

Mapping the sub-cellular proteome

Probabilistic modelling of protein sub-cellular localisation

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<http://lgatto.github.io/about>

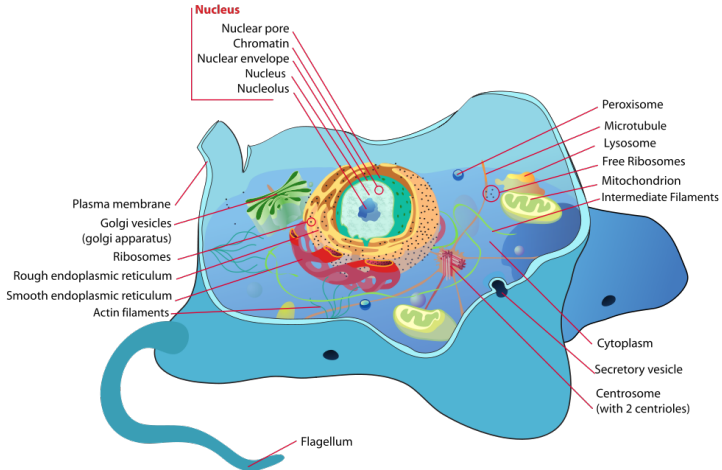
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Protein Folding and Stability

30 August 2019 – Liège

Cell organisation - localisation is function



Spatial proteomics is the systematic study of protein localisations.

Localisation(s) – re-localisation – mis-localisation

Fusion proteins and immunofluorescence

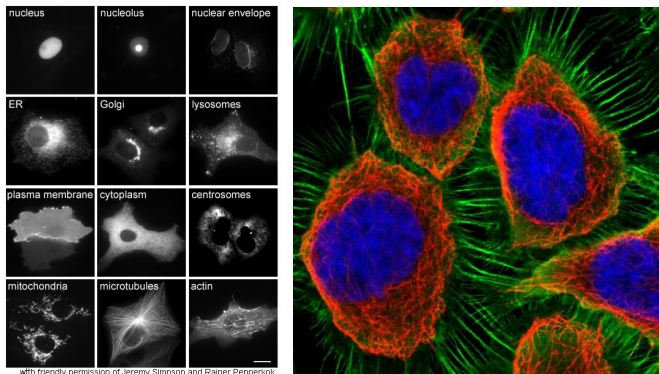
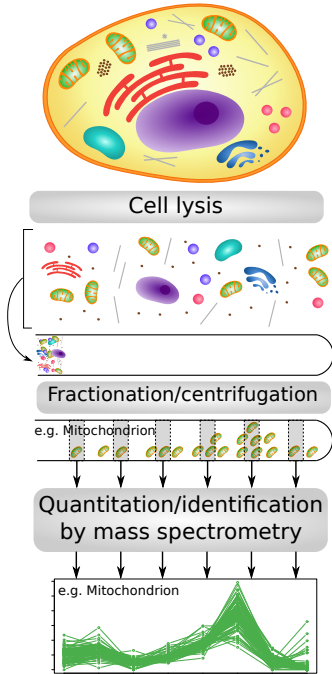


Figure: Targeted protein localisation. Example of discrepancies between IF and FPs as well as between FP tagging at the N and C termini (Stadler et al., 2013).

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](#)).



Quantitation data

	Fraction ₁	Fraction ₂	...	Fraction _L
x₁	x _{1,1}	x _{1,2}	...	x _{1,L}
x₂	x _{2,1}	x _{2,2}	...	x _{2,L}
x₃	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
x ₁	x _{1,1}	x _{1,2}	...	x _{1,L}	unknown
x ₂	x _{2,1}	x _{2,2}	...	x _{2,L}	<i>loc</i> ₁
x ₃	x _{3,1}	x _{3,2}	...	x _{3,L}	unknown
⋮	⋮	⋮	⋮	⋮	⋮
x _i	x _{i,1}	x _{i,2}	...	x _{i,L}	<i>loc</i> _k
⋮	⋮	⋮	⋮	⋮	⋮
x _N	x _{N,1}	x _{N,2}	...	x _{N, K}	unknown

Visualisation

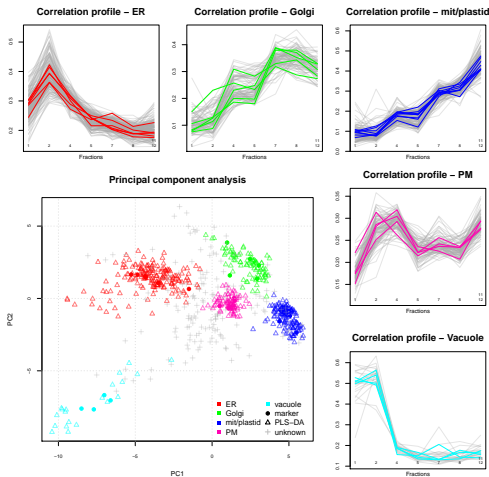


Figure: From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)

Supervised Machine Learning to infer **localisation**

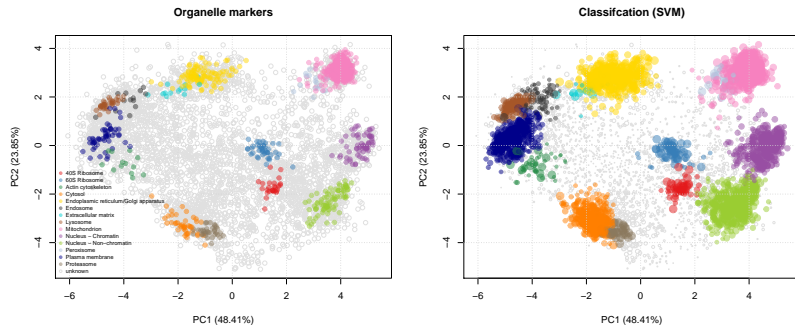
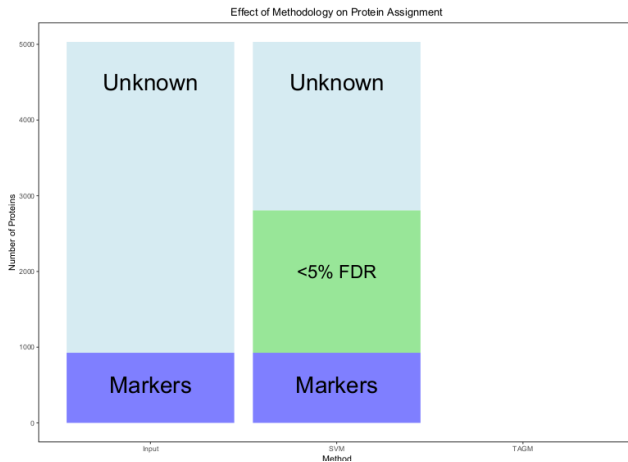


Figure: Support vector machines classifier (after 5% FDR classification cutoff) on the embryonic stem cell data from [Christoforou et al. \(2016\)](#).

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



A Bayesian Mixture Modelling Approach For Spatial Proteomics

- ▶ *T* Augmented Gaussian Mixture model (TAGM) is a **multivariate Gaussian generative model** for MS-based spatial proteomics data. It posits that each annotated sub-cellular niche can be modelled by a multivariate Gaussian distribution.

A Bayesian Mixture Modelling Approach For Spatial Proteomics

- ▶ *T Augmented Gaussian Mixture model (TAGM)* is a **multivariate Gaussian generative model** for MS-based spatial proteomics data. It posits that each annotated sub-cellular niche can be modelled by a multivariate Gaussian distribution.
- ▶ 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 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 .

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

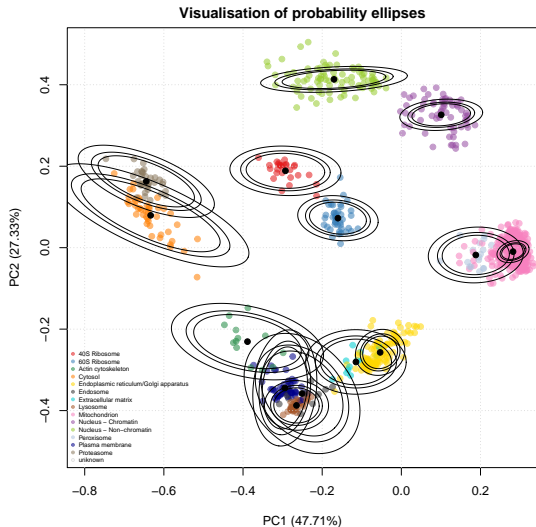


Figure: Illustration of how the TAGM model describes the pluripotent mouse embryonic stem cell data. Each ellipse contains a proportion of total probability of a particular multivariate Gaussian density. The outer ellipse contains 99% of the total probability whilst the middle and inner ellipses contain 95% and 90% of the probability respectively.

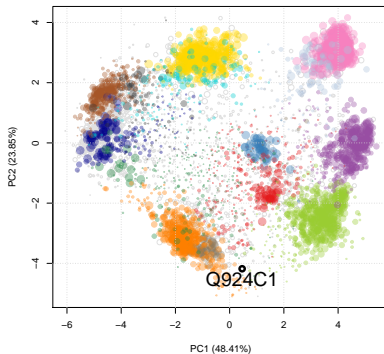
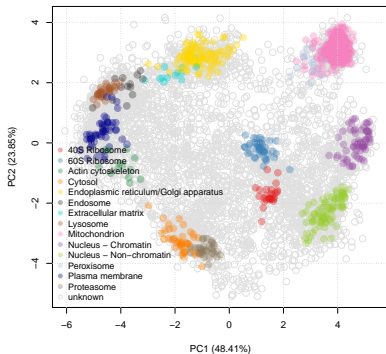
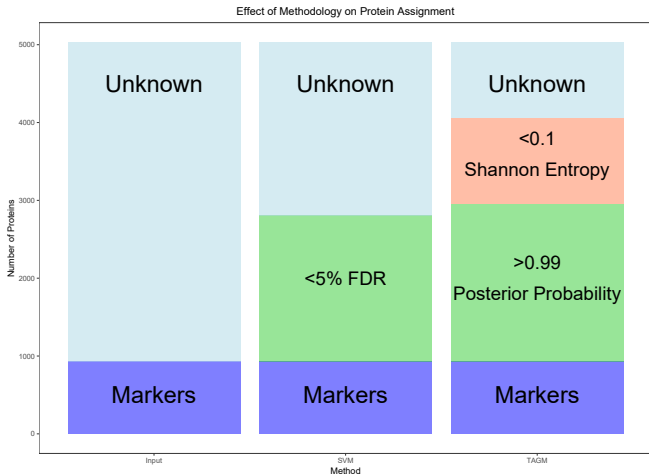


Figure: Assignment of proteins of *unknown* location to one of the annotated classes. The dots are scaled according to the protein assignment probabilities.



Multi-localisation: localisations

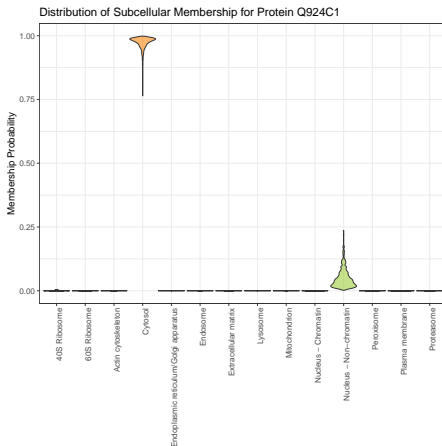
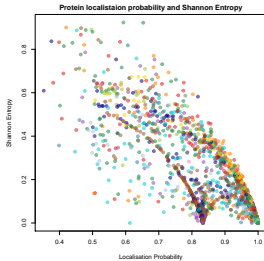
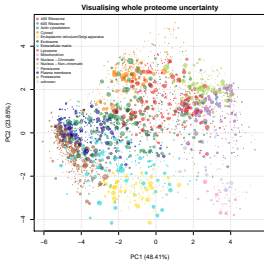
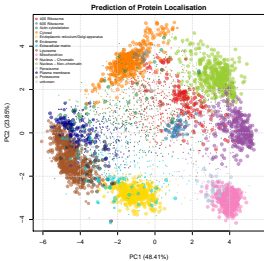


Figure: Exportin 5 (Q924C1) forms part of the micro-RNA export machinery, transporting miRNA from the nucleus to the cytoplasm for further processing. It then translocates back through the nuclear pore complex to return to the nucleus to mediate further transport between nucleus and cytoplasm. The model 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



Spatial dynamics

Trans-localisation event during monocyte to macrophage differentiation

Investigate the effect of lipopolysaccharides (LPS)-mediated inflammatory response in human monocytic cells (THP-1)

Data

- ▶ Triplicate **temporal** profiling (0, 2, 4, 6, 12, 24 hours).
- ▶ Triplicate **spatial** profiling (0 vs 12 hours) - early trafficking, before actual morphological differentiation at 24h.

Work lead by **Dr Claire Mulvey**, Cambridge.

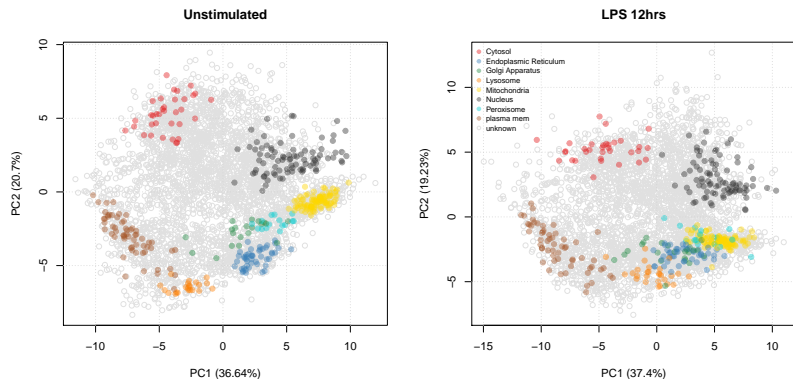


Figure: Spatial maps of unstimulated and LPS-treated cells (combined triplicates).

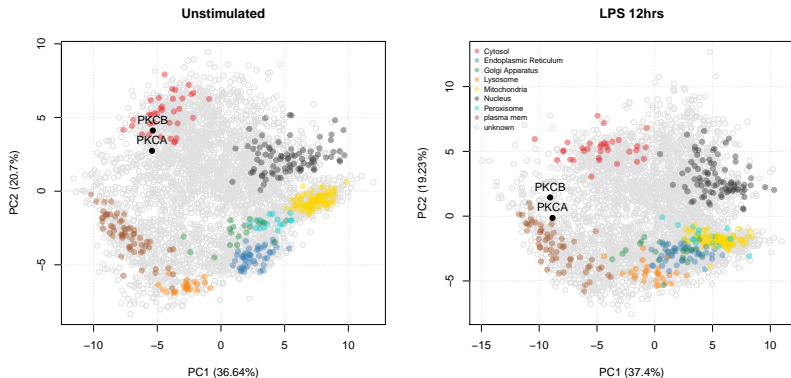


Figure: Relocation of Protein Kinase C α and β from the cytosol to the plasma membrane, **driving maturation into a differentiated macrophage phenotype.**

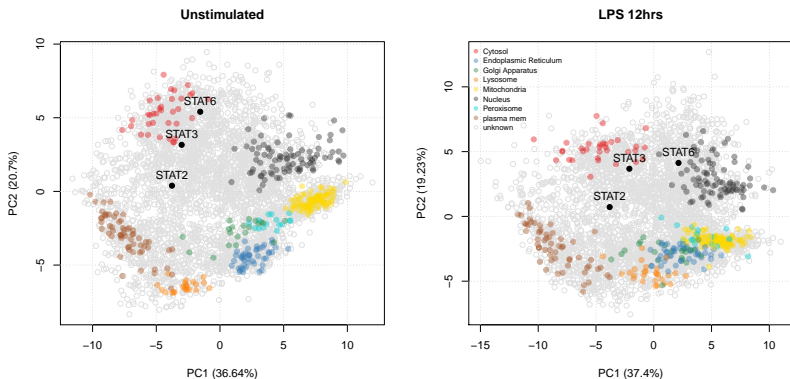


Figure: Relocation of Signal transducer and activator of transcription 6 (STAT6) from the cytosol to the Nucleus, **activating anti-bacterial and anti-viral-like response**. Validated by microscopy and see also [Chen et al. \(2011\)](#).

Folding and stability

- ▶ Effect of (sub-)cellular environment on protein folding.
- ▶ Different sub-cellular micro-environments driving different conformations.
- ▶ Re-localisation upon protein post-translational modification.
- ▶ Effect of localisation on protein structure.

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

Beyond the figures¹

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

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

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

³between and within domains/software

Beyond the figures¹

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- ▶ 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**.

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Open research: open source software

The screenshot shows the GitHub repository for **lgatto / pRoloc**. The repository description is "A unifying bioinformatics framework for organelle proteomics" with a link to <http://lgatto.github.io/pRoloc/>. It has 2,051 commits, 10 branches, 25 releases, 1 environment, and 14 contributors. The repository includes a file tree with folders like `R`, `data`, `inst`, `man`, `src`, `tests`, `vignettes`, and files like `_Rbuildignore`, `.editorconfig`, `gitignore`, `travis.yml`, `CONDUCT.md`, `DESCRIPTION`, `NAMESPACE`, `NEWS`, and `NEWS.md`. Each file has a commit message and a timestamp.

The screenshot shows the Bioconductor website for the **pRoloc** package. The Bioconductor logo is at the top, with the tagline "OPEN SOURCE SOFTWARE FOR BIOINFORMATICS". The navigation bar includes "Home", "Install", and "Help". The main content area shows the package name **pRoloc** and the version **3.8**. It includes statistics: 254 forks, 164 stars, 17 posts, 2/2/0 in BioC, and 6 years. The DOI is [10.18129/B3.bios.pRoloc](https://doi.org/10.18129/B3.bios.pRoloc). The description states: "The pRoloc package implements machine learning and visualisation methods for the analysis and interrogation of quantitative mass spectrometry data to reliably infer protein sub-cellular localisation." The authors are Laurent Gatto, Oliver Crook and Lisa M. Breckels. The citation is: Gatto L, Breckels LM, Wiecek S, Burger T, Lilley KS (2014). "Mass-spectrometry based spatial proteomics data analysis using pRoloc and pRolocdata." *Bioinformatics*.

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

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Assessing sub-cellular resolution in spatial proteomics experiments

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figure	Update for bioRxiv
alignore	add cover letter 2
train.pyd	add track file
Makelive	addressing more reviews comments
README.md	Update README.md
cover.pdf	add cover letter
cover.tex	add cover letter
cover2.pdf	add cover letter 2
r1data_rds	qrap assessment section with rls-cluster sims
t1data_rds	qrap assessment section with rls-cluster sims
mck.R	Calculate qrap distribution medians
mkswitch_qcap.pdf	incorporate Kathryn and Laura's comments
mkswitch-qcap.pdf	incorporate Kathryn and Laura's comments
mkswitch_rds	minor updates and change marker transfer paragraph
qcap.R	fix table
qcap.Prev	Update for bioRxiv
qcap.lib	changes to new part in col
qcap.pdf	Update for bioRxiv
qcap.tex	Update for bioRxiv
sims.pdf	incorporate Kathryn and Laura's comments
sims.R	incorporate Kathryn and Laura's comments



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Assessing sub-cellular resolution in spatial proteomics experiments

Laurent Gatlin, Lisa M Bruckley, Kathryn S Lilley
doi: <https://doi.org/10.1101/377930>

Now published in *Current Opinion in Chemical Biology* doi: [10.1016/j.cob.2016.11.015](https://doi.org/10.1016/j.cob.2016.11.015)

Abstract

Info-Pictory

Metrics

Preview PDF

Abstract

The sub-cellular localisation of a protein is vital in defining its function, and a protein's mis-localisation is known to lead to adverse effect. As a result, numerous experimental techniques and datasets have been published, with the aim of deciphering the localisation of proteins at various scales and resolutions, including high-profile mass spectrometry-based efforts. Here, we present a meta-analysis assessing and comparing the sub-cellular resolution of 29 such mass spectrometry-based spatial proteomics experiments using a newly developed tool termed QSep. Our goal is to provide a simple quantitative report of how well spatial proteomics resolve the sub-cellular niches they describe to inform and guide developers and users of such methods.

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The image is a screenshot of a web browser displaying the Elsevier article page for the paper "Assessing sub-cellular resolution in spatial proteomics experiments". At the top, there is a navigation bar with the Elsevier logo, a search bar, and links for "Outline", "Download", "Share", and "Export". Below this, the journal title "Current Opinion in Chemical Biology" is displayed, along with the volume and issue information: "Volume 48, February 2019, Pages 123-149". The article title "Assessing sub-cellular resolution in spatial proteomics experiments" is prominently featured in a large, bold font. Below the title, the authors "Laurent Gatto ^{1,2,3}", "Liza M. Brockels ²", and "Kathryn S. Lilley ²" are listed, followed by a "Show more" link. The article's DOI, "https://doi.org/10.1016/j.copbio.2018.11.015", and a link to "Get rights and content" are also visible. A Creative Commons license logo is present. The abstract section begins with the word "Abstract" in a large font, followed by a paragraph of text: "The **sub-cellular localisation** of a protein is vital in defining its function, and a protein's mis-localisation is known to lead to adverse effect. As a result, numerous **experimental techniques** and datasets have been published, with the aim of deciphering the localisation of proteins at various scales and resolutions, including high profile mass spectrometry-based efforts. Here, we present a meta-analysis assessing and comparing the sub-cellular resolution of 29 such mass spectrometry-based spatial **proteomics** experiments using a newly developed tool termed QSp. Our goal is to provide a simple quantitative report of how well spatial proteomics resolve the sub-cellular niches they describe to inform and guide developers and users of such methods."

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>).

Working with open and reproducible research in mind doesn't mean releasing everything prematurely, it means

- ▶ managing research in a way one can find data and results at every stage
- ▶ one can reproduce results, re-run/compare them with new data or different methods/parameters, and
- ▶ one can release data (or parts thereof) when/if appropriate.

Conclusions

- ▶ Protein sub-cellular localisation: *localisation is function*.
- ▶ Reliance on computational biology, statistics and dedicated software (for example MSnbase ([Gatto and Lilley, 2012](#)), pRoLoc ([Gatto et al., 2014a](#))) to interpret data and acquire biological knowledge (details not shown).
- ▶ Rigorous computational infrastructure and sound data analysis and interpretation is a **long term investment**.

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- ▶ Kathryn Lilley (U of Cambridge): spatial proteomics data.
- ▶ Funding: BBSRC (UK), Wellcome Trust (UK), FNRS (BE)

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Thank you for your attention