Probabilistic modelling of protein sub-cellular localisation

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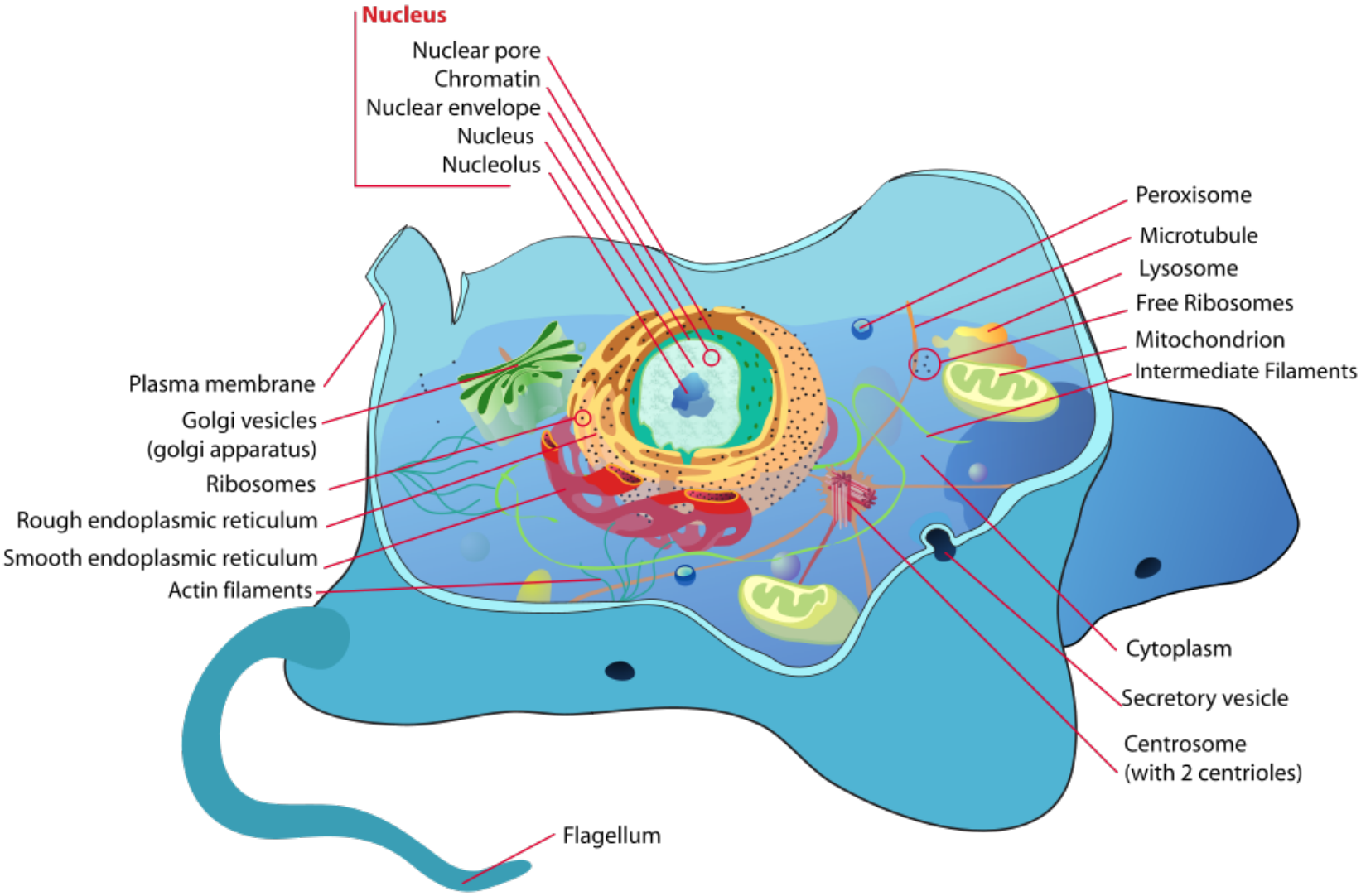
Abstract

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. **Assessing uncertainty** 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.

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

Spatial proteomics - Why?

- **Localisation is function**: Localisation and sequestration of proteins within sub-cellular niches is a fundamental mechanism for the post-translational regulation of protein function.
- **Re-localisation**: differentiation stem cells, activation of biological processes.
- **Mis-localisation**: Disruption of the targeting/trafficking process alters proper sub-cellular localisation, which in turn perturb the cellular functions of the proteins.

Spatial proteomics - How, experimentally

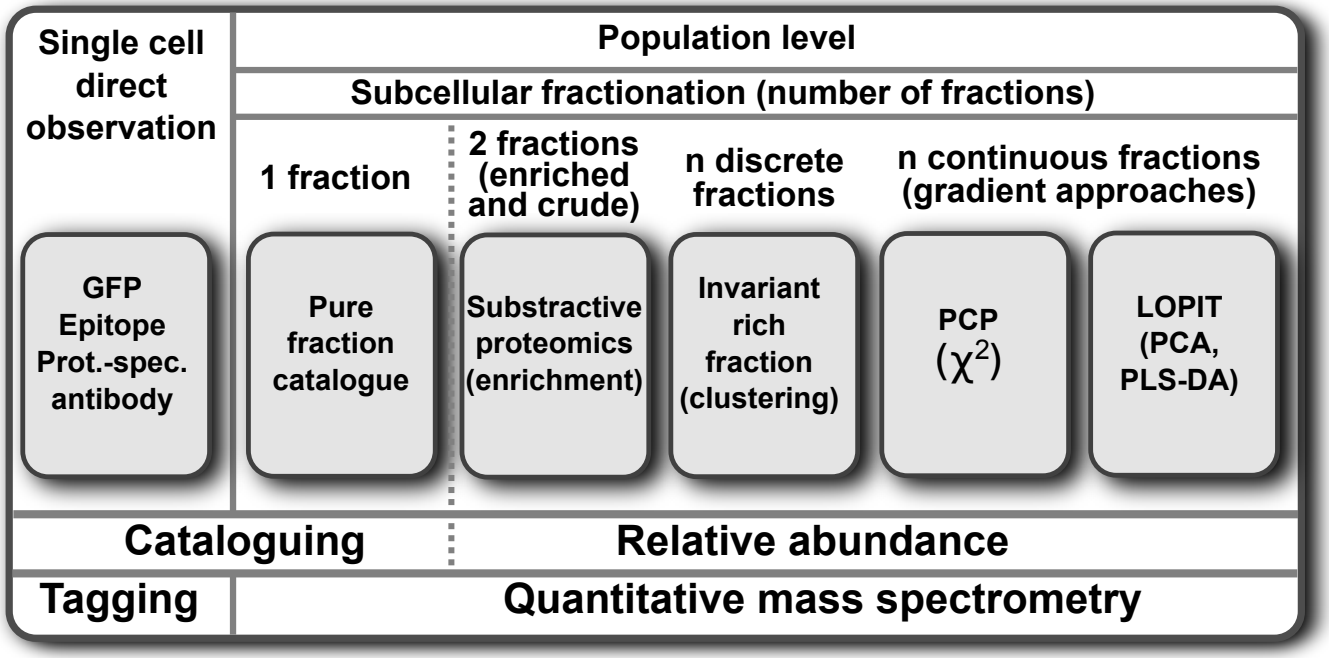
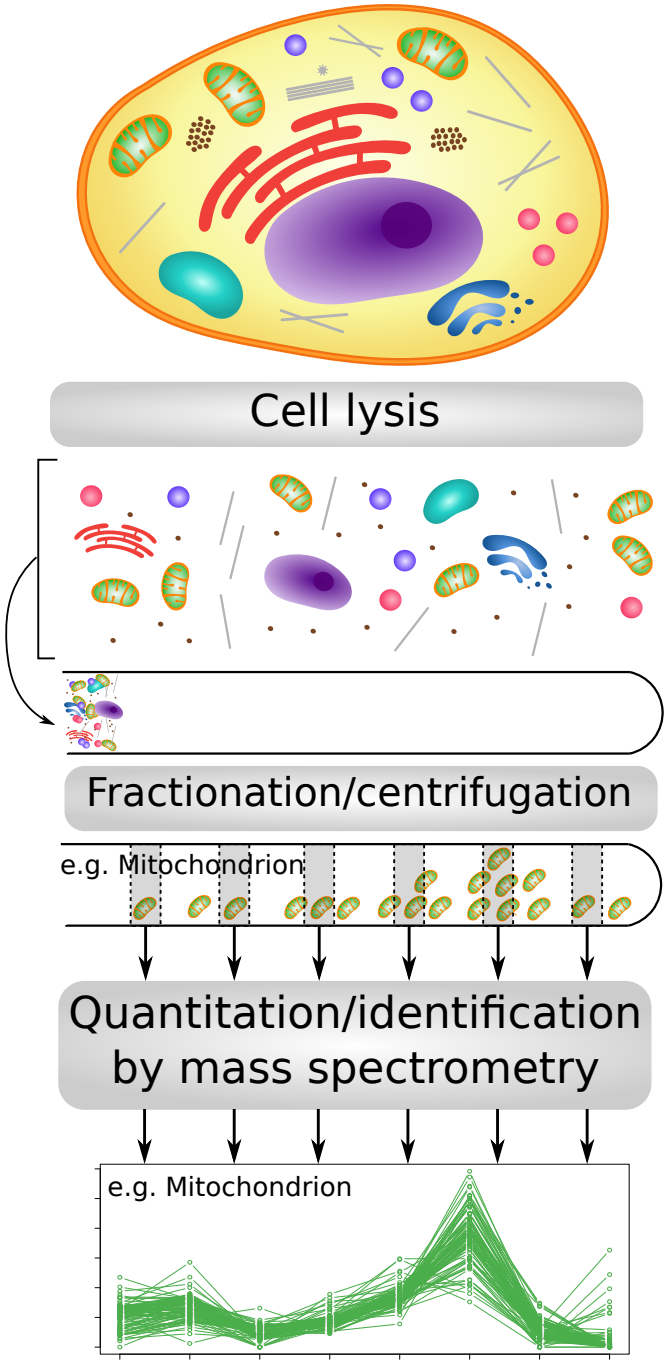


Figure: Organelle proteomics approaches (Gatto et al., 2010).

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_L
$\mathbf{x}_1$	$x_{1,1}$	$x_{1,2}$	...	$x_{1,L}$
$\mathbf{x}_2$	$x_{2,1}$	$x_{2,2}$	...	$x_{2,L}$
$\mathbf{x}_3$	$x_{3,1}$	$x_{3,2}$	...	$x_{3,L}$
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
$\mathbf{x}_i$	$x_{i,1}$	$x_{i,2}$	...	$x_{i,L}$
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
$\mathbf{x}_N$	$x_{N,1}$	$x_{N,2}$	...	$x_{N,\ L}$

Quantitation data and organelle markers

	Fraction_1	Fraction_2	...	Fraction_L	markers
$\mathbf{x}_1$	$x_{1,1}$	$x_{1,2}$	...	$x_{1,L}$	unknown
$\mathbf{x}_2$	$x_{2,1}$	$x_{2,2}$	...	$x_{2,L}$	loc_1
$\mathbf{x}_3$	$x_{3,1}$	$x_{3,2}$	...	$x_{3,L}$	unknown
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
$\mathbf{x}_i$	$x_{i,1}$	$x_{i,2}$	...	$x_{i,L}$	loc_k
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
$\mathbf{x}_N$	$x_{N,1}$	$x_{N,2}$	...	$x_{N,\ K}$	unknown

Data analysis

Visualisation

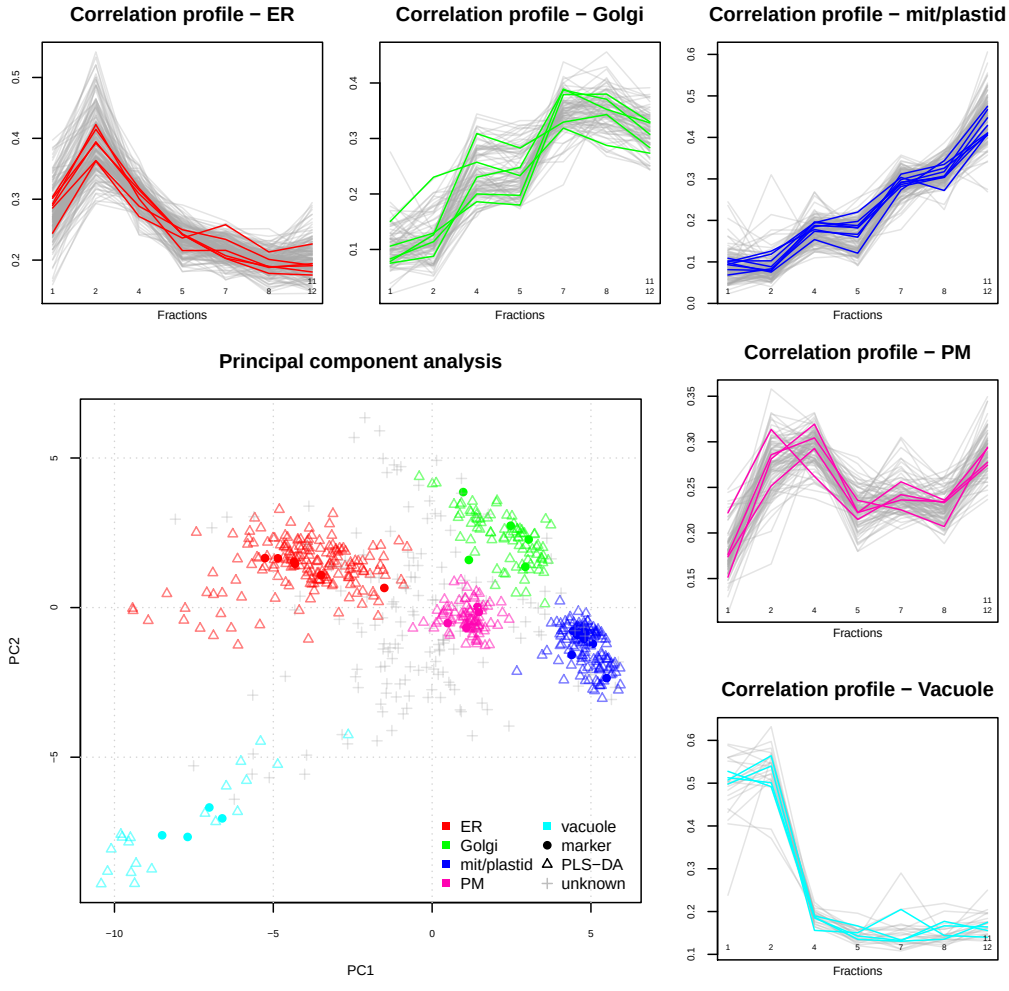


Figure: From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)

Supervised Machine Learning

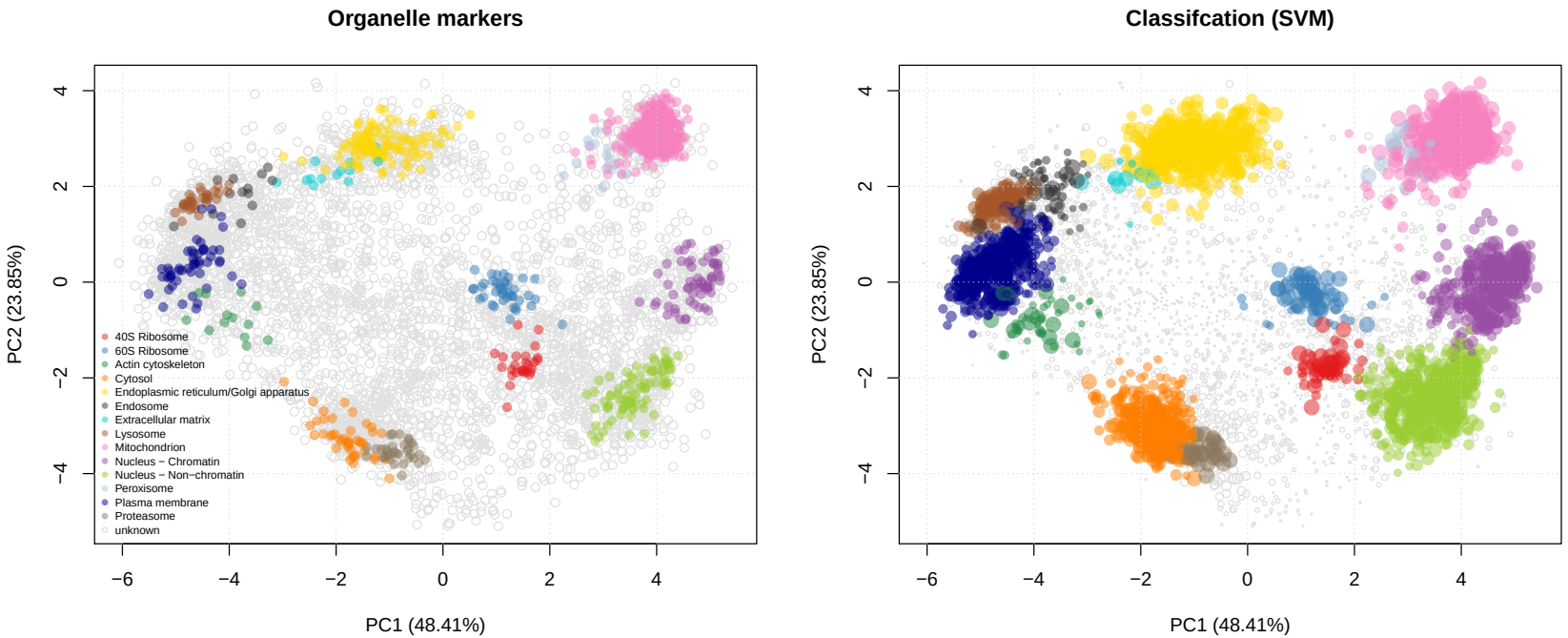
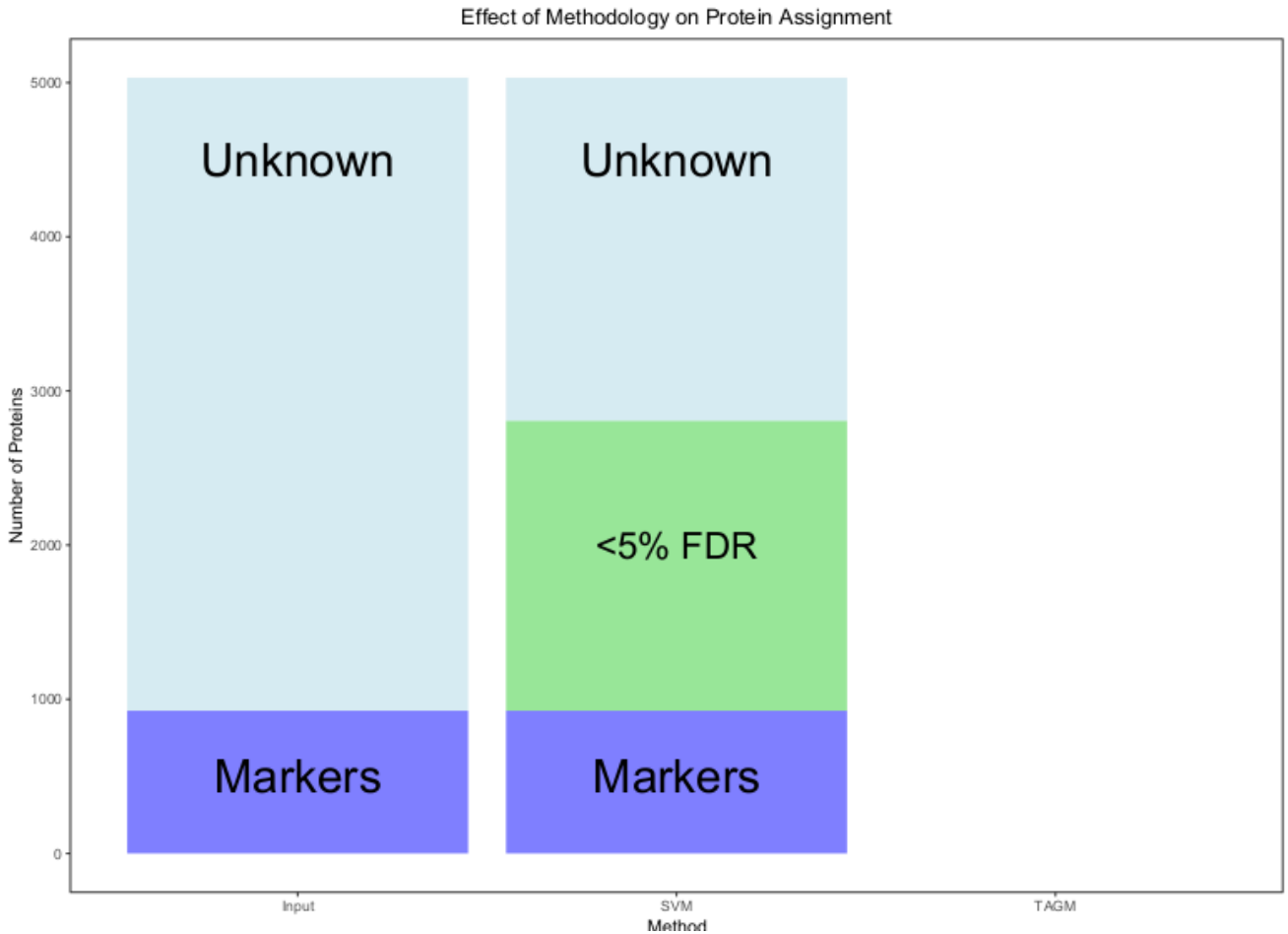
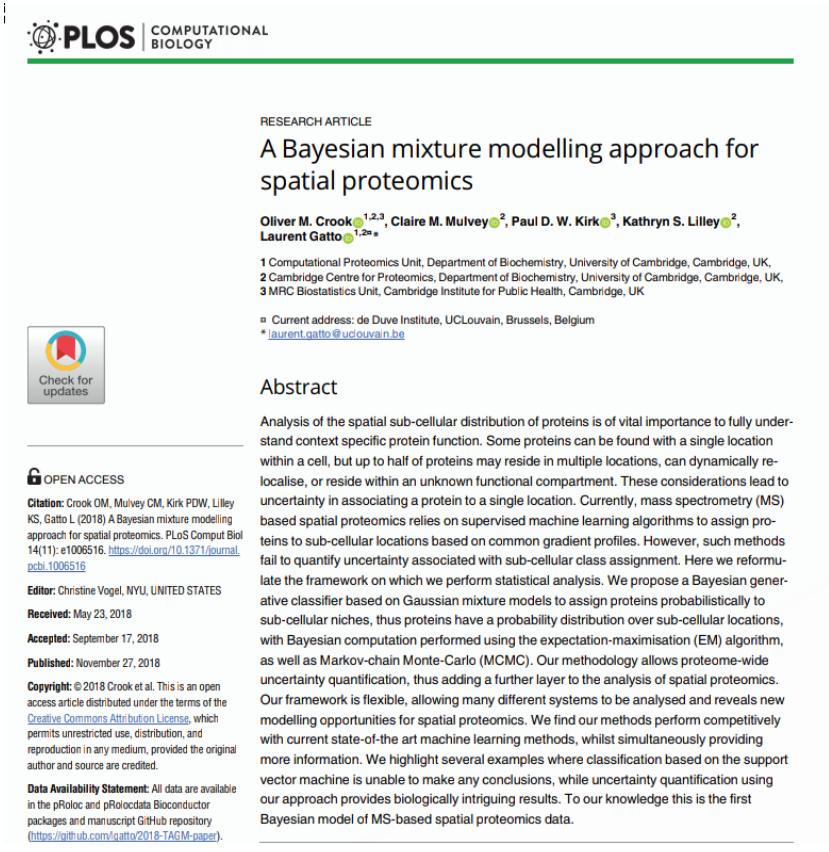


Figure: Support vector machines classifier (after 5% FDR classification cutoff) on the embryonic stem cell data from Christoforou et al. (2016).

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





PLOS COMPUTATIONAL BIOLOGY

RESEARCH ARTICLE

A Bayesian mixture modelling approach for spatial proteomics

Oliver M. Crook[1,2,3], **Claire M. Mulvey**[2], **Paul D. W. Kirk**[3], **Kathryn S. Lilley**[2], **Laurent Gatto**[1,2]*

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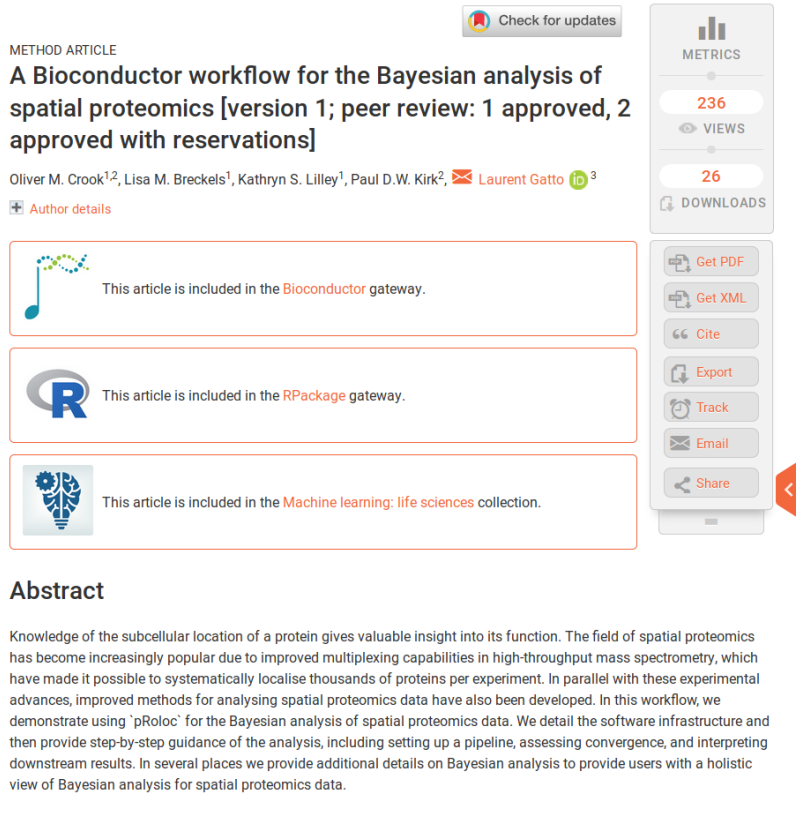
Data Availability Statement: All data are available in the pRoloc and pRolocdata Bioconductor packages and manuscript GitHub repository (https://github.com/lgatto/2018-TAGM-paper).

Abstract

Analysis of the spatial sub-cellular distribution of proteins is of vital importance to fully understand context specific protein function. Some proteins can be found with a single location within a cell, but up to half of proteins may reside in multiple locations, can dynamically re-localise, or reside within an unknown functional compartment. These considerations lead to uncertainty in associating a protein to a single location. Currently, mass spectrometry (MS) based spatial proteomics relies on supervised machine learning algorithms to assign proteins to sub-cellular locations based on common gradient profiles. However, such methods fail to quantify uncertainty associated with sub-cellular class assignment. Here we reformulate the framework on which we perform statistical analysis. We propose a Bayesian generative classifier based on Gaussian mixture models to assign proteins probabilistically to sub-cellular niches, thus proteins have a probability distribution over sub-cellular locations, with Bayesian computation performed using the expectation-maximisation (EM) algorithm, as well as Markov-chain Monte-Carlo (MCMC). Our methodology allows proteome-wide uncertainty quantification, thus adding a further layer to the analysis of spatial proteomics. Our framework is flexible, allowing many different systems to be analysed and reveals new modelling opportunities for spatial proteomics. We find our methods perform competitively with current state-of-the art machine learning methods, whilst simultaneously providing more information. We highlight several examples where classification based on the support vector machine is unable to make any conclusions, while uncertainty quantification using our approach provides biologically intriguing results. To our knowledge this is the first Bayesian model of MS-based spatial proteomics data.



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METHOD ARTICLE

A Bioconductor workflow for the Bayesian analysis of spatial proteomics [version 1; peer review: 1 approved, 2 approved with reservations]

Oliver M. Crook[1,2], Lisa M. Breckels[1], Kathryn S. Lilley[1], Paul D.W. Kirk[2], Laurent Gatto [3]

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Abstract

Knowledge of the subcellular location of a protein gives valuable insight into its function. The field of spatial proteomics has become increasingly popular due to improved multiplexing capabilities in high-throughput mass spectrometry, which have made it possible to systematically localise thousands of proteins per experiment. In parallel with these experimental advances, improved methods for analysing spatial proteomics data have also been developed. In this workflow, we demonstrate using `pRoloc` for the Bayesian analysis of spatial proteomics data. We detail the software infrastructure and then provide step-by-step guidance of the analysis, including setting up a pipeline, assessing convergence, and interpreting downstream results. In several places we provide additional details on Bayesian analysis to provide users with a holistic view of Bayesian analysis for spatial proteomics data.

Figure: See Crook et al. (2018) and Crook et al. (2019).

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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- 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 Σ_k, so that:

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

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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.

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

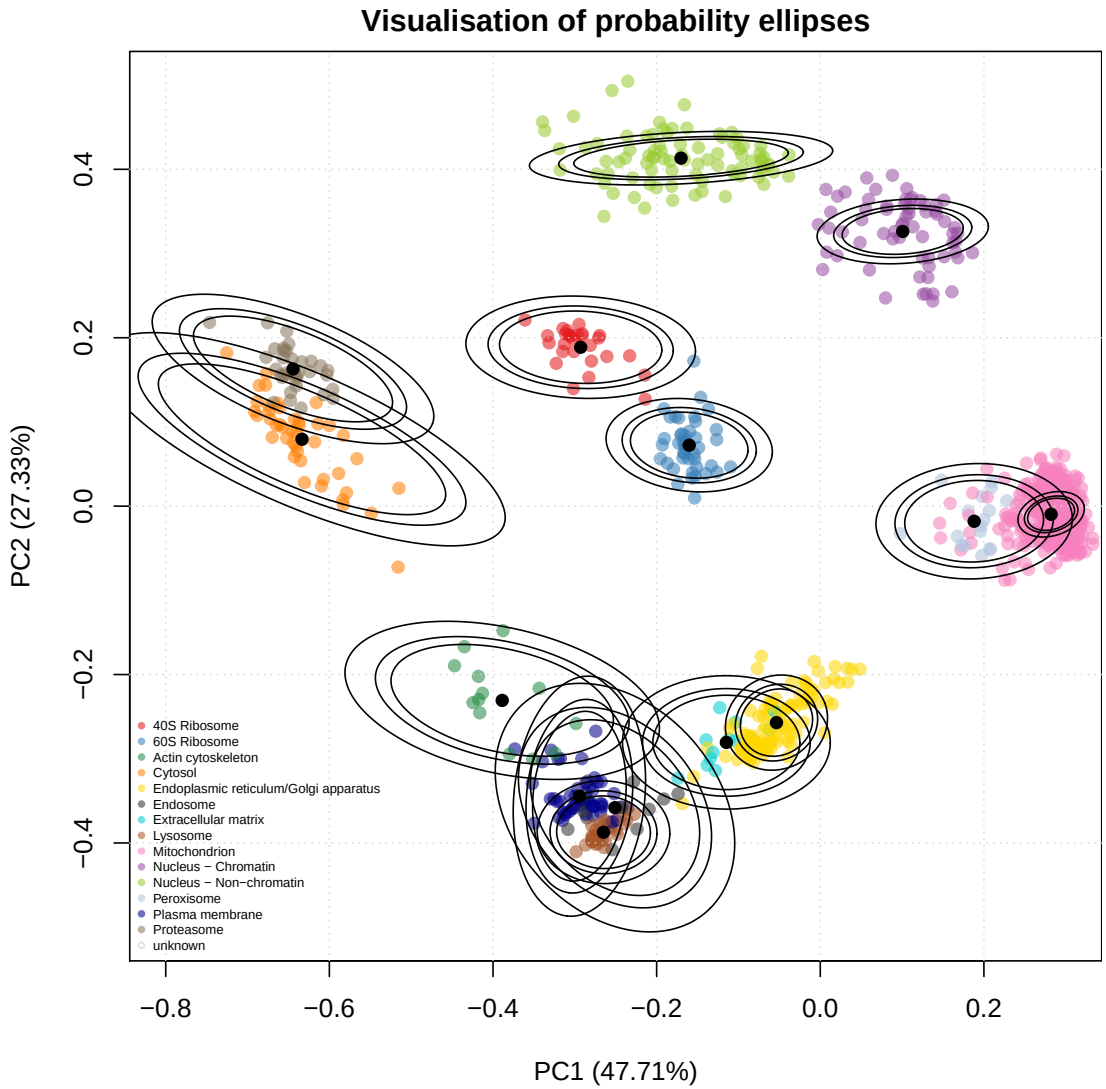


Figure: Illustration of how the TAGM model describes the pluripotent mouse embryonic stem cell data. Each ellipse contains a proportion of total probability of a particular multivariate Gaussian density. The outer ellipse contains 99% of the total probability whilst the middle and inner ellipses contain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

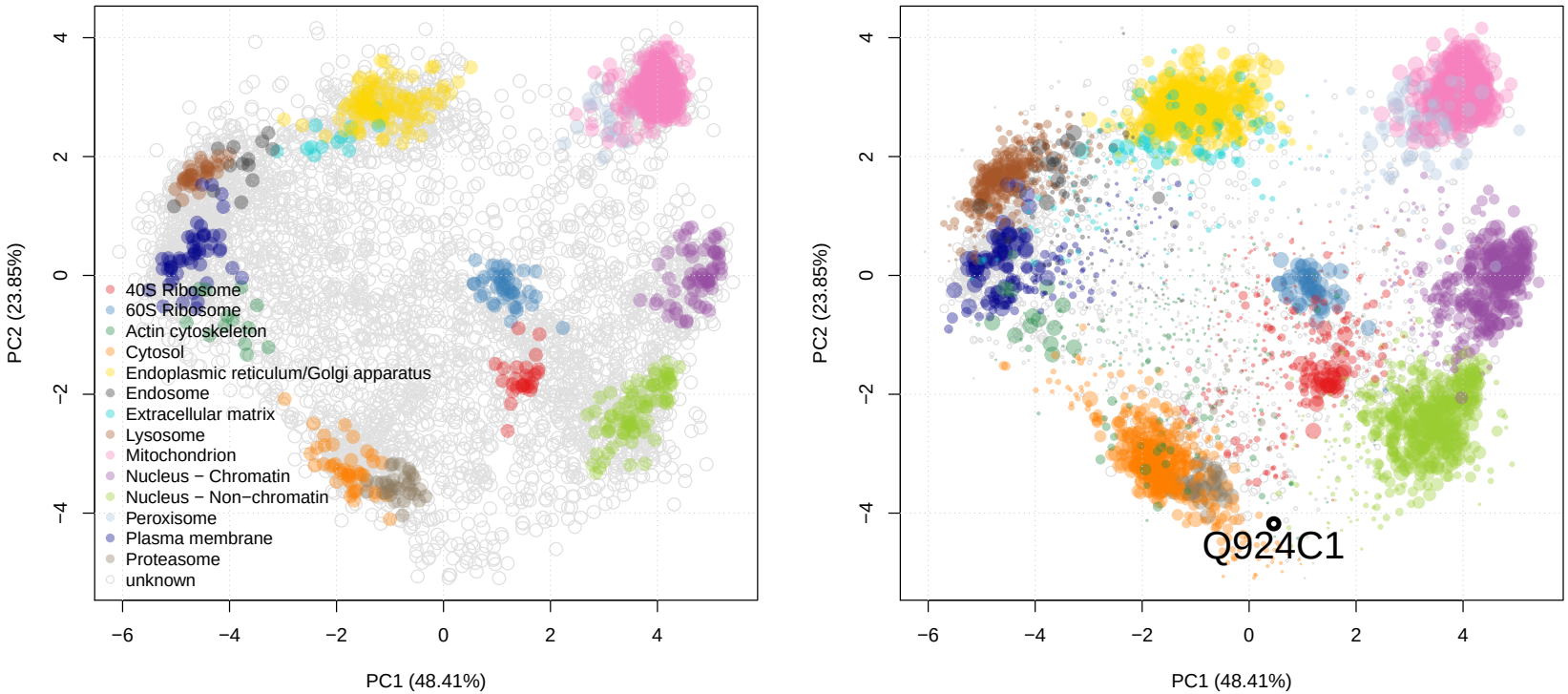
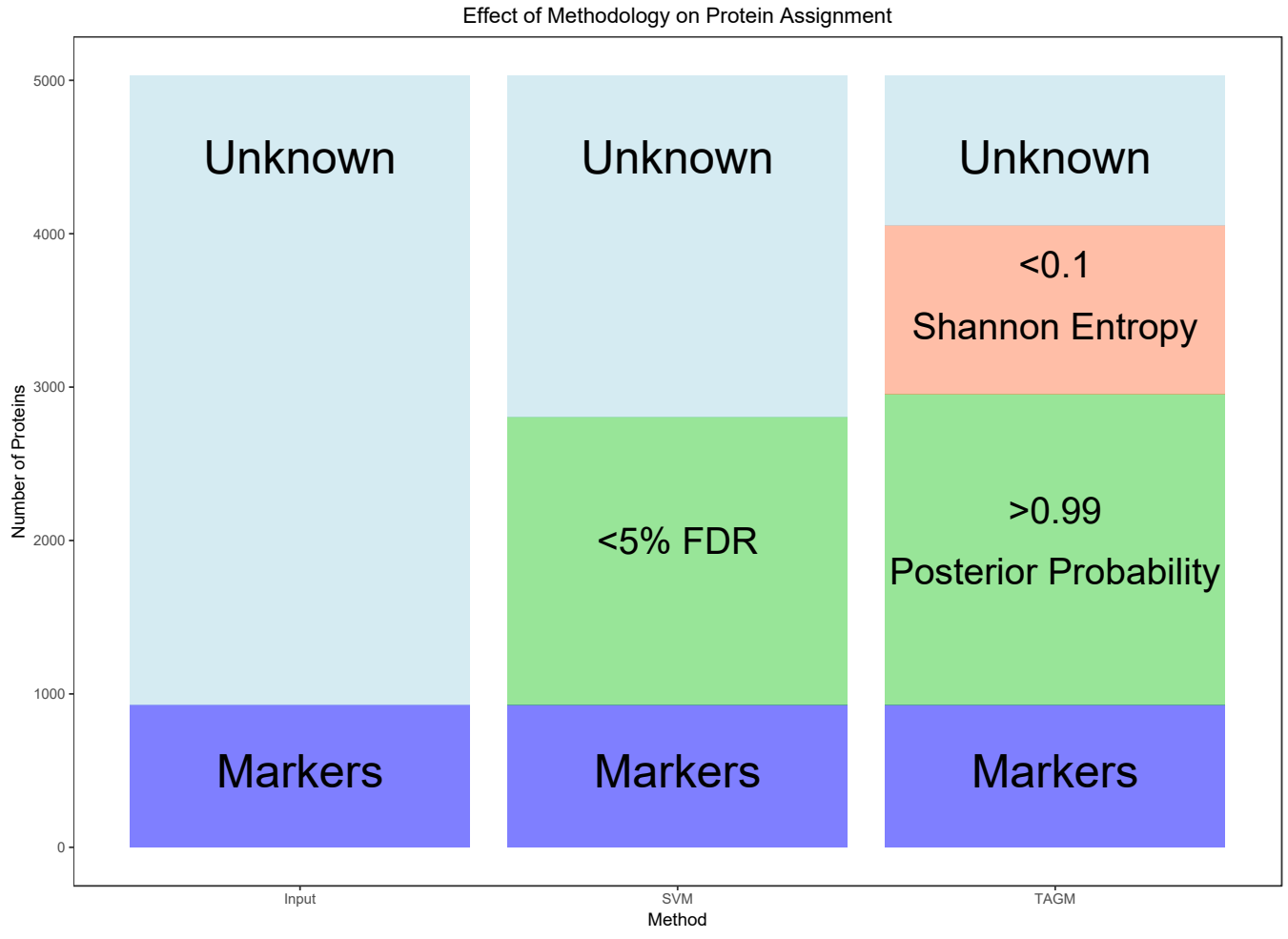


Figure: Assignment of proteins of *unknown* location to one of the annotated classes. The dots are scaled according to the protein assignment probabilities.


Effect of Methodology on Protein Assignment
Number of Proteins
5000
4000
3000
2000
1000
0
Unknown
Unknown
Unknown
<0.1
Shannon Entropy
<5% FDR
>0.99
Posterior Probability
Markers
Markers
Markers
Input
SVM
TAGM
Method

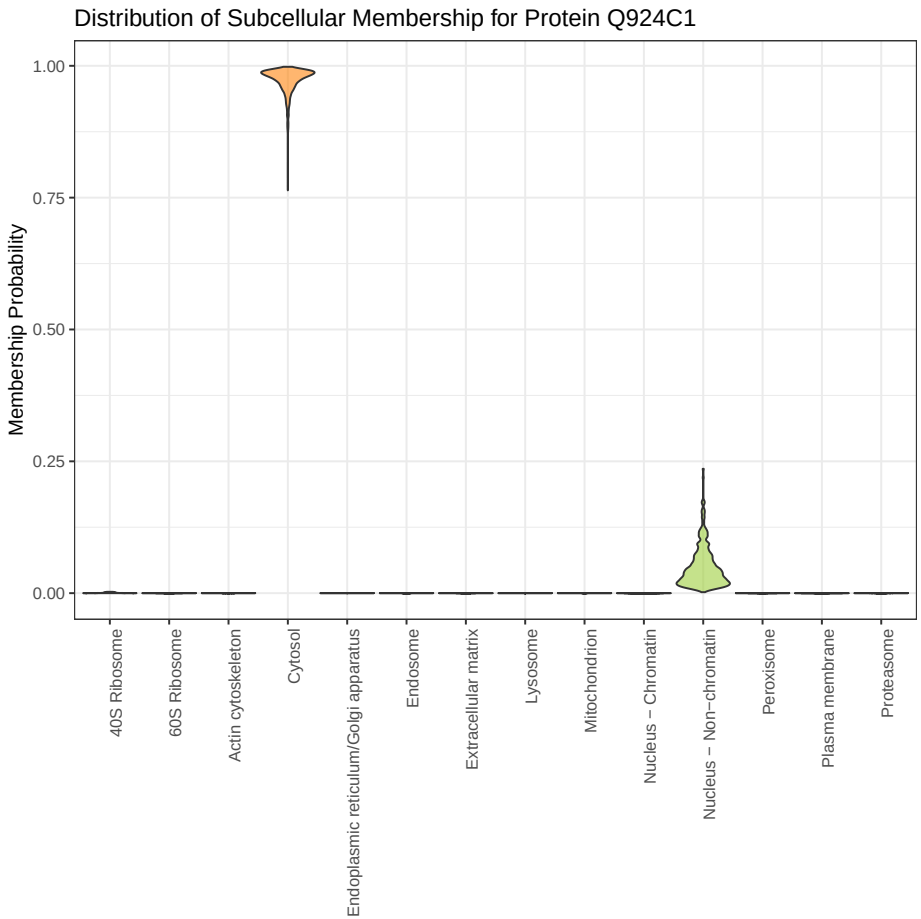
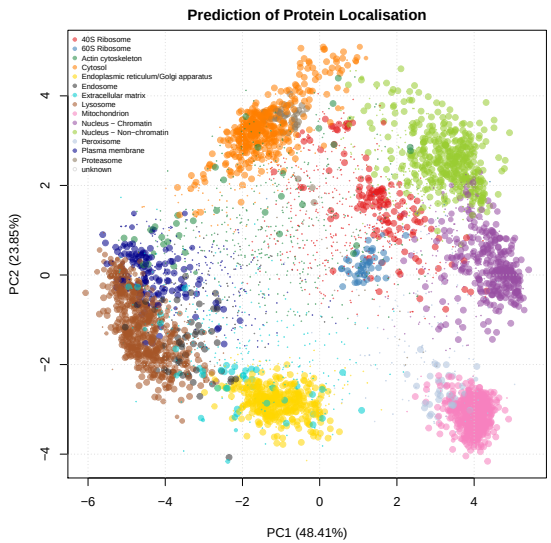
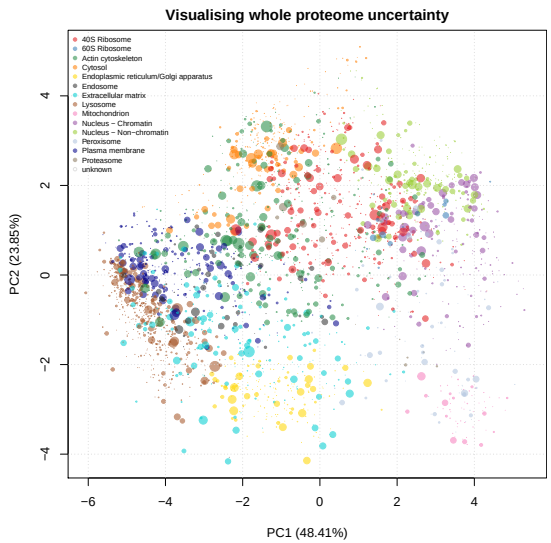
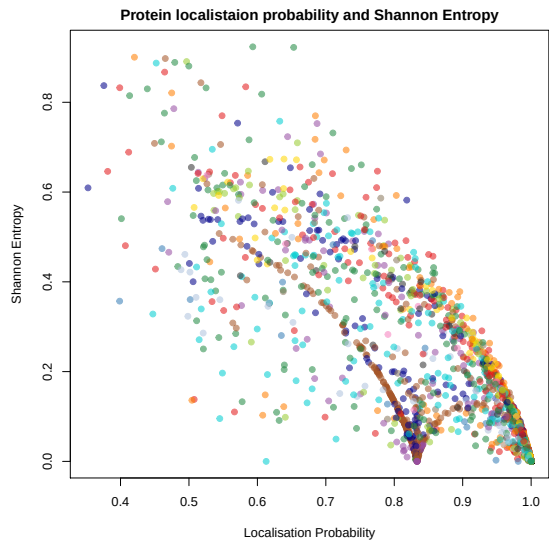


Figure: Exportin 5 (Q924C1) forms part of the micro-RNA export machinery, transporting miRNA from the nucleus to the cytoplasm for further processing. It then translocates back through the nuclear pore complex to return to the nucleus to mediate further transport between nucleus and cytoplasm. The model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







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

Beyond the figures[1]

- 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/

[3] between and within domains/software

Beyond the figures[1]

- 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**.

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[2] https://lgatto.shinyapps.io/christoforou2015/

[3] between and within domains/software

Open research: open source software

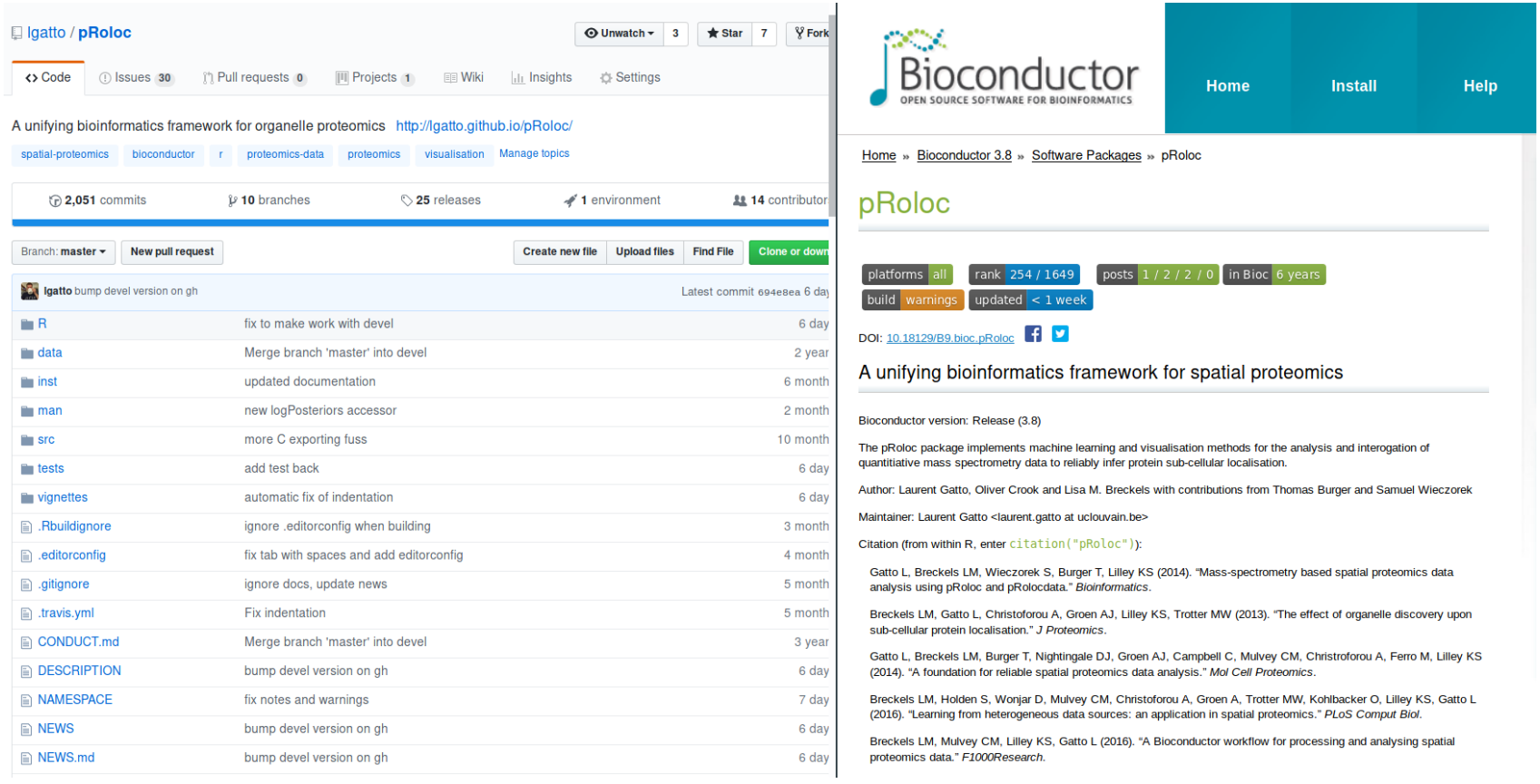

Figure: Gatto et al. (2014a) Left: Public repository for the `pRoloc` software (`https://github.com/lgatto/pRoloc`). Right: offical Bioconductor page.

Open and reproducible research

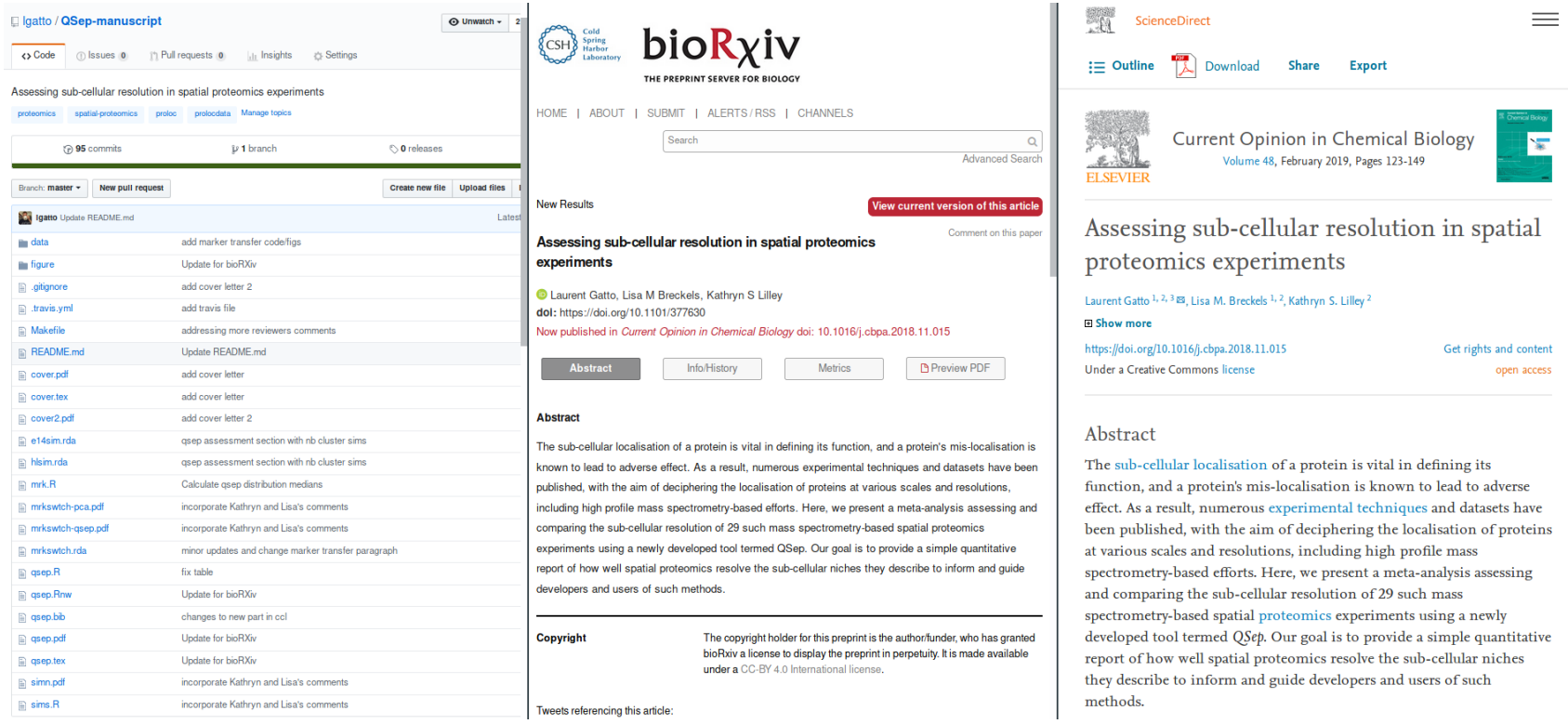

Figure: Gatto et al. (2018) reproducible document (https://github.com/lgatto/QSep-manuscript), preprint (https://doi.org/10.1101/377630) and paper (https://doi.org/10.1016/j.cbpa.2018.11.015).

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, 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**.

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- Funding: BBSRC, Wellcome Trust

Thank you for your attention