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Targeted profiling of inflammation related lipid mediators in biofluids by ultra-performance liquid chromatography – triple quadrupole mass spectrometry

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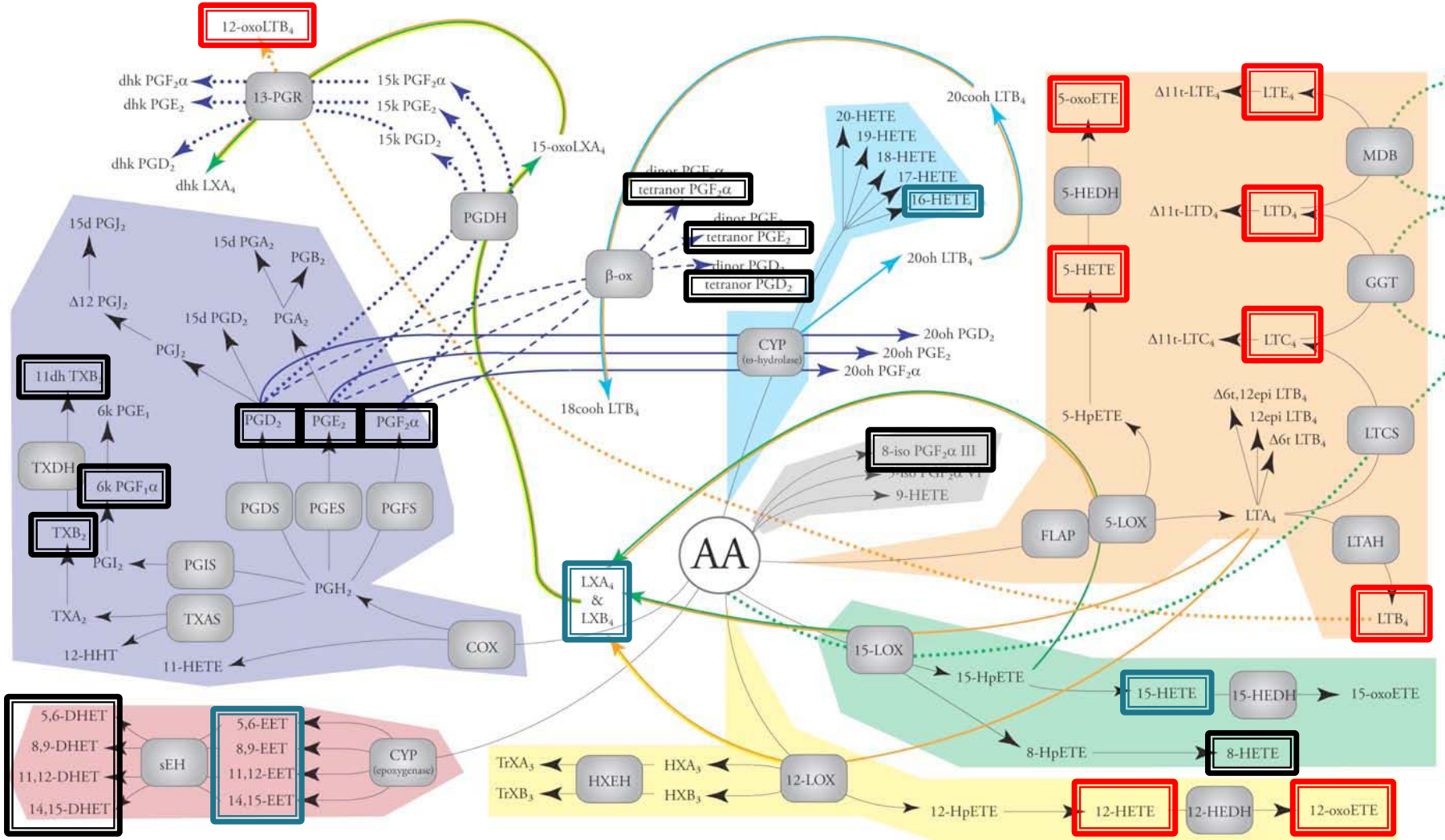
Inflammation

- Acute *vs* Chronic
  - Acute: sepsis, septic chock
  - Chronic: obesity, type 2 diabetes, atherosclerosis, Parkinson's disease, Alzheimer's disease

- Partners involved
  - Cytokines, chemokines
    - IL-1, IL-4, IL-6, IL-10, TNF-α, CRP...
  - Eicosanoids-Docosanoids
    - Structural properties
      - Oxidised metabolites of free fatty acids, mainly C20:4 and C22:6
      - Enzymatic (COX, LOX, CYP)/Chemical origin
    - Biological properties
      - Pro-inflammatory or Anti-inflammatory
      - Oxidative stress biomarkers

Metabolism of arachidonic acid



Adapted from Buczinski *et al, J. Lipid Res.*, 2009

Pro-inflammatory

Anti-inflammatory

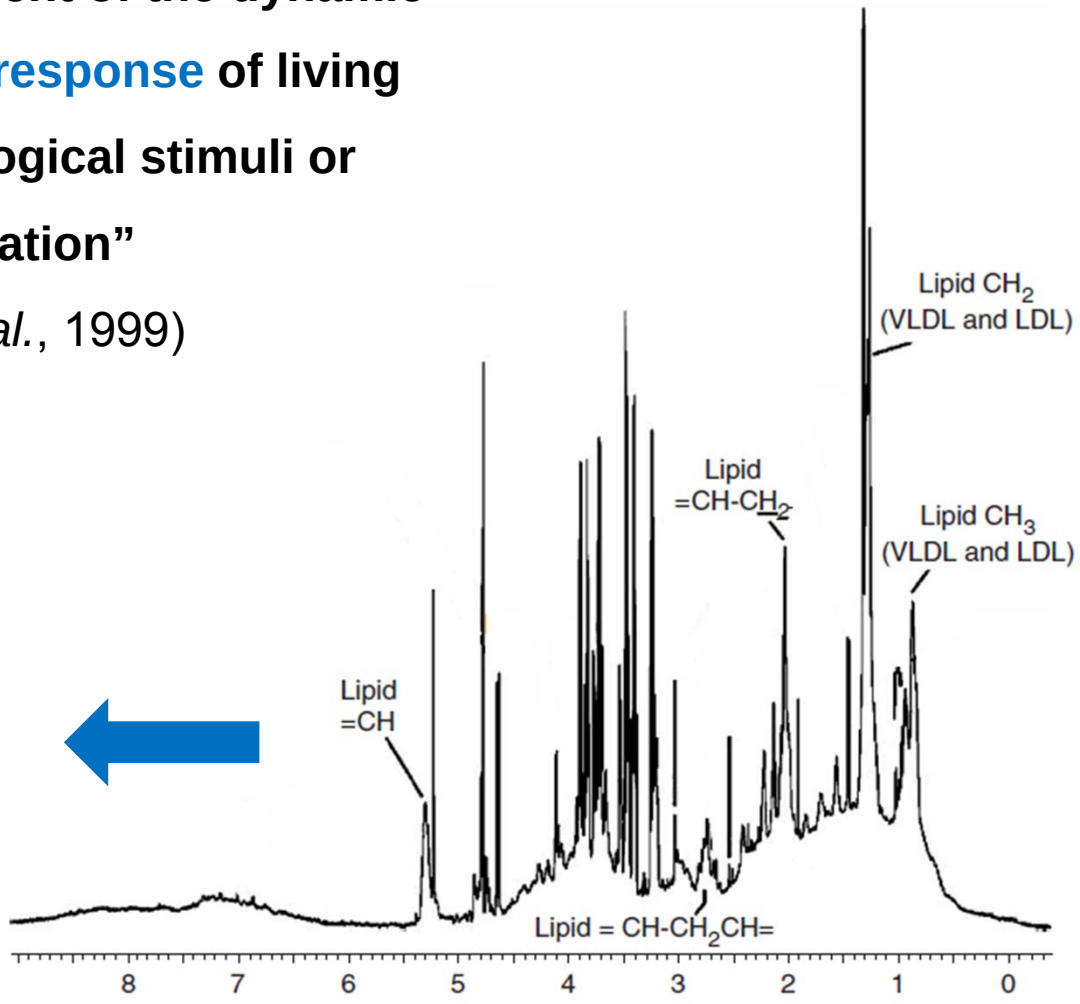
Others

NMR-based metabonomics

"The quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification"

(Nicholson J.K. *et al.*, 1999)

Alternatives to NMR for lipid analysis?



Beckonert *et al, Nat. Protoc.*, 2007

LC/MS-based metabonomics

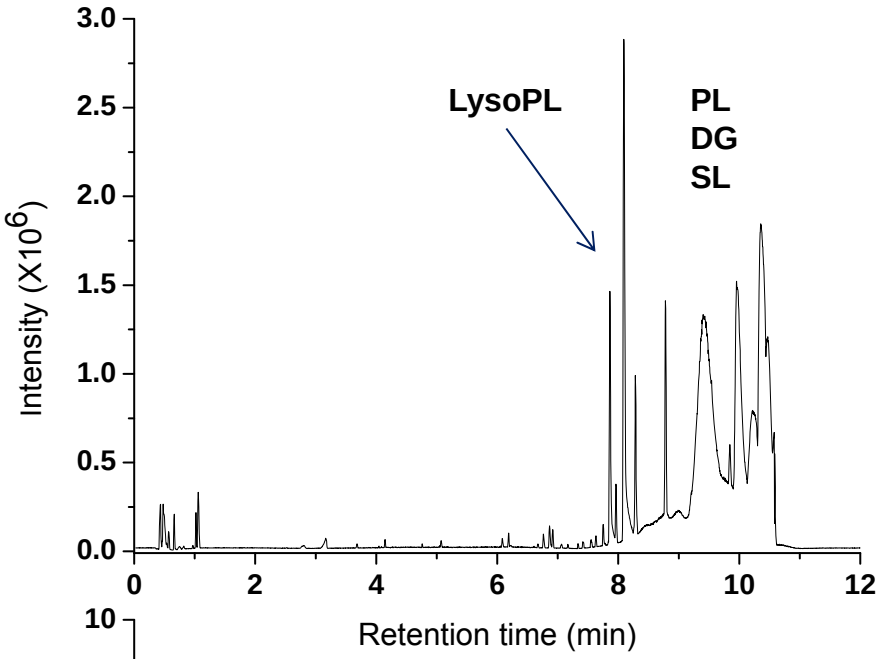
MS-based untargeted approaches

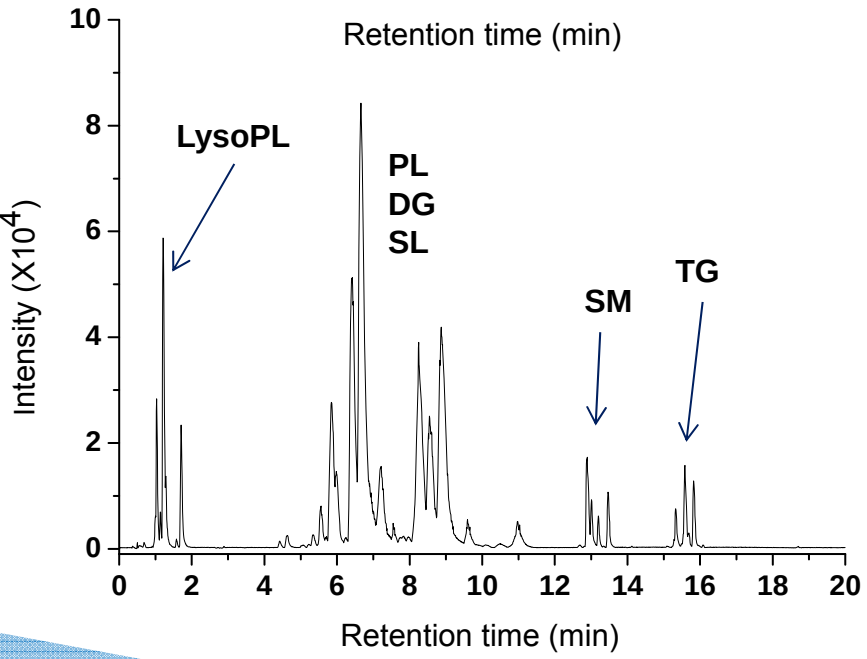
hydrophilic

Reversed-Phase LC or HILIC

hydrophobic

Dedicated Lipid Profiling method





From untargeted to targeted…

None of these methods untargeted methods is suitable for eicosanoid analysis

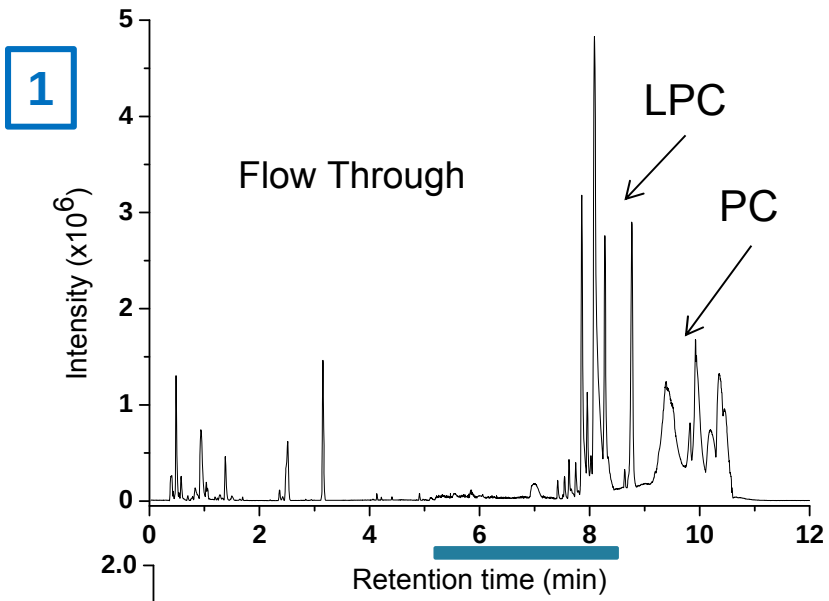
Selectivity issue
Isomers

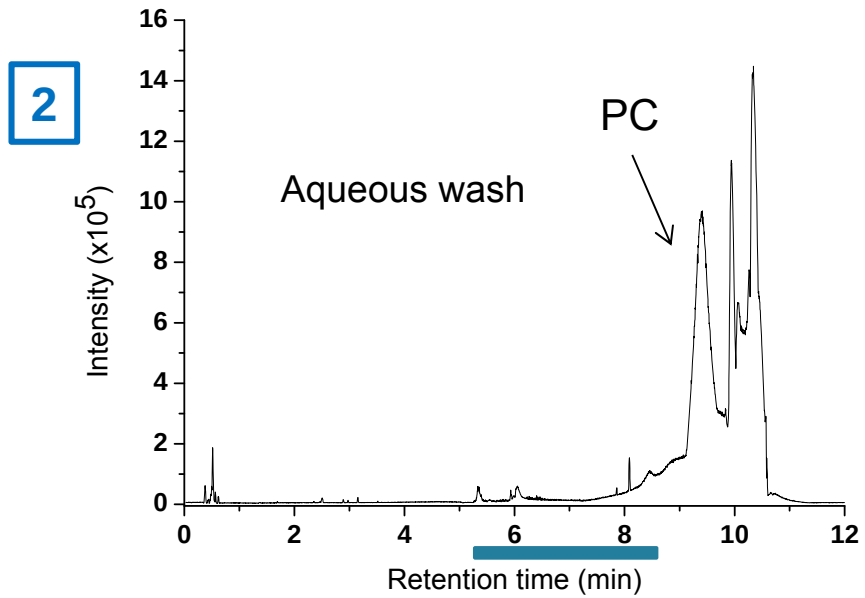
- Selective sample clean-up
- Optimised UPLC separation

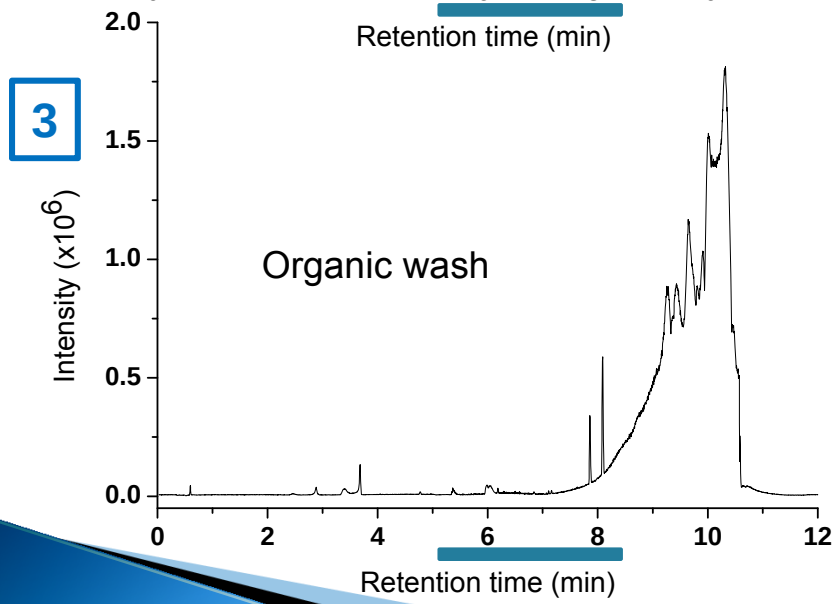
Sensitivity issue
Trace level in biofluids

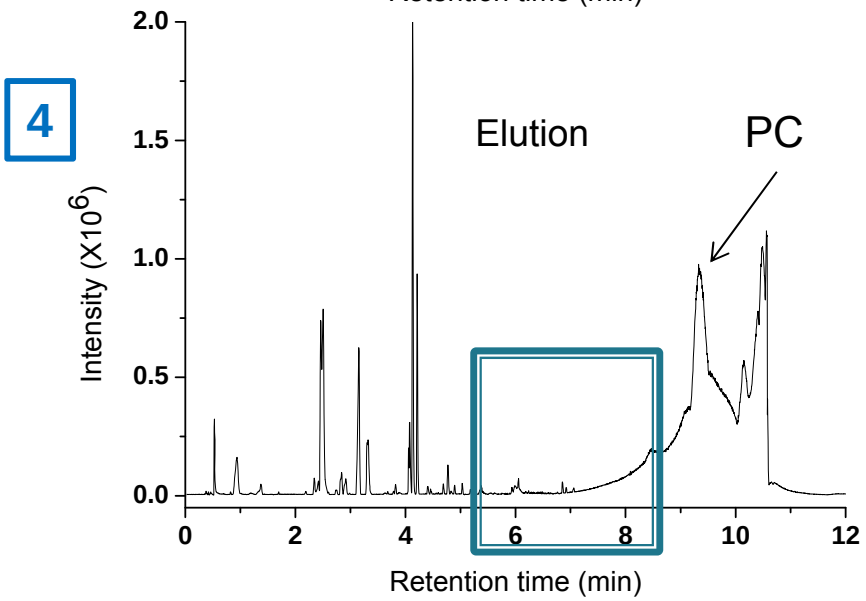
- Sample concentration
- Triple quadrupole mass spectrometer

Sample preparation: Anion exchange SPE









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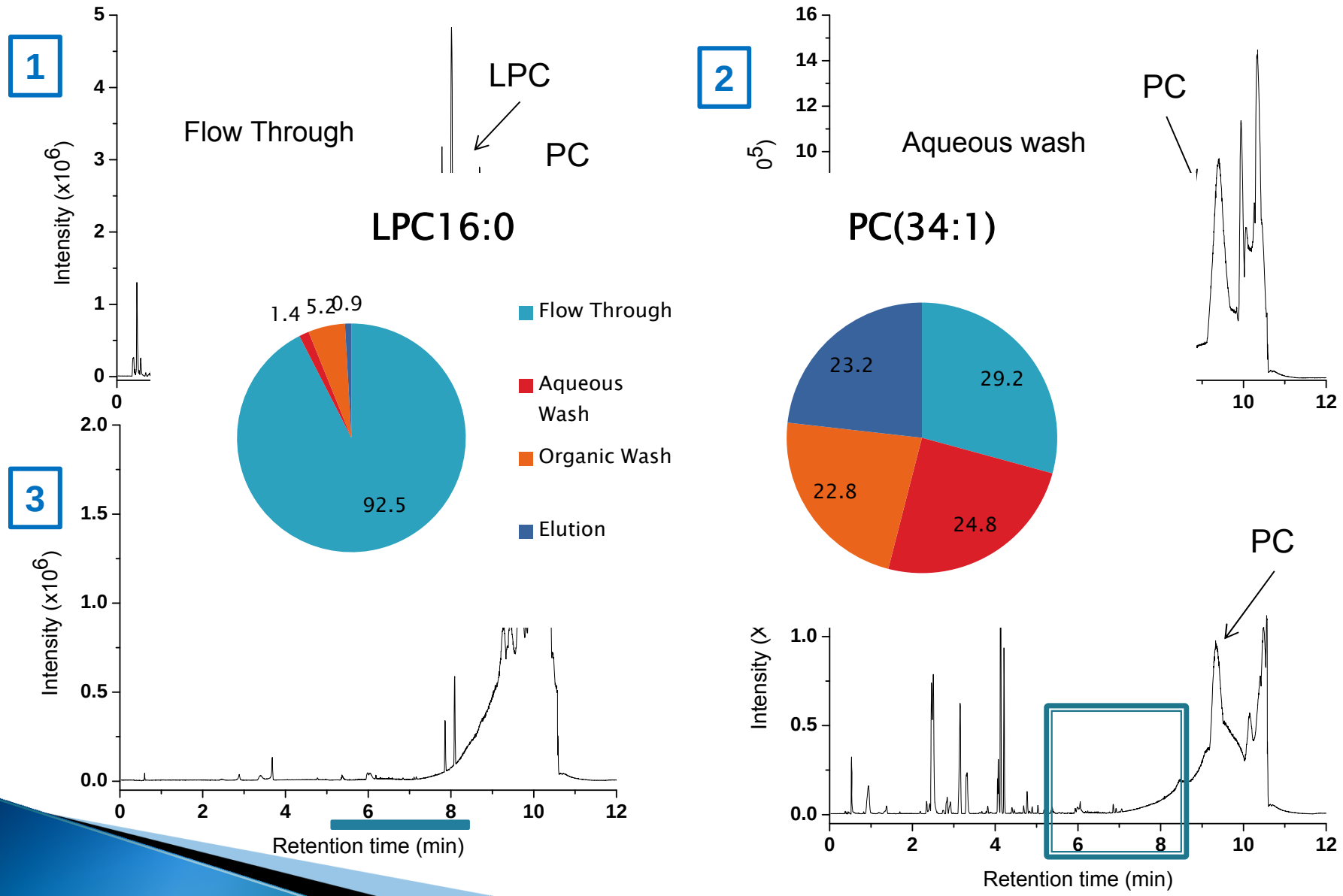
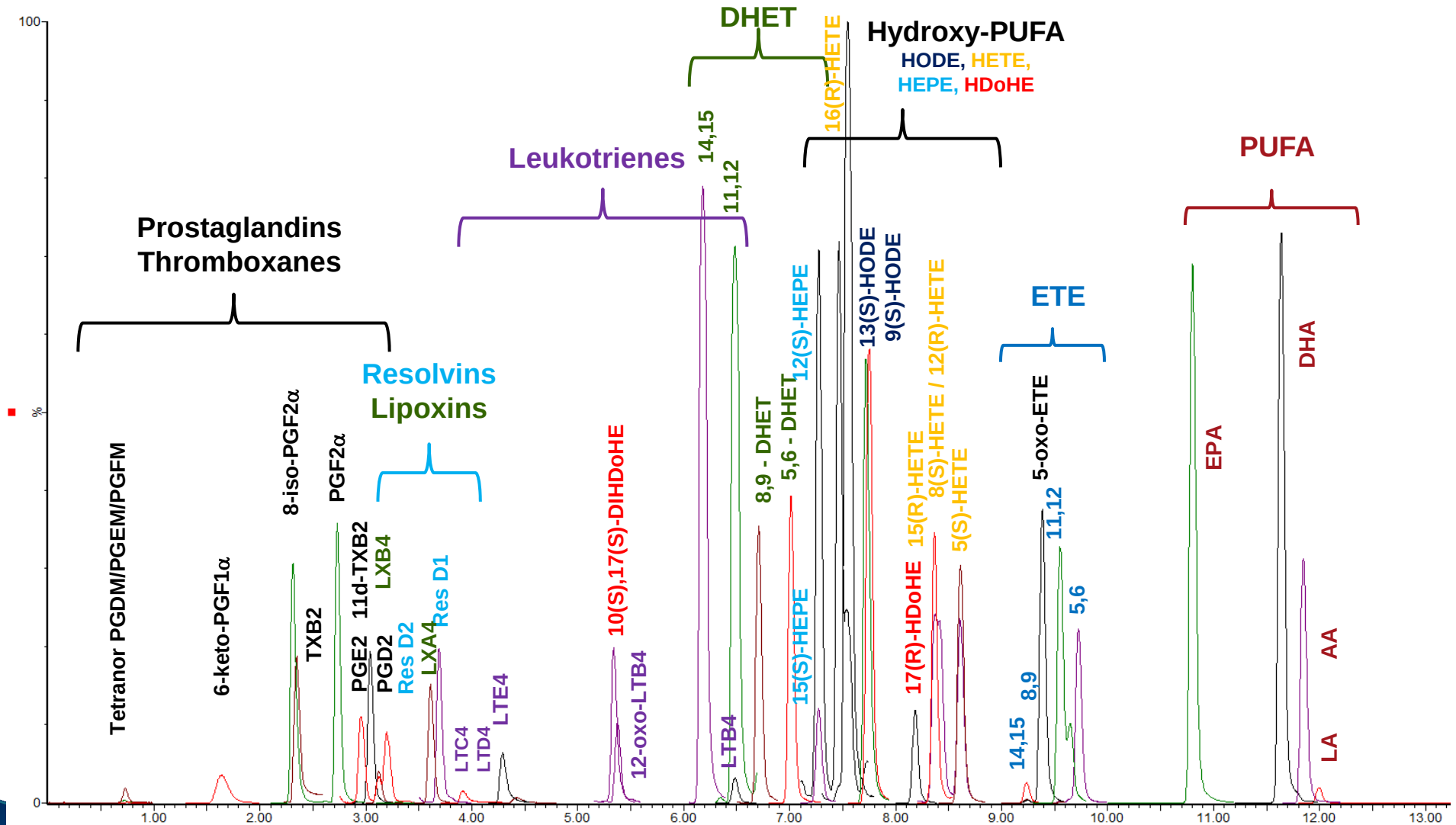
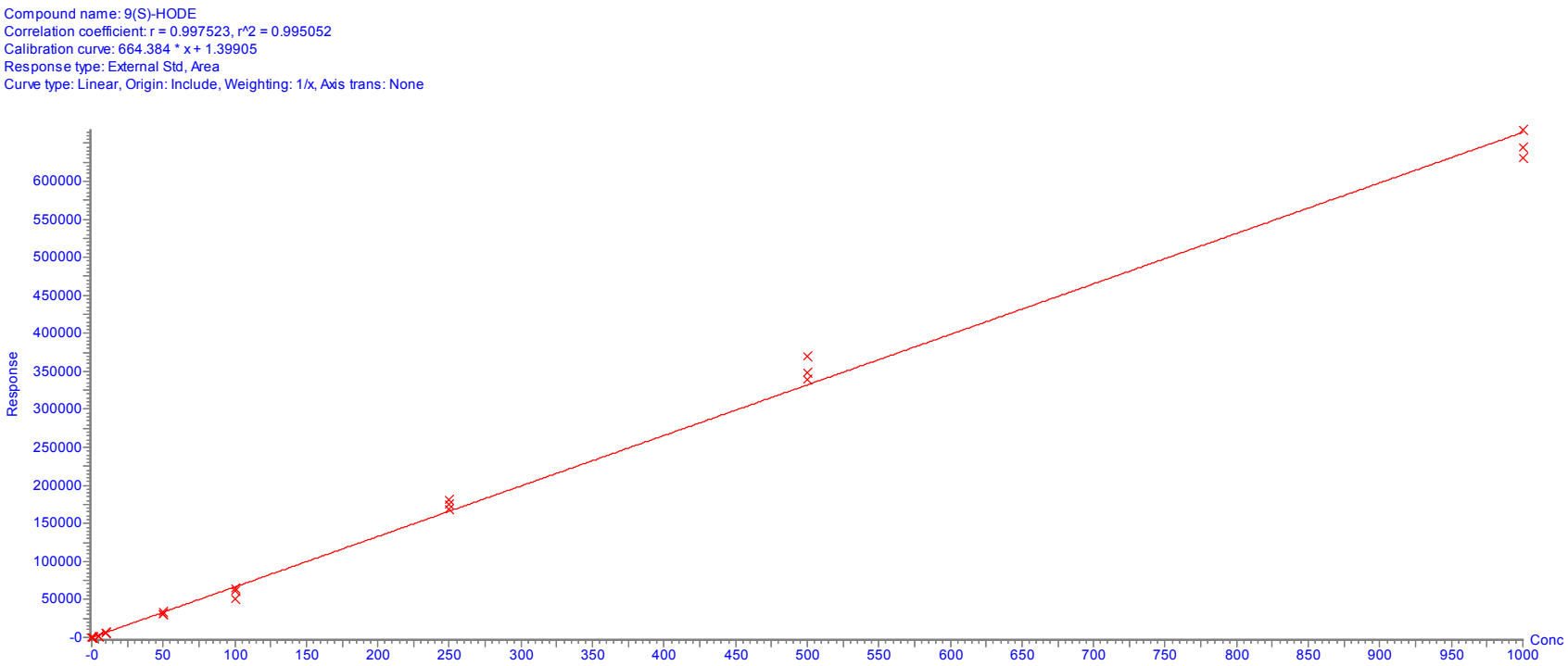


UPLC-MS/MS analysis



Validation features



	Curve
Linearity (R^2)	0.9848–0.9955
LOQ (pg/µL)	5–10

	High QC (500 pg/µL)
Recovery (%)	95% (PG)
Precision (%)	<14.6 ; <19.2 (LOQ)
Accuracy (%)	85.6 – 115.9

How to improve the surgical patient journey ?

A reality:

-operative theatres are largely free of chemical diagnostic information
- traditionnal clinical risk scores or clinical biochemistry lack sensitivity and/or specificity to predict patient outcome

Can metabolic phenotyping predict patients who are likely to have poor outcome after surgical intervention ?

Before intervention

Diagnosis
Prognosis
Risk stratification
Preoperative optimisation

During intervention

Diagnosis
Prognosis
Risk stratification
Real-time functional histology

After intervention

Post-operative care optimisation

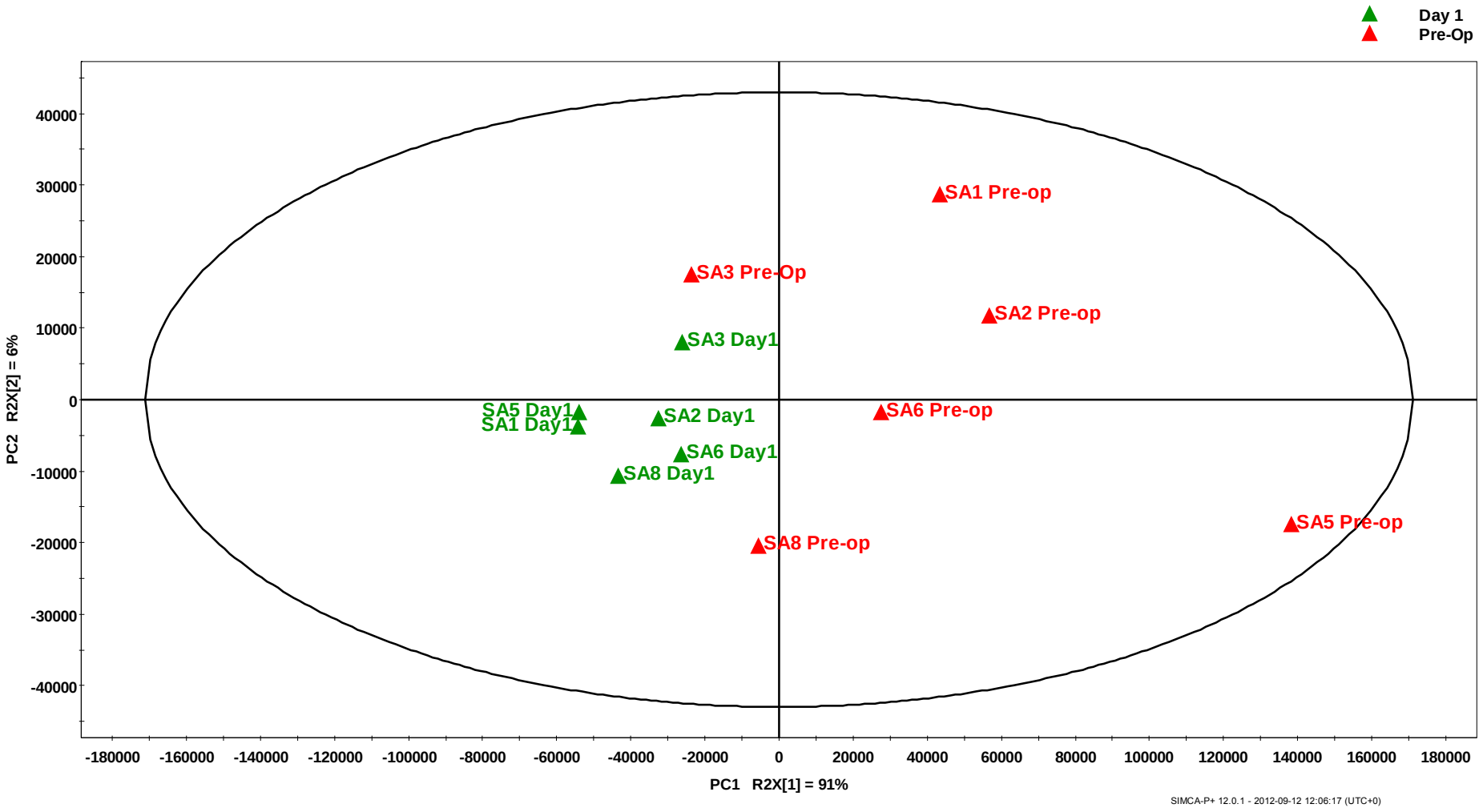
NMR and UPLC/MS of biofluids
MAS-NMR of biopsies
iKnife for real-time tissue classification

Longitudinal phenotyping of colorectal surgical patients

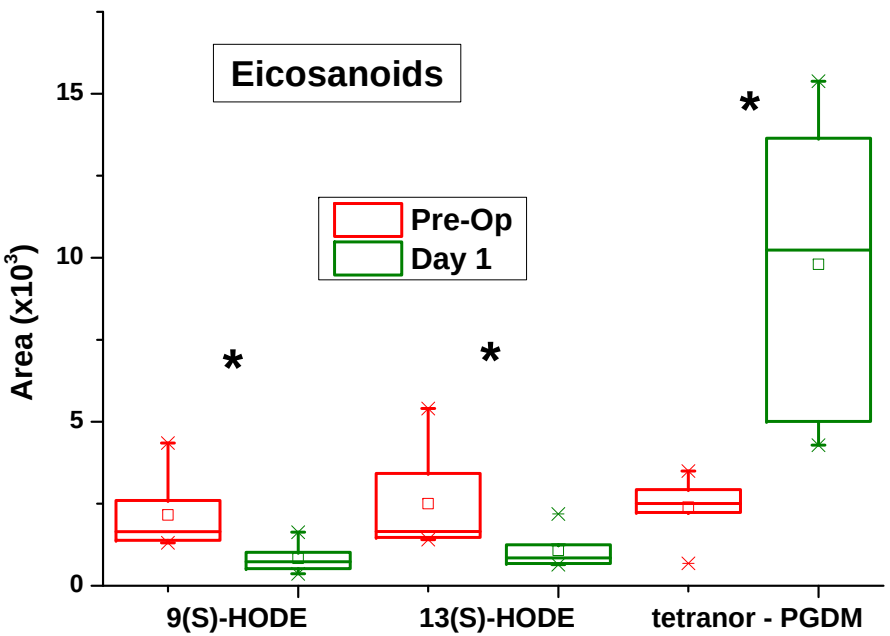
- Experimental design
  - Serum from 23 patients
  - Open *vs* laparoscopic surgery
  - Pre-operative sampling + 5 post-op time points

- Preliminary study
  - 3 Open vs 3 Lap
  - Pre-op vs Day+1

Longitudinal phenotyping of colorectal surgical patients

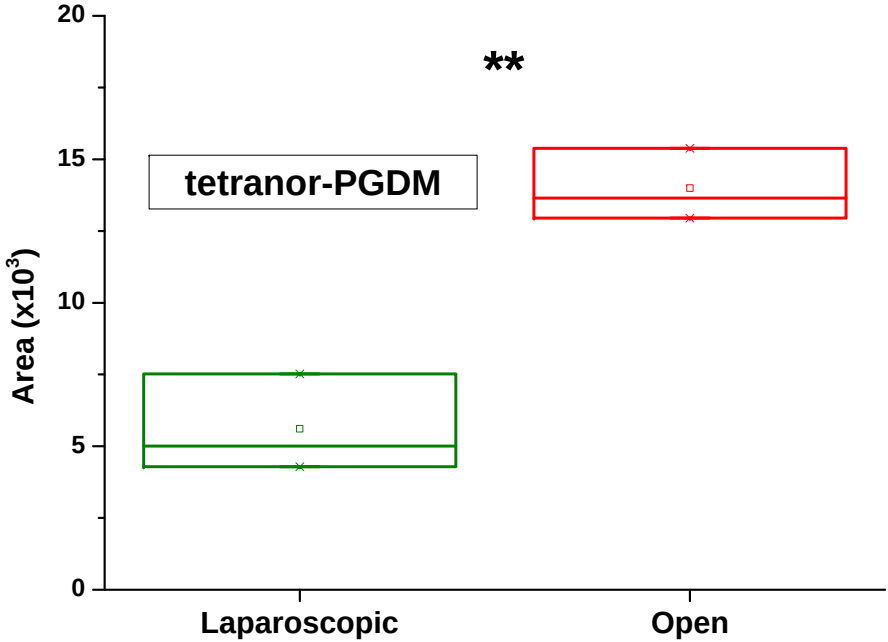


Longitudinal phenotyping of colorectal surgical patients



*: p<0.05 two-tailed unpaired t-test

Decrease in fibrinolysis *via* PPARγ activation
Increase in COX mediated pro-inflammatory signalling pathways



**: p<0.05 two-tailed unpaired t-test

Inflammatory response less pronounced in laparoscopic surgery

Conclusion and future works

- **Conclusion**
  - Method in compliance with initial goals (selectivity, sensitivity)
  - Good complementarity with untargeted profiling

- **Future works**
  - Further method validation
    - Long-term stability
    - Other matrices (plasma, urine, tissue extracts, cell cultures)
  - Analysis of serum samples from the whole cohort of colorectal surgical patients
  - Correlation with clinical data and cytokine profiling

Acknowlegments

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Thank you for your attention