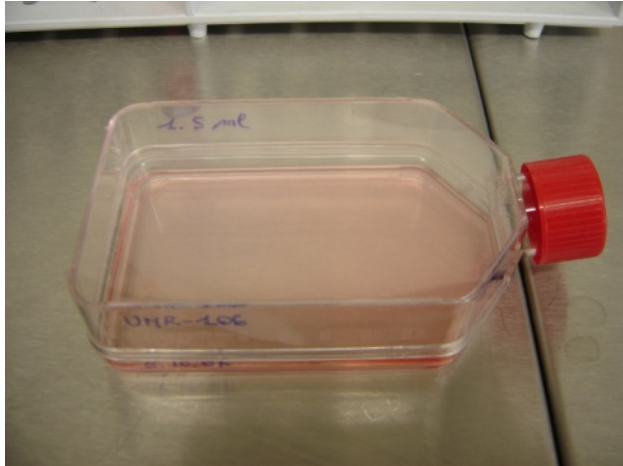


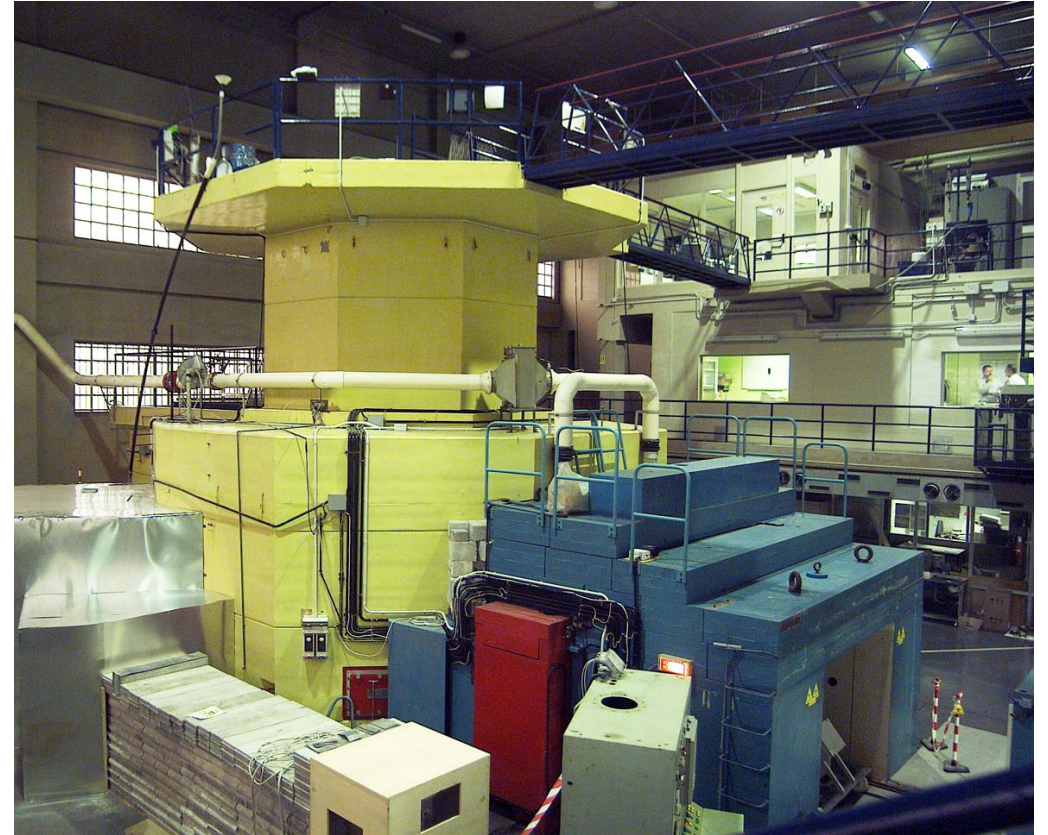
# Boron Concentration Measurements in cells by *neutron autoradiography*



1

Cells are exposed to **B in medium** for 4 hours

$$1 \text{ ppm of } B = \frac{\mu\text{g of boron}}{\text{g of medium}}$$



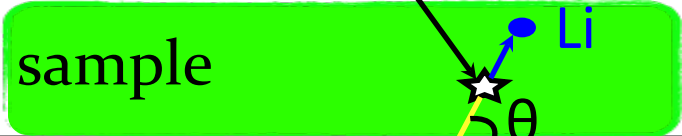
2



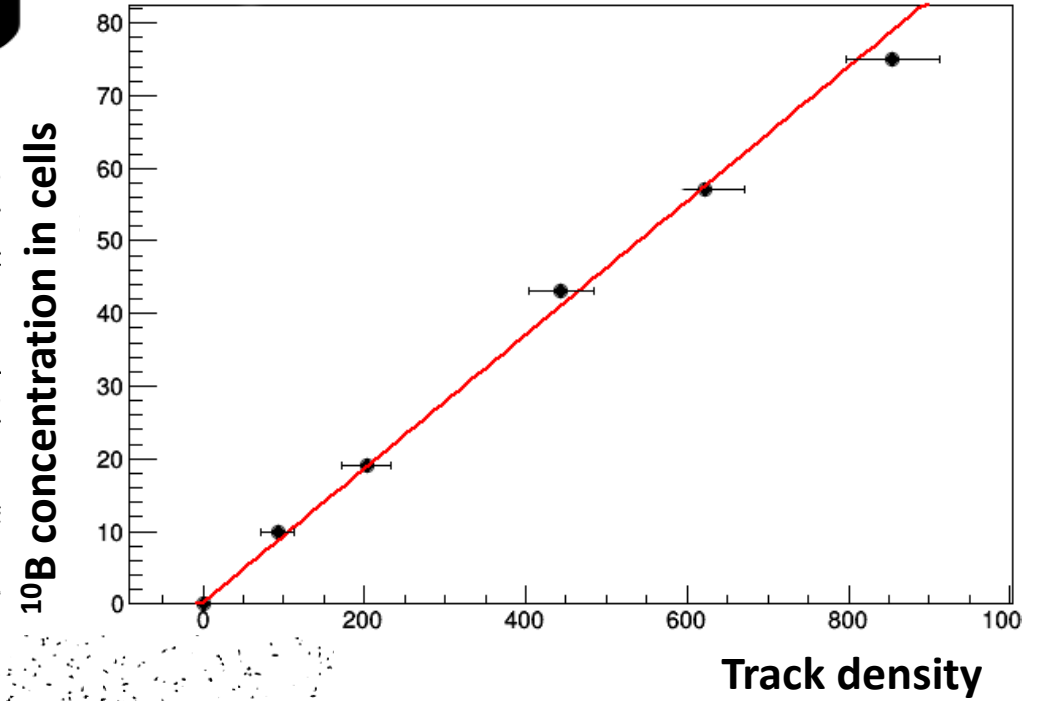
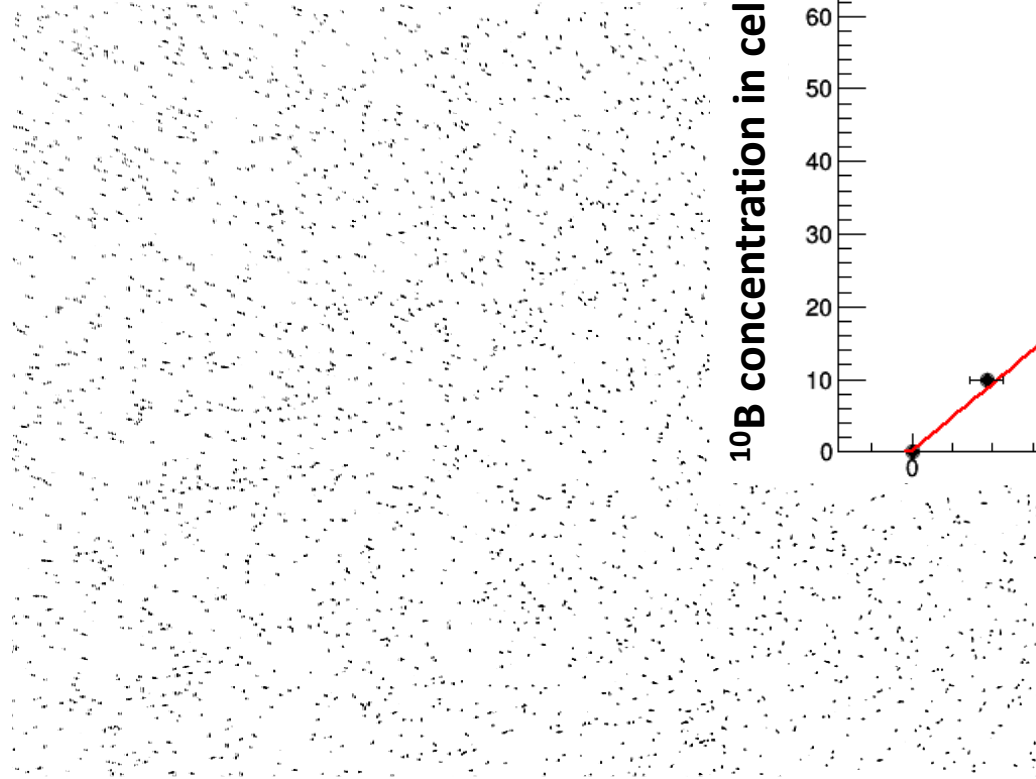
Cells are washed and deposited on a sensitive film

3

Films are irradiated with thermal neutrons; charged particles create latent tracks in film



CR-39 film



4 Films are chemically etched to enlarge tracks

5 Tracks are counted

6  $^{10}\text{B}$  concentration is inferred from previous calibration

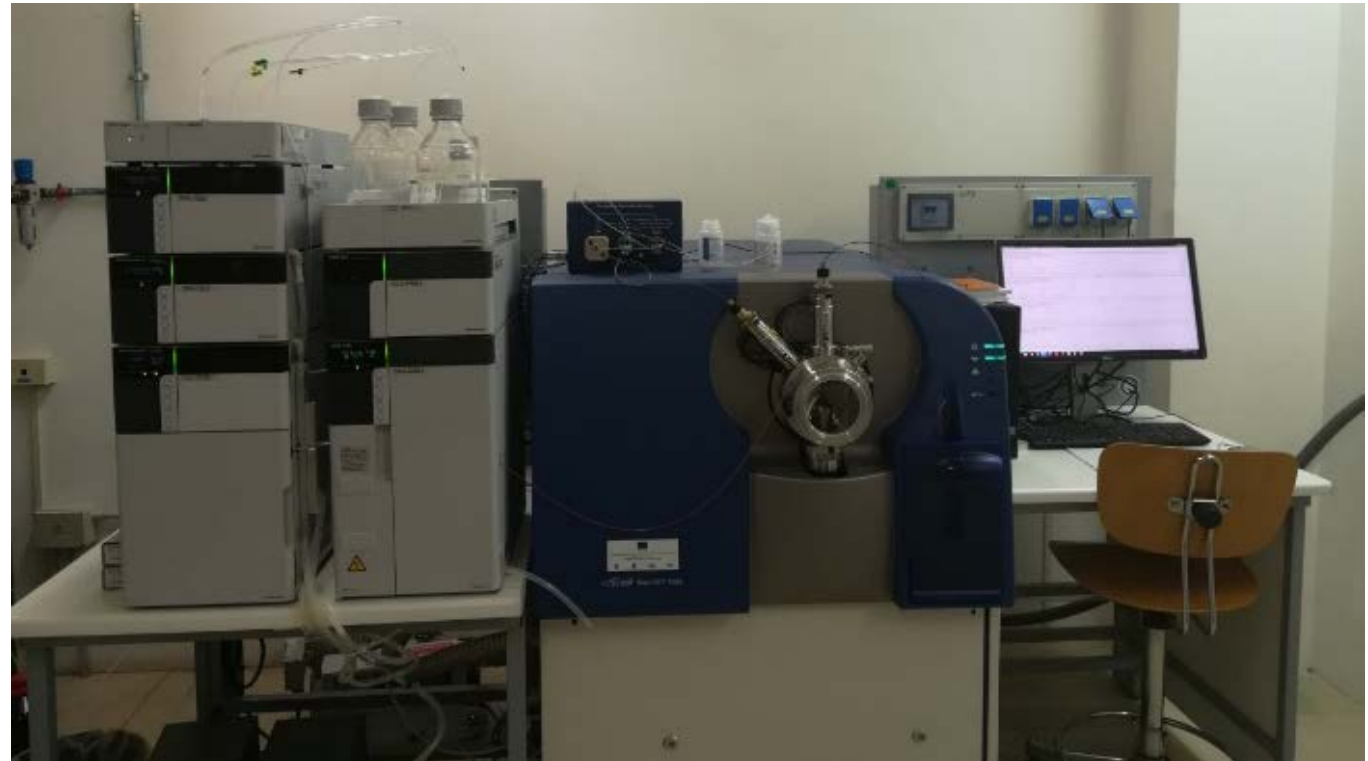
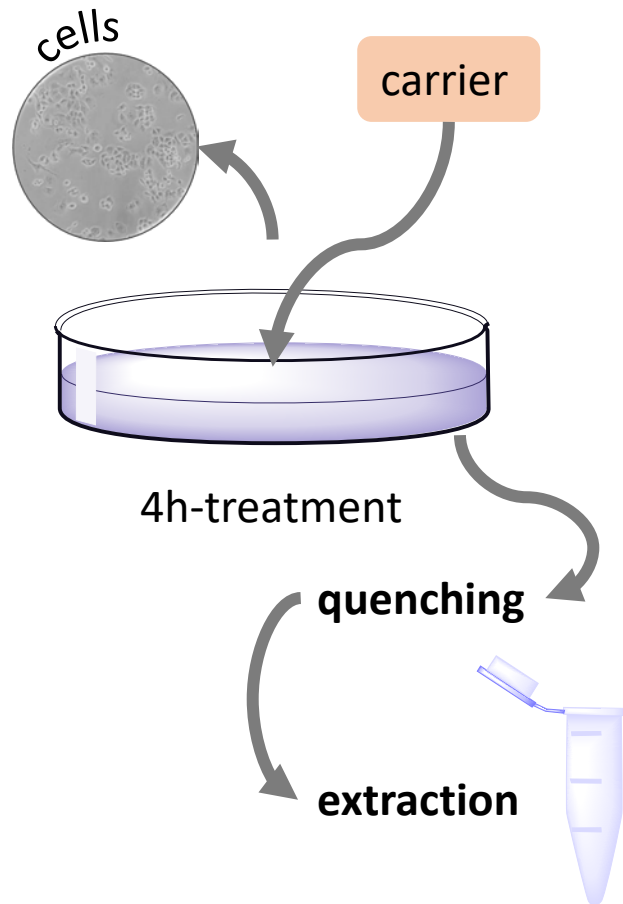
$$1 \text{ ppm of } B = \frac{\mu\text{g of boron}}{\text{g of cells}}$$

# IMPORTANT!

- The technique DETECTS ONLY  $^{10}\text{B}$ . To estimate  $^{11}\text{B}$ , we normalize for the isotopic composition. If BPA is 100% enriched in  $^{11}\text{B}$ , neutron autoradiography with neutrons cannot give information. However, one can use BPA enriched in  $^{10}\text{B}$  and measure the amount of boron internalized.
- In the same way, to measure F concentration, the molecule needs to contain  $^{10}\text{B}$ , too. Molecules containing only F cannot be measured with this technique
- The technique has been optimized to detect internalized boron only, for this reason, cells are washed before measurement.
- Adjusting the neutron fluence and the etching parameters, neutron autoradiography allows an imaging of boron distribution in the cell pellet, giving information on the availability of boron to every cell at the moment of irradiation.

# Boron/Fluorine Concentration Measurement in Cells by UHPLC-ESI-QqTOF-MS/MS- and UPLC-UV-DAD-based METABOLOMICS

**GOALS** low-molecular weight compounds in a biological sample

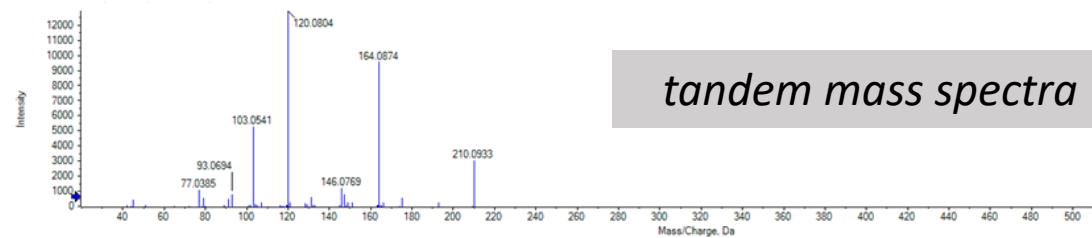
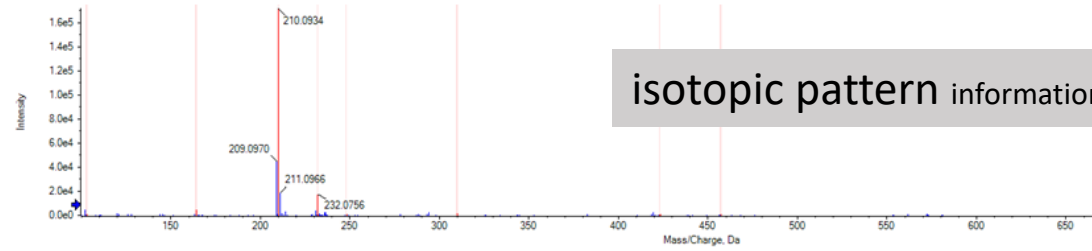
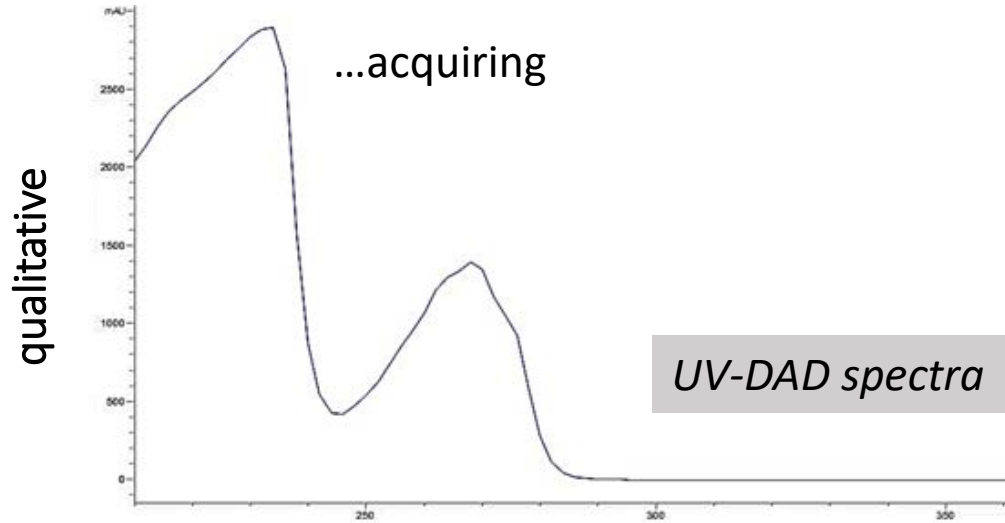


UHPLC-ESI-QqTOF-MS/MS and UPLC-UV-DAD analyses

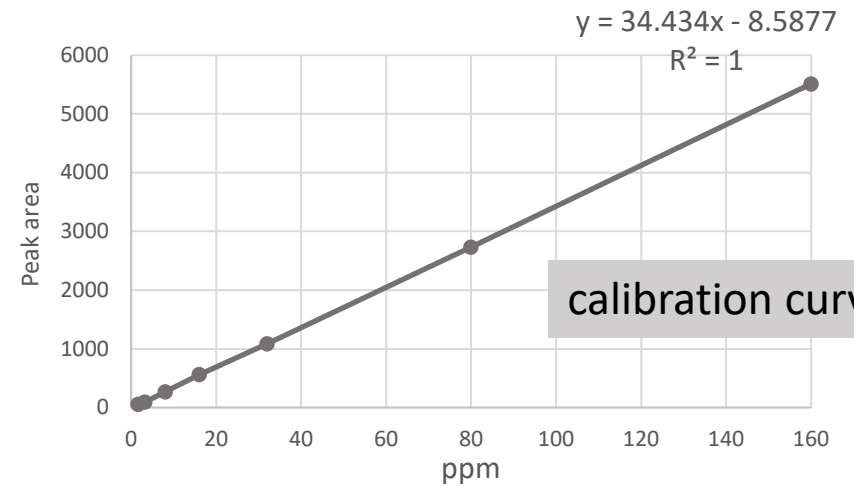
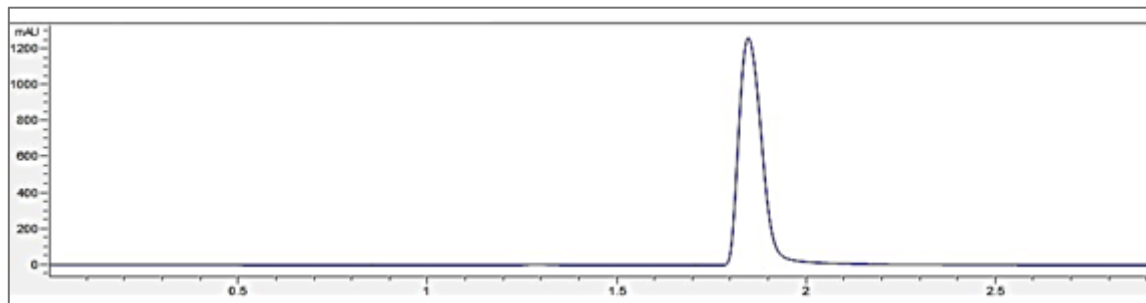


carrier

UHPLC-ESI-QqTOF-MS/MS analysis  
UPLC-UV-DAD analysis

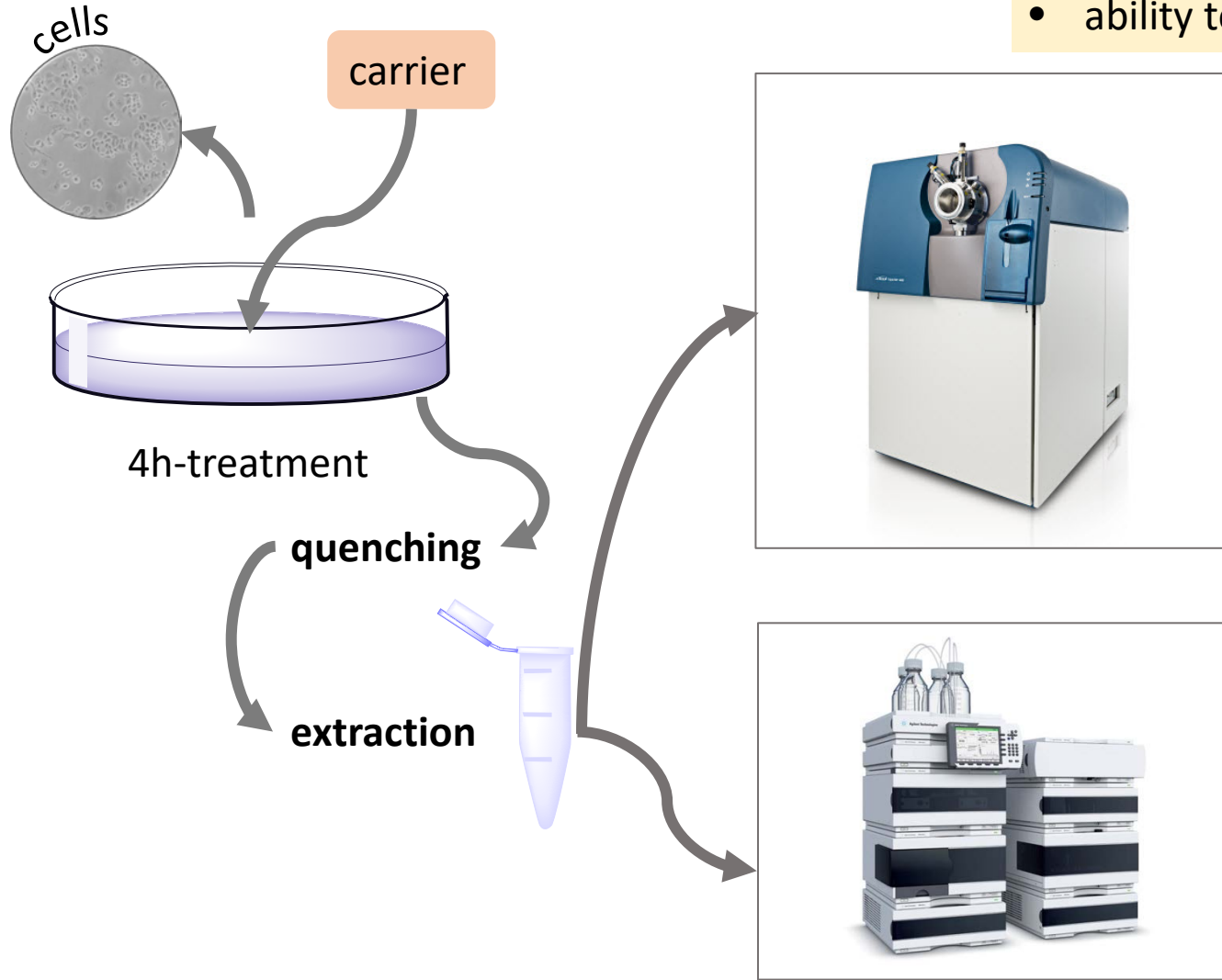


quantitative

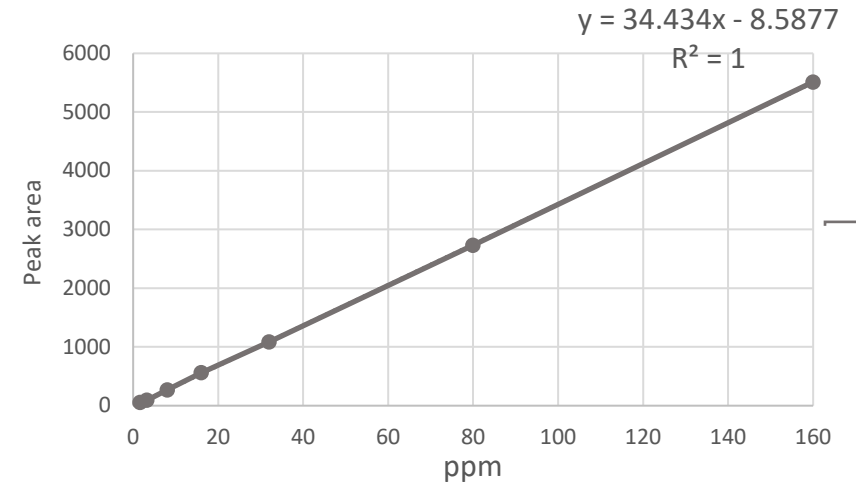


*take note*

- ability to estimate both  $^{11}\text{B}$  and  $^{10}\text{B}$
- ability to estimate fluorine in presence or absence of boron



*recognition*



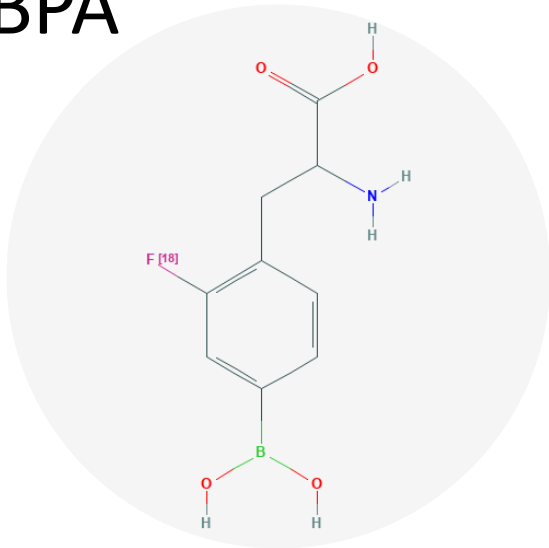
*quantification*

Treatment dose ( $^{11}\text{B}$ ppm)	Inside ( $^{11}\text{B}$ ppm)	Outside ( $^{11}\text{B}$ ppm)
120	34.6±0.83	59.2±0.24

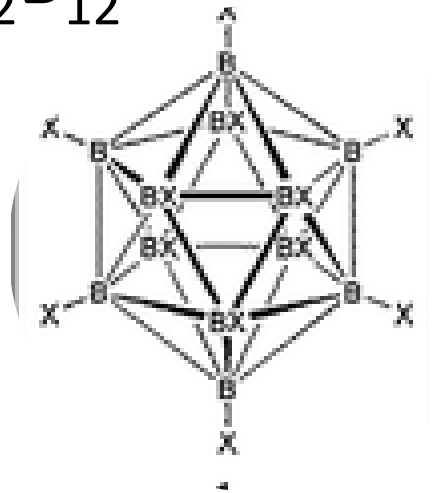
# Internalization measurements with MRS

Measure fraction internalized by looking to F-peak in magnetic resonance spectroscopy

F-BPA



$F_{12}B_{12}$



- 13.6 mM per 4h on PANC
- $C_{\text{internalized}} \sim 0.5 C_{\text{external}}$
- Qualitative agreement with previous results

- 1.2 mM per 22 h **not internalized**
- Toxicity at 13.6 mM (12xF)

