Selected Reaction Monitoring for targeted proteomics

Journal Club

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  - Investigating the abundance of proteins
  - Mass spectrometry
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- Outlook

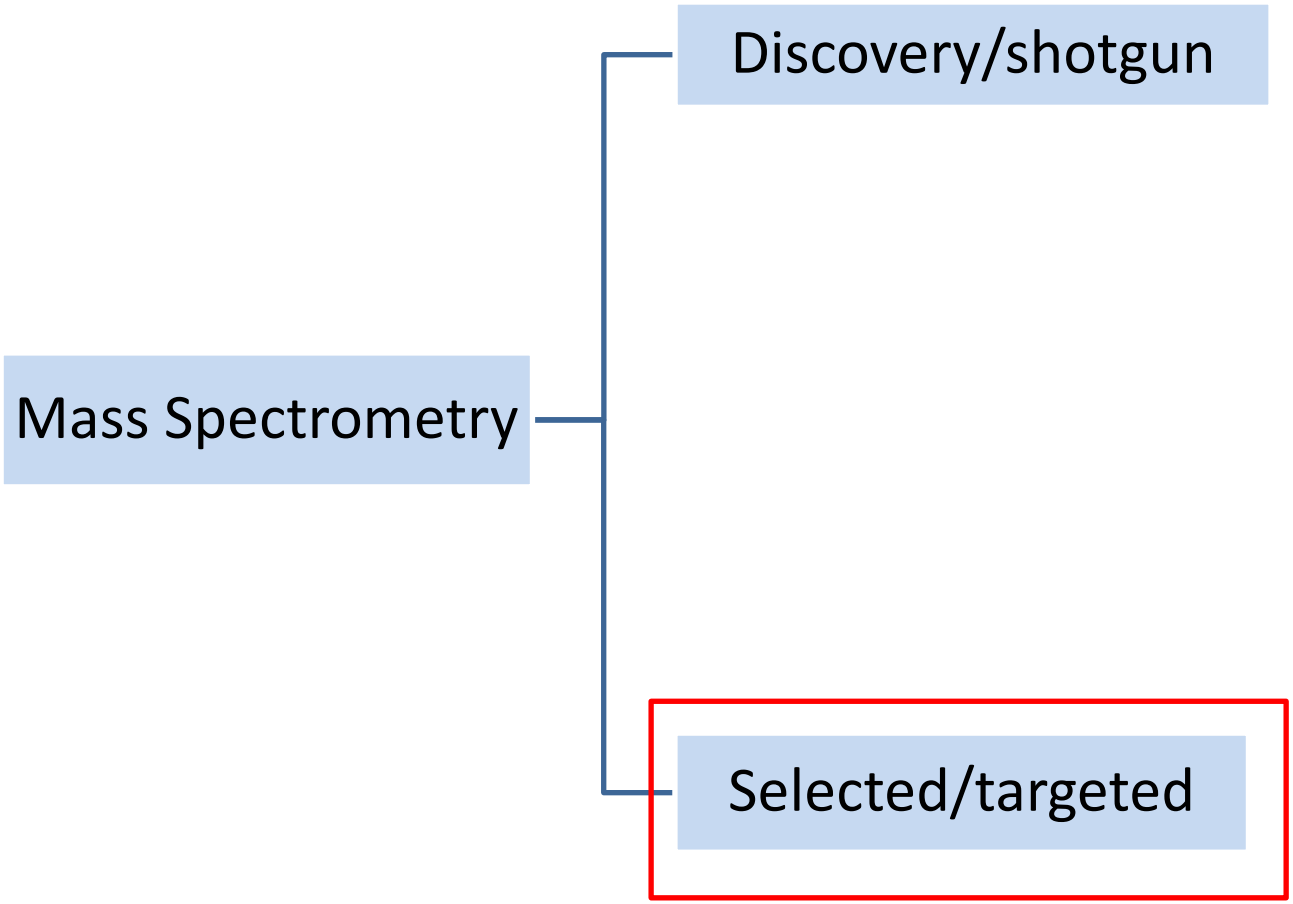
Investigating the abundance of proteins

Antibody based technologies

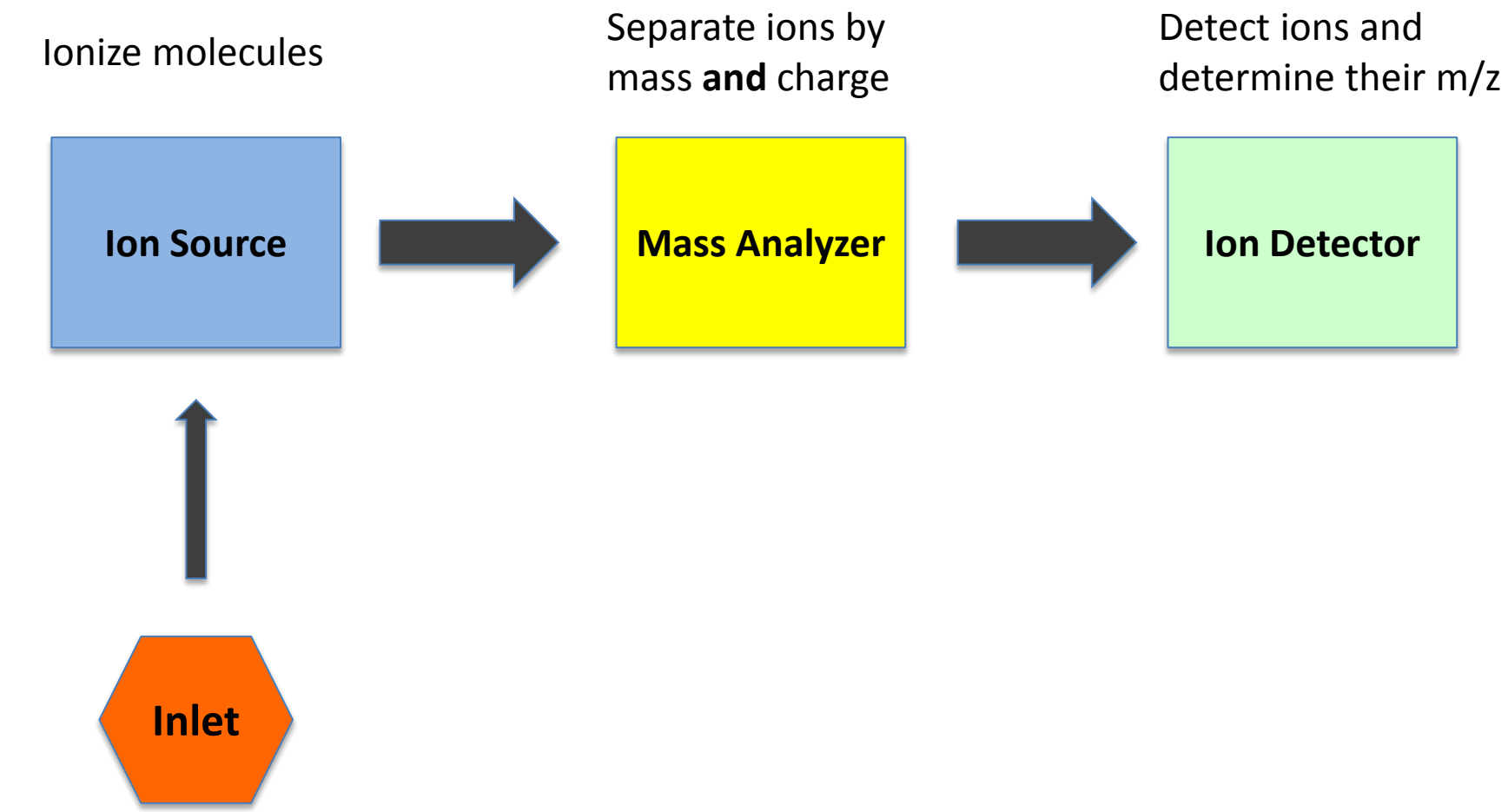
- Fluorescent imaging, WB, ELISA, IHC
- Antibody microarrays

Without antibody

- Mass spectrometry
  - Global discovery/shotgun
  - Targeted/selected


Discovery/shotgun
Mass Spectrometry
Selected/targeted

Overview of Mass spectrometers

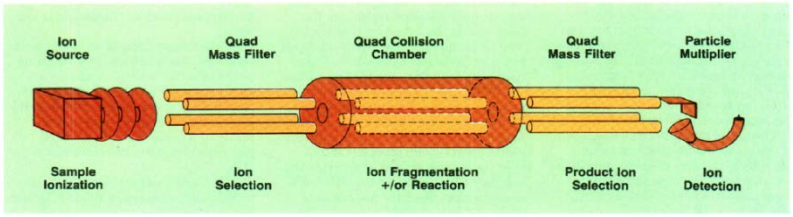


Modified from Carr, 2013

Selected Reaction Monitoring (SRM)

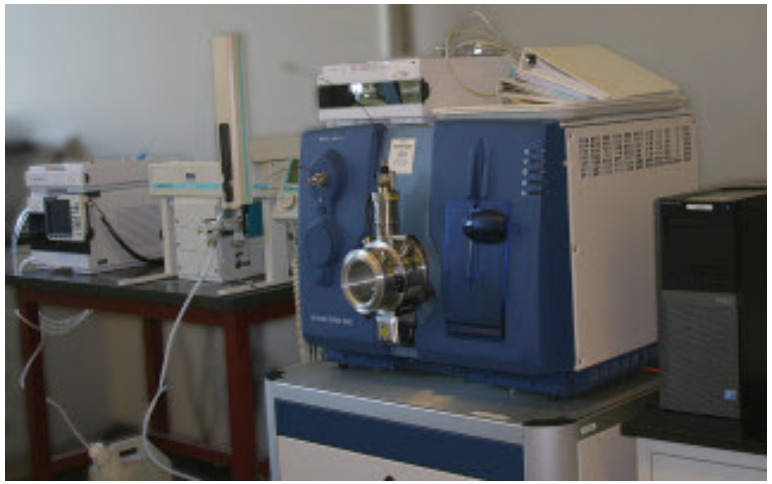
= multiple/plural reaction monitoring

Triple Quadrupole Mass Spectrometry for Direct Mixture Analysis and Structure Elucidation



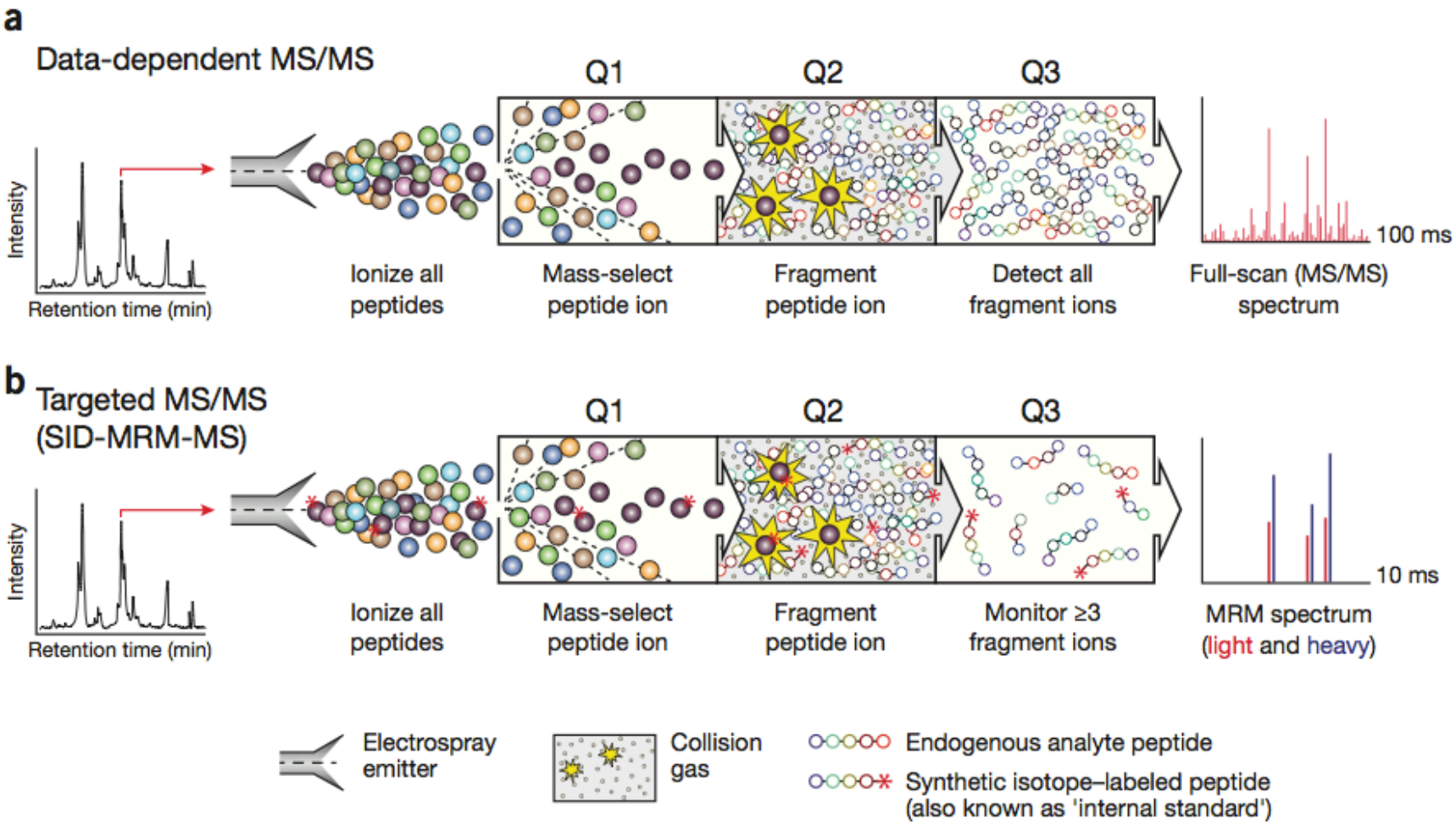
Yost and Enke, 1979

- Triple quadrupole MS (QQQ)
- 10-100 proteins in one experiment
- Suitable for cell lysates, tissue samples, bodyfluids, drug metabolites etc.



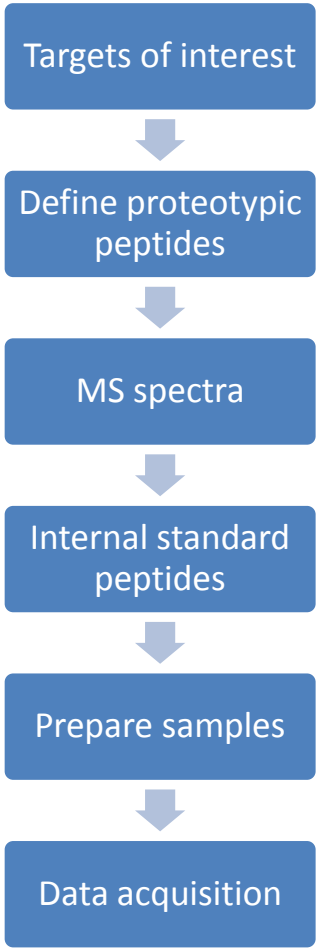
http://www.biosurplus.com/store/products/5023273-applied-biosystems-qtrap-5500-w-agilent-1290-infiity/

Differences between MS and SRM



Gillette and Carr, 2012

SRM workflow



10-100 proteins

Unique for given target (choose 3-5 for every protein)
Good response characteristics in MS
Lenght of 10-21 amino acids

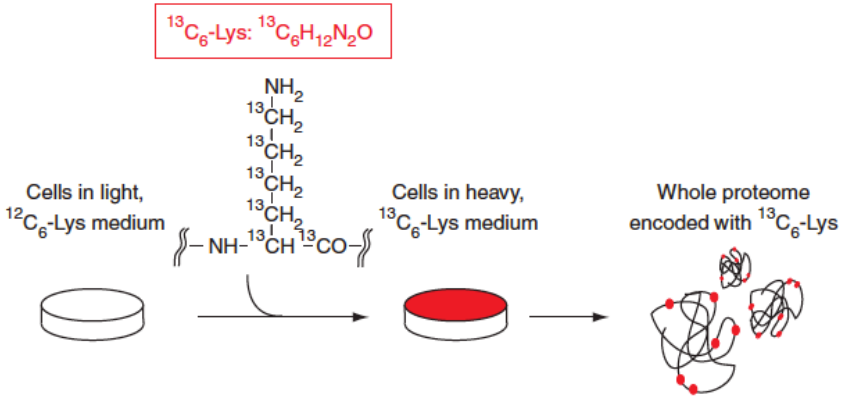
Fragmentation spectra database (PeptideAtlas, GPMDB, GAPP, Pride, Tranche)

Necessary for relative or absolute quantification

Trypsinize protein sample
Load tryptic peptides into HPLC

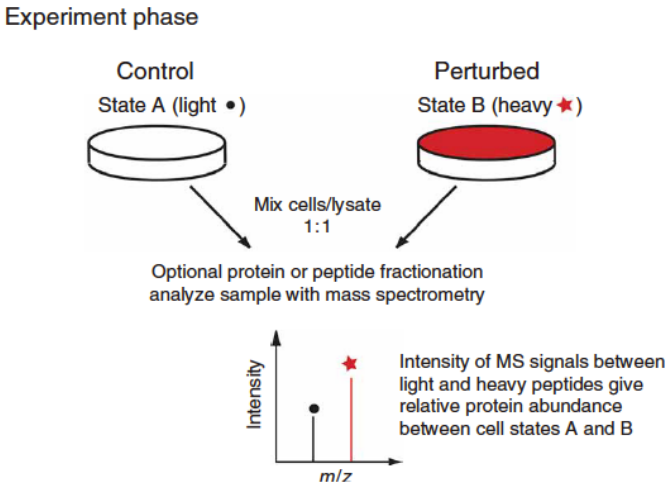
Internal standards for quantification

Quantification	Labeling	Quantification strategy
Relative (differential) quantification	Label-free	Label-free
	Metabolic stable-isotope labeling	[^{15}N]ammonium sulfate SILAC
	Chemical stable-isotope labeling	ICAT iTRAQ mTRAQ
	Enzymatic stable-isotope labeling	[^{18}O]water
Absolute quantification	Metabolic stable-isotope labeling	QconCAT PSAQ
	Chemical stable-isotope labeling	AQUA synthetic peptides

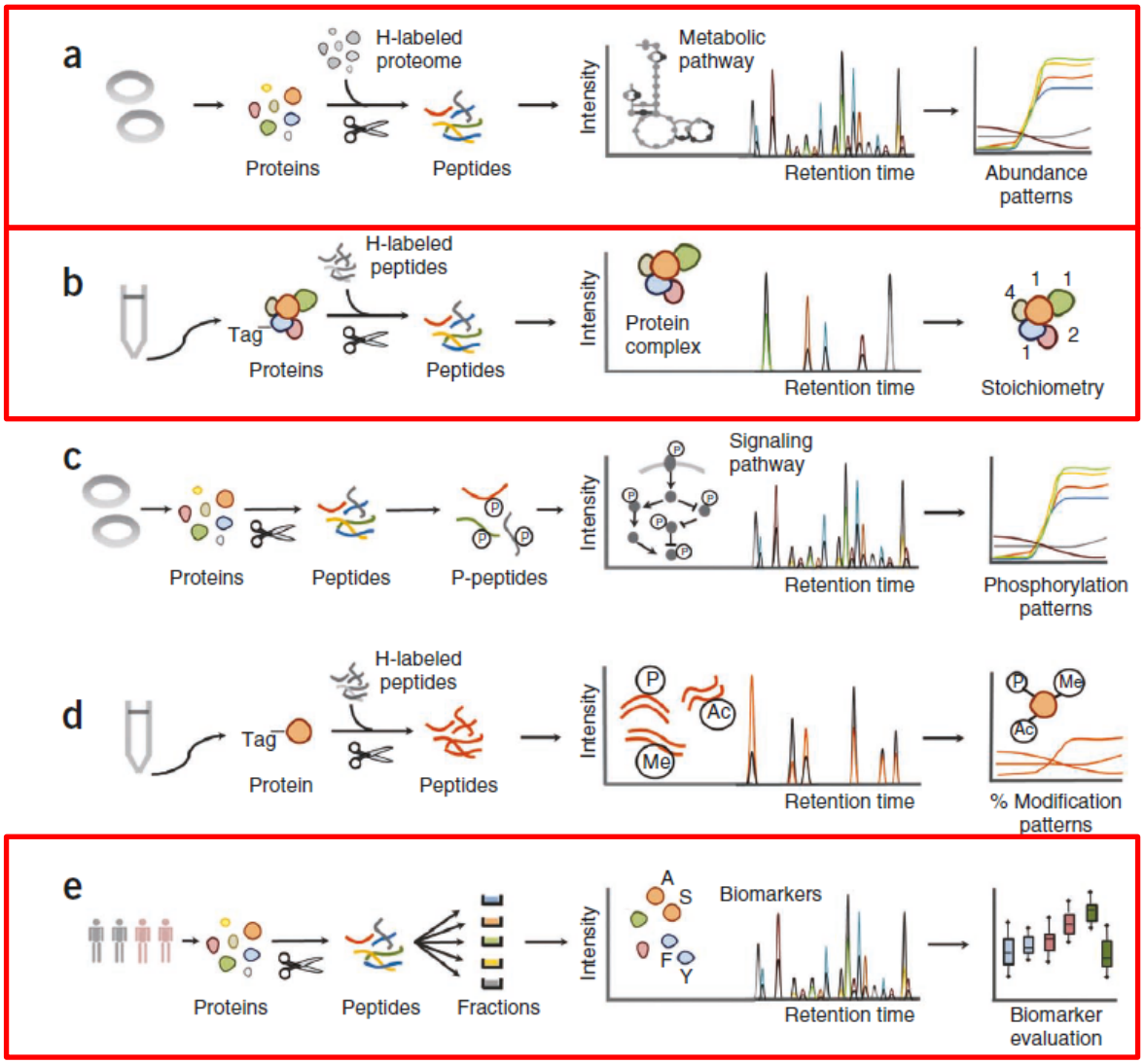


SILAC: stable isotope labeling by amino acids in cell culture

Natural («light») aa's are replaced by «heavy» SILAC aa's



Picotti and Abersold, 2012
Ong and Mann, 2006



Picotti and Aebersold, 2012

Absolute quantification of transcription factors during cellular differentiation using multiplexed targeted proteomics

Jovan Simicevic[1,5], Adrien W Schmid[2,5], Paola A Gilardoni[1,5], Benjamin Zoller[3], Sunil K Raghav[1], Irina Krier[1], Carine Gubelmann[1], Frédérique Lisacek[4], Felix Naef[3], Marc Moniatte[2] & Bart Deplancke[1]

[1]Laboratory of Systems Biology and Genetics, Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland. [2]Proteomics Core Facility, School of Life Sciences, EPFL, Lausanne, Switzerland. [3]Laboratory of Computational Systems Biology, Institute of Bioengineering, School of Life Sciences, EPFL, Lausanne, Switzerland. [4]Swiss Institute of Bioinformatics, Geneva, Switzerland. [5]These authors contributed equally to this work. Correspondence should be addressed to M.M. (marc.moniatte@epfl.ch) or B.D. (bart.deplancke@epfl.ch).

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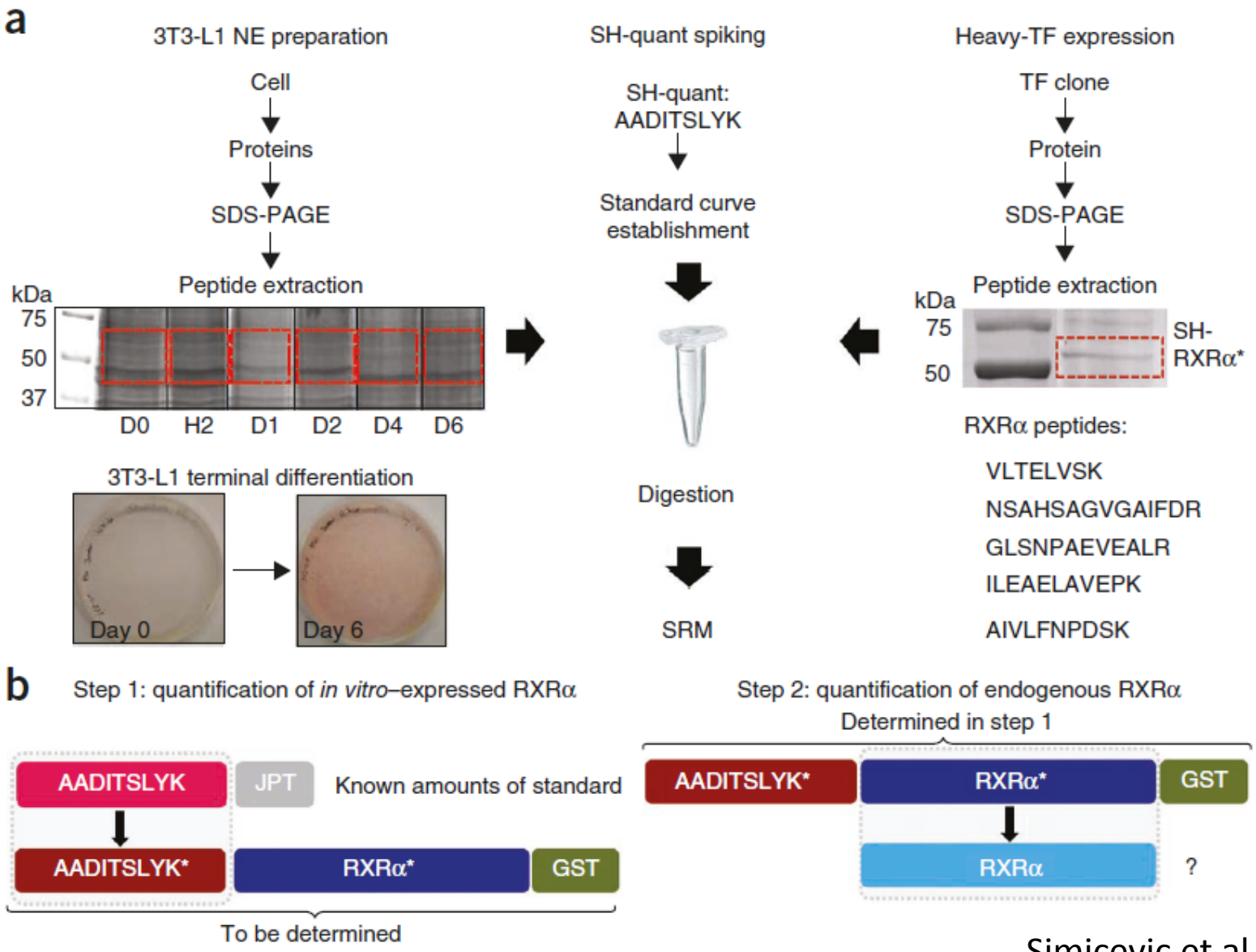
Aim: absolute levels of transcription factors (TF) during dynamic process

- Interest in models of gene regulatory networks predicting dynamic transcriptional mechanisms
- Little quantitative information so far
- Relatively low expression of TF in cells

➢ Screen 10 TF (including master regulators PPARγ and RXRα) during terminal phase of adipogenesis in mouse 3T3-L1 pre-adipocytes

Simicevic et al., 2013

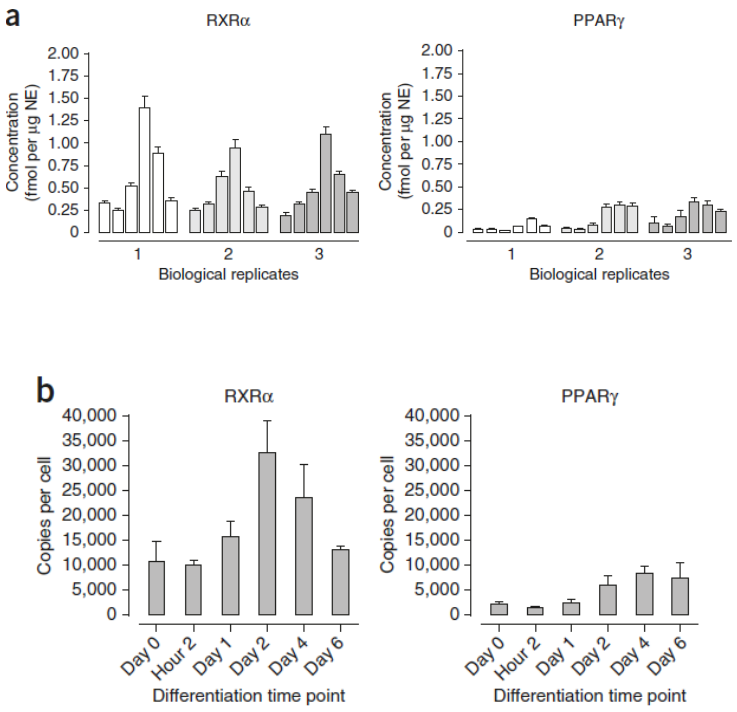
Workflow of quantifying TFs in 3T3-L1 cells



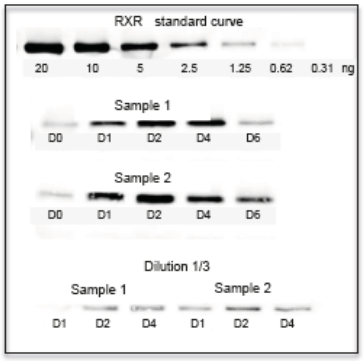
Simicevic et al., 2013

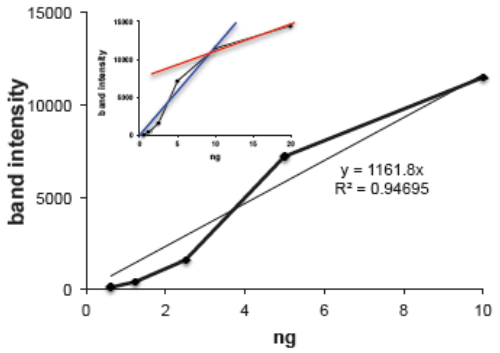
Quantification of PPARγ and RXRα

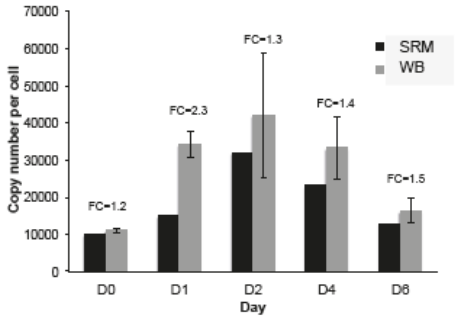
SRM

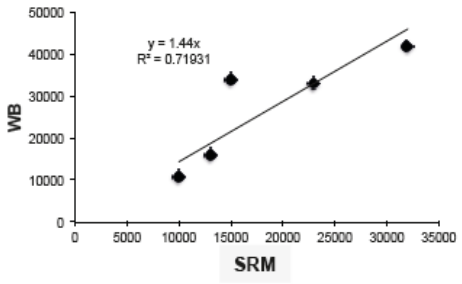


Semiquantitative WB for RXRα



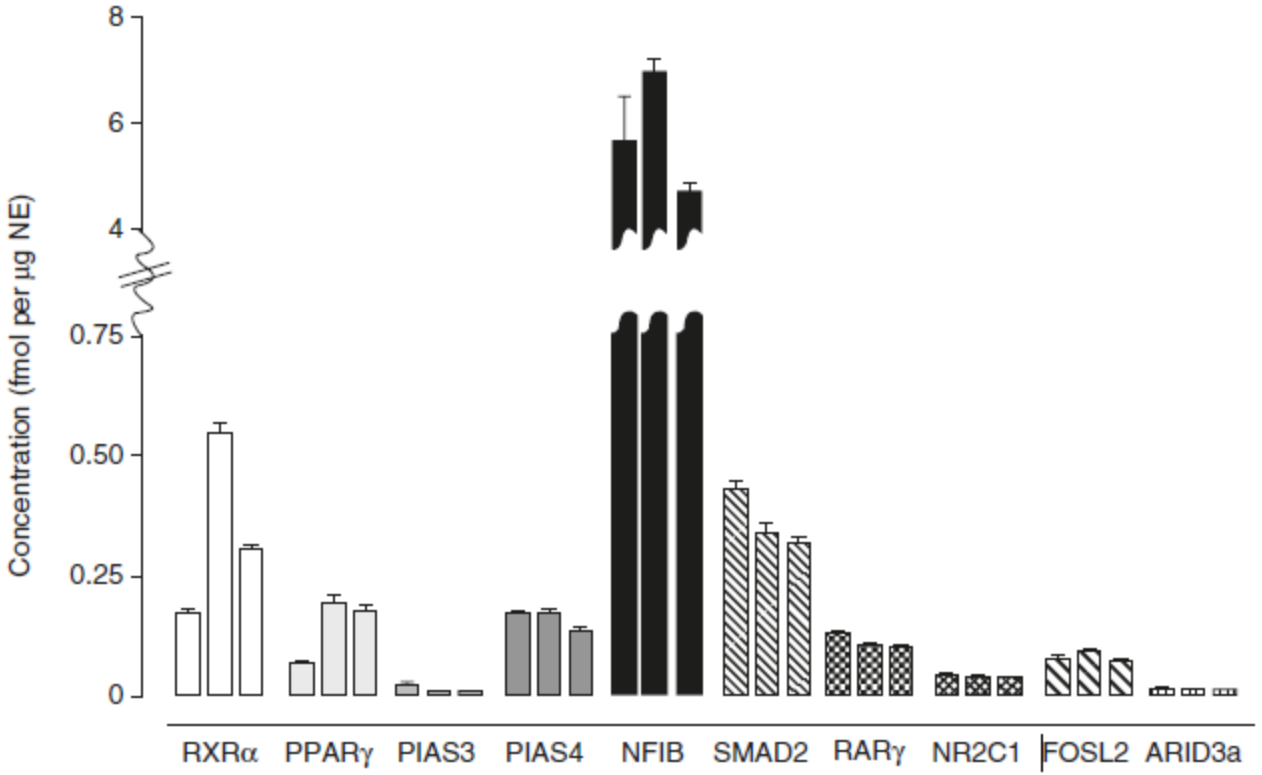






Simicevic et al., 2013

Levels of 10 TF at day 0, 2, and 4



Simicevic et al., 2013

Summary

- *In vitro* expression of internal standard protein
- Empirical selection of proteotypic peptides
- Run protein standards and endogenous counterpart in same assay, thus decreased variability
- Over 10 target proteins can be measured
- High accuracy for protein quantities above 2.5 ng
- Independence of antibodies

A sentinel protein assay for simultaneously quantifying cellular processes

Martin Soste[1], Rita Hrabakova[1,2], Stefanie Wanka[3], Andre Melnik[1], Paul Boersema[1], Alessio Maiolica[4], Timon Wernas[1], Marco Tognetti[1], Christian von Mering[3] & Paola Picotti[1]

[1]Institute of Biochemistry, Department of Biology, ETH Zurich, Zurich, Switzerland. [2]Laboratory of Applied Proteome Analyses, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic. [3]Institute of Molecular Life Sciences and Swiss Institute of Bioinformatics, University of Zurich, Zurich, Switzerland. [4]Institute of Molecular Systems Biology, Department of Biology, ETH Zurich, Zurich, Switzerland. Correspondence should be addressed to P.P. (paola.picotti@bc.biol.ethz.ch).

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Aim: proteomic screening to detect activation state of a given cellular process

Step 1: identify 570 potentially suitable sentinels for *S. cerevisiae*

Step 2: select proteins specific for one or more pathways

> activation, deactivation, phosphorylation, cleaved, induced expression

Step 3: biologically validated 'A-grade' sentinel (88% of the selection), predicted 'B-grade' sentinel (12% of the selection)

Step 4: establish quantitative SRM for 157 senitnel proteins and 152 sentinel phosphopeptides

> Multiplexed sentinel fingerprint assay (188 unique biological processes)

Soste et al., 2014

Defining environmental perturbations

Stationary phase

- Synthetic complete liquid medium, 2% glucose, 30°C

30-min and 60-min heat shock

- From 30°C to prewarmed 42°C medium

Heat-shock recovery

- Transferred back to 30°C medium

Osmotic stress

- Treated with 0.4 M NaCl for 10 min

Osmotic stress adaptation

- After 1.5h of salt stress

Rapamycin treatment

- 220 nM in 90% ethanol/10% Tween-20 for 3 h

Amino acid and nitrogen starvation

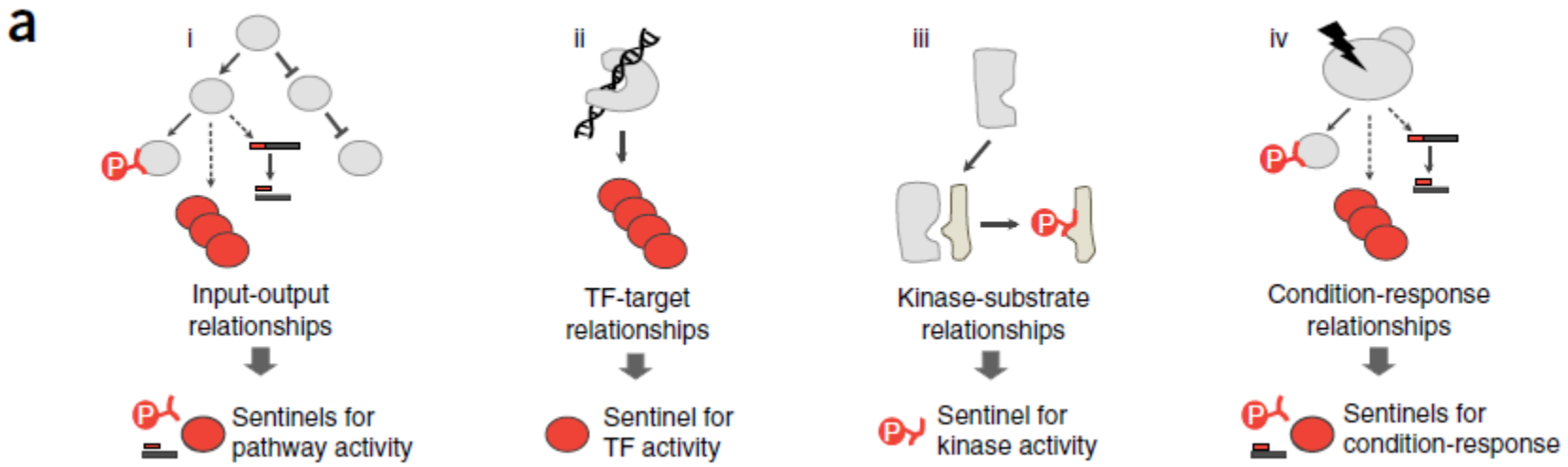
- Medium: 2% glucose w/o ammonium sulfate or amino acids for 4h

Soste et al., 2014

Criteria for ‘sentinel’ proteins or phosphopeptides

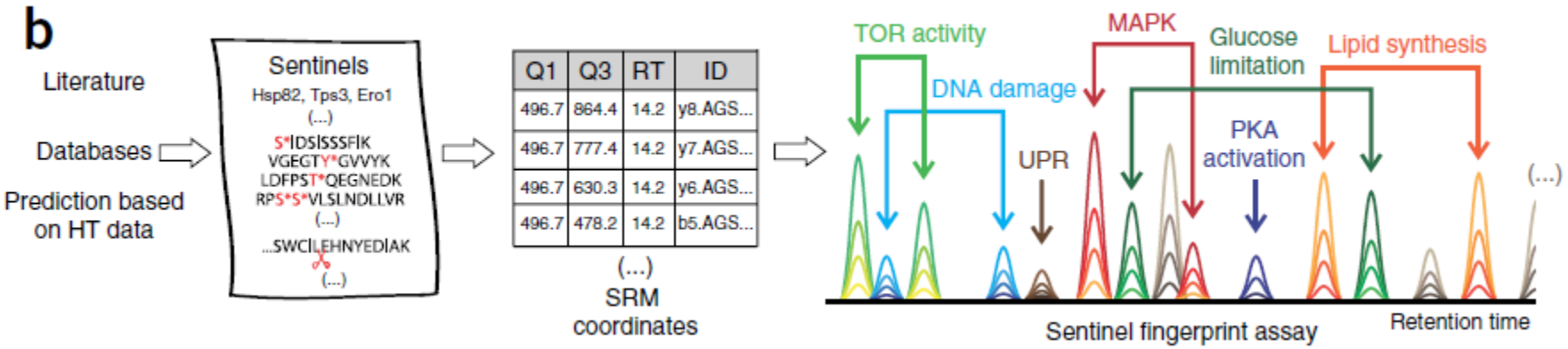
Find suitable proteins from literature, database, yeast biologists

- Activation, deactivation, phosphorylation, cleaved, induced expression
- Four classes of biological relationships



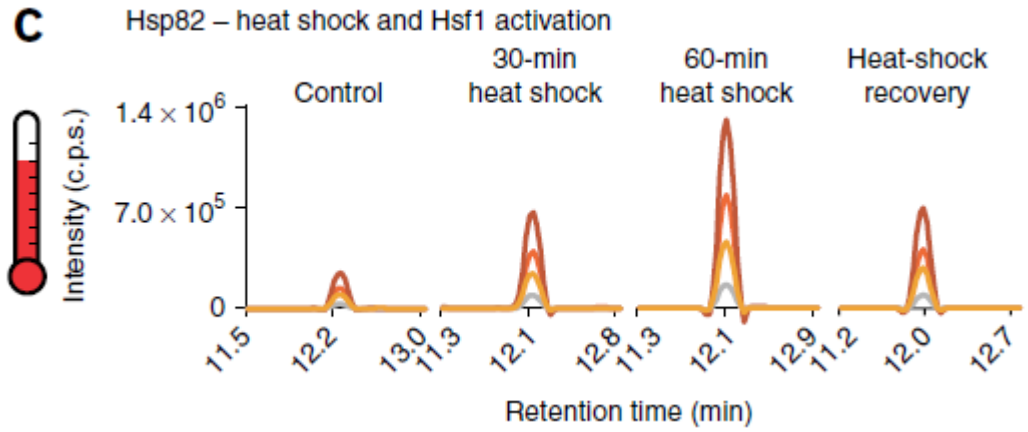
Soste et al., 2014

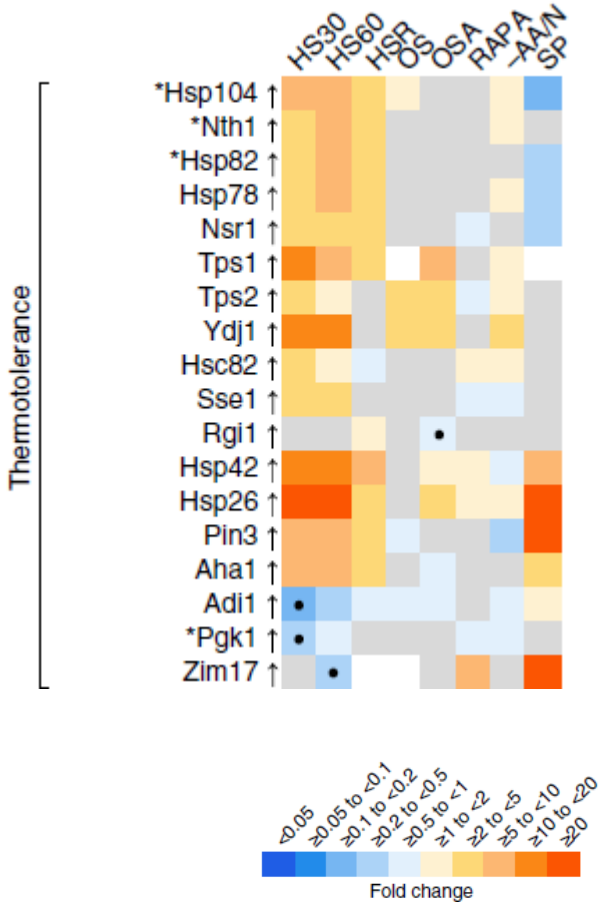
Sentinel fingerprint assay



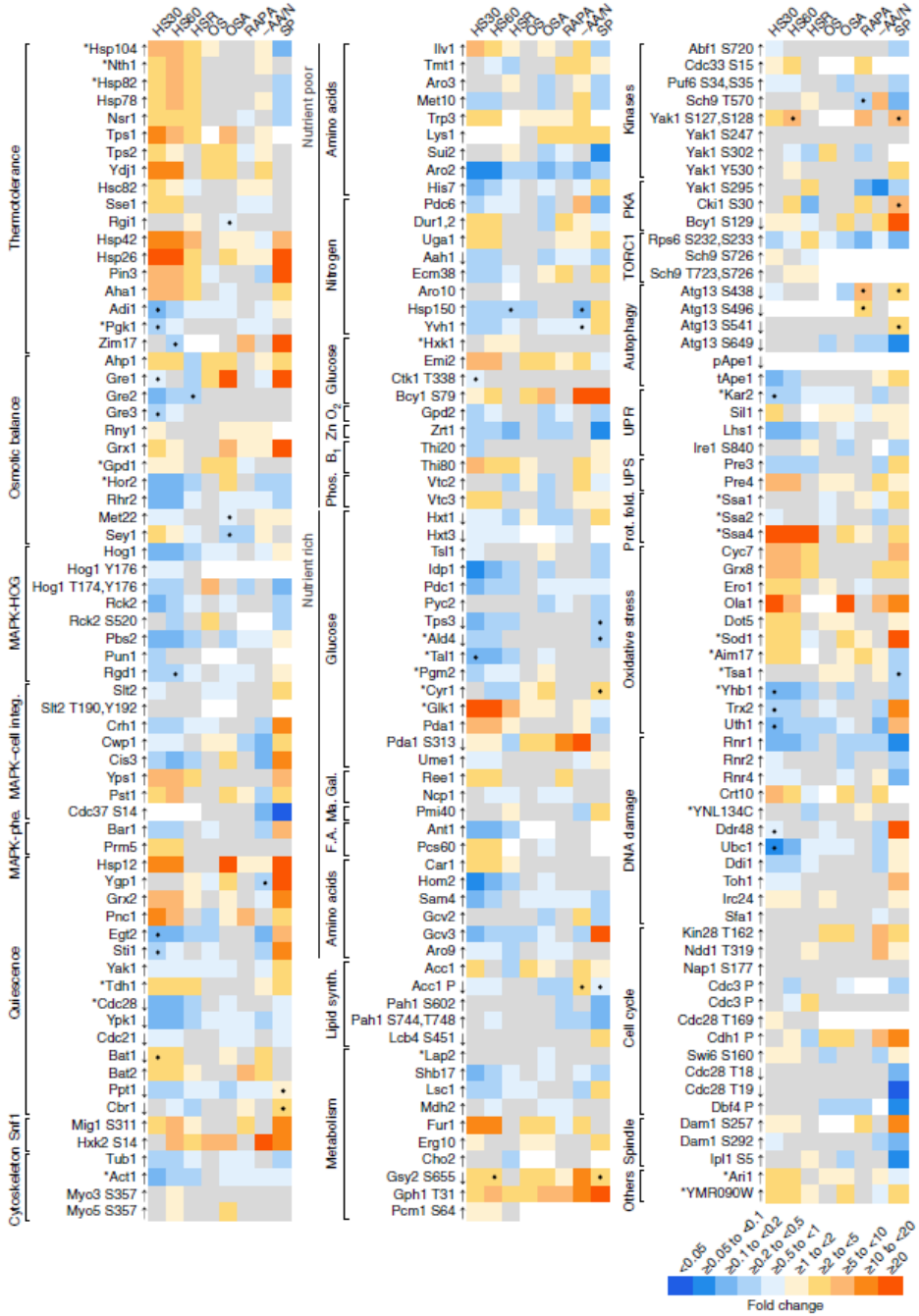
Soste et al., 2014

Response of yeast to heat shock





Soste et al., 2014



- Data reported 74% of expected responses
- Screen revealed also new responses

Soste et al., 2014

Summary

- Multiple markers tested simultaneously
- Detection of new pathways
- Adaptable for any protein
- Readout for activation status (no abundance)

- Only selection tested > other pathways precluded
- Cross-talk between biological pathways

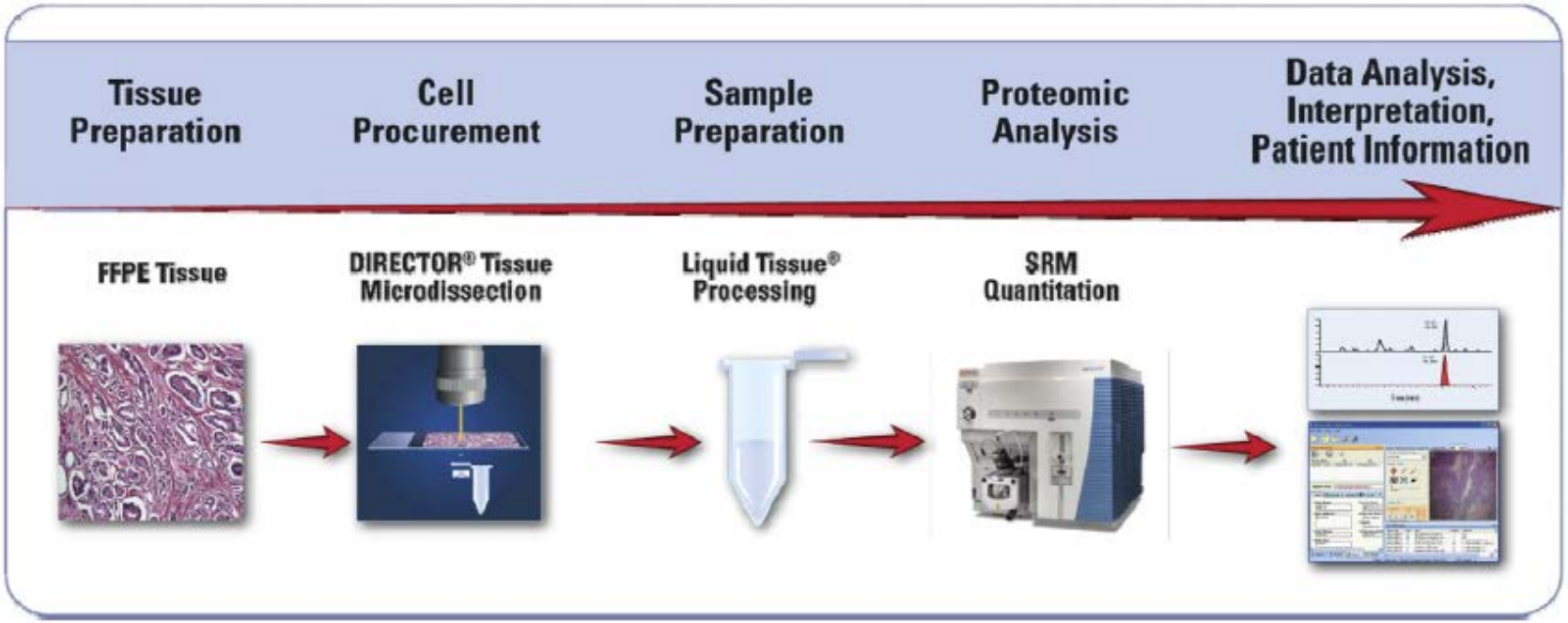
the Journal of Molecular Diagnostics

Application of Selected Reaction Monitoring for Multiplex Quantification of Clinically Validated Biomarkers in Formalin-Fixed, Paraffin-Embedded Tumor Tissue

Todd Hembrough,* Sheeno Thyparambil,* Wei-Li Liao,* Marlene M. Darfler,* Joseph Abdo,* Kathleen M. Bengali,* Stephen M. Hewitt,† Richard A. Bender,* David B. Krizman,* and Jon Burrows*

From OncoPlex Diagnostics, Inc., Rockville; and the Tissue Array Research Program,† the Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland*

The Journal of Molecular Diagnostics, Vol. 15, No. 4, July 2013



- Tumor tissue from infiltrating ductal carcinoma of the breast
- Measure expression level of oncological targets:

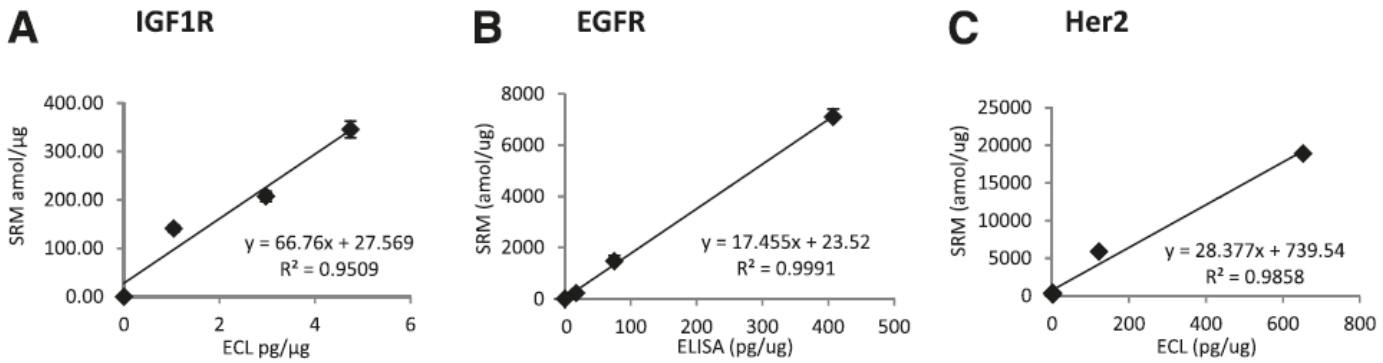
  Insulin-like growth factor 1 (IGF-1R),

  Human epidermal growth factor receptor 2 and 3 (HER2/3),
  Epidermal growth factor receptor (EGFR)

Hembrough et al., 2013

Correlation of SRM and immunoassay quantification of cell lines

- Cell lines expressing IGF1R, EGFR and HER2
  - ➢ fresh, detergent-solubilized cell lysates measured by ELISA/ECL
  - ➢ Formalin fixed and paraffin embedded cell blocks analysed by SRM



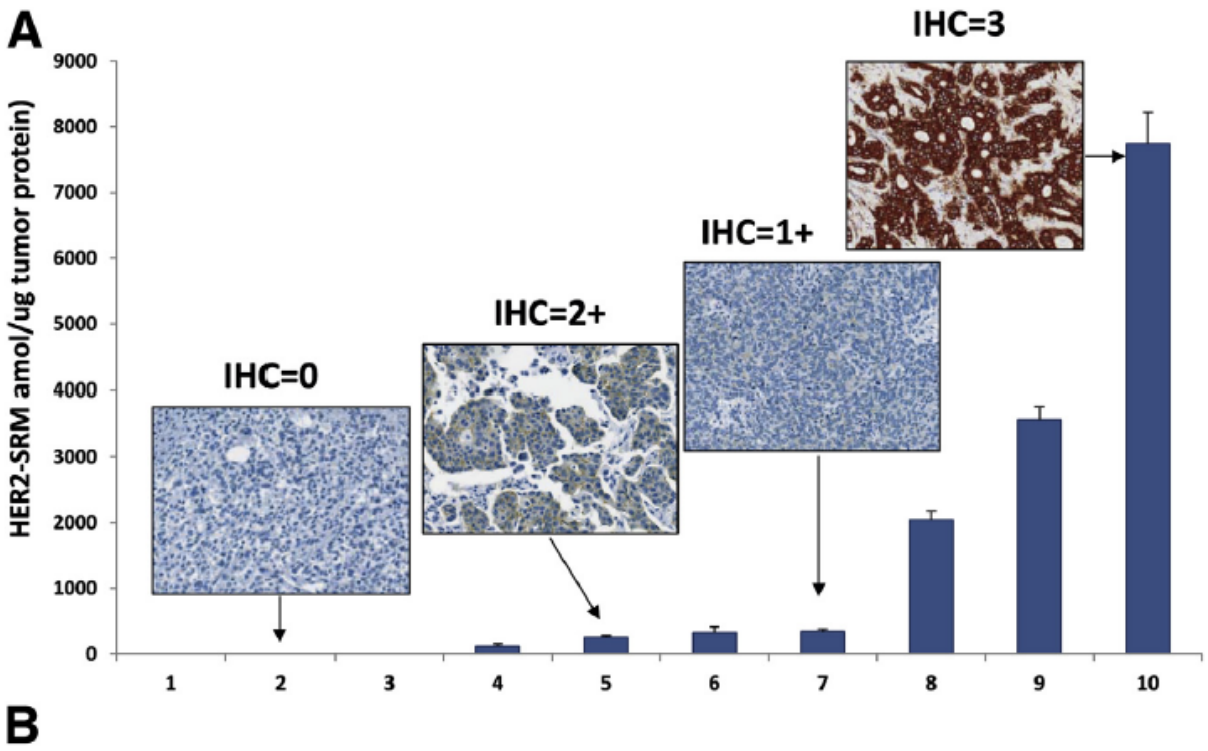
Cell	SRM amol/µg	ECL pg/ug
MDA-MB-231	0	0
HCC-827	141.10	1.04
A431	207.48	2.97
MCF7	354.84	4.74

Cell	SRM amol/µg	ELISA pg/ug
MCF7	0	0
HT29	227	17
MDA-MB-231	1,471	75
A431	7,106	407

Cell	SRM amol/µg	ECL pg/ug
MCF7	194	2
T47D	399	2
HT29	375	1
SKBR-3	5910	122
ZR75-30	18925	652

Hembrough et al., 2013

HER2 expression correlated to IHC score?

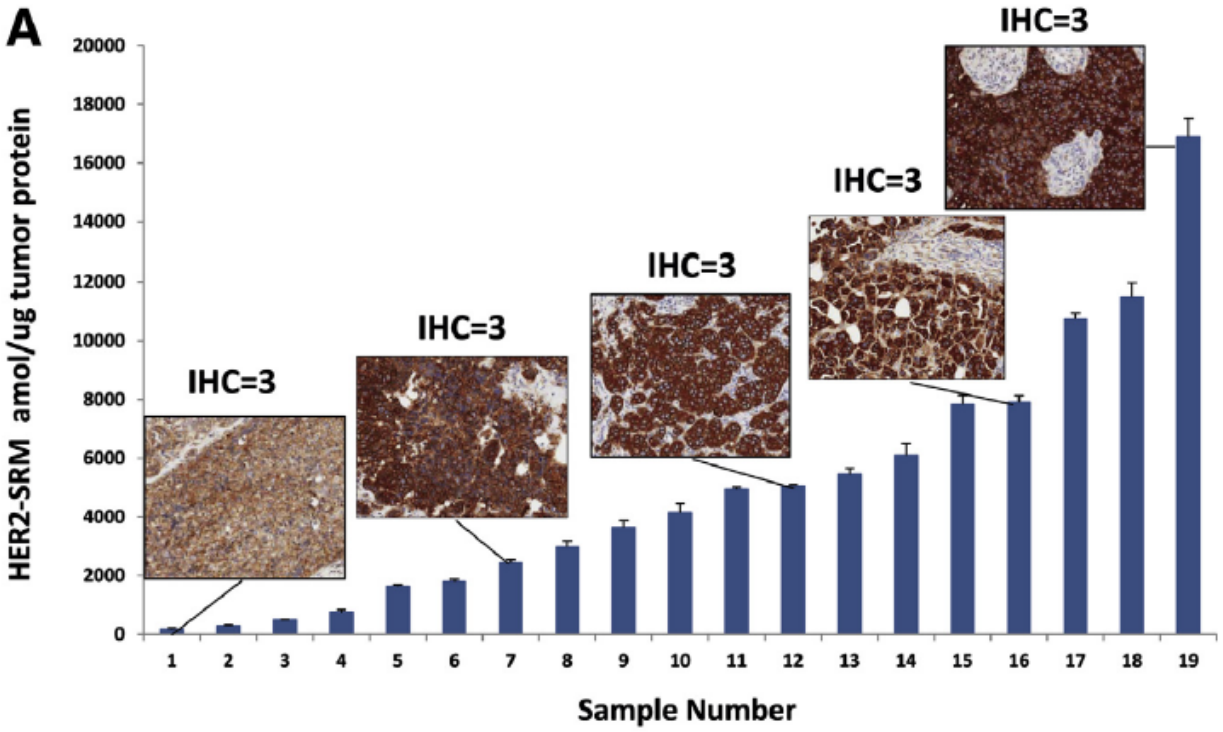


B

Sample	1	2	3	4	5	6	7	8	9	10
IHC Score	0	0	0	1	2	1	1	3	3	3
SRM amol/ug	0	0	0	121	252	329	344	2046	3569	7742

Hembrough et al., 2013

Different HER2 expression within the same IHC score



B

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
IHC Score	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
SRM amol/ug	175	290	486	753	1635	1788	2435	2976	3546	4155	4943	5058	5455	6107	7838	7908	10736	11502	16900

Hembrough et al., 2013

Summary

Quantitative expression levels of 4 proteins obtained from FFPE tumor samples

Advantages

- Many targets can be tested simultanously from limited amount of tissue
- Objective quantification
- Assay sensitivity 30- to 60-amol/μg

Limitation

- Subjectivity in Laser Microdissection
- Laborious preparation

Outlook

Can be performed on body fluids (plasma, serum, liquor), fixed tissue, drug meatbolites, food components etc.

Advantages

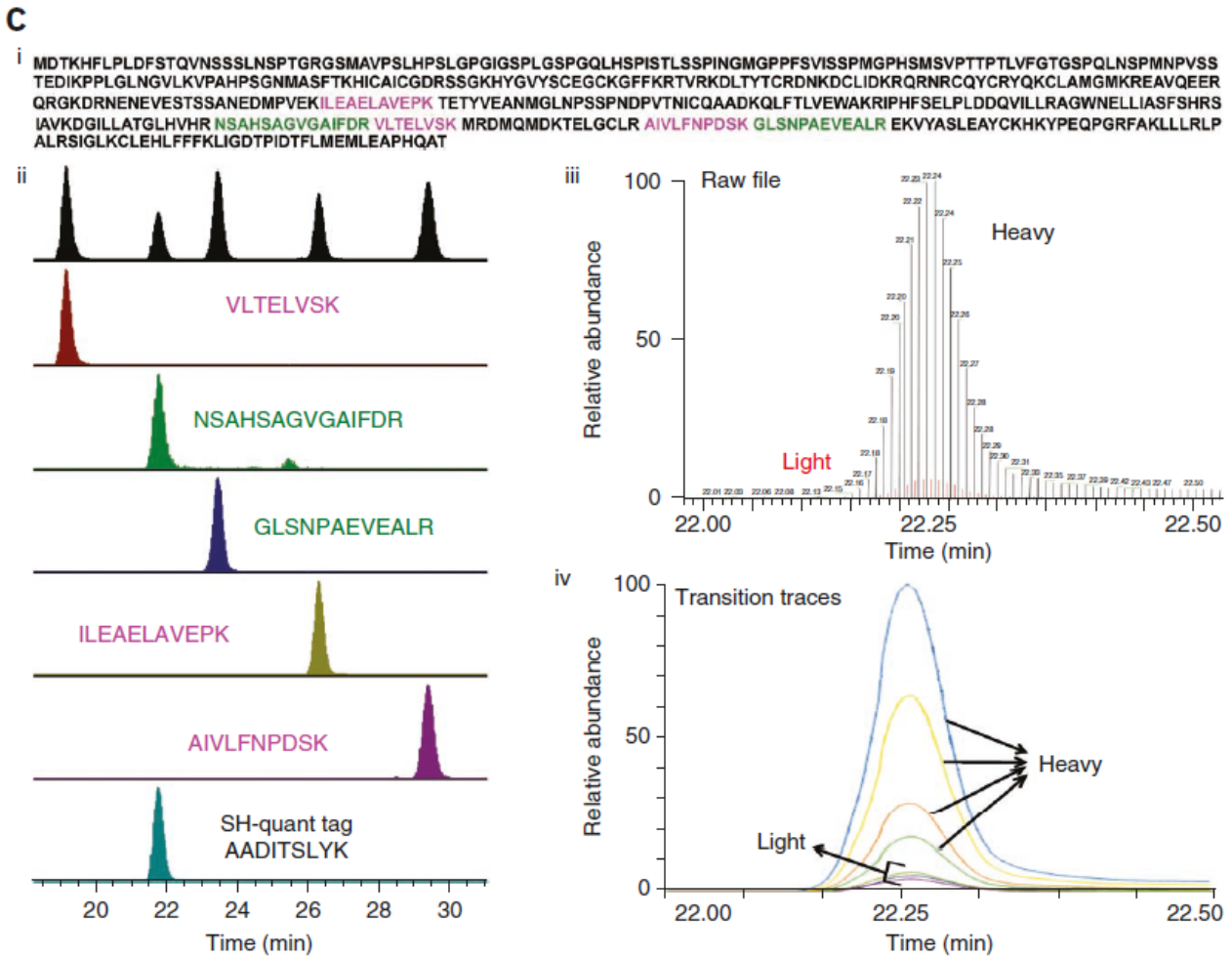
- PTM
- Multiplex analysis (50-100 proteins)
- Complex sample mixtures
- High molecular specificity
- mid-pg/mL to high ug/mL range
- High inter- and intralab assay reproducibility

Limitations

- Only average amount of protein in a sample
- No single cell, no histological context
- Small amounts only detectable witch further efford (IP, enrichment)

Thank you for your attention

RXRα sequence and proteotypic peptides



Simicevic et al., 2013