Mapping the sub-cellular proteome

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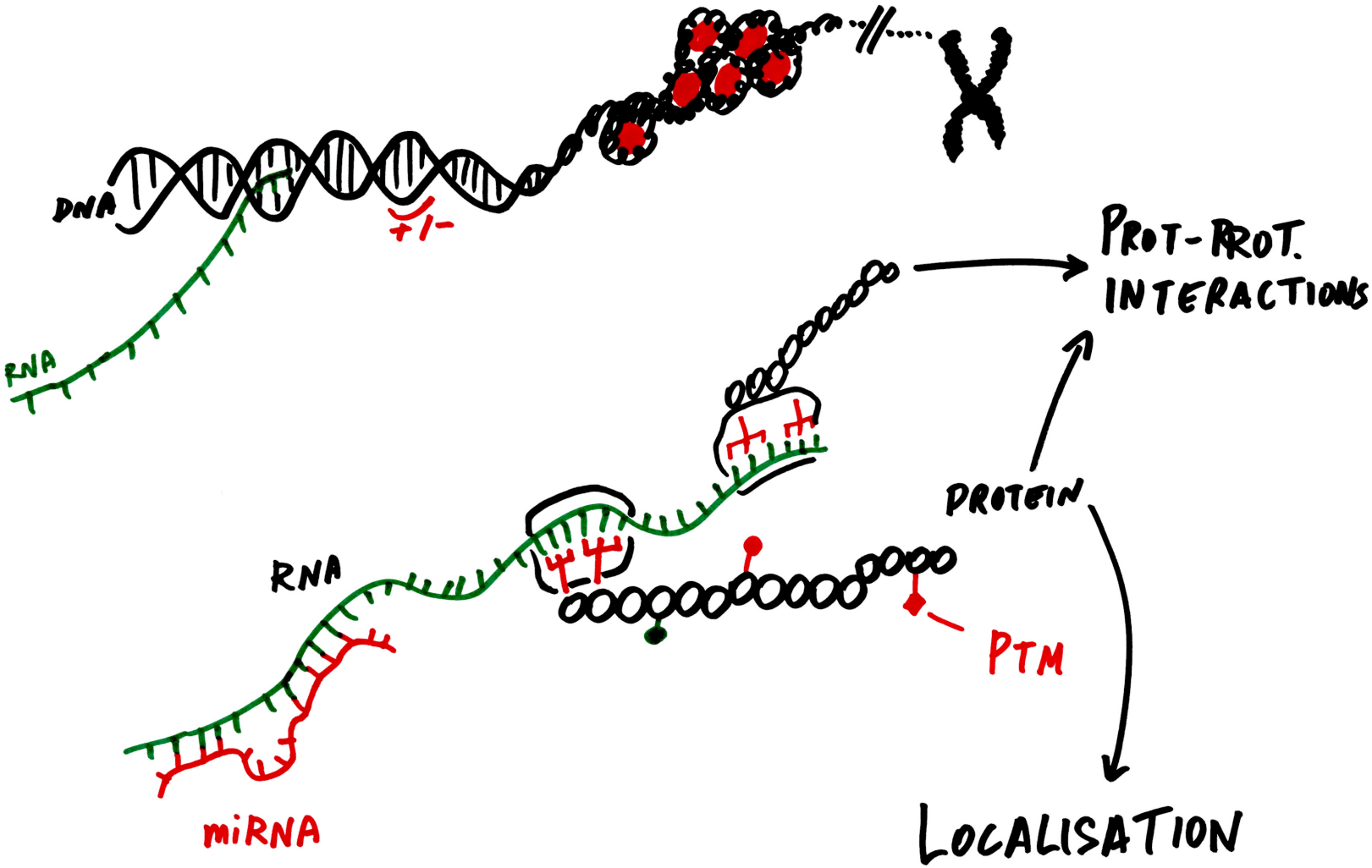
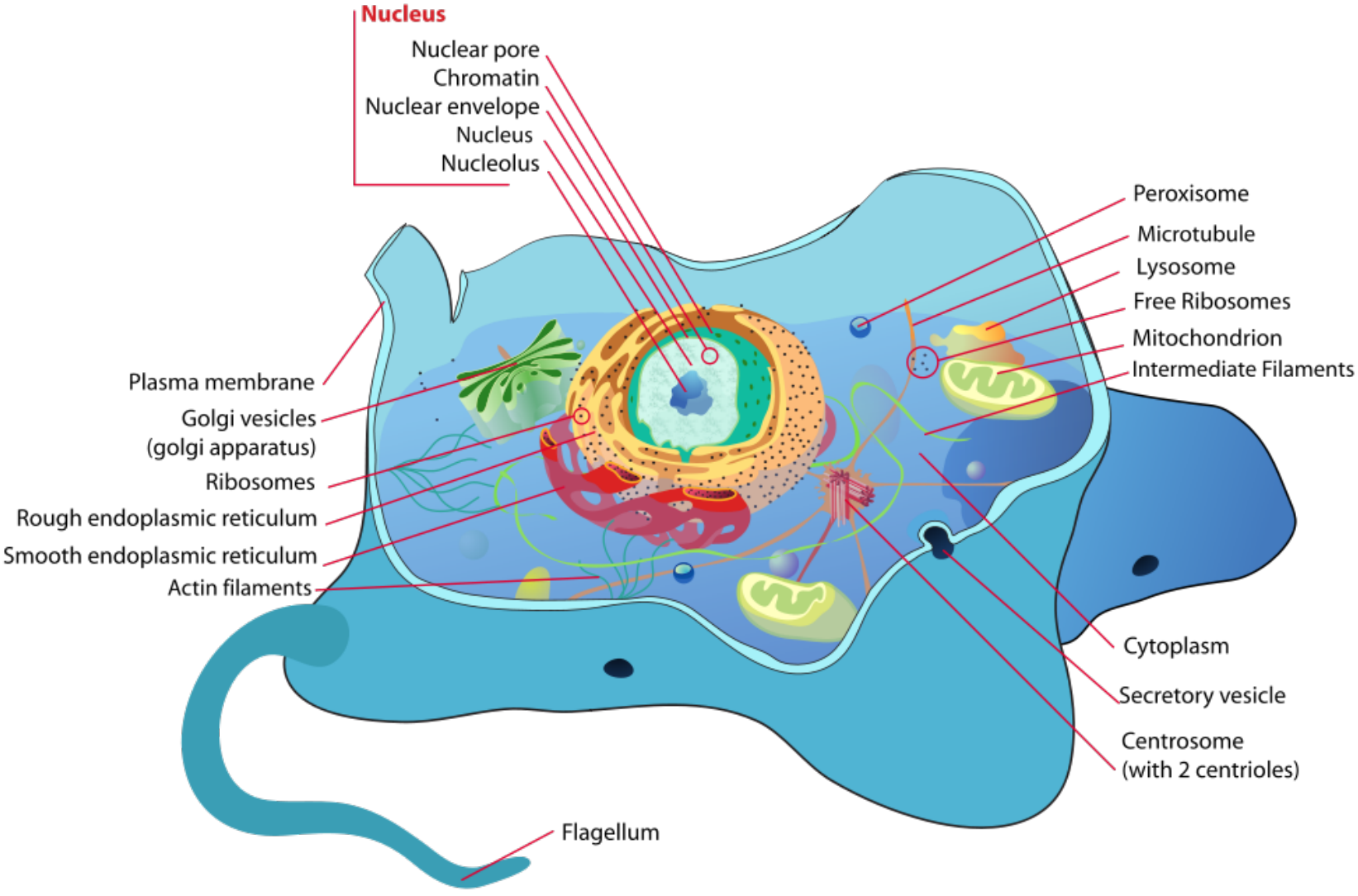
Plan

Regulations



Cell organisation



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

- 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.

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).

Re-localisation in

- Differentiation: Tfe3 in mouse ESC (Betschinger et al., 2013).
- Metabolism: changes in carbon sources, elemental limitations.

Spatial proteomics - How, experimentally

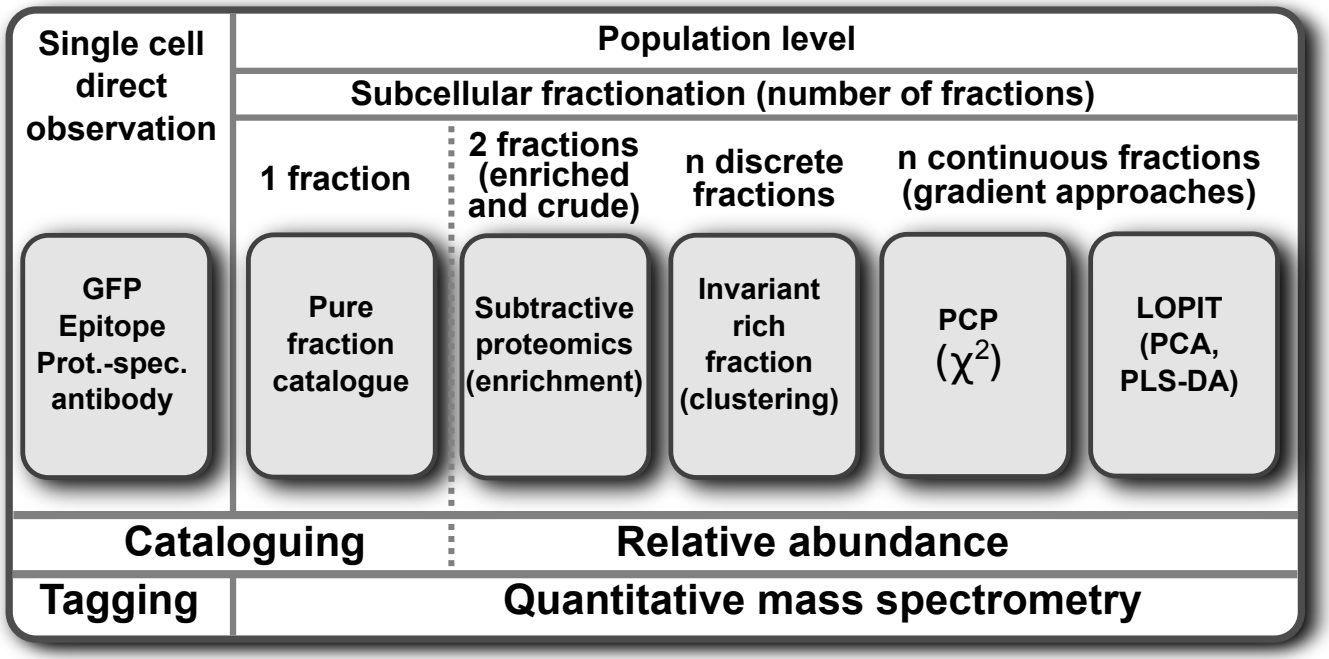
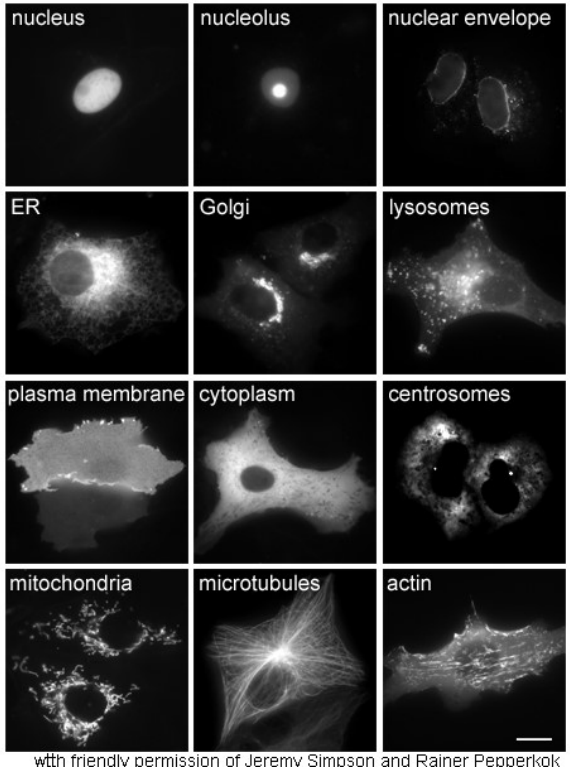
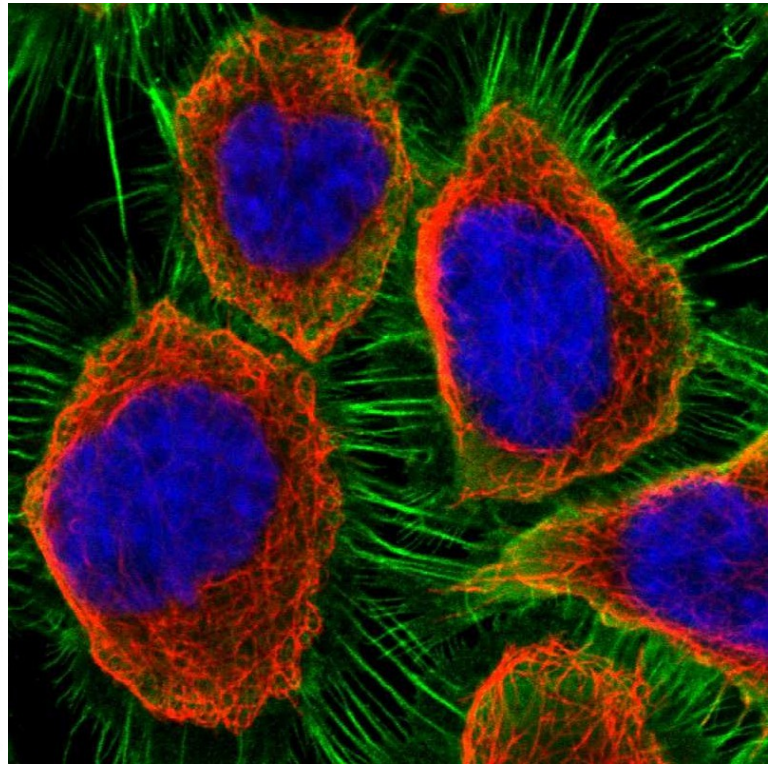


Figure : Organelle proteomics approaches (Gatto et al., 2010)

Fusion proteins and immunofluorescence



with friendly permission of Jeremy Simpson and Rainer Pepperkok



Fusion proteins and immunofluorescence

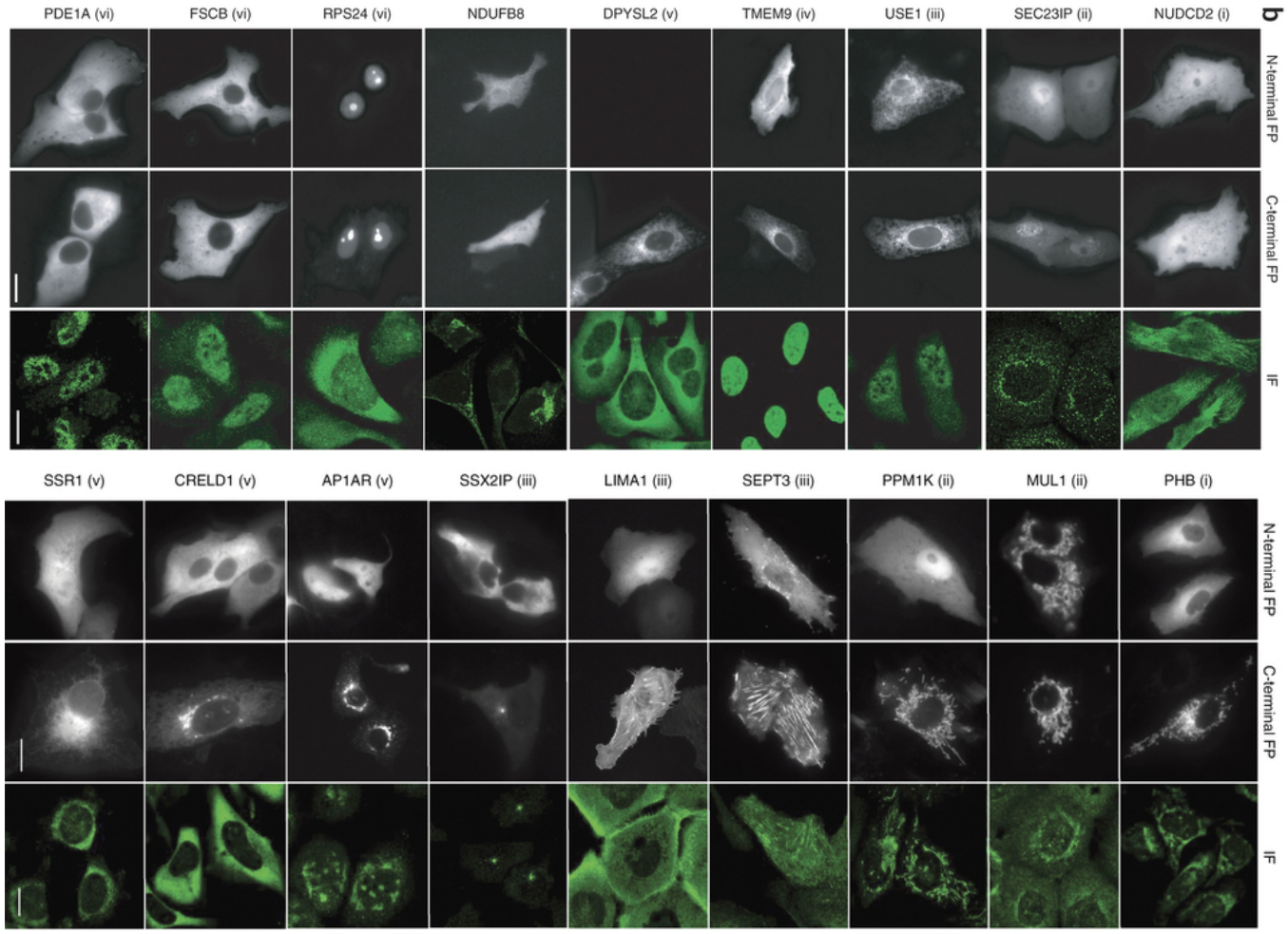


Figure : Example of discrepancies between IF and FPs as well as between FP tagging at the N and C termini (Stadler et al., 2013).

Spatial proteomics - How, experimentally

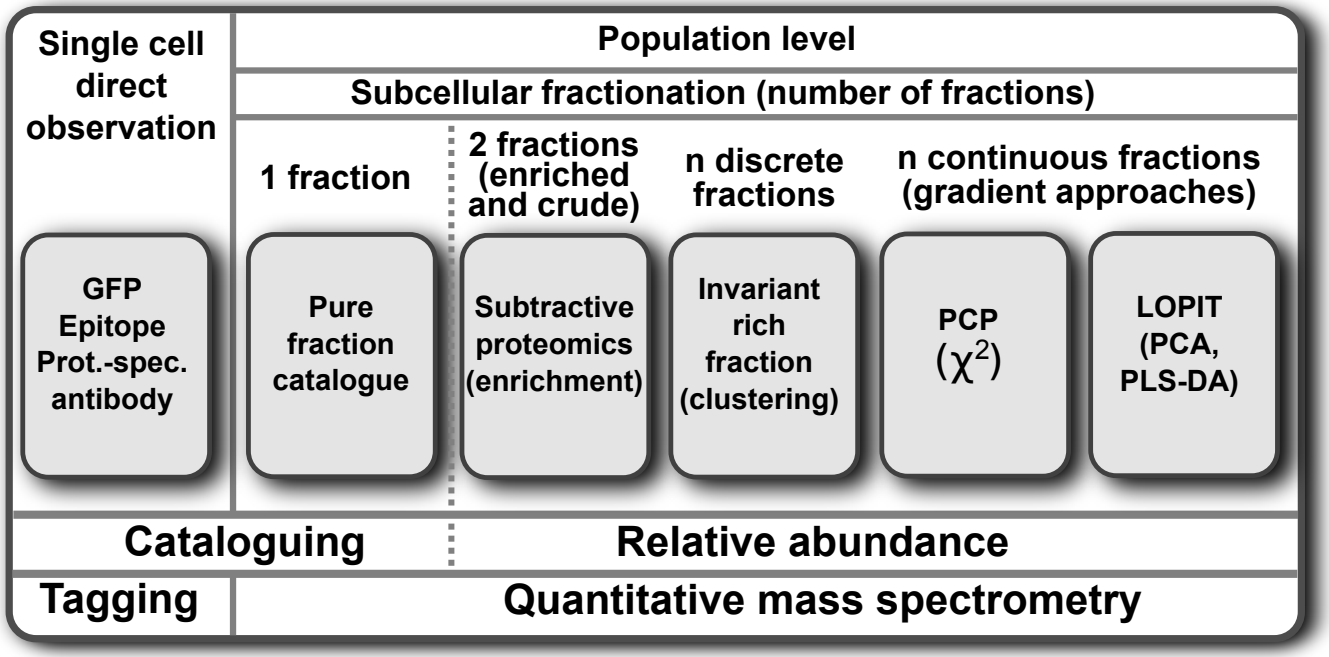
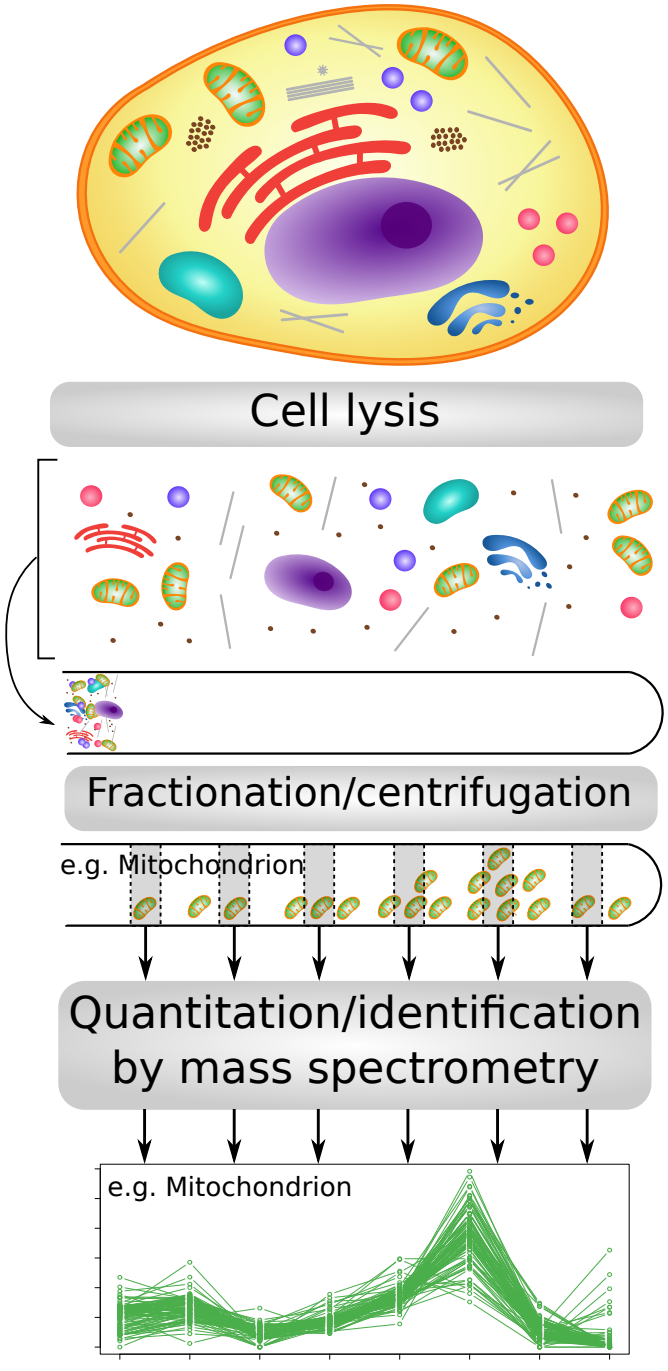


Figure : Organelle proteomics approaches (Gatto et al., 2010). Gradient approaches: Dunkley et al. (2006), Foster et al. (2006).

$\Rightarrow$ **Explorative/discovery approches**, **global localisation maps**.


Cell lysis
Fractionation/centrifugation
e.g. Mitochondrion
Quantitation/identification
by mass spectrometry
e.g. Mitochondrion

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	$q_{2,1}$	$q_{2,2}$	...	$q_{2,\,m}$	loc_1
p_3	$q_{3,1}$	$q_{3,2}$	...	$q_{3,\,m}$	unknown
p_4	$q_{4,1}$	$q_{4,2}$	...	$q_{4,\,m}$	loc_i
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
p_j	$q_{j,1}$	$q_{j,2}$	...	$q_{j,\,m}$	unknown

Visualisation and classification

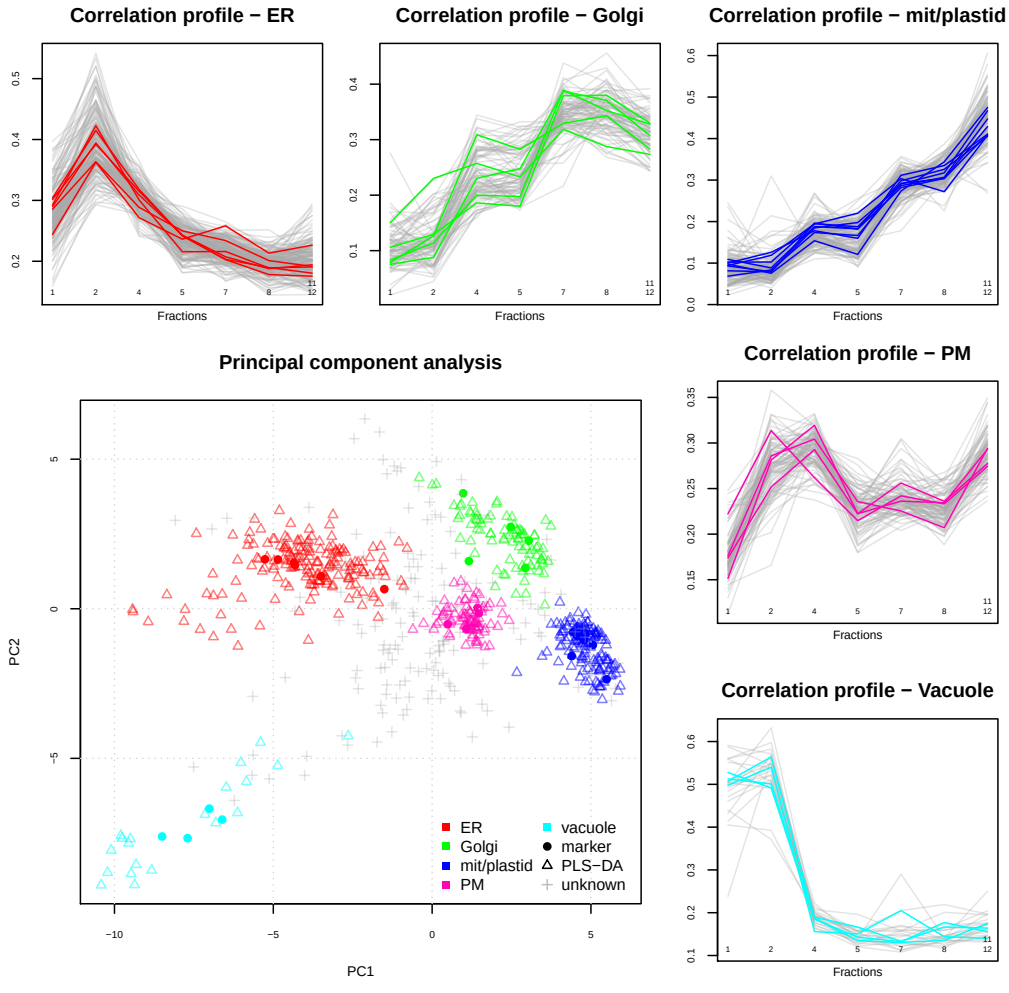


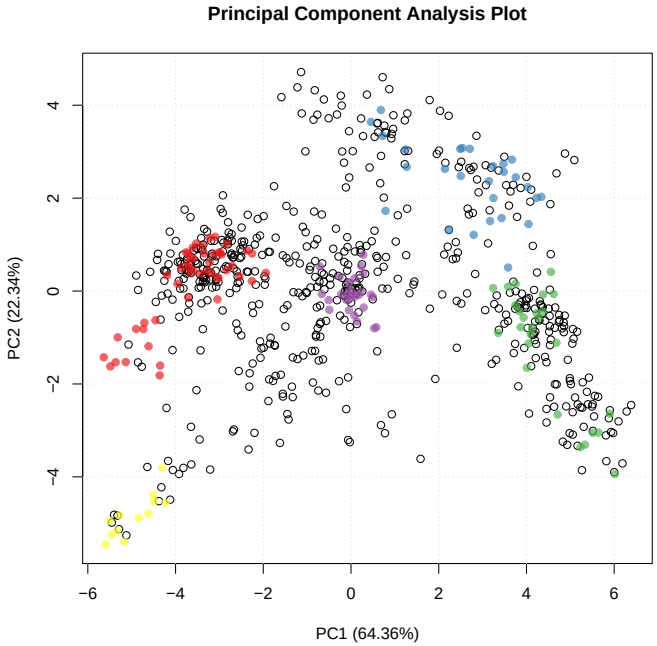
Figure : From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)

Data analysis

	Fraction_1	Fraction_2	...	Fraction_m
prot_1	$q_{1,1}$	$q_{1,2}$	...	$q_{1,\,m}$
prot_2	$q_{2,1}$	$q_{2,2}$	...	$q_{2,\,m}$
prot_3	$q_{3,1}$	$q_{3,2}$	...	$q_{3,\,m}$
prot_4	$q_{4,1}$	$q_{4,2}$	...	$q_{4,\,m}$
⋮	⋮	⋮	⋮	⋮
prot_i	$q_{i,1}$	$q_{i,2}$	...	$q_{i,\,m}$
⋮	⋮	⋮	⋮	⋮
prot_n	$q_{n,1}$	$q_{n,2}$	...	$q_{n,\,m}$

	markers	
...	*unknown*	...
	$organelle_1$	
	unknown	
	$organelle_2$	
⋮	⋮	⋮
	$organelle_k$	
⋮	⋮	⋮
...	*unknown*	

	Fraction_1	Fraction_2	...	Fraction_m
	...	...	...	...
	⋮	⋮	⋮	⋮
	...	...	...	...



Supervised machine learning

Using labelled marker proteins to match unlabelled proteins (of unknown localisation) with similar profiles and classify them as residents to the markers organelle class.

Supervised ML

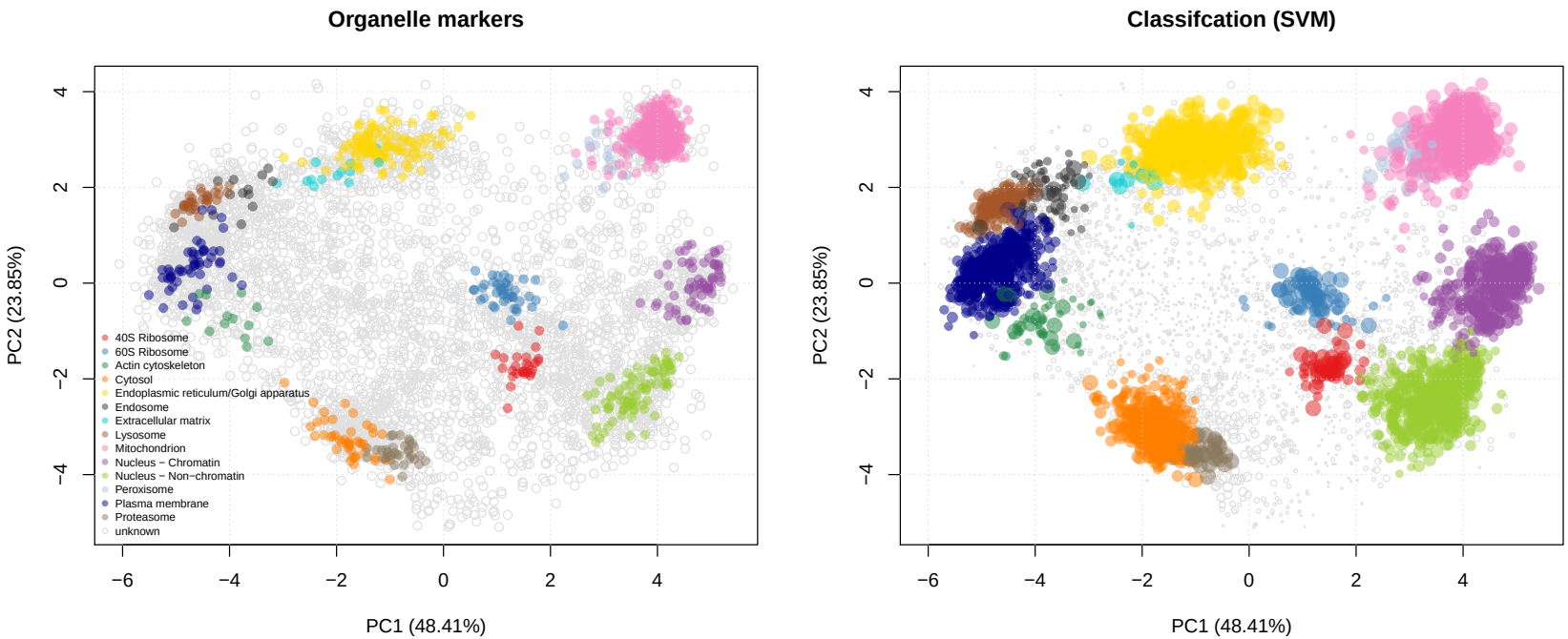
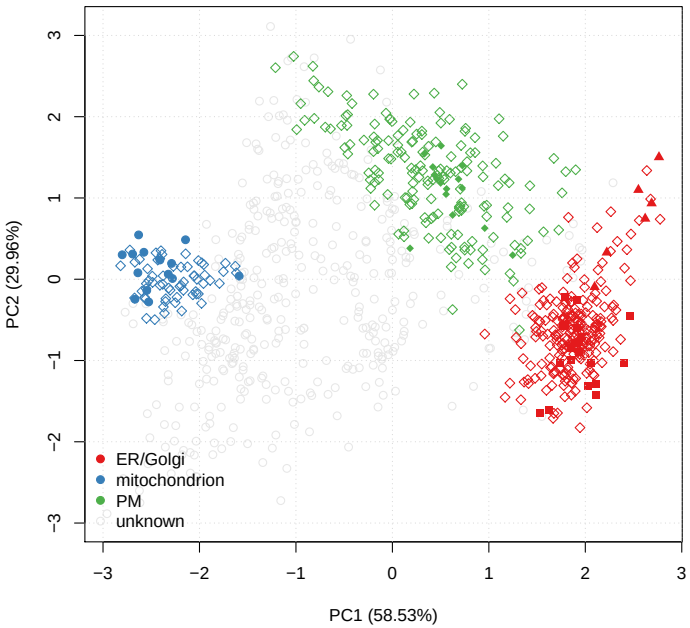
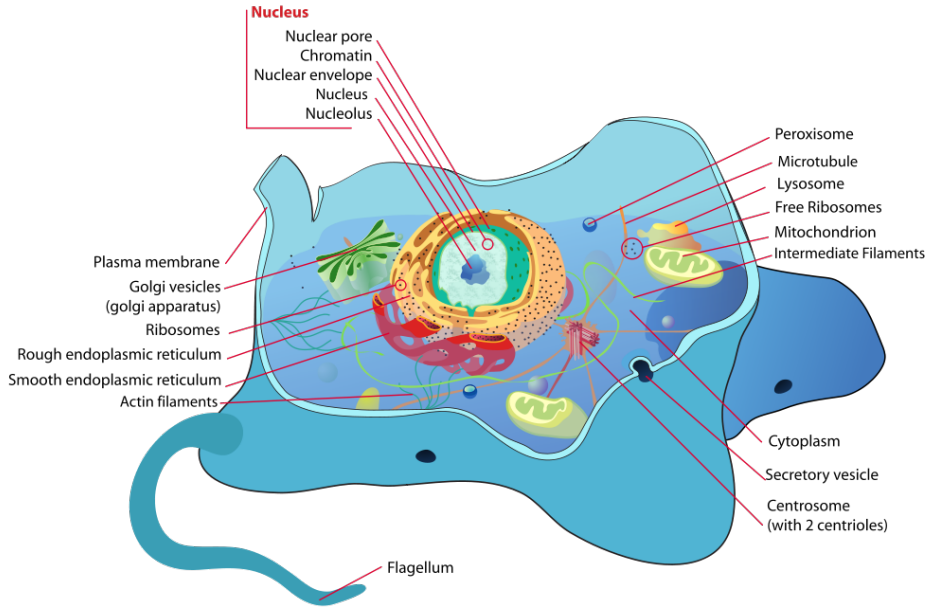


Figure : Support vector machines classifier on the embryonic stem cell data from Christoforou et al. (2016).

Limitations





Incomplete annotation, and therefore lack of training data, for many/most organelles. *Drosophila* data from Tan et al. (2009).

Novelty detection

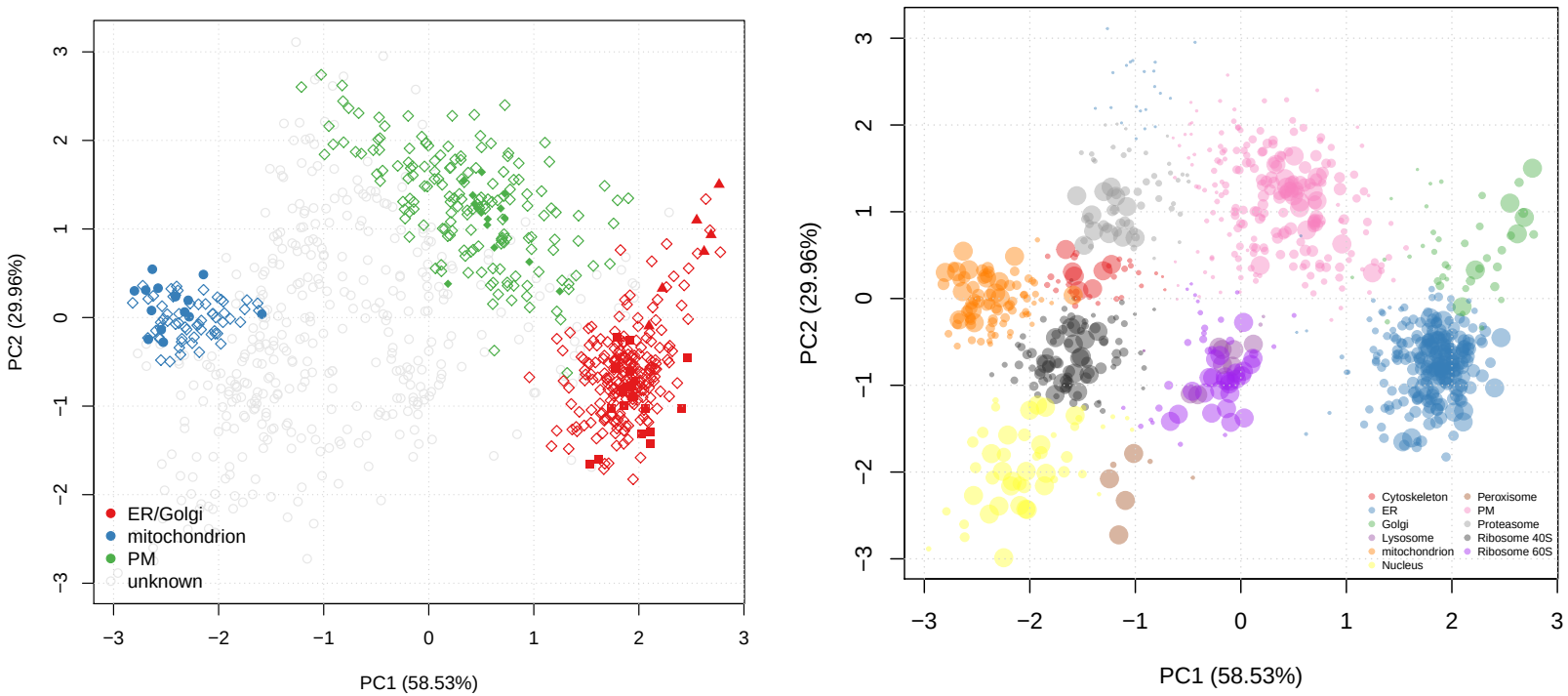


Figure : Left: *Drosophila* data from Tan et al. (2009). Right: Semi-supervised learning, Breckels et al. (2013).

Improving on LOPIT

Improving is obtaining better sub-cellular resolution to increase the number of protein that can be **confidently** assigned to a sub-cellular niche.

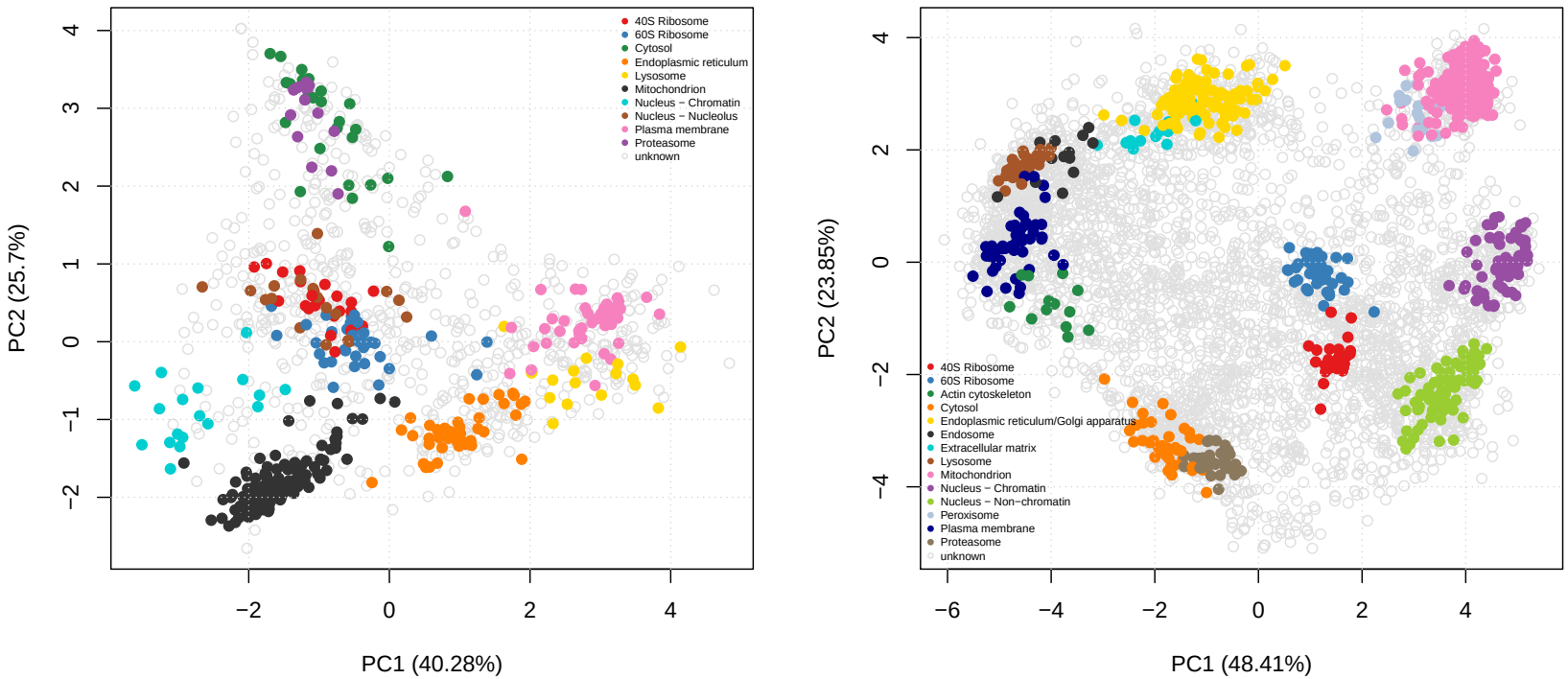


Figure : E14TG2a embryonic stem cells: old (left) *vs.* new, better resolved (right) experiments (Christoforou et al. (2016)).

Improving on LOPIT

LOPIT Dunkley et al. (2006)	**Computational**: *transfer learning* Breckels et al. (2016)
Experimental: *hyperLOPIT* Christoforou et al. (2016) Mulvey et al. (2017)	Biological discoveries

hyperLOPIT

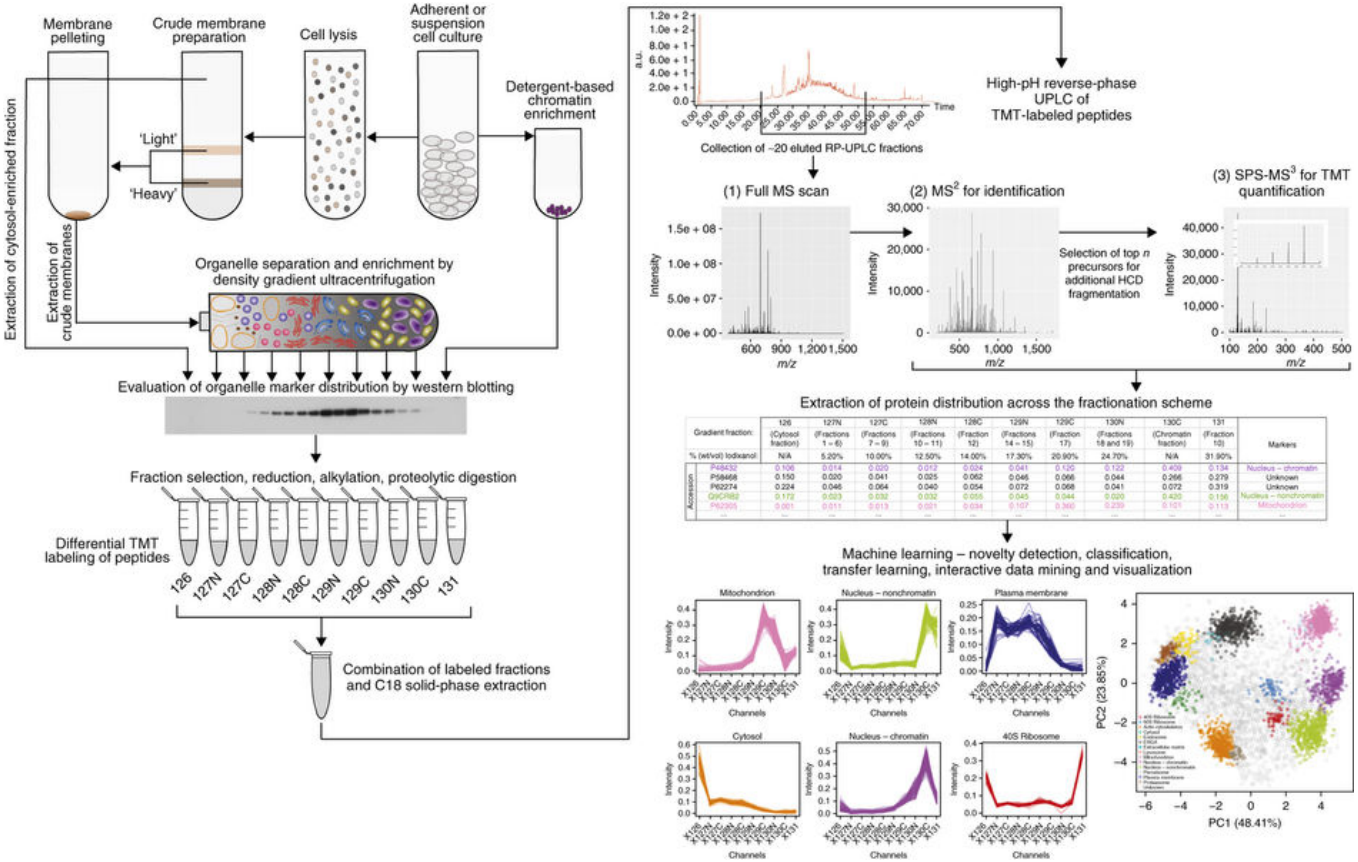


Figure : From Mulvey et al. (2017) *Using hyperLOPIT to perform high-resolution mapping of the spatial proteome*.

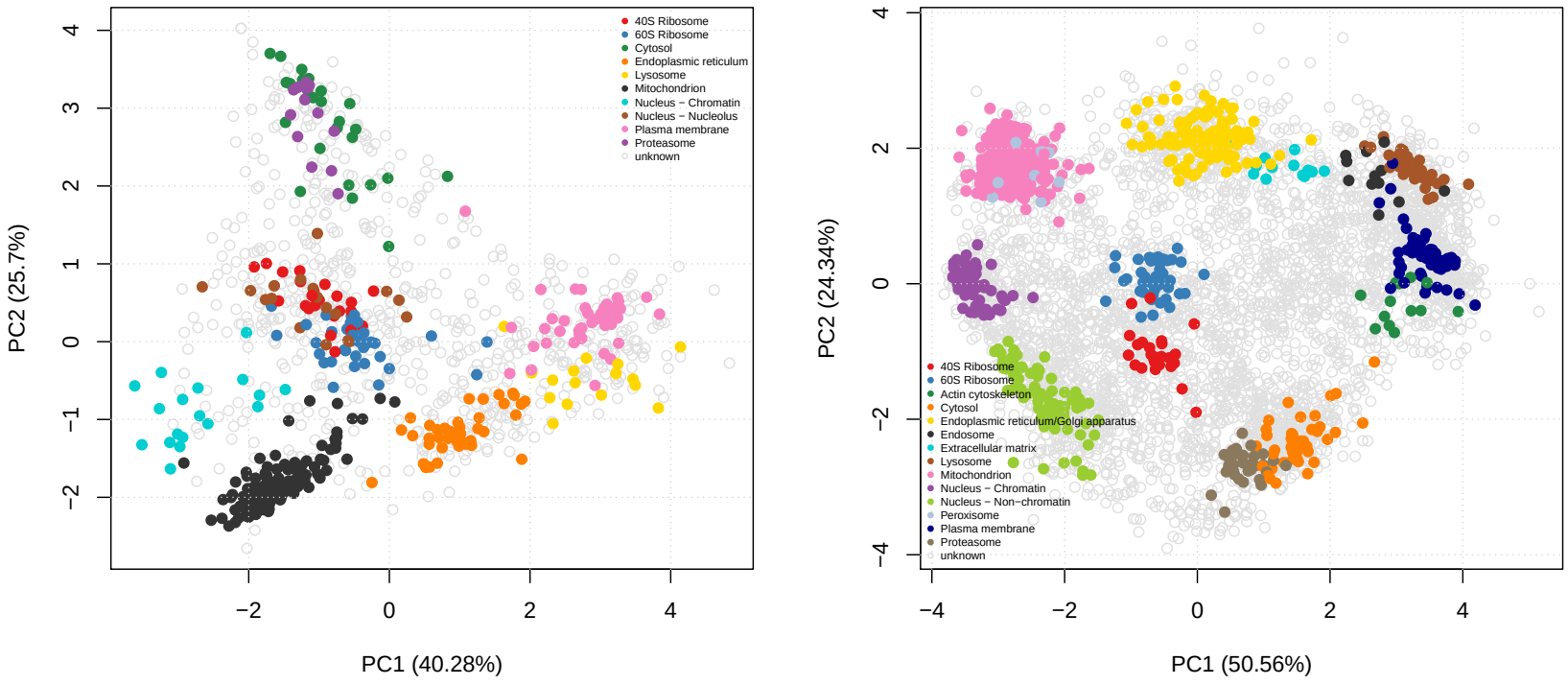


Figure : E14TG2a LOPIT on 8 fractions (using iTRAQ 8-plex) and 1109 proteins *vs.* hyperLOPIT on 10 fractions (using TMT 10-plex) and SPS-MS^3 for 5032 proteins.

Transfer learning

What about annotation data from repositories such as the Gene Ontogy (GO), sequence features, signal peptide, transmembrane domains, images, prediction software, ...

- From a user perspective: **"free/cheap"** vs. expensive
- 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**

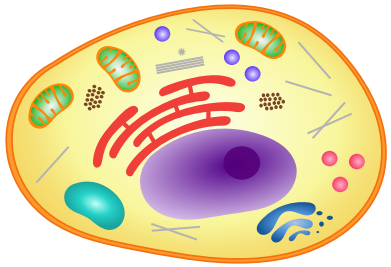
Transfer learning

What about annotation data from repositories such as the Gene Ontology (GO), sequence features, signal peptide, transmembrane domains, images, prediction software, ...

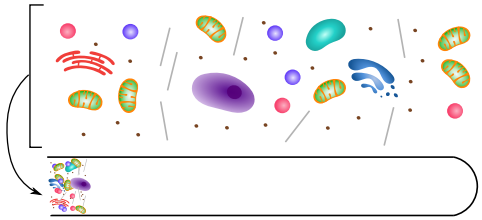
Transfer learning

Support/complement the **primary** target domain (experimental data) with **auxiliary** data (annotation, imaging, PPI, ...) features without compromising the integrity of our primary data (Breckels et al., 2016).

PRIMARY EXPERIMENTAL DATA

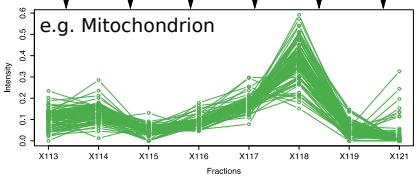


Cell lysis



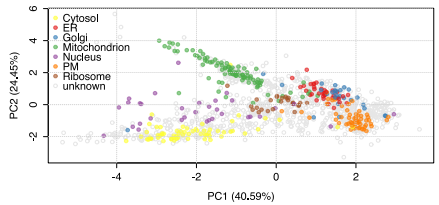
Fractionation/centrifugation

Quantitation/identification by mass spectrometry



	X113	X114	X115	X116	X117	X118	X119	X121
O00767	0.1361	0.150	0.1062	0.147	0.277	0.1429	0.0380	0.00338
P51648	0.1914	0.205	0.0566	0.165	0.237	0.0996	0.0180	0.02727
Q2TAA5	0.1297	0.201	0.0546	0.146	0.292	0.1463	0.0206	0.00902
Q9UKV5	0.0939	0.207	0.0419	0.204	0.344	0.1098	0.0000	0.00000

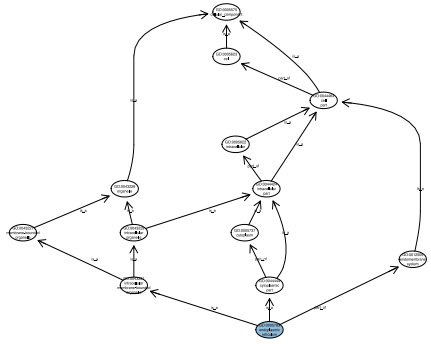
Visualisation



AUXILIARY DRY DATA

Database query

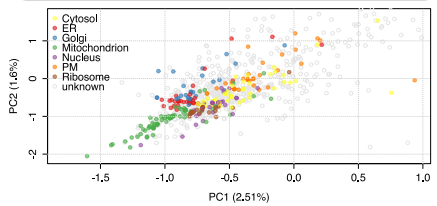
Extract GO CC terms



Convert terms to binary

	GO:0016021	GO:0005789	GO:0005783	...	...	...
O00767	1	1	1	...	...	...
P51648	1	1	0	...	...	...
Q2TAA5	1	1	0	...	...	...
Q9UKV5	0	0	0	...	...	...

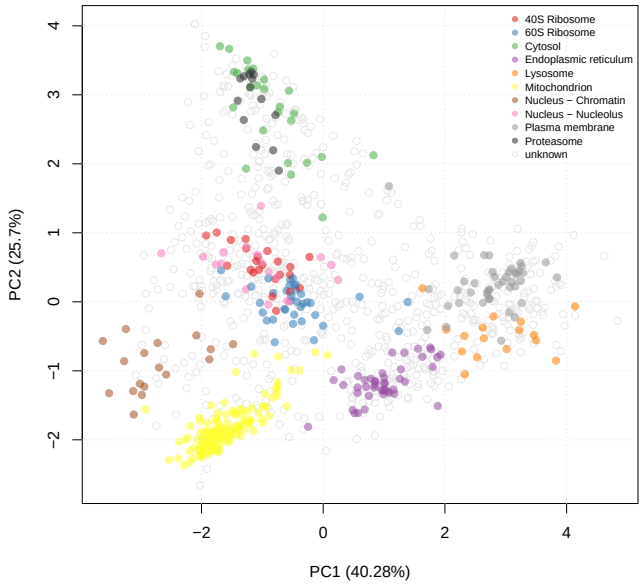
Visualisation



Transfer learnig, based on Wu and Dietterich (2004):

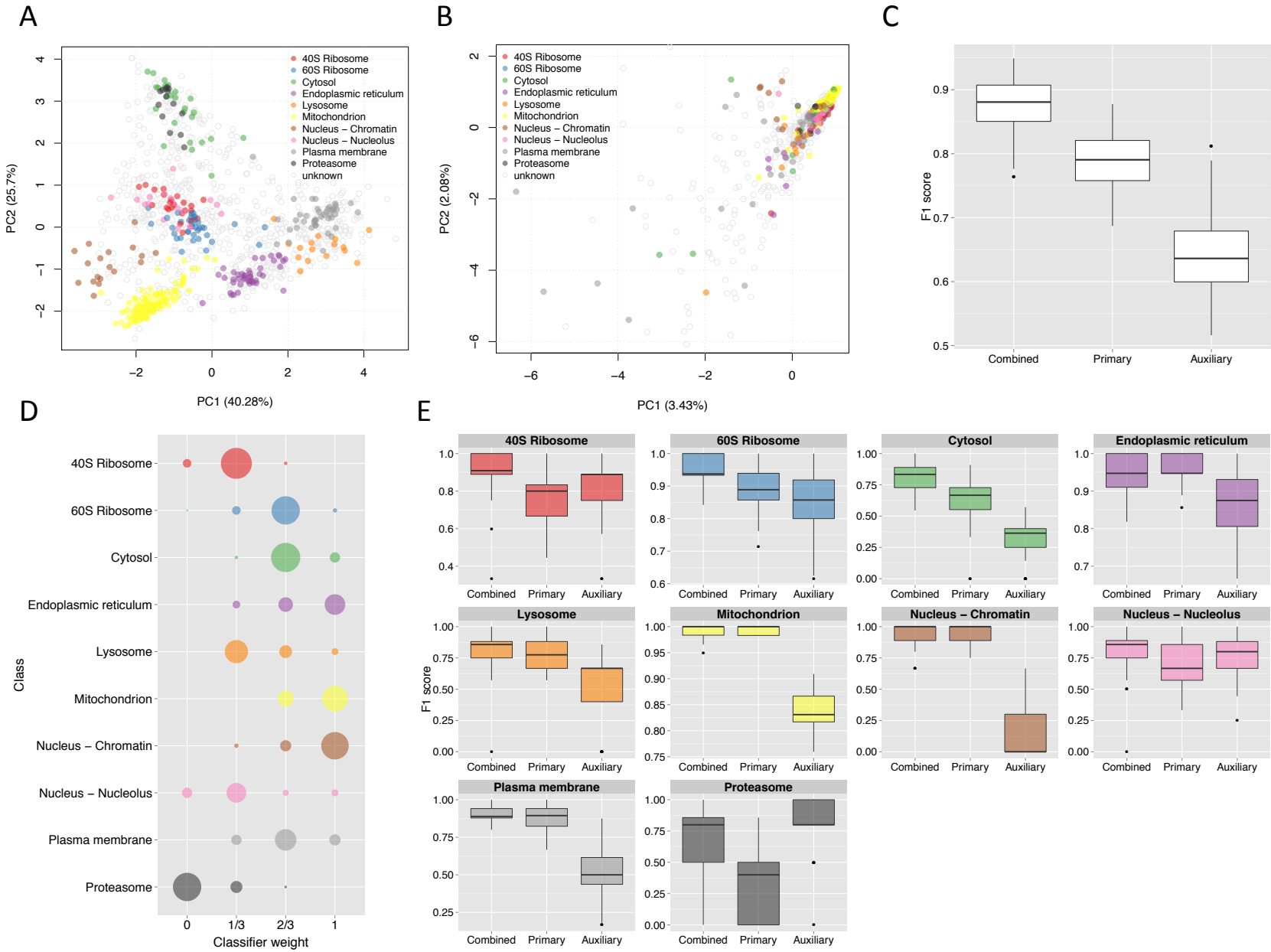
Class-weighted kNN

$$V(c_i)_j = \theta^* n_{ij}^P + (1 - \theta^*) n_{ij}^A$$



Linear programming SVM

$$f(\mathbf{x}, \mathbf{v}; \boldsymbol{\alpha}_P, \boldsymbol{\alpha}_A, b) = \sum_{l=1}^{m} y_l \left[\alpha_l^P K^P(\mathbf{x}_l, \mathbf{x}) + \alpha_l^A K^A(\mathbf{v}_l, \mathbf{v}) \right] + b$$



Data from mouse stem cells (E14TG2a).

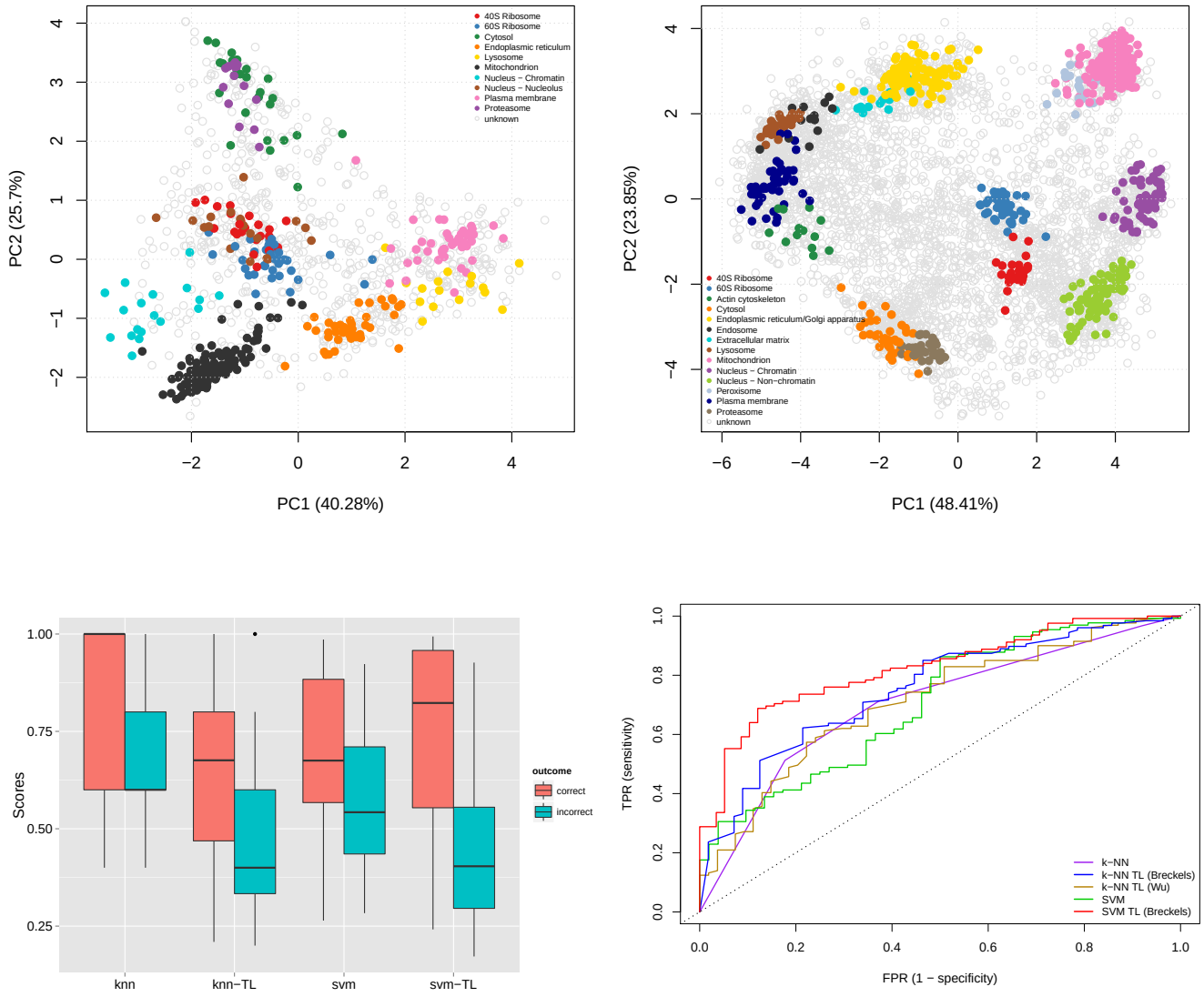


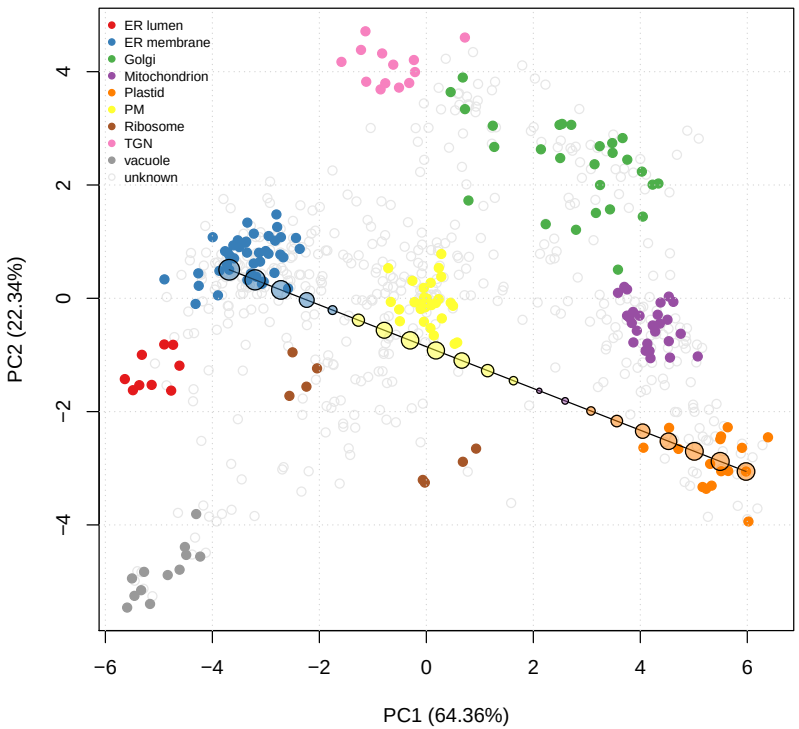
Figure : From Breckels et al. (2016) *Learning from heterogeneous data sources: an application in spatial proteomics*.

Biological applications

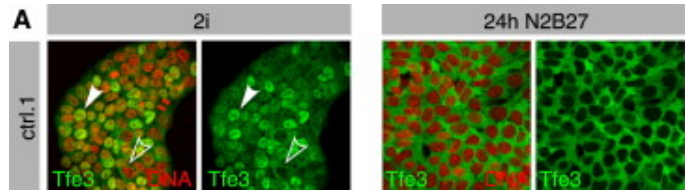
- Multi-localisation
- Trans-localisation

Dependent on good sub-cellular resolution.

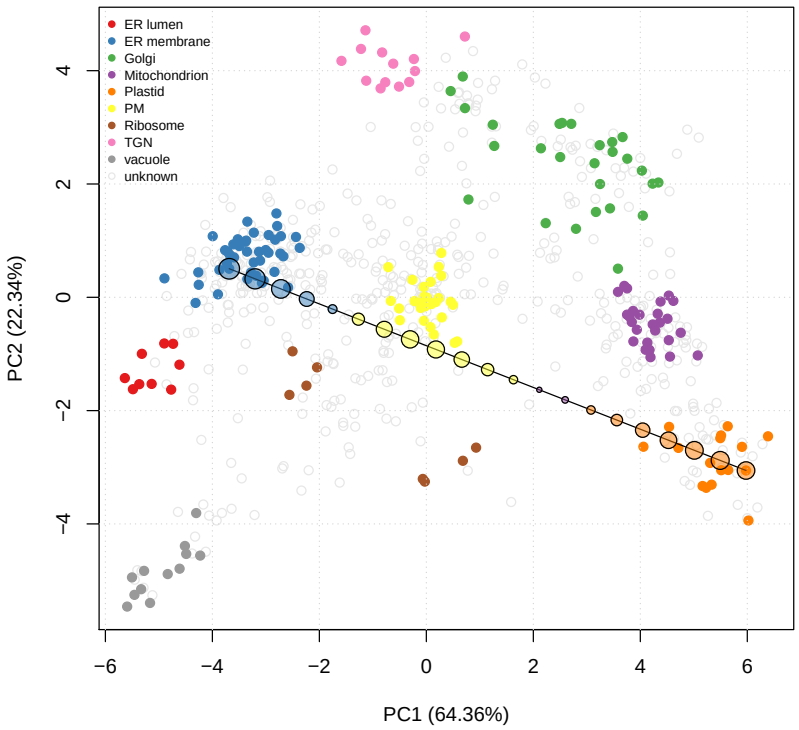
Dual-localisation Proteins may be present simultaneously in several organelles (e.g. trafficking). Example from embryonic stem cells (Christoforou et al., 2016).

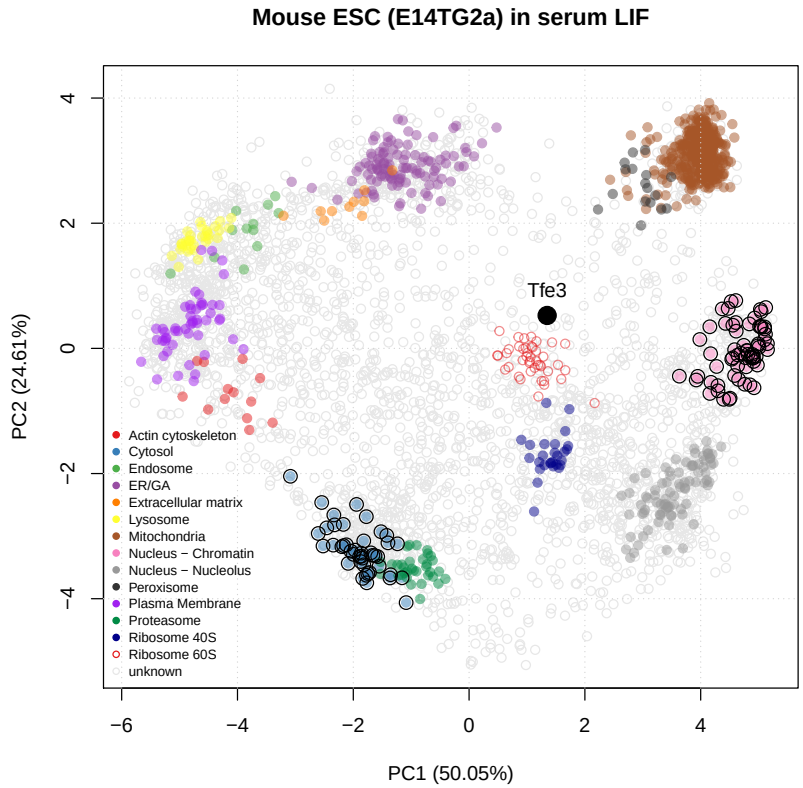


Dual-localisation Proteins may be present simultaneously in several organelles (e.g. trafficking). Example from embryonic stem cells (Christoforou et al., 2016).



From Betschinger et al. (2013)





Exampl

from

Spatial dynamics

Trans-localisation monocyte to macrophage differenciation

Goal: to investigate the effect of 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 components, before actual morphological differentiation at 24h.

Work lead by **Dr Claire Mulvey**, Cambridge Centre for Proteomics.

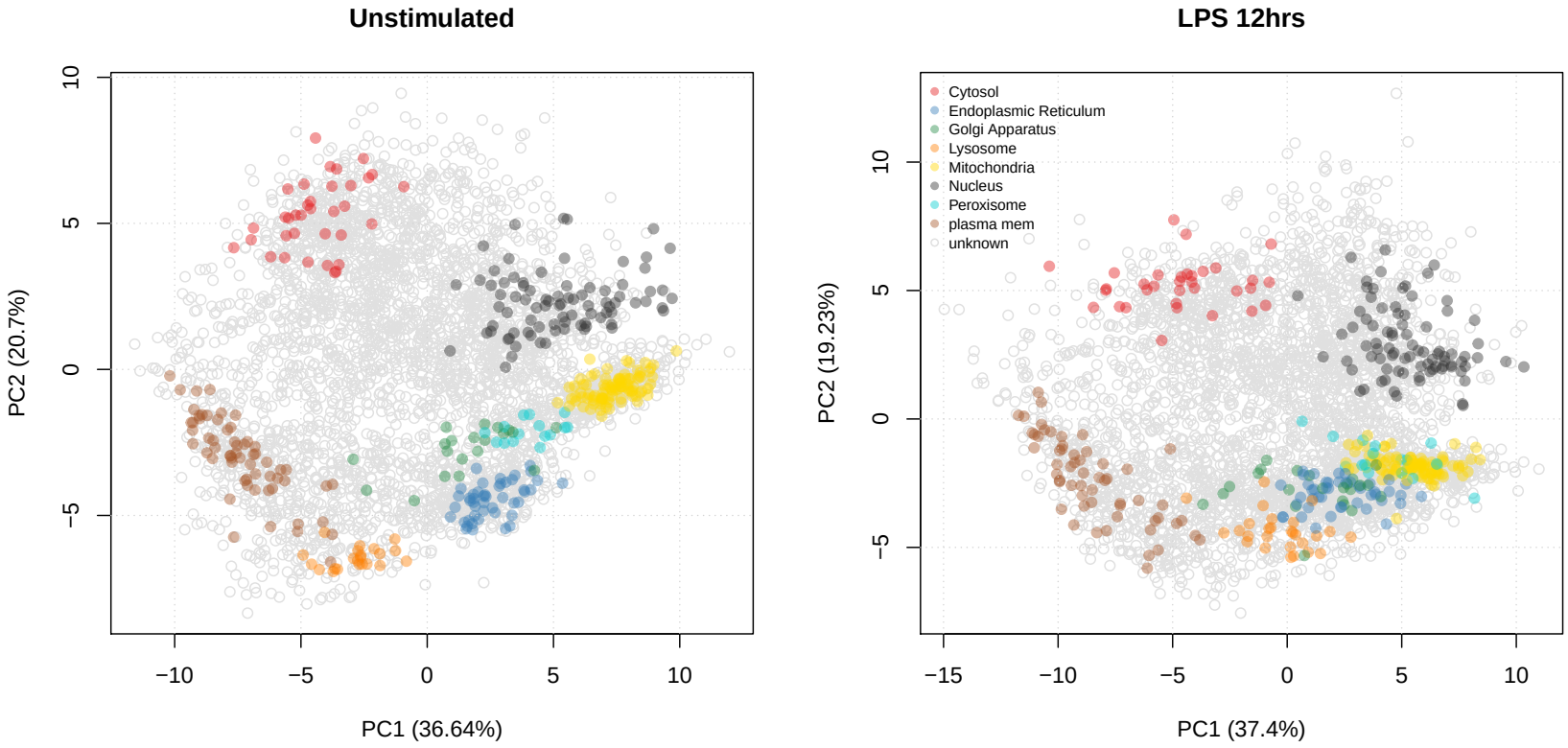


Figure : Spatial maps: unstimulated and LPS-treated.

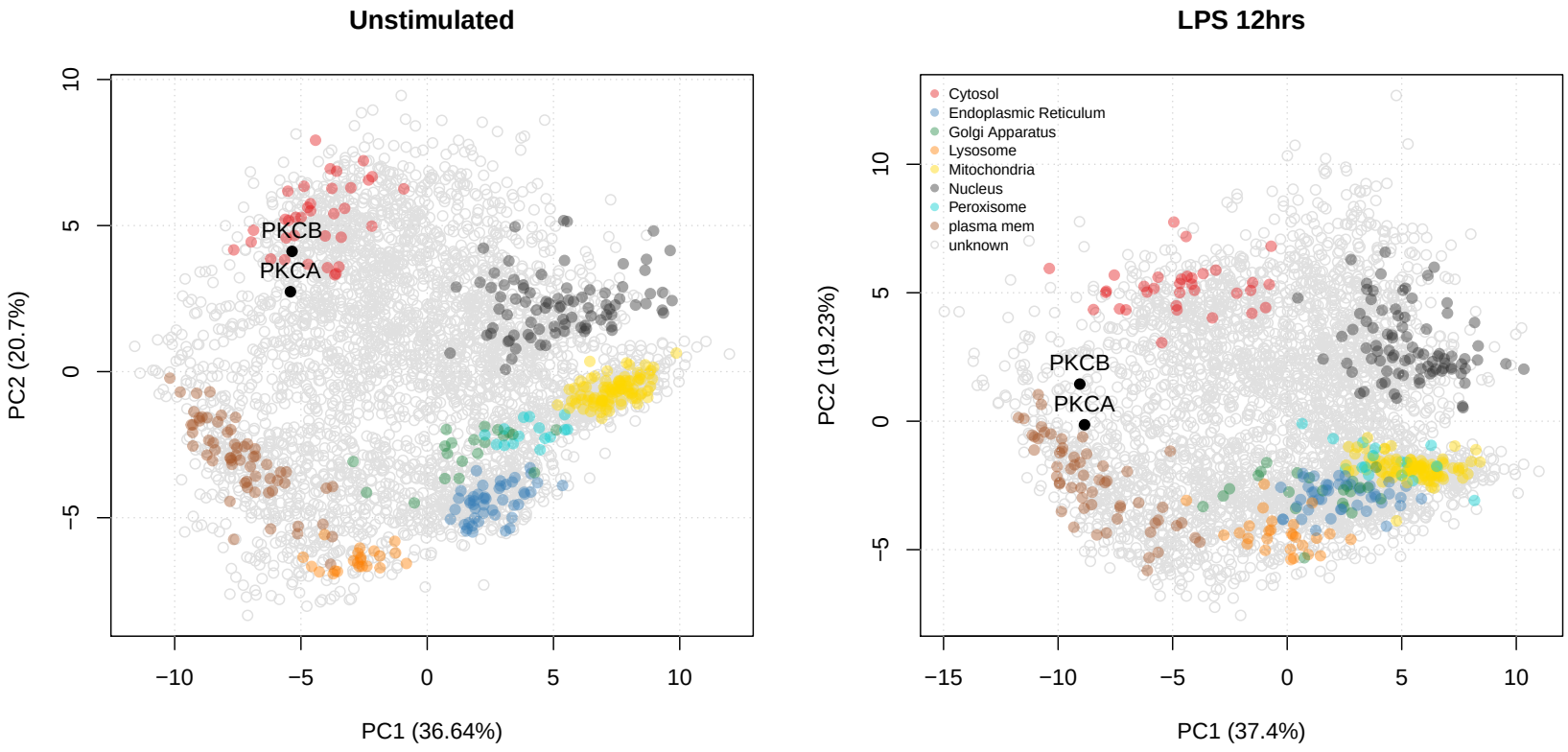


Figure : Relocation of Protein Kinase C alpha and beta from the cytosol to the plasma membrane, driving maturation into a differentiated macrophage phenotype.

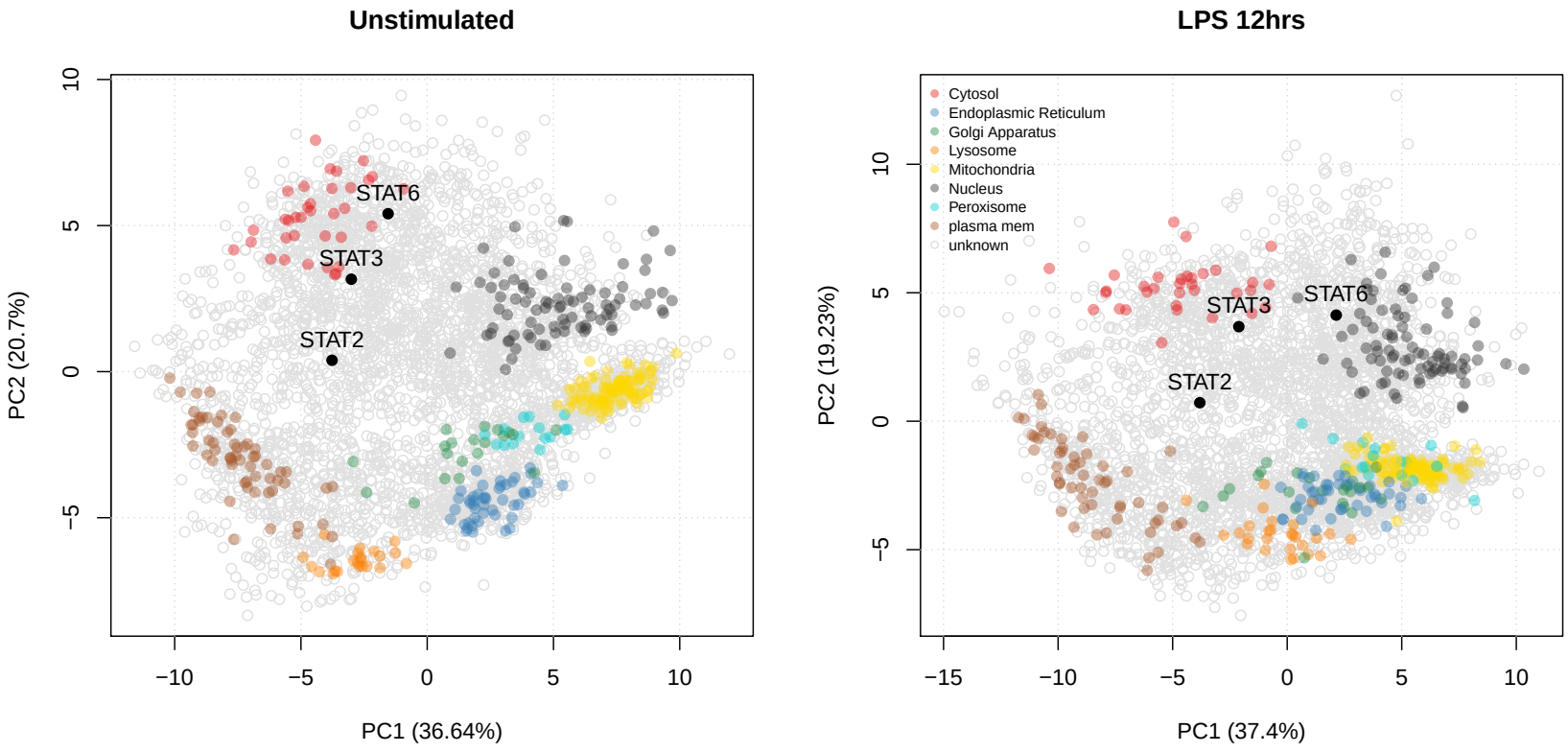


Figure : Relocation of STAT6 from the cytosol to the Nucleus, activating anti-bacterial and anti-viral-like response. Validated by microscopy and see also Chen et al. (2011).

Beyond organelles: application to PPI/Protein complexes

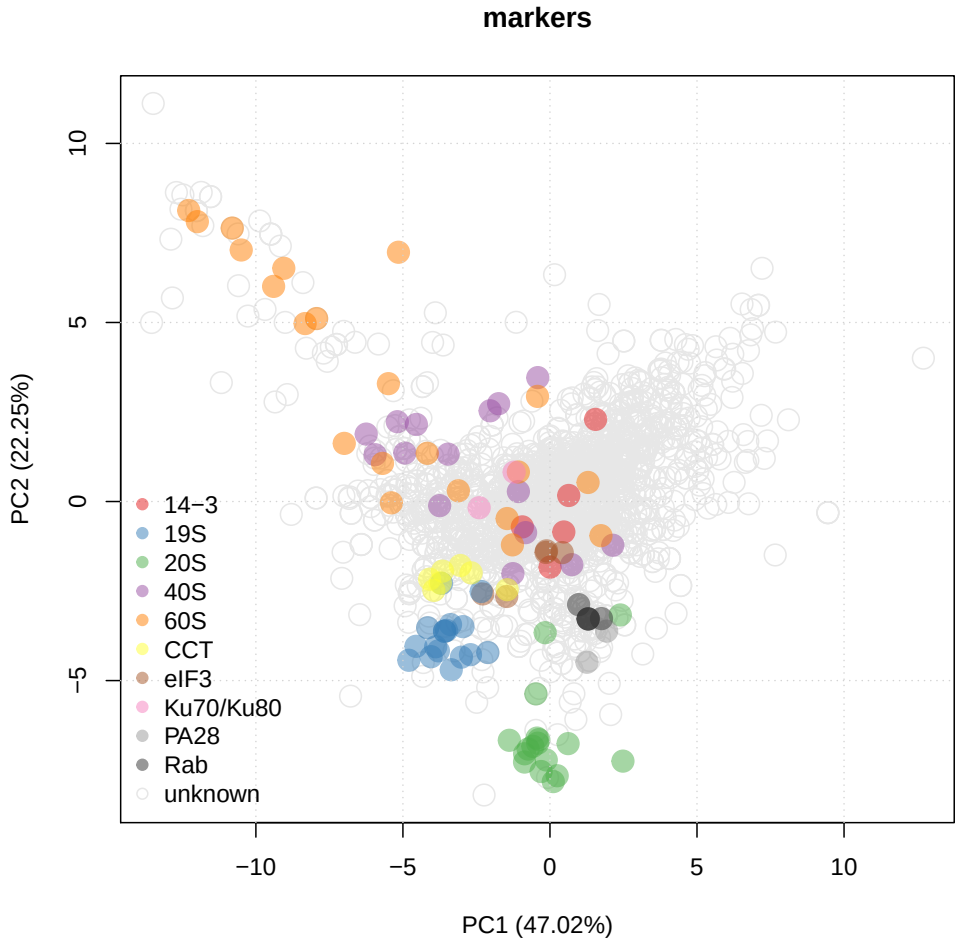


Figure : Data on proteasome complexes from Fabre *et al.* Mol Syst Biol (2015), DOI: 10.15252/msb.20145497

Plan

R/Bioconductor:

- Software for spatial proteomics.
- Ecosystem for high throughput biology data analysis and comprehension.

Software for mass spectrometry and (spatial) proteomics

Bioconductor Open source, enable **reproducible research**, enables understanding of the data (not a black box) and **drive scientific innovation**.

- `MSnbase` – infrastructure to handle quantitative data and meta-data (Gatto and Lilley, 2012) (~500 unique IP download/month in 2016).
- `pRoloc` and `pRolocGUI` – dedicated visualisation and ML infrastructure for spatial proteomics (Gatto et al., 2014) (~200 unique IP download/month in 2016).
- `pRolocdata` – structured and annotated spatial proteomics data (Gatto et al., 2014).
- And more generally `RforProteomics` (Gatto and Christoforou, 2014) (~160 unique IP download/month in 2016).

Plan

- What is Collaborative and open development?
- Use case: `MSnbase` and `mzR`: contributors and shared infrastructure for MS-based proteomics and metabolomics.

Use case

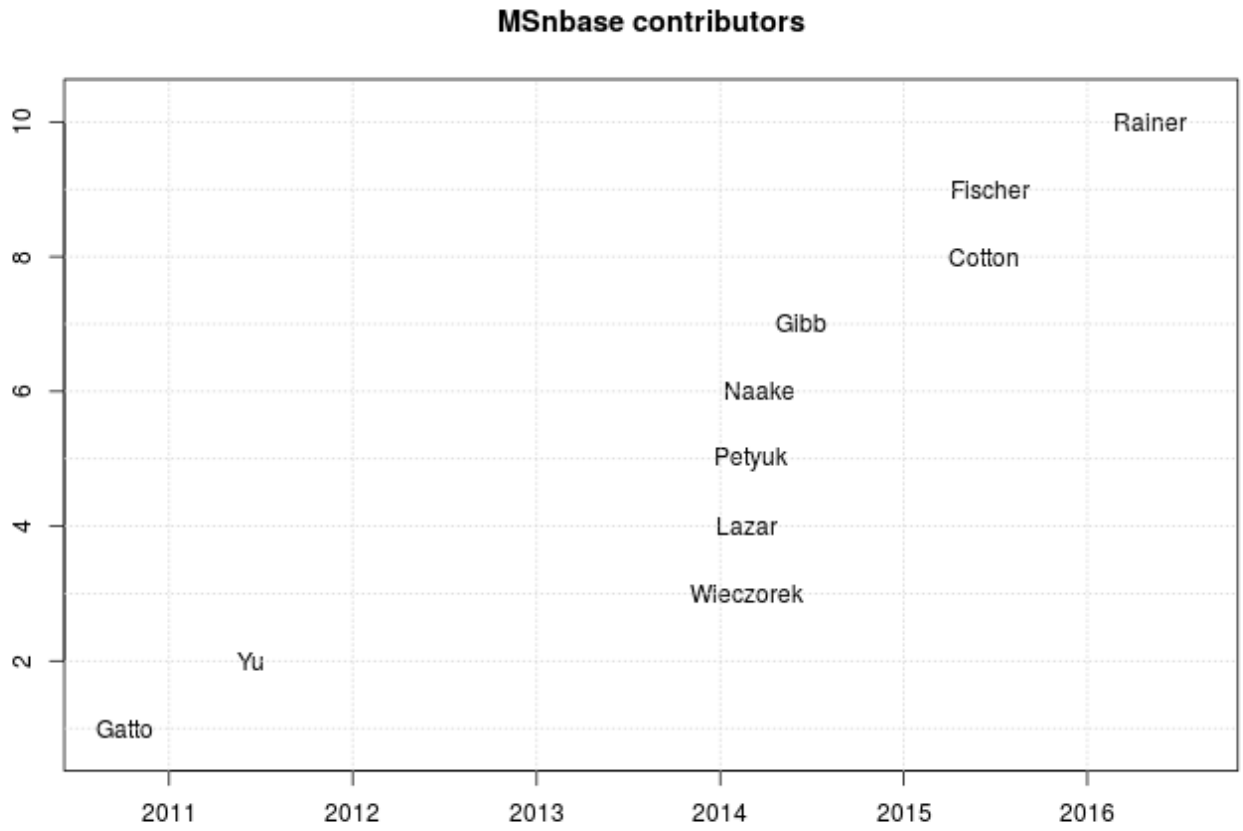


Figure : Contributions to the `MSnbase` package since its creation. More details: https://lgatto.github.io/msnbase-contribs/

References I

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