

Probabilistic mapping of the sub-cellular proteome

Slides available at: <http://bit.ly/ABLS2020>

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Dr Lisa Breckels (Cambridge):
novelty detection, transfer learning,
pRoloc and pRolocdata.



Mr Oliver Crook (Cambridge):
Bayesian spatial proteomics,
pRoloc and pRolocdata.



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Outline

Spatial proteomics

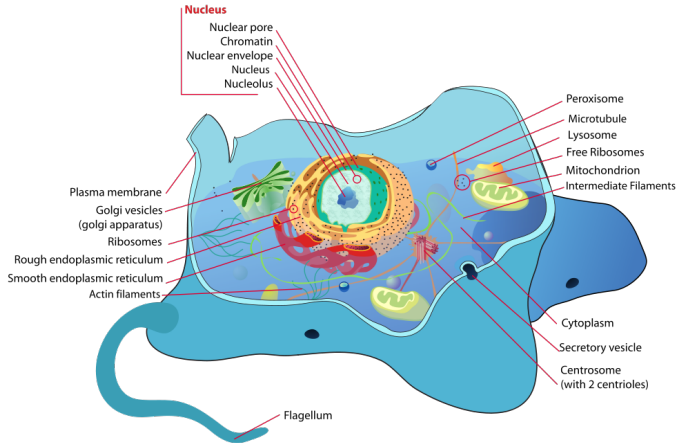
Data analysis (1)

Computational spatial proteomics (2)

Behind the scenes

Conclusions

Cell organisation - localisation is function



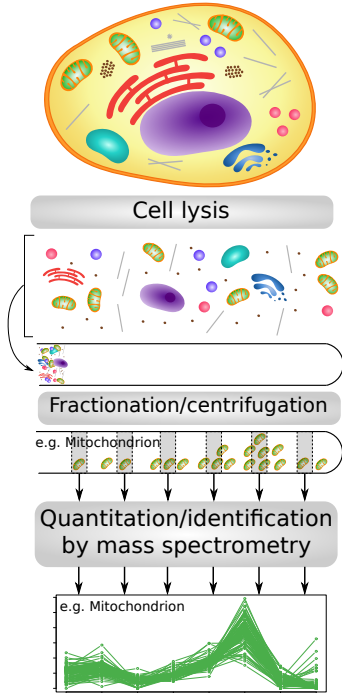
Spatial proteomics is the systematic study of protein localisations.

Localisation – re-localisation – mis-localisation

Image from Wikipedia [http://en.wikipedia.org/wiki/Cell_\(biology\)](http://en.wikipedia.org/wiki/Cell_(biology)).

Explorative/discovery approaches, **steady-state global localisation maps** (as opposed to targeted microscopy-based approaches).

Density gradient: PCP (Dunkley et al., 2006), LOPIT (Foster et al., 2006), hyperLOPIT (Christoforou et al., 2016; Mulvey et al., 2017) and **Differential centrifugation** Itzhak et al. (2016), LOPIT-DC (Geladaki et al., 2019).



Quantitation data

	Fraction ₁	Fraction ₂	...	Fraction _L
x ₁	x _{1,1}	x _{1,2}	...	x _{1,L}
x ₂	x _{2,1}	x _{2,2}	...	x _{2,L}
x ₃	x _{3,1}	x _{3,2}	...	x _{3,L}
⋮	⋮	⋮	⋮	⋮
x _i	x _{i,1}	x _{i,2}	...	x _{i,L}
⋮	⋮	⋮	⋮	⋮
x _N	x _{N,1}	x _{N,2}	...	x _{N, L}

Quantitation data and organelle markers

	Fraction ₁	Fraction ₂	...	Fraction _L	markers
x₁	x _{1,1}	x _{1,2}	...	x _{1,L}	unknown
x₂	x _{2,1}	x _{2,2}	...	x _{2,L}	<i>loc₁</i>
x₃	x _{3,1}	x _{3,2}	...	x _{3,L}	unknown
⋮	⋮	⋮	⋮	⋮	⋮
x_i	x _{i,1}	x _{i,2}	...	x _{i,L}	<i>loc_k</i>
⋮	⋮	⋮	⋮	⋮	⋮
x_N	x _{N,1}	x _{N,2}	...	x _{N, K}	unknown

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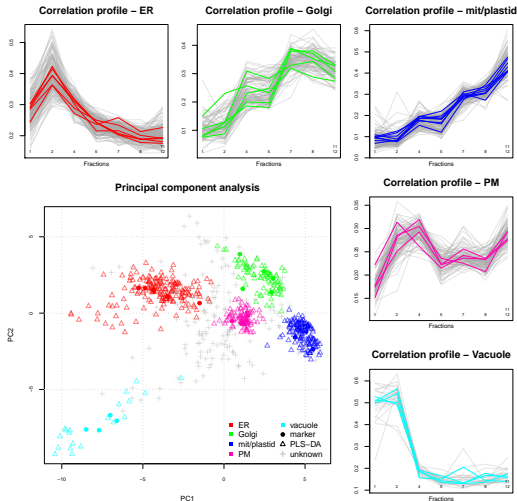


Figure: From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)

Quality control

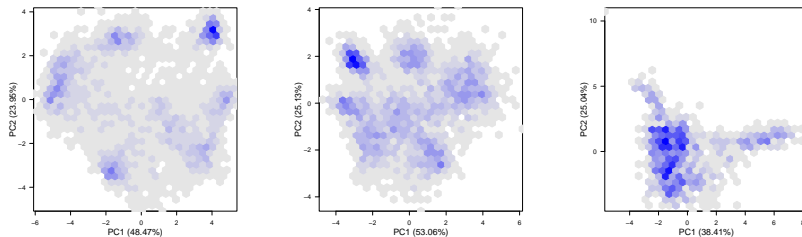


Figure: Assessing sub-cellular resolution in spatial proteomics experiments (Gatto et al., 2018)

Problem statement: classification

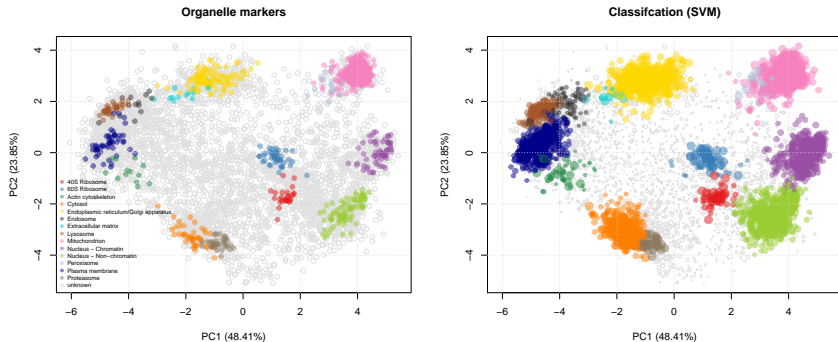


Figure: Support vector machines classifier (after 5% FDR classification cutoff) on the embryonic stem cell data from Christoforou et al. (2016).

- Visualisation (cluster, unsupervised learning)
- Classification (supervised learning)
- **Novelty detection** (semi-supervised learning)
- Data integration (transfer learning)
- **Uncertainty quantification**
- **Multi-localisation**
- **Spatial dynamics**

To uncover and understand biology

Outline

Spatial proteomics

Data analysis (1)

Computational spatial proteomics (2)

- Novelty detection

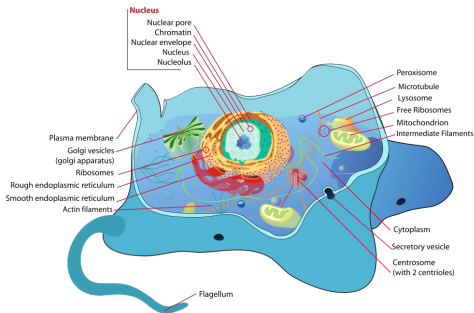
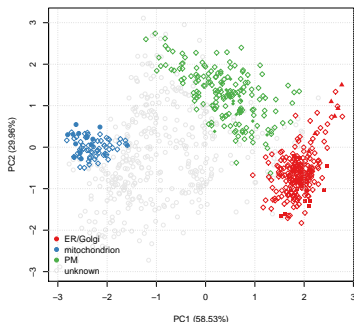
- Multi-localisation and uncertainly quantification

- Spatial dynamics

Behind the scences

Conclusions

Importance of annotation



Incomplete annotation, and therefore lack of training data, for many/most organelles. *Drosophila* data from Tan et al. (2009).

Semi-supervised learning: novelty detection

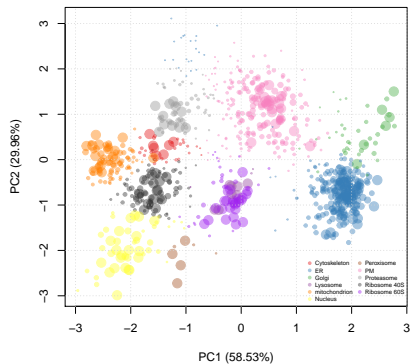
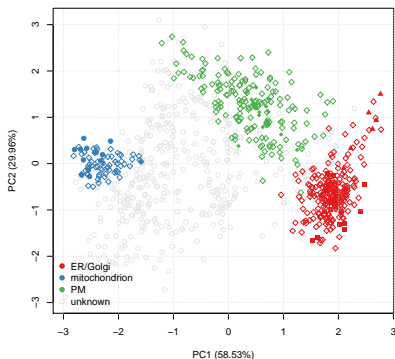
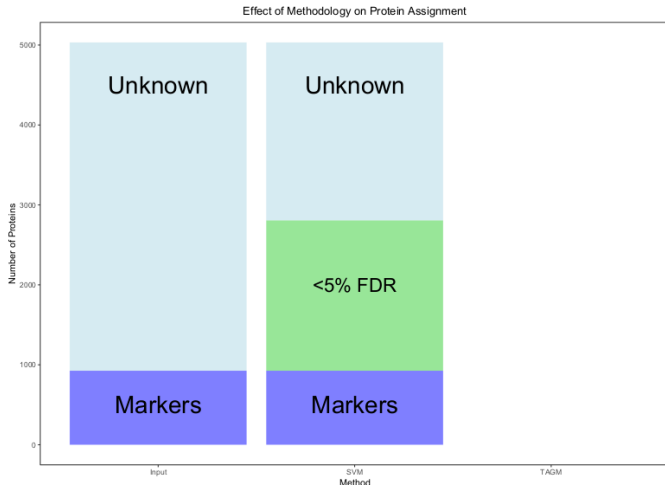


Figure: Left: Original *Drosophila* data from Tan et al. (2009). Right: After semi-supervised learning and classification, Breckels et al. (2013). Under development: Bayesian novelty detection (Novelty-TAGM, see below).

How much do we learn? How much do we miss?



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- With the prior knowledge that many proteins are not captured by known sub-cellular niches, we augment our model with an **outlier component**. Outliers are often dispersed and thus this additional component is described by a heavy-tailed distribution: the multivariate Student's t-distribution, leading us to a *T Augmented Gaussian Mixture model* (Crook et al., 2018, 2019b).

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- This methodology allows proteome-wide **uncertainty quantification**, thus adding a further layer to the analysis of spatial proteomics.

We initially model the distribution of profiles associated with proteins that localise to the k -th component as multivariate normal with mean vector $\boldsymbol{\mu}_k$ and covariance matrix $\boldsymbol{\Sigma}_k$, so that:

$$\mathbf{x}_i | z_i = k \sim \mathcal{N}(\boldsymbol{\mu}_k, \boldsymbol{\Sigma}_k) \quad (1)$$

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We extend it by introducing an additional *outlier component*. To do this, we augment our model by introducing a further indicator latent variable ϕ . Each protein \mathbf{x}_i is now described by an additional variable ϕ_i , with $\phi_i = 1$ indicating that protein \mathbf{x}_i belongs to a organelle derived component and $\phi_i = 0$ indicating that protein \mathbf{x}_i is not well described by these known components. This outlier component is modelled as a multivariate T distribution with degrees of freedom κ , mean vector \mathbf{M} , and scale matrix V .

$$\mathbf{x}_i | z_i = k, \phi_i \sim \mathcal{N}(\boldsymbol{\mu}_k, \boldsymbol{\Sigma}_k)^{\phi_i} \mathcal{T}(\kappa, \mathbf{M}, V)^{1-\phi_i} \quad (2)$$

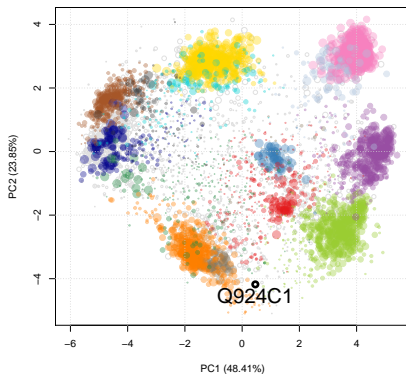
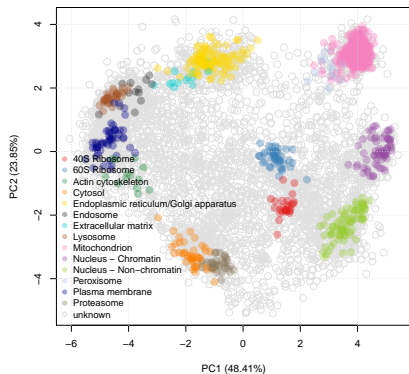
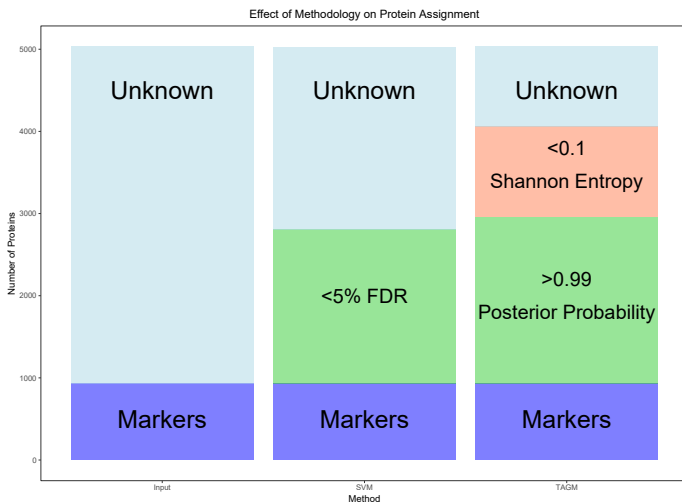


Figure: Assignment of proteins of *unknown* location to one of the annotated classes. The dots are scaled according to the protein assignment probabilities.



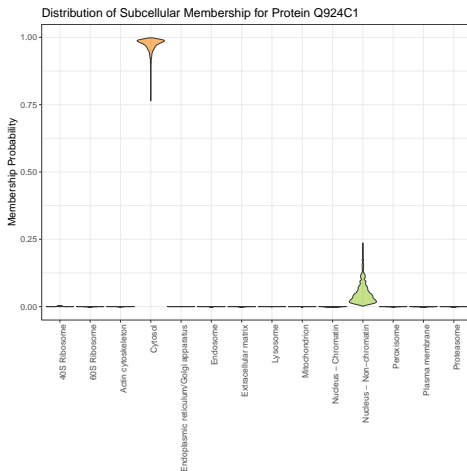
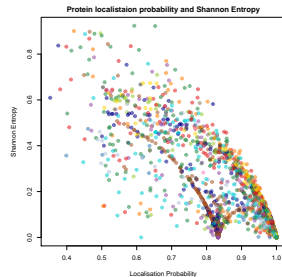
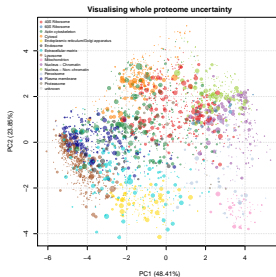
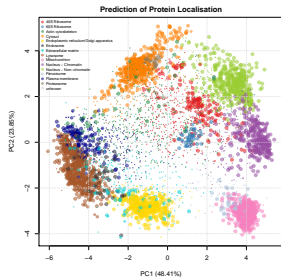


Figure: Exportin 5 (Q924C1) forms part of the micro-RNA export machinery, transporting miRNA from the nucleus to the cytoplasm for further processing. It then translocates back through the nuclear pore complex to return to the nucleus to mediate further transport between nucleus and cytoplasm. The model correctly infers that it most likely localises to the cytosol but there is some uncertainty with this assignment. This uncertainty is reflected in possible assignment of Exportin 5 to the nucleus non-chromatin and reflects the multi-location of the protein.

Whole sub-cellular proteome uncertainty



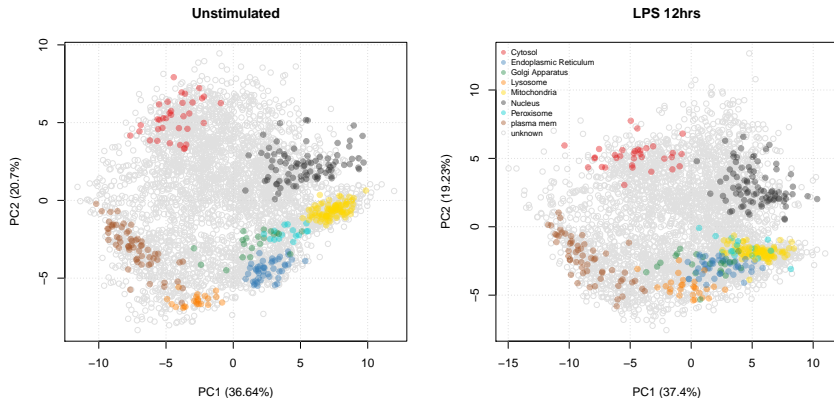


Figure: Spatial maps of unstimulated (**control**) and LPS-treated (**experimental condition**) cells (combined triplicates).

A probabilistic definition of differential localisation:

$$x_i = p(z_{i,1} \neq z_{i,2}) \quad (3)$$

with

- organelle-specific profiles modelled with mixtures of non-paramateric distributions (Crook et al., 2019a);
- explicit modelling of replicates and their variability;
- no assumption with regard to similarity of gradients between conditions;
- rigorous interpretation of the results with uncertainty quantification of differential localisation.

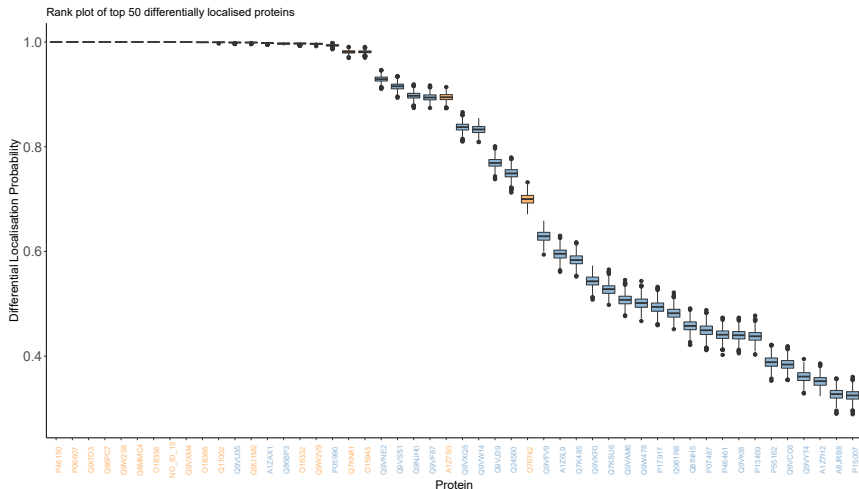


Figure: Proteins ranked based on their probability of being differentially localised, i.e. having been assigned different niches in the control and experimental condition. Orange: TP, blue: FP.

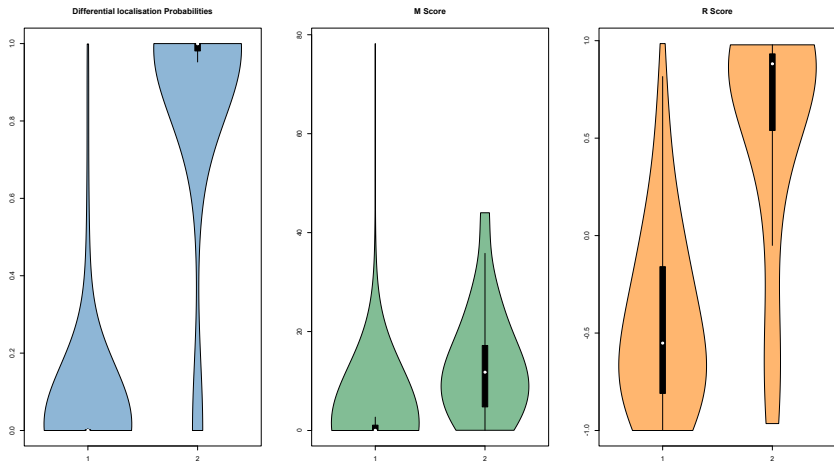


Figure: Differential localisation probabilities (left) provide excellent discrimination between static (1) and differentially localised proteins (2).

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Behind the scenes: **Applied** Bioinformatics Life Sciences - software/data structures and open research practice.

Beyond the figures¹

- Software: **infrastructure** (MSnbase, Gatto and Lilley (2012)), **dedicated machine learning** (pRoloc, Gatto et al. (2014)), **interactive visualisation**² (pRolocGUI, Breckels et al. (2017)) and **data** (pRolocdata, Gatto et al. (2014)) for spatial proteomics.
- The **Bioconductor** (Huber et al., 2015) ecosystem for high throughput biology data analysis and comprehension: **open source**, and **coordinated and collaborative**³ **open development**, enabling **reproducible research**, enables understanding of the data (not a black box) and **drive scientific innovation**.

¹... which are all reproducible, by the way.

²<https://lgatto.shinyapps.io/christoforou2015/>

³between and within domains/software

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- **Applied Bioinformatics:** Reliance on computational biology, statistics and dedicated software (pRoLoc *et al.*) to interpret data and acquire biological knowledge.
- **Life Sciences:** Protein sub-cellular localisation, technologies (hyperLOPIT) and opportunities.
- Rigorous computational infrastructure and sound data analysis and interpretation is a **long term investment**.

- Lisa Breckels, Thomas Naake, and Laurent Gatto. *pRolocGUI: Interactive visualisation of spatial proteomics data*, 2017. URL <http://ComputationalProteomicsUnit.github.io/pRolocGUI/>. R package version 1.11.2.
- LM Breckels, L Gatto, A Christoforou, AJ Groen, KS Lilley, and MW Trotter. The effect of organelle discovery upon sub-cellular protein localisation. *J Proteomics*, 88:129–40, Aug 2013.
- A Christoforou, C M Mulvey, L M Breckels, A Geladaki, T Hurrell, P C Hayward, T Naake, L Gatto, R Viner, A Martinez Arias, and K S Lilley. A draft map of the mouse pluripotent stem cell spatial proteome. *Nat Commun*, 7:8992, Jan 2016. doi: 10.1038/ncomms9992.
- Oliver M. Crook, Claire M. Mulvey, Paul D. W. Kirk, Kathryn S. Lilley, and Laurent Gatto. A bayesian mixture modelling approach for spatial proteomics. *PLOS Computational Biology*, 14(11):1–29, 11 2018. doi: 10.1371/journal.pcbi.1006516. URL <https://doi.org/10.1371/journal.pcbi.1006516>.
- Oliver M Crook, Kathryn S Lilley, Laurent Gatto, and Paul D W Kirk. Semi-Supervised Non-Parametric bayesian modelling of spatial proteomics. March 2019a.
- OM Crook, LM Breckels, KS Lilley, PDW Kirk, and L Gatto. A bioconductor workflow for the bayesian analysis of spatial proteomics [version 1; peer review: 1 approved, 2 approved with reservations]. *F1000Research*, 8(446), 2019b. doi: 10.12688/f1000research.18636.1.

- TPJ Dunkley, S Hester, IP Shadforth, J Runions, T Weimar, SL Hanton, JL Griffin, C Bessant, F Brandizzi, C Hawes, RB Watson, P Dupree, and KS Lilley. Mapping the Arabidopsis organelle proteome. *PNAS*, 103(17):6518–6523, Apr 2006.
- LJ Foster, CL de Hoog, Y Zhang, Y Zhang, X Xie, VK Mootha, and M Mann. A mammalian organelle map by protein correlation profiling. *Cell*, 125(1):187–199, Apr 2006.
- L Gatto and KS Lilley. MSnbase - an R/Bioconductor package for isobaric tagged mass spectrometry data visualization, processing and quantitation. *Bioinformatics*, 28(2): 288–9, Jan 2012.
- L Gatto, JA Vizcaino, H Hermjakob, W Huber, and KS Lilley. Organelle proteomics experimental designs and analysis. *Proteomics*, 2010.
- L Gatto, LM Breckels, T Burger, DJ Nightingale, AJ Groen, C Campbell, N Nikolovski, CM Mulvey, A Christoforou, M Ferro, and KS Lilley. A foundation for reliable spatial proteomics data analysis. *MCP*, 13(8):1937–52, Aug 2014.
- Laurent Gatto, Lisa M Breckels, and Kathryn S Lilley. Assessing sub-cellular resolution in spatial proteomics experiments. *bioRxiv*, 2018. doi: 10.1101/377630.
- Aikaterini Geladaki, Nina Kočevár Britovšek, Lisa M Breckels, Tom S Smith, Owen L Vennard, Claire M Mulvey, Oliver M Crook, Laurent Gatto, and Kathryn S Lilley. Combining LOPIT with differential ultracentrifugation for high-resolution spatial proteomics. *Nat. Commun.*, 10(1):331, January 2019.

- W Huber, V J Carey, R Gentleman, S Anders, M Carlson, B S Carvalho, H C Bravo, S Davis, L Gatto, T Girke, R Gottardo, F Hahne, K D Hansen, R A Irizarry, M Lawrence, M I Love, J MacDonald, V Obenchain, A K Oleś, H Pagès, A Reyes, P Shannon, G K Smyth, D Tenenbaum, L Waldron, and M Morgan. Orchestrating high-throughput genomic analysis with Bioconductor. *Nat Methods*, 12(2):115–21, Jan 2015. doi: 10.1038/nmeth.3252.
- D N Itzhak, S Tyanova, J Cox, and G H Borner. Global, quantitative and dynamic mapping of protein subcellular localization. *Elife*, 5, Jun 2016. doi: 10.7554/eLife.16950.
- C M Mulvey, L M Breckels, A Geladaki, N K Britovek, DJH Nightingale, A Christoforou, M Elzek, M J Deery, L Gatto, and K S Lilley. Using hyperlopit to perform high-resolution mapping of the spatial proteome. *Nat Protoc*, 12(6):1110–1135, Jun 2017. doi: 10.1038/nprot.2017.026.
- DJL Tan, H Dvinge, A Christoforou, P Bertone, A Arias Martinez, and KS Lilley. Mapping organelle proteins and protein complexes in *Drosophila melanogaster*. *J Proteome Res*, 8(6):2667–2678, Jun 2009.

Thank you for your attention

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