UNIVERSITÉ DE STRASBOURG

# Formation CE-MS :

# Couplage électrophorèse capillaire-spectrométrie de masse (CE-MS)

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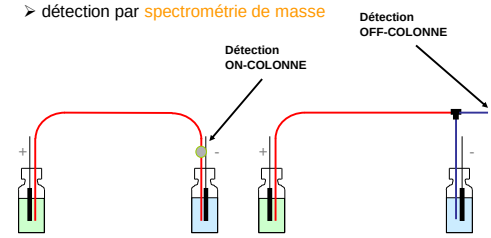
## DETECTION

**LES PLUS COURANTS:**

- détection UV
- détection par fluorescence
- détection par spectrométrie de masse

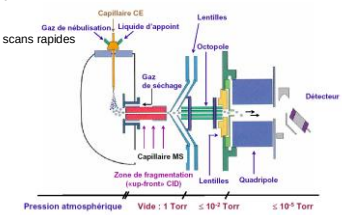



## OFF-COLONNE

- Détection par spectrométrie de masse

➘ **nécessite de concevoir une interface adaptée**

- ⊙ assurer le maintien du champ électrique
- ⊙ diminuer les effets d'aspiration
- ⊙ utiliser des analyseurs permettant des scans rapides

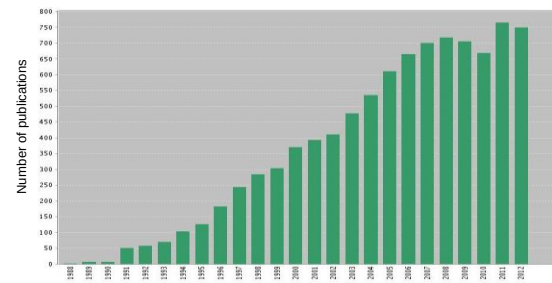



Interface basée sur le mode ESI/MS

appliquée aux sels d'ammonium, amines, dipeptides

ex : pour les ions simples, LOD = 10 amol

## CE–MS Coupling




*Web of science℠ search using term "capillary electrophoresis and mass spectrometry"*

## Couplage CE-MS

Advantages CE-MS ➡ Bonne efficacité
Ultra-low flow rate
Selectivité
Sensibilité
Information de structure

Inconvénients CE-MS ➡ Faible volume d'échatillon (haute concentration)
Compatibilité du BGE avec la MS
Adsorption protéine surface interne
**Difficulté pour maintenir le courant**

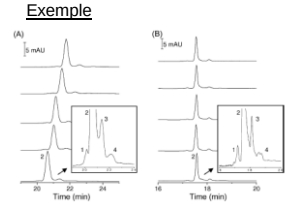
## Les réponses aux limitations

Adsorption des protéines à la surface interne du capillaire

➢Greffage des parois internes du capillaire.

✓Covalent (idéal pour MS)
✓Dynamique (compatible MS)

Exemple




Tampon Tris-Phosphate 400 mM pH 8,5, 30 kV

Répétition d'analyse CE-UV d'une solution d'hormone de croissance (3mg/mL).

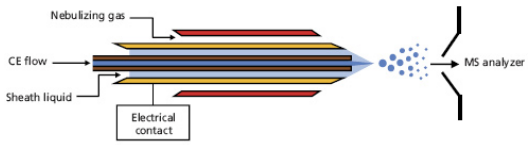
**(A) Capillaire Viège**
- Baisse de l'eof
- Baisse de la résolution

**(B) Capillaire greffé PB-PVS**
- RSD=0,68% (n=5)

***Catai et al. J. Chrom. B., 2006, 852, 160-166***

## Interface Sheath liquid CE-ESI-MS

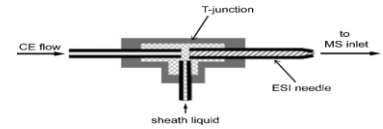
- Interface à liquide additionnel « Sheath liquid » CE-MS la plus communément utilisée
  - *Initially developed by Richard D. Smith group (Olivares et al., Anal. Chem.* ***1987****, 59, 1230-1232)*



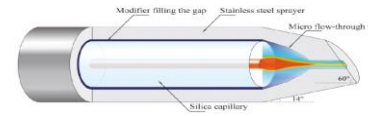

*Haselberg et al., LCGC North America* ***2012****, 30 (6), 504-525*

➢ Sheath liquid induces analytes dilution (~ 4 µl/min) → Significantly reduced sensitivity

## Interface à liquide de jonction « junction liquid »



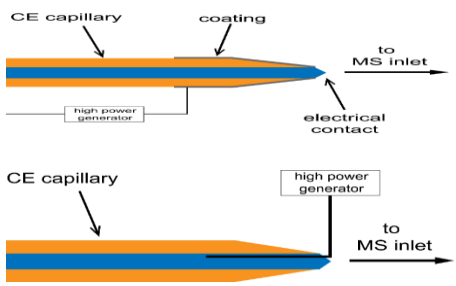

Maxwell, E.J. et al. Analytica Chimica Acta, 2008. **627**(1): p. 25-33.




Zhong, X. et al. Analytical Chemistry, 2011. **83**(12): p. 4916-4923.

➢ Débit > 300 nL/min → Réduction de la sensibilité
Développement instrumentale

## Interface sans liquide additionnel « Sheathliquid »

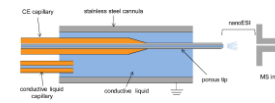



➢ Débit < 100 nL/min → Augmentation de la sensibilité
Modification du capillaire

## Interface CESI-MS

- CESI-MS allows to be operated using nano flowrates

Favorable to ESI ionization




- CESI-MS showed improved sensitivity compared to sheath liquid interface
  - *Faserl et al., Anal. Chem.* ***2011****, 83, 7297-7305*
  - *Busnel et al., Anal. Chem.* ***2010****, 82, 9476-9483*

*Diagram and picture of the CESI interface*

**Optimisation du couplage CE-MS**

## CE-ESI-MS Coupling

**Advantages of CE-MS**

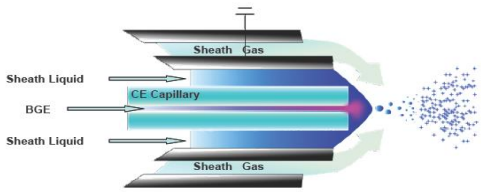
- Great efficiency
- Ultra-low flow rate
- Selectivity
- Sensitivity
- Structural information

**Les réponses aux limitations**

436 Anal. Chem. 1988, 60, 436–441

## Capillary Zone Electrophoresis–Mass Spectrometry Using an Electrospray Ionization Interface

Richard D. Smith,* José A. Olivares,† Nhung T. Nguyen, and Harold R. Udseth
*Chemical Methods and Separations Group, Chemical Sciences Department, Pacific Northwest Laboratory, Richland Washington 99352*




Jusqu'à présent, l'interface « sheathliquid » a été la plus utilisée

## CE-ESI-MS Coupling

CE is a miniaturized technique performing ultra-low flow rates

Decreasing the flow allows for increased sensitivity in the ESI-MS[1]

**"Ultra-low flow" CESI-MS**

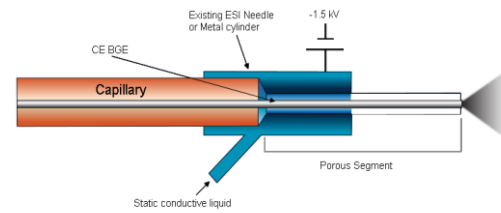
[1]Wilm, Mann International Journal of Mass Spectrometry 1994, 136, 167–180

## Flow rates comparison

| Separation Technology | Column Diameter | Flow-rate (nL/min) |
|---|---|---|
| High flow LC-MS | 2.1 – 4.6 mm | 200,000 – 2,000,000 |
| Microbore LC-MS | 1 mm | 50,000-200,000 |
| Microflow LC-MS | 0.3-0.5 mm | 2,000-50,000 |
| CE-MS | 50-100 µm | 2,000 – 4,000 |
| Nanoflow LC-MS | 50-200 µm | 100-1500 |
| **CESI-MS** | **30 µm** | **< 30** |

CESI allows performing real nano flowrates

## CESI Interface



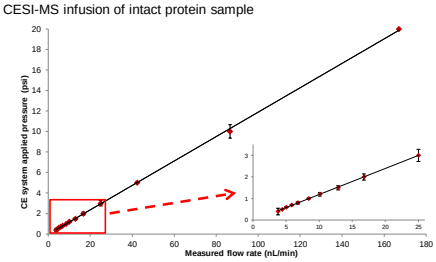

30 µm ID separation capillary with outlet portion etched by HF, provides electrical contact

*Originally developed by M. Moini at U. of Texas and further developed by Beckman Coulter Inc.*

## *What are the accessible flow rates?*

## CESI Interface Achievable Flow rates

- CESI-MS infusion of intact protein sample

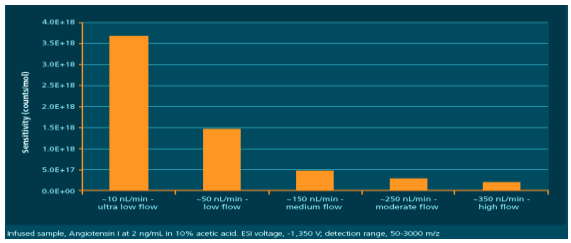



*Conditions : Myoglobin 1 µM (in 10% acetic acid), Flow rates 3, 7 - 170 nL/min, Capillary voltage: -1400V, Investigated m/z : 848,94*

Spray could be obtained using flow rate as low as 4 nL/min

*Gahoual et al, Analytical and Bioanalytical Chemistry 2014, 406 (4), 1029-1038*

## Influence of Flowrates on Sensitivity



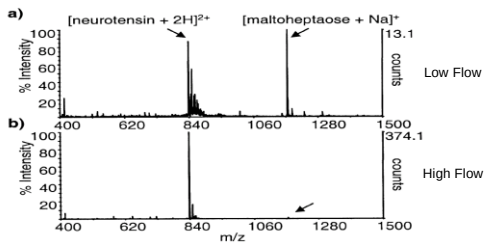

*Evolution of the peak intensity of Angiotensin I (a) and detection sensitivity as a function of the flow rate (b) Experimental conditions: capillary electrophoresis; bare fused silica capillary with a porous tip, total length 88.5 cm×30 μm.i.d.×150 μmo.d.; Infused sample, Angiotensin I at 2 ng/mL in 10% acetic acid. Mass spectrometry; capillary voltage, -1350 V; detection range, 50-3000m/z;*

Decreasing the flowrate from 350 to around 10 nL/min, sensitivity increased by a factor of 20

***Busnel et al, Analytical Chemistry 2010, 82, 9476-9483***

***Decrease of the Ion Suppression Phenomenon at Very Low Flow Rates?***

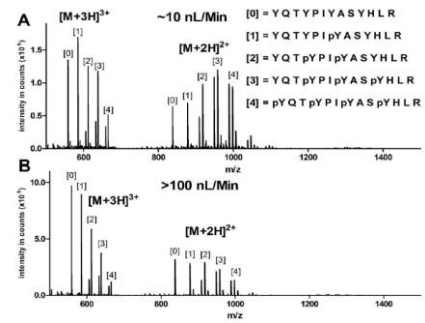
## Importance of Flow Rates in ESI-MS: Ion Suppression




**"Analyte suppression is practically absent at minimal flow rates below 20 nL/min"**

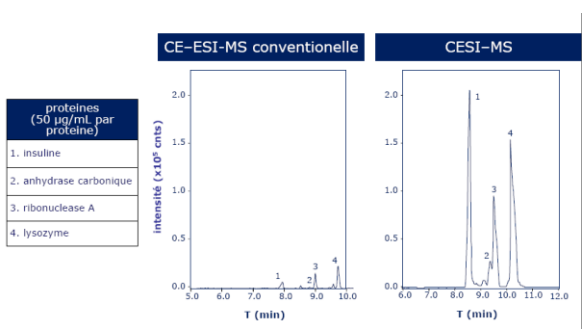
***Schmidt, Karas, Dulcks , J Am Soc Mass Spectrom 2003, 14, 492–500***

## Infusion Pattern as a Function of Infusion Flow Rates




***Heemskerk et al, Analytical Chemistry 2012, 84, 4552-4559***

**Comparaison CE/MS et CESI/MS**




| proteines (50 µg/mL par protéine) |
|---|
| 1. insuline |
| 2. anhydrase carbonique |
| 3. ribonuclease A |
| 4. lysozyme |

***Haselberg et al, J. Chrom., 2010, 1217, 7605-7611***

**Comparaison CE/MS et CESI/MS**

| limites de détection (nM) | | |
|---|---|---|
| protéine | CESI–MS | CE–ESI-MS |
| insuline | 1.3 | 106 |
| anhydrase carbonique | 0.58 | 79 |
| ribonuclease A | 0.62 | 33 |
| lysozyme | 0.50 | 41 |

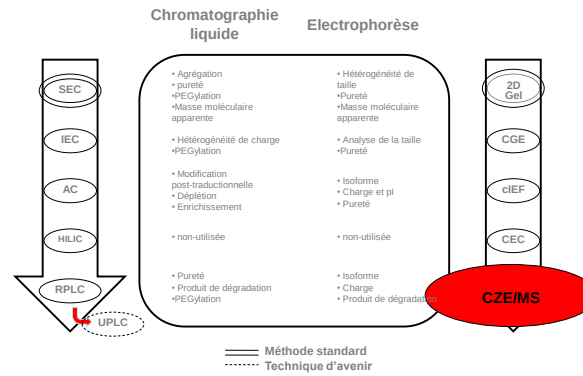
- Limites de détection sub-nM
- 50-135 X de sensibilité

| | RSDs, n=15 | | linéarité |
|---|---|---|---|
| protéine | T de migration | peak area | $R^2$ |
| insuline | 0.63% | 8.5% | 0.999 |
| anhydrase carbonique | 0.61% | 6.3% | 0.989 |
| ribonuclease A | 0.68% | 8.4% | 0.992 |
| lysozyme | 0.74% | 7.0% | 0.997 |

- **Faible limite de détection**
- **Répétabilité sur les temps de migration**
- **Bonne linéarité**

# Applications

**Applications**




*Staub et al, J. of Pharm. Biomed. Anal., 2011, 55, 810-822*

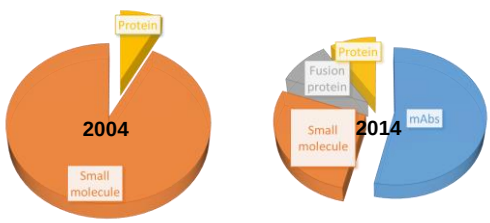
**Le couplage CZE-ESI/MS**

- Séparation suivant des différences de mobilités électrophorétiques
- Nature de l'électrolyte support :
  - ✓ Volatile
  - ✓ Peu concentré en sel
  - ✓ Compatible pour des études en non dénaturant
- Efficacité inversement proportionnelle au coefficient de diffusion des molécules.
- Grand champ d'applications :
  - Séparation d'isoformes
  - Détermination de PTMs (Glycolsylation, phosphorlation…)
  - Analyse des produits de dégradation
  - Détermination de constante de stabilité

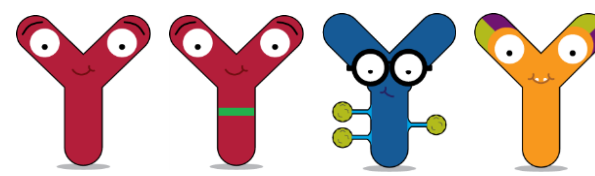
Application to the characterization of biotherapeutics using CE-MS

## Biotherapeutic revolution

Pharmaceutical market – Top 10 sales




## mAbs and related products

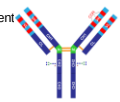


**Monoclonal antibody** | **Biosimilar antibody** | **Antibody Drug conjugate** | **Bispecific antibody**

https://www.gene.com/stories/genentech-antibodies-at-

## Therapeutic proteins: origin, importance and market

- Monoclonal antibody (mAb) therapeutics attract the most interest due to their strong therapeutic poter
  - In January 2017, 68 were marketed
  - more than 50 mAbs in clinical trial phase III
- mAbs specificity for its antigen opens new avenues for therapeutic treatment
  - oncology
  - autoimmune diseases
  - Transplant rejection prevention

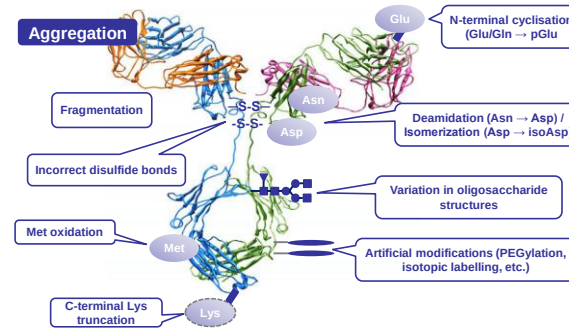


- mAbs are complex and heterogeneous glycoproteins representing a challenge to analytical sciences
  - Characterization on different level of the mAbs
  - Necessity of precise and high throughput characterization

*FDA, 2017/08*
*Zhang Z. et al., Mass Spec. Rev., **2009** (28), 147-176*



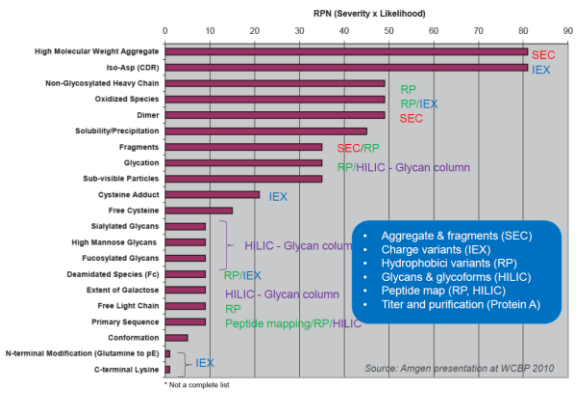
## mAbs heterogeneity



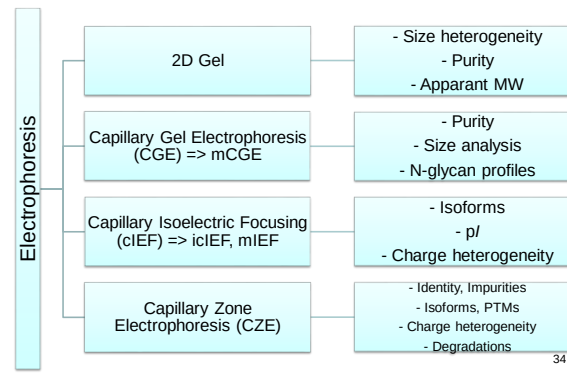

Adapté de TOSOH BIOSCIENCE

**Isofoms influence therapeutic efficiency**

## Significance of each mAbs isoforms



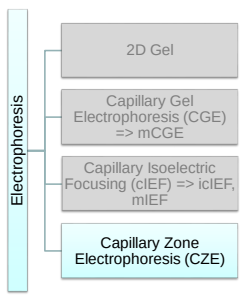

## Electrophoretic approaches to characterize biopharmaceuticals






## Electrophoretic approaches to characterize biopharmaceuticals

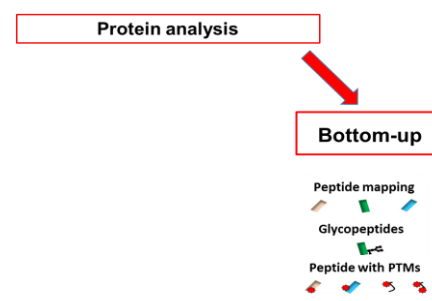
CE **can be coupled to MS** to characterize proteins in different levels (bottom, middle, top)

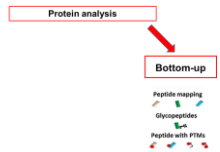





*Fekete et al., Anal. Chem. 2016, 88, 480−507*
*Gahoual R. et al., J. Chromatrogr. B. 2016, In press*

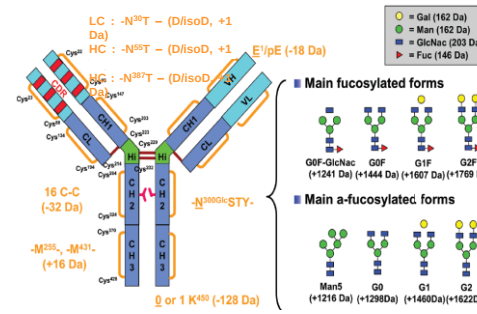
## mAbs characterization Workflow






1. Monoclonal antibodies primary structure characterization by CE-ESI-MS
2. Biosimilarity assessment by CE-ESI-MS
3. Antibody-drug-conjugates primary structure and drug loaded-peptide characterization by CE-ESI-MS

## Monoclonal Antibody

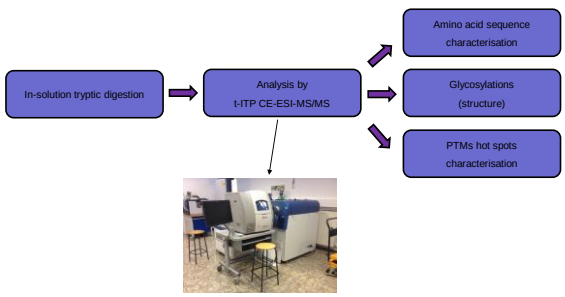



Average mass: 148,057 Da (1,328 a.a.)

*A. Beck et al., Anal. Chem. 2012, 84, 4637-4646*

## mAbs characterisation workflow

- Primary structure characterisation workflow based on bottom-up proteomics strategy




*CESI8000 coupled to 5600 TripleTOF MS*

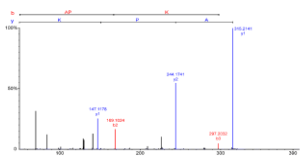
## Amino acid sequence characterisation

- MS/MS amino acid sequence characterisation (trastuzumab)


100% sequence coverage achieved in a single injection through **only purely tryptic unmodified peptides**

*Gahoual R. et al., Anal. Chem., **2014** (86), 9074-9081*

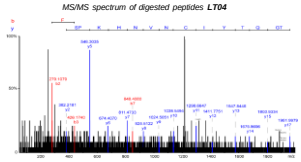
## Amino acid sequence characterisation



*MS/MS spectrum of digested peptides **LT04***

**APK**

(m/z 315.2039 ; 2+)



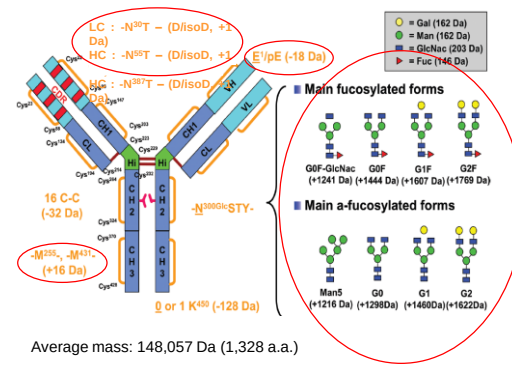
*MS/MS spectrum of digested peptides **HT15***

**DYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTK**

(63 amino acids ; m/z 1119.898 ; 6+)

Implementation of CE allows separation and successful detection of a larger variety of peptides

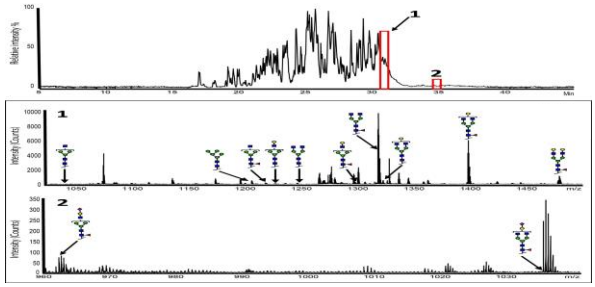
## Trastuzumab (Herceptin)




Average mass: 148,057 Da (1,328 a.a.)

*A. Beck et al., Anal. Chem. 2012, 84, 4637-4646*

## Trastuzumab glycosylation characterization



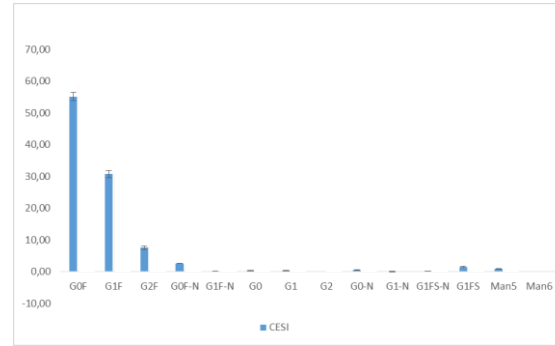
- Overall results in one injection

Detection of 14 different glycosylations (MS) → 9 structures characterized (MS/MS)

*Gahoual R. et al., Anal. Chem.*, ***2014*** (86), 9074-9081

## Glycosylations characterization

- Glycopeptides MS signal intensity used to estimate glycoforms relative abundances




*Gahoual R. et al., Anal. Chem.*, ***2014*** (86), 9074-9081

## Glycosylations characterization

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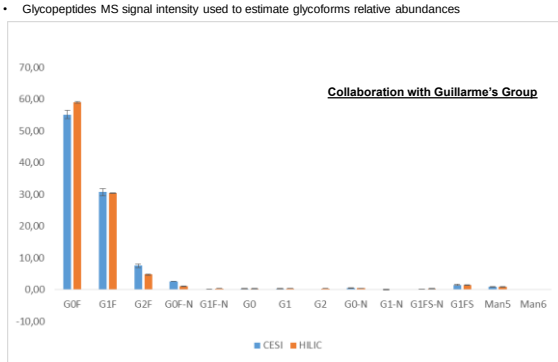
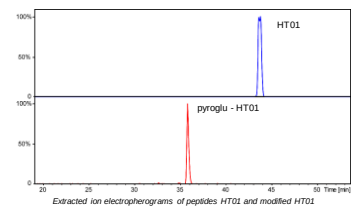



*Giorgetti J. et al., Talanta*, ***2018,*** 178, 530-537

## PTMs hot spots characterization

N-terminal glutamic acid cyclization characterization



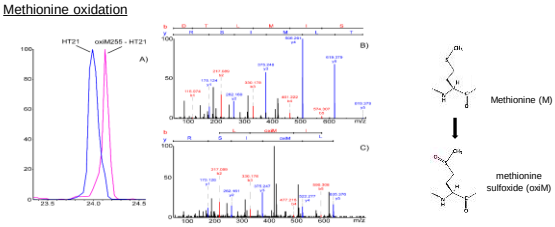

- CE mechanism separates of peptide with N-terminal glutamic acid cyclization from the unmodified peptide

Results suggest partial modification of sample → Favorable conditions to estimate sample modification level

*Gahoual R. et al., Anal. Chem.*, ***2014*** (86), 9074-9081

## PTMs hot spots characterization

Methionine oxidation



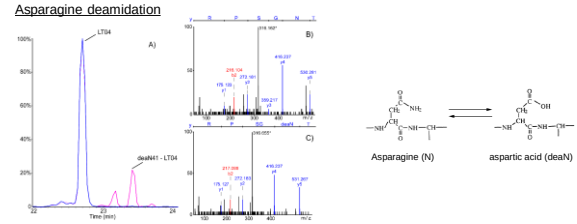

- Methionine oxidation causes peptide mass shift (+15.99 Da) leading to the separation of the modified peptide in CZE

confirmed by MS/MS spectra

*Gahoual R. et al., Anal. Chem.*, ***2014*** (86), 9074-9081

## PTMs hot spots characterization

Asparagine deamidation



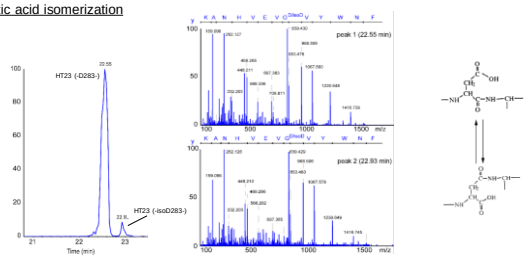

- Deamidation (+ 0.98 Da) involves mobility change in CZE enabling the separation of the unmodified peptide

CE separation of deamidated peptides eases the identification of the modification by MS

*Gahoual R. et al., Anal. Chem.*, ***2014*** (86), 9074-9081

## PTMs hot spots characterization

Aspartic acid isomerization



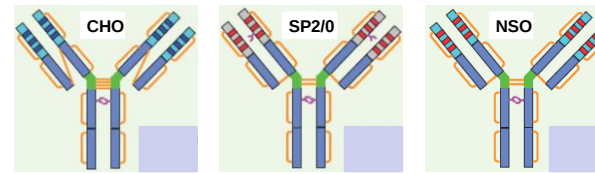

*EIEs and MS/MS spectra of peptides HT23 (intact and modified)*

CE separation prior to MS analysis allows in this particular case to include aspartic acid isomerization in the overall characterization workflow

*Gahoual R. et al., Journal of Mass Spectrometry, **2016** (51), 150-158*

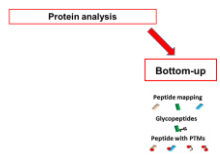
## Application of the CE-ESI-MS workflow

Characterization of 12 differents mAbs




- 100% amino acid sequence characterization
- 14 glycoforms characterization and quantification
- All PTMs hot spots characterization

Results consistencies demonstrate the robustness of the characterization strategy




1. Monoclonal antibodies primary structure characterization by CE-ESI-MS
2. Biosimilarity assessment by CE-ESI-MS
3. Antibody-drug-conjugates primary structure and drug loaded-peptide characterization by CE-ESI-MS

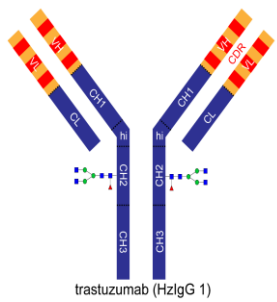
## mAbs biosimilarity assessment

- As several mAbs patent are ending in the next few months/years, other companies should have the possibility to commercialize « unprotected » mAbs
- mAbs complexity and production process (cell line selection) makes it nearly impossible to produce strictly the same product as the innovator company
- FDA and EMA are introducing guidelines to help biopharma companies to determine the key features needed for a biosimilarity between two products in term of structure, PK and PD => reducing clinical trials

Guidelines → biosimilars approval

*EMA, CHMP/437/04*
*EMA, CHMP/437/04 Rev 1*

## Monoclonal Antibody




*Gahoual R. et al., mAbs **2014**, 6, 1464-1473*

*1st case*

## trastuzumab vs. candidate biosimilar

Herceptin trastuzumab

## Amino acid sequence similarity

- Complete sequence coverage obtained for trastuzumab
- Biosimilar candidate sequence could be successfully identified except HC **K$^{217}$**

Suggesting an amino acid substitution between the two samples


*Gahoual R. et al., mAbs **2014**, 6, 1464-1473*

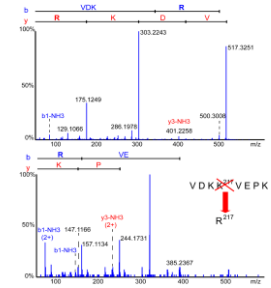
## Amino acid sequence similarity

Interpretation of unidentified MS/MS spectra

Unambiguous characterisation of the amino acid substitution of biosimilar candidate

V D K **R$^{217}$** V E P K

rejected candidate

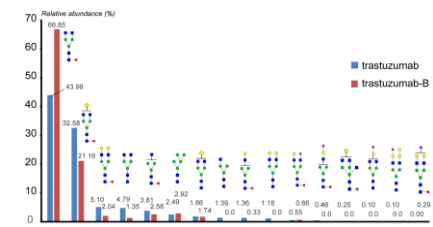



*CESI-MS/MS spectra of trastuzumab biosimilar candidate*

*Gahoual R. et al., mAbs **2014**, 6, 1464-1473*

## Glycoforms characterisation

- Glycosylation distribution evaluated for each sample using CESI-MS/MS data




Identification of a significant number of glycoforms → Minor differences of glycoforms could be distinguished

*Gahoual R. et al., mAbs **2014**, 6, 1464-1473*

## PTMs characterisation

| position | sequence | PTM | Trastuzumab distribution | | Trastuzumab-B distribution | |
|---|---|---|---|---|---|---|
| | | | unmodif | modif | unmodif | modif |
| 1 -19 | EVQLVESGGGLVQPGGSLR | E$^{1}$ / pE$^{1}$ | 98.2 | 1.8 | 97.3 | 2.7 |
| 51 - 59 | IYPTNGYTR | N$^{55}$ / deaN$^{55}$ | 89.4 | 10.6 | 92.3 | 7.7 |
| 99 - 124 | WGGDGFYAMDYWGQGTLVTVSSASTK | D$^{102}$ / isoD$^{102}$ | 84.9 | 15.1 | 86.0 | 14.0 |
| 252 - 258 | DTLMISR | M$^{255}$ / oxiM$^{255}$ | 95.3 | 4.7 | 94.5 | 5.5 |
| 259 - 277 | TPEVTCVVVDVSHEDPEVK | D$^{272}$ / isoD$^{272}$ | 94.4 | 5.6 | 94.5 | 5.5 |
| 278 - 291 | FNWYVDGVEVHNAK | D$^{283}$ / isoD$^{283}$ | 92.0 | 8.0 | 96.5 | 3.5 |
| 374 - 395 | GFYPSDIAVEWESNGQPENNYK | N$^{387}$ / deaN$^{387}$ | 85.8 | 14.3 | 100.0 | 0.0 |
| 420 - 442 | WQQGNVFSCSVMHEALHNHYTQK | M$^{431}$ / oxiM$^{431}$ | 97.8 | 2.2 | 95.2 | 4.8 |
| 396 - 412 | TTPPVLDSDGSFFLYSK | D$^{404}$ / isoD$^{404}$ | 93.0 | 7.0 | 93.9 | 6.1 |
| 25 - 42 | ASQDVNTAVAWYQQKPGK | N$^{30}$ / deaN$^{30}$ | 96.1 | 3.9 | 96.0 | 4.0 |
| 150 - 169 | VDNALQSGNSQESVTEQDSK | D$^{167}$ / isoD$^{167}$ | 64.0 | 36.0 | 91.6 | 8.4 |
| 170 - 183 | DSTYSLSSTLTLSK | D$^{170}$ / isoD$^{170}$ | 86.6 | 13.4 | 92.9 | 7.1 |

*Gahoual R. et al., mAbs **2014**, 6, 1464-1473*

## PTMs characterisation

N-Terminal glutamic cyclization and methionine oxidation

| position | sequence | PTM | Trastuzumab distribution | | Trastuzumab-B distribution | |
|---|---|---|---|---|---|---|
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| 51 - 59 | IYPTNGYTR | N$^{55}$ / deaN$^{55}$ | 89.4 | 10.6 | 92.3 | 7.7 |
| 99 - 124 | WGGDGFYAMDYWGQGTLVTVSSASTK | D$^{102}$ / isoD$^{102}$ | 84.9 | 15.1 | 86.0 | 14.0 |
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*Gahoual R. et al., mAbs **2014**, 6, 1464-1473*

## PTMs characterisation

Asparagine deamidation

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|---|---|---|---|---|---|---|
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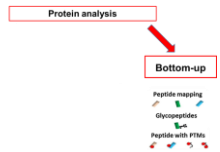
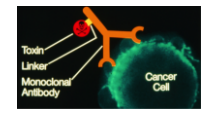
*Gahoual R. et al., mAbs **2014**, 6, 1464-1473*




1. Monoclonal antibodies primary structure characterization by CE-ESI-MS
2. Biosimilarity assessment by CE-ESI-MS
3. Antibody-drug-conjugates primary structure and drug loaded-peptide characterization by CE-ESI-MS

## ADC characterisation by CE-ESI-MS/MS

- Antibody drug conjugates (ADCs): New class of biopharmaceutical drugs for cancer treatment




http://adcreview.com/knowledge-center/adcs-101/how-do-antibody-drug-conjugates-work/

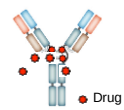
- Three ADCs were approved by the US Food and drug Administration
  - Brentuximab vedotin (Adcetris)
  - ado-trastuzumab emtasine (Kadcyla)
  - Inotuzumab ozogamicin (Besponsa)
- More than 50 are investigated in clinical trials.
- ADCs are complex and heterogeneous glycoproteins representing a challenge to analytical sciences
  - Characterization on different level of the ADCs
  - Necessity of precise and high throughput characterization

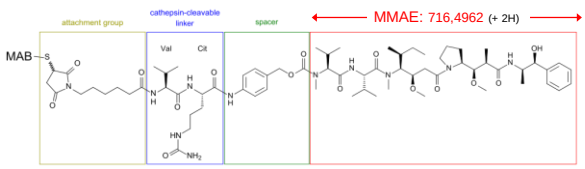
## Brentuximab vedotin

**Cysteine-linked ADCs**

Use for the treatment of people with cancer (since 2011):

- for treatment of Hodgkin lymphoma (HL)
- systemic anaplastic large cell lymphoma (ALCL)



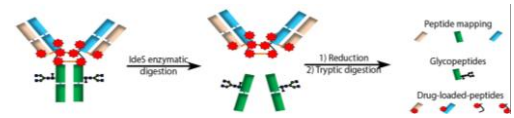




MMAE: monomethyl auristatin E

## ADC characterisation by CE-ESI-MS/MS

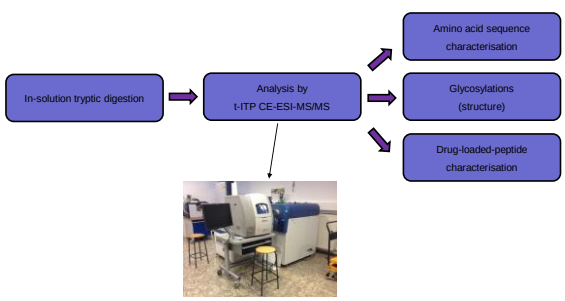
**Objectives :**

**Characterization**
- Amino acid sequence
- N-glycosylation
- Drug-loaded-peptides




## mAbs characterisation workflow

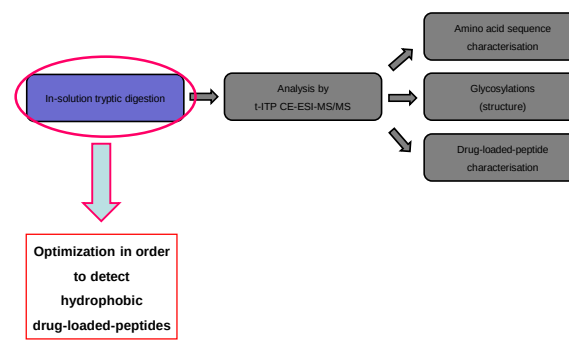
- Primary structure characterisation workflow based on bottom-up proteomics strategy




*CESI8000 coupled to 5600 TripleTOF MS*

## mAbs characterisation workflow

- Primary structure characterisation workflow based on bottom-up proteomics strategy



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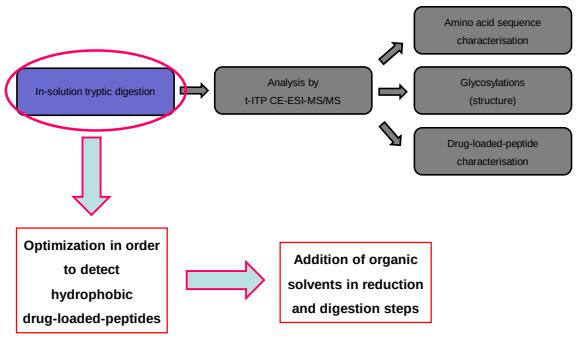


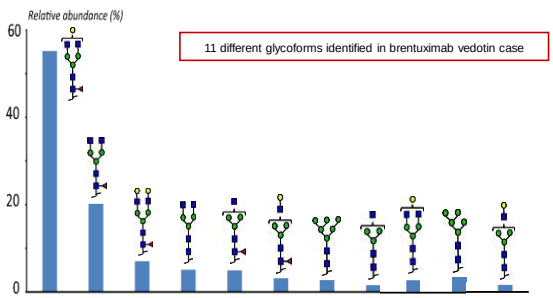

## Amino acid sequence characterisation

- MS/MS amino acid sequence characterization (Brentuximab Vedotin)


*Said et al, Analytica Chimica Acta 2016, 918, 50-59.*

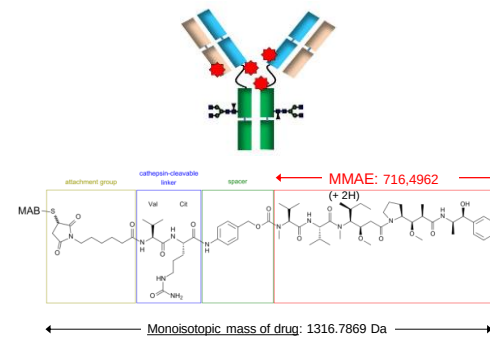
## Glycosylations characterization

- Glycopeptides MS signal intensity used to estimate glycoforms relative abundances




Modification of the enzymatic protocol, especially the presence of acetonitrile and isopropanol did not affect the characterization of glycopeptides

## Drug-loaded-peptide characterization




MMAE: monomethyl auristatin E

## Drug-loaded-peptide localization

QIQLQQSGPEVVKPGASVKISCKASGYTF DIVLTQSPASLAVSLGQRATISCKASQSVD
TDYYITWVKQKPGQGLEWIGWIYPGSGN FDGDSYMNWYQQKPGQPPKVLIYAASN
TKYNEKFKGKATLTVDTSSSTAFMQLSSLT LESGIPARFSGSGSGTDFTLNIHPVEEEDA
SEDTAVYFCANYGNYWFAYWGQGTQVT ATYYCQQSNEDPWTFGGGTKLEIKRTVAA
VSAASTKGPSVFPLAPSSKSTSGGTAALG PSVFIFPPSDEQLKSGTASVVCLLNNFYPR
CLVKDYFPEPVTVSWNSGALTSGVHTFPA EAKVQWKVDNALQSGNSQESVTEQDSK
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVN DSTYSLSSTLTLSKADYEKHKVYACEVTHQ
HKPSNTKVDK KVEPKSCDK THTCPPCPA GLSSPVTKSFNRGEC
PELLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYR VVSVLTVLHQDWLNGK E
YKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG
Drug-loaded Peptide
Position of the drug

| Chain | Disulfide interchain | Séquences | Nb of vcMMAE |
|---|---|---|---|
| HC | LC-HC | $^{219}$SCDK$^{222}$ | 1 |
| LC | LC-HC | $^{212}$GEC$^{214}$ | 1 |
| HC | HC-HC | $^{223}$THTCPPCPAPELLG$^{237}$ | 1 |
| HC | HC-HC | $^{223}$THTCPPCPAPELLG$^{237}$ | 2 |

*Said et al, Analytica Chimica Acta 2016, 918, 50-59.*

## Drug-loaded-peptide characterization

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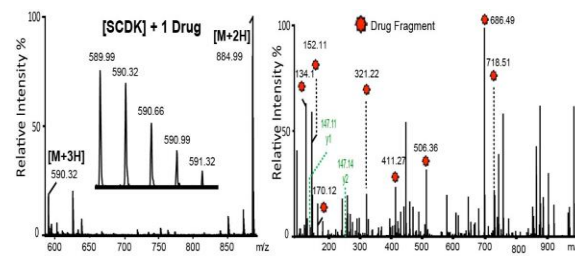


*Said et al, Analytica Chimica Antica 2016, 918, 50-59.*

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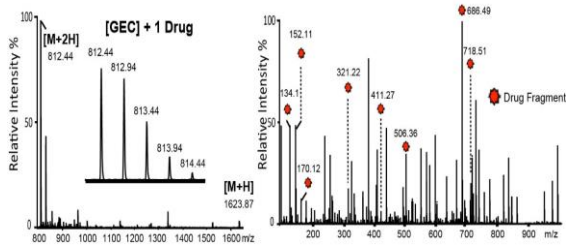


*Said et al, Analytica Chimica Acta 2016, 918, 50-59.*

## Drug-loaded-peptide characterization

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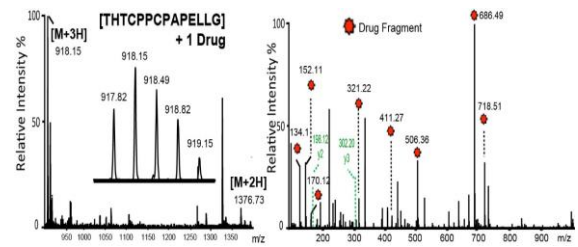


*Said et al, Analytica Chimica Acta 2016, 918, 50-59.*

## Drug-loaded-peptide characterization

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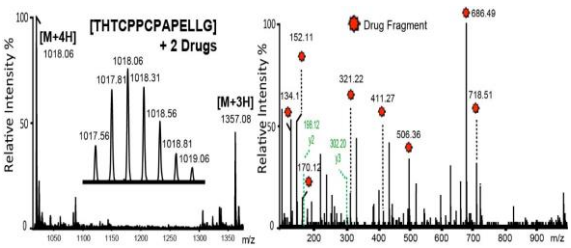
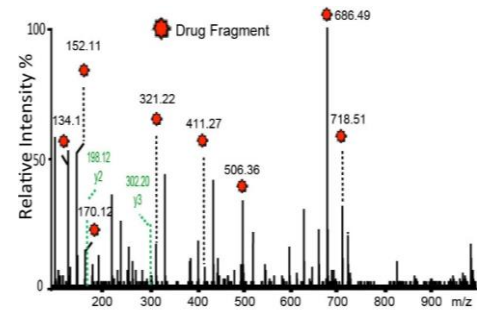



*Said et al, Analytica Chimica Acta 2016, 918, 50-59.*

## Drug-loaded-peptide characterization




Presence of diagnostic ions in the MS/MS spectra

## Conclusion

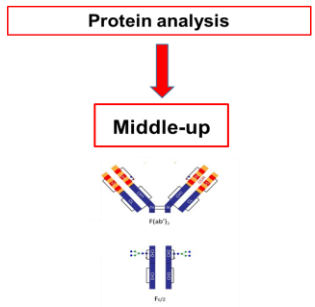
### Primary Struture Characterization

- **Monoclonal antibodies primary structure characterization by CE-ESI-MS**
- **CE-ESI-MS/MS enabled biosimilarity assessment regarding primary structure and PTMs**
- **Primary structure characterization of ADCs**
- **Structural characterization of drug-loaded-peptide**

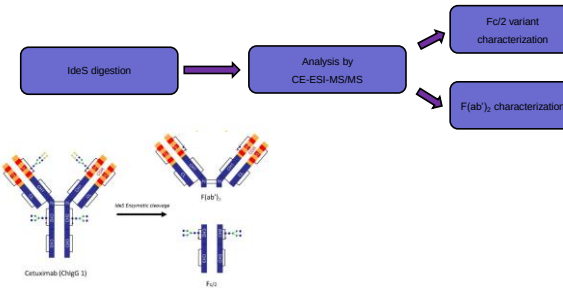
## mAbs characterization Workflow

**Protein analysis**

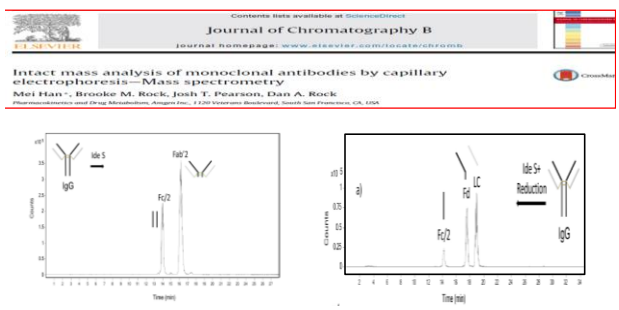
## Middle up approach




## mAbs characterization workflow



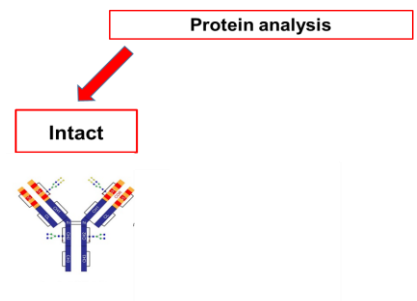

## Middle up approach




Sample: 2 g/L of IgG1 in 15% acetic acid, 15% acetonitrile
Capillary: LPA-coated fused silica (50µm ID, 70 cm lenght), outlet etched to 180 µm OD
BGE: 10% acetic acid; sheath liquid, 0.5% formic acid, 50% MeOH
Separation: 18 kV with 1 mbar pressure on capillary inlet.

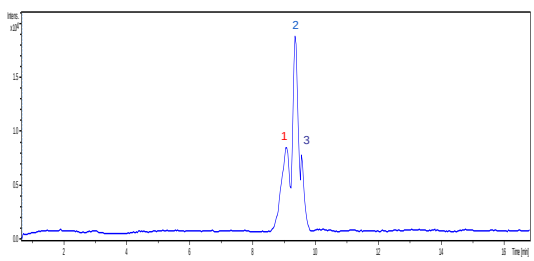
*Han et al, J. Chromatogr. B 2016, 1011, 24-32.*

## Intact protein approach




## Intact mAbs analysis

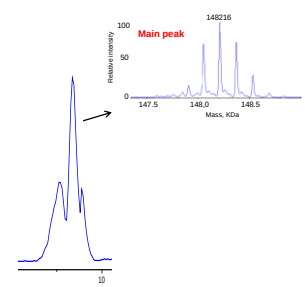
- Analysis of Intact Trastuzumab using positively coated capillary (PEI)




Trastuzumab 1 g/L in MeOH/AA 3% (30/70), BGE AA 3%; 30 kV, injected volume 3 nL

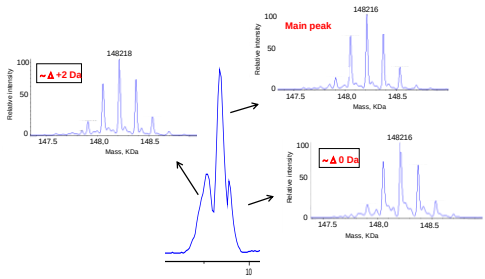
## Intact mAbs analysis

- Deconvoluted MS of Trastuzumab charge variants



## Intact mAbs analysis

- Deconvoluted MS of Trastuzumab charge variants




Potential deamidation of asparagine and isomerization of aspartic acids

## Major issues!!!

- Classical condition described in the literature[1,2]

➢400 mM ε-amino-caproic acid pH 5.7
➢Triethylenetetramine as additive

**Not compatible with direct ESI-MS detection**

**setting up of alternative strategy**

[1]He et al, Anal. Chem. 2010, 82, 3222-3230
[2]Gassner et al, Electrophoresis 2013, 34, 2718-2724

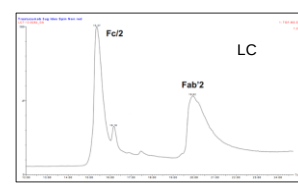
## Couplage CE-MALDI-MS

### Couplage CE/MS

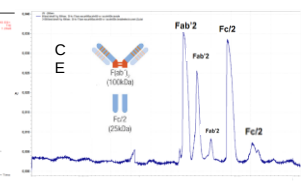
INTERET

Electrophorèse capillaire (CE)

➢ Principe de séparation (Electrocinétique)
➢ **Bonne efficacité**




Séparation sur colonne C4 d'un digestat IdeS de mAbs par LC-MS




Séparation d'un digestat IdeS de mAbs par CE-UV/MALDI-MS

### Couplage CE/MS

INTERET

Electrophorèse capillaire (CE)

➢ Principe de séparation (Electrocinétique)
➢ **Bonne efficacité**

MS

➢ Sélectivité / Précision (qq ppm)
➢ Sensibilité (fmol-Amol)

DIFFICULTES

➢ Maintien du courant
➢ Capacité de chargement du capillaire (qq nL)
➢ Sels Détergents

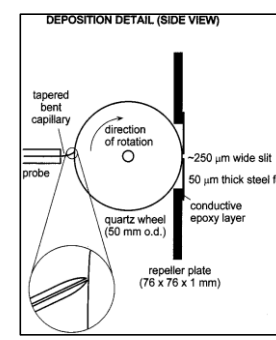
⇨ **MALDI- MS plus de tolérance aux sels**

### Couplage CE/MALDI-MS Direct

***Preisler, Foret, Karger***
***Anal.Chem. 1998, 70, 5278-5287***

**Dépot sur un disque tournant ou sur une boule en rotation**

**Bonne sensibilité mais difficile à mettre en place et très faible robustesse**



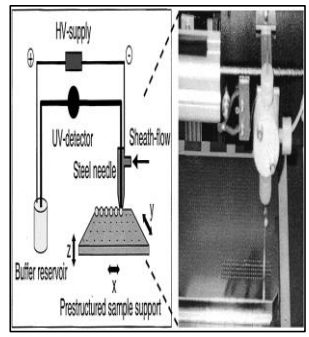
**Couplage indirect**

***Collecteur de fraction avec liquide additionnel***

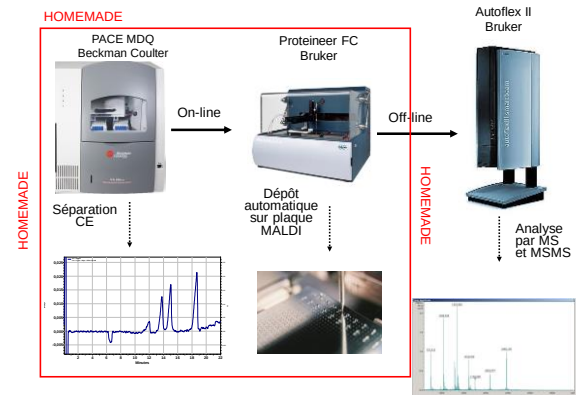
Johnson, Bergquist, Ekman, Nordhoff, Schürenberg, Klöppel, Müller, Lehrach, Gobom
*Anal. Chem.* **2001**, *73*, 1670-1675

**Dépot sur plaque MALDI avec liquide additionnel**

- ✓ **Bonne sensibilité**
- ✓ **Bonne efficacité**
- ✓ **Bonne robustesse**
- ✓ **Capillaire fixe**




**Couplage CE/MALDI-MS indirect**




**Couplage indirect**

***Collecteur de fraction avec liquide additionnel***


**Couplage indirect**

***Collecteur de fraction avec liquide additionnel***

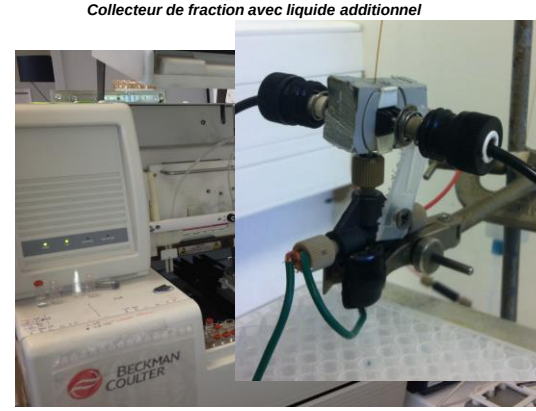
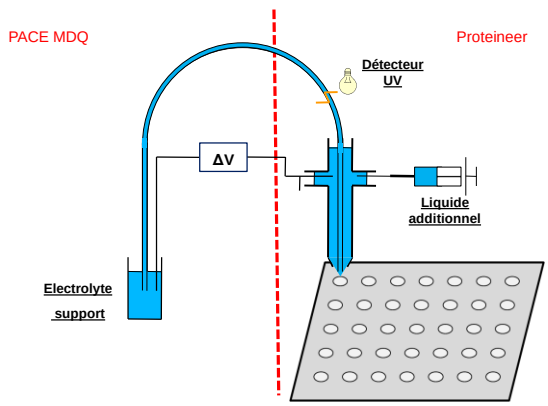
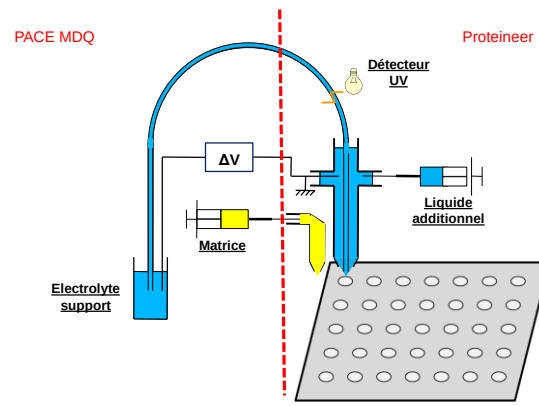



**Schéma du couplage CE-UV/MALDI-MS**



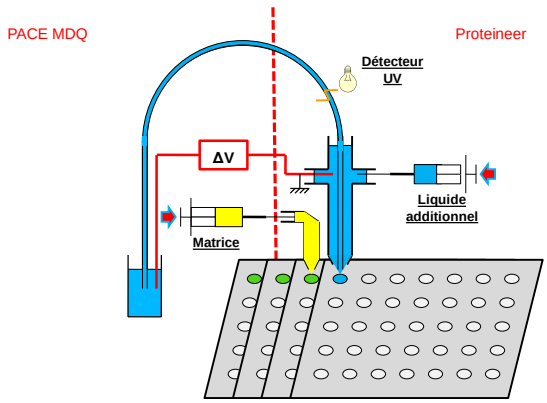

M. Biacchi et al. *Electrophoresis* **2014**, online available

**Schéma du couplage CE-UV/MALDI-MS**




M. Biacchi et al. *Electrophoresis* **2014**, online available

**Schéma du couplage CE-UV/MALDI-MS**



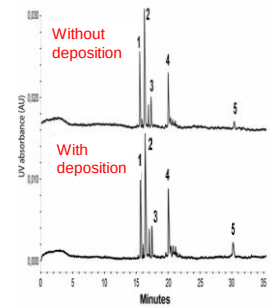

M. Biacchi et al. *Electrophoresis* **2014**, online available

## Intact proteins application

CE separation

Model proteins:

- 1 Cytochrome C
- 2 Lysozyme
- 3 Myoglobine
- 4 Ribonucléase A
- 5 α-lactoglobuline



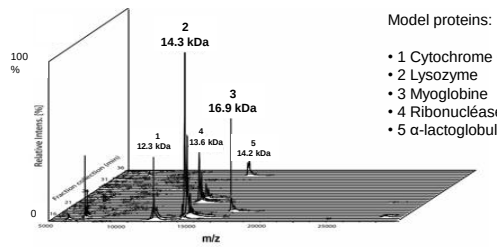

UV Electropherogram without and with fraction collection) of a five protein sample by **CE-UV/MALDI-MS**. Experimental conditions: HPC coated capillary, 50 µm d.i. x 60 cm (detection cell, 60 cm); BGE: 83.3mM ionic strength ammonium acetate (pH 4.0); Voltage: 20 kV; Temperature: 25°C; UV Detection: 200 nm; Injection: 3 kV, 8 min

**No influence of the deposition process**
**Repeatability RSD < 2%**

M. Biacchi et al. *Electrophoresis* **2014**, 35 (20), 2986-2995

## Intact proteins application

CE-MALDI mass spectra




Model proteins:

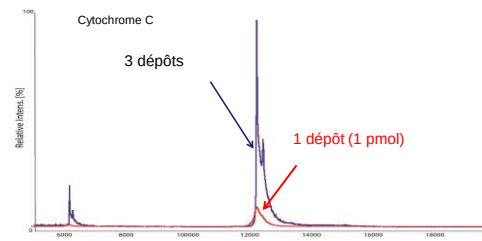
- 1 Cytochrome C
- 2 Lysozyme
- 3 Myoglobine
- 4 Ribonucléase A
- 5 α-lactoglobuline

CE-MALDI mass spectra of 5 proteins sample. Experimental conditions: HPC coated capillary, 50 µm d.i. x 60 cm (detection cell, 60 cm); BGE: 83 mM ionic strength ammonium acetate (pH 4.0); Voltage: 20 kV; Temperature: 25°C; UV Detection: 200 nm; Injection: 3 kV, 8 min

**Respect of separation order**
**No contamination fraction by fraction**
**No carryover**

M. Biacchi et al. *Electrophoresis* **2014**, 35 (20), 2986-2995

**Séparation, Enrichissement, Top-Down d'un mélange de 5 protéines**



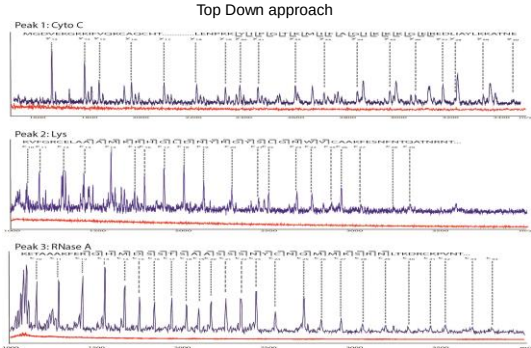

*Superposition des spectres de masse du cytochrome C à 1 (Rouge) et 3 (Bleu) répétitions ; Puissance laser : 71% Matrice : 2,5-dihydroxybenzoïque (DHB) (saturé) dans du TA30 (0.1%TFA, 70% eau et 30% acétonitrile).*

**Absence d'effet mémoire**
**Augmentation du signal en fonction du nombre du dépôt**

**Enrichissement**

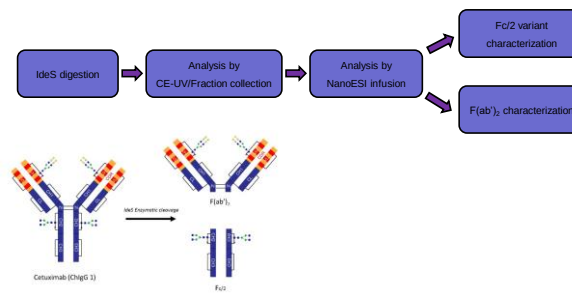
## Intact proteins application

Top Down approach

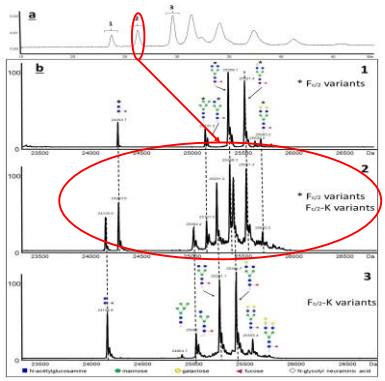



**CE-MALDI-MS allowed Top Down approach by enrichement fraction**

## mAbs characterisation workflow



## Characterisation of $F_{C/2}$ variants



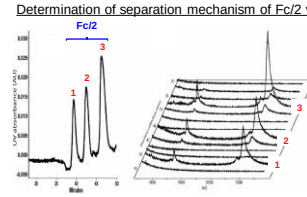
- Characterization of 7 Fc/2 glycoforms.
- Separation due to C-terminal lysine truncation
  - Peak 1 $F_{c/2}$
  - Peak 3 $F_{c/2}$-K

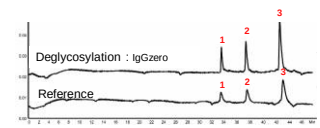
Peak 2: Mixture $F_{c/2}$ and $F_{c/2}$-K

M. Biacchi et al. *Anal. Chem.* **2015**, 87, 6240-6250

## Characterisation of $F_{C/2}$ variants

Determination of separation mechanism of Fc/2 variants:





**Problems of fractionation?**

Analysis by Off-line CE-UV/MALDI-MS :

- No diffusion phenomena.
- No memory effect.

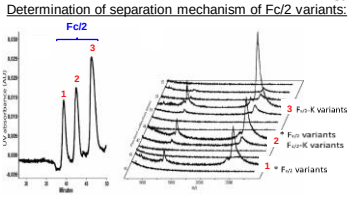
**Separation mechanism?**

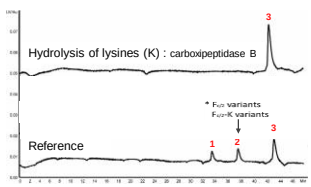
- No effect of glycoforms.

M. Biacchi et al. *Anal. Chem.* **2015**, 87, 6240-6250

## Characterisation of $F_{C/2}$ variants

Determination of separation mechanism of Fc/2 variants:





**Problems of fractionation?**

Analysis by Off-line CE-UV/MALDI-MS :

- No diffusion phenomena.
- No memory effect.

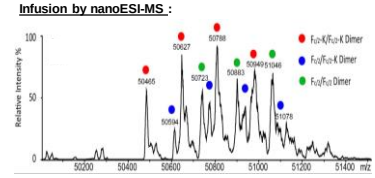
**Separation mechanism?**

- Loss of peak1 and 2 after hydrolysis.
- Separation of peak1 and 3 due to lysine loss.
- Possibility to have interactions between $*F_{c/2}$ et $F_{c/2}$ – K variants of peak2.

M. Biacchi et al. *Anal. Chem.* **2015**, 87, 6240-6250

## Characterisation of $F_{C/2}$ variants

**Infusion by nanoESI-MS :**

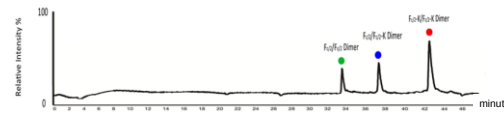


**In CE-UV separation condition :**

25 mM AcONH4+, pH 5.7

⟹ Glycoformes profil of dimers.

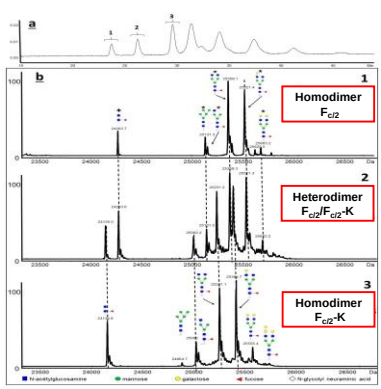
**Separation by CE-UV :**



Separation of $F_{c/2}$ variants by CE-UV due to a separation of dimers.

M. Biacchi et al. *Anal. Chem.* **2015**, 87, 6240-6250

## Characterisation of $F_{C/2}$ variants



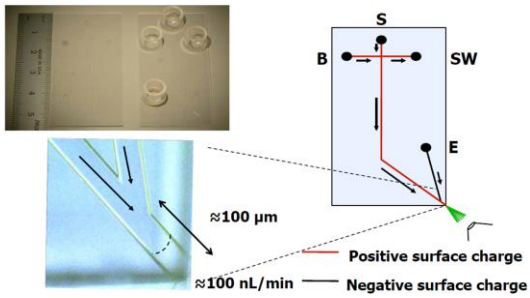
- Characterization of 7 Fc/2 glycoforms.
- Separation due to C-terminal lysine truncation
  - Peak 1 $F_{c/2}$
  - Peak 3 $F_{c/2}$-K

Separation of $F_{c/2}$ dimers

M. Biacchi et al. *Anal. Chim. Acta* **2015**, Submitted

**Couplage µChips-MS**

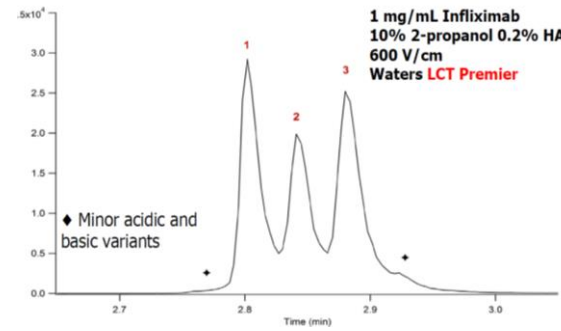
## Microchip CE-ESI Device




**Directly inspired from Ramsey 's presentation in CASSS MS 2016**

J.S. Mellors et al., *Anal. Chem*. **2008.** *80*, p. 6881-6887

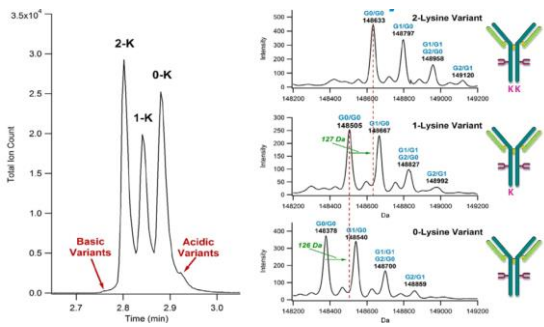
## Separation of intact mAbs




**Directly inspired from Ramsey 's presentation in CASSS MS 2016**

E.A. Redman et al., *Anal. Chem*. **2015.** *87*, p. 2264-2272.

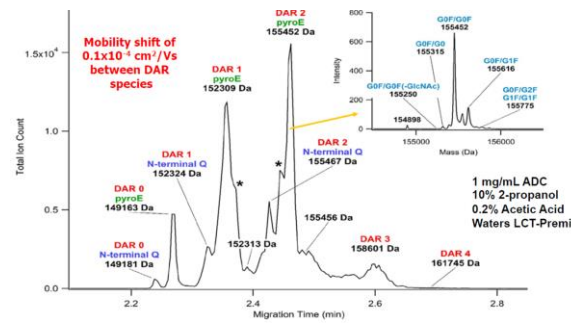
## Separation of intact mAbs




**Directly inspired from Ramsey 's presentation in CASSS MS 2016**

E.A. Redman et al., *Anal. Chem*. **2015.** *87*, p. 2264-2272.

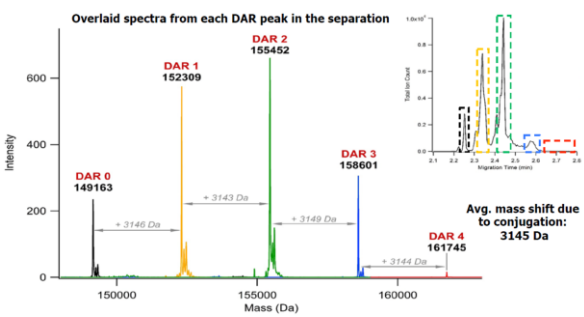
## Separation of Intact ADC




**Directly inspired from Ramsey 's presentation in CASSS MS 2016**

Redman, E. A et al., *Anal. Chem*. 2016, 88 (4), 2220-2226.

## Deconvoluted DAR Mass Spectra



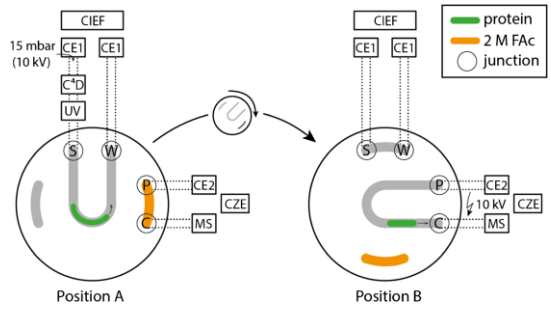

**Directly inspired from Ramsey 's presentation in CASSS MS 2016**

Redman, E. A et al., *Anal. Chem*. 2016, 88 (4), 2220-2226.

**Vers l'infini, et au-delà!!!**

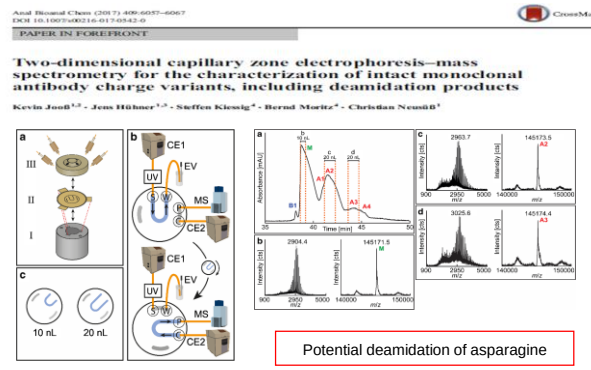
**La 2D-CE-MS**

## 2D CE-ESI-MS




J. Hühner et al., *Anal. Bioanal. Chem*. **2016.** *408*, p. 4055-4061

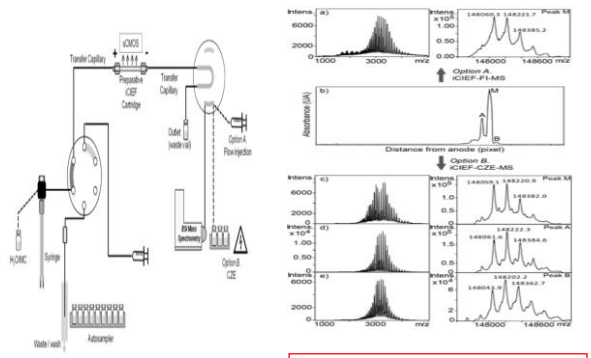
## 2D CZE-ESI-MS




Potential deamidation of asparagine

K. Jooss et al., *Anal. Bioanal. Chem*. **2017.** *409*, p. 6057-6067

## 2D CIEF-CZE-ESI-MS




Potential deamidation of asparagine and succinimide formation (-17 Da) or partial cyclisation of N-terminal glutamic acid (-18 Da)

C. Montealegre et al., *Electrophoresis* **2018.** online available

## Acknowledgments

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*Antony LECHNER*

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*Nina POURHASSAN*
*Alicia BURR*
*Clotilde DEVAUX*
*Camille LINKER*
*Jordan KIRMANN*
*Charly RENARD*
*Stéphanie GROSS*
*Estelle FLIEDEL*
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*Ricky BHAJUN*
*Heidi SOUFFARY*
*Marc LIBERATORE*
*Jasmine HERTZOG*
*Sarah Deras*
*Julie CANONGE*

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*C. MAZOHIE, M. METZELER*
*I. EL MASOUSI, J. HEISER, S. PASHINA*
*T. GUGGENBUHL, C. BALHONZER*

***Strasbourg***

## *Thank you for your attention*