Mapping the sub-cellular proteome

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Slides @ http://goo.gl/SZRMjg
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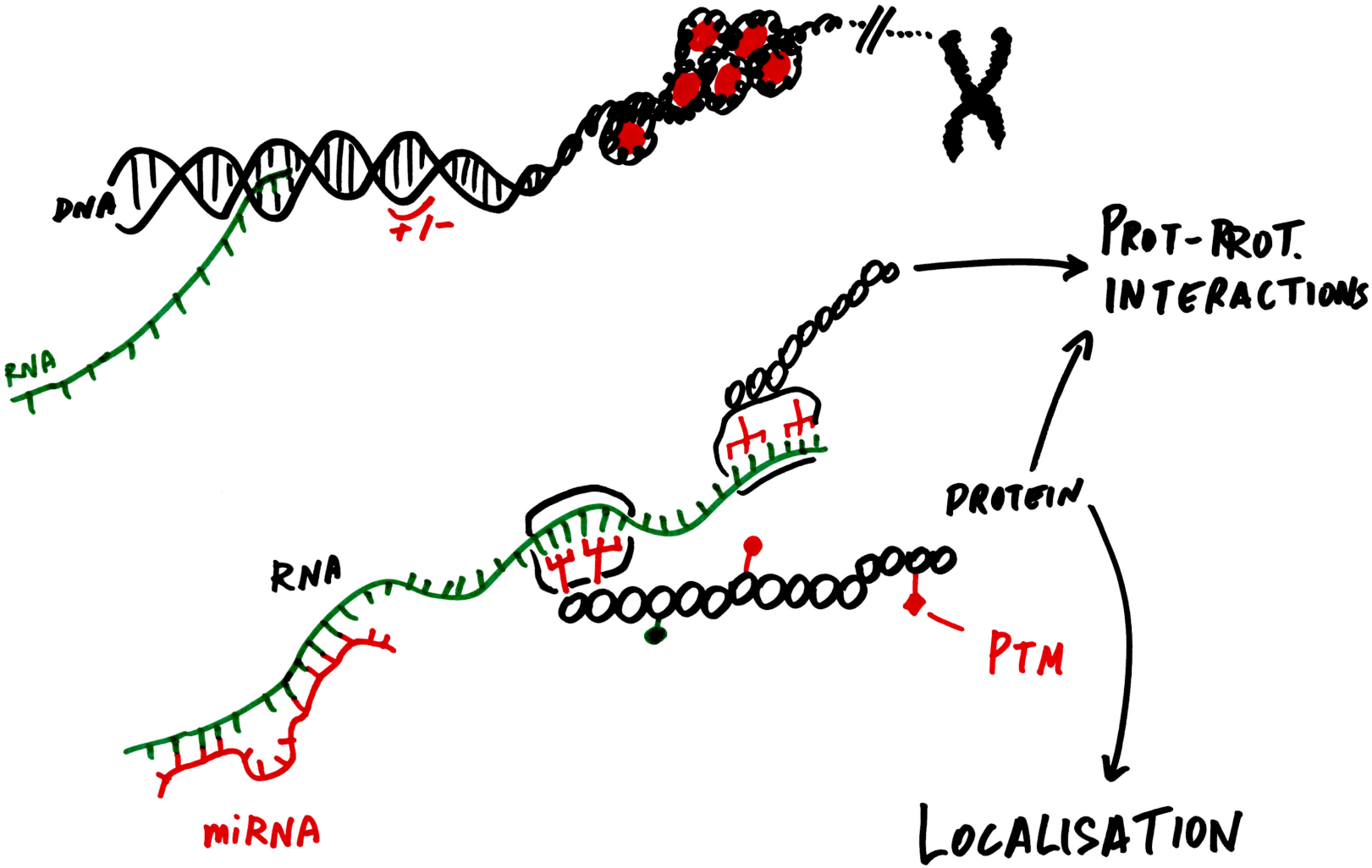
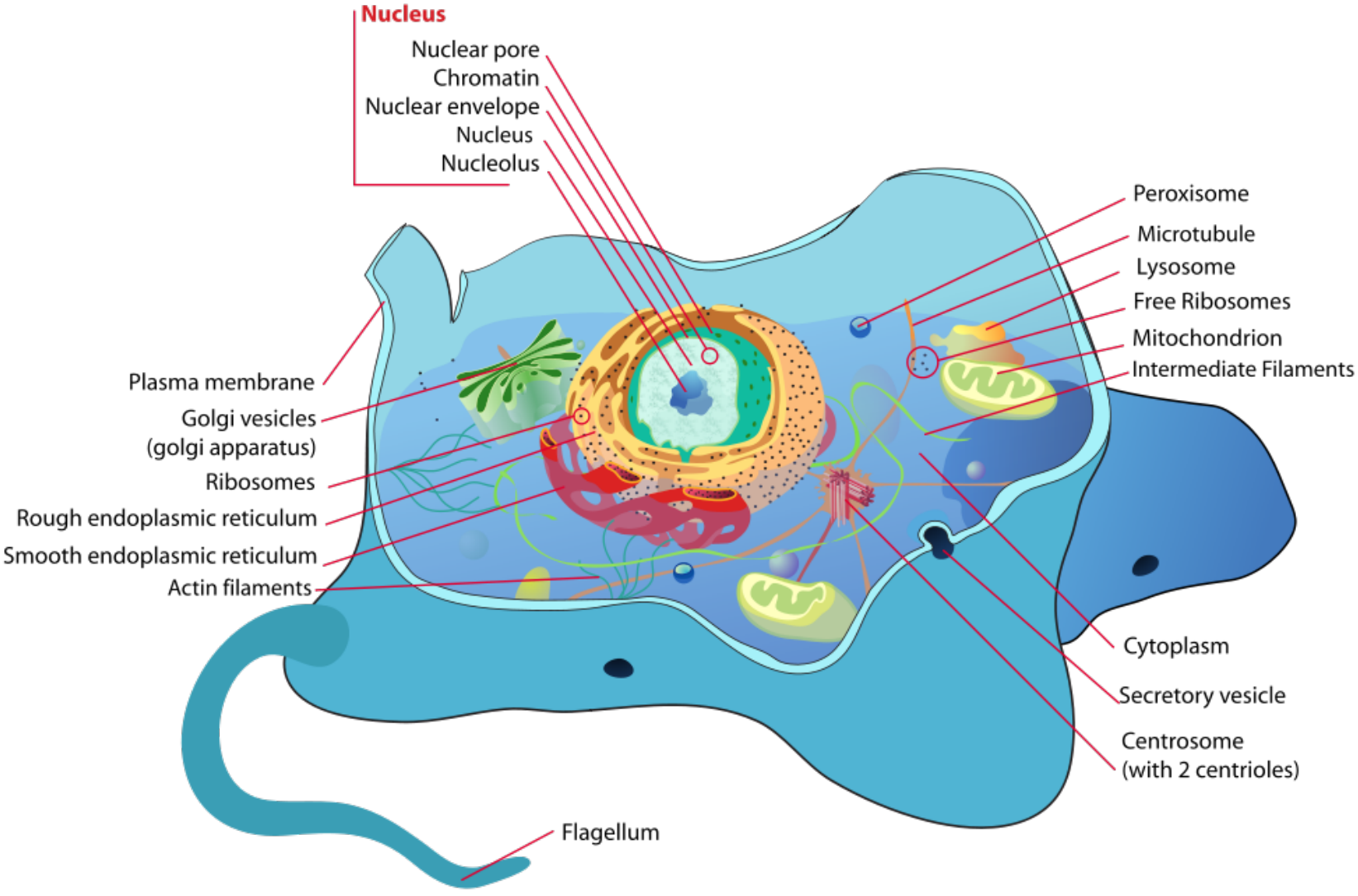
Plan

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

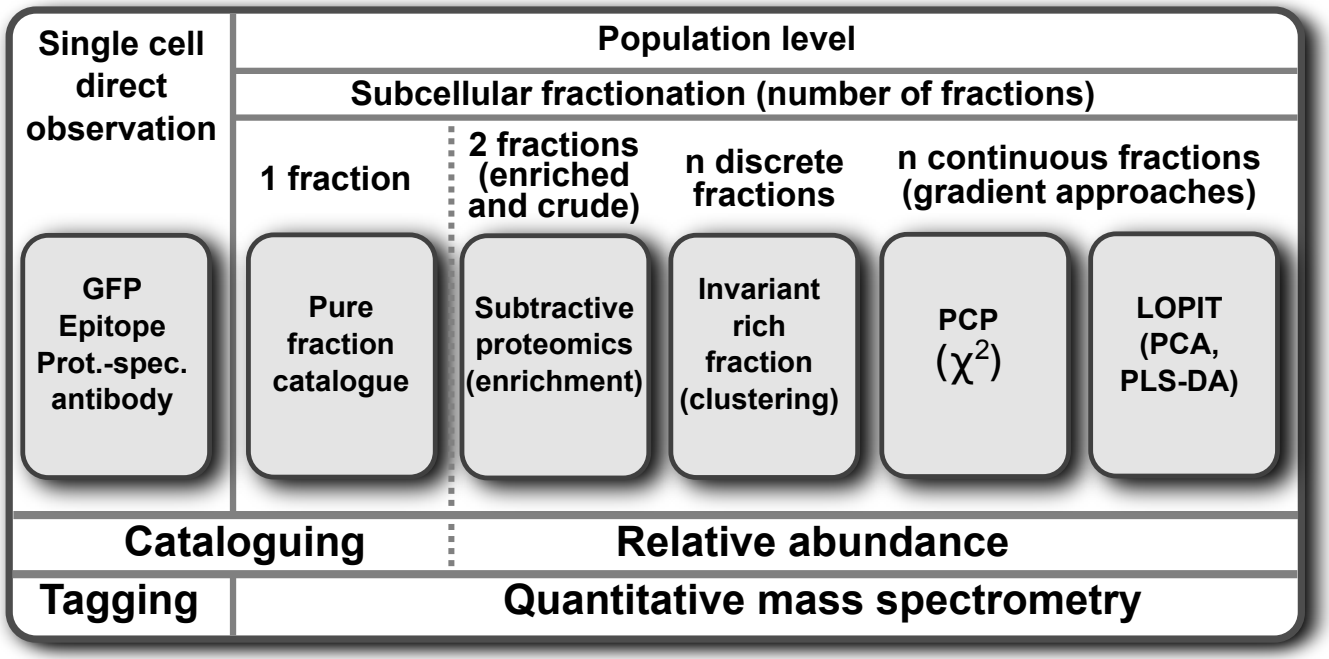
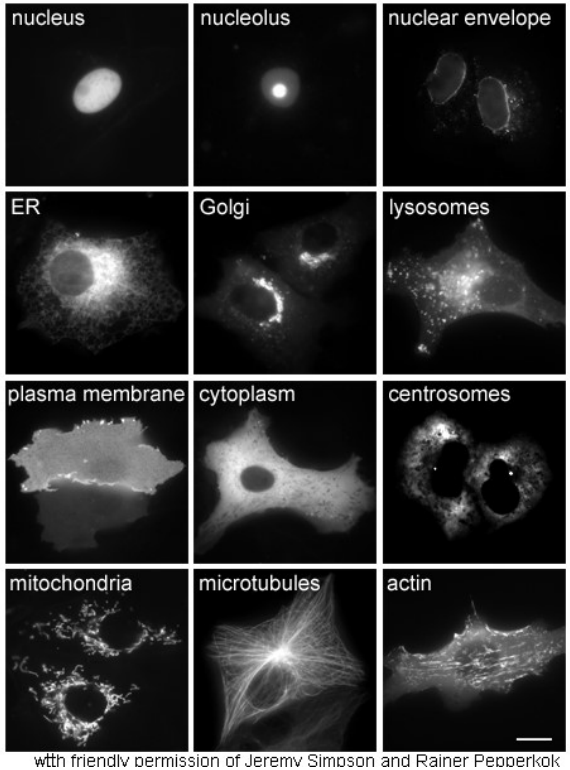
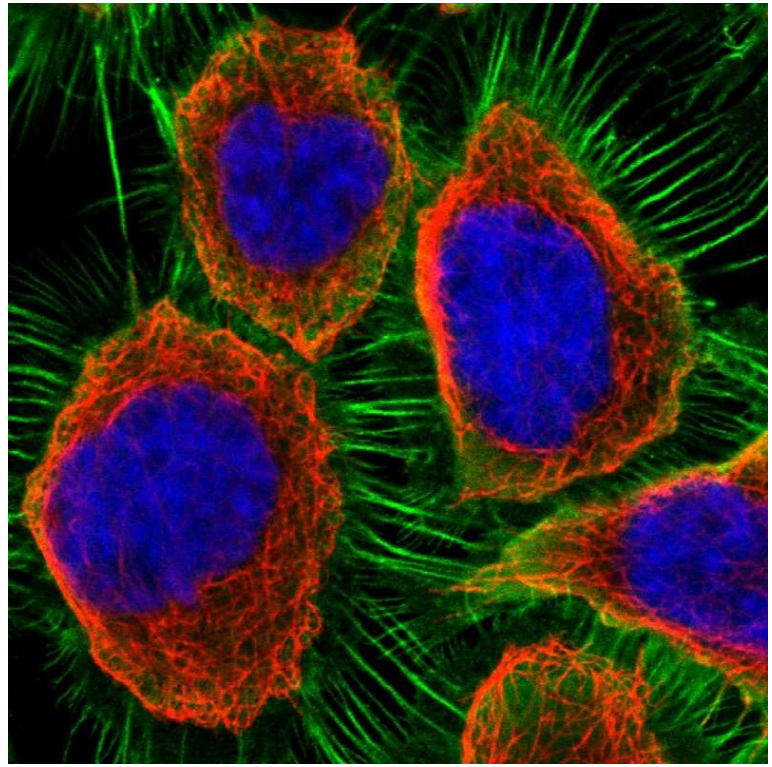


Figure : Organelle proteomics approaches (Gatto et al., 2010)

Fusion proteins and immunofluorescence



with friendly permission of Jeremy Simpson and Rainer Pepperkok



Fusion proteins and immunofluorescence

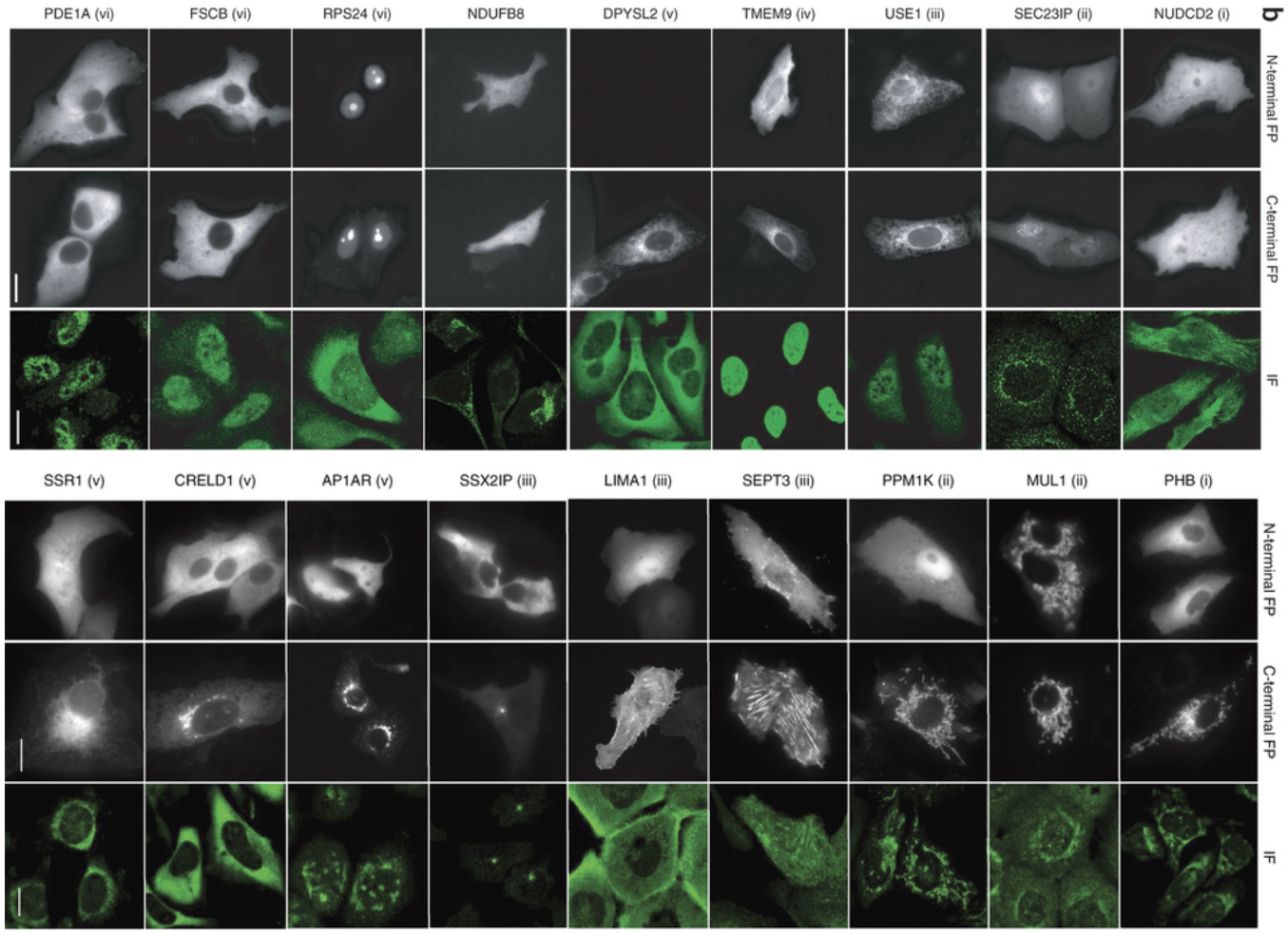


Figure : Example of discrepancies between IF and FPs as well as between FP tagging at the N and C termini (Stadler et al., 2013).

Spatial proteomics - How, experimentally

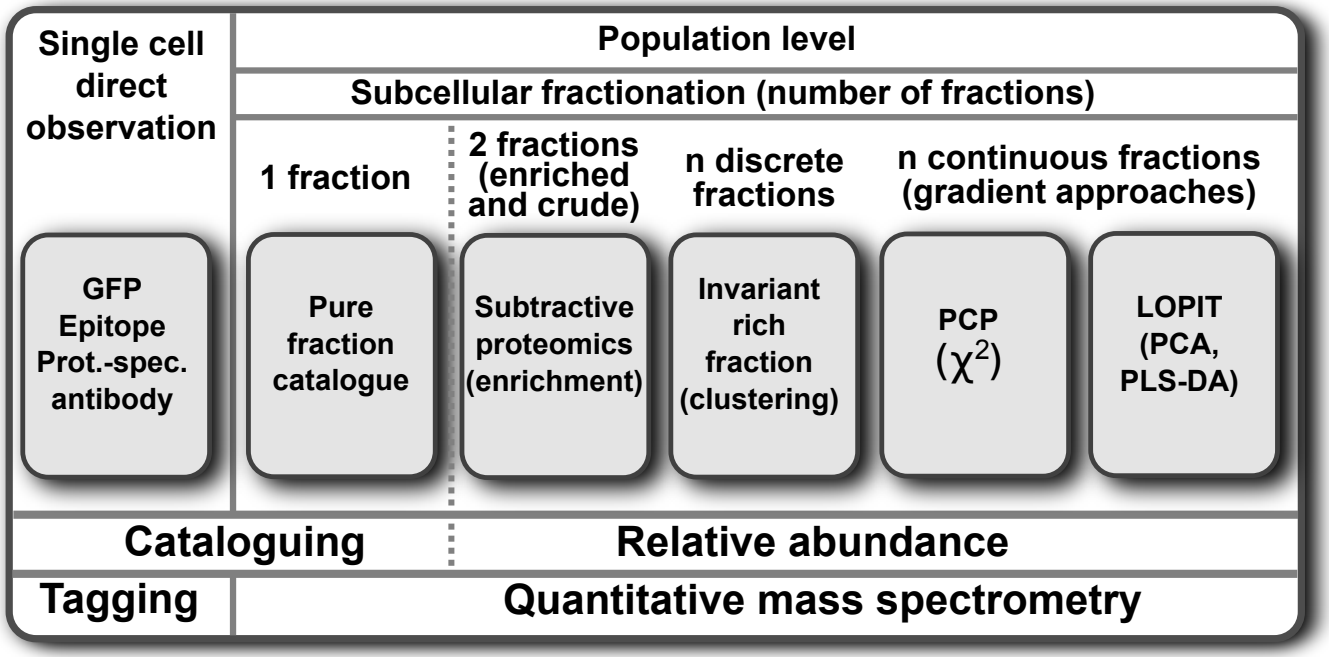
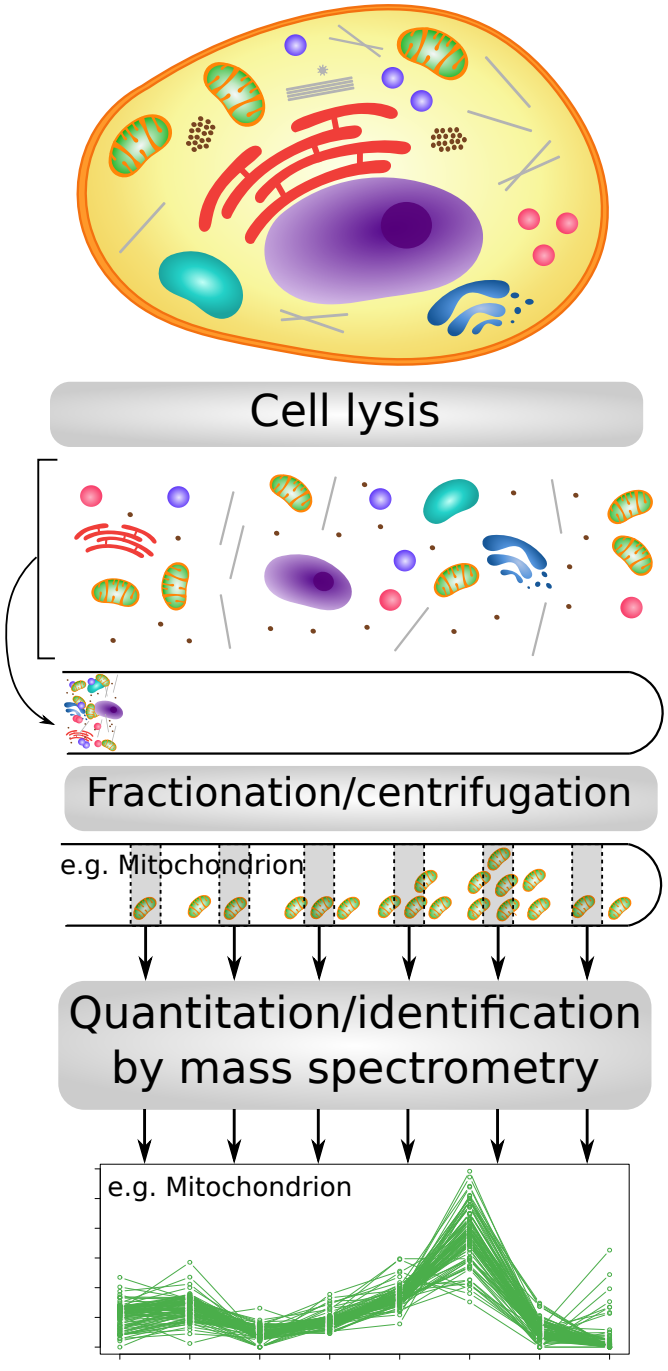


Figure : Organelle proteomics approaches (Gatto et al., 2010). Gradient approaches: Dunkley et al. (2006), Foster et al. (2006).

$\Rightarrow$ **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

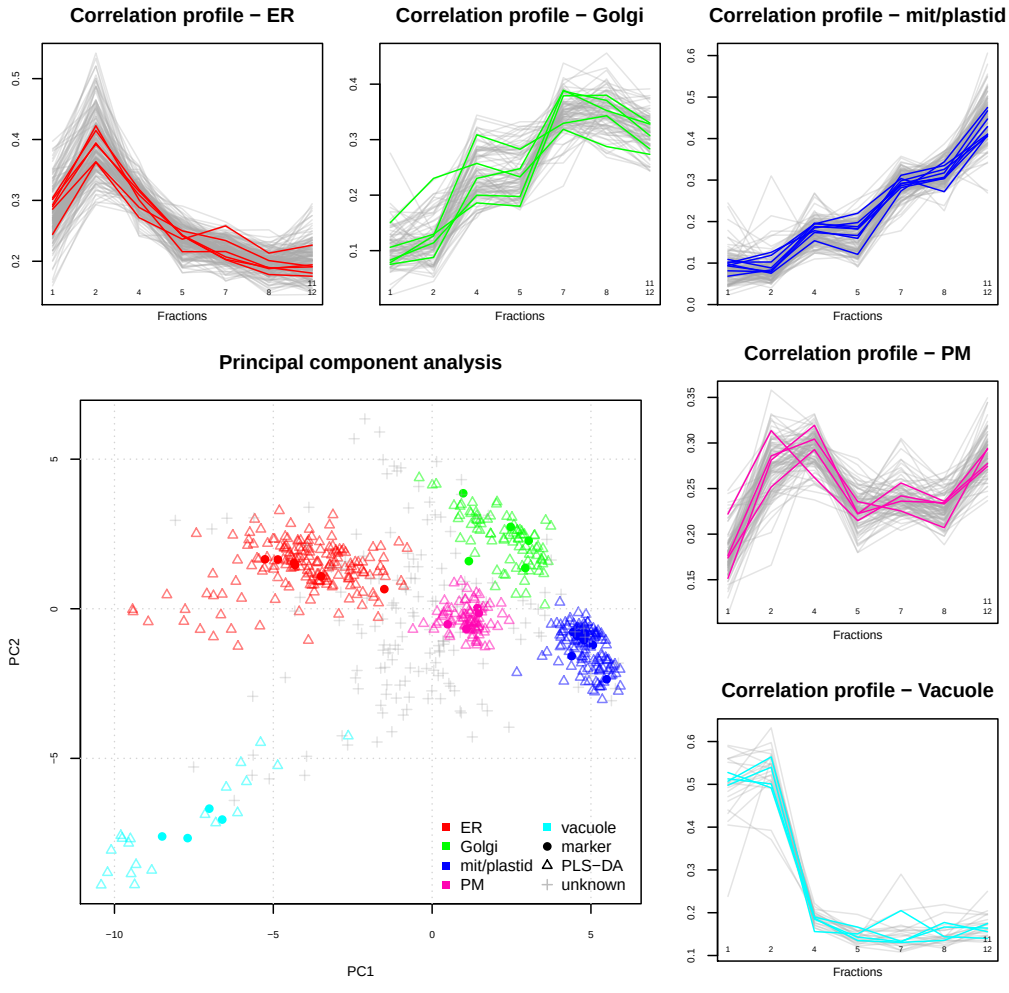


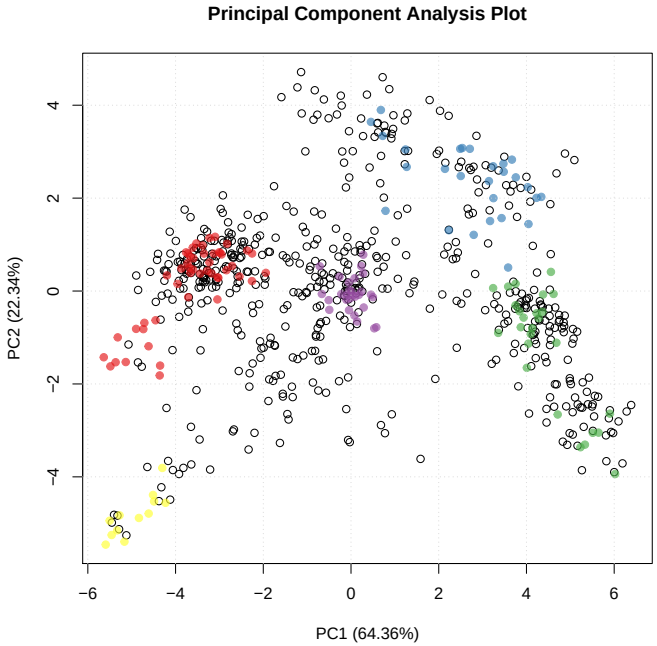
Figure : From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)

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.

Current approaches - supervised ML

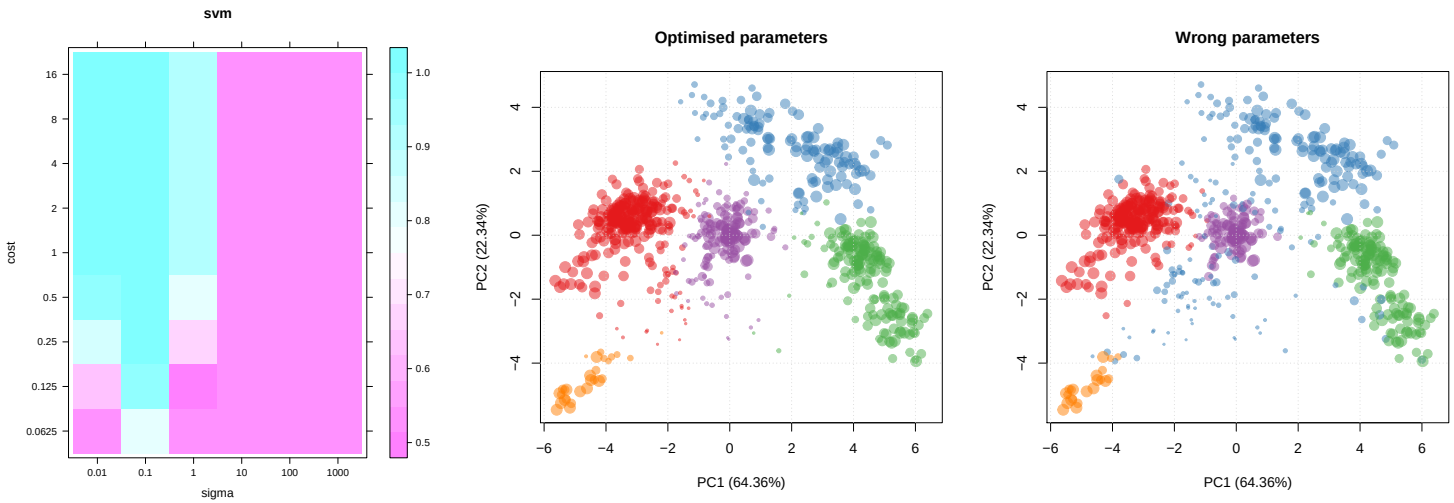
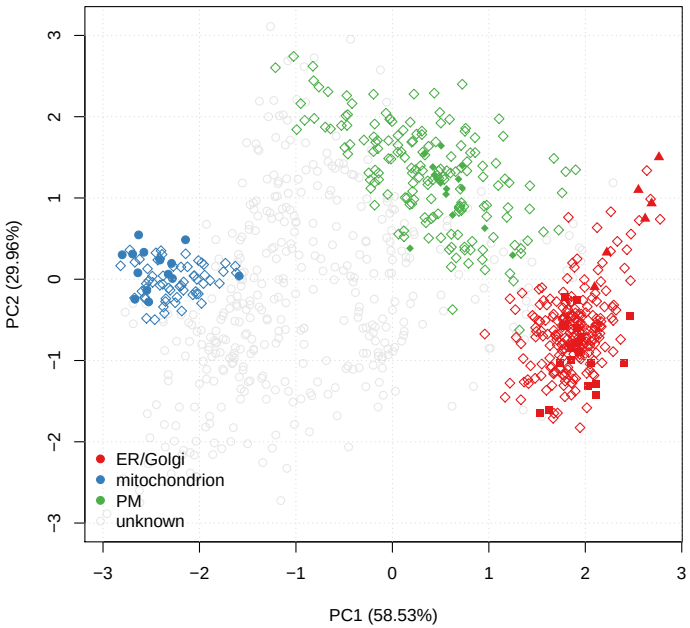
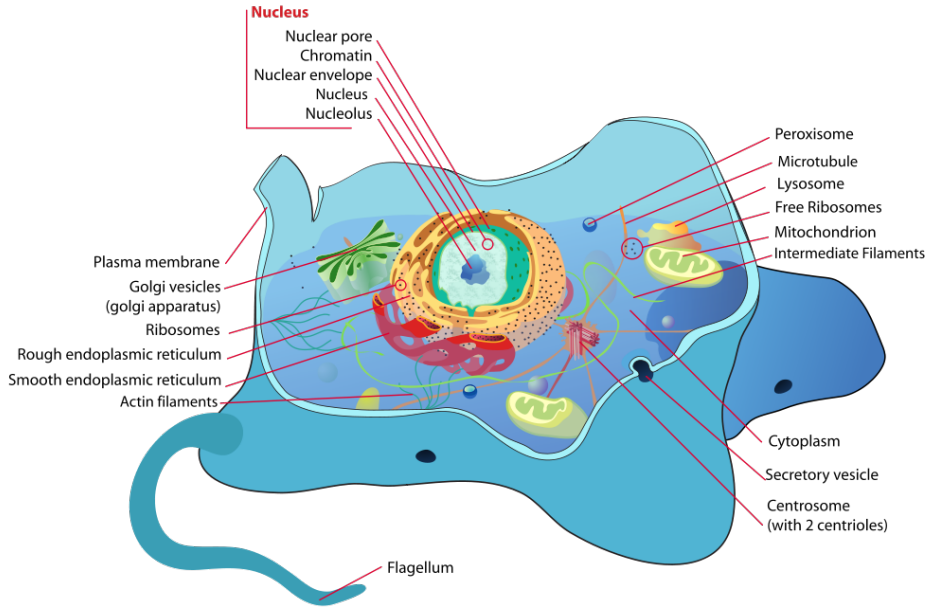


Figure : Support vector machines classifier with a radial basis kernel function, using the pRoloc Bioconductor package[1] (Gatto et al., 2014a).

[1] www.bioconductor.org/packages/release/bioc/html/pRoloc.html

Limitations





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

Novelty detection

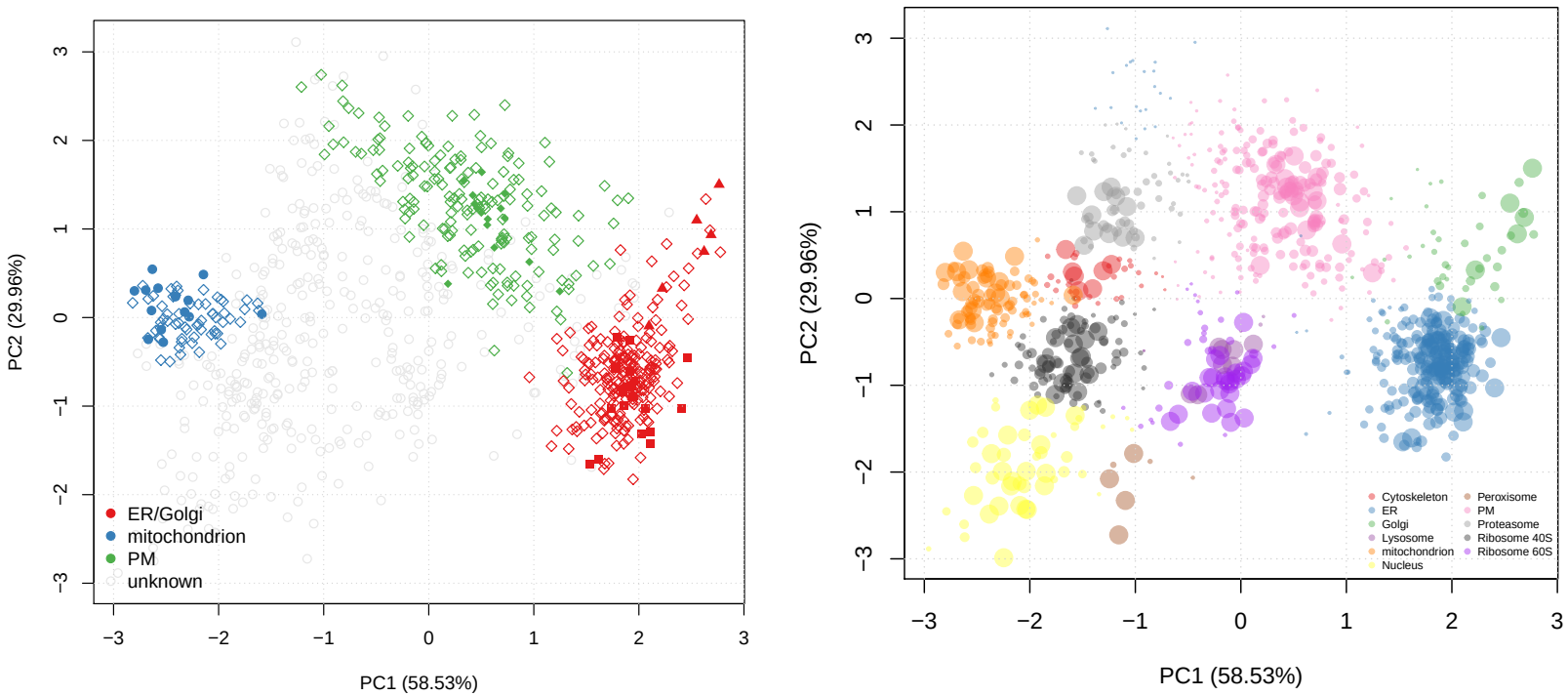


Figure : Left: *Drosophila* data from Tan et al. (2009). Right: Semi-supervised learning, Breckels et al. (2013).

Improving on LOPIT

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

What about annotation data from repositories such as 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**

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- **Static** vs. **dynamic**

number GO features $\gg$ experimental fractions
$\Rightarrow$ dilution of experimental data

Goal

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

Updated experimental design for

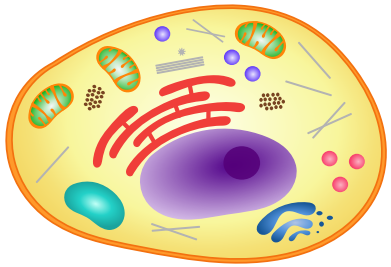
- primary/experimental data

and

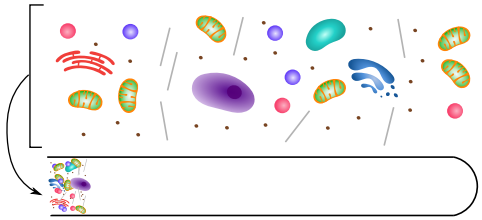
- auxiliary/annotation data

Breckels et al. (2016) *Learning from heterogeneous data sources: an application in spatial proteomics.* PLoS Comput Biol. DOI:http://dx.doi.org/10.1371/journal.pcbi.1004920.

PRIMARY EXPERIMENTAL DATA

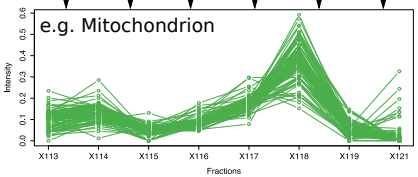


Cell lysis



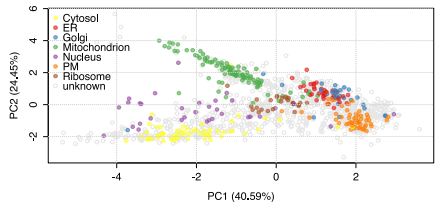
Fractionation/centrifugation

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

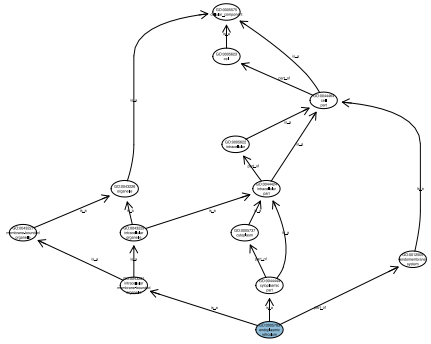
Visualisation



AUXILIARY DRY DATA

Database query

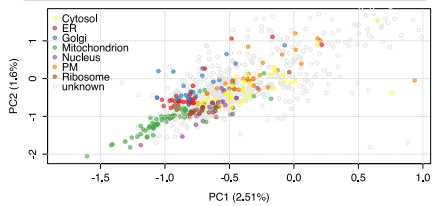
Extract GO CC terms



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	...	...	...

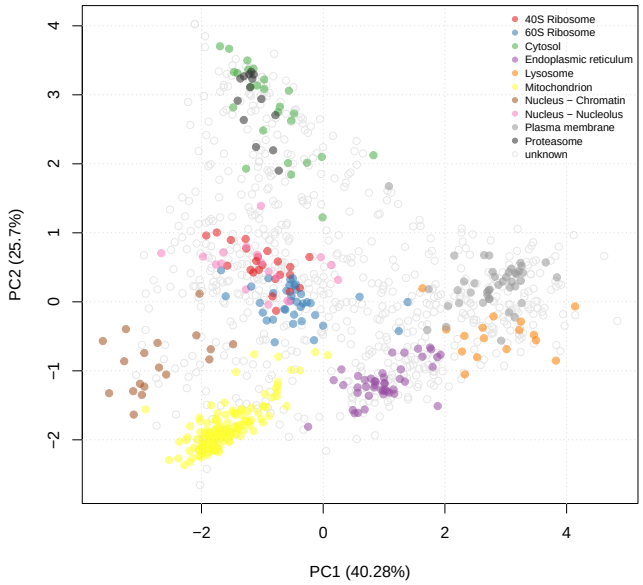
Visualisation



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

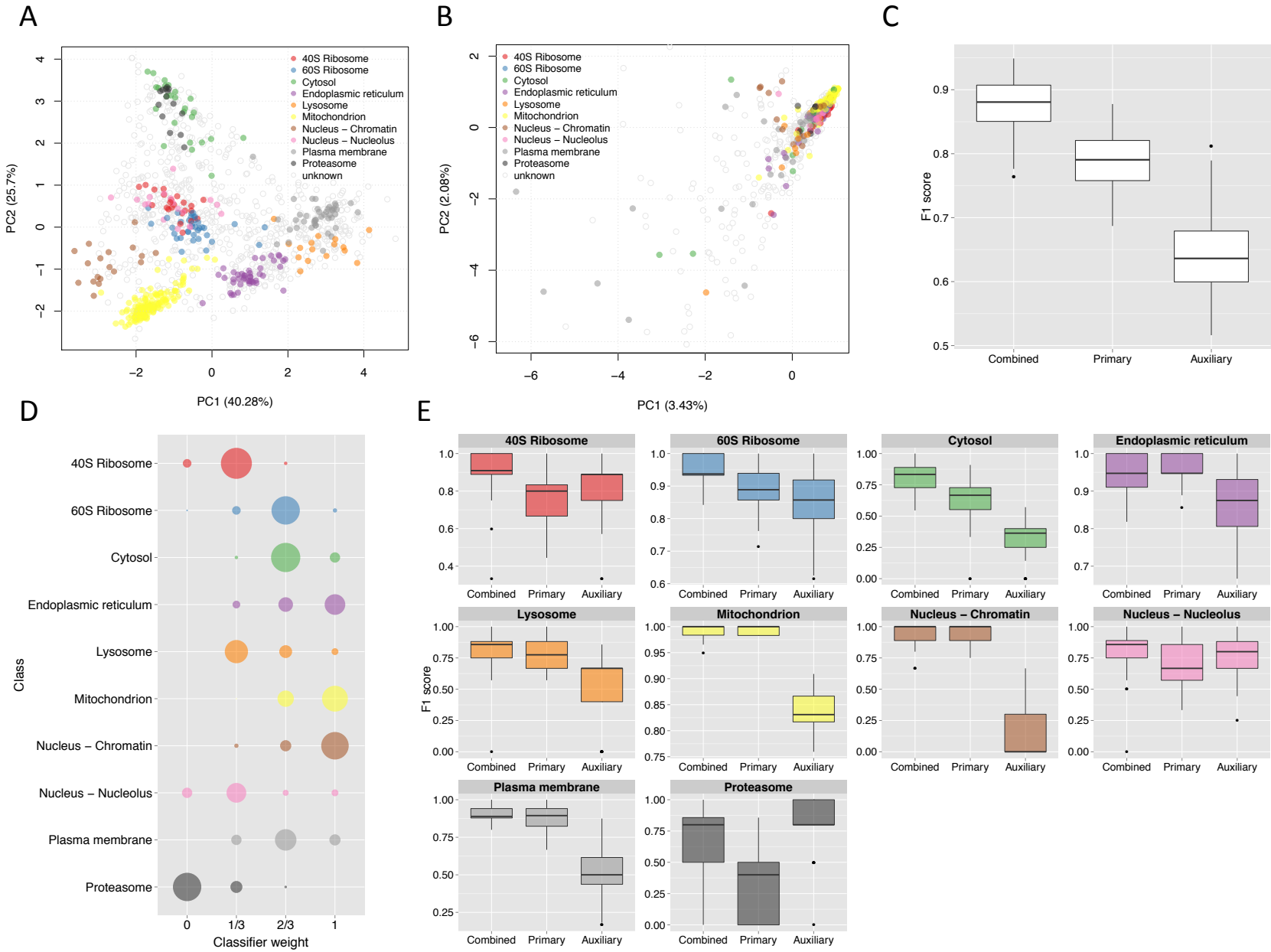
Class-weighted kNN

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

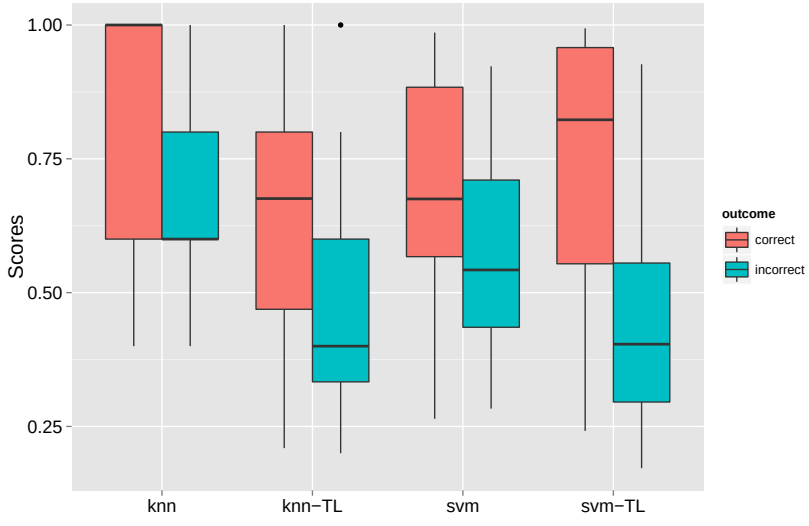


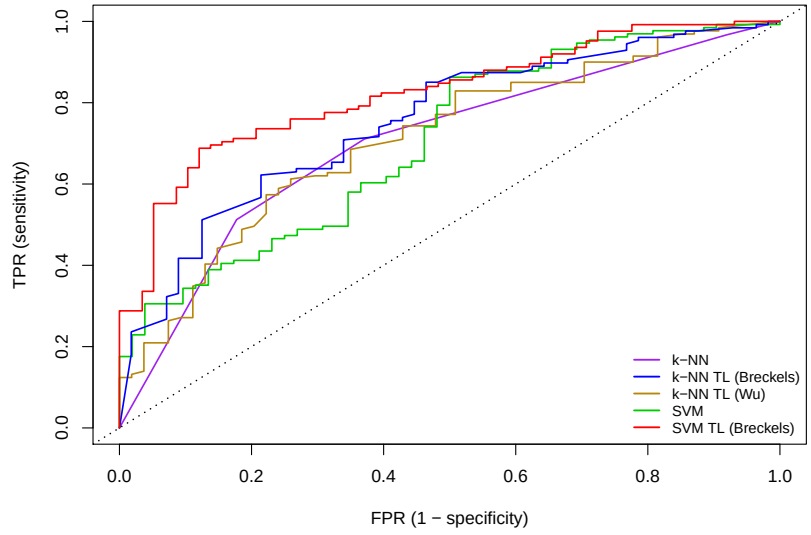
Linear programming SVM

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



Data from mouse stem cells (E14TG2a).

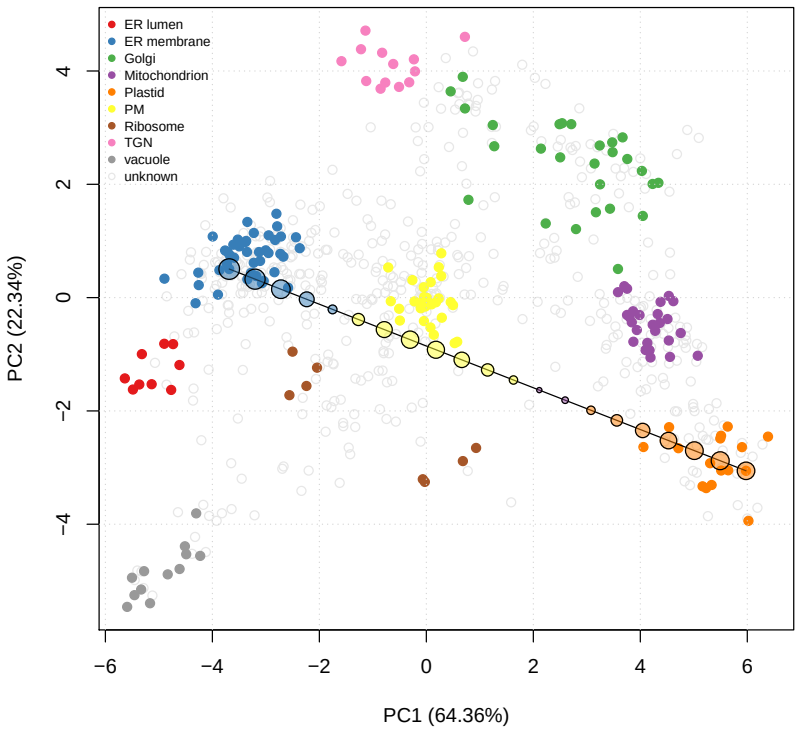

1.00
0.75
0.50
0.25
Scores
knn
knn−TL
svm
svm−TL
outcome
correct
incorrect


TPR (sensitivity)
0.0
0.2
0.4
0.6
0.8
1.0
FPR (1 − specificity)
k−NN
k−NN TL (Breckels)
k−NN TL (Wu)
SVM
SVM TL (Breckels)

- Multi-localisation
- Trans-localisation

Defined in Gatto et al. (2014b), A foundation for reliable spatial proteomics data analysis.

Dual-localisation Proteins may be present simultaneously in several organelles (e.g. trafficking).



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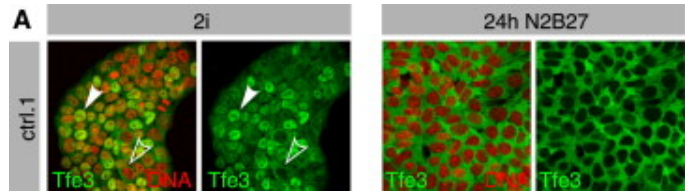
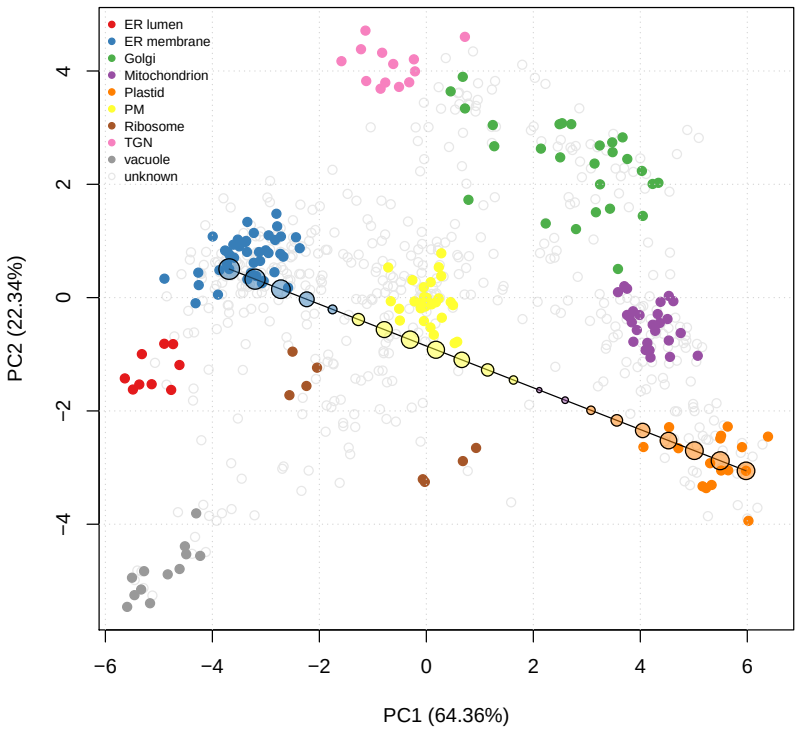
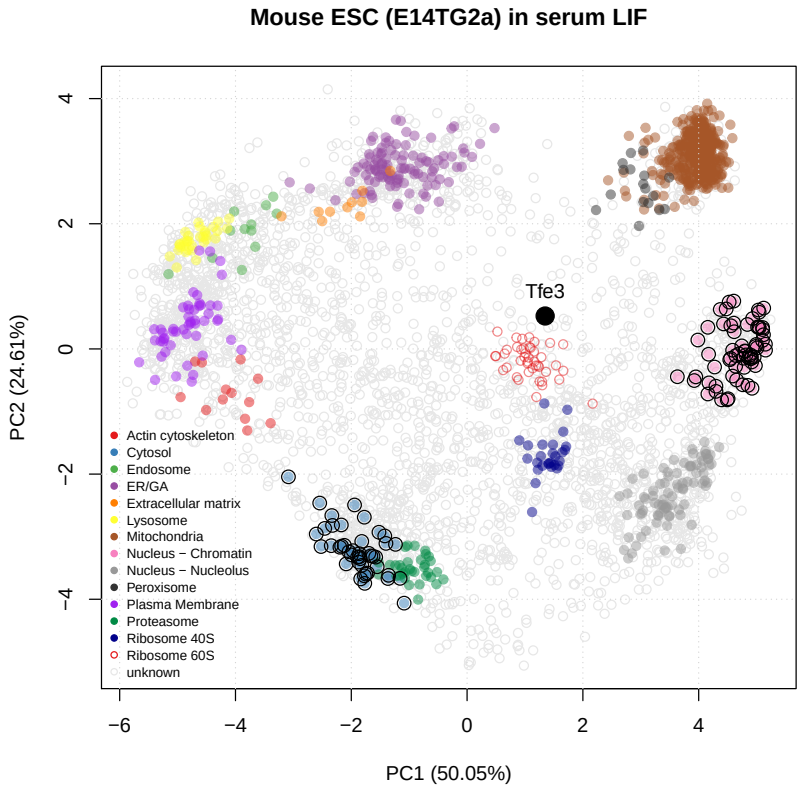


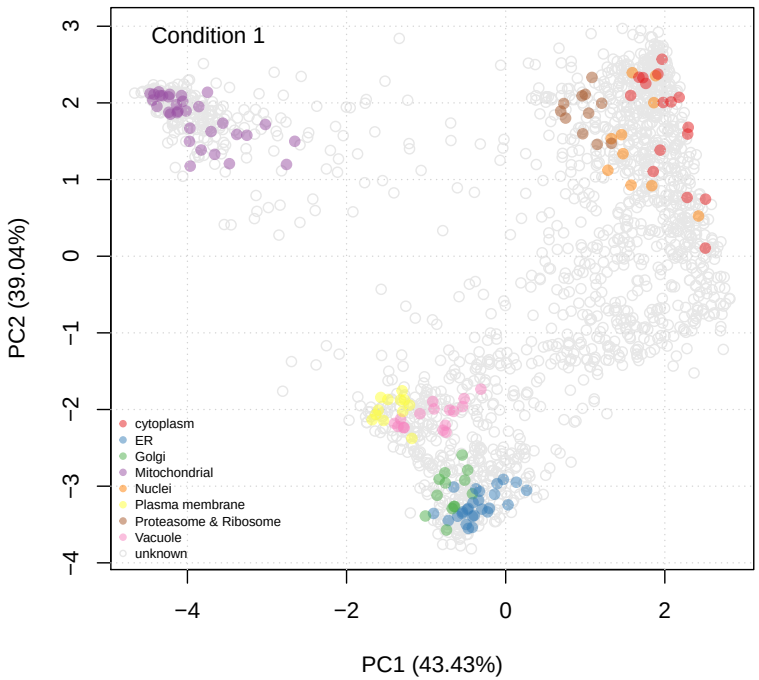
From Betschinger et al. (2013)

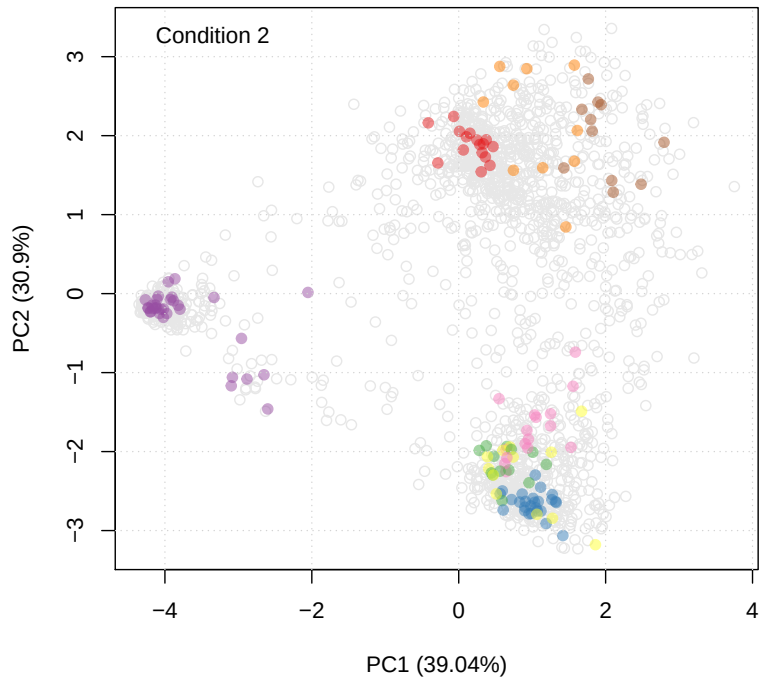




Spatial dynamics

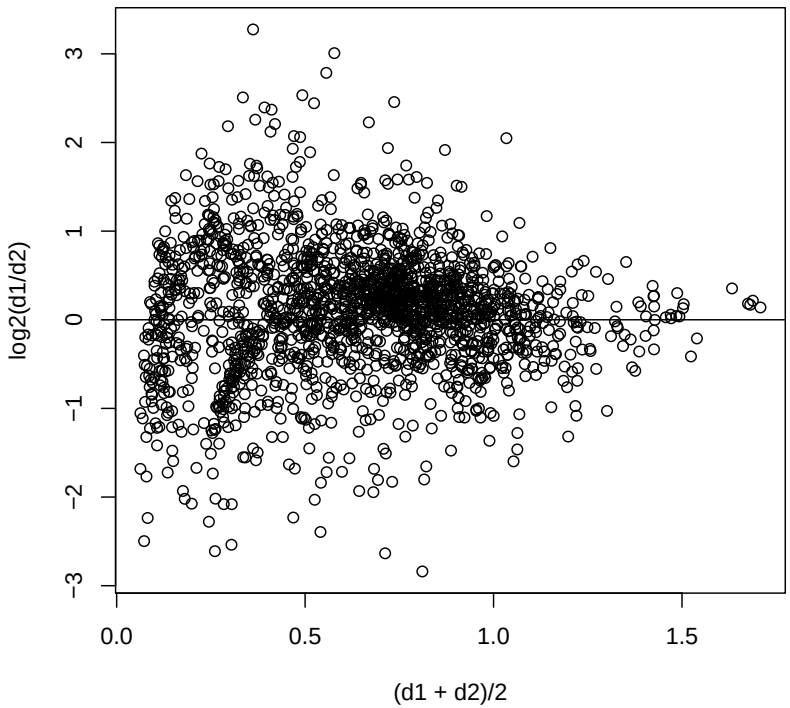
Trans-localisation Changes in localisation upon perturbations.

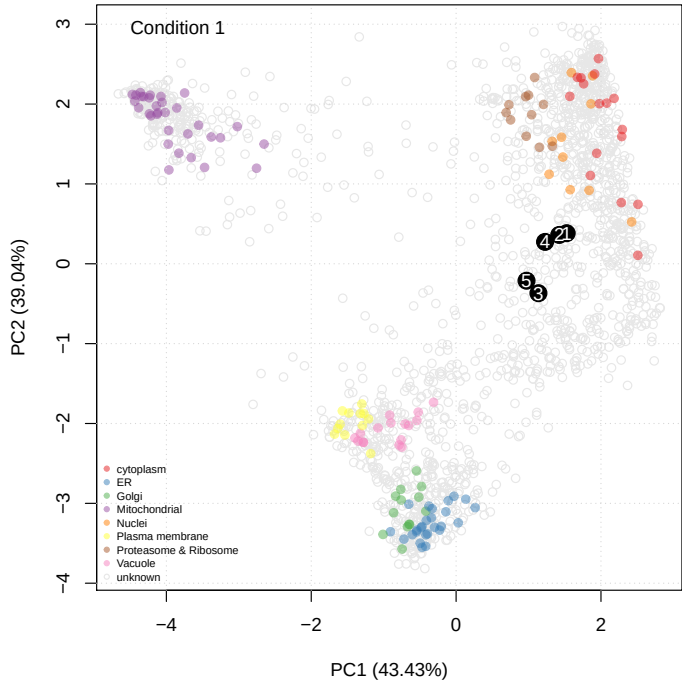


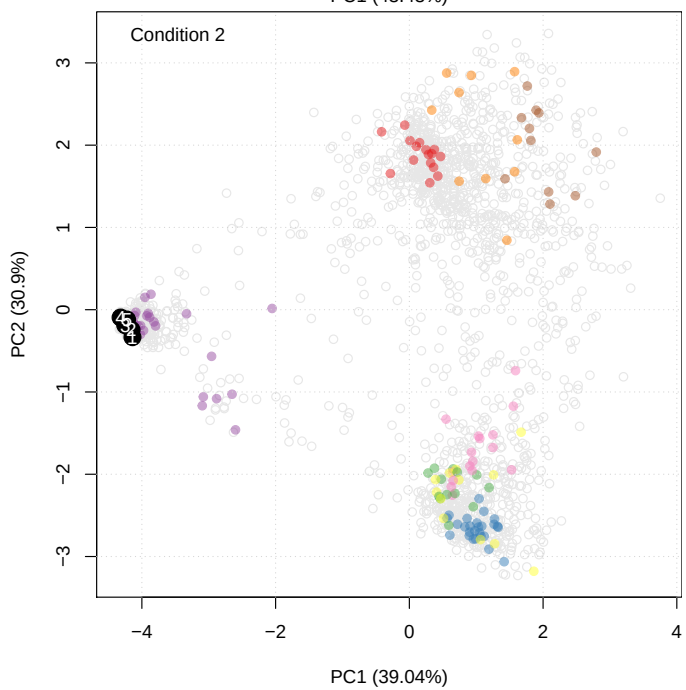


Spatial dynamics

$$d_1 = dist(profile^{rep_1}_{condition_1}, profile^{rep_1}_{condition_2})$$
$$d_2 = dist(profile^{rep_2}_{condition_1}, profile^{rep_2}_{condition_2})$$







Beyond organelles: application to PPI/Protein complexes

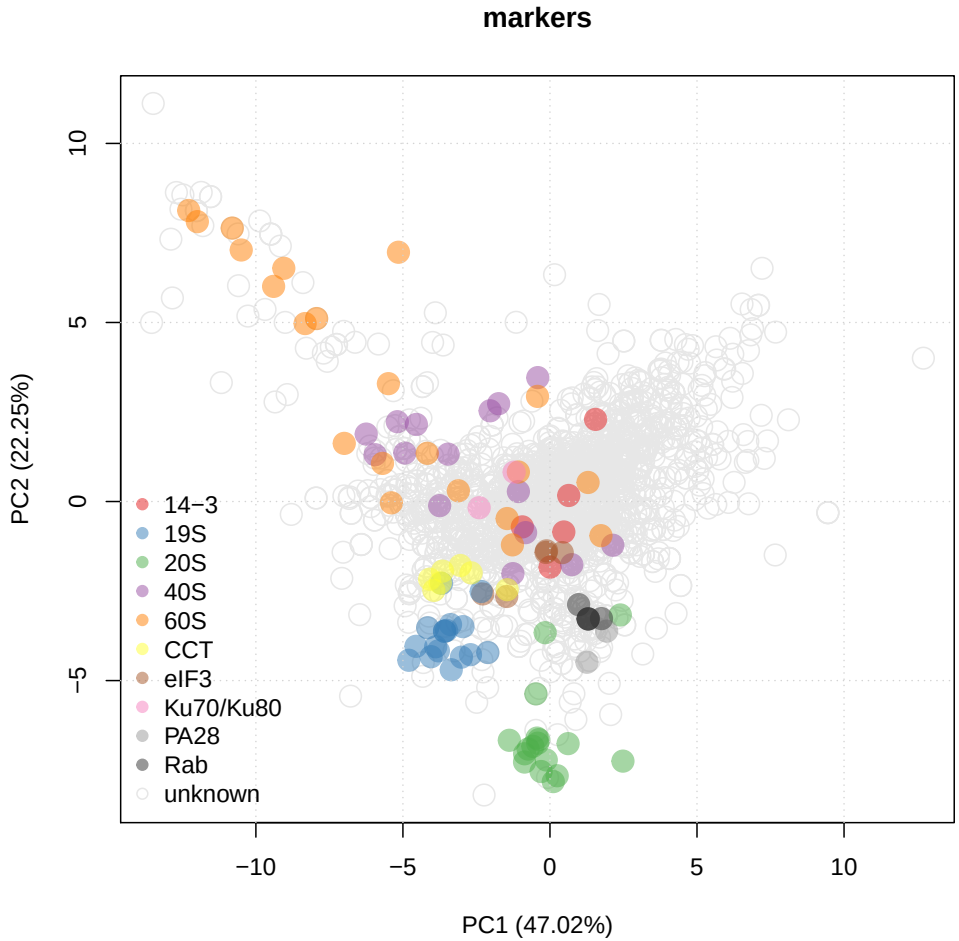


Figure : Data on proteasome complexes from Fabre *et al.* Mol Syst Biol (2015), DOI: 10.15252/msb.20145497

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., 2014a) (~200 unique IP download/month in 2016).
- `pRolocdata` – structured and annotated spatial proteomics data (Gatto et al., 2014a).
- 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.

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