

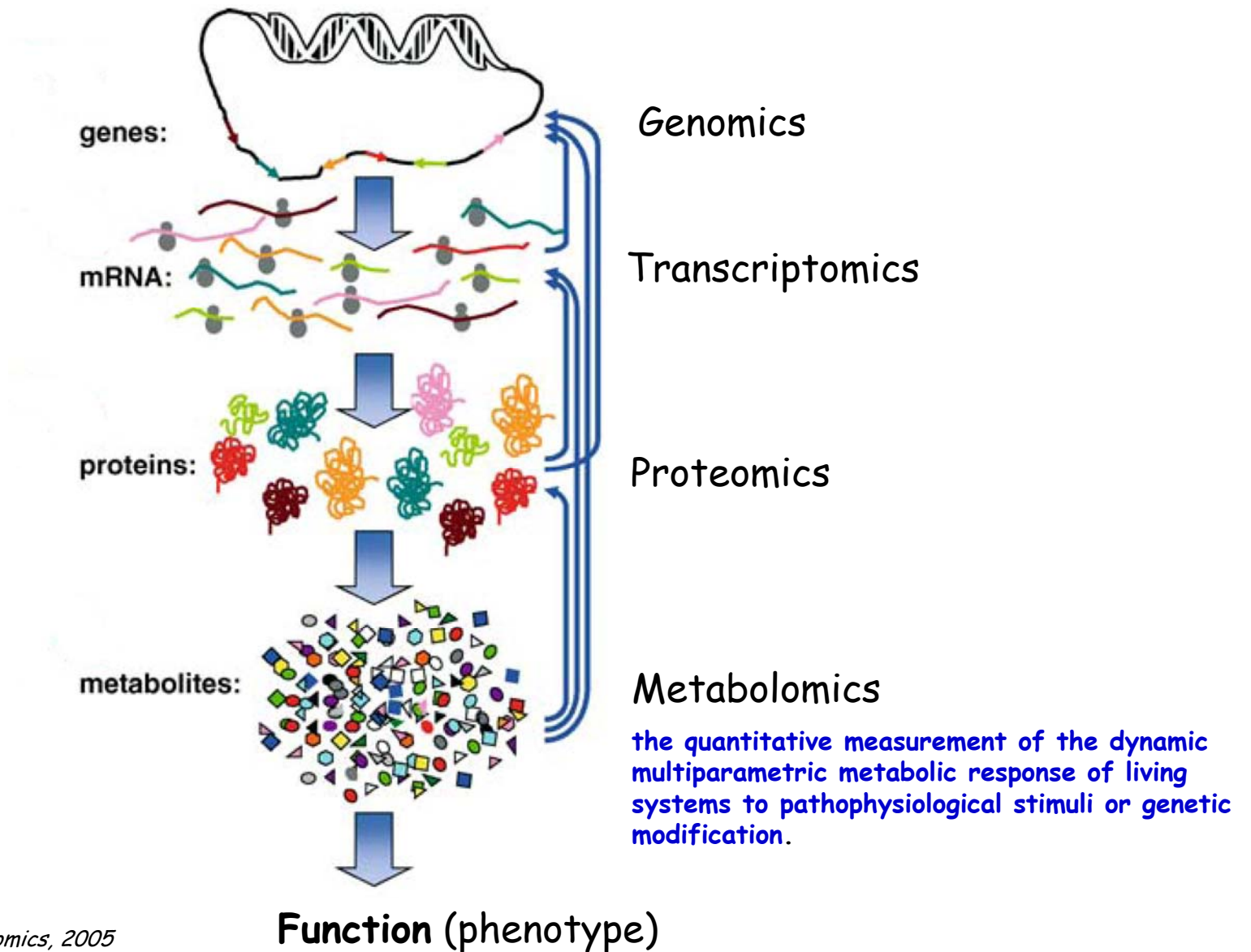


# Global Metabolomics of Breast Cancer Cells

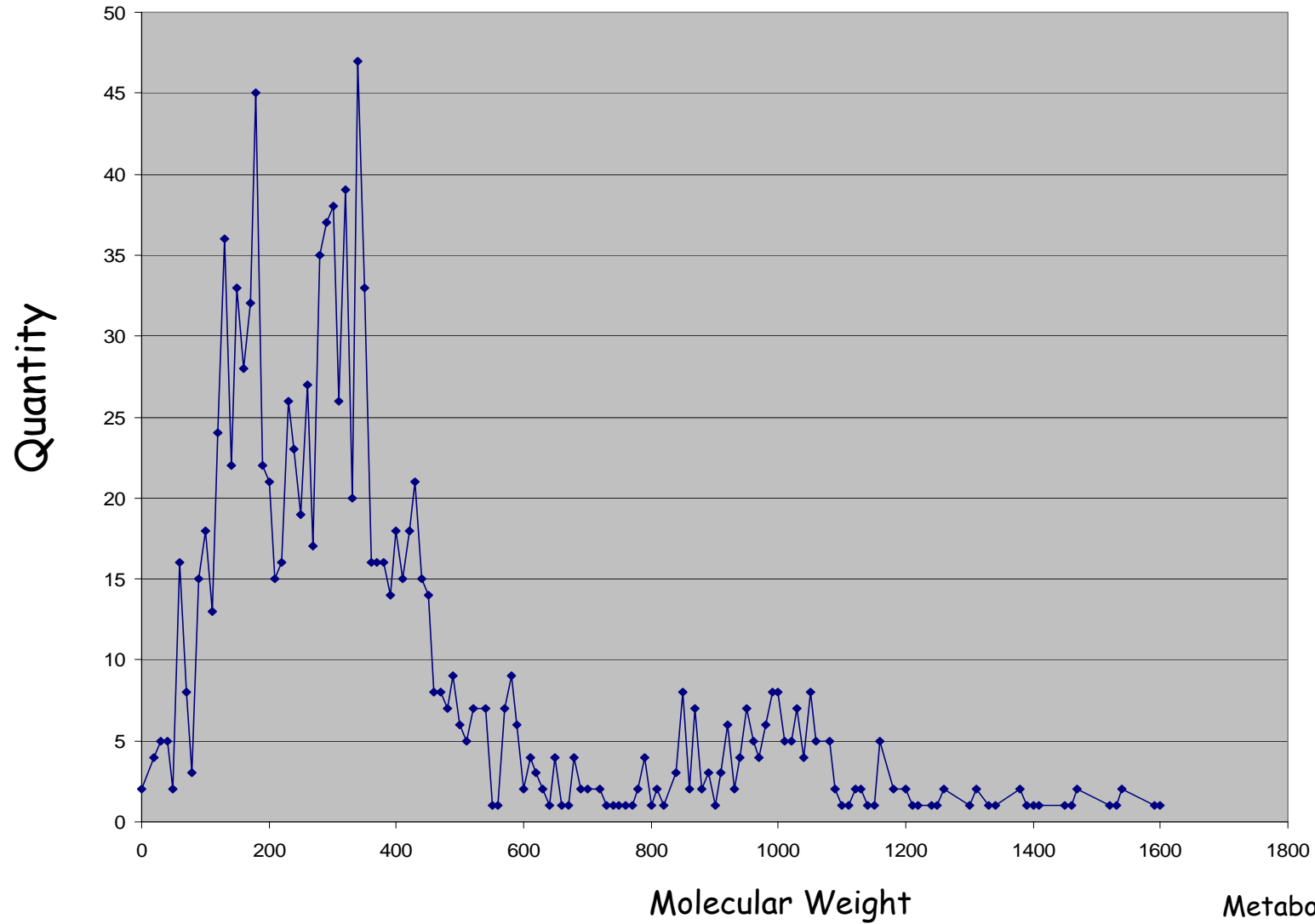
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# What is Metabolomics?



# Mass Distributions in the Human Metabolome



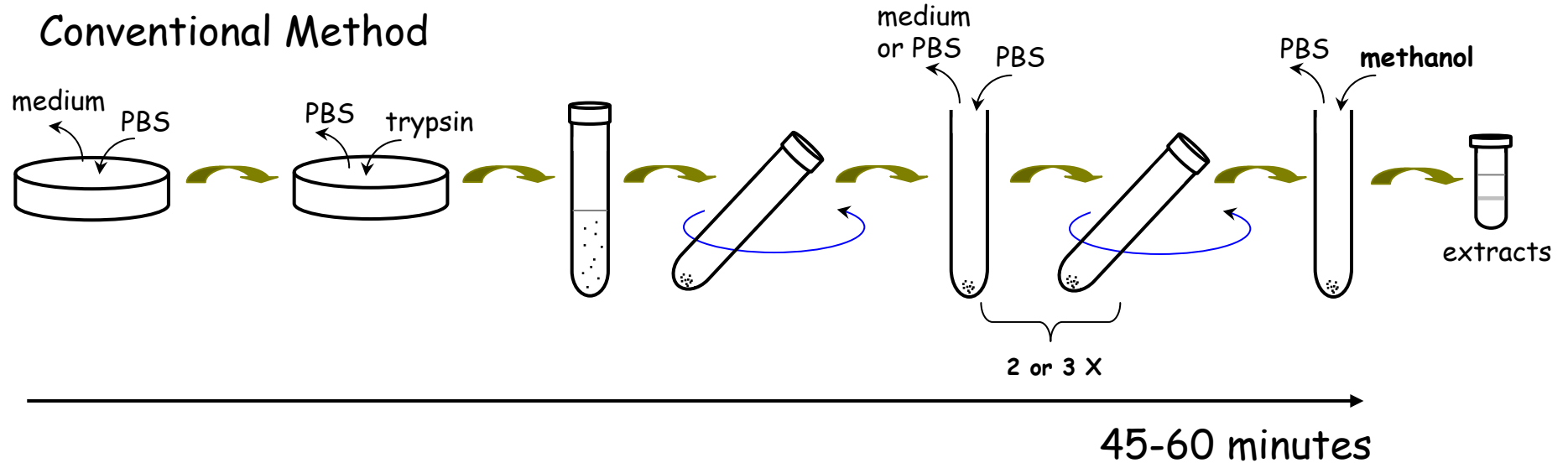
# What is Metabolomics?

- Chemical exposure often affects the biochemical pathways in a cellular or biological system (cell, organ or organism).
- As a result, changes in the normal composition of endogenous metabolites in biofluids, tissues and cells occur.
- Analysis of these biofluids, tissues or cells with techniques, such as NMR and MS, can provide insights into the nature of the toxicity.

# Why Cell Culture-Based Metabolomics?

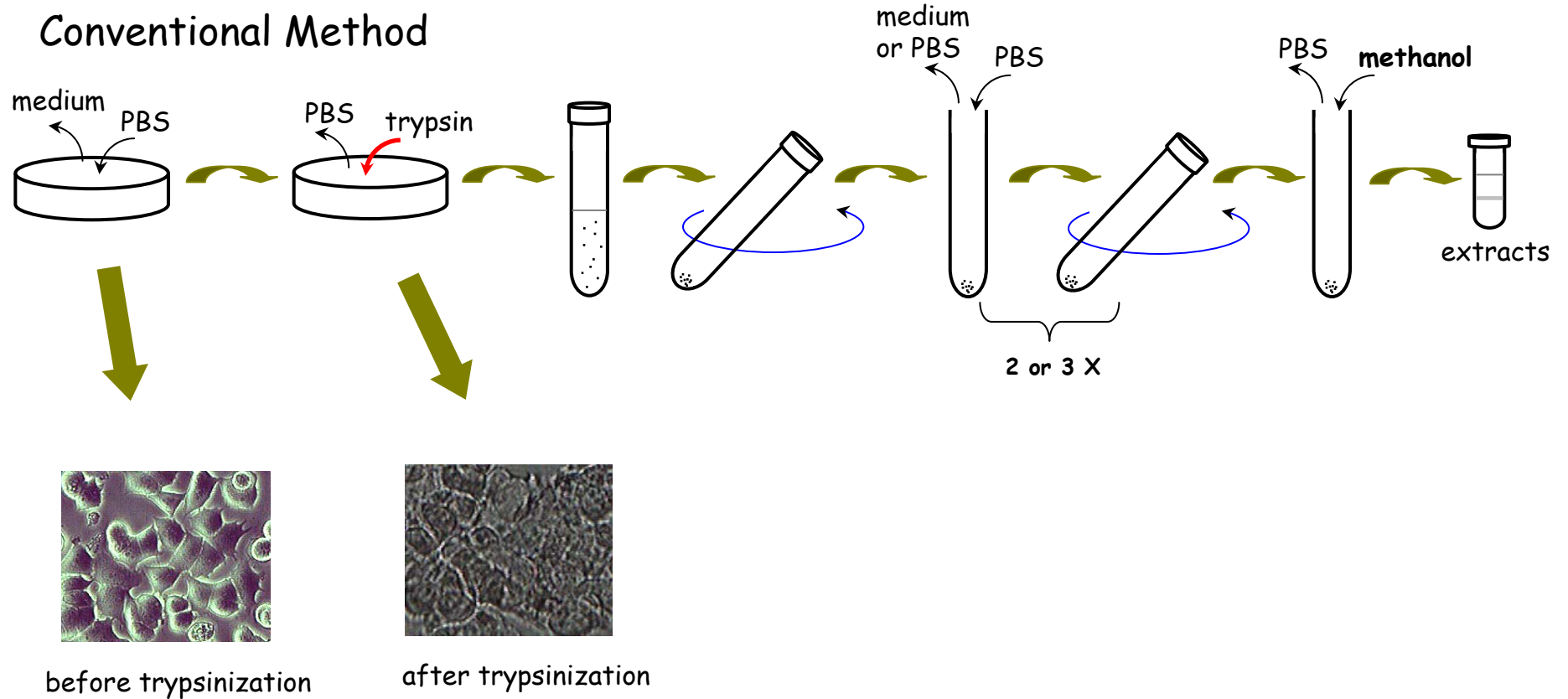
- Cell culture is an alternative system to study the metabolic responses to stress (such as chemical exposure, drug testing) in a well-controlled experimental environment.
- Can reduce the use of animals (effective extrapolation to whole organism responses is required).
- Can be rapid, inexpensive and highly automated.
- Human cell lines can be employed in order to avoid cross-species extrapolations.

# Cell Quench Methods



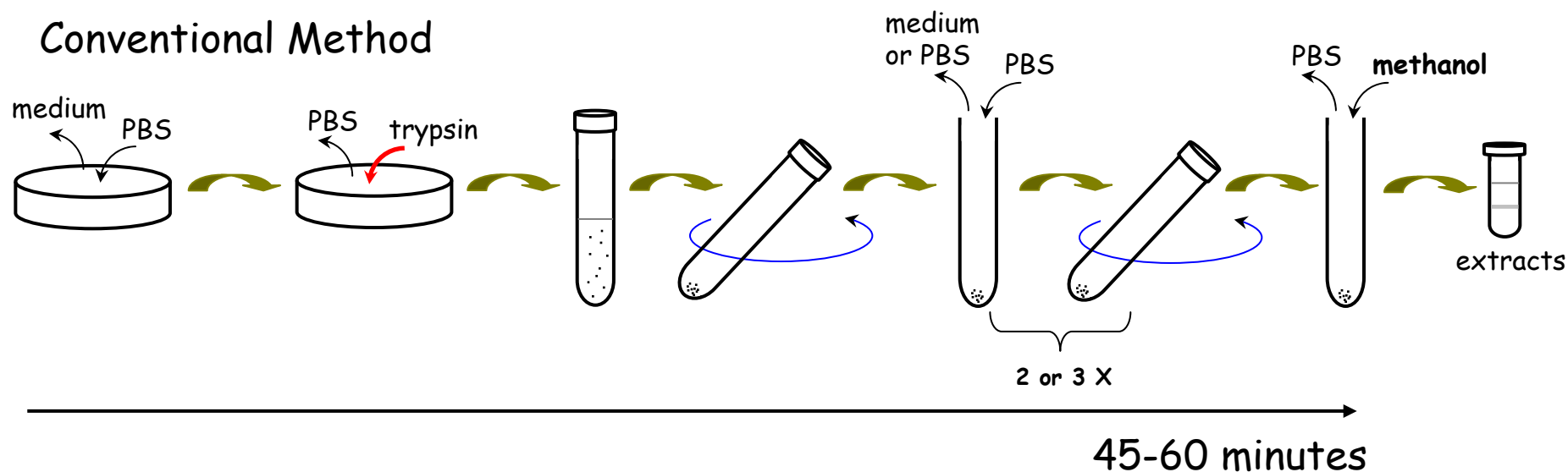
- The turn-over rate of small molecule metabolites is short, from a few seconds to minutes.
- A considerable portion of metabolites are secreted into solution during trypsinization and wash/centrifuge steps.

# Cell Quench Methods

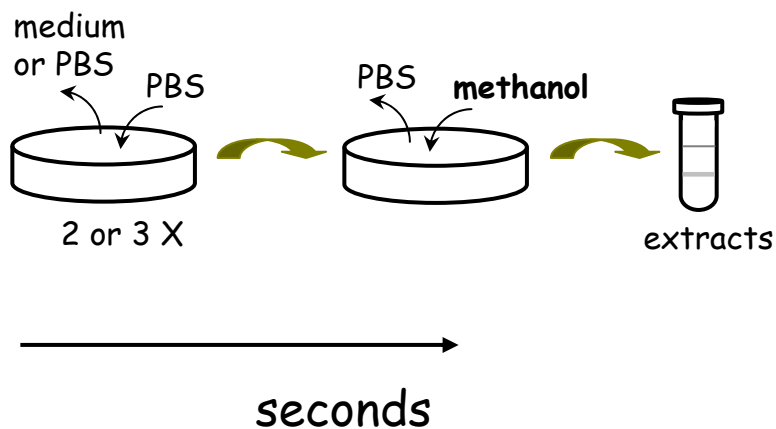


# Cell Quench Methods

## Conventional Method



## Direct Quench Method

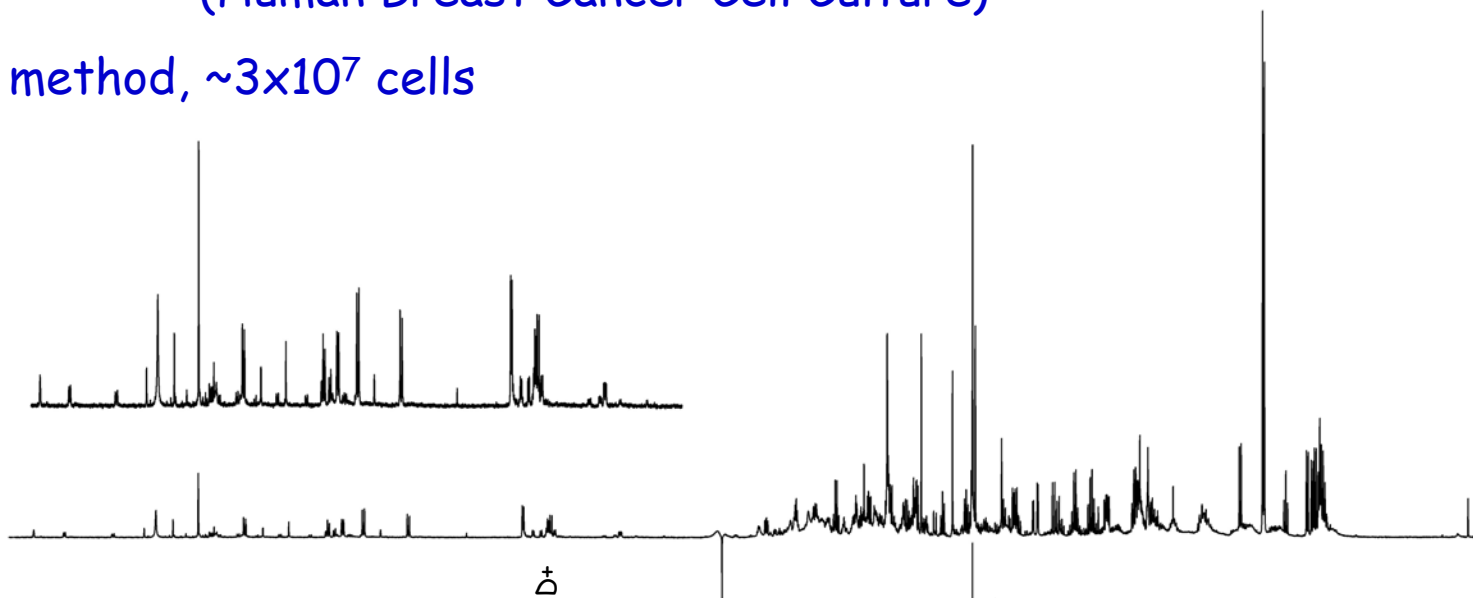




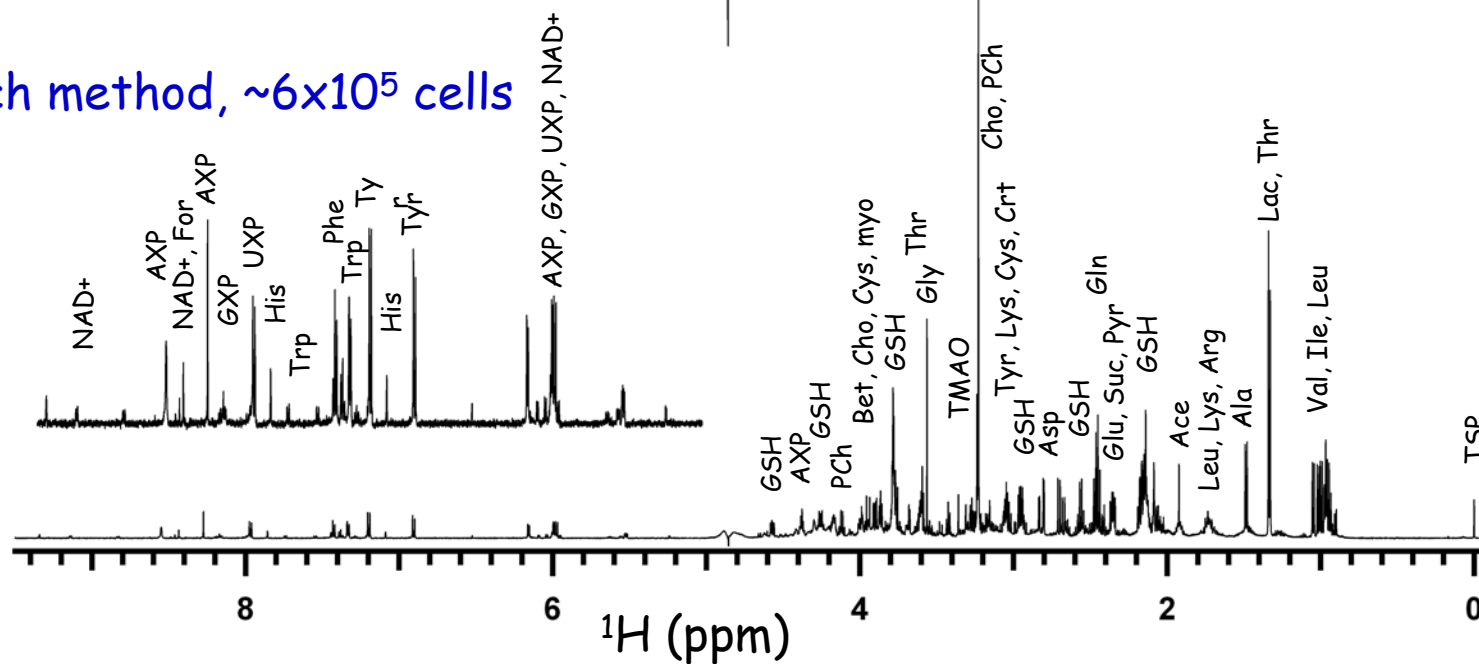
# $^1\text{H}$ NMR of MCF-7 Intracellular Extracts

(Human Breast Cancer Cell Culture)

Conventional method,  $\sim 3 \times 10^7$  cells



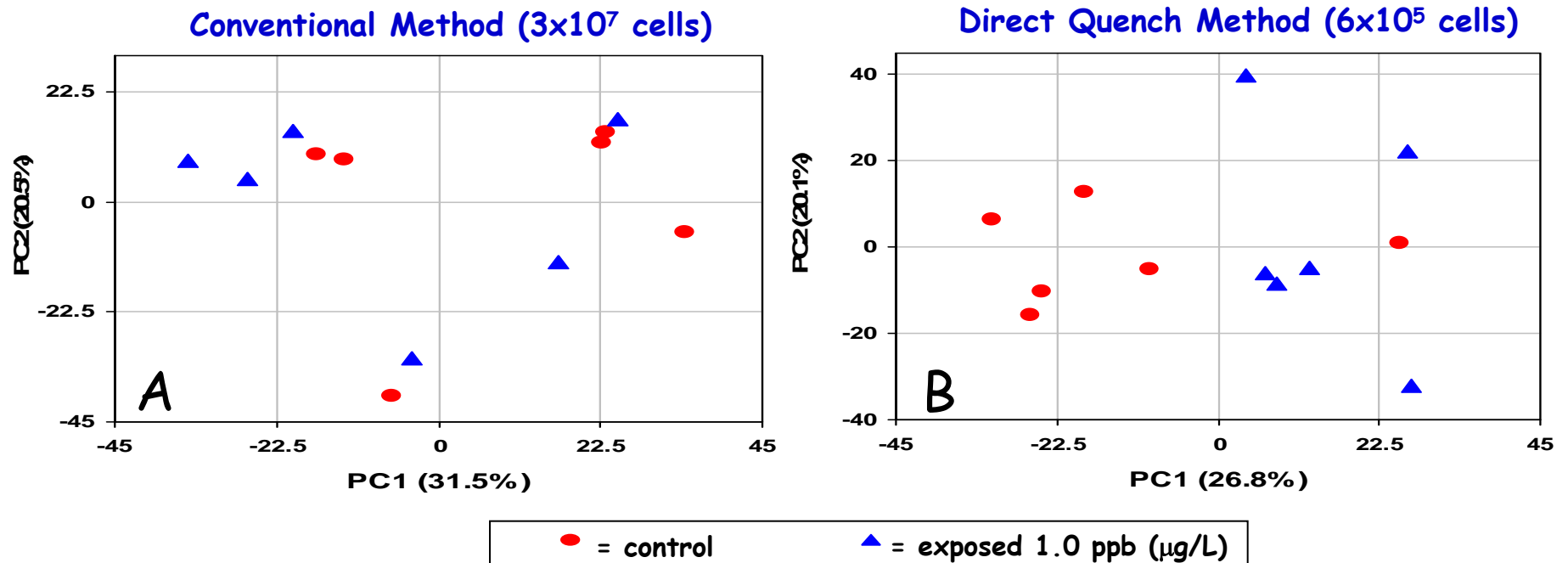
Direct quench method,  $\sim 6 \times 10^5$  cells



# Exposure of MCF-7 cells to 17 $\alpha$ -ethynylestradiol

- MCF-7 human breast cancer cell line is estrogen-receptor positive (ER<sup>+</sup>).
- ER $\alpha$  is an important predictive and prognostic marker in human breast cancer, being expressed in over 60% of cases.
- 17 $\alpha$ -Ethynylestradiol (EE2) is a potent synthetic estrogen and hormonally effective by activating the estrogen receptor. It is an endocrine disruptor.

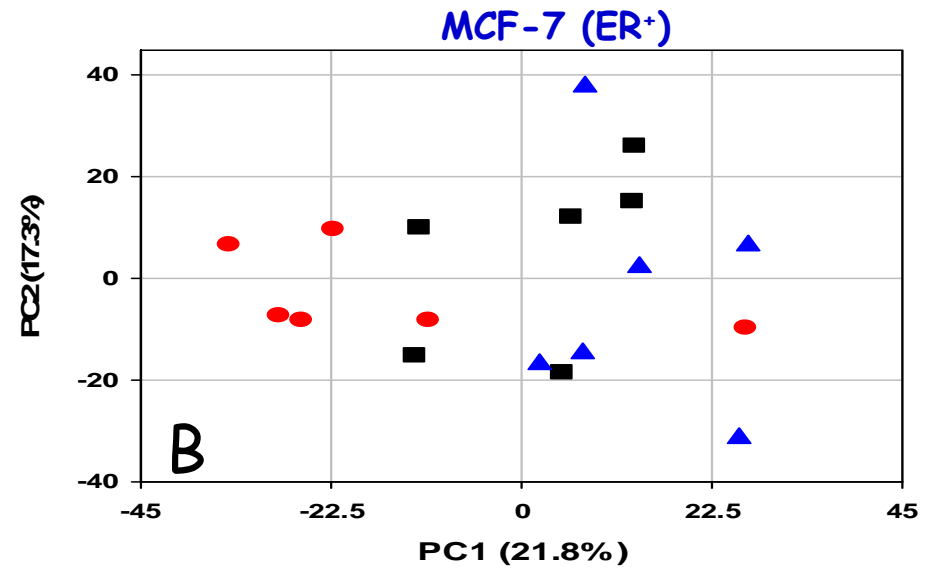
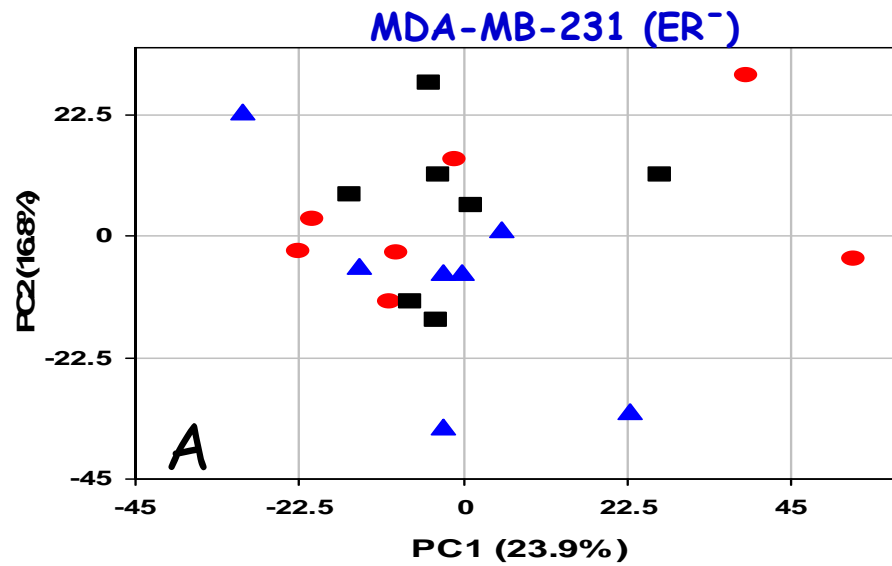
# Principle Component Analysis (PCA) Score Plots



## Exposure of MCF-7 and MDA-MB-231 cells to EE2

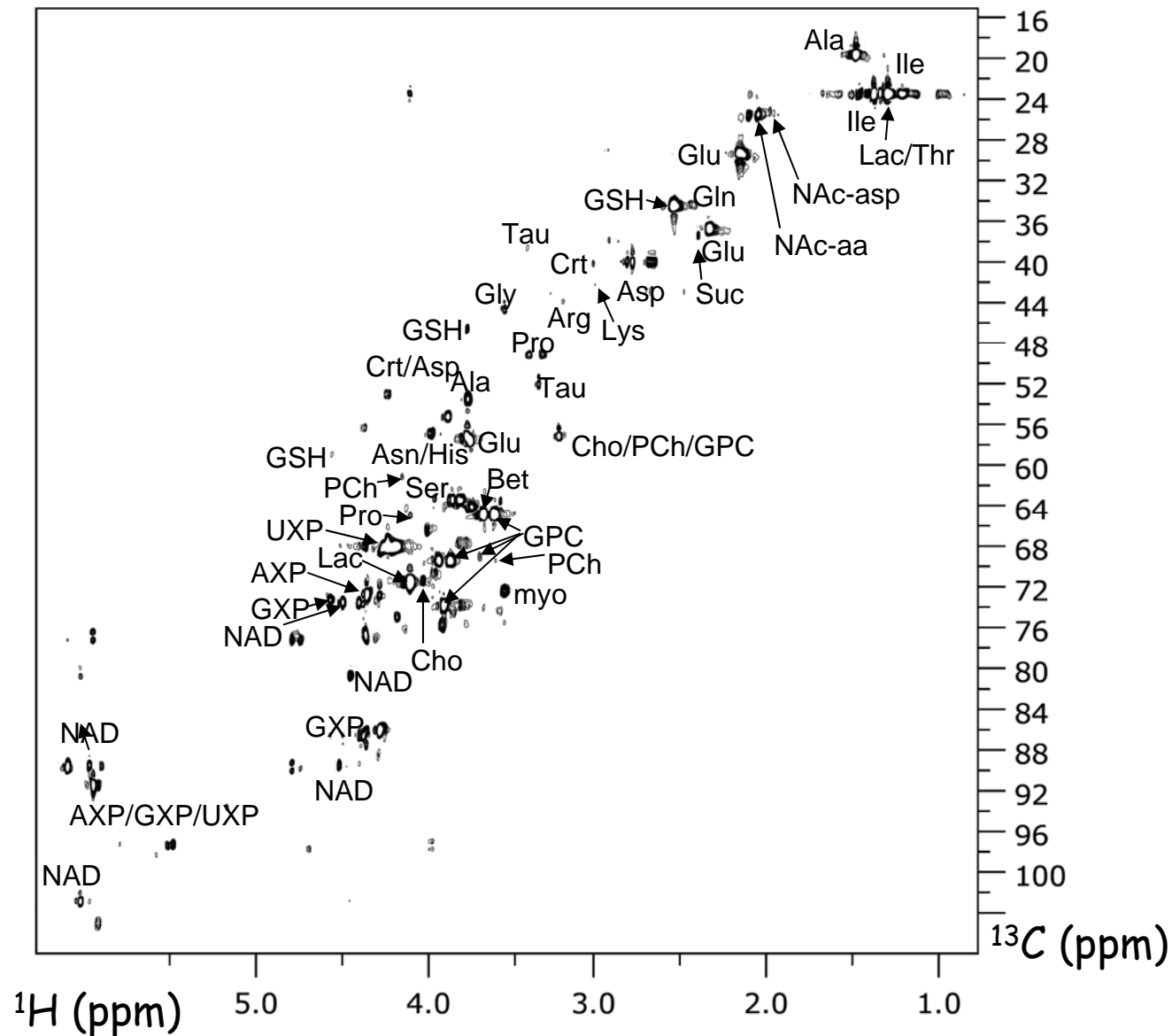
- MCF-7 human breast cancer cell line is estrogen-receptor positive (ER<sup>+</sup>).
- MDA-MB-231 human breast cancer cell line is estrogen-receptor negative (ER<sup>-</sup>).
- 48 hour exposure with three dose levels: 0.0, 0.5 and 1.0 ppb ( $\mu\text{g/L}$ ).

# PCA Score Plots

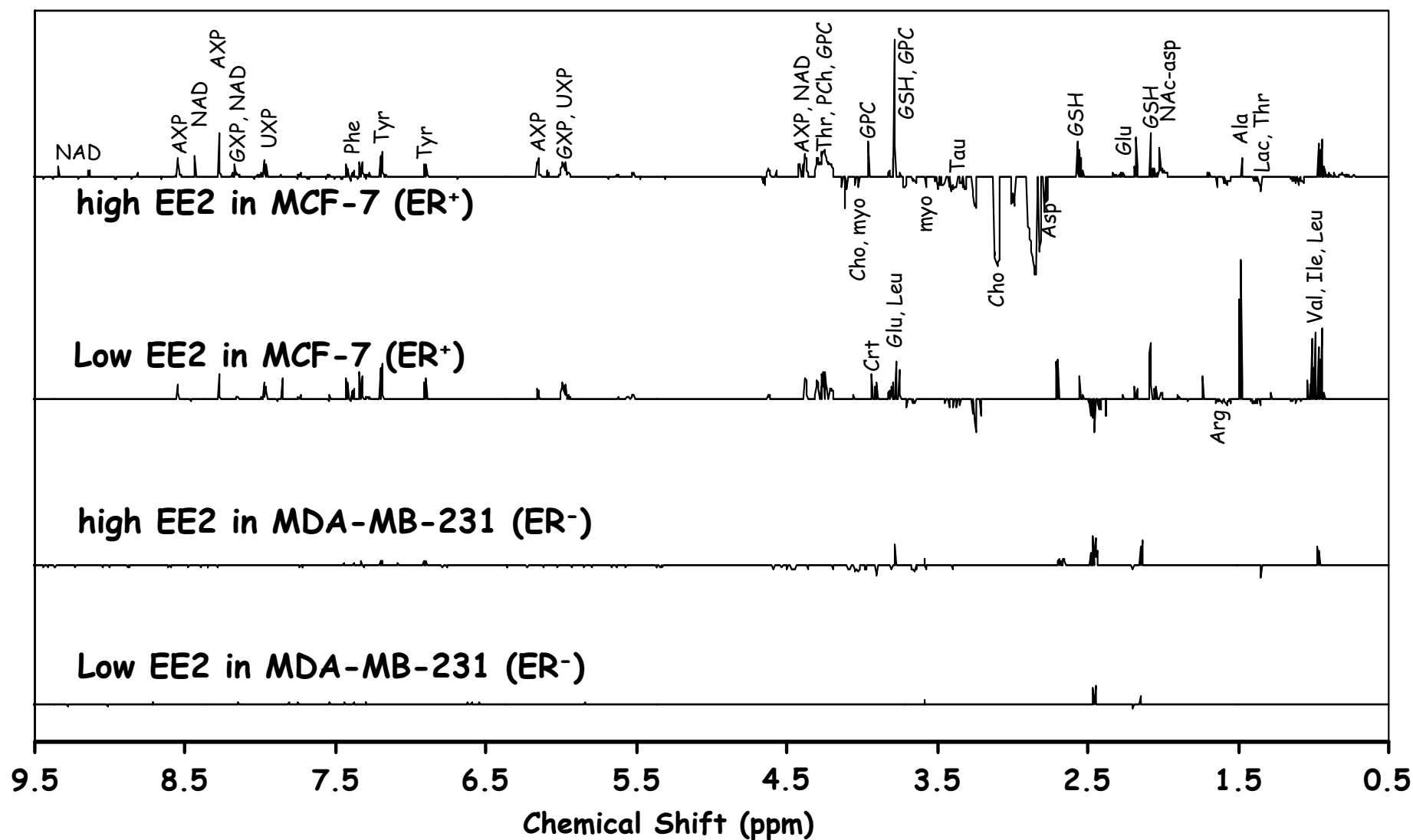


● = control    ■ = 0.5 ppb    ▲ = 1.0 ppb

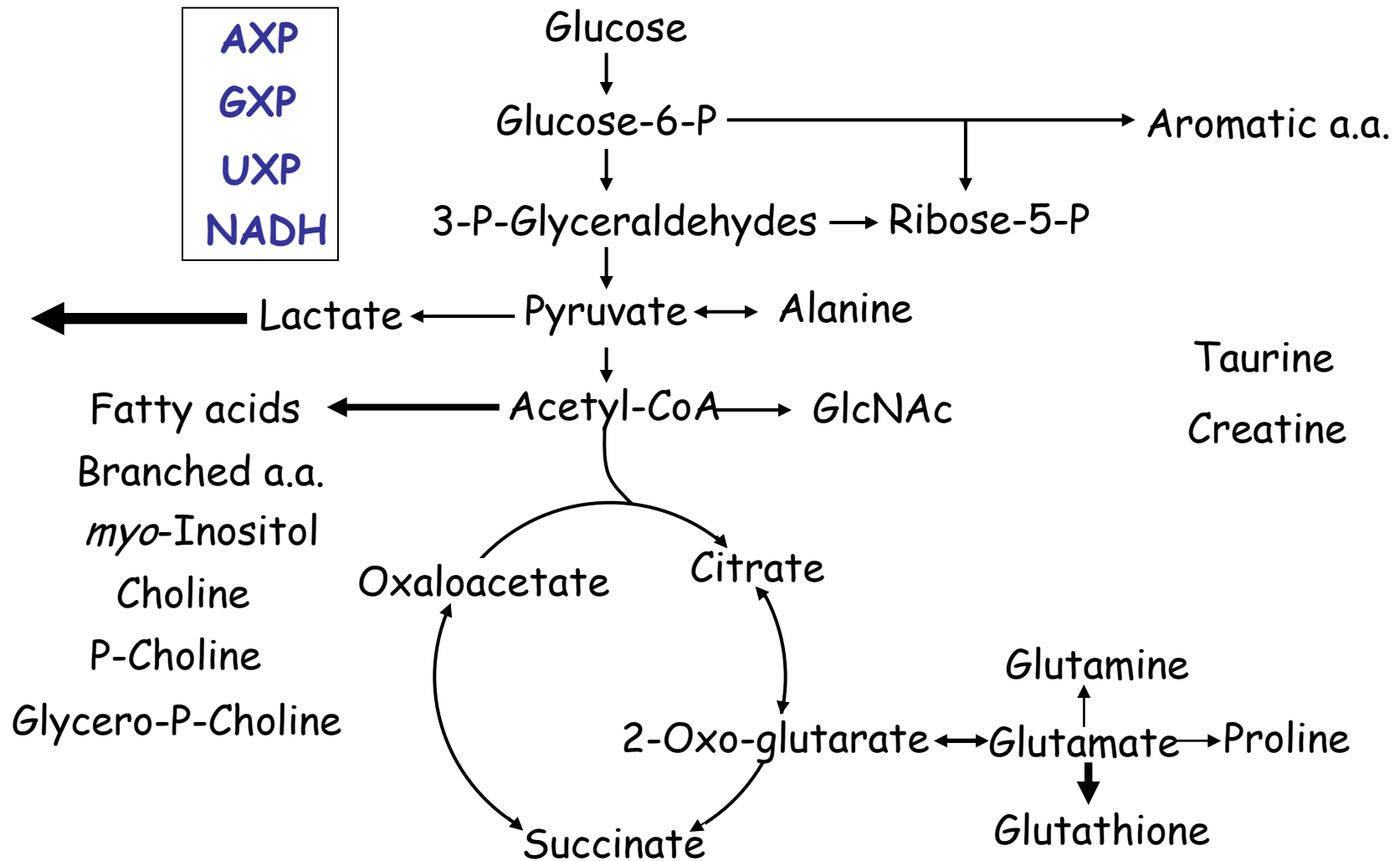
# HSQC of MCF-7 Intracellular Extract



# T-test Filtered Difference Spectra

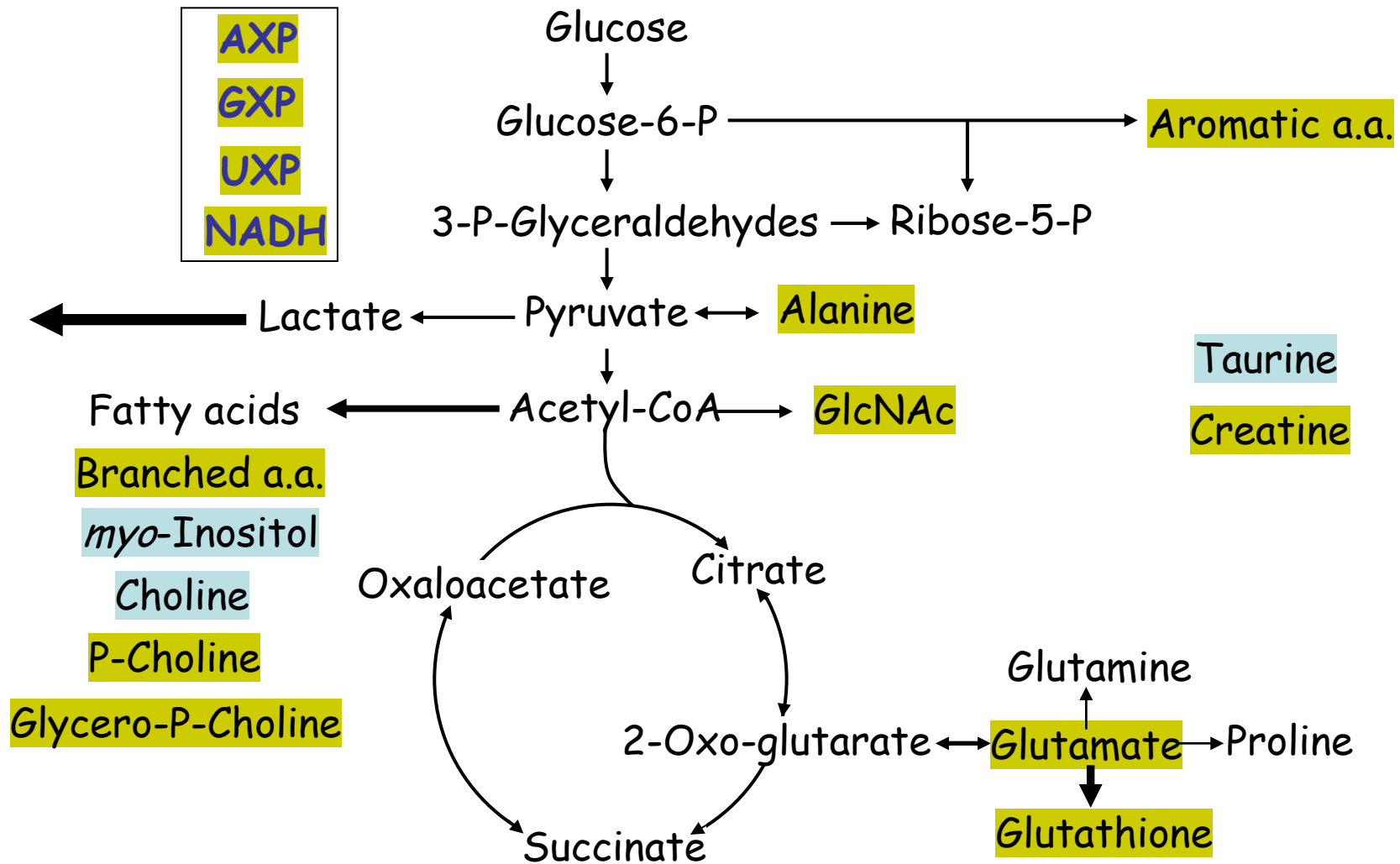


# Metabolic Profile Changes of MCF-7 Cells





# Metabolic Profile Changes of MCF-7 Cells



## Conclusions

- The direct cell quenching method is rapid and effective, with a recovery rate 50 fold higher than the conventional method, which makes it possible to use cell lines for metabolomics.
- Cell culture based metabolomics offers the ability to obtain biochemical information rapidly and at relatively low cost (per sample).
- Use of cell culture based metabolomics provides an excellent platform for studying cellular responses to chemical exposure(s) and for testing toxicity.

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