

# **Probabilistic mapping of the sub-cellular proteome**

**Laurent Gatto**  
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**Abstract:** In biology, localisation is function - understanding the sub-cellular localisation of proteins is paramount to comprehend the context and full extend of their functions. Shotgun mass spectrometry-based spatial proteomics method are orthogonal to widely used targeted microscopy-based assay. In conjunction with contemporary machine learning, the former enable to build proteome-wide protein localisation maps, informing us on the location of thousands of proteins. When studying these proteome-wide spatial maps, one can learn that while some proteins can be found in a single location within a cell, up to half of the proteins may reside in multiple locations, can dynamically re-localise, or reside within an unknown functional compartment, leading to considerable uncertainty in associating proteins to their sub-cellular location. Recent Bayesian modelling approaches enable us to mine these data, and in particular the dynamic fraction of the spatial proteome, in much greater depth. We are now in a position to (1) probabilistically model protein localisation as well as quantify the uncertainty in the location assignments, and (2) compute a probability for, and quantify uncertainty in, whether a protein is differentially localised upon cellular perturbation. These computational approaches lead to better and more trustworthy biological interpretation of these rich spatial proteomics data.

# Acknowledgements

- Mr Oliver Crook (Cambridge)
- Dr Lisa Breckels (Cambridge)

# Outline

Spatial proteomics

Data analysis

Computational challenges

Novelty detection

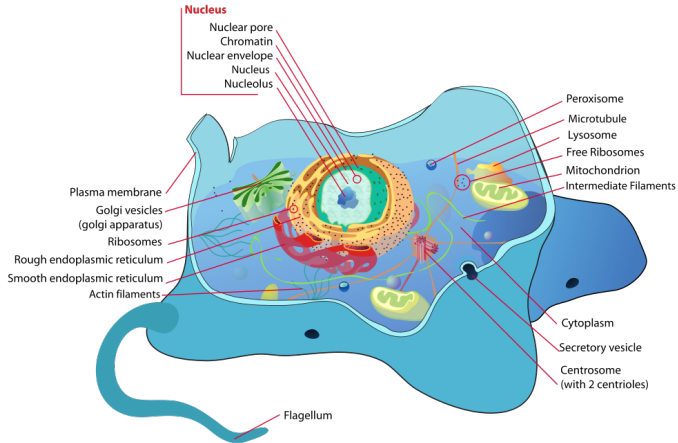
Multi-localisation and uncertainly quantification

Spatial dynamics

Behind the scences

Conclusions

# Cell organisation - localisation is function



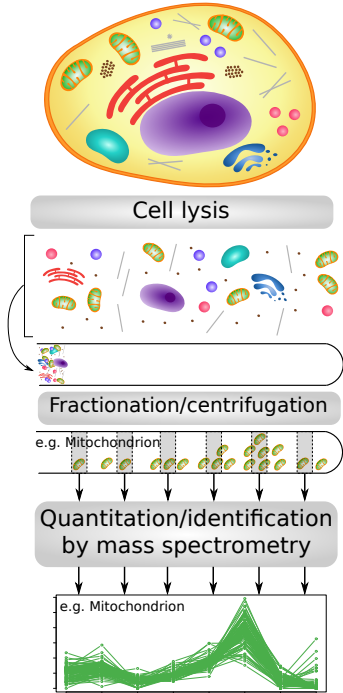
**Spatial proteomics** is the systematic study of protein localisations.

## 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 microscopy-based targeted 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., 2018).



	Fraction <sub>1</sub>	Fraction <sub>2</sub>	...	Fraction <sub>L</sub>
<b>x<sub>1</sub></b>	x <sub>1,1</sub>	x <sub>1,2</sub>	...	x <sub>1,L</sub>
<b>x<sub>2</sub></b>	x <sub>2,1</sub>	x <sub>2,2</sub>	...	x <sub>2,L</sub>
<b>x<sub>3</sub></b>	x <sub>3,1</sub>	x <sub>3,2</sub>	...	x <sub>3,L</sub>
⋮	⋮	⋮	⋮	⋮
<b>x<sub>i</sub></b>	x <sub>i,1</sub>	x <sub>i,2</sub>	...	x <sub>i,L</sub>
⋮	⋮	⋮	⋮	⋮
<b>x<sub>N</sub></b>	x <sub>N,1</sub>	x <sub>N,2</sub>	...	x <sub>N, L</sub>

# Quantitation data and organelle markers

	Fraction <sub>1</sub>	Fraction <sub>2</sub>	...	Fraction <sub>L</sub>	markers
<b>x<sub>1</sub></b>	x <sub>1,1</sub>	x <sub>1,2</sub>	...	x <sub>1,L</sub>	unknown
<b>x<sub>2</sub></b>	x <sub>2,1</sub>	x <sub>2,2</sub>	...	x <sub>2,L</sub>	<i>loc<sub>1</sub></i>
<b>x<sub>3</sub></b>	x <sub>3,1</sub>	x <sub>3,2</sub>	...	x <sub>3,L</sub>	unknown
⋮	⋮	⋮	⋮	⋮	⋮
<b>x<sub>i</sub></b>	x <sub>i,1</sub>	x <sub>i,2</sub>	...	x <sub>i,L</sub>	<i>loc<sub>k</sub></i>
⋮	⋮	⋮	⋮	⋮	⋮
<b>x<sub>N</sub></b>	x <sub>N,1</sub>	x <sub>N,2</sub>	...	x <sub>N, K</sub>	unknown



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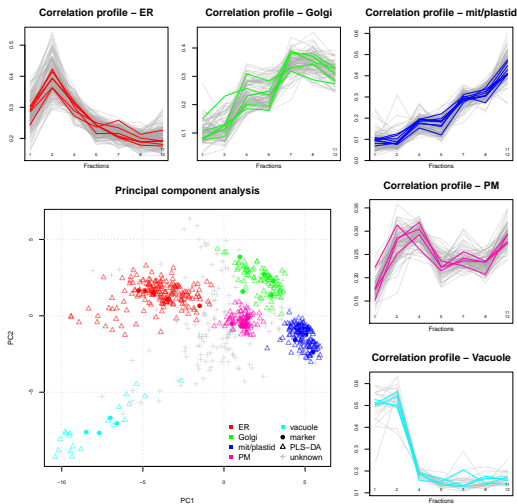
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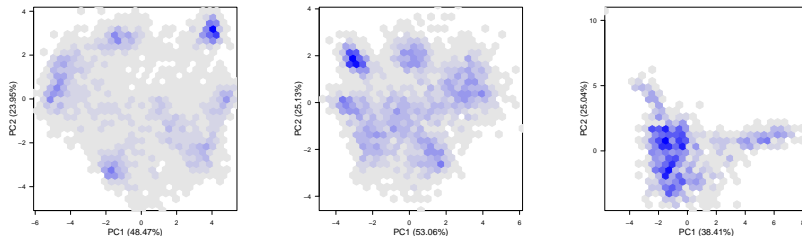
Spatial dynamics

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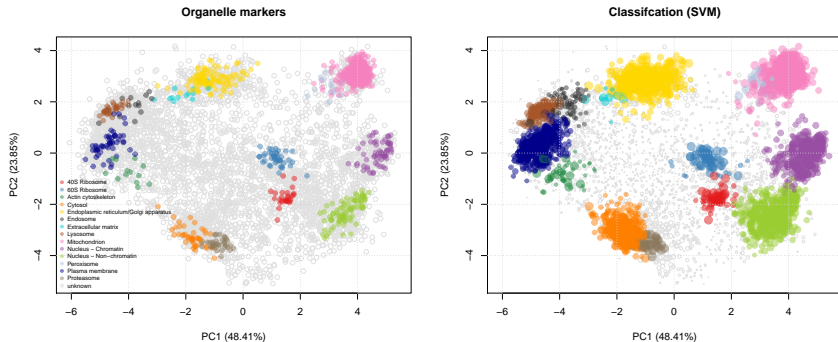


**Figure:** From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)



**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).

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

To uncover and understand biology

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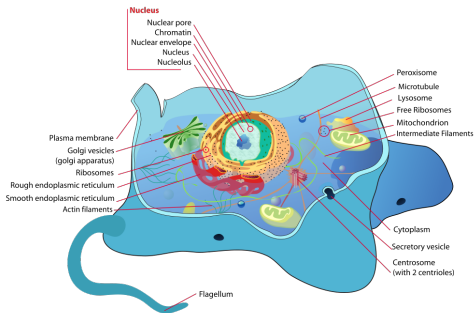
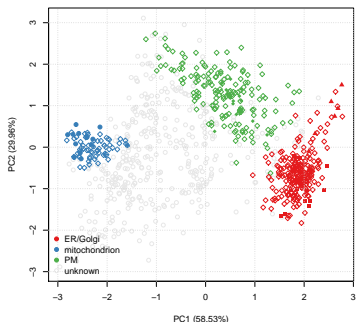
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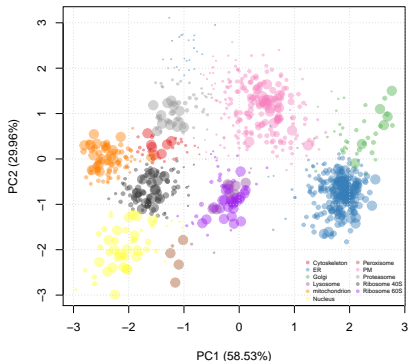
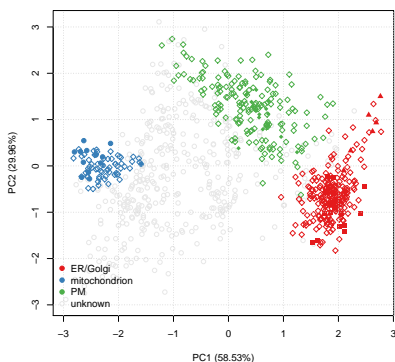
# Importance of annotation



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).

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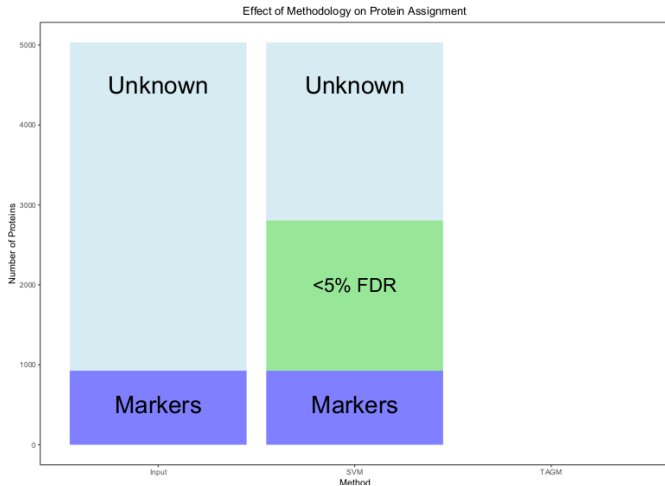
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# How much do we learn? How much do we miss?



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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, 2019).

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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:

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

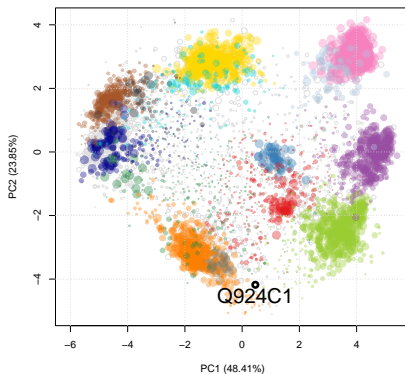
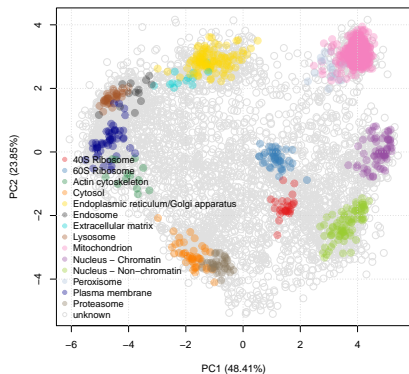
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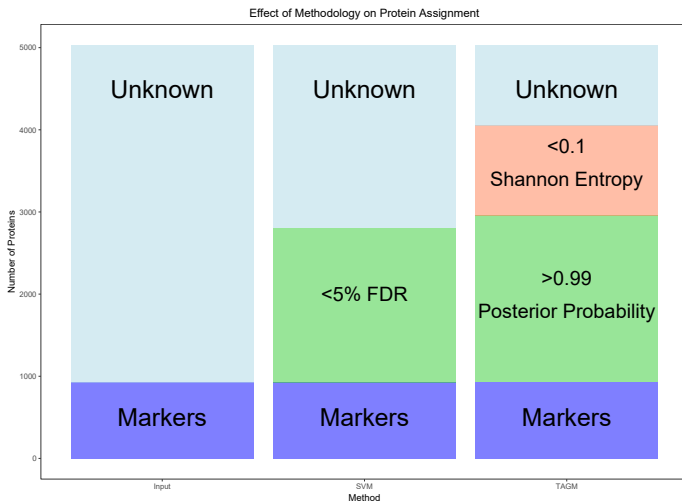
We extend it by introducing an additional *outlier component*. To do this, we augment our model by introducing a further indicator latent variable  $\phi$ . Each protein  $\mathbf{x}_i$  is now described by an additional variable  $\phi_i$ , with  $\phi_i = 1$  indicating that protein  $\mathbf{x}_i$  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  $\kappa$ , mean vector  $\mathbf{M}$ , and scale matrix  $V$ .

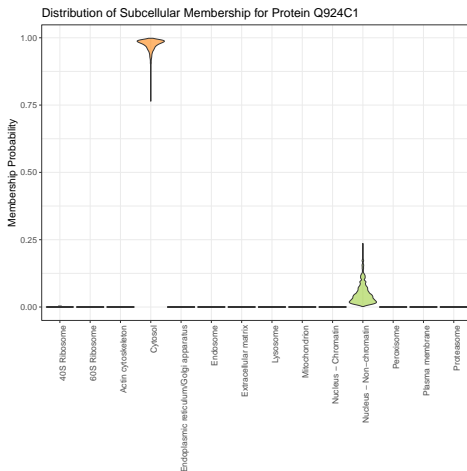
$$\mathbf{x}_i | z_i = k, \phi_i \sim \mathcal{N}(\boldsymbol{\mu}_k, \boldsymbol{\Sigma}_k)^{\phi_i} \mathcal{T}(\kappa, \mathbf{M}, V)^{1-\phi_i} \quad (2)$$





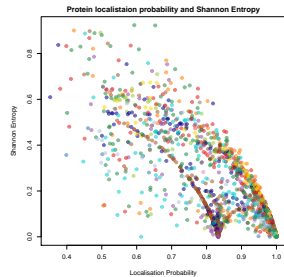
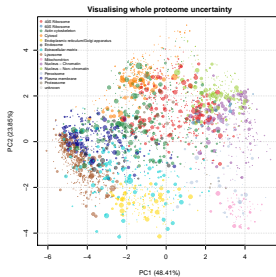
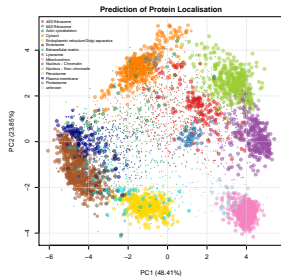
**Figure:** Assignment of proteins of *unknown* location to one of the annotated classes. The dots are scaled according to the protein assignment probabilities.





**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



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**Behind the scenes:** software/data structures and  
open research practice.



## Beyond the figures<sup>1</sup>

- Software: **infrastructure** (MSnbase, Gatto and Lilley (2012)), **dedicated machine learning** (pRoloc, Gatto et al. (2014b)), **interactive visualisation**<sup>2</sup> (pRolocGUI, Breckels et al. (2017)) and **data** (pRolocdata, Gatto et al. (2014b)) for spatial proteomics.

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<sup>1</sup>... which are all reproducible, by the way.

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

<sup>3</sup>between and within domains/software

## Beyond the figures<sup>1</sup>

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

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# Open research: open source software

The image displays two side-by-side screenshots. The left screenshot shows the GitHub repository for 'lgatto / pRoloc'. It includes the repository name, navigation tabs (Code, Issues, Pull requests, Projects, Wiki, Insights, Settings), a description 'A unifying bioinformatics framework for organelle proteomics', and statistics like 2,051 commits, 10 branches, 25 releases, 1 environment, and 14 contributors. Below this is a table of recent commits with columns for file changes, commit message, and time since last commit.

File	Commit Message	Time
R	fix to make work with devel	6 day
data	Merge branch 'master' into devel	2 year
inst	updated documentation	6 month
man	new logPosterior accessor	2 month
src	more C exporting fuss	10 month
tests	add test back	6 day
vignettes	automatic fix of indentation	6 day
.Rbuildignore	ignore .editorconfig when building	3 month
.editorconfig	fix tab with spaces and add editorconfig	4 month
.gitignore	ignore docs, update news	5 month
.travis.yml	Fix indentation	5 month
CONDUCT.md	Merge branch 'master' into devel	3 year
DESCRIPTION	bump devel version on gh	6 day
NAMESPACE	fix notes and warnings	7 day
NEWS	bump devel version on gh	6 day
NEWS.md	bump devel version on gh	6 day

The right screenshot shows the Bioconductor website. It features the Bioconductor logo and navigation links (Home, Install, Help). Below is the 'pRoloc' package page, which includes the package name, version (3.8), a description, author information (Laurent Gatto, Oliver Crook, and Lisa M. Breckels), maintainer information (Laurent Gatto), citation information, and a list of references.

Bioconductor version: Release (3.8)

The pRoloc package implements machine learning and visualisation methods for the analysis and interpretation of quantitative mass spectrometry data to reliably infer protein sub-cellular localisation.

Author: Laurent Gatto, Oliver Crook and Lisa M. Breckels with contributions from Thomas Burger and Samuel Wiecek

Maintainer: Laurent Gatto <laurent.gatto at uclouvain.be>

Citation (from within R, enter `citation("pRoloc")`):

Gatto L, Breckels LM, Wiecek S, Burger T, Lilley KS (2014). "Mass-spectrometry based spatial proteomics data analysis using pRoloc and Proteodata." *Bioinformatics*.

Breckels LM, Gatto L, Christoforou A, Groen AJ, Lilley KS, Trotter MW (2013). "The effect of organelle discovery upon sub-cellular protein localisation." *J Proteomics*.

Gatto L, Breckels LM, Burger T, Nightingale DJ, Groen AJ, Campbell C, Mulvey CM, Christoforou A, Ferro M, Lilley KS (2014). "A foundation for reliable spatial proteomics data analysis." *Mol Cell Proteomics*.

Breckels LM, Holden S, Worjir D, Mulvey CM, Christoforou A, Groen A, Trotter MW, Kohlbacker O, Lilley KS, Gatto L (2016). "Learning from heterogeneous data sources: an application in spatial proteomics." *PLoS Comput Biol*.

Breckels LM, Mulvey CM, Lilley KS, Gatto L (2016). "A Bioconductor workflow for processing and analysing spatial proteomics data." *F1000Research*.

Figure: Gatto et al. (2014a) Left: Public repository for the pRoloc software (<https://github.com/lgatto/pRoloc>). Right: official Bioconductor page.

# Open and reproducible research

The figure consists of three side-by-side screenshots illustrating open and reproducible research practices.

- Left Screenshot (GitHub):** Shows the repository `lgatto / QSep-manuscript`. It displays the repository structure with files like `data`, `figs`, `glgpro`, `travis.yml`, `Makefile`, `README.md`, `cover.pdf`, `cover.tex`, `cover2.pdf`, `e143n1a`, `figs`, `mk.R`, `rekaetch.pptx`, `rekaetch.pptx.pdf`, `rekaetch.rmd`, `qsep.R`, `qsep.Rnw`, `qsep.bib`, `qsep.pdf`, `qsep.tex`, `simu.pdf`, and `simu.R`. The repository has 85 commits and 1 branch.
- Middle Screenshot (bioRxiv):** Shows the preprint `Assessing sub-cellular resolution in spatial proteomics experiments` by Laurent Gatto, Lisa M Broekels, and Kathryn S Lilley. It includes the bioRxiv logo, a search bar, and a list of new results. The preprint is published in *Current Opinion in Chemical Biology* (doi: 10.1016/j.cbpa.2018.11.015).
- Right Screenshot (Current Opinion in Chemical Biology):** Shows the full paper `Assessing sub-cellular resolution in spatial proteomics experiments` by Laurent Gatto, Lisa M Broekels, and Kathryn S Lilley. It includes the Elsevier logo, the journal title, volume, issue, and page information, and a Creative Commons license.

**Figure:** Gatto et al. (2018) reproducible document (<https://github.com/lgatto/QSep-manuscript>), preprint (<https://doi.org/10.1101/377630>) and paper (<https://doi.org/10.1016/j.cbpa.2018.11.015>).

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- Protein sub-cellular localisation: technologies (hyperLOPIT) and opportunities.
- Reliance on computational biology, statistics and dedicated software (pRoLoc *et al.*) to interpret data and acquire biological knowledge.
- 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.
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- 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.
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- 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**

Contact:

`laurent.gatto@uclouvain.be – lgatto.github.io/about`