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Probabilistic mapping of 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 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

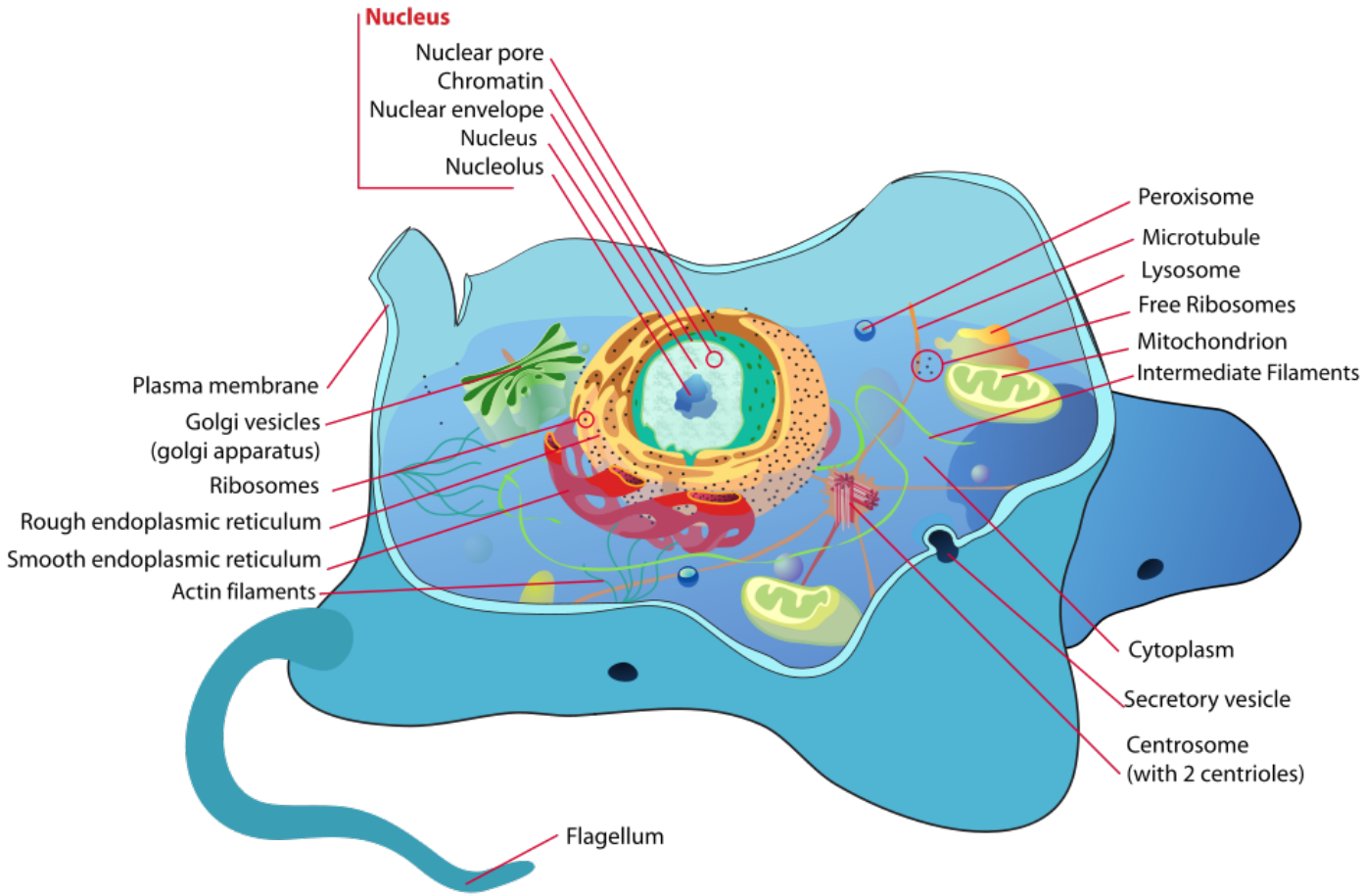
Dr Lisa Breckels, Cambridge: novelty detection, transfer learning, `pRoloc` and `pRolocdata`.

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

Funding: BBSRC, Wellcome Trust

Outline

Cell organisation - **localisation is function**



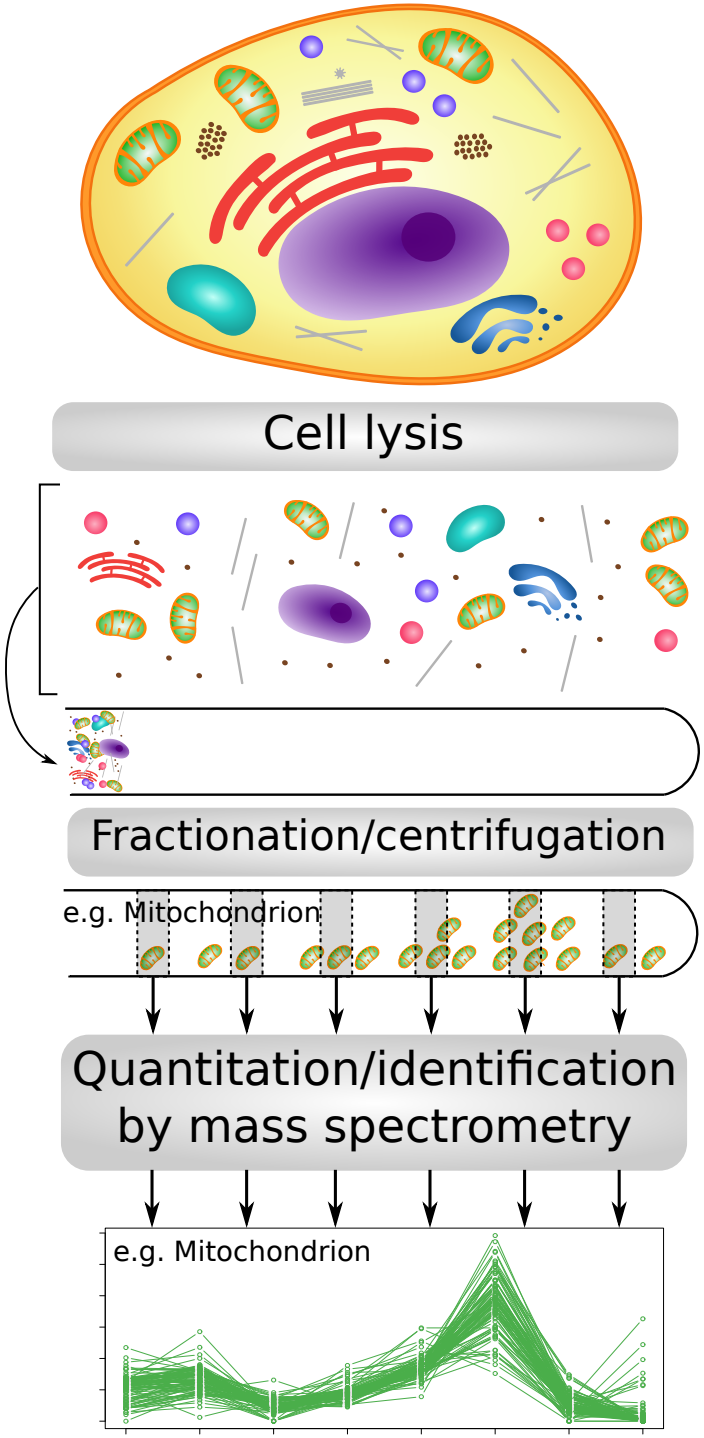
Spatial proteomics is the systematic study of protein localisations.

Localisation – re-localisation – mis-localisation

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

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., 2019).



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

Outline

Visualisation

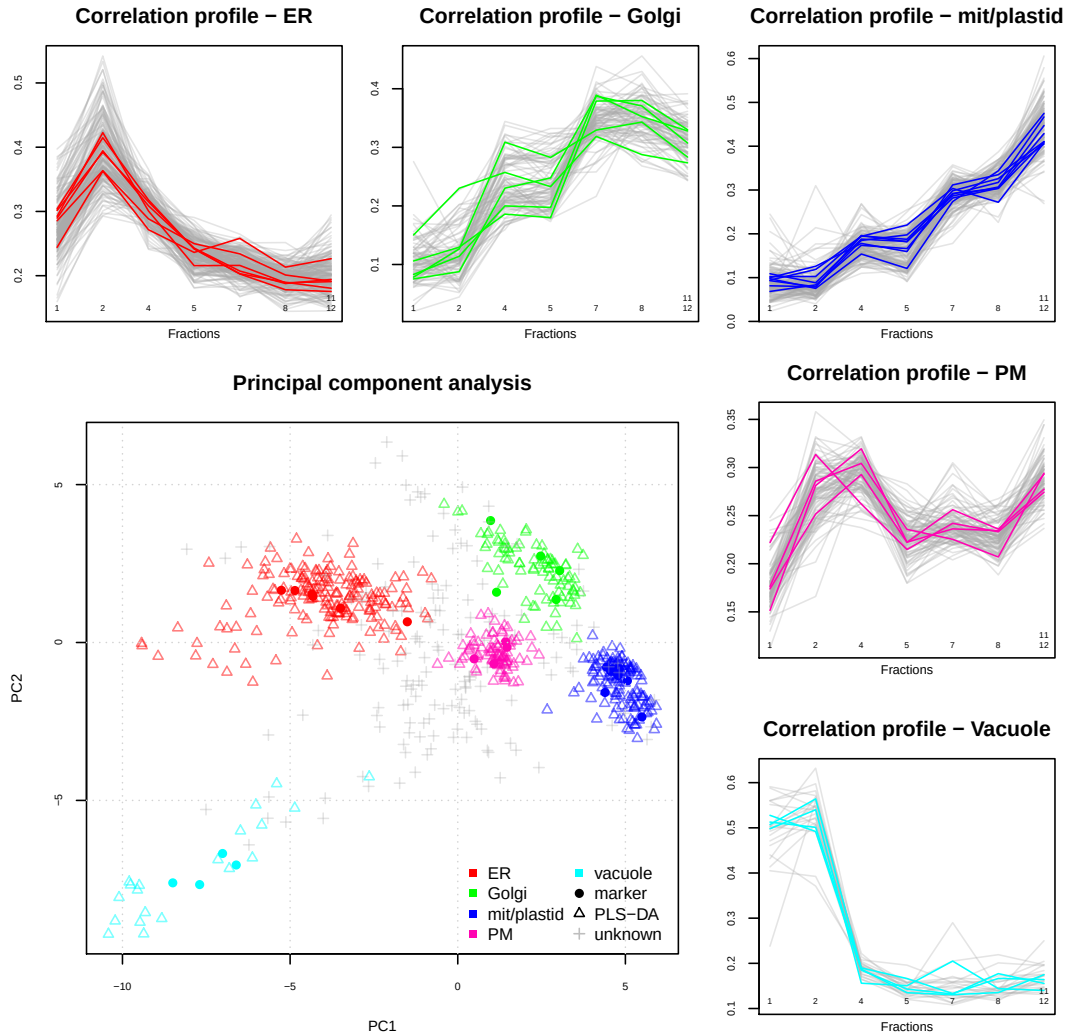


Figure: From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)

Quality control

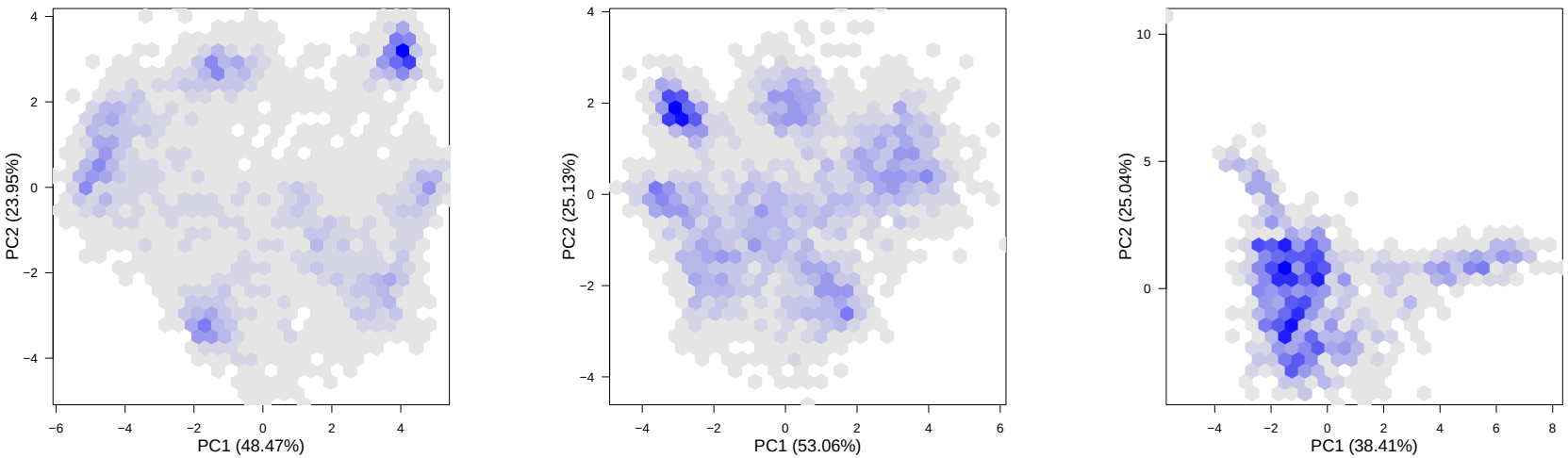


Figure: Assessing sub-cellular resolution in spatial proteomics experiments (Gatto et al., 2018)

Problem statement: classification

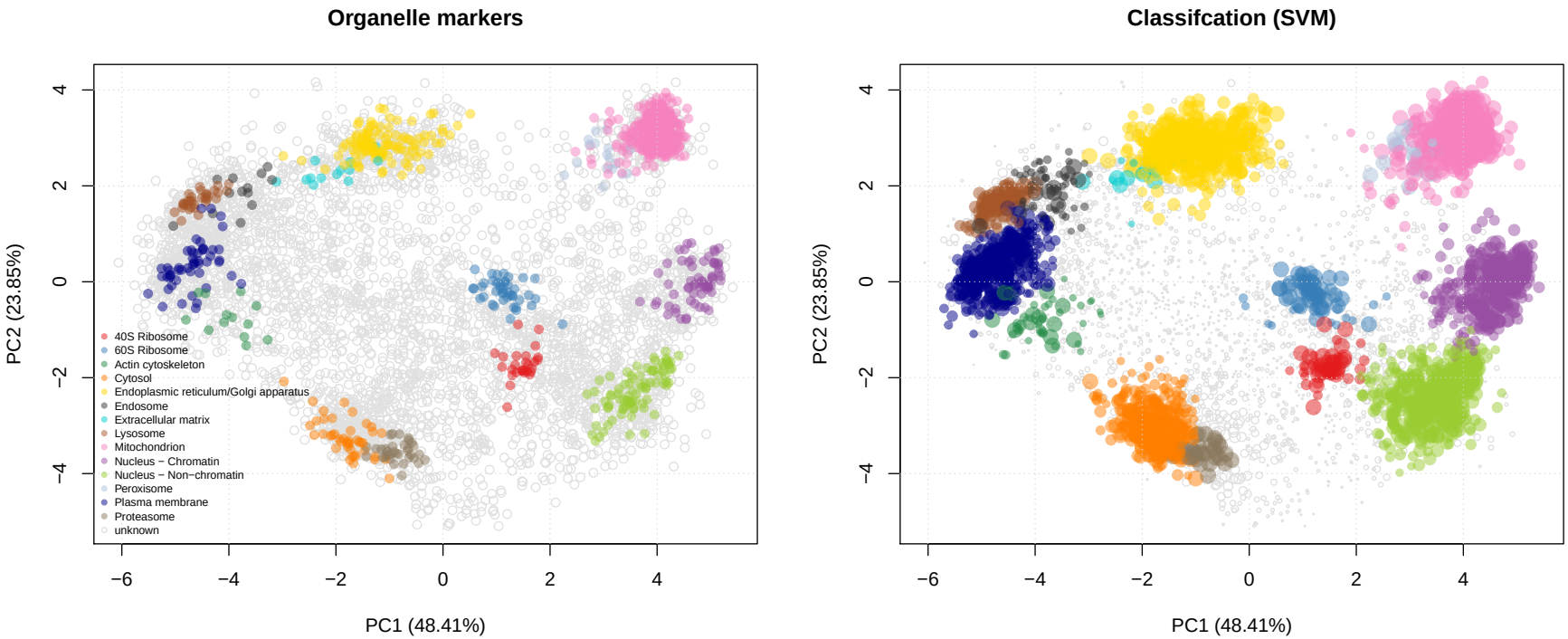


Figure: Support vector machines classifier (after 5% FDR classification cutoff) on the embryonic stem cell data from Christoforou et al. (2016).

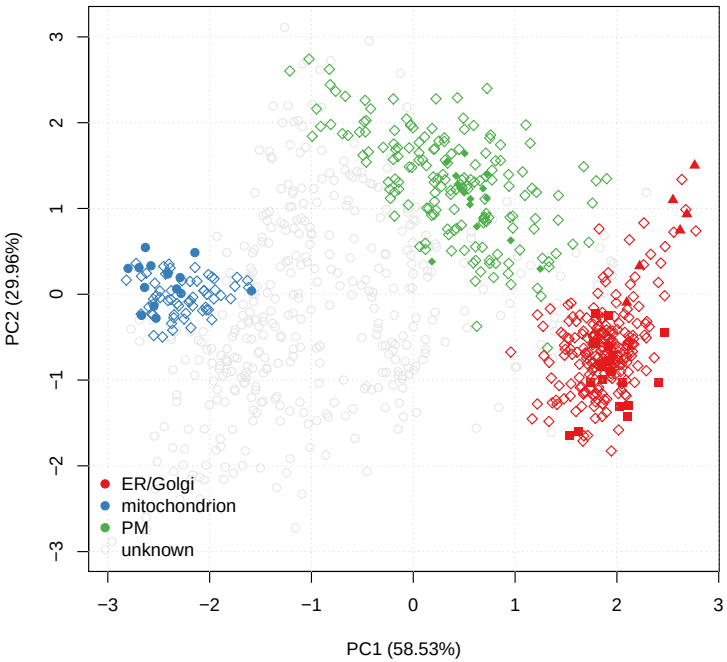
Computational challenges

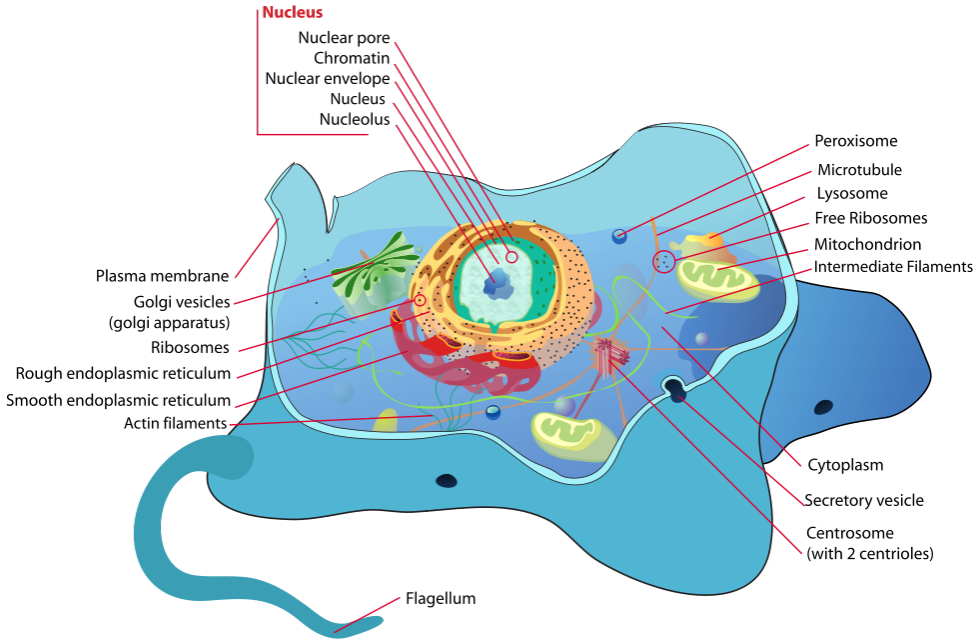
- Visualisation (cluster, unsupervised learning)
- Classification (supervised learning)
- **Novelty detection** (semi-supervised learning)
- Data integration (transfer learning)
- **Unvertainty quantification**
- **Multi-localisation**
- **Spatial dynamics**

To uncover and understand biology

Outline

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

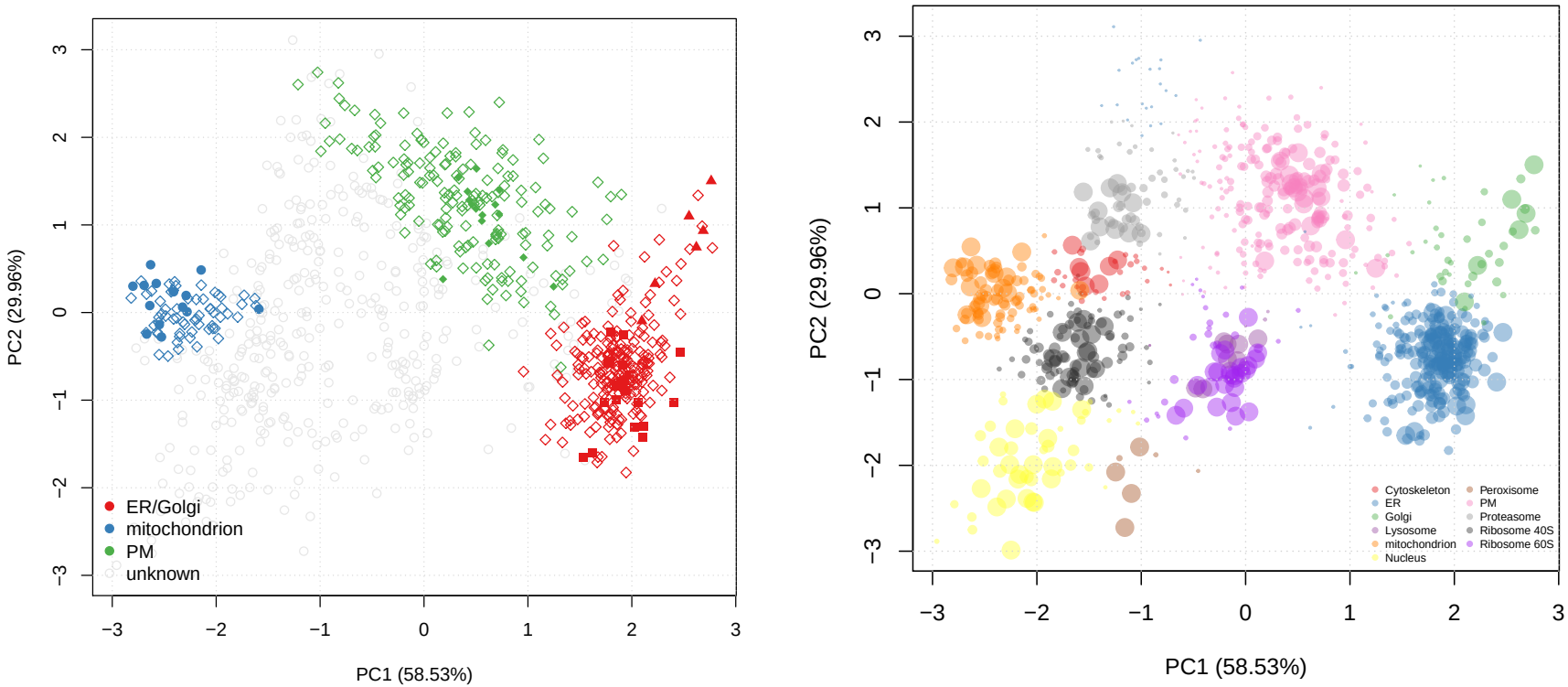
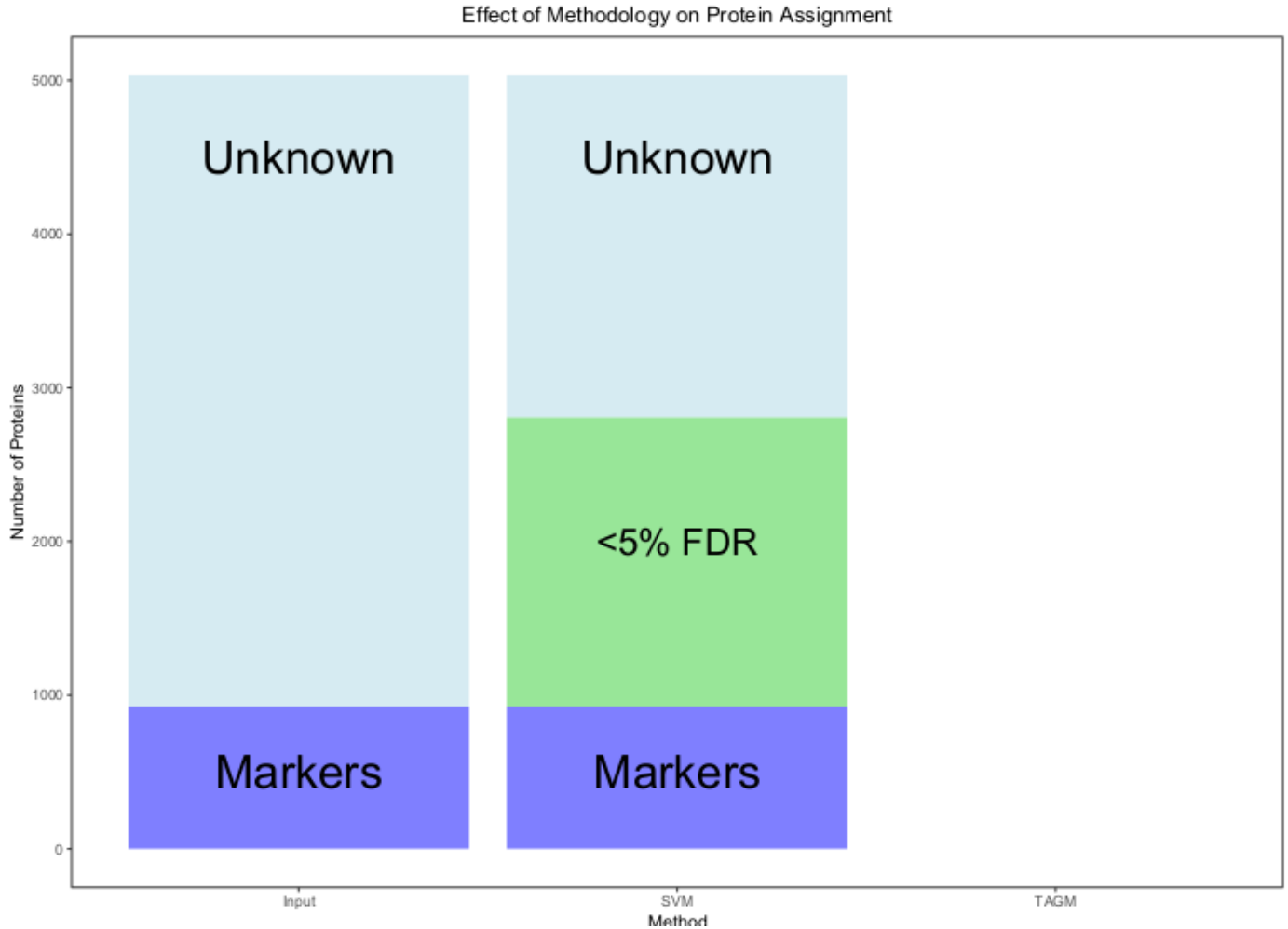


Figure: Left: Original *Drosophila* data from Tan et al. (2009). Right: After semi-supervised learning and classification, Breckels et al. (2013). Under development: Bayesian novelty detection (Novelty-TAGM).

Outline

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



Bayesian Mixture Modelling 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.

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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 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.

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

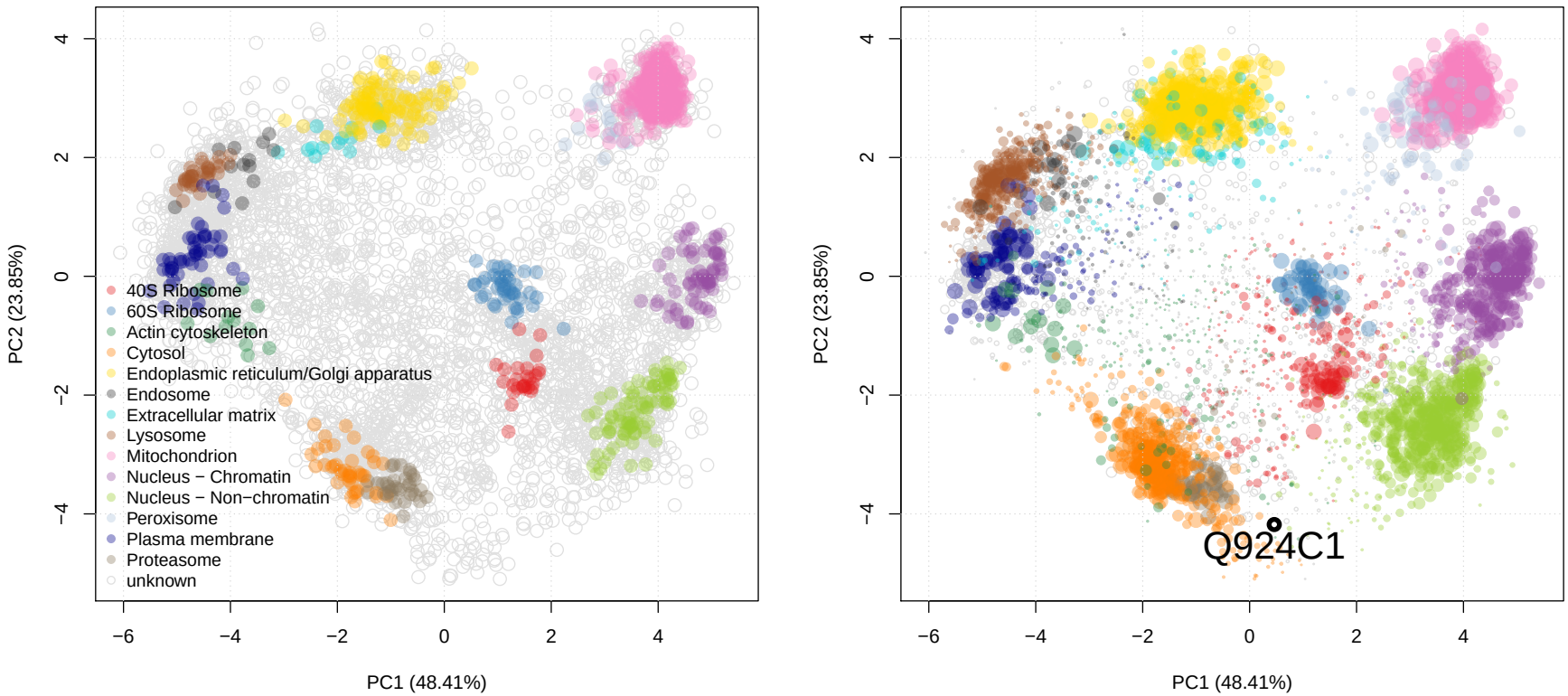
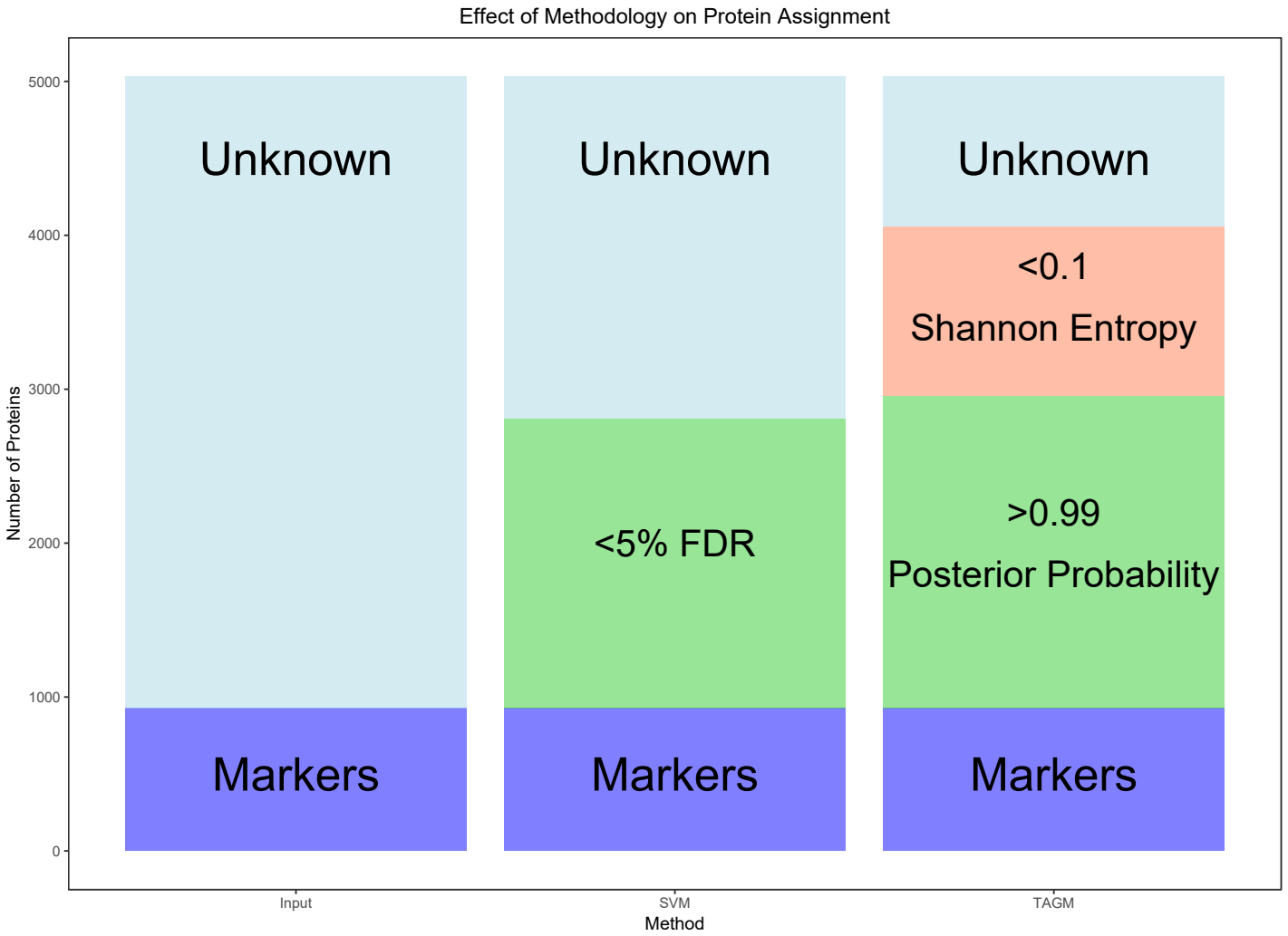


Figure: Assignment of proteins of *unknown* location to one of the annotated classes. The dots are scaled according to the protein assignment probabilities.


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

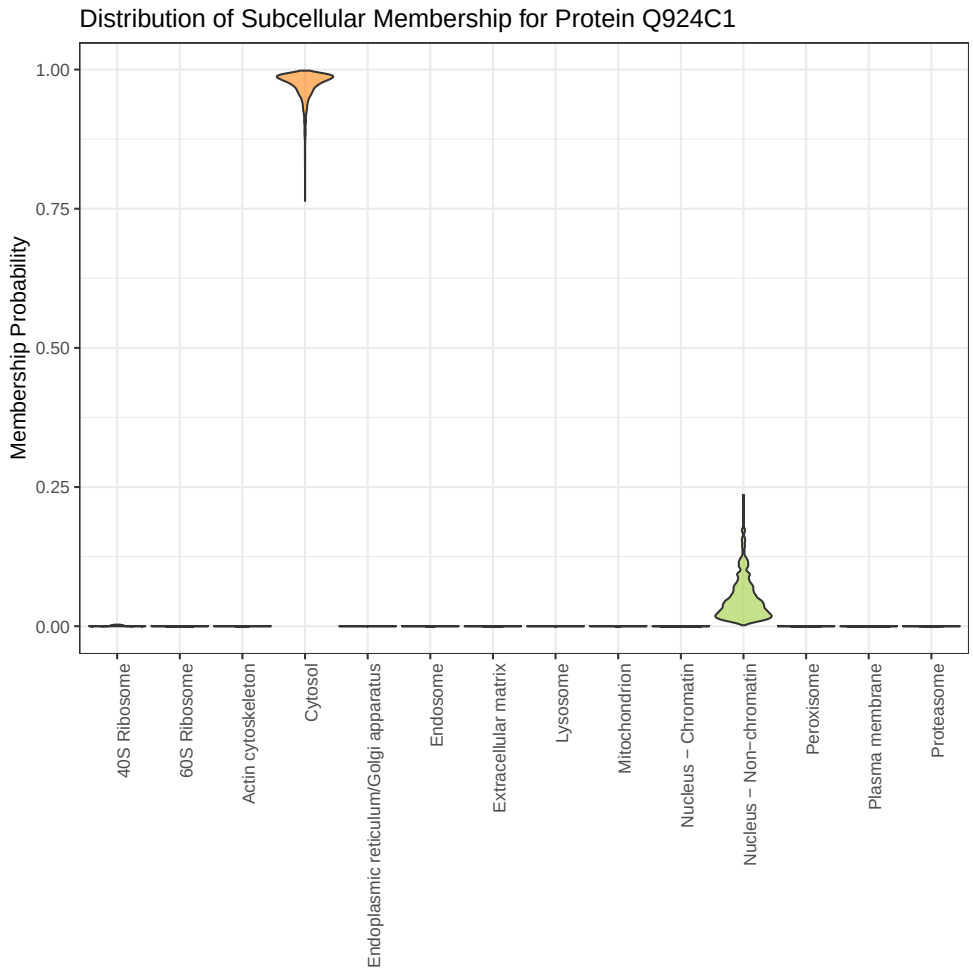
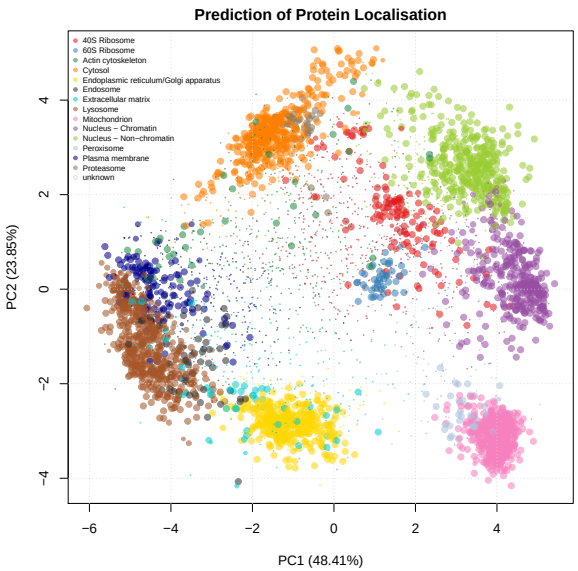
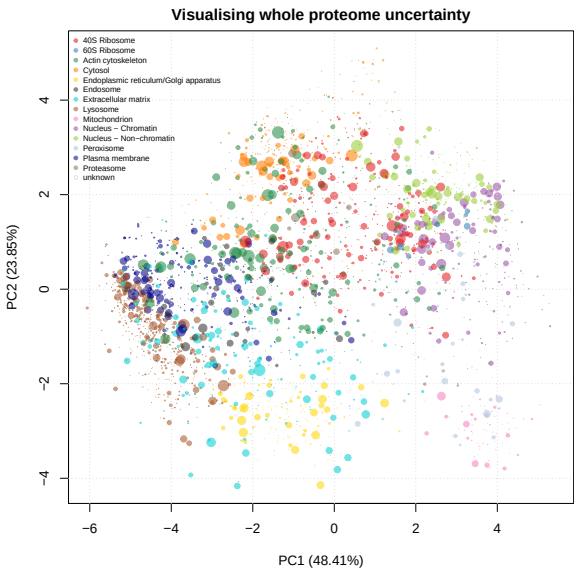
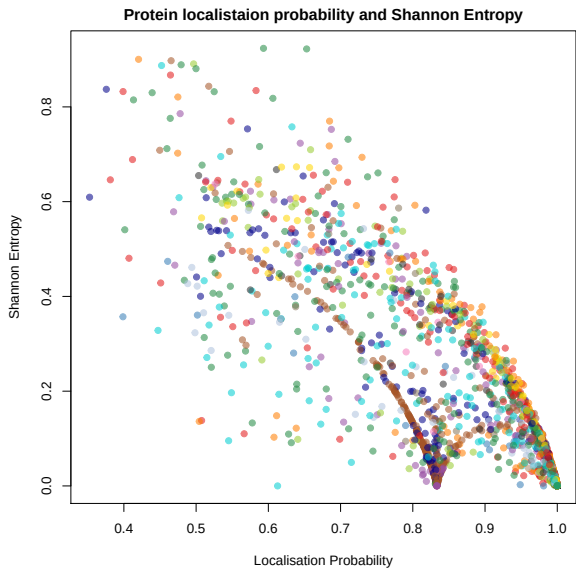


Figure: Exportin 5 (Q924C1) forms part of the micro-RNA export machinery, transporting miRNA from the nucleus to the cytoplasm for further processing. It then translocates back through the nuclear pore complex to return to the nucleus to mediate further transport between nucleus and cytoplasm. The model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







Outline

Spatial dynamics

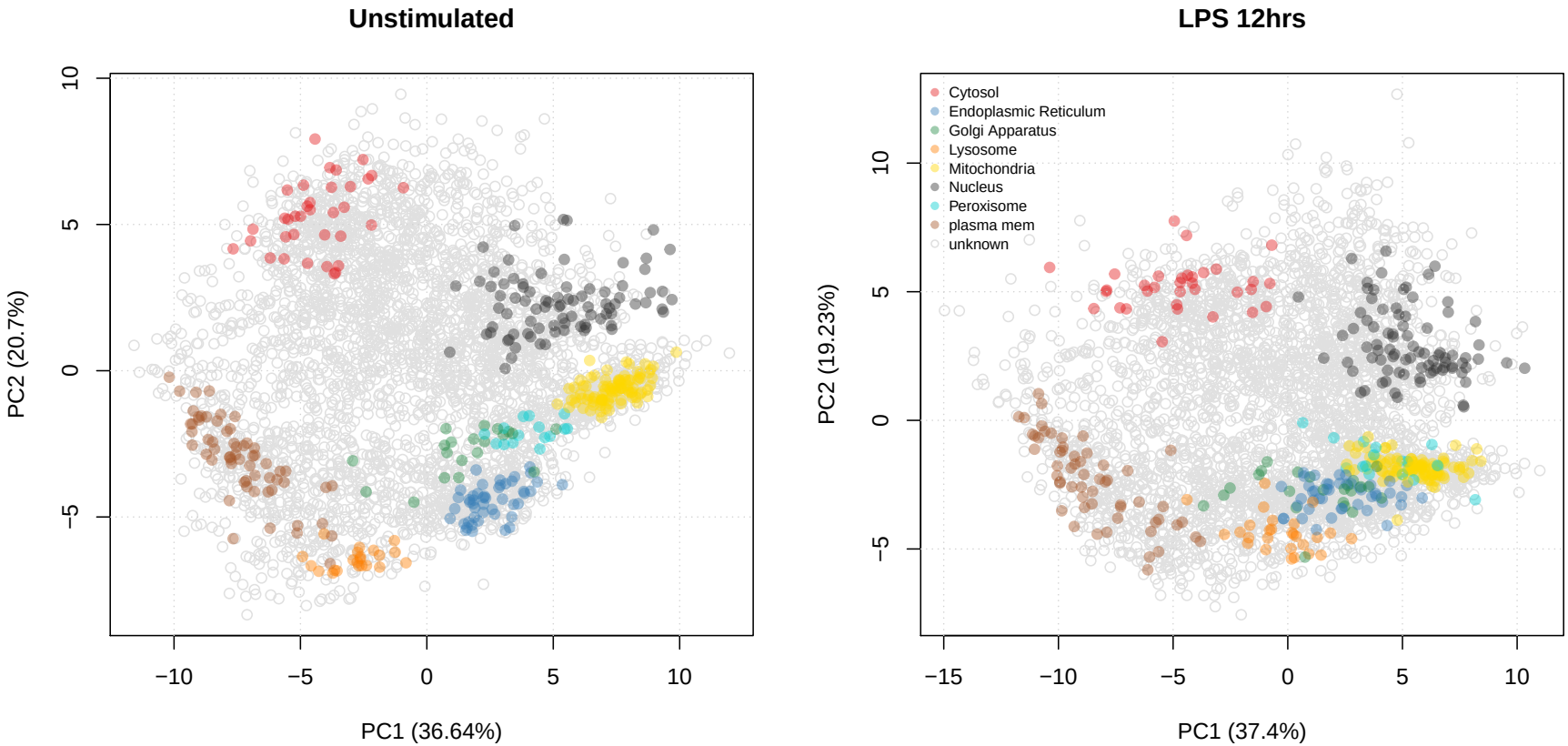


Figure: Spatial maps of unstimulated (**control**) and LPS-treated (**experimental condition**) cells (combined triplicates).

A probabilistic definition of differential localisation:

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

with

- organelle-specific profiles modelled with mixtures of Gaussian process regressions (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.

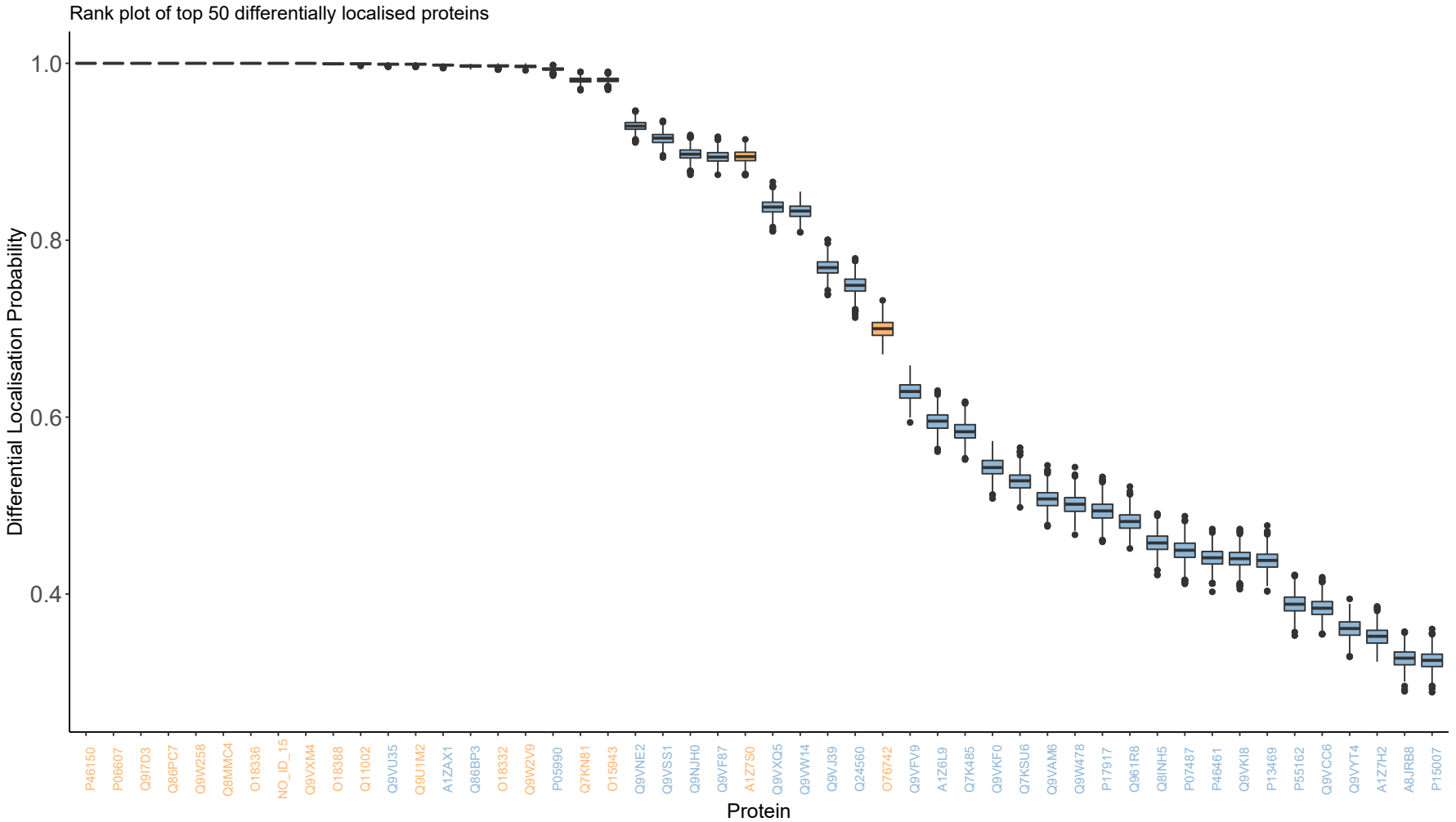


Figure: Proteins ranked based on their probability of being differentially localised, i.e. having been assigned different niches in the control and experimental condition.

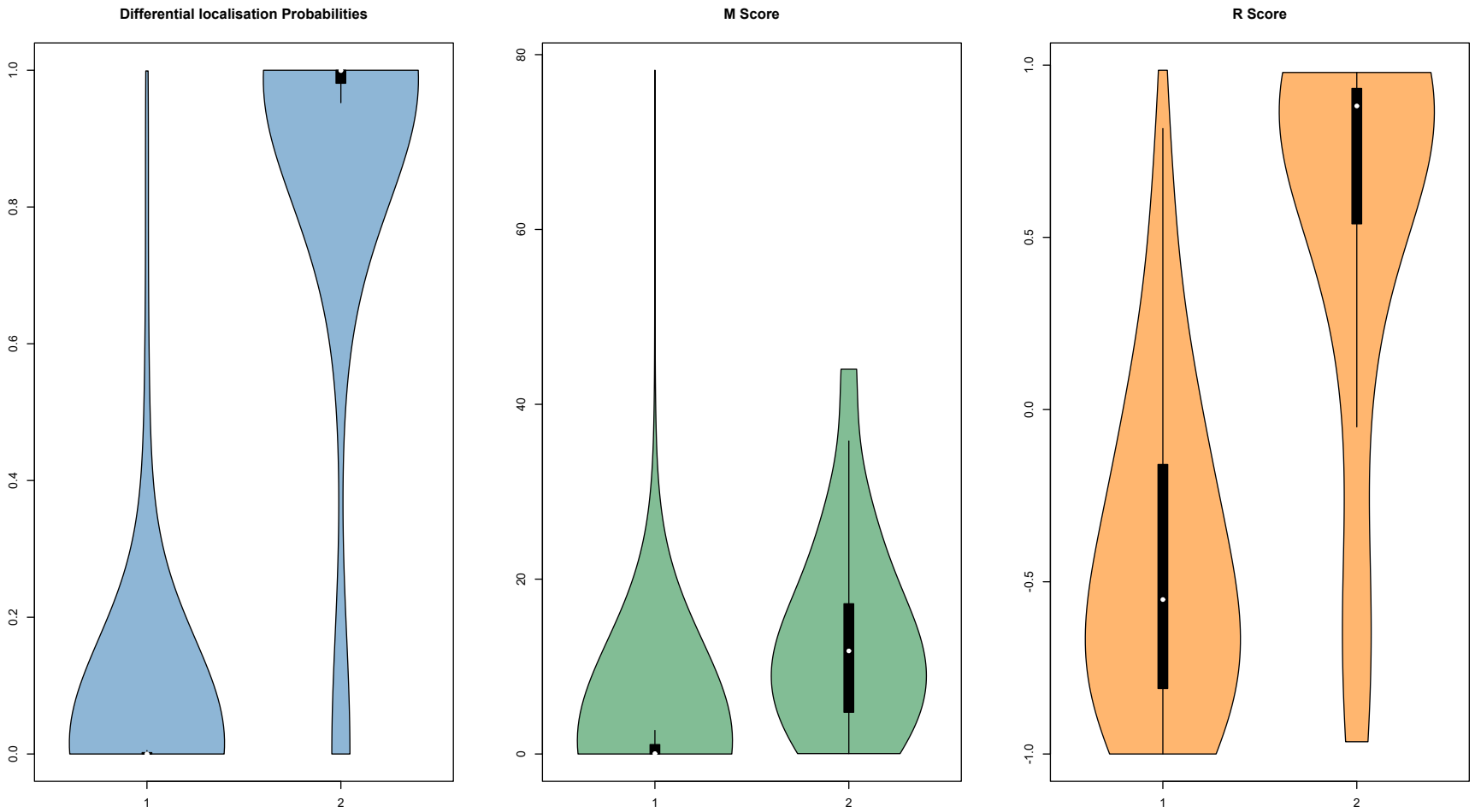


Figure: **Differential localisation probabilites** (**left**) provide excellent discrimination betwee static (1) and differentially localised proteins (2).

Outline

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. (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/`

[3] between and within domains/software

Outline

Conclusions

Applied Bioinformatics in Life Sciences.

- Life Sciences: Protein sub-cellular localisation, technologies (hyperLOPIT) and opportunities.
- Bioinformatics: 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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Thank you for your attention

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