

Mapping the sub-cellular proteome

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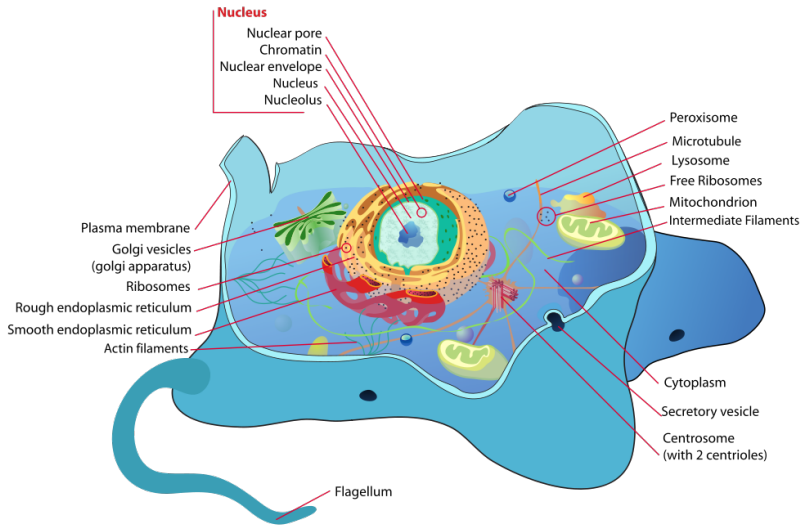
Abstract

In biology, **localisation is function** - understanding the sub-cellular localisation of proteins is paramount to comprehend the context of their functions. Mass spectrometry-based **spatial proteomics** and contemporary **machine learning** enable to build proteome-wide spatial maps, informing us on the location of thousands of proteins. Nevertheless, while some proteins can be found in a single location within a cell, up to half of proteins may reside in multiple locations, can dynamically re-localise, or reside within an unknown functional compartment, leading to considerable **uncertainty** in associating a protein to their sub-cellular location. Recent advances enable us to **probabilistically** model protein localisation as well as quantify the uncertainty in the location assignments, thus leading to better and more trustworthy biological interpretation of the data.

1. **Use case:** spatial proteomics.
2. Novel **computational biology research and developments** to acquire reliable biological knowledge.
3. **Behind the scenes:** software/data structures and open research practice.

Use case: spatial proteomics.

Cell organisation - regulation of protein localisation



Spatial proteomics is the systematic study of protein localisations.

Spatial proteomics - Why?

Localisation is function

- ▶ The cellular sub-division allows cells to establish a range of distinct micro-environments, each favouring different biochemical reactions and interactions and, therefore, allowing each compartment to fulfil a particular functional role.
- ▶ Localisation and sequestration of proteins within sub-cellular niches is a fundamental mechanism for the post-translational regulation of protein function.

Re-localisation in

- ▶ **Differentiation** stem cells.
- ▶ **Activation** of biological processes.

Spatial proteomics - Why?

Mis-localisation

Disruption of the targeting/trafficking process alters proper sub-cellular localisation, which in turn perturb the cellular functions of the proteins.

- ▶ Abnormal protein localisation leading to the **loss of functional** effects in diseases ([Laurila and Vihinen, 2009](#)).
- ▶ Disruption of the nuclear/cytoplasmic transport (nuclear pores) have been detected in many types of **carcinoma cells** ([Kau et al., 2004](#)).
- ▶ Sub-cellular localisation of MC4R with ADCY3 at neuronal primary cilia underlies a common pathway for genetic predisposition to **obesity** ([Siljee et al., 2018](#)).

Spatial proteomics - How, experimentally

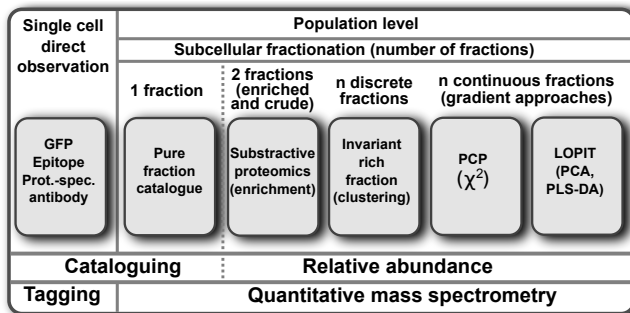


Figure: Organelle proteomics approaches ([Gatto et al., 2010](#))

Fusion proteins and immunofluorescence

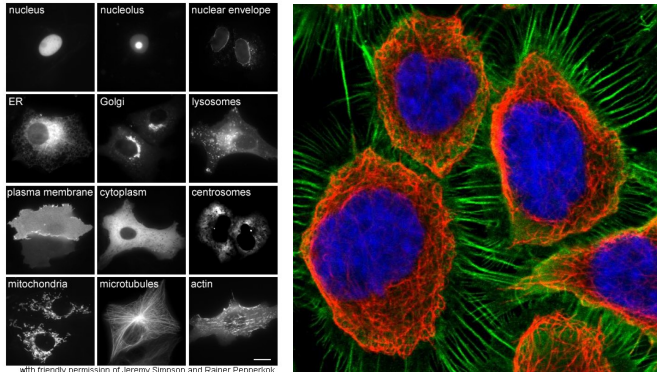


Figure: Targeted protein localisation. Example of discrepancies between IF and FPs as well as between FP tagging at the N and C termini (Stadler et al., 2013).

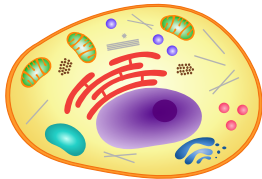
Spatial proteomics - How, experimentally

Single cell direct observation	Population level				
	Subcellular fractionation (number of fractions)				
	1 fraction	2 fractions (enriched and crude)	n discrete fractions	n continuous fractions (gradient approaches)	
	GFP Epitope Prot.-spec. antibody	Pure fraction catalogue	Subtractive proteomics (enrichment)	Invariant rich fraction (clustering)	PCP (χ^2)
Cataloguing		Relative abundance			
Tagging	Quantitative mass spectrometry				

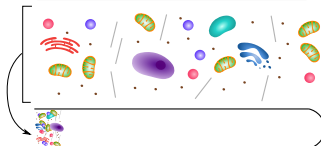
Figure: Organelle proteomics approaches ([Gatto et al., 2010](#)).

Gradient approaches: [Dunkley et al. \(2006\)](#), [Foster et al. \(2006\)](#).

Explorative/discovery approaches, [steady-state global localisation maps](#).

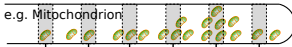


Cell lysis



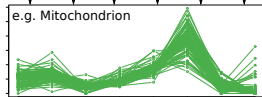
Fractionation/centrifugation

e.g. Mitochondrion



Quantitation/identification
by mass spectrometry

e.g. Mitochondrion



Quantitation data

	Fraction ₁	Fraction ₂	...	Fraction _m
p ₁	q _{1,1}	q _{1,2}	...	q _{1,m}
p ₂	q _{2,1}	q _{2,2}	...	q _{2,m}
p ₃	q _{3,1}	q _{3,2}	...	q _{3,m}
p ₄	q _{4,1}	q _{4,2}	...	q _{4,m}
⋮	⋮	⋮	⋮	⋮
p _j	q _{j,1}	q _{j,2}	...	q _{j, m}

Quantitation data and organelle markers

	Fraction ₁	Fraction ₂	...	Fraction _m	markers
p ₁	q _{1,1}	q _{1,2}	...	q _{1,m}	unknown
p ₂	q _{2,1}	q _{2,2}	...	q _{2,m}	<i>loc₁</i>
p ₃	q _{3,1}	q _{3,2}	...	q _{3,m}	unknown
p ₄	q _{4,1}	q _{4,2}	...	q _{4,m}	<i>loc_i</i>
⋮	⋮	⋮	⋮	⋮	⋮
p _j	q _{j,1}	q _{j,2}	...	q _{j, m}	unknown

Data analysis

Data analysis

- ▶ Visualisation (cluster, unsupervised learning)
- ▶ Classification (supervised learning)
- ▶ Novelty detection (semi-supervised learning)
- ▶ Data integration (transfer learning)
- ▶ Multi-localisation (Bayesian spatial proteomics)
- ▶ Spatial dynamics

To uncover and understand biology

Visualisation

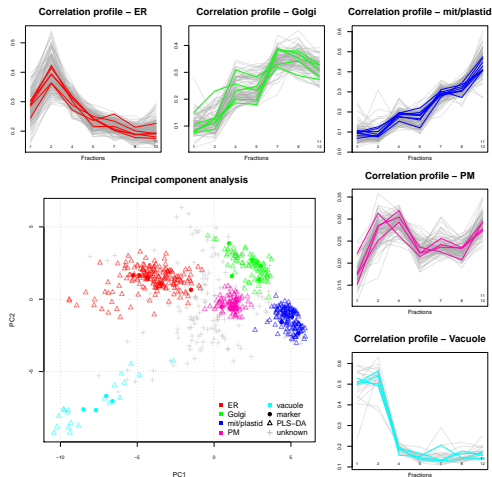


Figure: From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)

Supervised Machine Learning

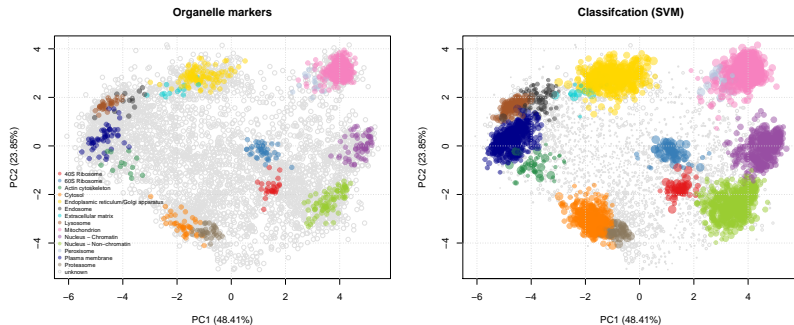
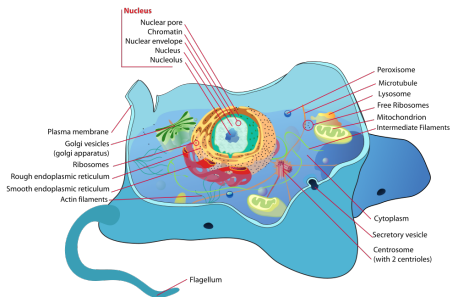
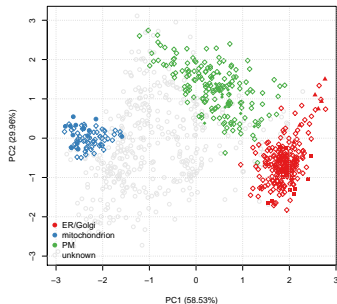


Figure: Support vector machines classifier (after 5% FDR classification cutoff) on the embryonic stem cell data from [Christoforou et al. \(2016\)](#).

Novel **computational biology research and developments** to acquire reliable biological knowledge.

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

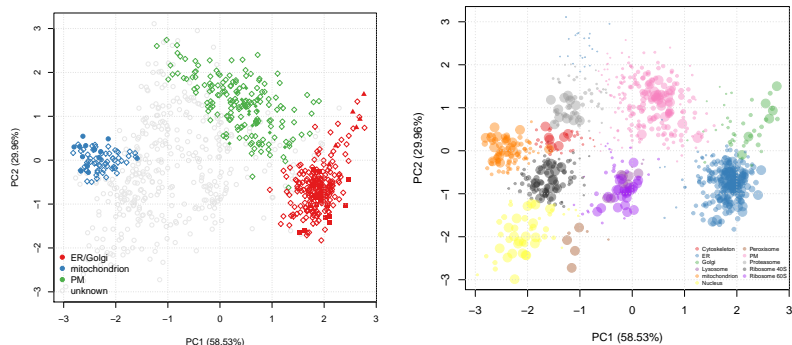


Figure: Left: Original *Drosophila* data from Tan et al. (2009). Right: After semi-supervised learning and classification, Breckels et al. (2013).

Computational advances: Transfer learning

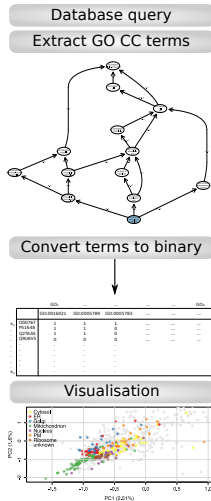
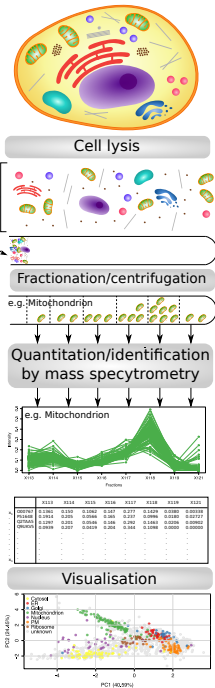
What about using **addition data**, such as annotations from the Gene Ontology (GO), sequence features (pseudo aminoacid composition), signal peptide, trans-membrane domains (length, number, ...), images (IF, FP), interaction data, prediction software, ...

- ▶ From a user perspective: "**free/cheap**" vs. expensive and time-consuming experiments.
- ▶ Abundant (all proteins, 100s of features) vs. (experimentally) limited/**targeted** (1000s of proteins, 6 – 20 of features)
- ▶ For localisation in system at hand: *low* vs. high **quality**
- ▶ **Static** vs. **dynamic**

Transfer learning

Support/complement the **primary** target domain (experimental data) with **auxiliary** data (annotation, imaging, PPI, ...) features without compromising the integrity of our primary data.

PRIMARY EXPERIMENTAL DATA



AUXILIARY DRY DATA

Transfer learning results

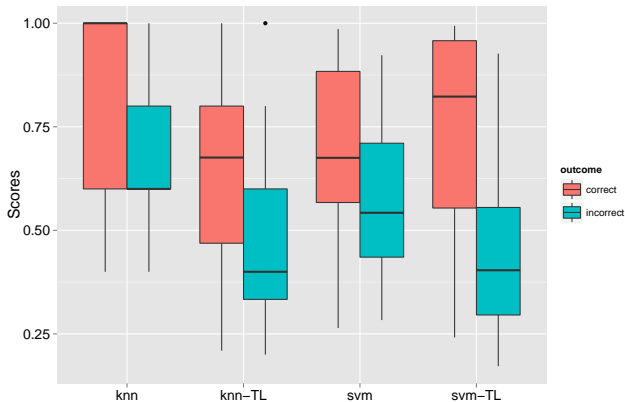
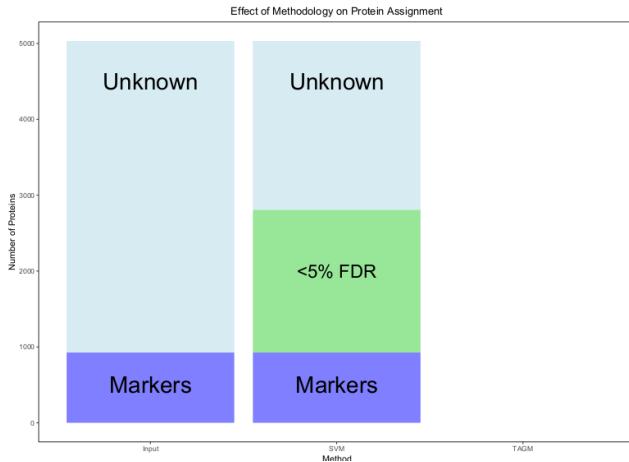


Figure: From Breckels et al. (2016) *Learning from heterogeneous data sources: an application in spatial proteomics*.

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*.

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

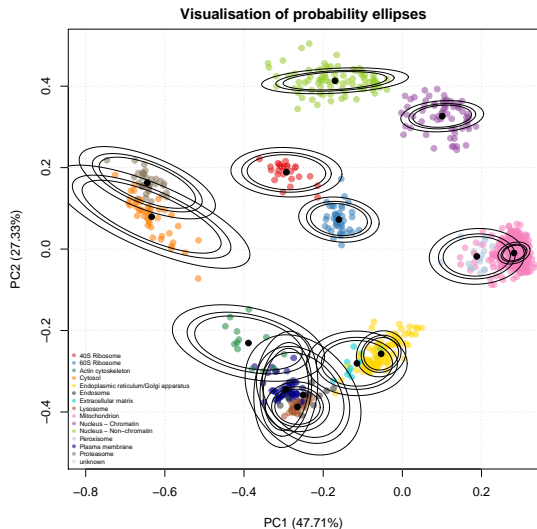


Figure: Illustration of how the TAGM model describes the pluripotent mouse embryonic stem cell data. Each ellipse contains a proportion of total probability of a particular multivariate Gaussian density. The outer ellipse contains 99% of the total probability whilst the middle and inner ellipses contain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

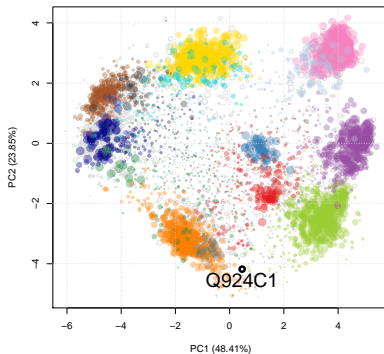
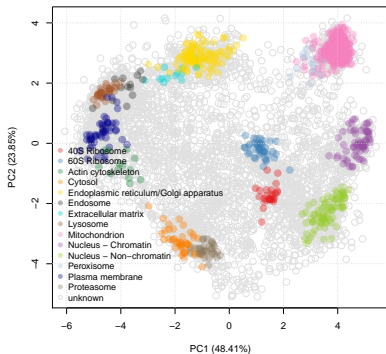
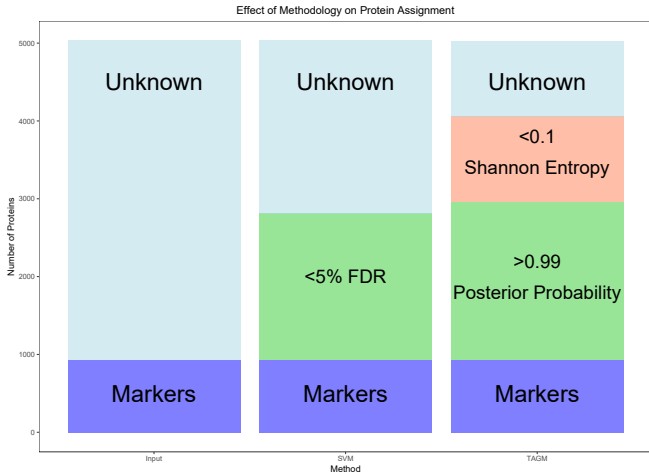


Figure: Assignment of proteins of *unknown* location to one of the annotated classes. The dots are scaled according to the protein assignment probabilities.



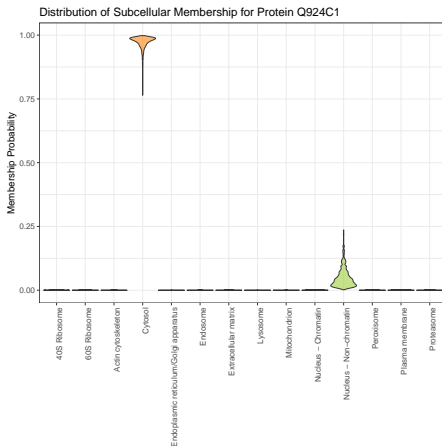
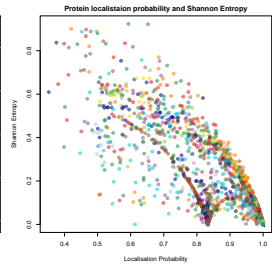
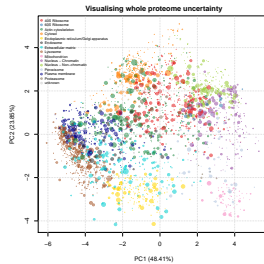
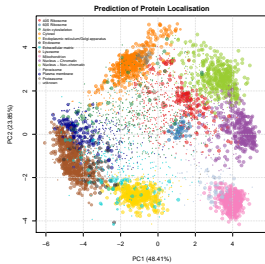


Figure: Exportin 5 (Q924C1) forms part of the micro-RNA export machinery, transporting miRNA from the nucleus to the cytoplasm for further processing. It then translocates back through the nuclear pore complex to return to the nucleus to mediate further transport between nucleus and cytoplasm. The model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

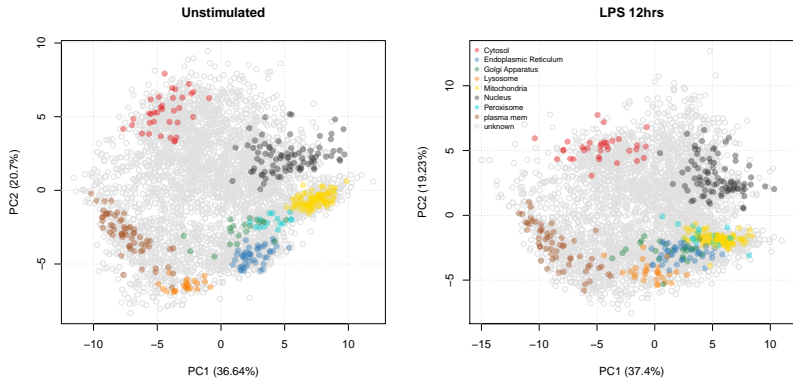


Figure: Spatial maps of unstimulated and LPS-treated cells (combined triplicates).

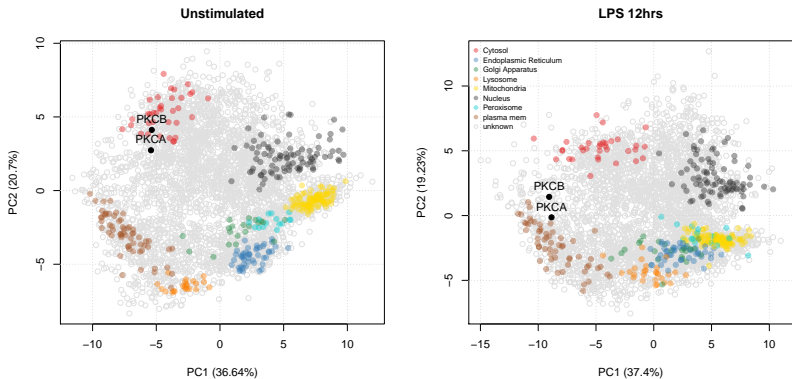


Figure: Relocation of Protein Kinase C α and β from the cytosol to the plasma membrane, **driving maturation into a differentiated macrophage phenotype.**

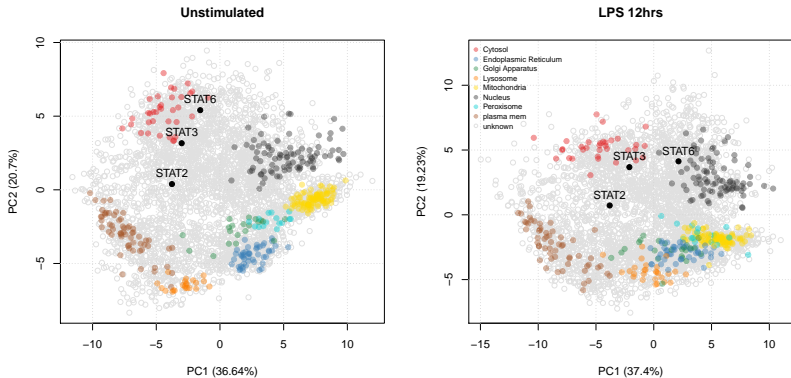


Figure: Relocation of Signal transducer and activator of transcription 6 (STAT6) from the cytosol to the Nucleus, **activating anti-bacterial and anti-viral-like response**. Validated by microscopy and see also [Chen et al. \(2011\)](#).

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

- ▶ 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.
- ▶ 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

Open research: open source software

The screenshot shows the GitHub repository for **lgatto / pRoloC**. The repository is described as "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 is in the **master** branch. A table of files is shown below:

File	Description	Latest commit	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 logPosterioris 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
NAMESPAC	fix notes and warnings		7 day
NEWS	bump devel version on gh		6 day
NEWS.md	bump devel version on gh		6 day

The screenshot shows the Bioconductor website for the **pRoloC** package. The Bioconductor logo is at the top left, and the navigation bar includes **Home**, **Install**, and **Help**. The package page shows the version **3.8** and the description: "A unifying bioinformatics framework for spatial proteomics". The package is available on **platforms: all**, has a **Rank: 254 / 1649**, **posts: 1 / 2 / 2 / 0**, and is **in Bioc: 6 years**. The **build** status is **warnings** and it was **updated < 1 week**. The **DOR** is [10.18129/J9.2000.pRoloC](https://doi.org/10.18129/J9.2000.pRoloC). The package description states: "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." The authors are Laurent Gatto, Oliver Crook and Lisa M. Breckels, with contributions from Thomas Burger and Samuel Wleczorek. The citation is: "Gatto L, Breckels LM, Wleczorek S, Burger T, Lilley KS (2014). 'Mass-spectrometry based spatial proteomics data analysis using pRoloC and pRoloCdata.' Bioinformatics." The package is available on **platforms: all**, has a **Rank: 254 / 1649**, **posts: 1 / 2 / 2 / 0**, and is **in Bioc: 6 years**.

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-ocular resolution in spatial proteomics experiments

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[Manage tags](#)

[195](#) commits
 [p1](#) branch
 [0](#) releases

[Branch: master](#)
[New pull request](#)
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File	Commit	Message
figs	figs	Update README.md
figs	figs	add data
figs	figs	Update for bioRxiv
figs	figs	add cover letter 2
figs	figs	add cover letter 1
figs	figs	add Travis file
figs	figs	addressing more reviewers comments
figs	figs	add Travis file
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figs	figs	incorporate Kallrithy and Lavin's comments
figs	figs	minor updates and change marker transfer paragraph
figs	figs	fix table
figs	figs	Update for bioRxiv
figs	figs	changes to new part in col
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figs	figs	incorporate Kallrithy and Lavin's comments



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Assessing sub-cellular resolution in spatial proteomics experiments

Laurent Gatto, Lisa M Breckels, Kathryn S Lilley
<https://doi.org/10.1101/277830>

Now published in *Current Opinion in Cellular Biology* doi: 10.1016/j.cob.2018.11.015

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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 QSub. 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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A screenshot of the Elsevier website. At the top, there is a navigation bar with the Elsevier logo, the text 'ScienceDirect', and several icons for navigation: a magnifying glass, a document, a folder, and a shopping cart. Below the navigation bar, there is a large banner area. On the left, there is a small image of a classical building. To its right, the text 'Current Opinion in Chemical Biology' is displayed in a large, bold font. Below this, the text 'Volume 48, February 2015, Pages 123-149' is shown. On the right side of the banner, there is a small thumbnail image of the article cover. Below the banner, the article title 'Assessing sub-cellular resolution in spatial proteomics experiments' is prominently displayed in a large, bold font. Underneath the title, the authors 'Laurent Gatto ^{1,2,3,4}, Lisa M. Breckels ², Kathryn S. Lilley ²' are listed. To the right of the authors, there is a link 'Get rights and content' and a button 'open access'. Below the authors, the URL 'https://doi.org/10.1016/j.cop.2015.11.015' is provided. Underneath the URL, there is a link 'Under a Creative Commons license'. Below this, the word 'Abstract' is displayed in a large, bold font. The abstract text follows, starting with '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 QSeq. Our goal is to provide a simple quantitative overview 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: technologies (hyperLOPIT) and opportunities.
- ▶ Reliance on computational biology 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**.

References I

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