Slice SILAC : coupling isotope labeling with electrophoretic migration to study protein processing events.

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INTRODUCTION

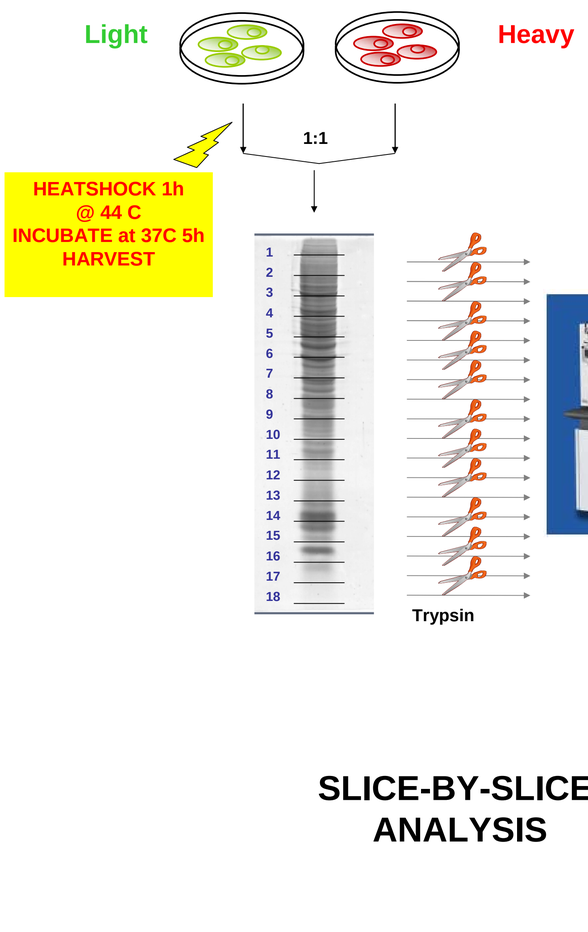
Metabolic isotope labeling of proteins (e.g. SILAC) is commonly used in conjunction with LC-MS/MS for quantitative proteomics. To decrease complexity, samples are often fractionated at the peptide level after total digestion. Alternatively, 1D SDS-PAGE can be used to separate proteins based on molecular weight, followed by in-gel digestion of gel slices (geLC-MS workflow).

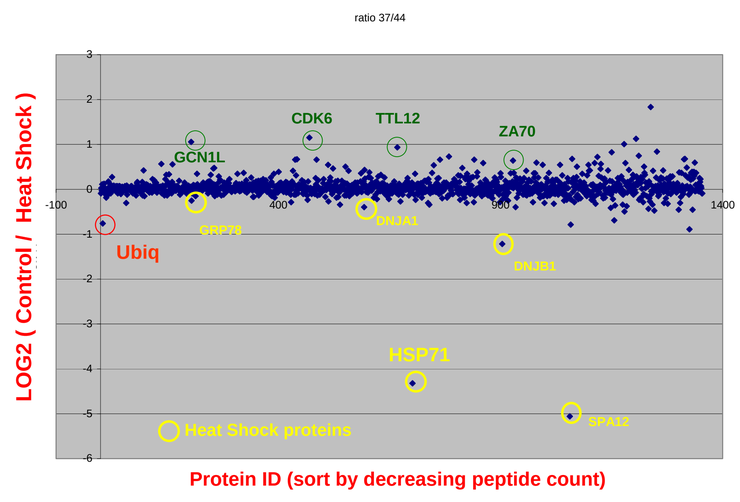
While showing some drawbacks for global quantification, the geLC-MS workflow allows to retain information on protein mass and thus has the potential to reveal post-translational processing events normally invisible with a total digestion / peptide fractionation approach.
We analysed 1D gel-separated SILAC mixtures of lysates from heat-shocked (44C) vs. control Jurkat cells. Data were analysed with MaxQuant (1) first by global quantification then slice-by-slice and the results were compared.

CONCLUSIONS

Compilation of spectral counts as a function of gel slice provided a low resolution *virtual* western blot profile for most (>1400) identified proteins. The addition of SILAC ratio information per slice allows to detect changes in protein mobility induced by the treatment. We detected significant heat shock-induced mobility shifts for 35 proteins. The pattern detected was consistent with a proteolytic cleavage for 32 proteins, of which 22 were previously known caspase substrates (2), thus revealing the onset of an apoptotic cascade.

Therefore a slice SILAC analysis can offer a completely different image (more based on post-translational modifications) of the cellular reaction to a stimulus than the one obtained with a global protein quantitation analysis.



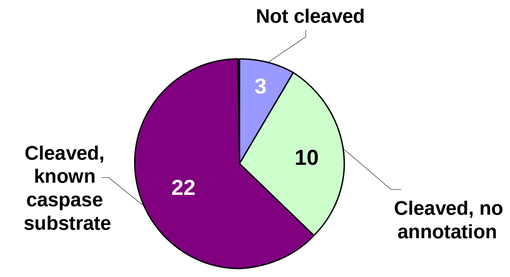


GLOBAL ANALYSIS

- Increased Ubiquitination
- Heat Shock response

SLICE SILAC

- Increased ubiquitination
- Caspase activation (=> apoptosis)



35 PROTEINS w. GEL MOBILITY SHIFT

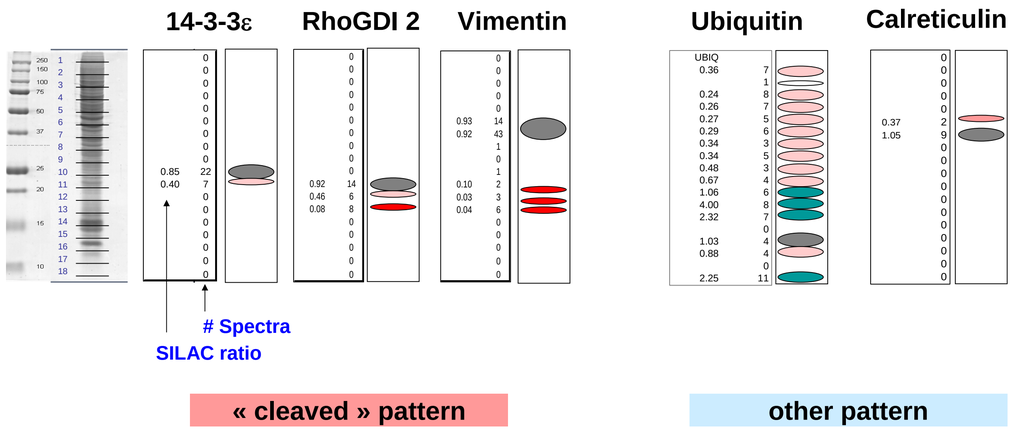
FILTER:

Protein in two or more slices

- ***with 1st SILAC ratio : r < 0.8***
  AND
- ***with 2nd SILAC ratio : r > 1.0***

Script

CASBAH db
UNIPROTkb



LARGE SCALE DETECTION OF STIMULUS-INDUCED GEL MOBILITY SHIFTS

- Ratio below average (stronger in Heat Shock)
- Ratio close to average (0.78)
- Ratio above average (stronger in control)

References : (1) Cox, J. and Mann, M. , **MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies …**, Nature Biotechnology 26, 1367 - 1372 (2008)

(2) Lüthi, a U., & Martin, S. J. (2007). **The CASBAH: a searchable database of caspase substrates**. *Cell death and differentiation*, *14*(4), 641-50. http://bioinf.gen.tcd.ie/casbah/