

Nouveaux développements et applications dans l'analyse de protéines intactes par CE-TOF/MS

Aline Staub

14^{èmes} Journées Scientifiques du ccCTA

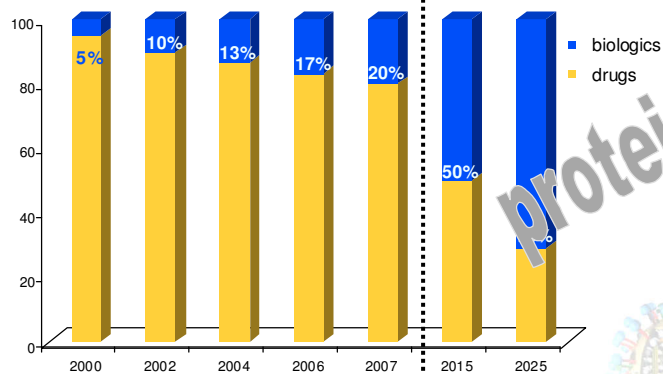
Les Diablerets | 16.09.2010



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ANALYTIQUE PHARMACEUTIQUE

Biologics vs. Drugs

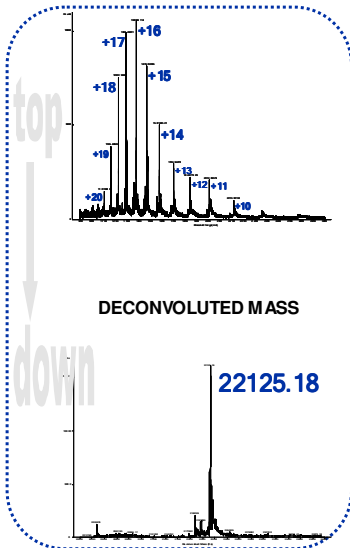
Pharmaceutical blockbusters



Biologics are produced by any technological application that uses **biological systems** or **living organisms** to make or modify products or processes for specific use.



The top-down approach



- The **intact** protein is analyzed by **ESI-MS** with high mass measurement accuracy.
- The identity of the original protein is determined by **deconvolution** of mass spectra from **multiply charged ions**.



Sample preparation is simple
Complete protein sequence is potentially accessible
 High sequence recovery permits to **locate and characterize PTMs**

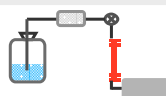
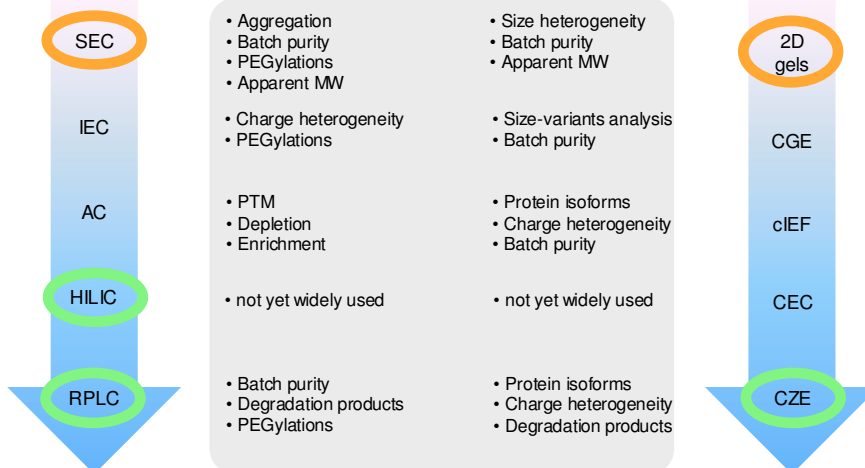


Very **complex mass spectra** are generated by multiply charged protein
 Approach **limited to isolated protein or simple mixtures**



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Intact protein analysis: separative techniques



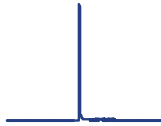
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Strategy for intact protein analysis by CE-MS (1)



High efficiency

$$N = \frac{\mu \cdot I \cdot U}{2 \cdot D \cdot L}$$



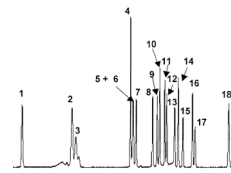
Separation



- Green chemistry
- Ease of operation and maintaining
- No mechanical constraints

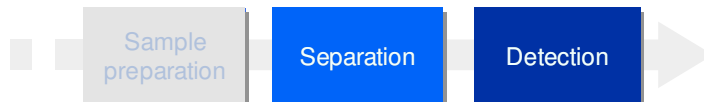
High selectivity

$$\mu = \frac{q}{6 \cdot \pi \cdot \eta \cdot r}$$



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Strategy for intact protein analysis by CE-MS (2)



High efficiency

$$N = \frac{\mu \cdot I \cdot U}{2 \cdot D \cdot L}$$

High selectivity

$$\mu = \frac{q}{6 \cdot \pi \cdot \eta \cdot r}$$



- Green chemistry
- Ease of operation and maintaining
- No mechanical constraints



- High dynamic range
- Identification based on true isotopic pattern

High resolution

$$Rs(FWHM) = \frac{m/z}{W_{50\%}}$$

$Rs(FWHM) = 9000 (m/z 616)$

High accuracy

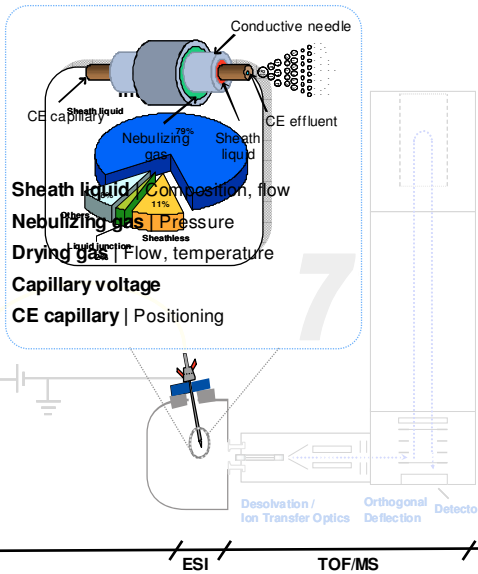
$$bias(ppm) = \frac{\Delta m_{theo-exp}}{m_{theo}}$$



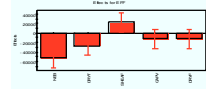
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CE-ESI-TOF/MS | coupling issue

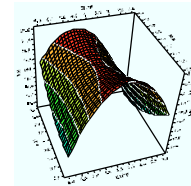
ESI



1. Screening

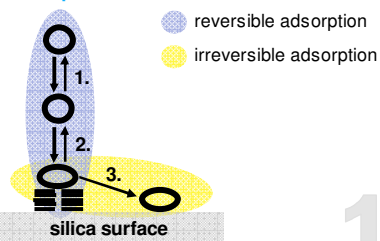


2. Modelization



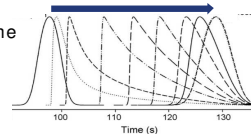
CE-ESI-TOF/MS | adsorption issue

Principles

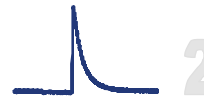


Drawbacks

- Migration time irrepeatability



- Peak tailing
- Loss in efficiency



Classical methods for adsorption minimization

Small molecules

NC(CO)CO

Surfactants

Coatings

New method for direct MS-coupling

- Organic solvent directly in the background electrolyte

Modification of :

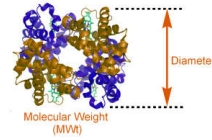
- Electrostatic interactions
- Hydrophobic interactions
- Protein conformation

CE of intact proteins : applications

1

Fundamental studies

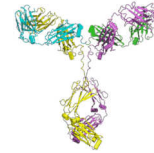
Drug-protein interactions | Protein sizing
Post-translational modifications



2

Pharmaceutical analysis

Quality control | Protein aggregation
Drug seizure | Counterfeits



3

Clinical analysis

Biomarkers discovery | Diagnostics
Toxicology | Forensics | Doping control



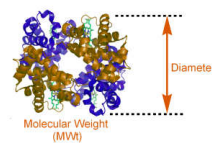
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CE-ESI-TOF/MS of intact proteins : applications

1

Fundamental studies

Drug-protein interactions | Protein sizing
Post-translational modifications



2

Pharmaceutical analysis

Quality control | Protein aggregation
Drug seizure | Counterfeits



3

Clinical analysis

Biomarkers discovery | Diagnostics
Toxicology | Forensics | **Doping control**



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Drug seizure : human growth hormone (hGH)

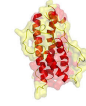


hGH



- polypeptide hormone secreted by anterior pituitary gland
- protein chain of 191 aa | pI 5.1
- endogenous hGH presents several isoforms | **22 kDa** (most abundant one), 20 kDa, 17 kDa, ...
- physiologic function | to stimulate growth and cell reproduction
- pulsating secretion | 20-30 min half-life

rhGH

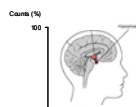


- production by recombinant DNA technology
- recombinant hGH possesses an identical sequence to the naturally occurring **22 kDa** hormone
- therapeutic use | to treat shortness due to numerous reasons
- illegal use in doping | abuse in esthetics



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hGH | CE-ESI-TOF/MS

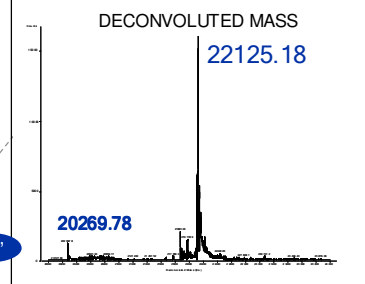
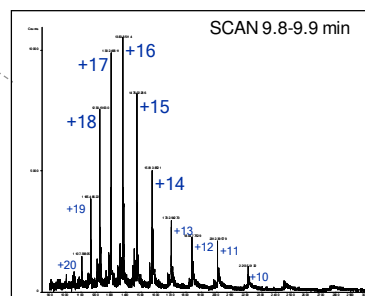


N = 750'000

CE-ESI-TOF/MS

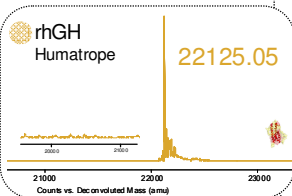
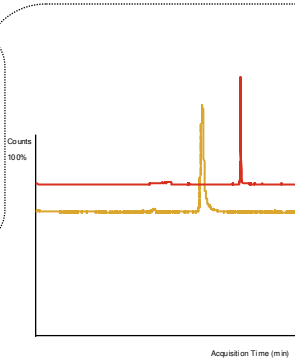
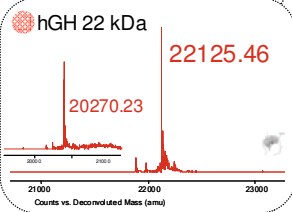
- unambiguous identification
- 22 kDa / 20 kDa ratio | ca. 5%

10'



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hGH vs. rhGH | CE-ESI-TOF/MS



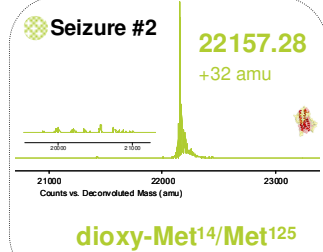
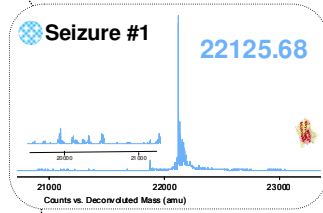
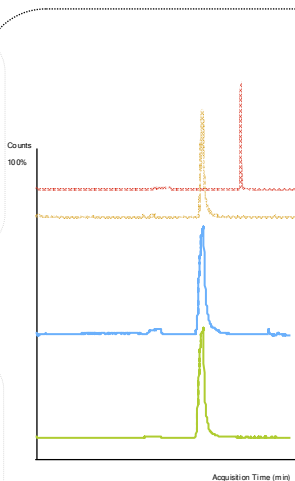
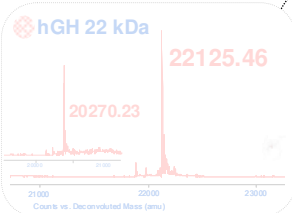
CE-ESI-TOF/MS

- 2 selectivity levels
- hGH vs. rhGH discrimination



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Seized samples analysis | CE-ESI-TOF/MS

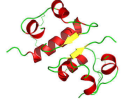


Slaub et al., Electrophoresis (in press)



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Quality control: identification and quantitation

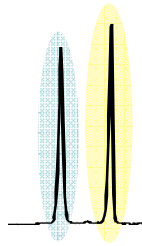


- Biopharmaceutical formulations
- Quality control | concentration, identification
- Counterfeits | lower/higher concentration, no/wrong active principle,...

TOF

- **Identification** | major multicharged ions and deconvoluted mass spectrum
- **Quantitation** | internal standard choice ?

- Stable isotopically labeled (SIL) IS
- Structural analogues



Double injection
Standard of the protein



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Quantitation : multiple injection technique

- each run / two injections:

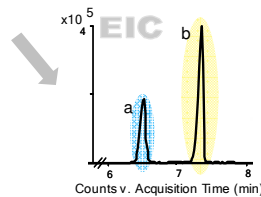
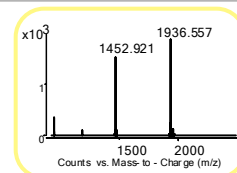
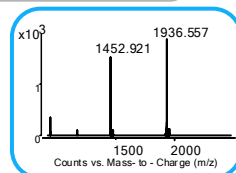
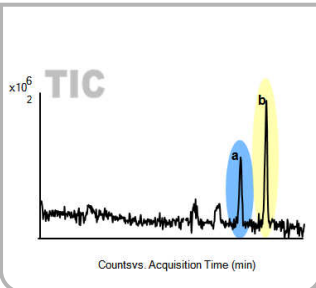
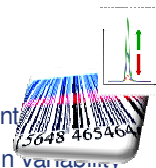
- 1st injection = protein standard
- 2nd injection = protein to be quantified

Protein ID - exact masses / comparison with standard

Protein Quantification - Ratio peak area / standard peak area

Double injection

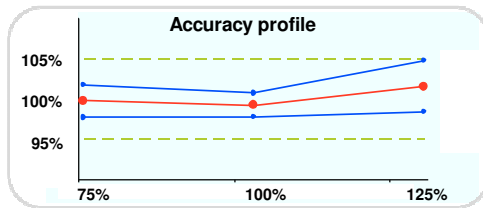
correct ionization
variability



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Quantitation : validation results

	Trueness <i>Relative bias</i>	Repeatability <i>RSD</i>	Intermediate precision <i>RSD</i>
80 % (131 ppm)	+0.3 %	0.7 %	1.0 %
100 % (175 ppm)	+0.7 %	0.4 %	0.8 %
120 % (219 ppm)	+1.6 %	1.2 %	1.6 %



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Doping control : HBOC



Hb
Hemoglobin



HBOC
Hemoglobin-based
oxygen carriers

OXYGL[®] BIN[®] approved for veterinary use
in US and Europe
hemoglobin glutamer-200 (bovine)

Hemopure[®] phase III trials
hemoglobin glutamer-200 (bovine)

PolyHeme[®] phase III trials

HEMO SPAN[®] phase III trials

→ Polymerized **bovine** hemoglobin

→ Polymerized **human** hemoglobin

avoid organ

ood oxygen

ase oxygen

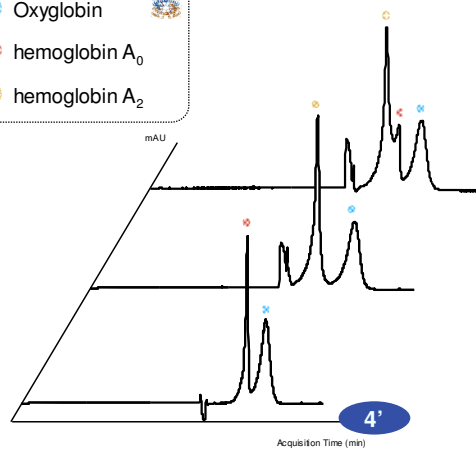
X3



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CE-UV/VIS | pH optimization

- Oxyglobin
- hemoglobin A₀
- hemoglobin A₂



Alkaline buffer

- no adsorption
- OXY vs. Hb selectivity
- short analysis time

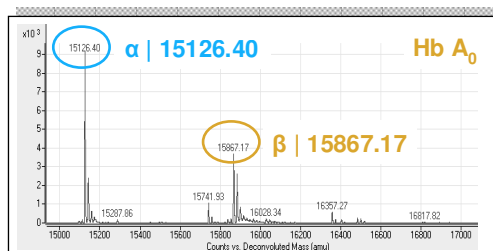
→ LOD (UV/VIS)

0.10 g/dL | 6.7 μM
528 fmol (8 ng) injected



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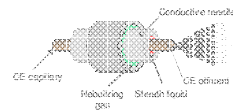
CE-ESI-TOF/MS | interface feature



CE separation

- migration under their intact form

ESI-TOF/MS detection

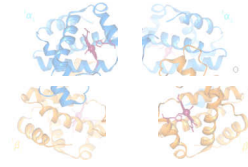
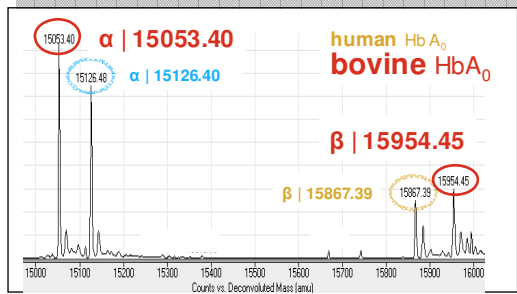


- sheath liquid | acid, organic solvent
- in-Taylor cone dissociation
- individual globine detection
accuracy < 4 ppm



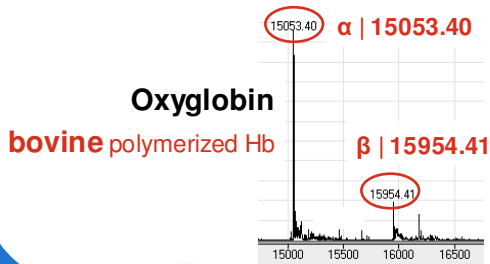
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CE-ESI-TOF/MS | human and bovine Hb and OXY



ESI-TOF/MS detection

- species selectivity
- OXY vs. Hb selectivity



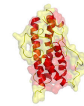
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Conclusions

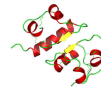


Mass accuracy

Isoforms identification | hGH seizure



Identification and quantitation | protein quality control



Species selectivity | HBOC doping control



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Swiss Centre for Applied Human Toxicology

