

University of Nottingham



nmRC CASE STUDY

CHARACTERISING PROTEINS ON SURFACES

nmRC_CS_14





Protein Analysis on Surfaces

3D Orbitrap Secondary Ion Mass Spectrometry Case Study



PROTEIN ANALYSIS AT SURFACES - APPLICATIONS



- Protein analysis is needed to:
 - Understand disease in tissue
 - Develop new medical devices resistant to infection.
 - Analyse bio-materials to prevent foreign body responses.
 - Understand protein-protein interactions
- Typically done with MS requiring a chemical matrix or digestion of protein before analysis.
- Need a technique which can analyse proteins in their native state.







• Imaging capabilities of analyte on surfaces







PROTEIN ANALYSIS ON A BIOCHIP

University of Nottingham Nanoscale and Microscale Research Centre





- 3D OrbiSIMS analysis at -150 C showed increased ion intensity and speciation of higher mass species, compared to room temperature analysis.
- Importantly, fragments originate from highly specific C- and N- termini of the protein.



Sequence coverage

ALBU_HUMAN1n

DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQCPFEDHVKLVNEVTEFAK TCVADESAENCDKSLHTLFGDKLCTV ATLRETYGEMADCCAKQEPERNECF LQHKDDNPNLPRLVRPEVDVMCTAF HDNEETFLKKYLYEIARRHPYFYAPE LLFFAKRYKAAFTECCQAADKAACL LPKLDELRDEGKASSAKQRLKCASL **QKFGERAFKAWAVARLSQRFPKAEFA EVSKLVTDLTKVHTECCHGDLLECA** DDRADLAKYICENQDSISSKLKECCE **KPLLEKSHCIAEVENDEMPADLPSLA** ADFVESKDVCKNYAEAKDVFLGMFL **YEYARRHPDYSVVLLLRLAKTYETTL** EKCCAAADPHECYAKVFDEFKPLVEE PONLIKONCELFEQLGEYKFONALLV RYTKKVPQVSTPTLVEVSRNLGKVGS KCCKHPEAKRMPCAEDYLSVVLNQL **CVLHEKTPVSDRVTKCCTESLVNRRP CFSALEVDETYVPKEFNAETFTFHAD** ICTLSEKERQIKKQTALVELVKHKPKA TKEQLKAVMDDFAAFVEKCCKADDK ETCFAEEGKKLVAASQAALGL



PROTEINS IN SKIN



Image credit: Human Protein Atlas, http://www.proteinatlas.org

- The layers of the skin assigned based on the abundance of a phospholipid marker, PO₃⁻ (Starr *et al.* 2019)
- Protein locations based on the tissue sections gathered in the Protein Atlas (Uhlén M *et al.*, 2015)
- Sequences characteristic to corneodesmosin,
 keratin and collagen in the skin layers where they
 are known to be exclusive or predominant



PROTEIN ANALYSIS ON A BIOCHIP





- Protein monolayer immobilised on thiol βcyclodextrin (TCD) as developed by Di Palma *et al.*, 2019
- Maximum amount of lysozyme molecules in the analysed area:
 - 2.18 × 10¹⁰ (40 femtomoles)
- Seven distinct lysozyme fragments

detected



For more details on the work showcased in this case study see the following publications:

A. M. Kotowska, G. F. Trindade, P. M. Mendes, P. M. Williams, J. W. Aylott, A. G. Shard, M. R. Alexander and D. J. Scurr, Protein identification by 3D OrbiSIMS to facilitate in situ imaging and depth profiling, *Nature Communications*, 2020, **11**, 5832.

The Orbitrap Secondary Ion Mass Spectrometry documented here was performed in the Nanoscale and Microscale Research Centre at the University of Nottingham.



Advanced Materials & Healthcare Technologies



- We hope the information provided in this case study is of interest.
- If you wish to get in touch with us to discuss any of the information provided, raise a query/concern or provide feedback then please use any of the methods listed below:

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