Analysis of Tissue Biopsies by PCT-SWATH

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B/D-HPP cancer

The research gap

- **The challenges for analyzing tissue biopsies:**
  - Small amount
  - Measure proteome
  - Large number of tissues

- **Ideal method:**
  - Minimal requirement of tissues
  - Simple and robust protocol
  - Proteomic scale
  - Quantitatively reproducible

Our solution

PCT-SWATH

Pressure cycling technology (PCT)-assisted tissue lysis and digestion

tissue biopsy
~1mg

All procedures
in single tube

Batch processing
Minimize variations

Pressure cycling technology
fast and efficient digestion

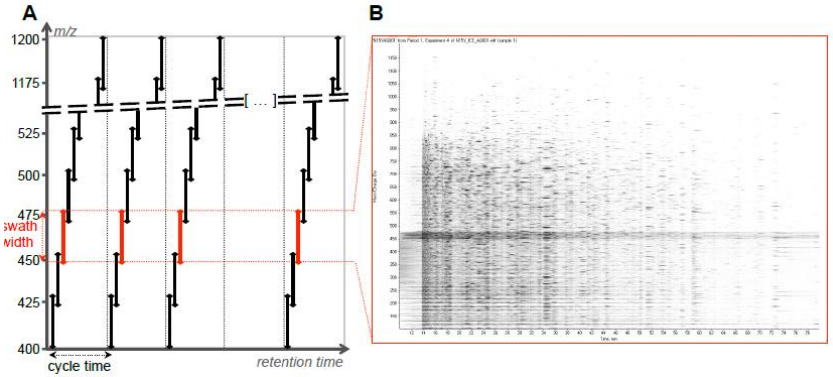
Yield:
~50 ug
peptides per
1 mg wet
tissue

2ug peptides
sufficient for
a swath
analysis

6~8 hrs to convert 6 pieces of tissues into clean peptides that are ready for MS analysis

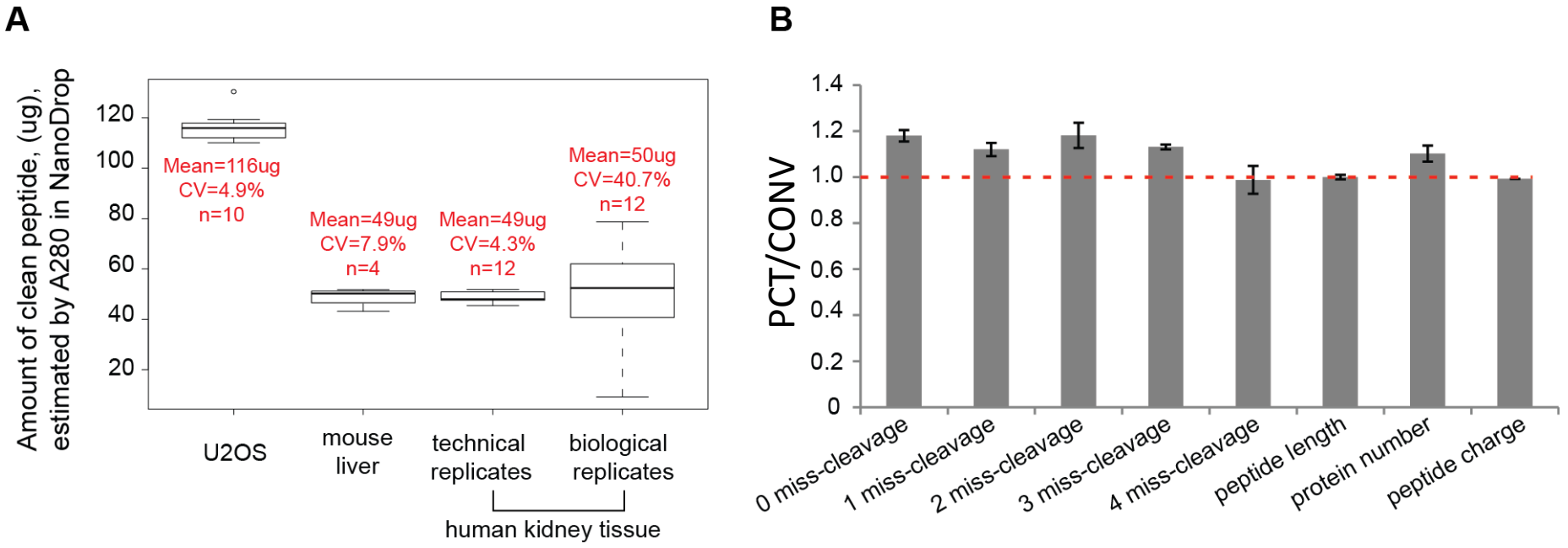
<12hr

Proteomic analysis using SWATH-MS



Gillet, et al. 2012.

PCT-assisted tissue lysis and digestion: Yield and digestion efficiency

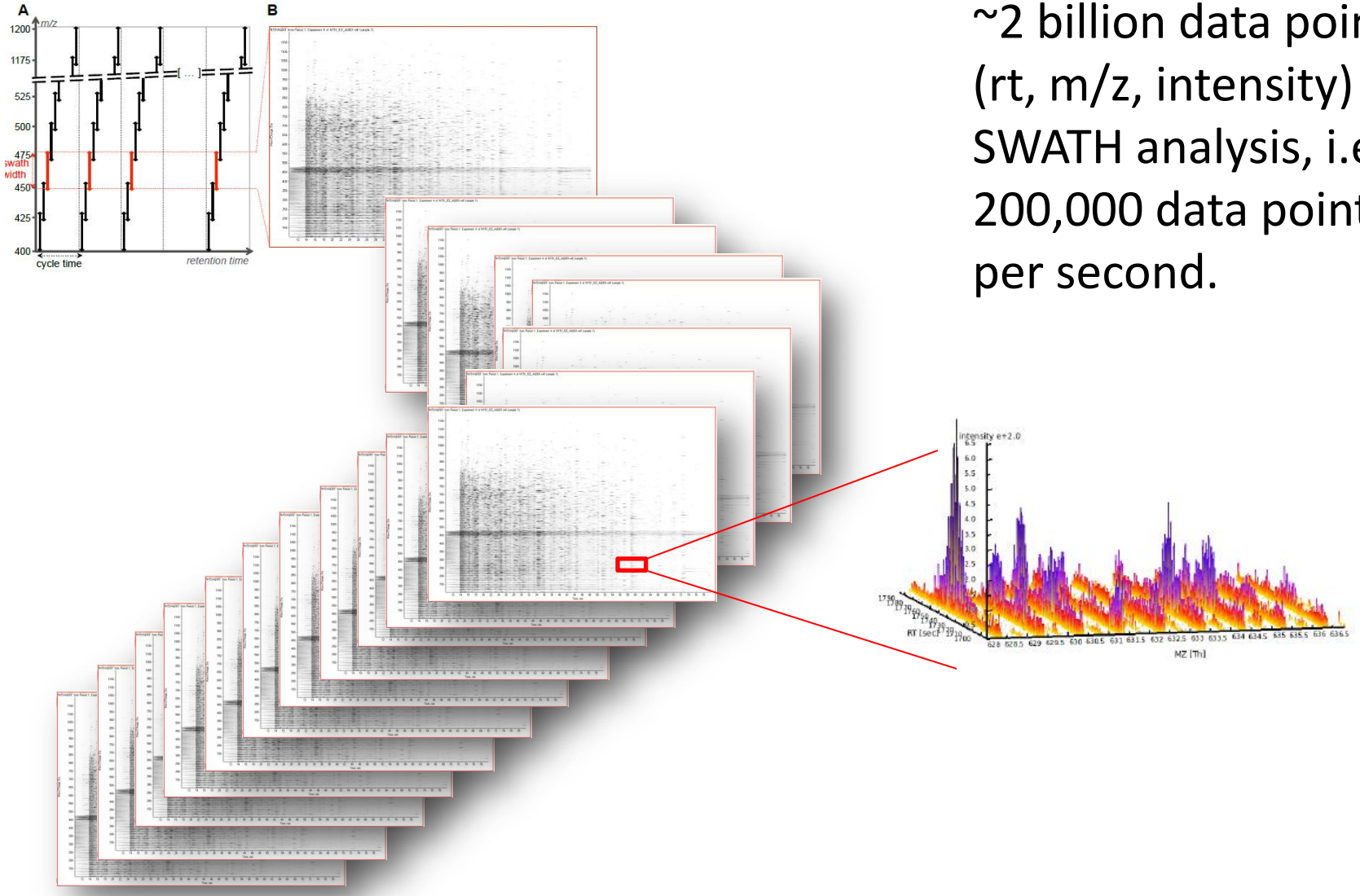


SWATH-MS

- Single-shot (~2hr gradient) proteomic analysis
- Permanent record of all ionized peptides: ideal for integrative OMICS analysis
- Highly reproducible: comparable to SRM (Liu, Y, et al. Proteomics. 2012.13:1247)

SWATH results: big data

Every SWATH analysis generates data sets for 32 swath windows and MS1



~2 billion data points (rt, m/z, intensity) per SWATH analysis, i.e. 200,000 data points per second.

Performance of PCT-SWATH in analyzing human kidney tissues

AB SCIEX

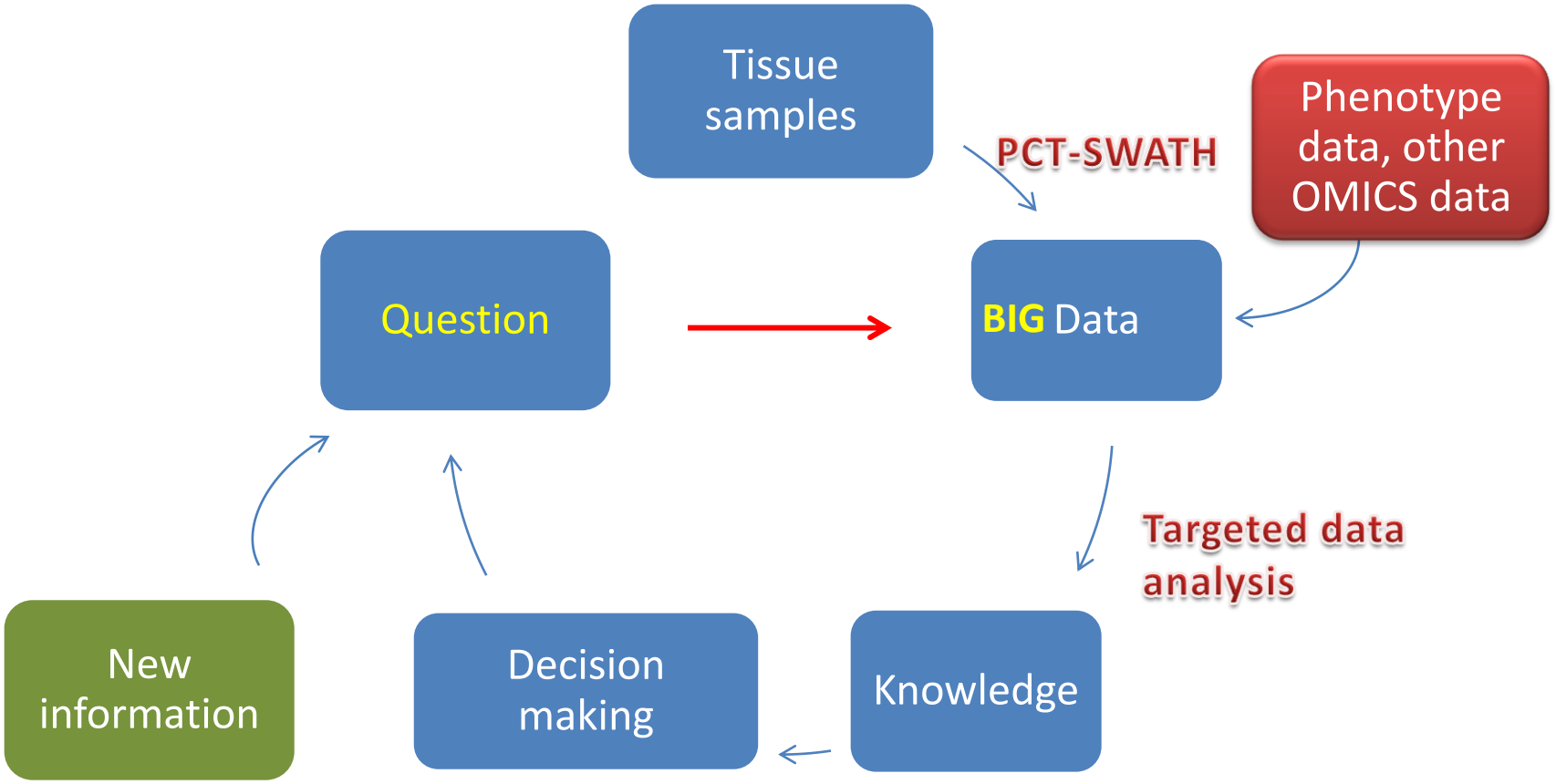
Experimental design:

- **Spectral library (**5893 protein groups)
  - ➢ 18 human kidney tissues, DDA analysis
  - ➢ Pool of 18 human kidney tissues, 1D-gel separation, DDA analysis
  - ➢ spiked with iRT peptides (Biognosis)

- **PCT-SWATH**
  - ➢ A test human kidney tissue: processed by PCT-SWATH for 12 times
  - ➢ 18 human kidney tissues from 9 renal cell carcinoma patients (control versus tumor tissues from each patient)

- **Peptide/protein identification and quantification using OpenSWATH analysis (**www.**openswath**.org**)**

Conclusion

- PCT-SWATH is a practical method to analyze proteome of biopsy tissues
- Benefits
  - Fast
  - Small amount of tissues
  - Reproducible
  - Correctly classifies tissues
  - Applicable to large number of samples
  - Permanent digital records of tissue proteome

New paradigm for protein biomarker discovery