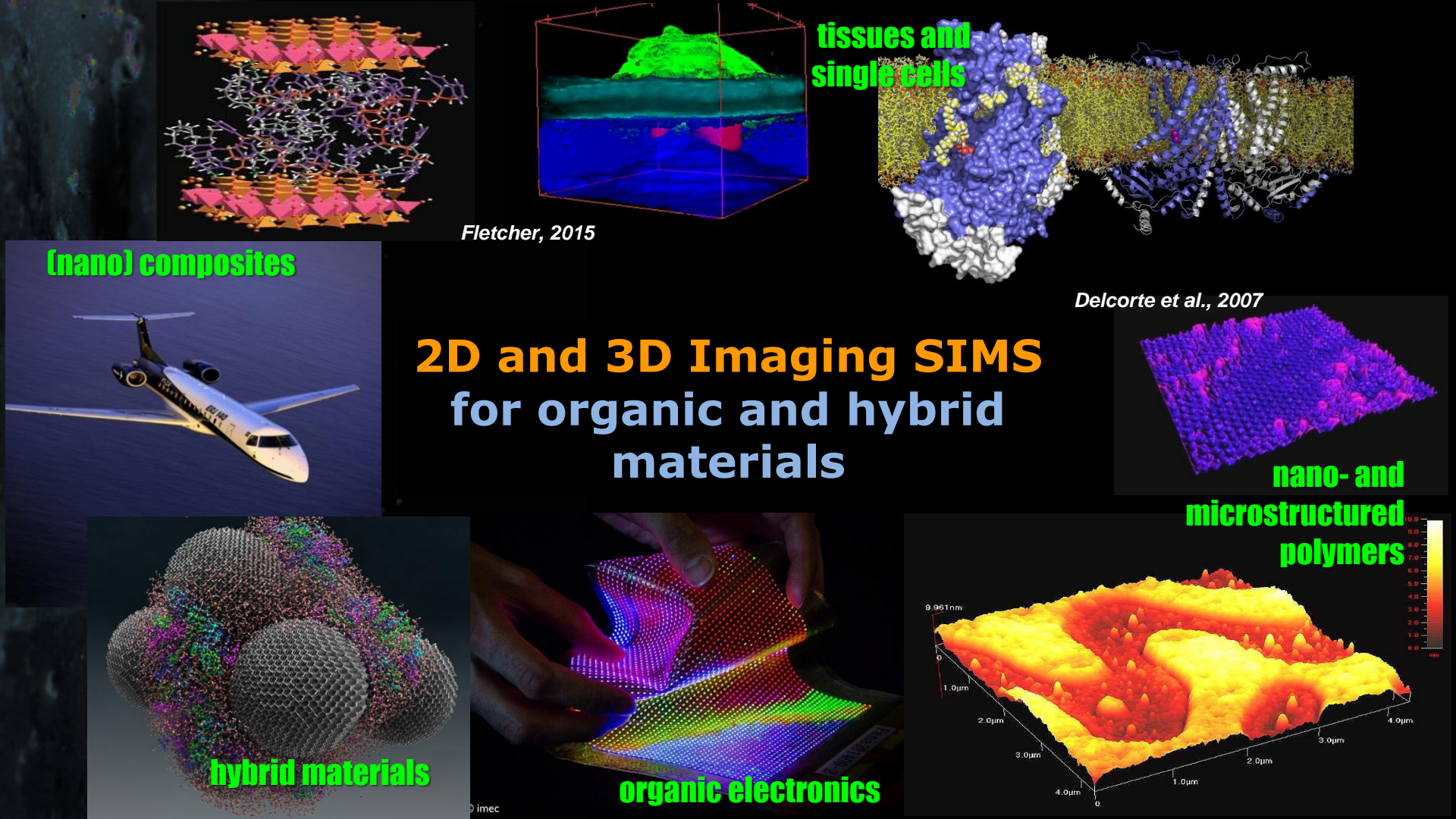


New perspectives for soft matter analysis by SIMS

Arnaud Delcorte
Thomas Daphnis
Benjamin Tomasetti
Vincent Delmez
Shadi Bazazordeh
Samuel Bertolini
Jaroslaw Mazuryk
Christine Dupont-Gillain



(nano) composites

Fletcher, 2015

**tissues and
single cells**

Delcorte et al., 2007

2D and 3D Imaging SIMS for organic and hybrid materials

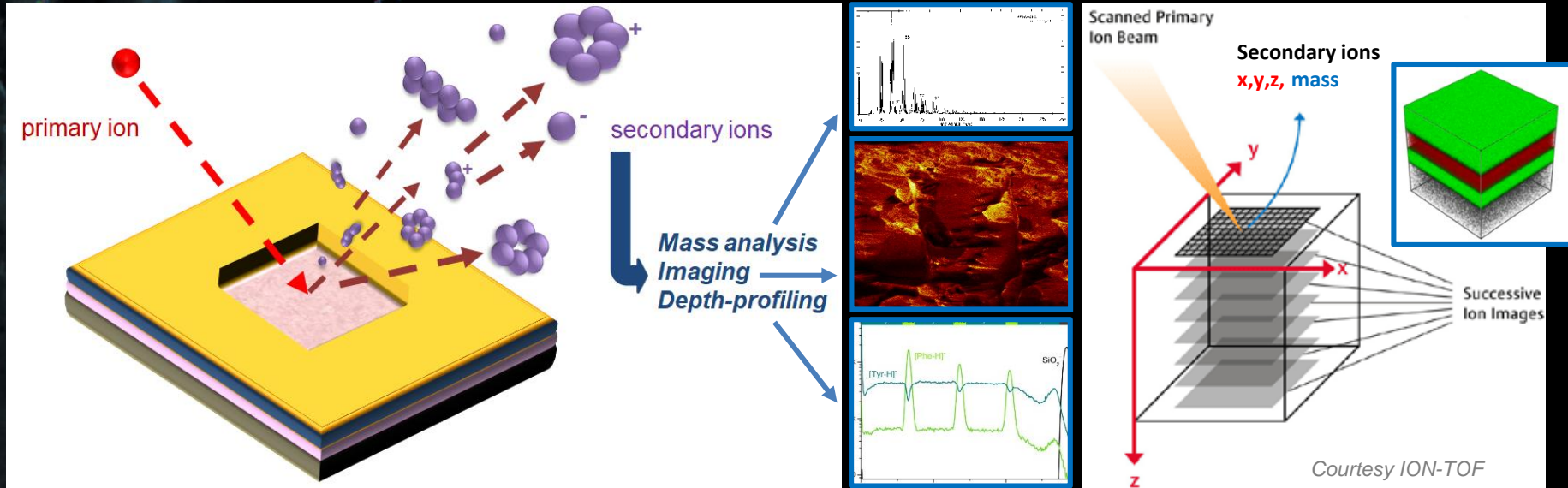
**nano- and
microstructured
polymers**

hybrid materials

organic electronics

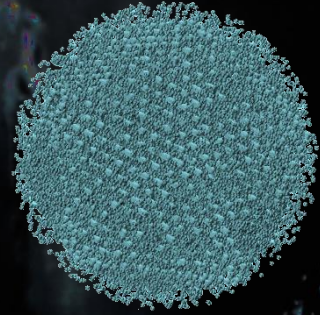
© imec

Secondary ion mass spectrometry



molecular information and localization with μm lateral resolution and nm depth resolution

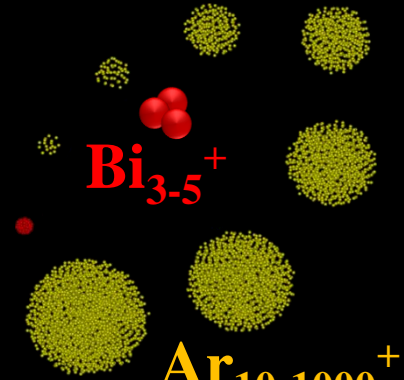
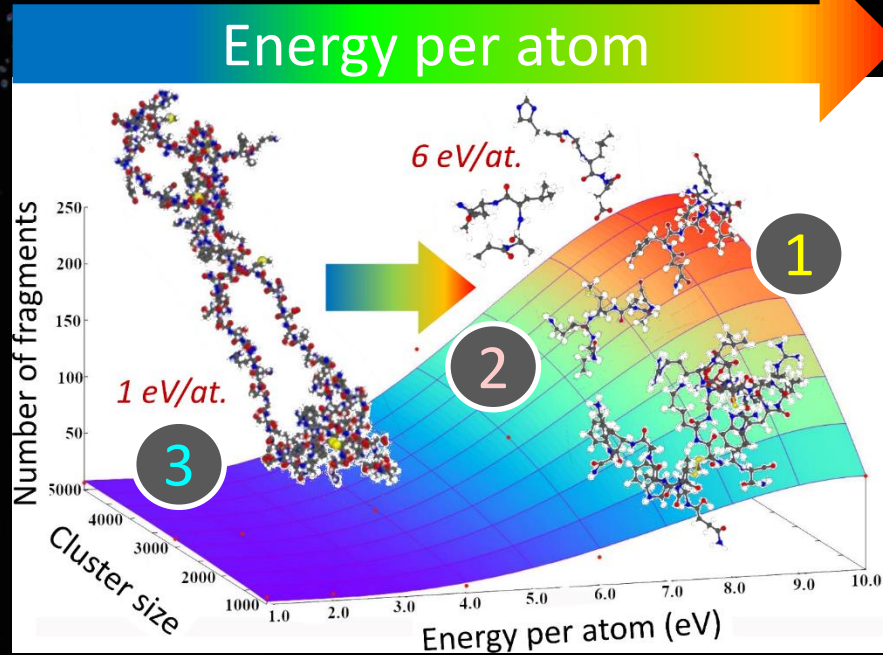
Cluster ions for SIMS



$\text{Ar}_{5000-10000}^+$



*(Large)
intact
molecules*



Bi_{3-5}^+

$\text{Ar}_{10-1000}^+$



*Small
molecules and
fragments*

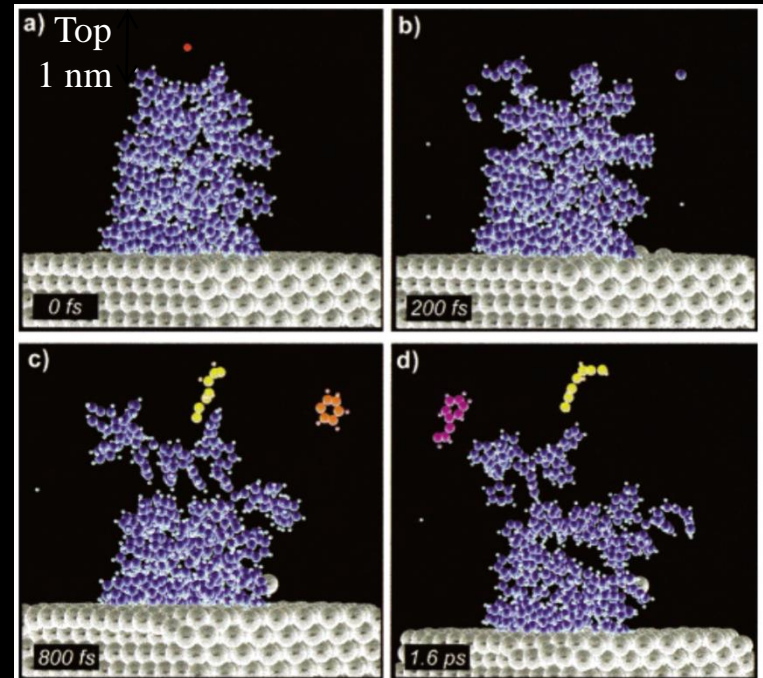
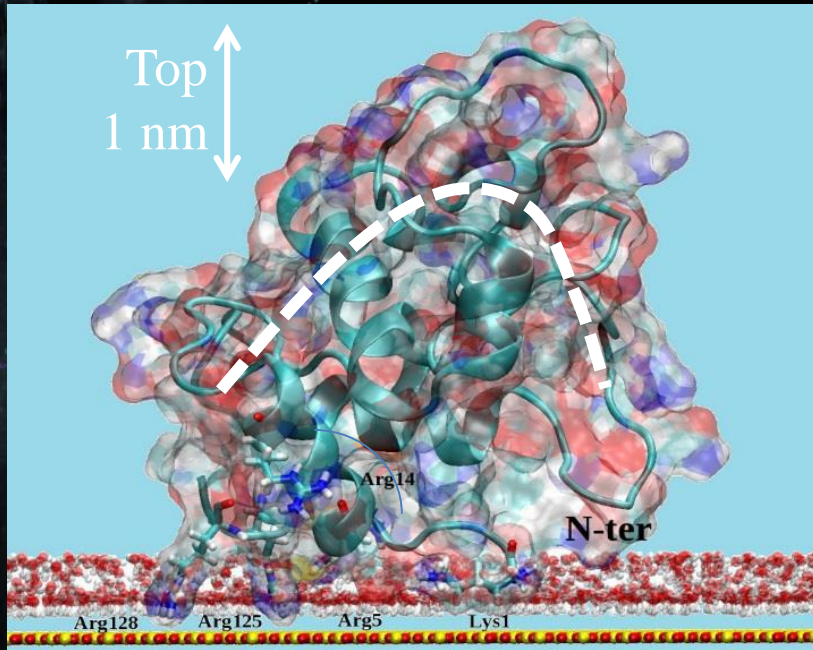
1. Fragments for orientation and sequencing
2. Molecular imaging (with surface sensitivity)
3. Desorption and transfer of intact molecules



The image shows a 3D visualization of a polymer network. A dense, greyish-purple mesh of polymer chains is shown at the bottom, representing a substrate. From this substrate, several long, thin, yellow polymer chains extend upwards and outwards. A 3D coordinate system is visible in the upper left, with a blue arrow pointing up (z-axis), a red arrow pointing right (x-axis), and a green circle indicating the y-axis. The background is dark, highlighting the polymer structure.

1. Fragments for orientation and sequencing

Concept: using AA fragments to probe the exposed molecular surface
=> molecule should be larger than the average sampling depth



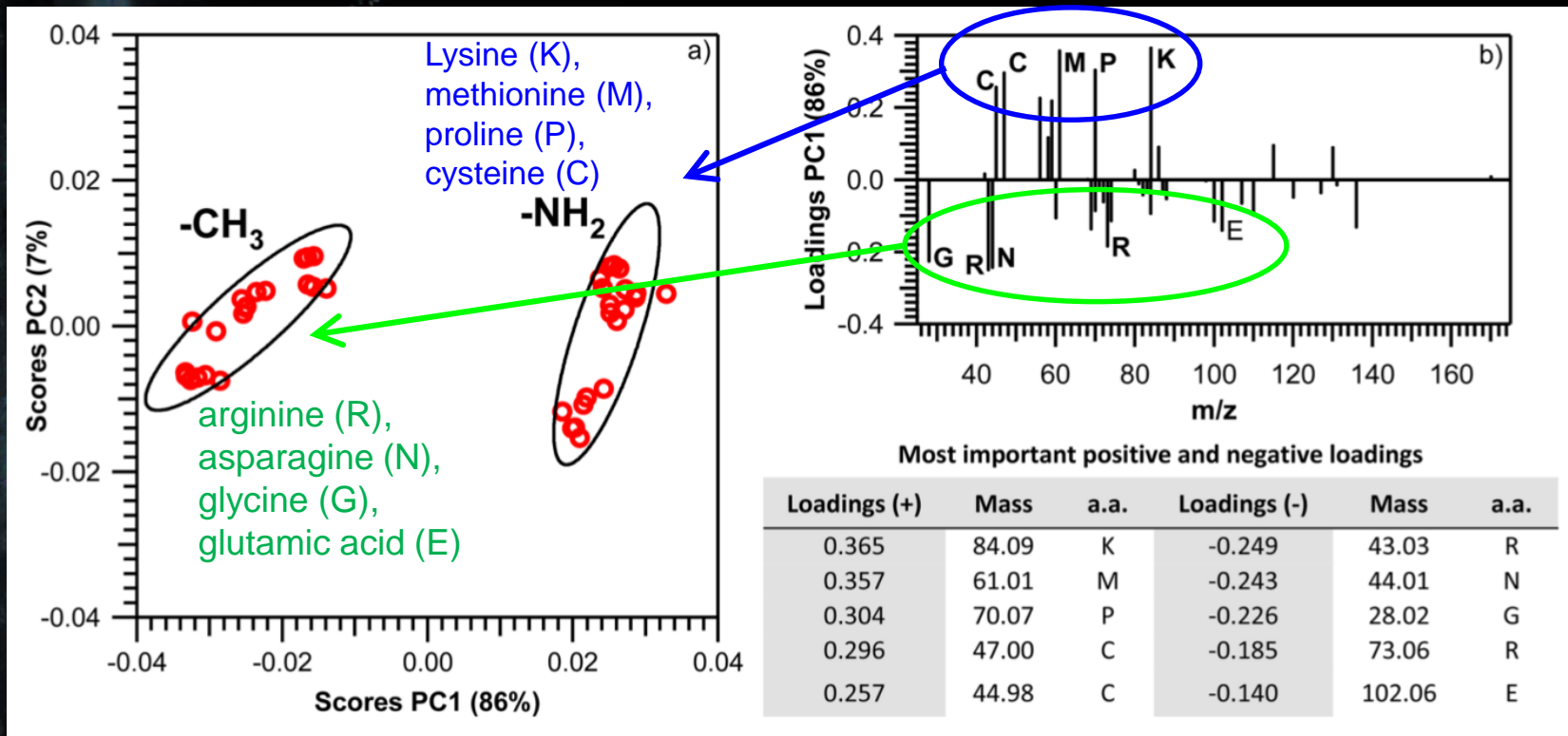


Victor

Biomolecule orientation: β -lactoglobulin

β -lactoglobulin adsorbed on $-\text{CH}_3$ and $-\text{NH}_2$ terminated self-assembled monolayers

Ion source: 30 keV Bi^+

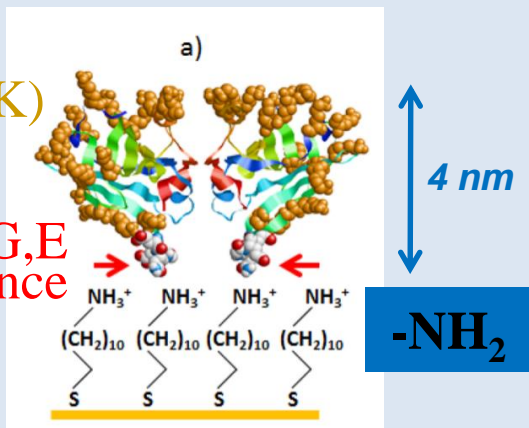


*NESAC/BIO MVA toolbox; 44 AA peaks; normalized by sum; mean-centered.

V. Lebec,^{†,‡} J. Landoulsi,[‡] S. Boujday,[‡] C. Poleunis,[†] C.-M. Pradier,[‡] and A. Delcorte* *J. Phys. Chem. C* 2013, 117, 11569–11577

Lysine (K)

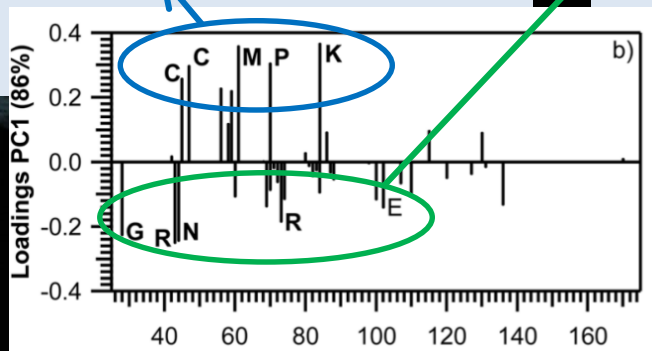
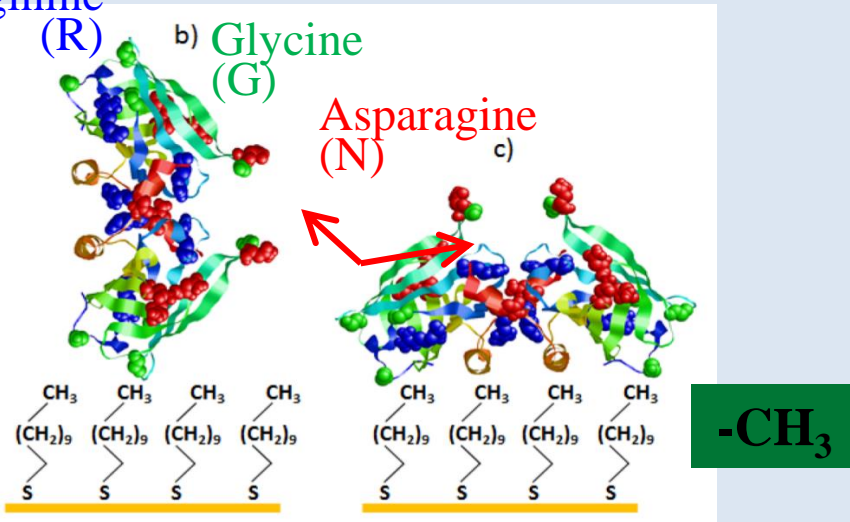
E,N,G,E
sequence



Arginine
(R)

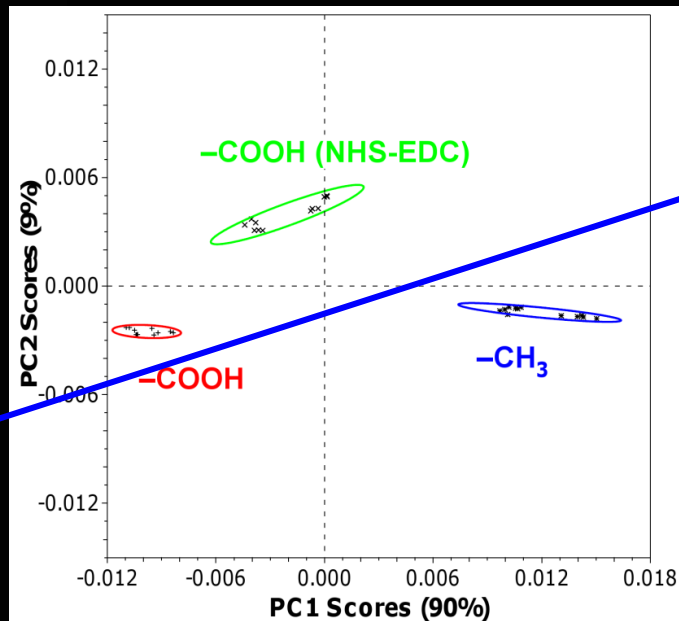
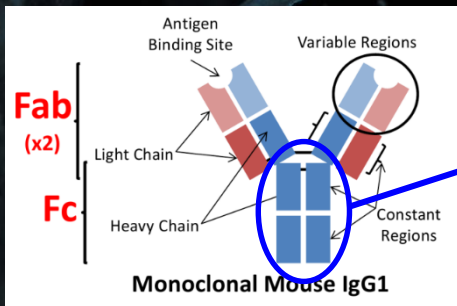
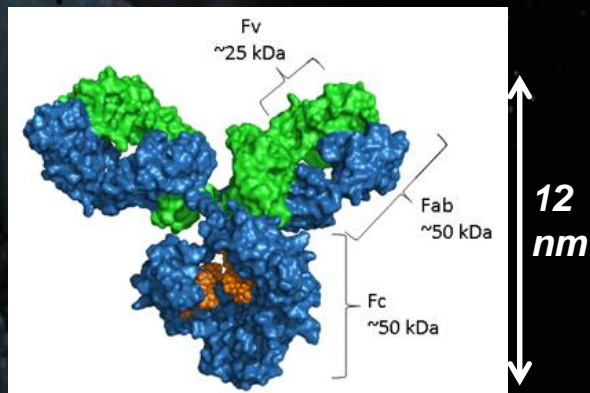
Glycine
(G)

Asparagine
(N)



β -lactoglobulin adsorbed on $-\text{CH}_3$ and $-\text{NH}_2$ terminated self-assembled monolayers

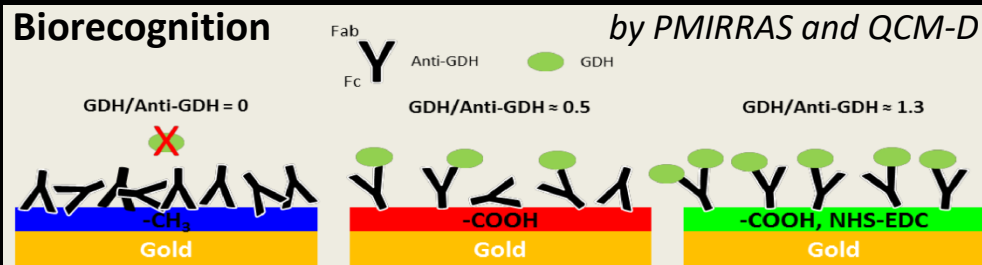
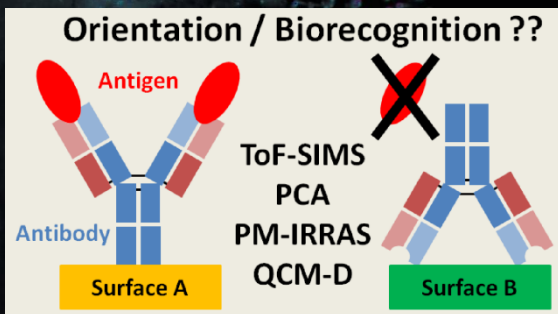
Biomolecule orientation: anti-GDH



PC1 (90% of variance) loadings					
Pos.	Mass	a.a.	Neg.	Mass	a.a.
0.83	70.07	Pro	-0.05	46.99	Cys
0.38	72.08	Val	-0.04	43.02	Arg
0.16	110.08	Arg/his	-0.01	127.10	Arg
0.16	60.05	Ser	-0.01	58.99	Cys
0.15	74.06	Thr	-0.01	61.01	Met

PC2 (9% of variance) loadings					
Pos.	Mass	a.a.	Neg.	Mass	a.a.
0.63	72.08	Val	-0.35	46.99	Cys
0.19	81.04	His	-0.27	69.03	Thr
0.14	110.07	Arg/His	-0.26	74.06	Thr
0.13	127.10	Arg	-0.23	70.07	Pro
0.13	56.05	Lys/Phe/Met	-0.18	61.01	Met

antiglutamate dehydrogenase



Lebec *et al.*, *J. Phys. Chem. C* **2014**, 118, 2085-2092

Protein identification by 3D OrbiSIMS to facilitate in situ imaging and depth profiling

Anna M. Kotowska¹, Gustavo F. Trindade¹, Paula M. Mendes², Philip M. Williams¹, Jonathan W. Aylott¹, Alexander G. Shard³, Morgan R. Alexander¹ & David J. Scurr^{1,3*}

Contents lists available at ScienceDirect

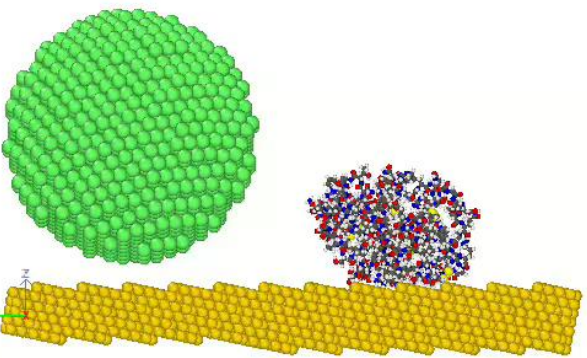
Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

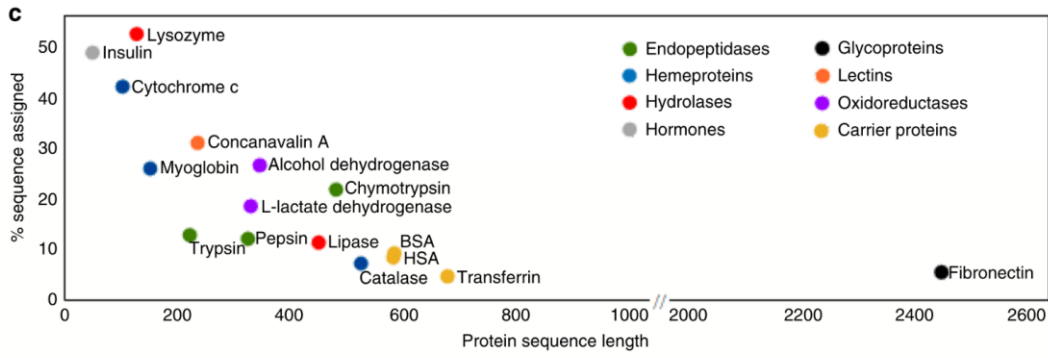
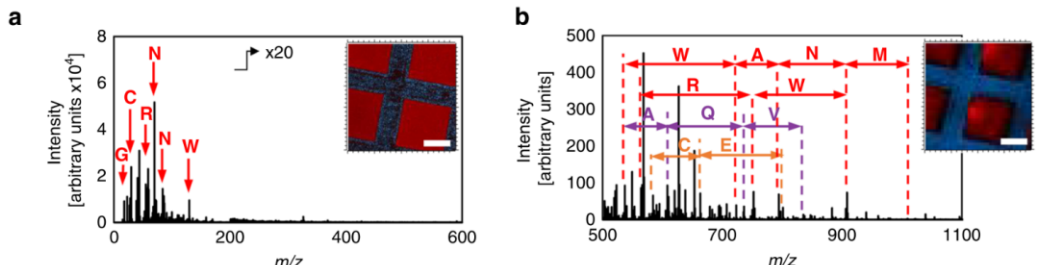
Full Length Article

Reactive molecular dynamics simulations of lysozyme desorption under Ar cluster impact

Samuel Bertolini⁺, Arnaud Delcorte⁺



Perspective: protein sequencing

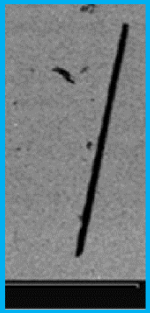


20 keV Ar₃₀₀₀⁺ : 6 eV/atom

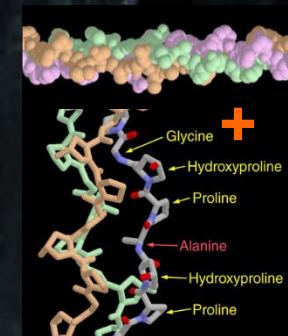
The image displays a molecular imaging scan. On the left, a grayscale image shows a biological structure, possibly a bone or a piece of wood, with some internal texture. Overlaid on this is a color-coded scan. The top portion of the scan is bright blue, while the lower portion is a dark red or orange. The background is black. A semi-transparent dark gray horizontal bar is positioned across the middle of the image, containing the text '2. Molecular imaging (with surface sensitivity)' in a bright green font.

2. Molecular imaging (with surface sensitivity)

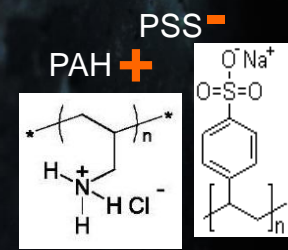
Lateral and depth resolution



SEM

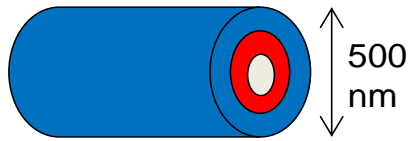


Collagen triple helix

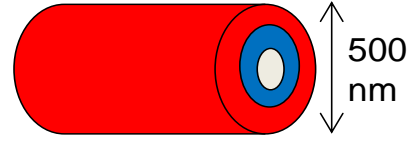


ToF-SIMS

A) Collagen inside



B) Collagen outside



C) Collagen outside (microtubes)

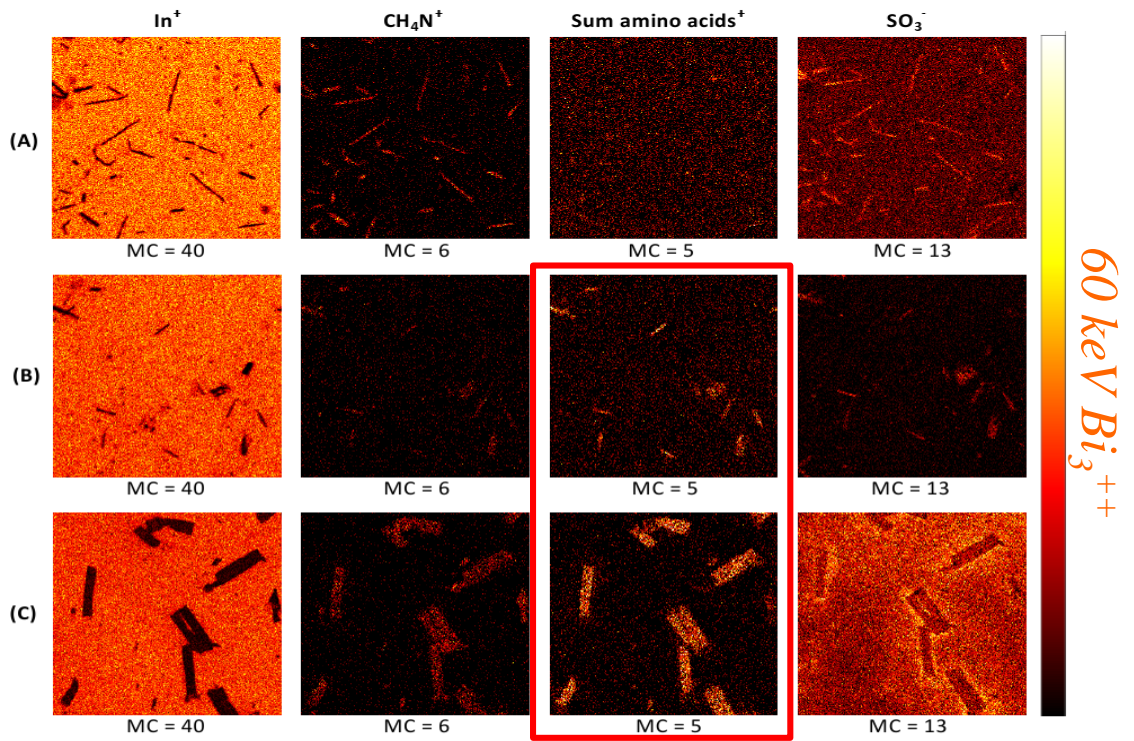
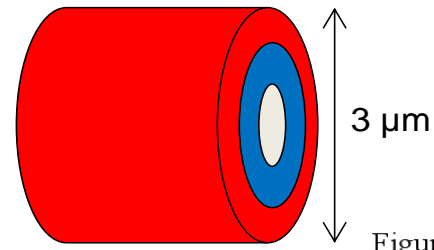
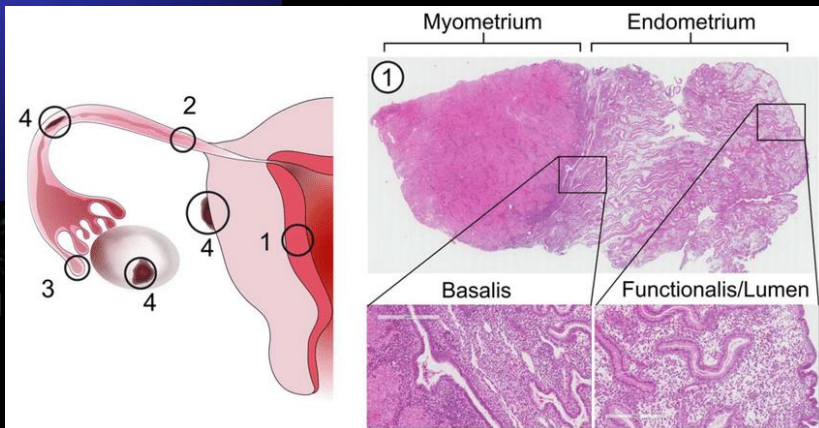


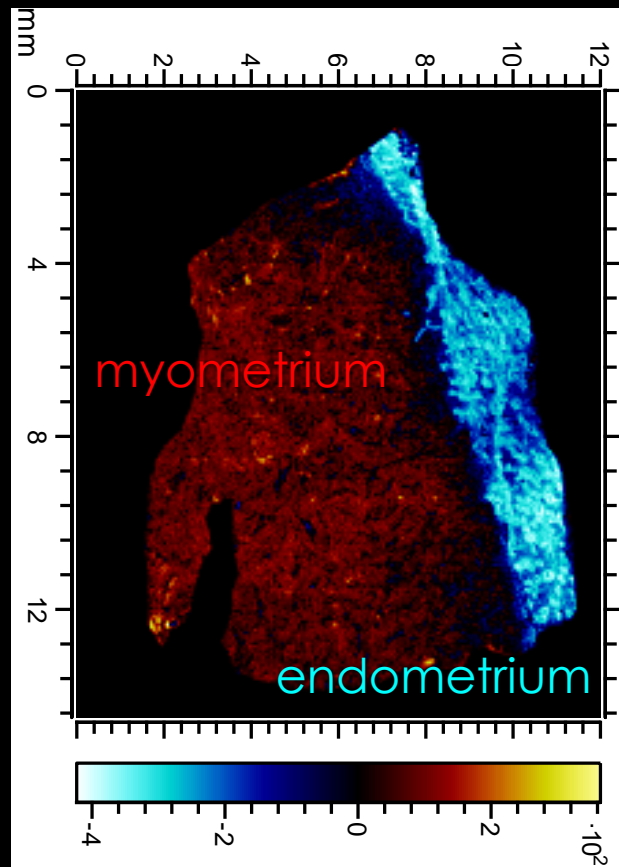
Figure 3. Positive and negative ToF-SIMS images (field of view $100 \mu m^2$) of ITO glass coverslips used as working electrode for nano-microtubes collection by EPD. (A) (Flu-PAH/PSS)₃/(COL/PSS)₃ nanotubes; (B) (COL/PSS)₃/(Flu-PAH/PSS)₃ nanotubes; (C) (COL/PSS)₃/(Flu-PAH/PSS)₃ microtubes. MC means maximum intensity counts. Sum of amino acids⁺ corresponds to the sum of the images provide by the ions $C_2H_6N^+$, $C_4H_8N^+$ and $C_4H_8NO^+$.



Samples PFA fixated, cryo-preserved with sucrose, embedded in carboxymethyl cellulose (CMC), frozen and cryomicrotomed. Sections defrozed for analysis.

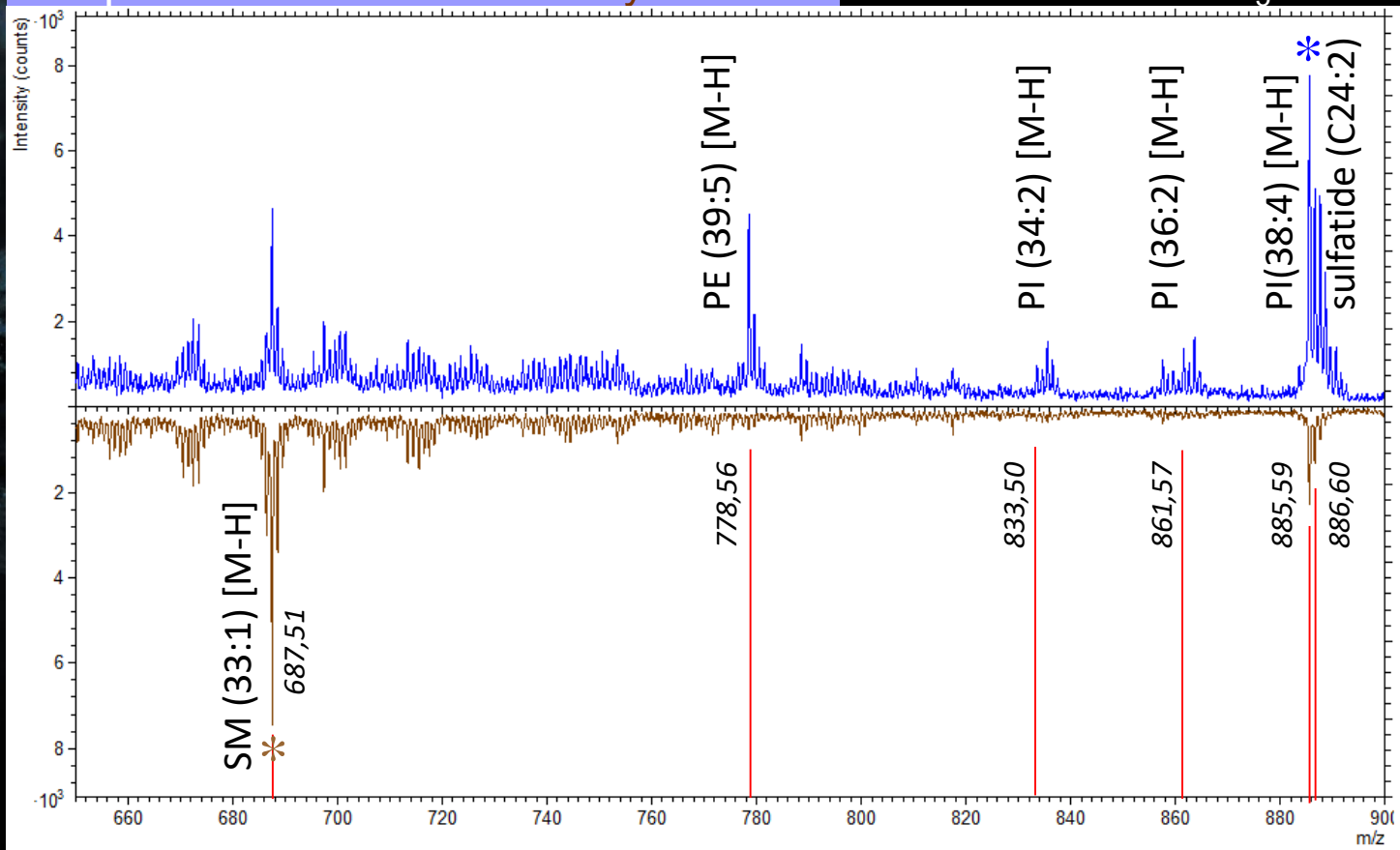


Multivariate analysis (PCA) on the SIMS images of the tissue cross-sections discriminates **endometrium** and **myometrium**



Comparison Endometrium / Myometrium

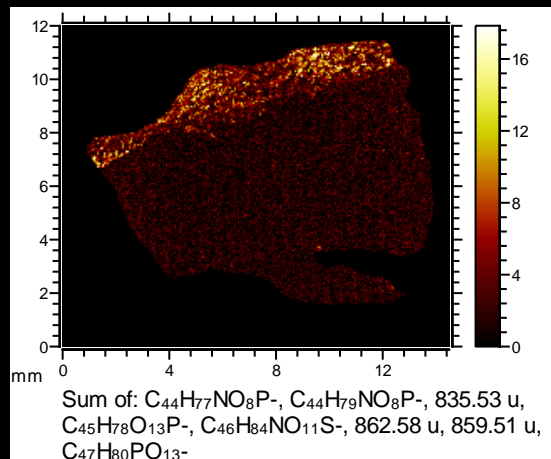
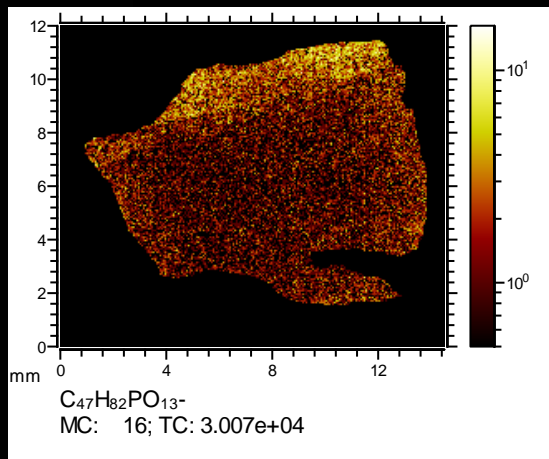
30 keV Bi₅⁺ – ITO



endometrium and myometrium present different distributions of lipids

endometrium

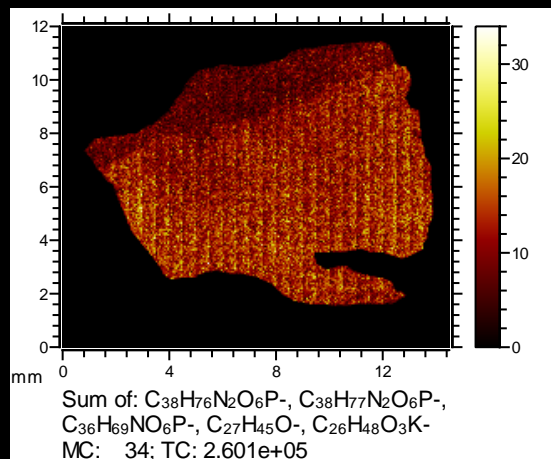
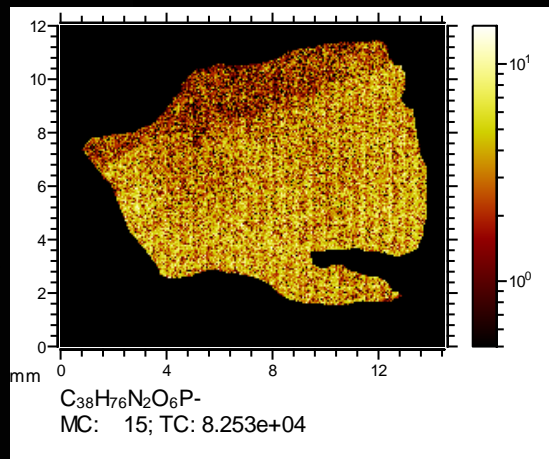
✧ Phosphatidyl inositol 38:4
m/z = 885



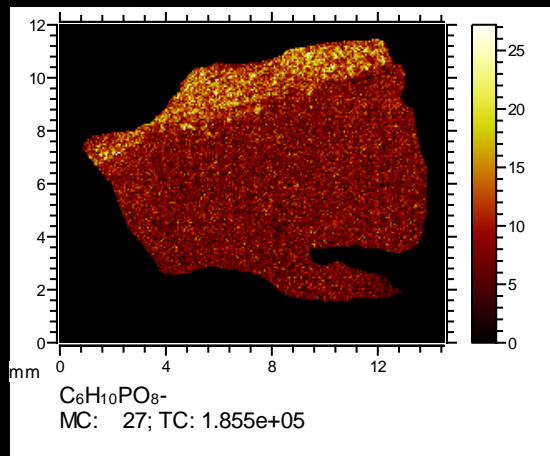
Information is richer in the negative SIMS

myometrium

✧ Sphingomyelin 33:1
m/z = 687

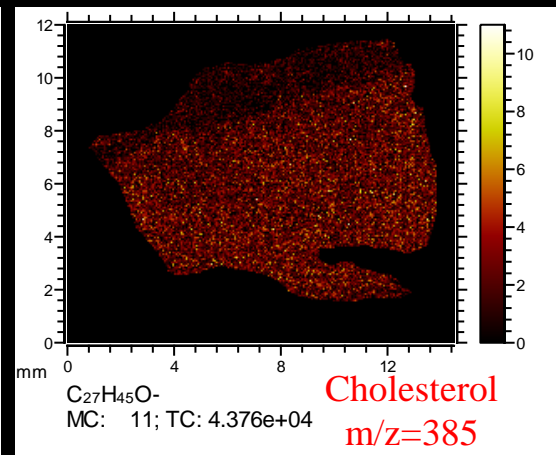
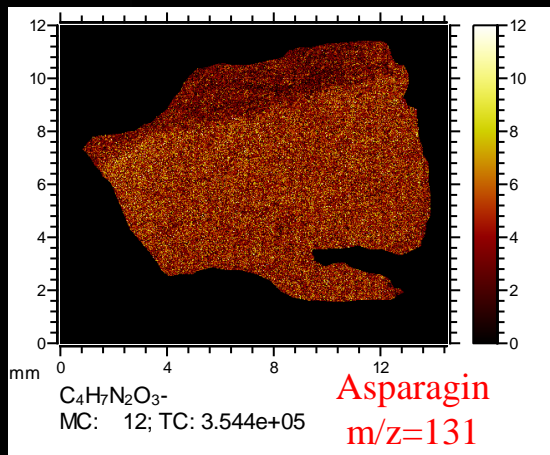


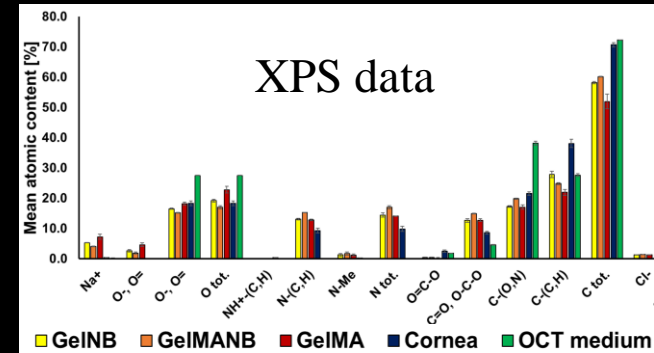
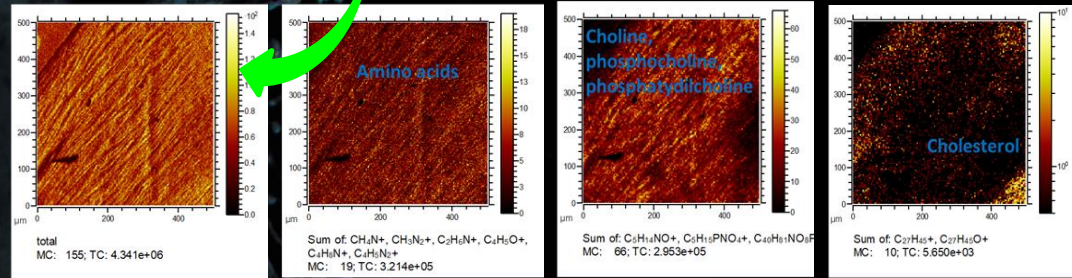
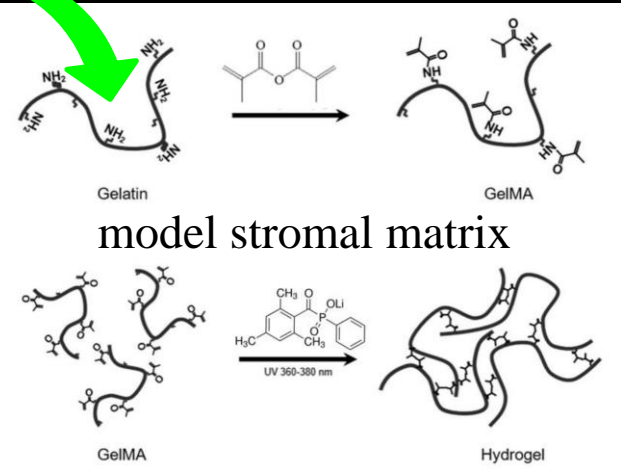
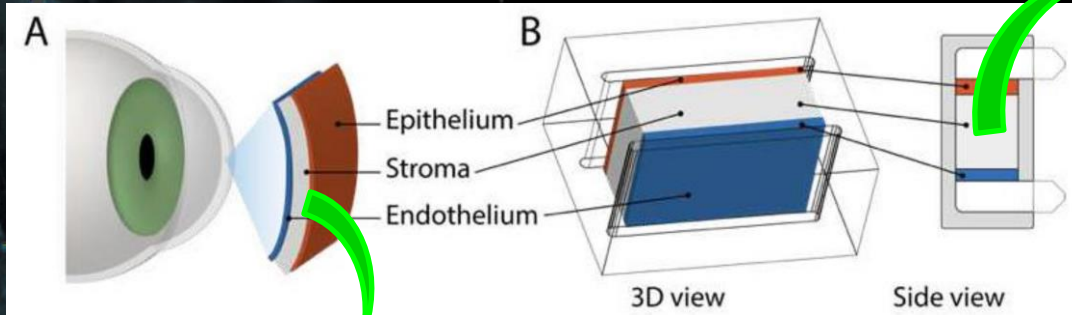
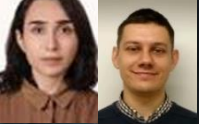
endometrium and
myometrium present
different distributions
of lipids
endometrium



Phosphatidyl inositol headgroup
m/z= 241

Information is richer in
the negative SIMS
myometrium





“Artificial Lithographic MODEL for COrneal drug Screening” (AL MOD CONS)

1. Flash-freezing of the chip
2. Transfer to precooled sample holder (-110°C)
3. Keep at low T° through sample analysis

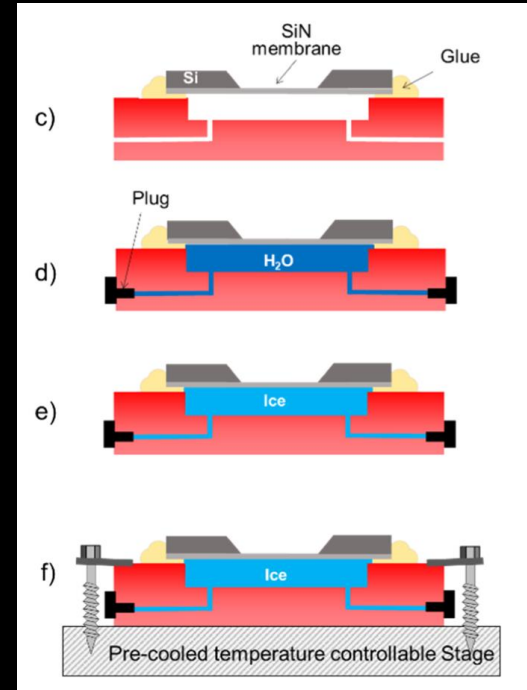
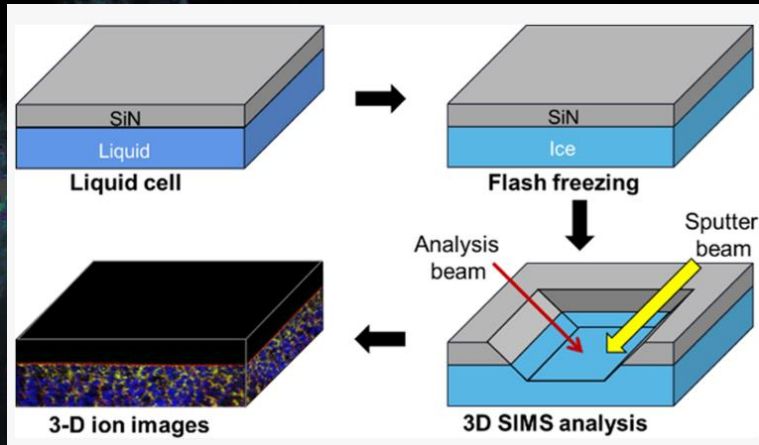
Three-Dimensional Mass Spectrometric Imaging of Biological Structures Using a Vacuum-Compatible Microfluidic Device

Wenxiao Guo, Michal Kanski, Wen Liu, Mikolaj Goluński, Yadong Zhou, Yining Wang, Cuixia Cheng, Yingge Du, Zbigniew Postawa,* Wei David Wei,* and Zihua Zhu*

Cite This: *Anal. Chem.* 2020, 92, 13785–13793

 Read Online

analytical
chemistry

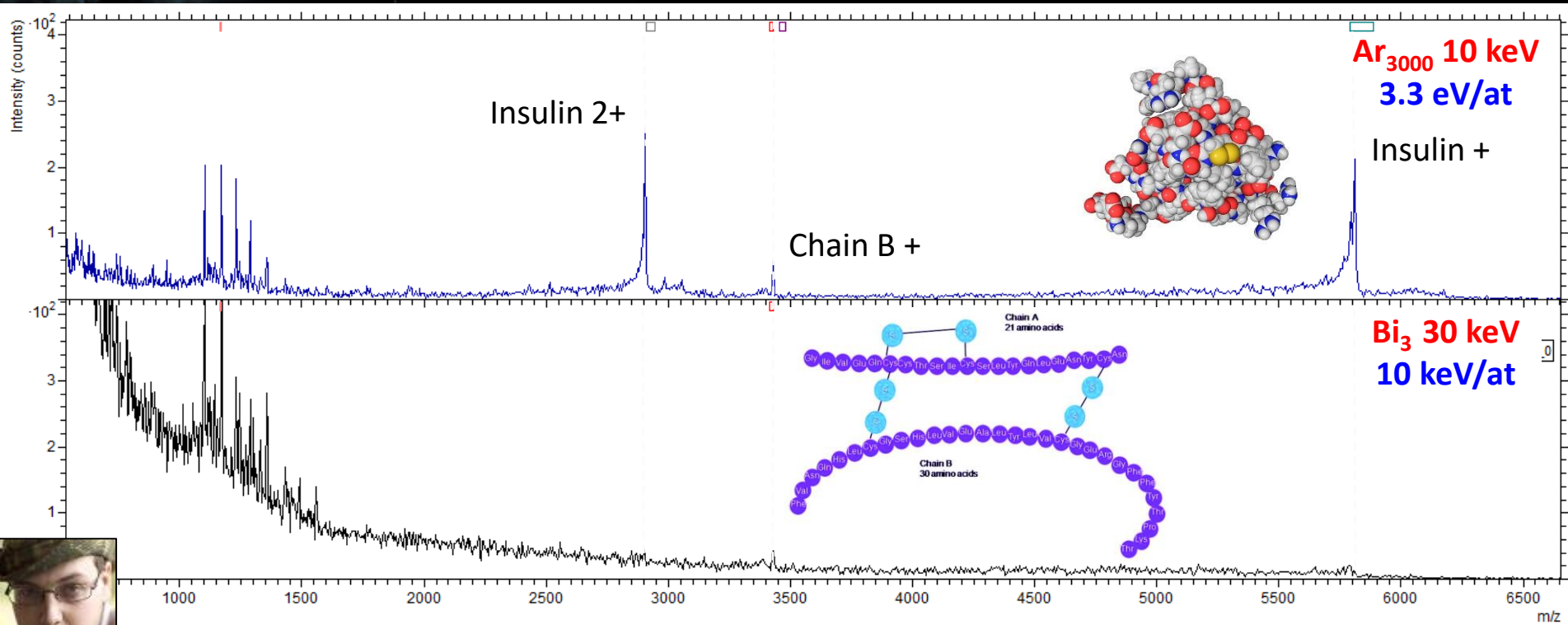




3. Desorption and transfer of intact molecules

Molecular analysis with Ar clusters

- 6 kDa insulin without special sample preparation (spin-coating on Si)
- Ratio molecule/fragment $\nearrow \nearrow \nearrow$ with Ar_n^+

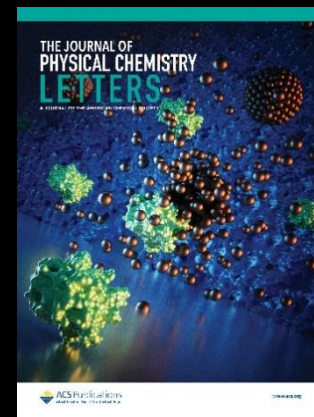


measurements by K. Moshkunov

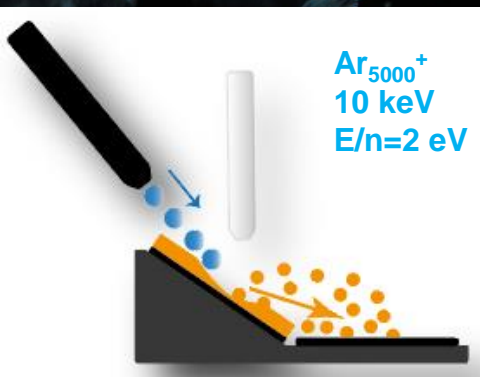
A. Delcorte et al., *Phys.Chem.Chem.Phys.*, 2020, 22, 17427 (REVIEW ARTICLE) 20

Transferring proteins in vacuo

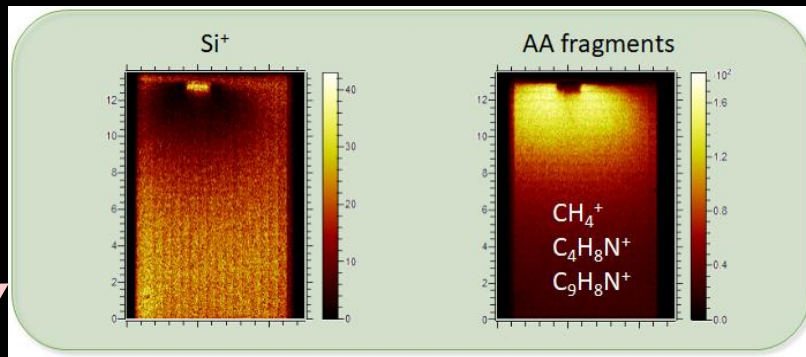
V. Delmez et al. *J. Phys. Chem. Lett.* **2021**, 12, 952–957



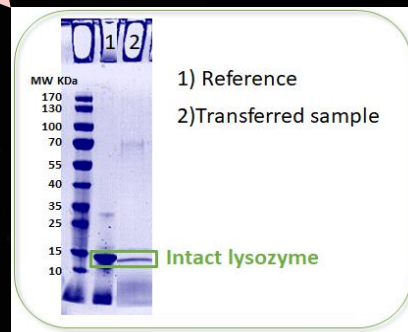
LYSOZYME
129 amino acids; 14.3 kDa



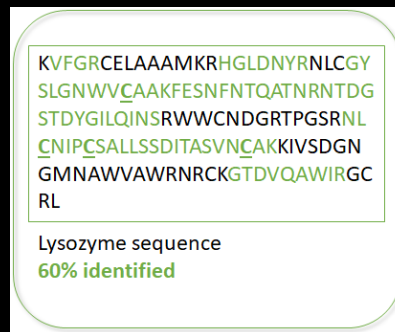
1. ToF-SIMS analysis



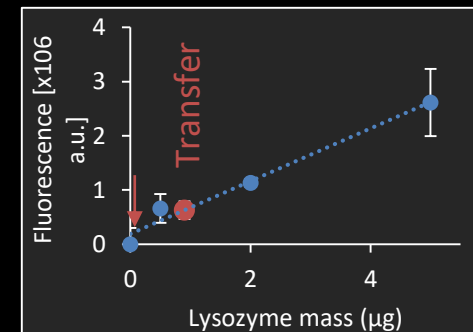
2. SDS-PAGE gel electrophoresis for mass estimation



3. ESI-MS of digested molecule for sequence ID



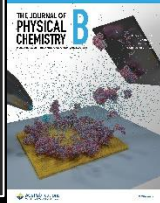
4. Fluorescence bioassay to check enzyme activity



Vincent



S. Bertolini,
A. Delcorte,
JPC:B, 2023
in press.



Possibility to desorb larger molecules

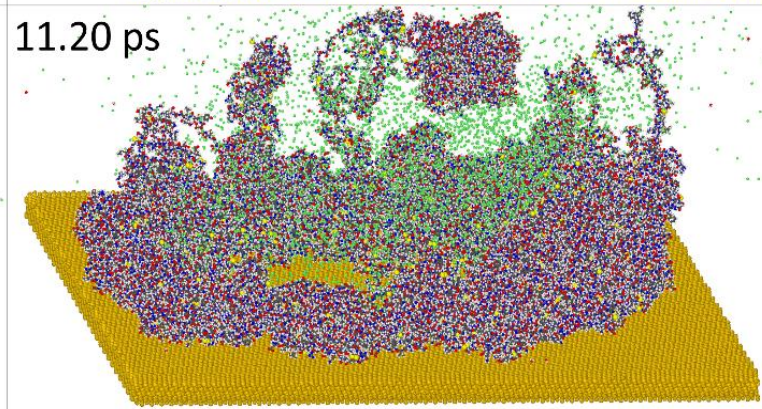
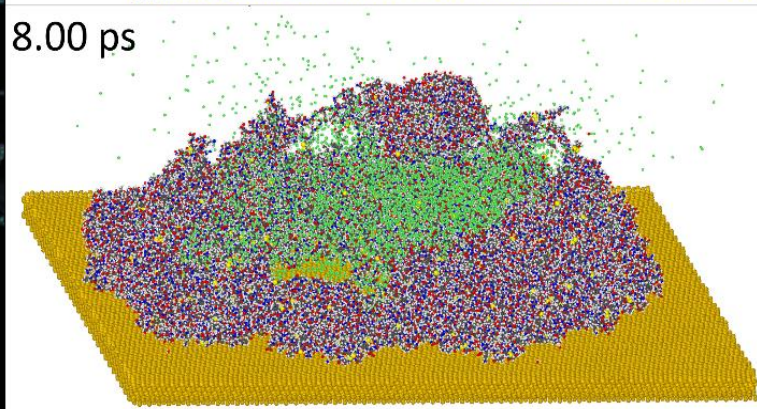
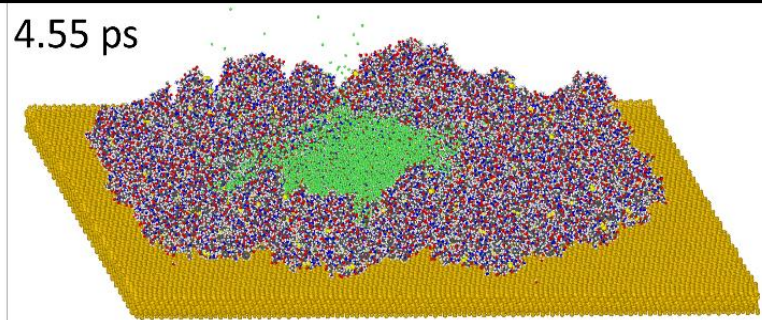
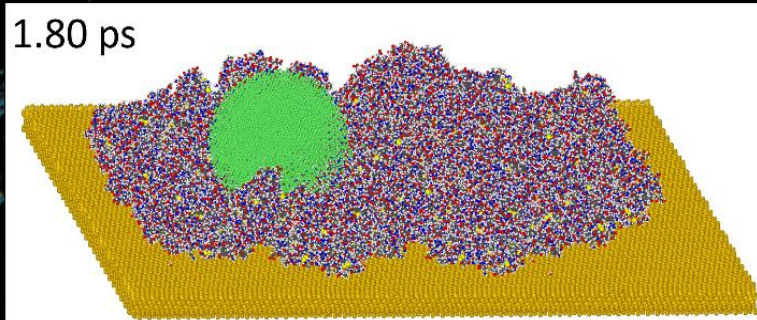
MD simulations predict larger protein desorption!

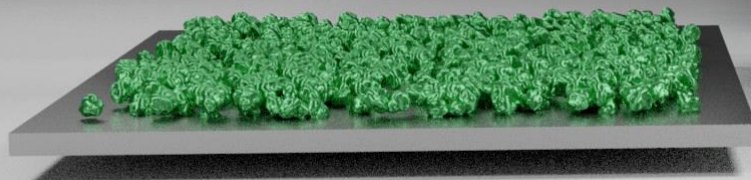
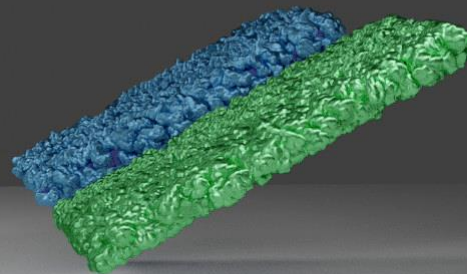
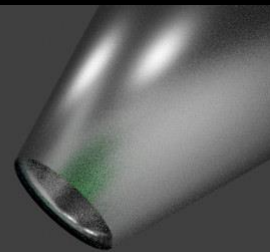
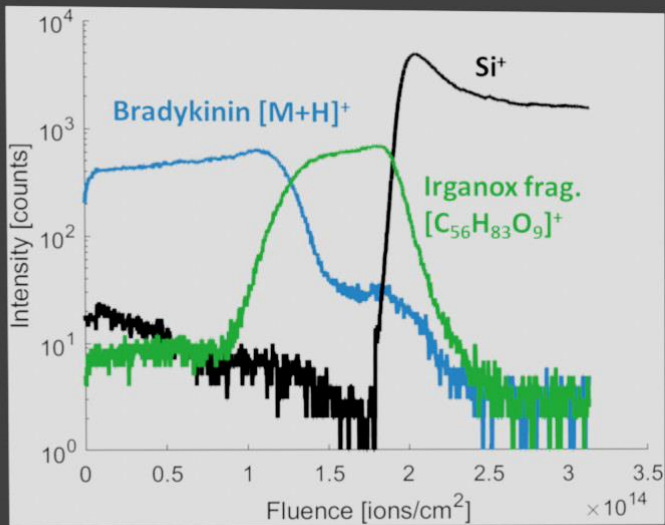
Samuel

**65 kDa
glucose oxidase
molecule on a
lysozyme layer
adsorbed
on gold**

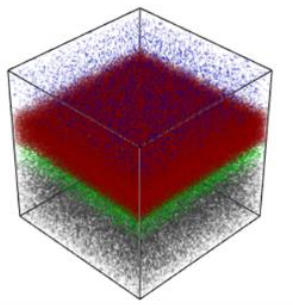


**15 keV Ar₅₀₀₀
bombardment
(3 eV/atom)**





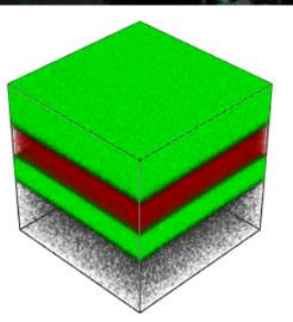
Deposition of multilayers



Neurotensin [M-H]⁻

Irganox 1010 [M]⁻

Bradykinin [M-H]⁻

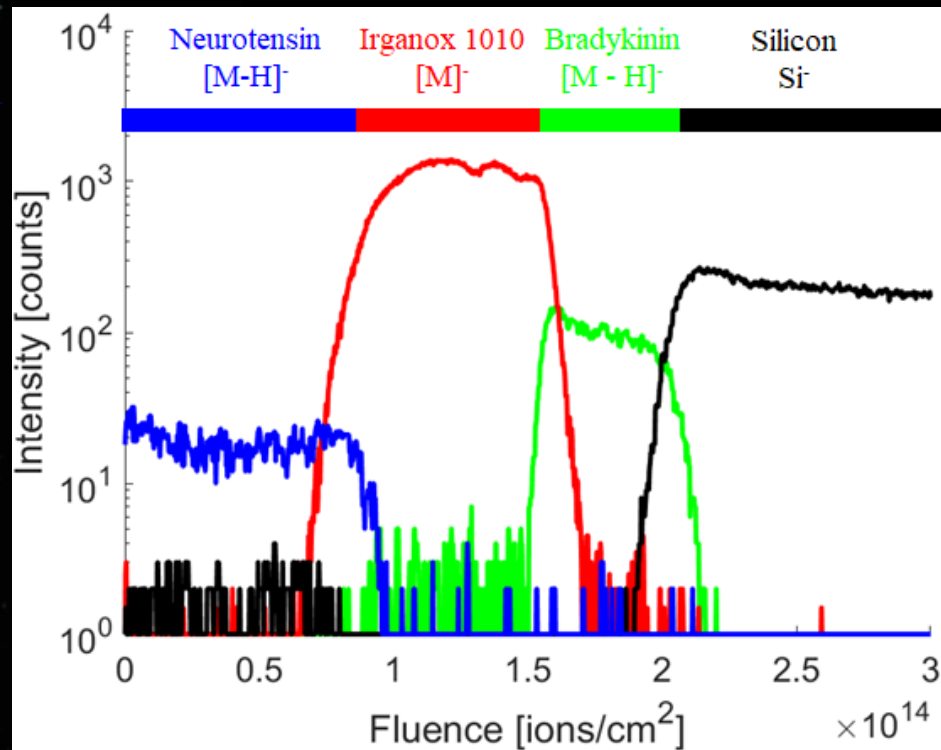


CNO⁻

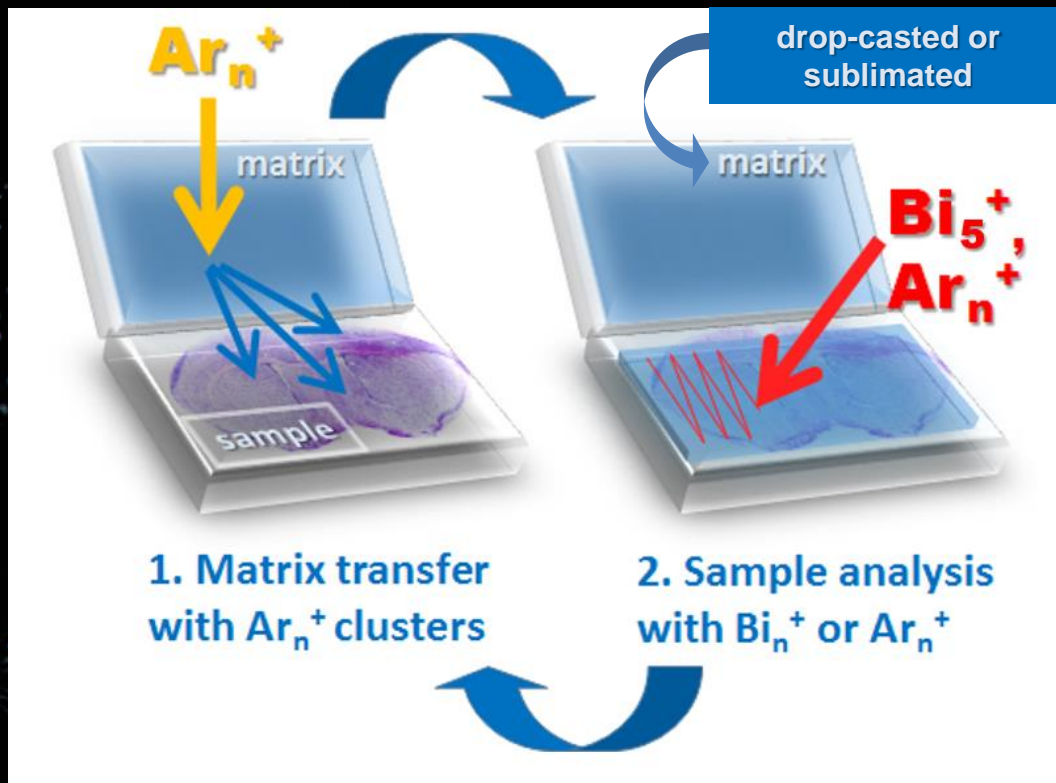
Irganox 1010 [M]⁻

CNO⁻

Silicon

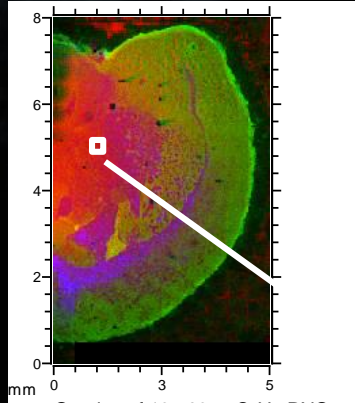


- Multilayers of virtually any kind of non-volatile molecules can be achieved
- Not feasible in solution
- Compatible with any substrate



Here the goal is to increase SIMS sensitivity by transferring MALDI matrix in-situ the ToF-SIMS

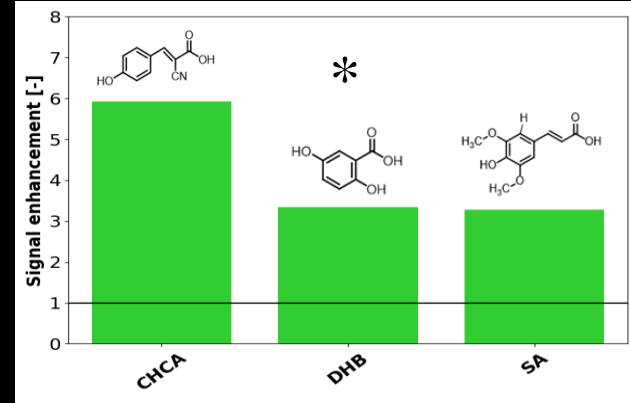
Sample holder geometry inspired by NPL



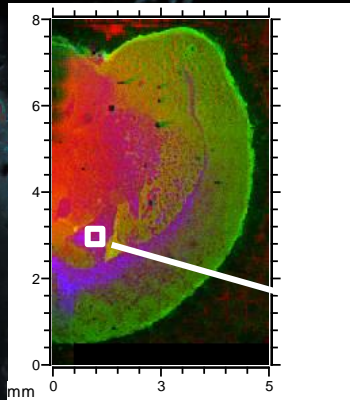
Grey matter

11 high mass lipids identified

200/200µm analyzed zone



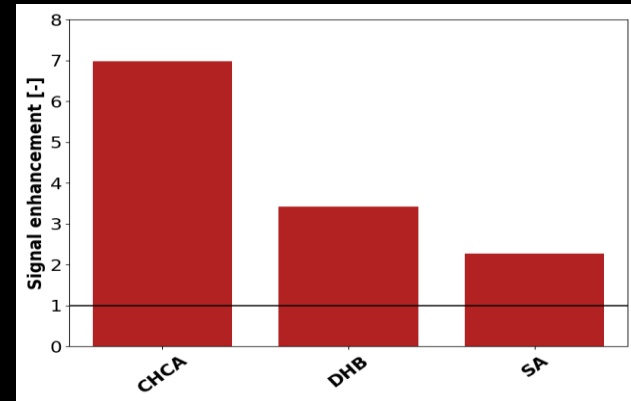
30 keV
Bi⁵⁺
analysis



White matter

24 high mass lipids identified

200/200µm analyzed zone

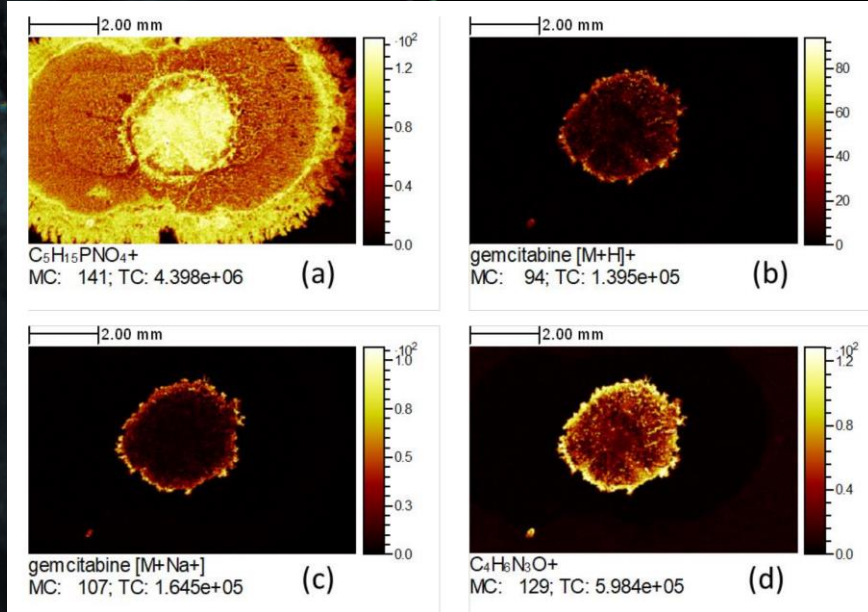


T. Daphnis
et al.,
JASMS
2023,
in press.

* α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (DHB), sinapinic acid (SA)

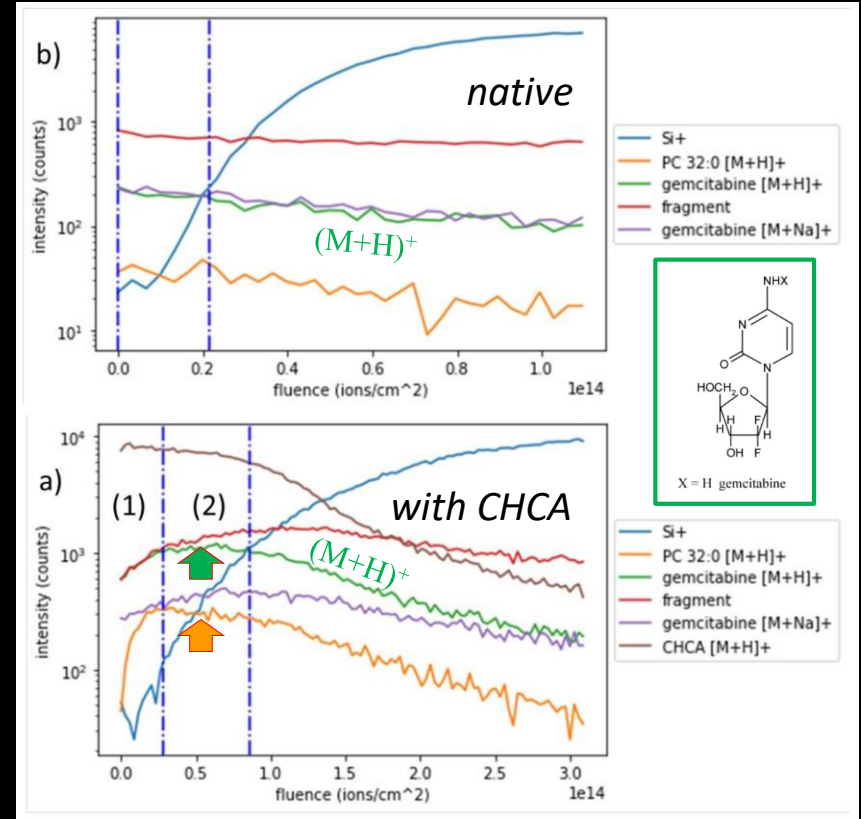
Enhancement of drug signal in the brain

- Gemcitabine, anticancer prodrug, hydrophilic ($\log P = -1.4$)
- Nucleoside analog used as the standard treatment against advanced pancreatic cancer and other solid tumors



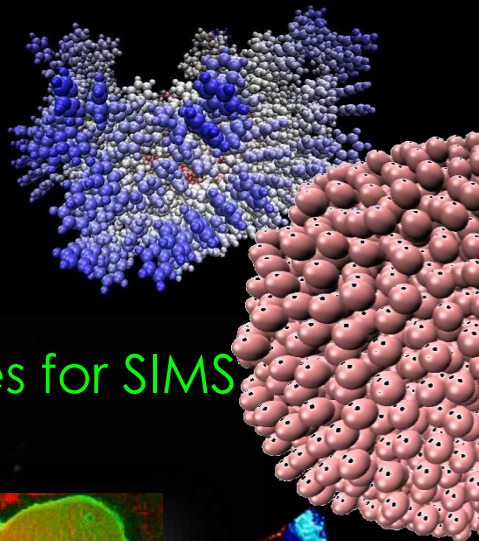
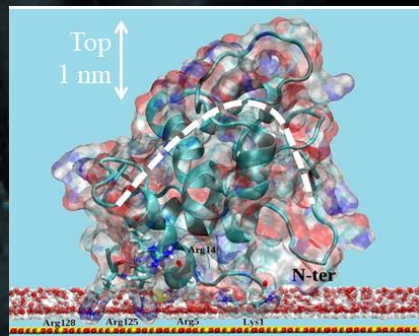
- 2 μ l drops (2-10 g/L) were spotted on brain cross-sections
- Imaging was conducted prior and after CHCA transfer

Master thesis Colin Nicolay

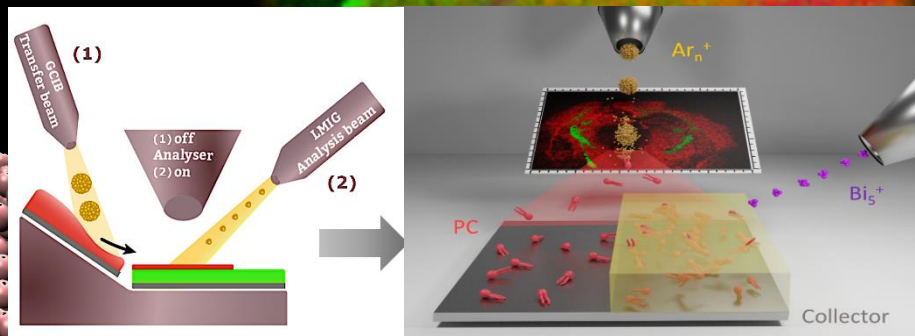


CHCA preferentially enhances $(M+H)^+$, even with a very small layer thickness (5-10nm)

Use of fragments for orientation and sequencing

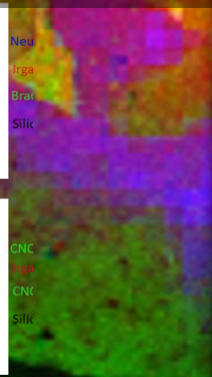
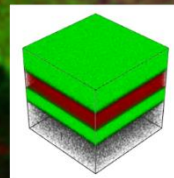
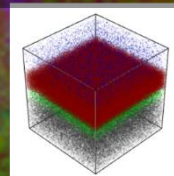
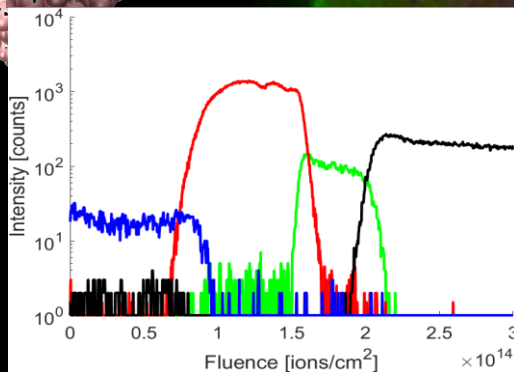
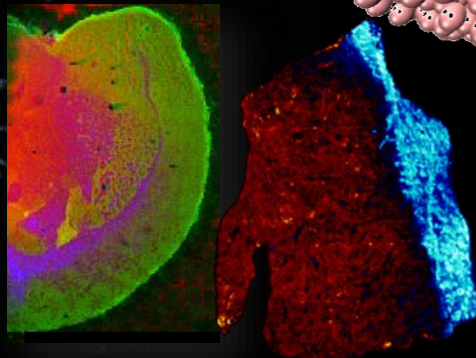
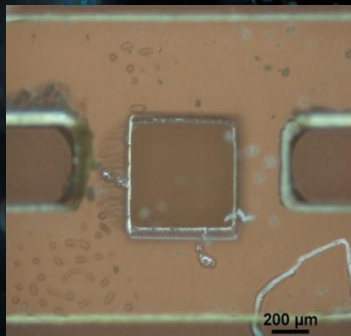


Molecular transfer for microanalysis



New opportunities for SIMS imaging

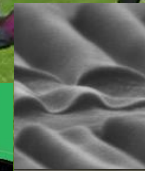
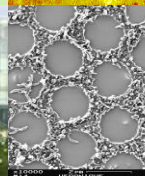
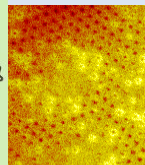
Peptide and protein multilayers can be built in vacuo





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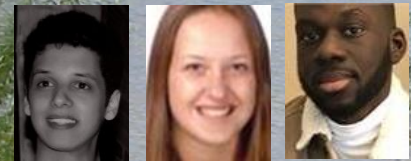
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