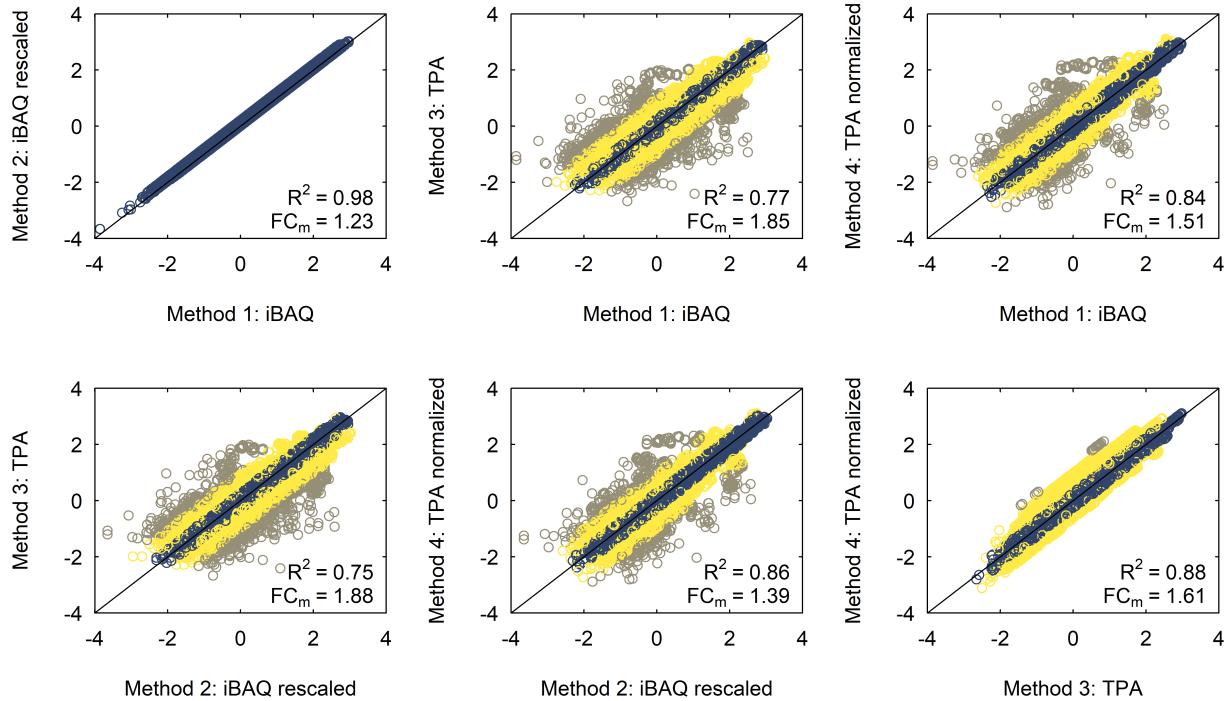


Figure S19: Comparison of predictions [fmol/µg protein] between all methods (on log10 scale). Blue is a FC lower than 2, yellow between 2 and 10, and gray over 10.

5.4.4 Sequence length

Finally, let's see the predicted abundances of all samples compared to sequence length:

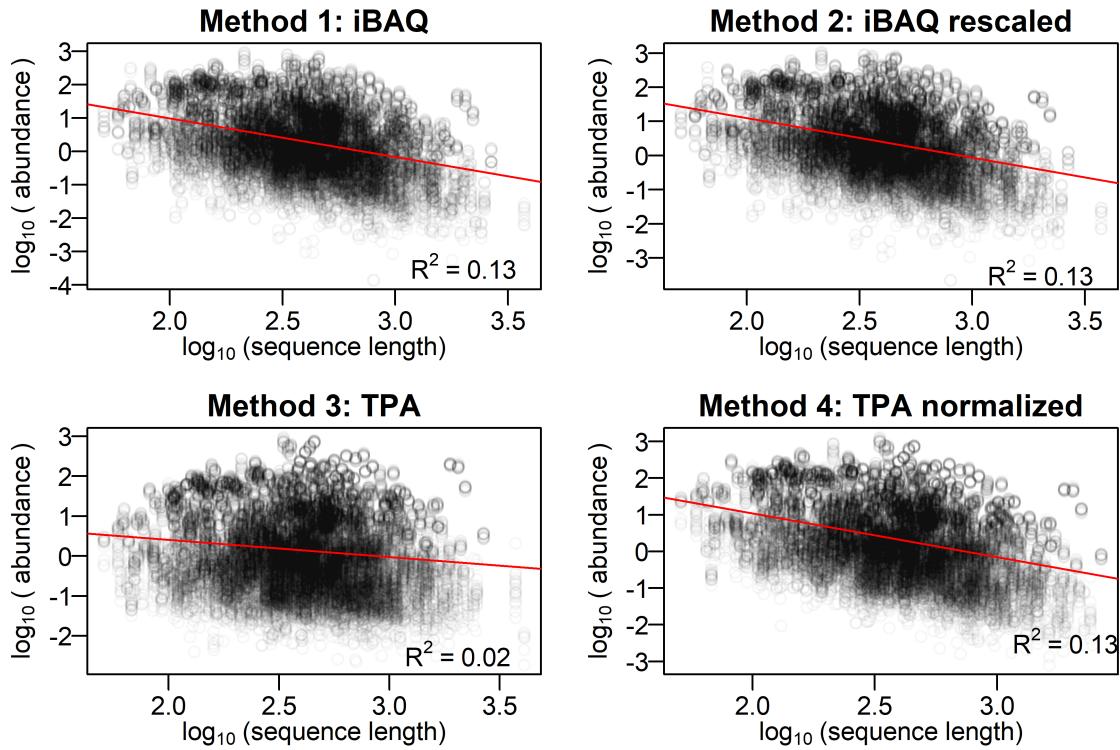


Figure S20: Predicted abundances [fmol/ μ g protein] Vs sequence length (both in the \log_{10} space) for all proteins and all methods.

We can see that methods 1, 2 and 4 all have similar correlation values, while method 3 correlates less than the other 3 methods to protein length. This is expected, because methods 1, 2 and 4 all normalize by the number of theoretical peptides, which correlates well with sequence length:

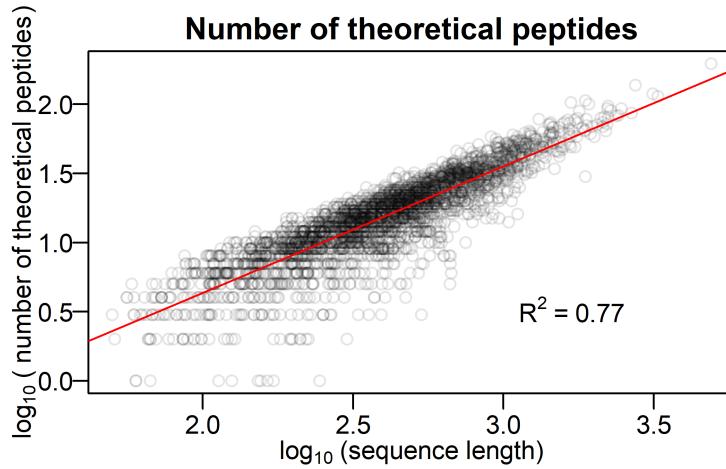


Figure S21: Number of theoretical peptides Vs sequence length for all proteins (both in the \log_{10} space).

With this we also see that sequence length can work as a good proxy for the number of theoretical peptides (if the latter is not available).

References

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