

A mass spectrometry workflow for measuring protein turnover rates in vivo

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Supplementary Table 1: Exemplary labeling schemes including basic guidelines to optimize labeling

Feeding scheme number	Brief description of the feeding scheme	Suggested number of time points	Number of mice per time point	Total number of mice	Estimated food use (grams)	Estimated food cost (€/€)	Suggested 1st feeding time point (days)	Suggested 2nd time point (days)	Suggested 3rd time point (days)	Suggested 4th time point (days)	Suggested 5th time point (days)	Estimated MS time for two technical replicates (days)
1	Minimal design: limits food consumption and animal number	3	1	3	~150	~1500	5	14	21	---	---	6
2	Standard design: allows to fit most of the proteins with good precision	3	3	9	~450	~4500	5	14	21	---	---	18
3	Design optimized for short-living proteins* : allows to optimize results for short-living proteins	3	3	9	~270	~2700	3	5	14	---	---	18
4	Design optimized for long-living proteins* : allows to optimize results for long-living proteins	3	3	9	~1200	~12000	14	30	60	---	---	18
5	Deluxe design: allows to obtain the most reliable results but requires very long MS machine times	5	4	9	~1700	~17000	3	5	14	30	60	40

* = lifetimes can be estimated from the Supplementary Data 1 from the key reference of this protocol:

Fornasiero, E. et al. Precisely measured protein lifetimes in the mouse brain reveal differences across tissues and subcellular fractions. Nat. Commun. (2018)

[Supplementary Data 1](#)

Supplementary Table 2: Influence of biological and technical replicates on the lifetime determination

	Reference (3 bio. and 3 tech. replicates)	3 bio. replicates and 1 tech. replicate	1 bio. replicate and 3 tech. replicates	1 bio. replicate and 1 tech. replicate
Number of proteins with heavy vs. light ratios	2428	2185	2188	1931
Average number of measures /protein	13.45	5.10	5.20	2.03
Precisely determined lifetimes	2096	1839	1640	1410
Precisely determined lifetimes as % of reference	100.00	87.74	78.24	67.27

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<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

- Raw LC-MS data sets were analyzed using MaxQuant
- Raw GC-MS data sets were analyzed using MSD ChemStation D.01.02.16 (Agilent)

Data analysis

- MATLAB (version 2014b or more recent) with Optimization and Statistics Toolbox
- Microsoft Office or Open Office
- All new software developed for this protocol is publicly available at GitHub: <https://github.com/malevra/protein-turnover>. The password-protected .zip file can be open using the password "turnover321".

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

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Not applicable

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Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Not applicable
Validation	Not applicable

Eukaryotic cell lines

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Cell line source(s)	Not applicable
Authentication	Not applicable
Mycoplasma contamination	Not applicable
Commonly misidentified lines (See ICLAC register)	Not applicable

Palaeontology

Specimen provenance	Not applicable
Specimen deposition	Not applicable

Dating methods

Not applicable

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Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Mice (*Mus musculus*)

Wild animals

Not applicable

Field-collected samples

Not applicable

Ethics oversight

All mouse experiments were approved by the local authority, the Lower Saxony State Office for Consumer Protection and Food Safety (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit)

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