

# MALDI-2-MS, chemical derivatization and Ion Mobility on the timsTOF-flex-MS for enhanced MSI to assess Vit-D metabolism and androgen intracrinology

Diego Cobice<sup>1</sup>, Jens Soltwisch<sup>2-3</sup>, Bram Heijts<sup>2</sup>, Karl Smith<sup>4</sup>, Annika Koch<sup>5</sup>, Klaus Dreisewerd<sup>2-3</sup> and C. Logan Mackay<sup>6\*</sup>,

<sup>1</sup>Mass Spectrometry Centre, Ulster University, Coleraine, Northern Ireland, UK · <sup>2</sup>Institute of Hygiene, University of Muenster, Muenster, NRW, Germany. <sup>3</sup>Interdisciplinary Centre for Clinical Research (IZKF), Muenster, NRW, Germany. <sup>4</sup>National High Magnetic Field Laboratory (NHFML), Tallahassee, FL, USA. <sup>5</sup>Bruker Daltonics, Bremen, Germany. <sup>6</sup>SIRCAMs, EastChem School of Chemistry, University of Edinburgh, Scotland, UK. Corresponding author: [lmackay@ed.ac.uk](mailto:lmackay@ed.ac.uk)

Institut für Hygiene

medizinische fakultät  
Westfälische  
Wilhelms-Universität Münster



Ulster University



## INTRODUCTION

- Growth of many prostate cancer tumors are dependent on active androgens such as testosterone (T) and dehydroepiandrosterone (DHEA).
- Vitamin D's active metabolite (1,25-(OH)<sub>2</sub>-D<sub>3</sub>), modulates the androgen intracrine pathway

### Aims of the study

- To evaluate the application of ion mobility and MALDI-2 on a timsTOF flex for determining the spatial distribution of derivatized androgens and vitamin D metabolites to improve sensitivity, and separate biological active isobaric species.

## MATERIALS AND METHODS

- Instrument was a MALDI-2-IM-MSI using a timsTOF-flex in-house modified with a 1 kHz, frequency-quadrupled Nd:YAG post-ionization laser (266 nm).
- Several derivatization reagents for both androgens (Girard-T and Dansyl Hydrazine) and VitD metabolite (PTAD and DMEQ-TAD) were screened to assess ionization enhancement and mobility separation on standards and tissue sections
- On-tissue chemical derivatization (OTCD) was performed by the Bruker ImagePrep using prostate tumour (10µm) tissue and matrix was applied using a modified 3D printer
- The source and TIMS pressures, as well as the CCS were calibrated using a low-molecular weight tune-mix.

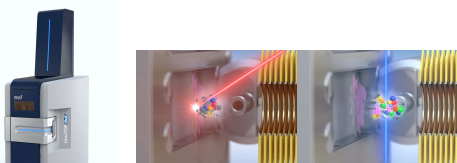


Figure 1. MALDI-2 timsTOF flex instrument

## RESULTS

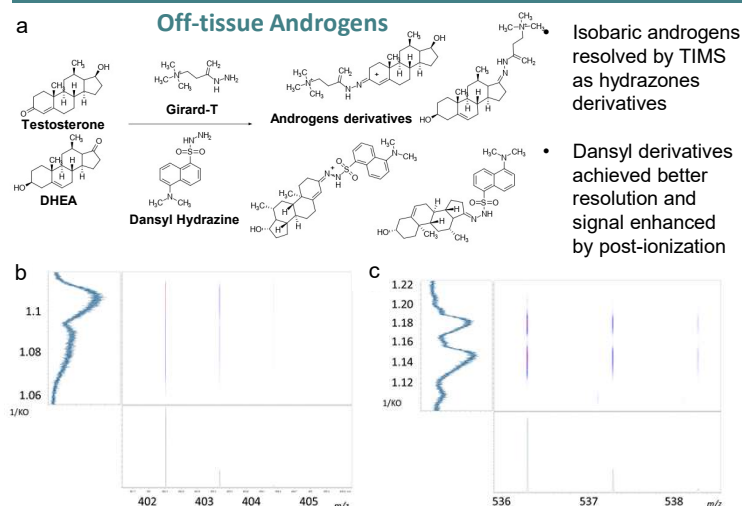


Figure 2. a) Androgens (T and DHEA) derivatization reaction scheme using Hydrazine-type reagents. Off-tissue Mobilogram of equimolar T/DHEA mix standard as b) Girard derivatives, c) Dansyl derivatives

### Androgens imaging on prostate tissue section

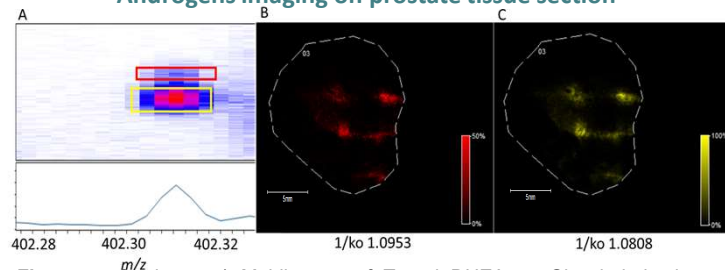


Figure 4. On-tissue a) Mobilogram of T and DHEA as Girard derivatives. Spatial distribution of b) Testosterone and c) DHEA as Girard derivatives.

### Off-tissue Vitamin D metabolite

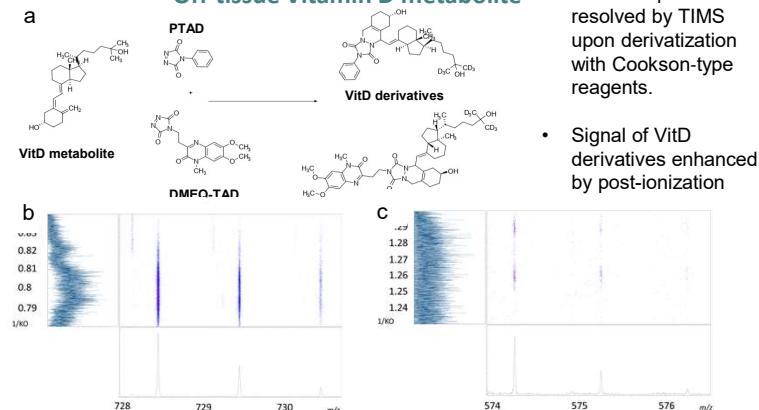


Figure 3. a) VitD 1,25 (OH)<sub>2</sub>-D<sub>3</sub> derivatization reaction scheme using Cookson-type reagents. Off-tissue Mobilogram of equimolar 1,25 (OH)<sub>2</sub>-D<sub>3</sub>/C<sub>3</sub>-epimer standard mix as b) DMEQ-TAD derivatives c) PTAD derivatives

## CONCLUSIONS

- Increase in ionization efficiency and isobaric separation of Dansyl DHEA/T derivatives achieved using MALDI-2-TIMS
- MALDI-2-MS shown an increase in sensitivity for azodicarbonyls VitD derivatives and C<sub>3</sub>-epimers successfully resolved using TIMS.
- First time spatial distribution of isobaric androgens achieved at tissue level by OTCD-MALDI-2-IM-MSI.

## REFERENCES

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