SILAC In Proteomics

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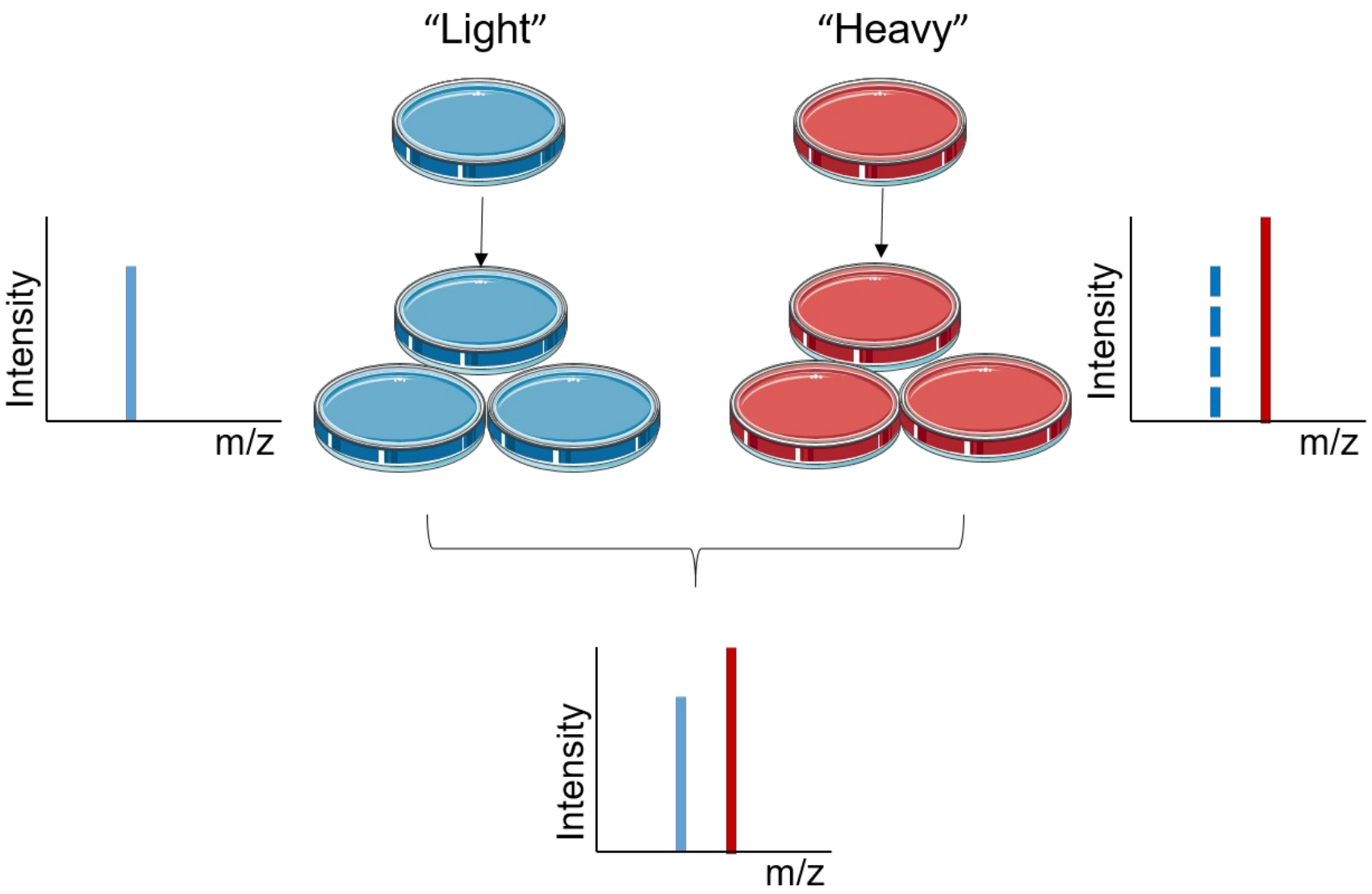
What is SILAC?

- It's the method of Stable Isotope Labelling by amino acids in Cell Culture, is a mass spectrometry technique that uses non-radioactive isotopic labelling to detect changes in protein abundance between samples.
- tool for studying close proteome change under various treatments.
- It is used to compare protein expression levels in two or three cell samples.


"Light"
"Heavy"
Intensity
m/z
Intensity
m/z
Intensity
m/z

Who invented SILAC?

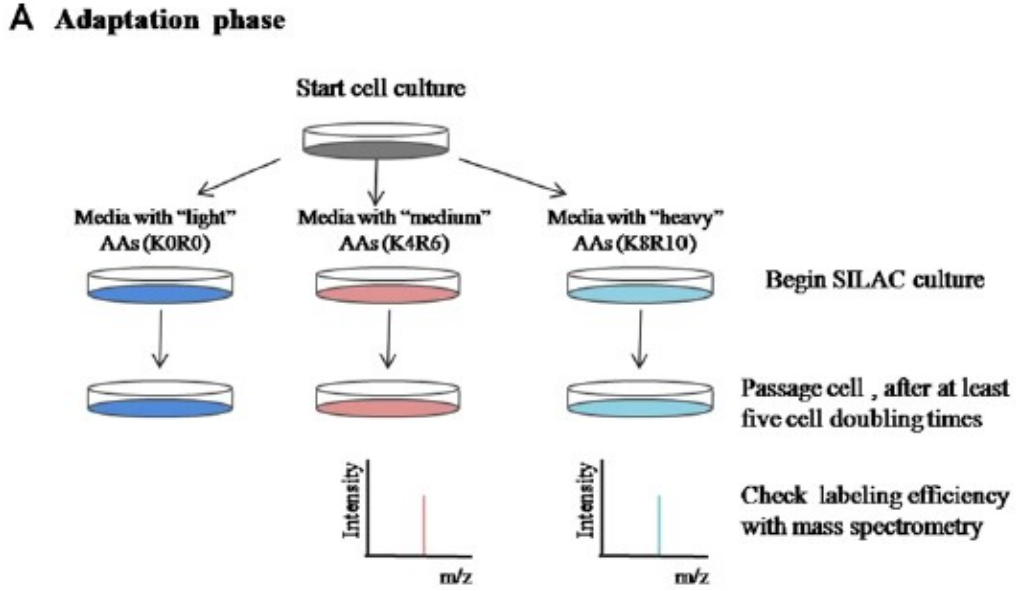
- Mantthias Mann's group created the stable isotope labelling by amino acids in cell culture (SILAC) in 2002.
- In 2008, they explained that SILAC can be used to isotope label a mouse, search for the dynamics of phosphorylation in the cell cycle, search for the effects of microRNA on cellular proteomes of haploid and diploid yeast, to name few applications.

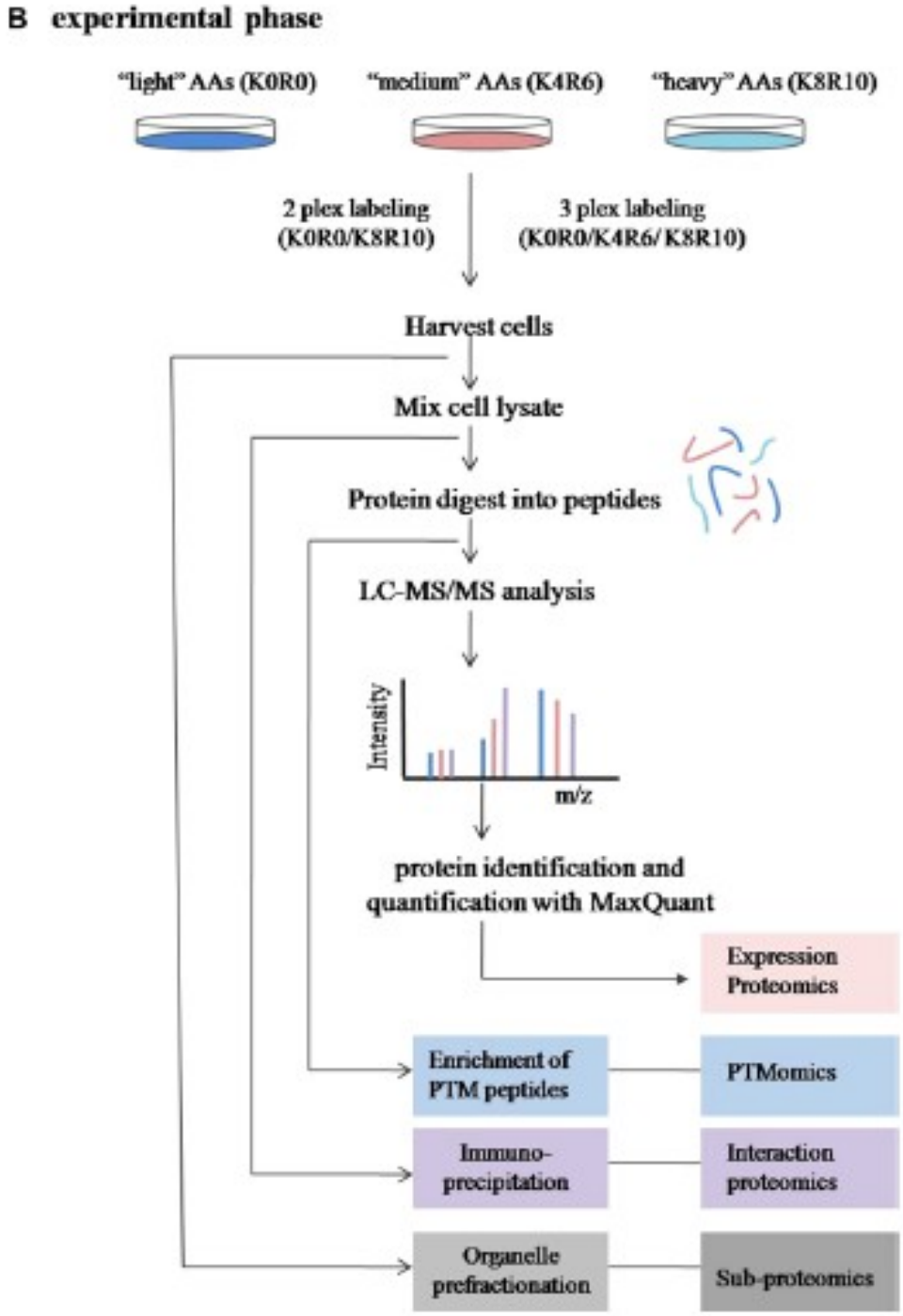
How SILAC works?

- In SILAC, there's two samples that are combined with 'light' or 'heavy' version of the amino acid.
- Two cell populations are cultivated in specific conditions that are the same save for existence of 'light' and 'heavy' version of amino acid in one of them (12C and 13C labelled L-lysine).
- Because the two isotopically tagged amino acids are chemically similar, their integration has small effect on growing normal cell while getting out protein and peptides that can be identified by mass, make them perfect for mass spectrometry study.
- Changes in post-translational modifications are suitable to SILAC techniques.

Applications of SILAC

- Expression Proteomics
- Dynamic changes of proteins
- Interactions between proteins
- Protein turnover
- cell culture, cell therapy, proteomics analysis




B experimental phase
"light" AAs (K0R0)
"medium" AAs (K4R6)
"heavy" AAs (K8R10)
2 plex labeling (K0R0/K8R10)
3 plex labeling (K0R0/K4R6/ K8R10)
Harvest cells
Mix cell lysate
Protein digest into peptides
LC-MS/MS analysis
Intensity
m/z
protein identification and quantification with MaxQuant
Expression Proteomics
Enrichment of PTM peptides
PTMomics
Immuno-precipitation
Interaction proteomics
Organelle prefractionation
Sub-proteomics

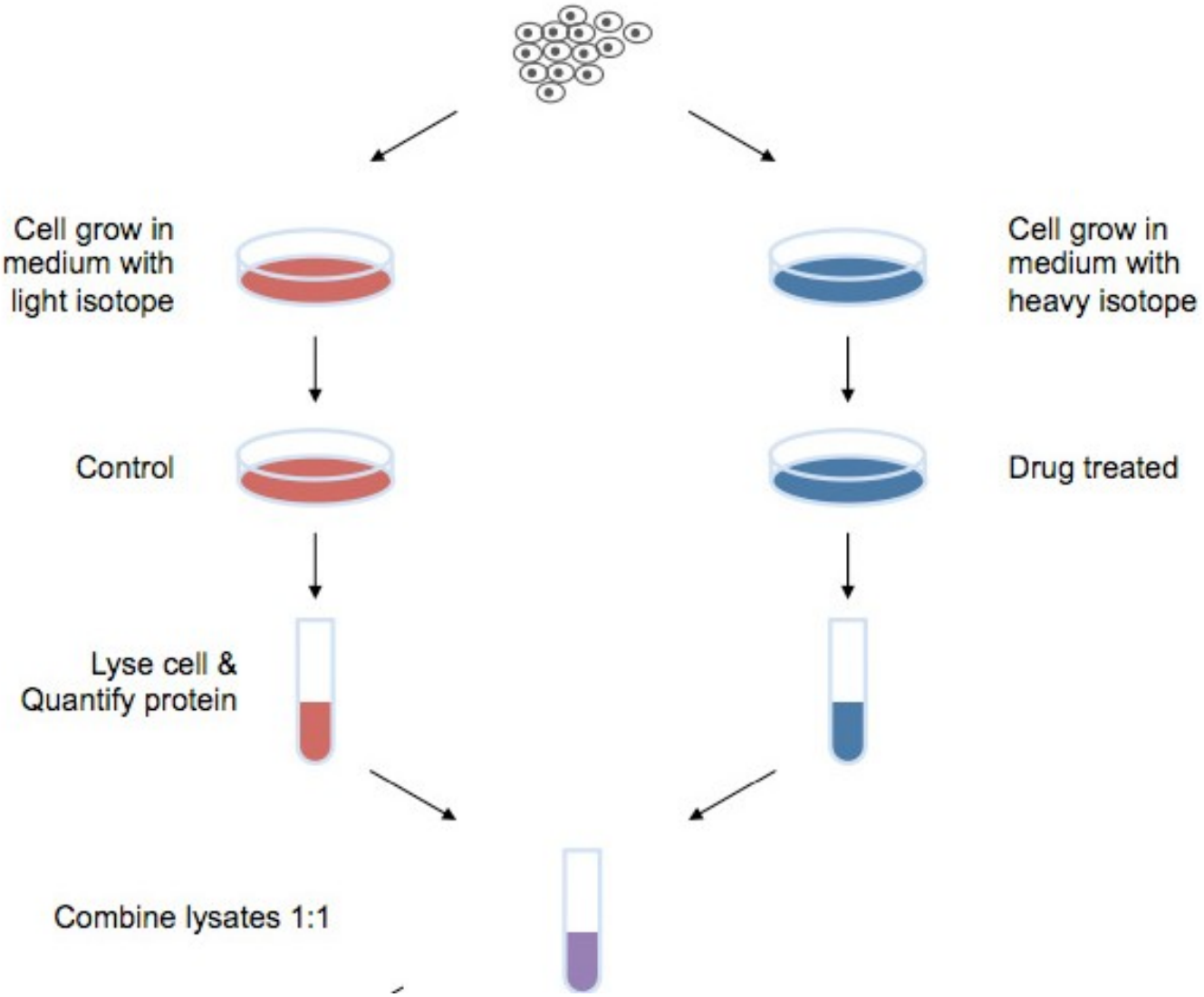
SILAC-based proteomic analysis

SILAC can be used to cell culture, cell therapy, proteomics analysis, then examine the relative proteome change under different treatments by SILAC and mass spectrometry.

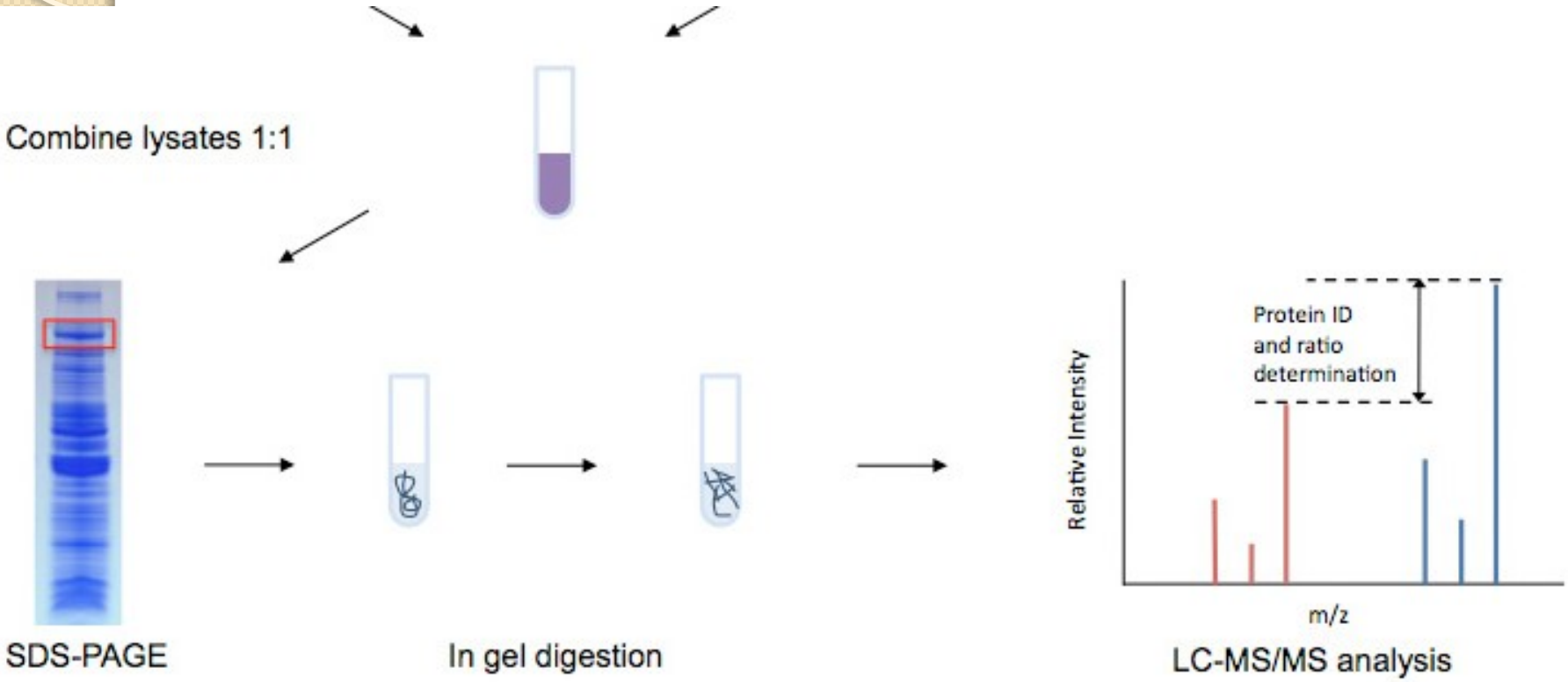
It also can give protein-protein interaction and post-translational modification study, which is not possible with other technologies.

In quantitive proteomic search, the capacity to multiplex two or three samples per analysis provide for better throughput and save costs, as well as improve relative quantification.

Workflow of SILAC-based Proteomics Analysis



Cell culturing and cell labelling are two techniques used to study cells.



Steps in the sample preparation process

- Protein extraction
- Mix unlabeled (light) and labelled (heavy) samples.
- Trypsin digestion
- Peptide fractionation
- Analysis using LC-MS/MS.
- For reliable results, data analysis is performed using expert tools and a database.
- Report in great detail.

Sample Requirement

- We accept non-labeled cell samples ‘light’ as well as ones that have been SILAC labelled ‘heavy’.
- Cell samples that haven't been tagged
- Samples of labelled cells
- Samples of proteins

Advantages of SILAC

- High effiency in labelling: lysate doesn't affect on labelling efficiency, which is high 100%.
- High sensitivity: the sample size is small, tens of microrgams of protein per sample.
- Mass spectrometry has high throughput capability, allow it to detect and quantify hundreds of proteins at the same time.
- High precision: multiple samples are combined, digested and identified at the same time. The sample is affected in the same way sequential tests, which decrease the impact of experimental operation and equipment and improve precision and iteration.
- High activity: the label is created by vivo labelling technology and more representative of the sample's true state.

How SILAC differs from iTRAQ?

- SILAC labels intact proteins, but iTRAQ labels peptides, and peptides are quantified during MS analysis in SILAC, while quantification happen during fragmentation in iTRAQ.

- SILAC is based on the direct insertion of selected stable isotope amino acids into the cell culture meduim, allow for superior quantitative search of the cellular proteome.