

Probabilistic mapping of the sub-cellular proteome

Laurent Gatto

February 10, 2020

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.

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

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

Outline

Spatial proteomics

Data analysis

Novelty detection

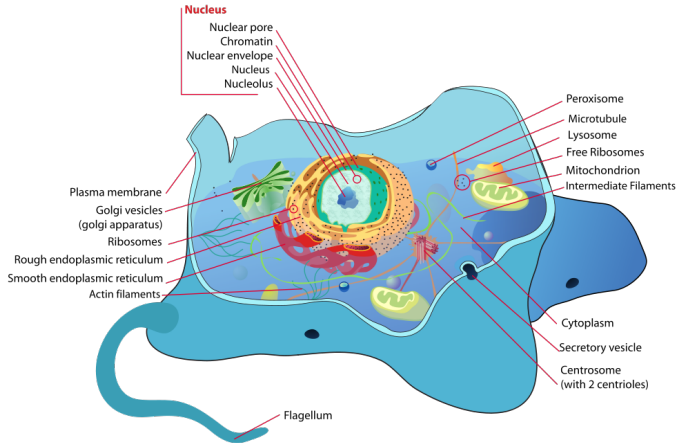
Multi-localisation and uncertainly quantification

Spatial dynamics

Behind the scences

Conclusions

Cell organisation - localisation is function



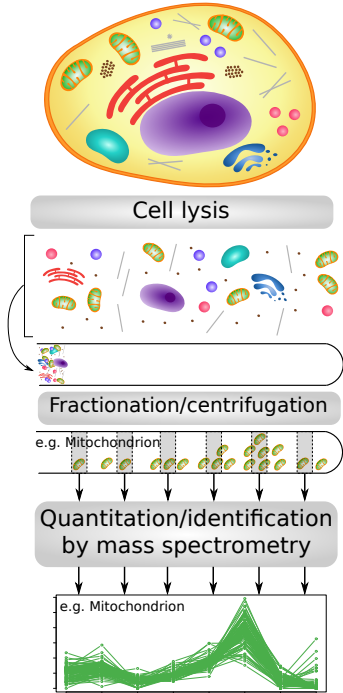
Spatial proteomics is the systematic study of protein localisations.

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., 2019).



	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
x₁	x _{1,1}	x _{1,2}	...	x _{1,L}	unknown
x₂	x _{2,1}	x _{2,2}	...	x _{2,L}	<i>loc₁</i>
x₃	x _{3,1}	x _{3,2}	...	x _{3,L}	unknown
⋮	⋮	⋮	⋮	⋮	⋮
x_i	x _{i,1}	x _{i,2}	...	x _{i,L}	<i>loc_k</i>
⋮	⋮	⋮	⋮	⋮	⋮
x_N	x _{N,1}	x _{N,2}	...	x _{N, K}	unknown

Outline

Spatial proteomics

Data analysis

Novelty detection

Multi-localisation and uncertainly quantification

Spatial dynamics

Behind the scences

Conclusions

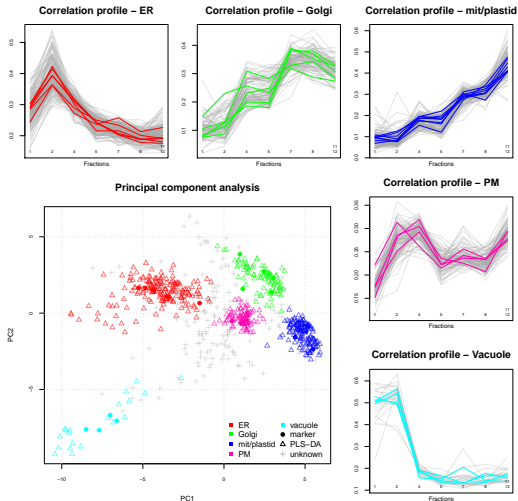


Figure: From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)

Quality control

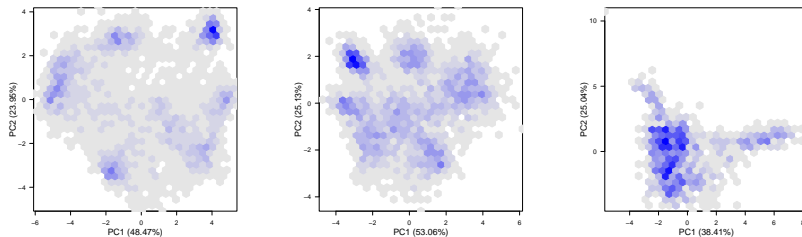


Figure: Assessing sub-cellular resolution in spatial proteomics experiments (Gatto et al., 2018)

Problem statement: classification

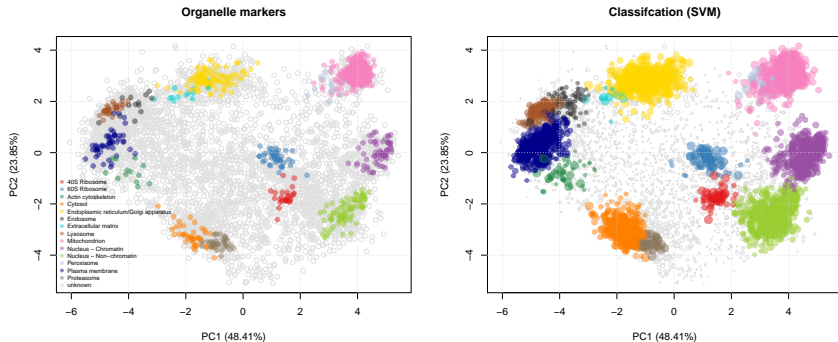


Figure: Support vector machines classifier (after 5% FDR classification cutoff) on the embryonic stem cell data from Christoforou et al. (2016).

- 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

Spatial proteomics

Data analysis

Novelty detection

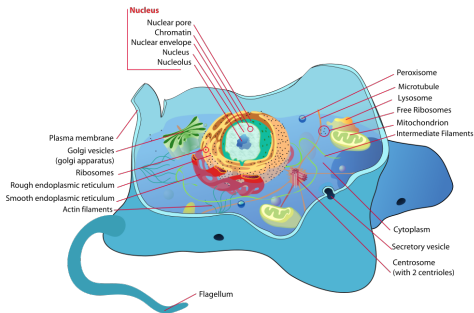
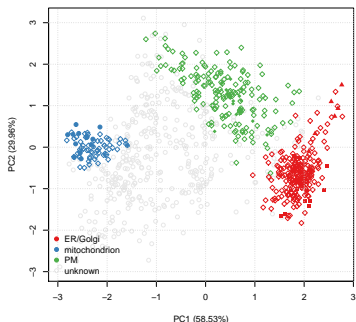
Multi-localisation and uncertainly quantification

Spatial dynamics

Behind the scences

Conclusions

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

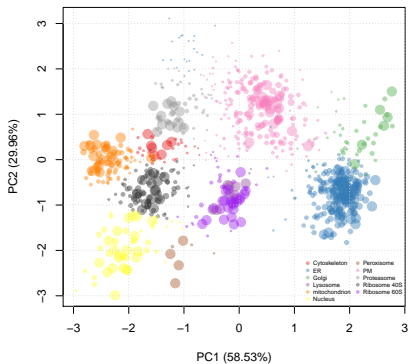
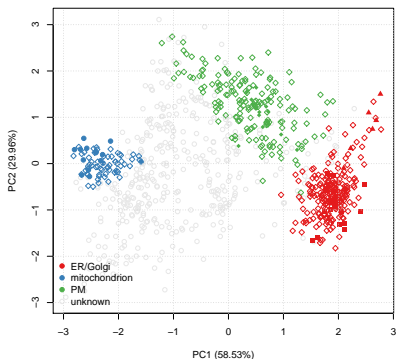


Figure: Left: Original *Drosophila* data from Tan et al. (2009). Right: After semi-supervised learning and classification, Breckels et al. (2013). Under development: Bayesian novelty detection (Novelty-TAGM).

Outline

Spatial proteomics

Data analysis

Novelty detection

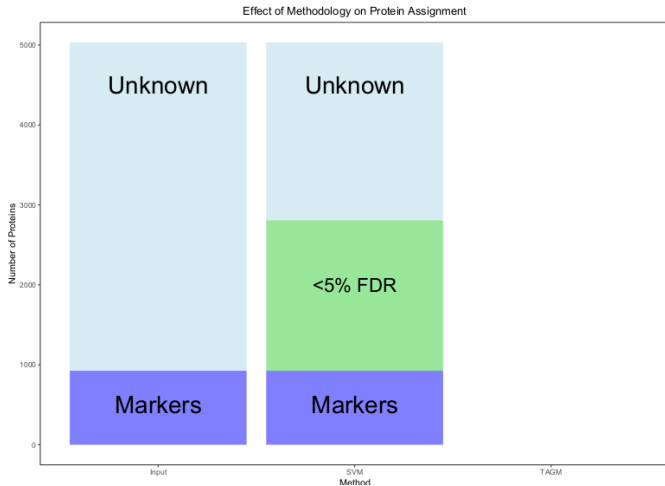
Multi-localisation and uncertainly quantification

Spatial dynamics

Behind the scences

Conclusions

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



- *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.

- *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).

- *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).
- 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:

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

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:

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

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 .

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

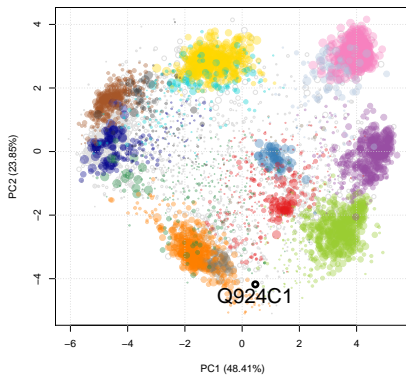
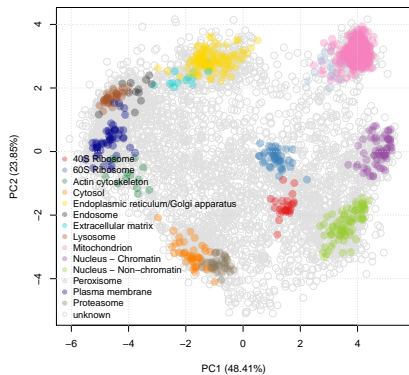
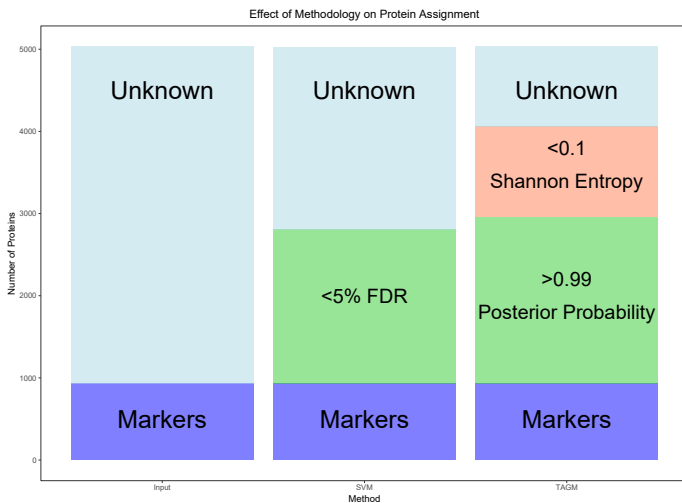


Figure: Assignment of proteins of *unknown* location to one of the annotated classes. The dots are scaled according to the protein assignment probabilities.



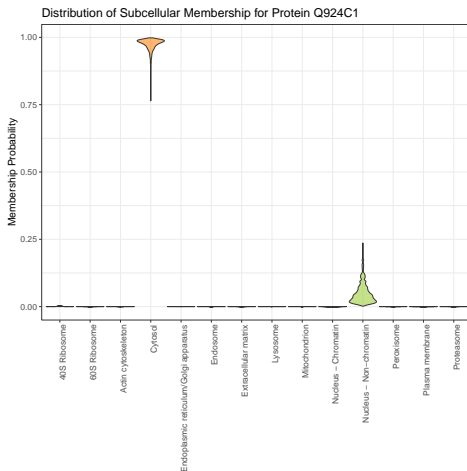
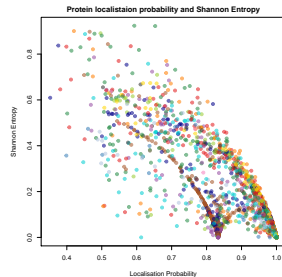
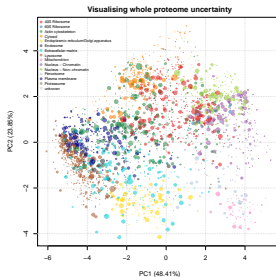
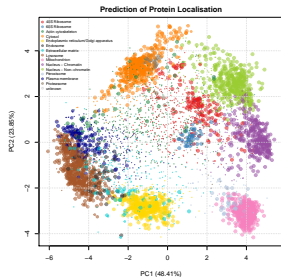


Figure: Exportin 5 (Q924C1) forms part of the micro-RNA export machinery, transporting miRNA from the nucleus to the cytoplasm for further processing. It then translocates back through the nuclear pore complex to return to the nucleus to mediate further transport between nucleus and cytoplasm. The model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



Outline

Spatial proteomics

Data analysis

Novelty detection

Multi-localisation and uncertainly quantification

Spatial dynamics

Behind the scences

Conclusions



Outline

Spatial proteomics

Data analysis


Novelty detection

Multi-localisation and uncertainly quantification

Spatial dynamics

Behind the scences

Conclusions



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. (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/>

³between and within domains/software

Outline

Spatial proteomics

Data analysis

Novelty detection

Multi-localisation and uncertainly quantification

Spatial dynamics

Behind the scences

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**.

- 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.
- Oliver M. Crook, Claire M. Mulvey, Paul D. W. Kirk, Kathryn S. Lilley, and Laurent Gatto. A bayesian mixture modelling approach for spatial proteomics. *PLOS Computational Biology*, 14(11):1–29, 11 2018. doi: 10.1371/journal.pcbi.1006516. URL <https://doi.org/10.1371/journal.pcbi.1006516>.
- OM Crook, LM Breckels, KS Lilley, PDW Kirk, and L Gatto. A bioconductor workflow for the bayesian analysis of spatial proteomics [version 1; peer review: 1 approved, 2 approved with reservations]. *F1000Research*, 8(446), 2019. doi: 10.12688/f1000research.18636.1.

- TPJ Dunkley, S Hester, IP Shadforth, J Runions, T Weimar, SL Hanton, JL Griffin, C Bessant, F Brandizzi, C Hawes, RB Watson, P Dupree, and KS Lilley. Mapping the Arabidopsis organelle proteome. *PNAS*, 103(17):6518–6523, Apr 2006.
- LJ Foster, CL de Hoog, Y Zhang, Y Zhang, X Xie, VK Mootha, and M Mann. A mammalian organelle map by protein correlation profiling. *Cell*, 125(1):187–199, Apr 2006.
- L Gatto and KS Lilley. MSnbase - an R/Bioconductor package for isobaric tagged mass spectrometry data visualization, processing and quantitation. *Bioinformatics*, 28(2): 288–9, Jan 2012.
- L Gatto, JA Vizcaino, H Hermjakob, W Huber, and KS Lilley. Organelle proteomics experimental designs and analysis. *Proteomics*, 2010.
- L Gatto, LM Breckels, T Burger, DJ Nightingale, AJ Groen, C Campbell, N Nikolovski, CM Mulvey, A Christoforou, M Ferro, and KS Lilley. A foundation for reliable spatial proteomics data analysis. *MCP*, 13(8):1937–52, Aug 2014.
- Laurent Gatto, Lisa M Breckels, and Kathryn S Lilley. Assessing sub-cellular resolution in spatial proteomics experiments. *bioRxiv*, 2018. doi: 10.1101/377630.
- Aikaterini Geladaki, Nina Kočevár Britovšek, Lisa M Breckels, Tom S Smith, Owen L Vennard, Claire M Mulvey, Oliver M Crook, Laurent Gatto, and Kathryn S Lilley. Combining LOPIT with differential ultracentrifugation for high-resolution spatial proteomics. *Nat. Commun.*, 10(1):331, January 2019.

- 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.
- D N Itzhak, S Tyanova, J Cox, and G H Borner. Global, quantitative and dynamic mapping of protein subcellular localization. *Elife*, 5, Jun 2016. doi: 10.7554/eLife.16950.
- 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
Slides available at <http://bit.ly/ABLS2020>