Is the natural shape of ions in the gas phase spherical ?

The allegory of the cave (Plato) applied in mass spectrometry

Dr Johann Far, Jean Haler, Christopher Kune, Dr Denis Morsa and Pr Edwin de Pauw
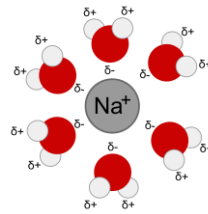
University of Liege

Paint: Plato´s Allegory of the Cave.

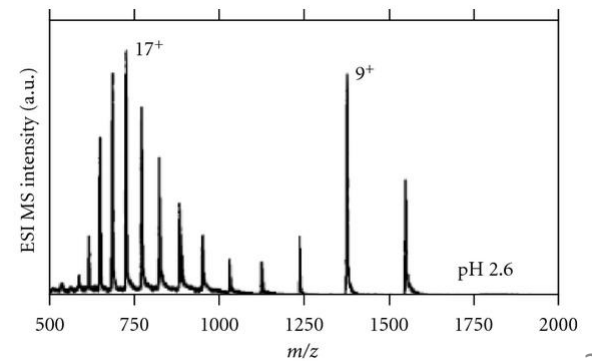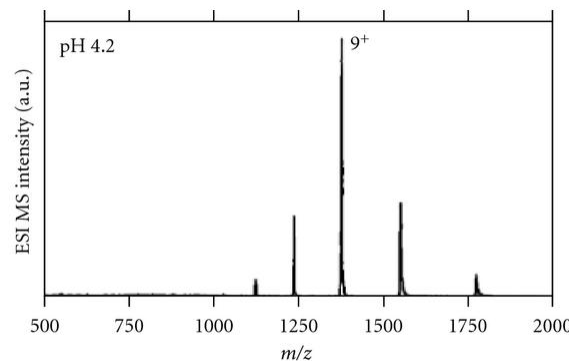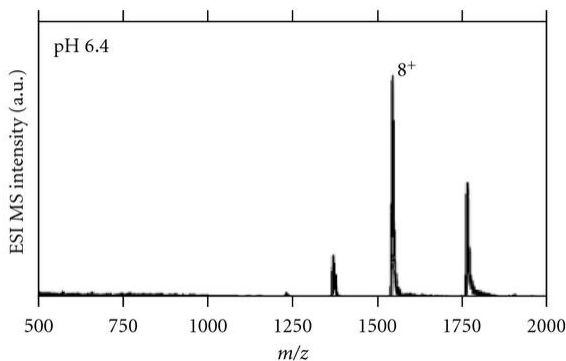33rd Informal Meeting on Mass Spectrometry
Szczyrk, Poland

The shape of ions in solution from their « shadow » in the gas phase

- The shape of ions in solution are driven by the balance between intra and intermolecular solvatation.

- Clearly changes in solution alter the mass spectrum like for example the charge state distribution increasing at acidic pH following denaturation.

Konermann and coworkers, Biochemistry (1997), vol. 36, no. 40, pp. 12296–12302. Cytochrome C electrospray mass spectra

The shape of ions in solution from their « shadow » in the gas phase

- The question has been since years adressed through the extent of the memory effect.

- We will approach here the question from a radically different point of view:

  Let us assume that the best shape in the gas phase is the sphere to maximize intramolecular solvatation

- What could be the origin of a deviation from this shape ?

The shape of ions in the gas phase

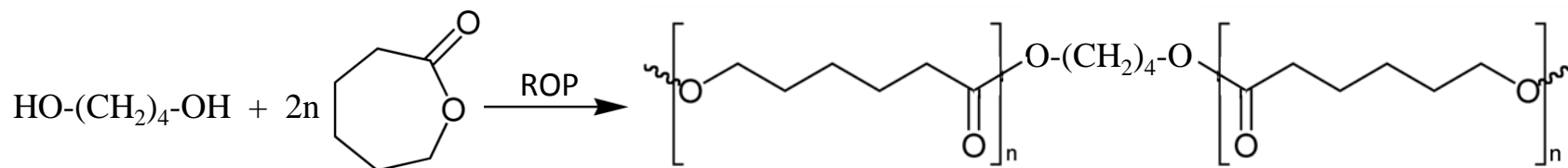The electrostatic intramolecular interactions are responsible for structural organization.

- Our experimental model will start with homopolymers in which no specific strong interaction can lead to shape modeling,

- We will introduce local organisation using cations (coordination),

- We will introduce covalent topology changes to provide a more rigid system.
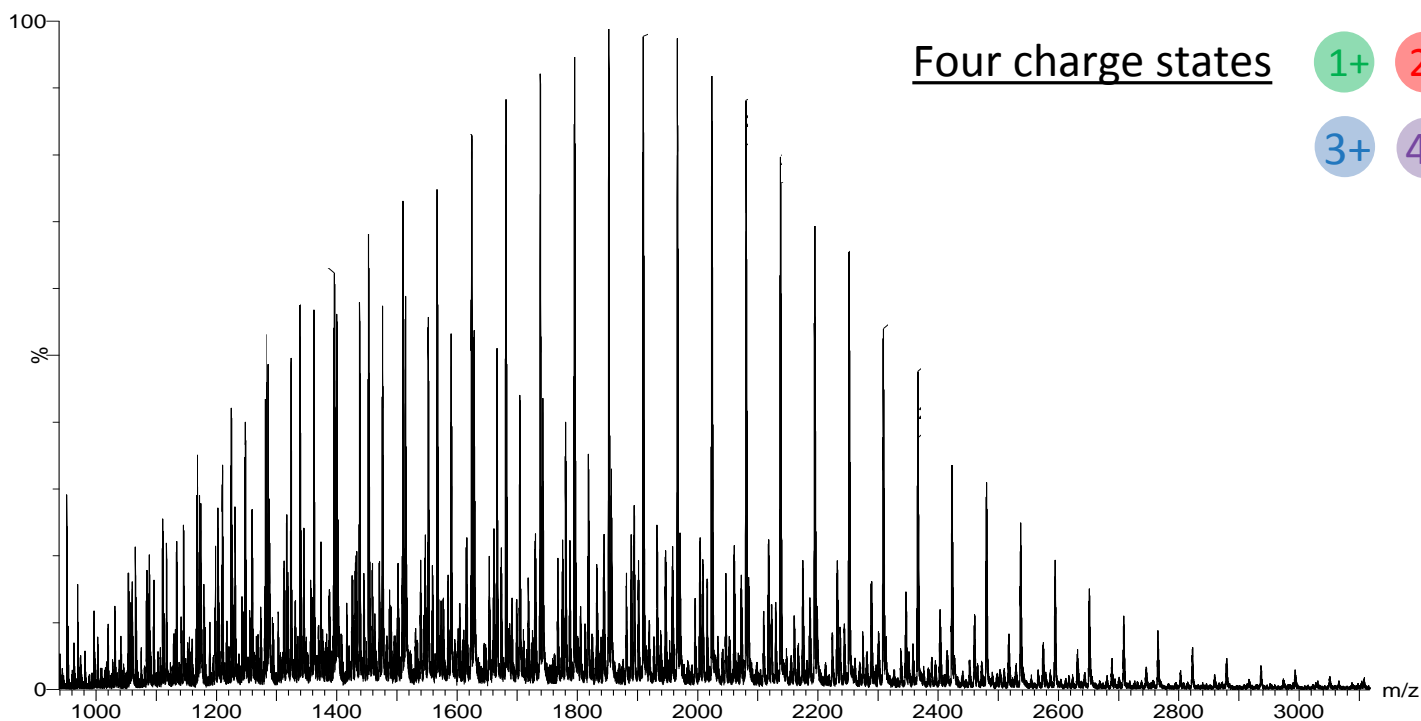
Synthetic polymers as model

1. Linear poly-ε-caprolactone (PCL)
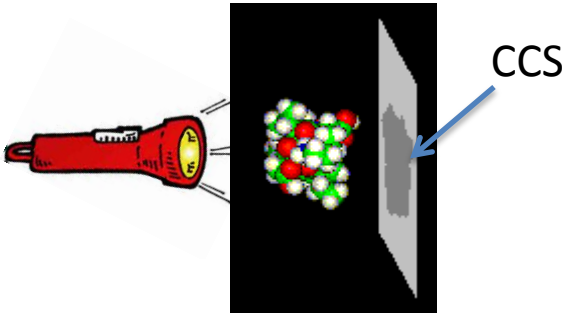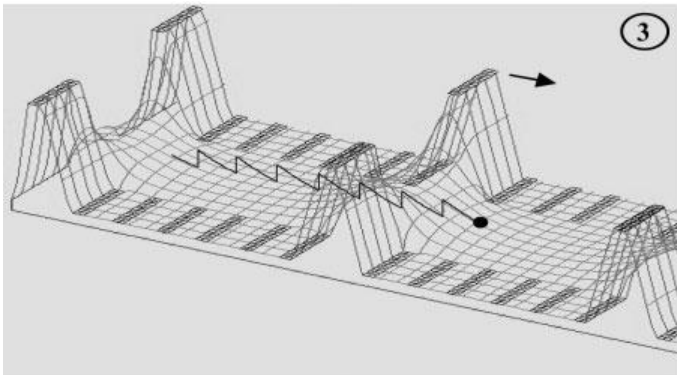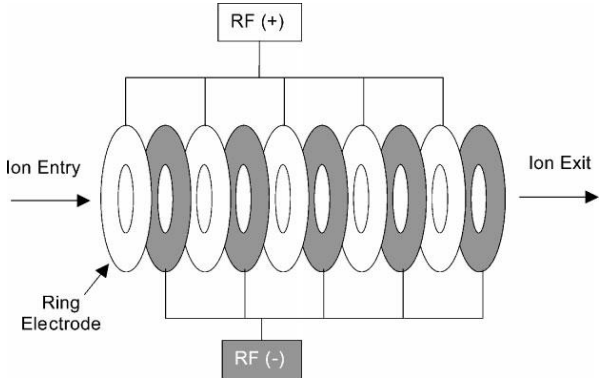
Floppy polymer model

Linear

ESI-MS

Four charge states   1+   2+   3+   4+

Shape dimension brought by ion mobility (IM) separation

ESI-IM-MS

Waters Synapt G2

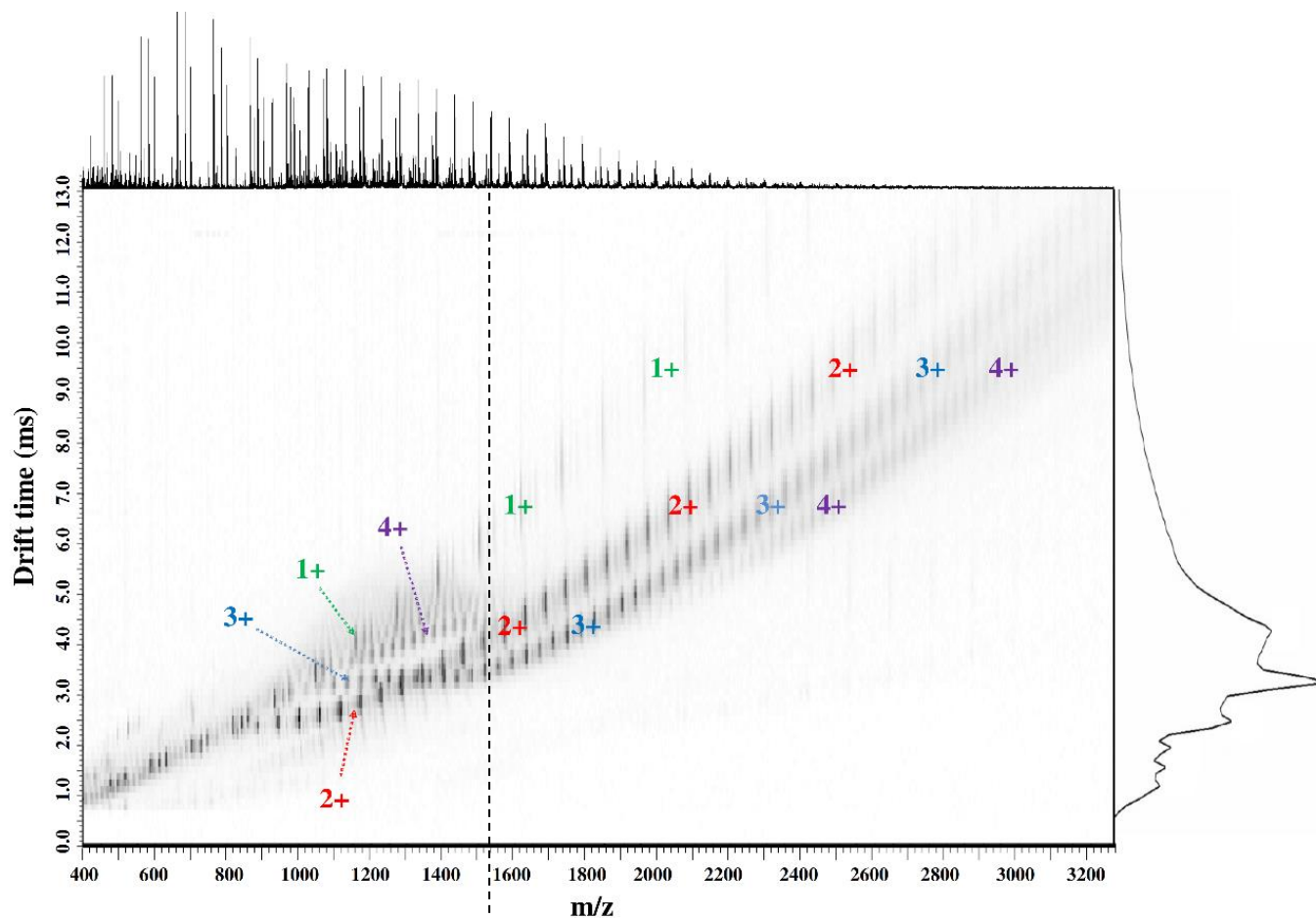Quad.   Trap   IMS   Transf.   TOF MS

Trap   IMS   Transf.

He cell

RF (+)

Ion Entry

Ion Exit

Ring Electrode

RF (-)

CCS
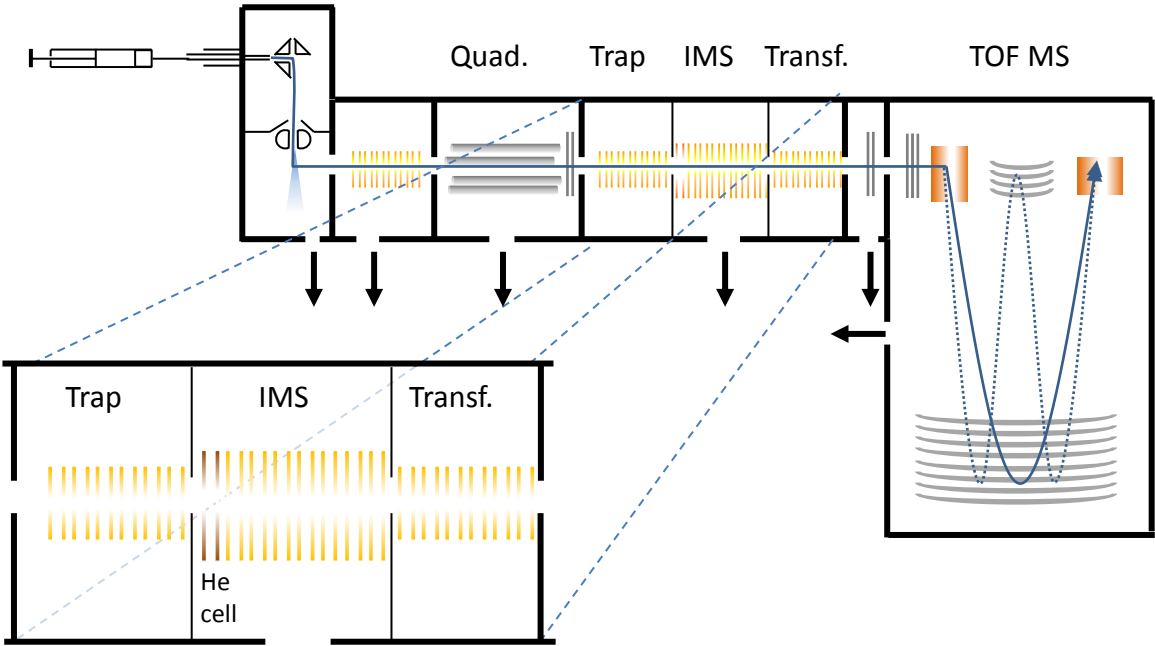
Stacked rings and Travelling Wave Ion Guide
(Giles and coworkers, Rapid Commun. Mass Spectrom. (2004) 18: 2401–2414)

http://www.indiana.edu/~clemmer/Research/Intro.php

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ESI-IM-MS

Drift time (ms)

m/z

Sequential breaks
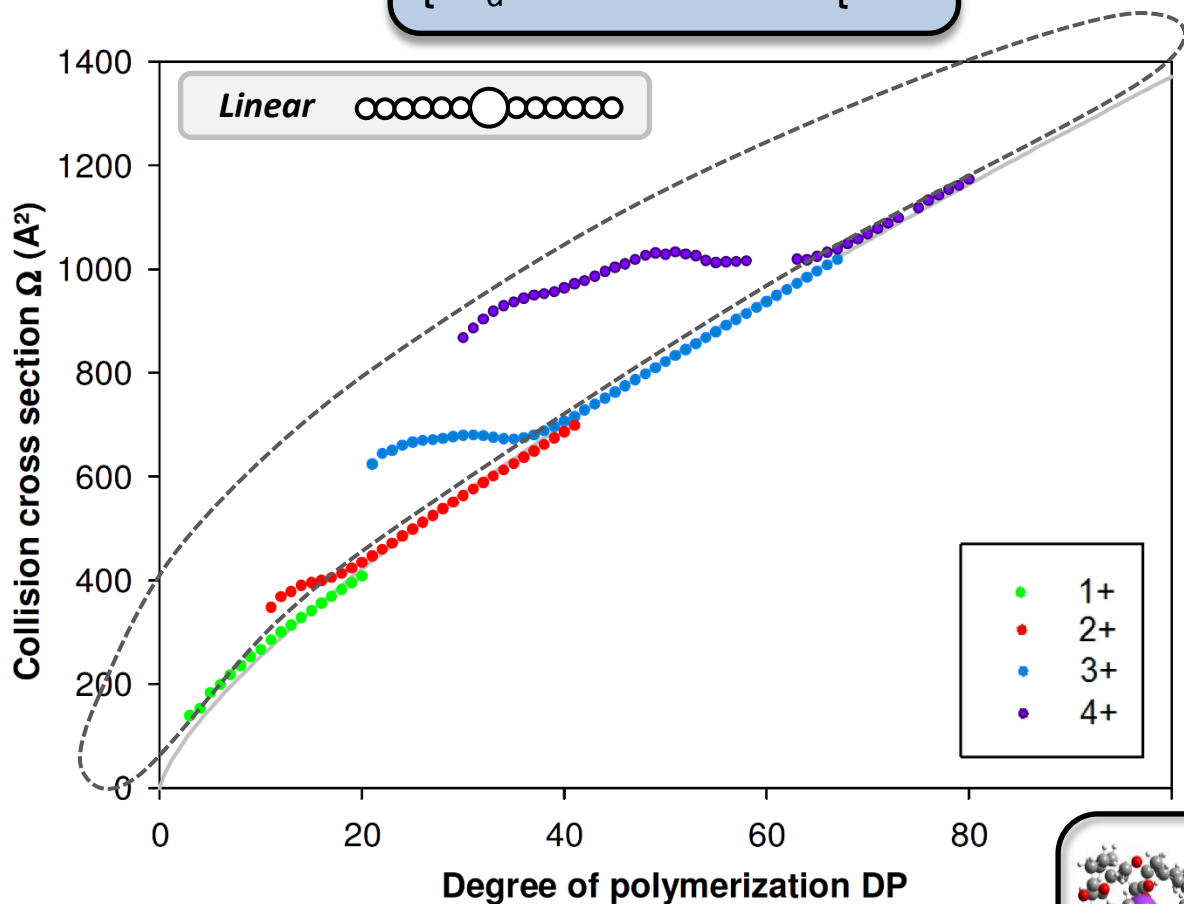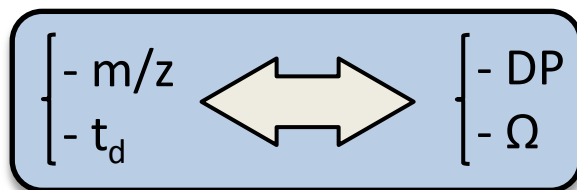
Low m/z region

High m/z region

Steady evolution

1st 4+   2nd 3+

3rd 2+

4+   3+   2+   1+

z

t_d

From arrival drift time distribution to collision cross section (ATD to CCS)

$$\begin{cases} -\ m/z \\ -\ t_d \end{cases} \Longleftrightarrow \begin{cases} -\ DP \\ -\ \Omega \end{cases}$$

Two main conformations

1) Spherical shape
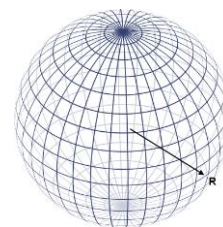
→ High DP/z ratios

V_{sphere}
$= 4/3\pi R^3$
$= n.V_{mono}$

$\Omega \propto R^2 \propto N^{2/3}$

Fit → $N^{\sim 0.7}$

- Z = 2
- DP = 36
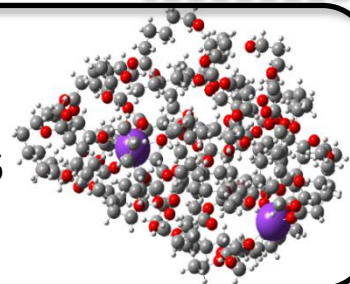
2) Elongated shape

→ Low DP/z ratios

$\Omega_{exp} > \Omega_{sph}$

- Z = 4   - DP = 36

Linear ⚬⚬⚬⚬⚬◯⚬⚬⚬⚬⚬

Legend:
- 1+
- 2+
- 3+
- 4+

Y-axis: **Collision cross section Ω (Å^2)** (0, 200, 400, 600, 800, 1000, 1200, 1400)

X-axis: **Degree of polymerization DP** (0, 20, 40, 60, 80)

Morsa and coworkers, Anal. Chem. (2014), **19**: 9693–9700
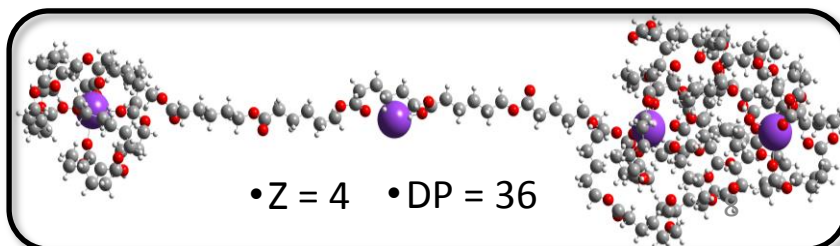
Implication of the polymer topology
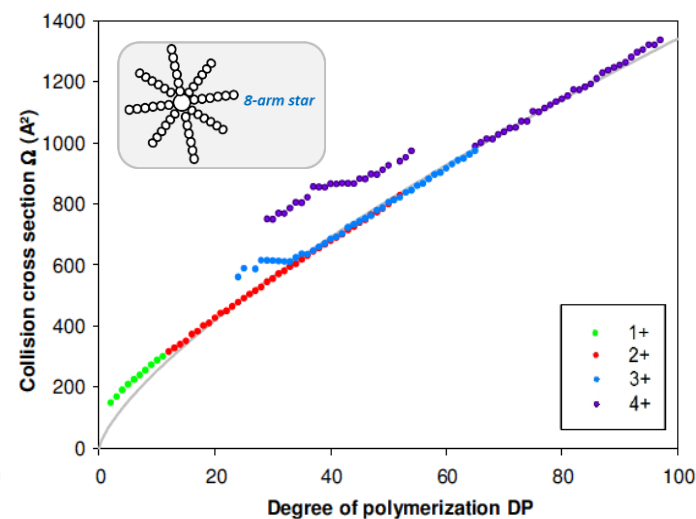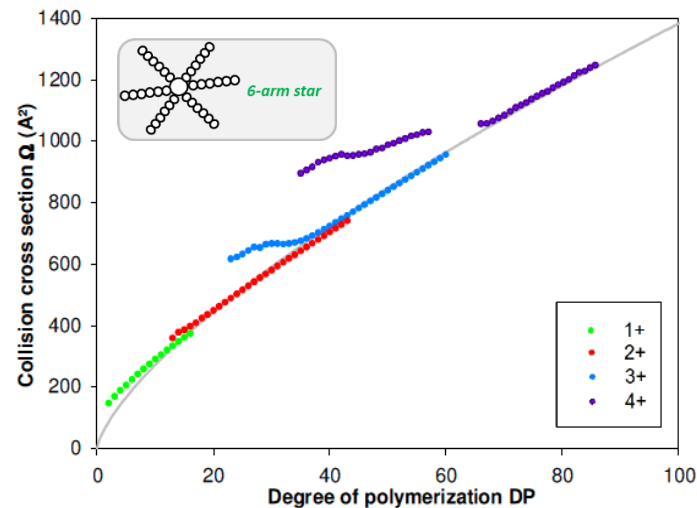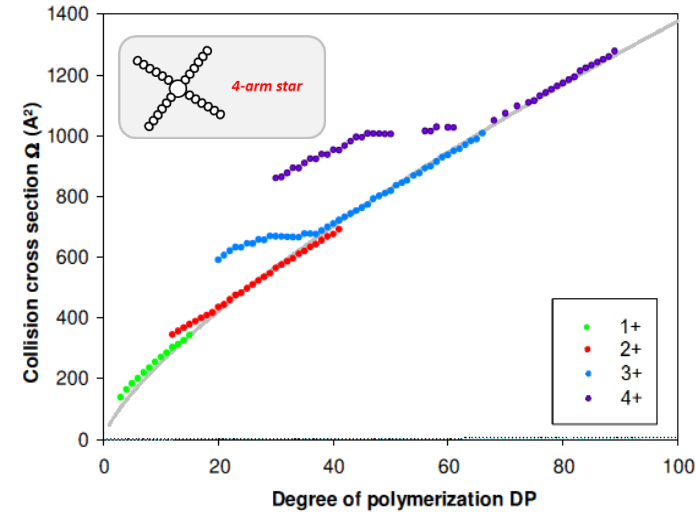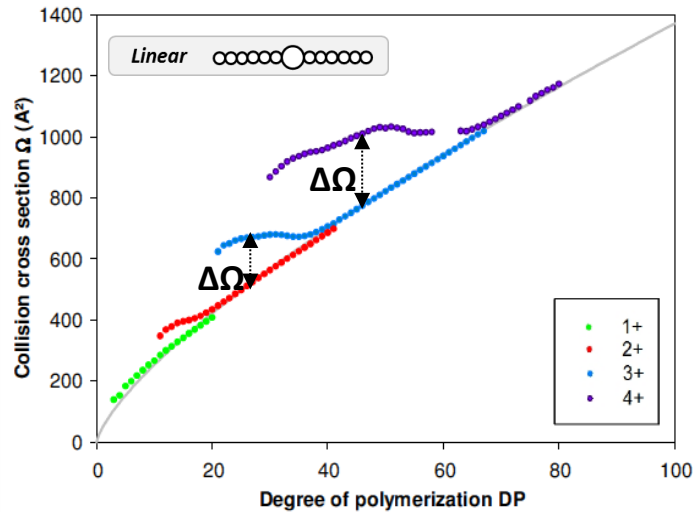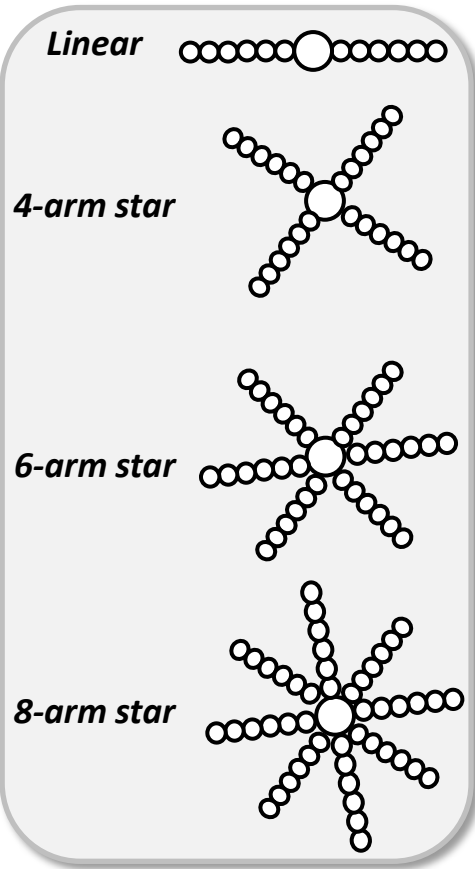
→ **High DP/z ratios**
Common spherical structure basis

→ **Low DP/z ratios**
Differentially elongated structures?

The CCS expansion as an indicator of the topology

$$\Delta\Omega = \Omega_{exp} - \Omega_{theory}$$
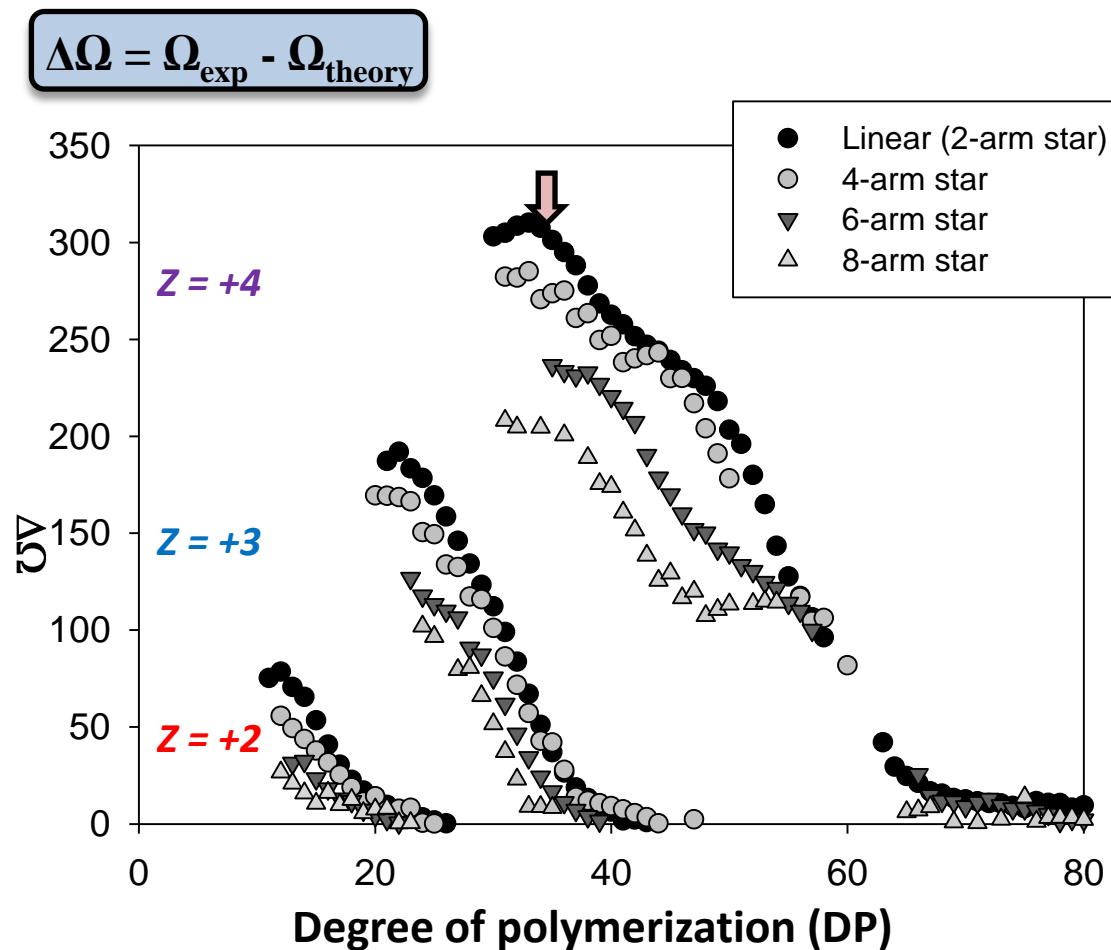
For all topologies

$$\Delta\Omega_{2+} < \Delta\Omega_{3+} < \Delta\Omega_{4+}$$

→ *Influence of the electrostatic repulsions*

For a same DP

$$\Delta\Omega_{2\text{-arm}} > \Delta\Omega_{4\text{-arm}} > \Delta\Omega_{6\text{-arm}} > \Delta\Omega_{8\text{-arm}}$$

→ *Influence of the chain flexibility*

Calculation of the difference between experimental CCS and the perfect sphere CCS

Homopolymers with improved steric hindrance per monomer

- Models are polymers where substituents on the backbone are increased
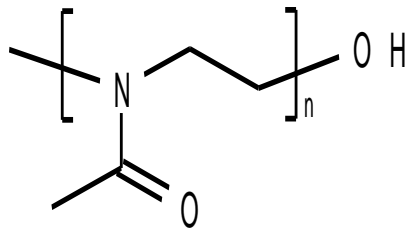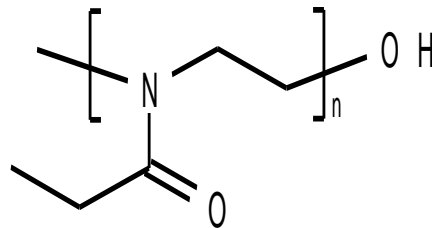
polymethyl   polyethyl   polypropyl
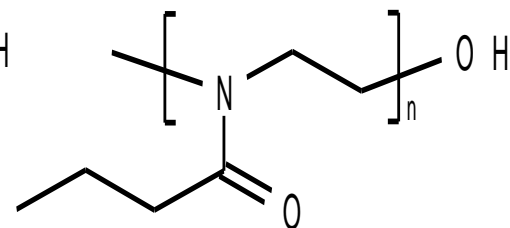
- Let's compare the shape evolution of these homopolymers according to their degree of polymerisation and the number of carried charges by IM-MS

Is the sphere model still valide ?

Rearrangement with compaction effect

ΔCCS_{sphere}

Sphere model fit

Collision Cross-Section ($\AA^2$)

D.P.

Collision cross section Ω ($\AA^2$)

Degree of polymerization DP

- 1+
- 2+
- 3+
- 4+
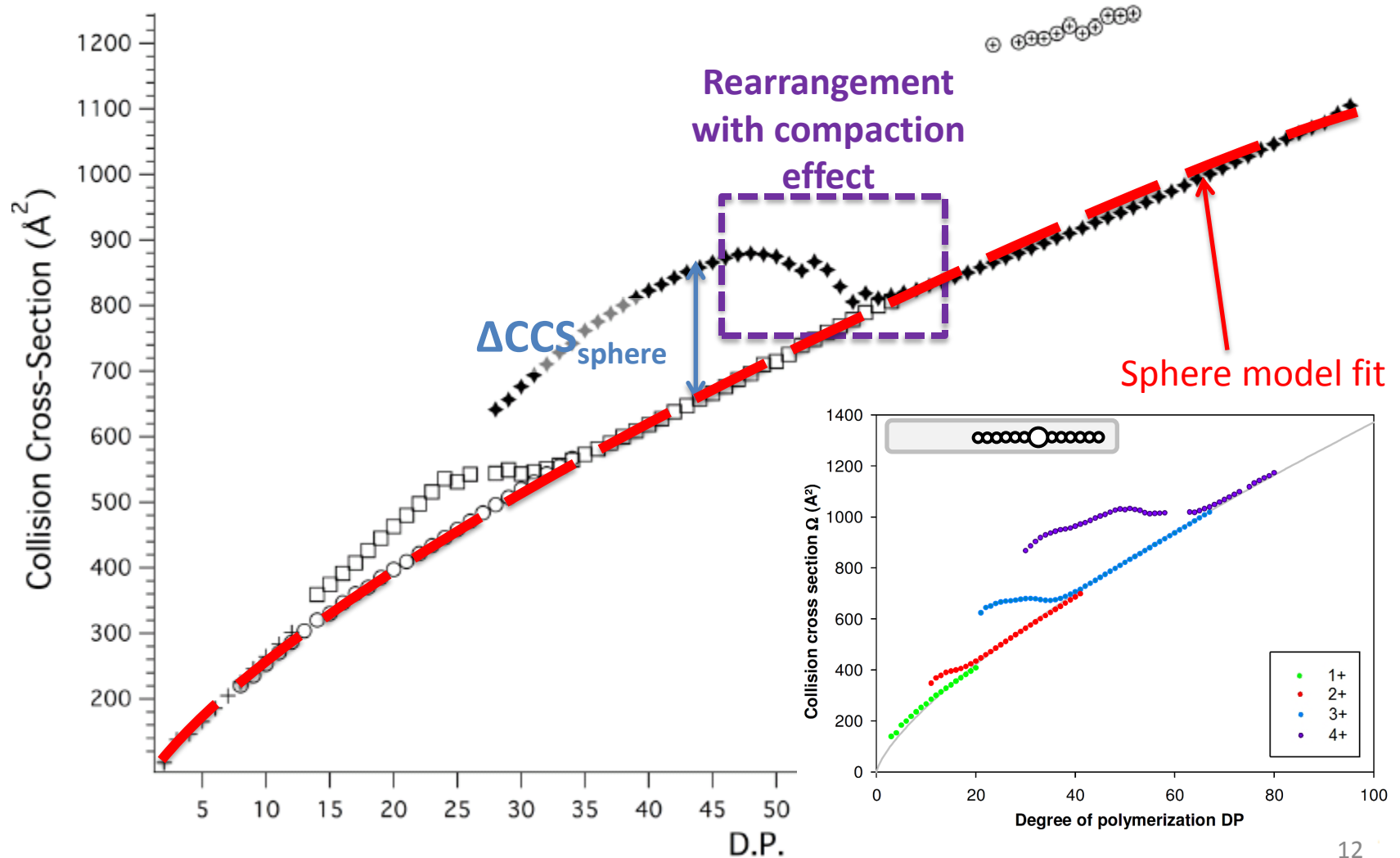
Modifying the steric hindrance of the monomer : polymethyl to polypropyl

3 different spheres fit
Each homopolymer having its own fit

Legend:
- + PEtOx_50_100_1Na
- ○ PEtOx_50_100_2Na
- □ PEtOx_50_100_3Na
- ✦ PEtOx_50_100_4Na
- ⊕ PEtOx_50_100_5Na
- + PMeOx_50_100_1Na
- ○ PMeOx_50_100_2Na
- □ PMeOx_50_100_3Na
- ✦ PMeOx_50_100_4Na
- + PnPrOx_50_100_1Na
- ○ PnPrOx_50_100_2Na
- ✦ PnPrOx_50_100_4Na
- ⊕ PnPrOx_50_100_5Na

Axes: Collision Cross-Section ($\mathring{A}^2$) vs D.P.
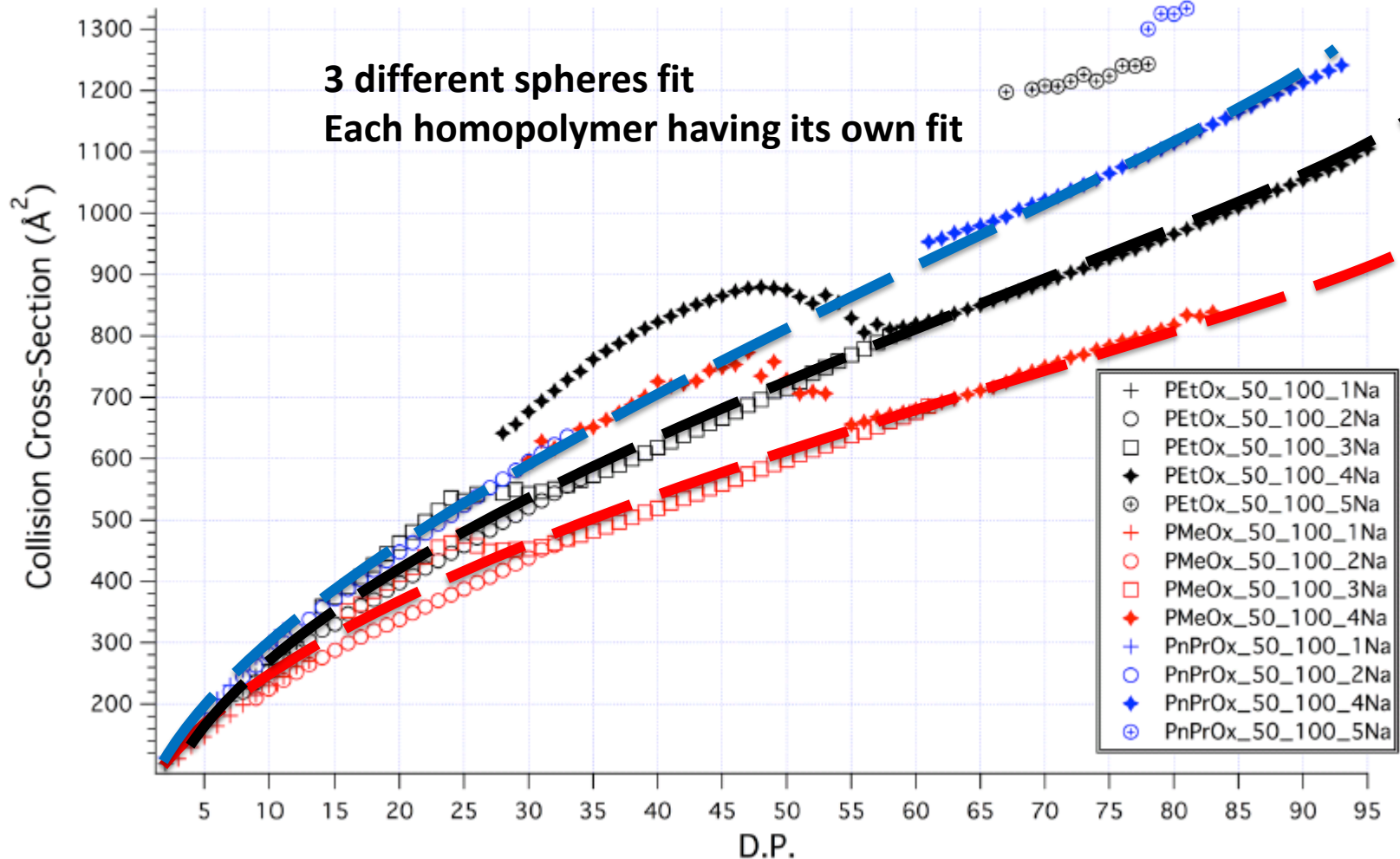
The CCS expansion as Coulomb repulsion indicator: Self charge solvation when DP increase
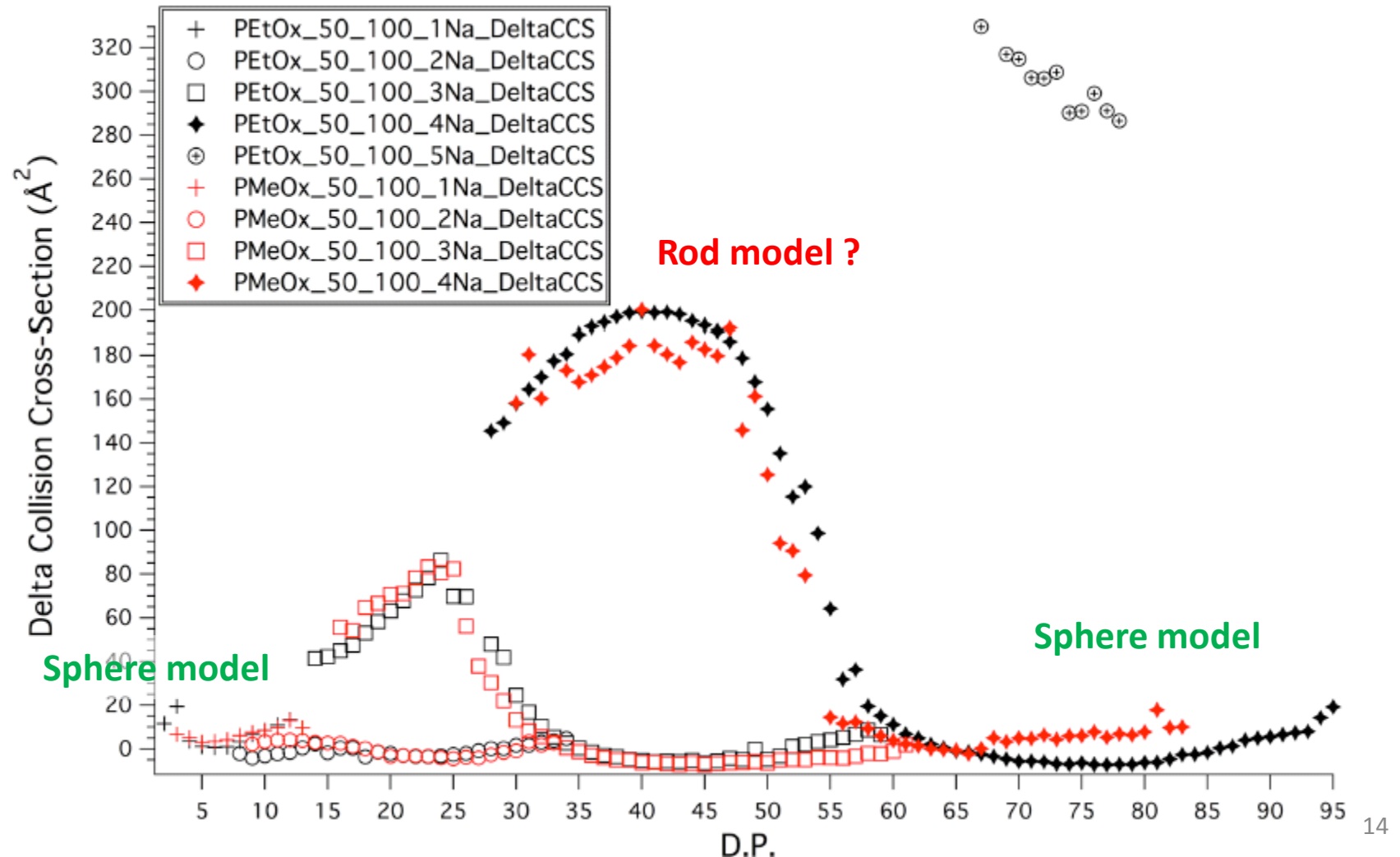
Conformational evolution from solution to the gas phase

In solution (THF)

→ **Solvent-swollen random coil conformations**

Size ? Probed by <u>static light scattering</u>

Morsa and coworkers, Anal. Chem. (2014), **19**: 9693–9700

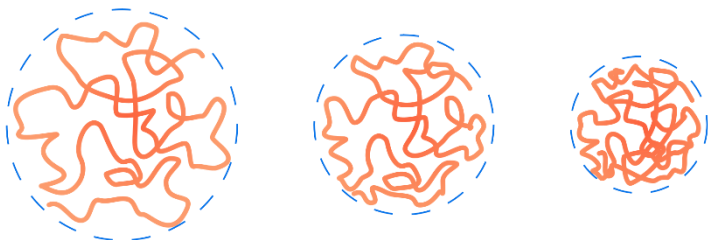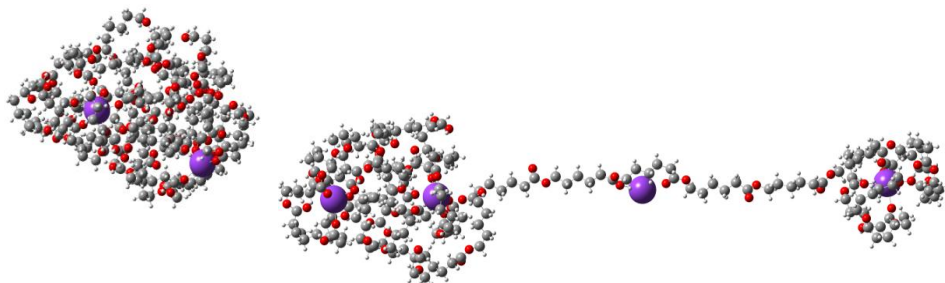|	R_g (DP = 65) Solution (THF)
2 arm star	**3.03 nm**
4 arm star	**2.81 nm**
6 arm star	**2.67 nm**
8 arm star	**2.52 nm**

Radii compaction after desolvatation

In the gas phase

→ **Dense near-spherical/elongated conformations**

Size ? Probed by <u>ion mobility spectrometry</u>

	R (DP = 65, z= 4) Gas phase	Ω (DP = 36, z = 4) Gas phase
2 arm star	**1.81 ± 0.02 nm**	**943 ± 13 Å²**
4 arm star	**1.83 ± 0.02 nm**	**926 ± 12 Å²**
6 arm star	**1.83 ± 0.02 nm**	**906 ± 15 Å²**
8 arm star	**1.79 ± 0.02 nm**	**821 ± 15 Å²**

Charge effect was not considered during the liquid to gas phase radii comparison

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Mobility in solution compared to mobility in gas phase could help ?

Used model was biologically relevant heteropolymers: the peptides

- Does desolvating change the conformation ? What about a memory effect?
- Need a method to probe the mobility of ion in solution: capillary electrophoresis (CE)
- Introducing the coupling between capillary electrophoresis and ion mobility spectrometry

Work in progress
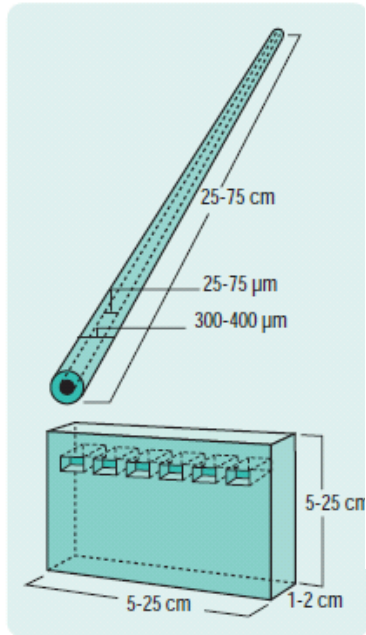
Capillary Electrophoresis in brief

25-75 cm

25-75 μm

300-400 μm

5-25 cm

5-25 cm   1-2 cm

Figure 1.1
Comparison of gel used for slab gel
electrophoresis and capillary for CE.

Detector

Computer
control

Capillary

Inlet
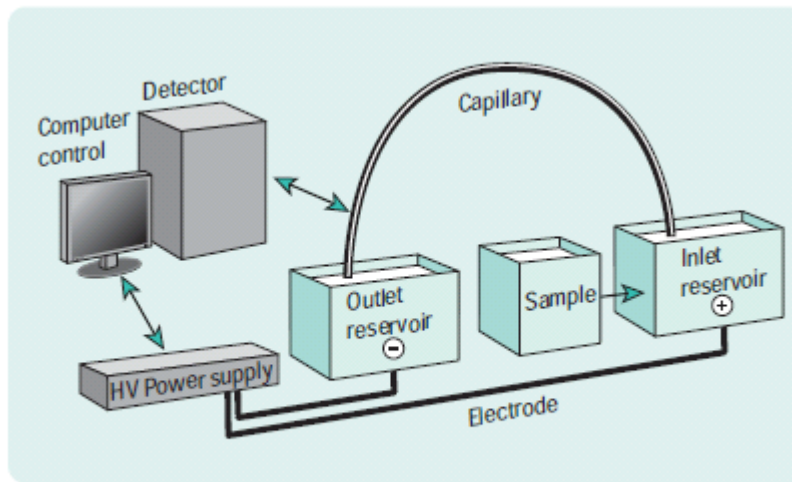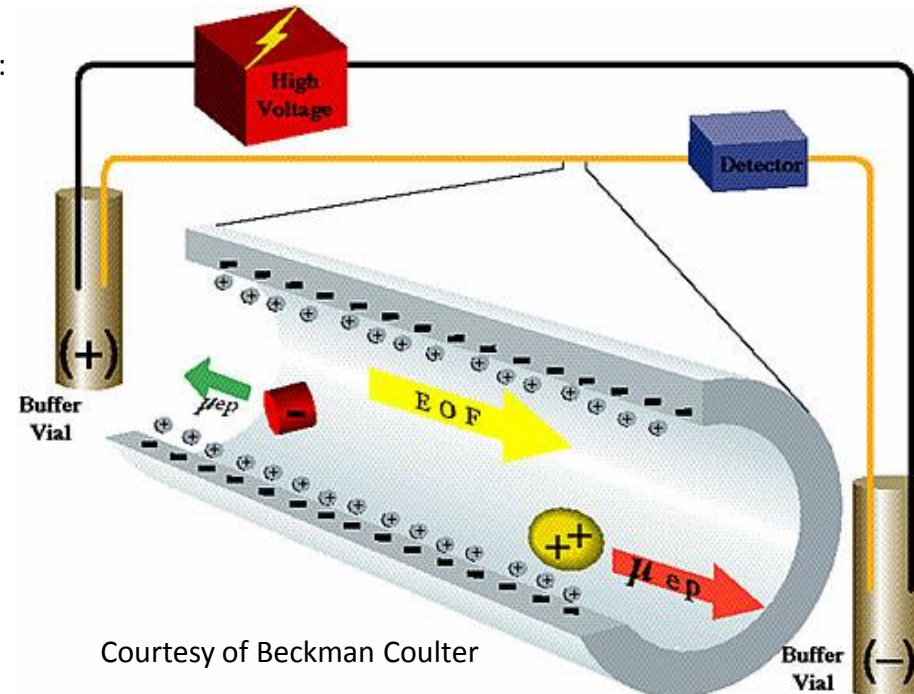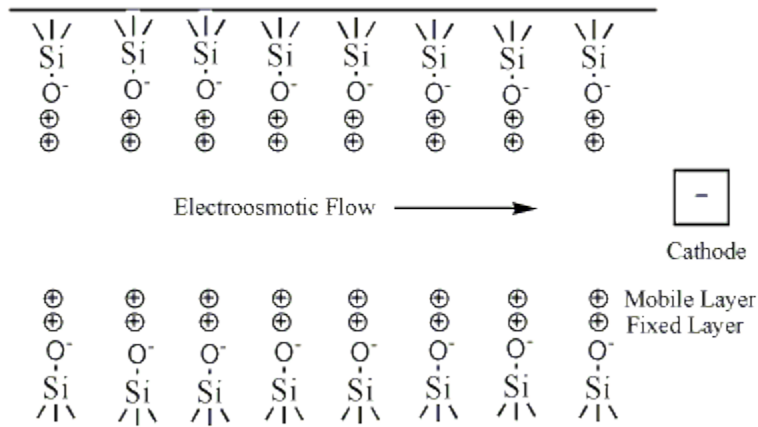reservoir
⊕

Outlet
reservoir
⊖

Sample

HV Power supply

Electrode

Figure 1.2
Basic components of CE instrumentation.

High Performance Capillary Electrophoresis:
A primer (Agilent Technologies)

Si Si Si Si Si Si Si Si
O⁻ O⁻ O⁻ O⁻ O⁻ O⁻ O⁻ O⁻
⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕
⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕

+   Electroosmotic Flow ⟶   –

Anode   Cathode

⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ Mobile Layer
⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ ⊕ Fixed Layer
O⁻ O⁻ O⁻ O⁻ O⁻ O⁻ O⁻ O⁻
Si Si Si Si Si Si Si Si

http://pixshark.com/electroosmotic-flow.htm

High
Voltage

Detector

Buffer
Vial

(+)

μ_{ep} EOF

$+^+$

μ_{ep}

Buffer (−)
Vial

Courtesy of Beckman Coulter

Bovine Serum Albumin digested by trypsine injected in CE-IM-MS

- Generating a list of peptides with known sequences
- pH electrolyte → Charge of peptides in solution determination (q)
- Viscosity determination of electrolyte (η)
- Electrophoretic mobility (µ) obtained from migration times
- Hydrodynamic radii (r) calculation from µ
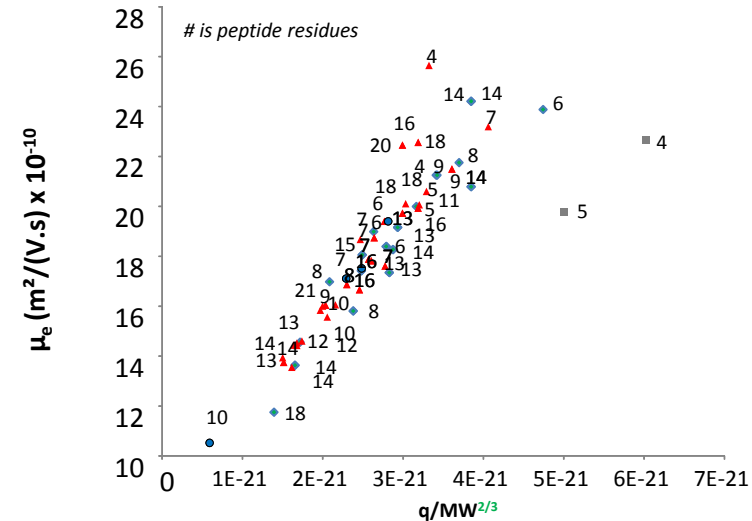- CCS of peptides from BSA tryptic digest are available (Ω)

Valentine and coworkers, J. Am. Soc. Mass Spectrom. (1999), **10**:1188-1211

Base Peak Electropherogram
$100\% = 1{,}66 \times 10^{+4}$ cps

$$\mu = \frac{q}{6\pi\eta r}$$
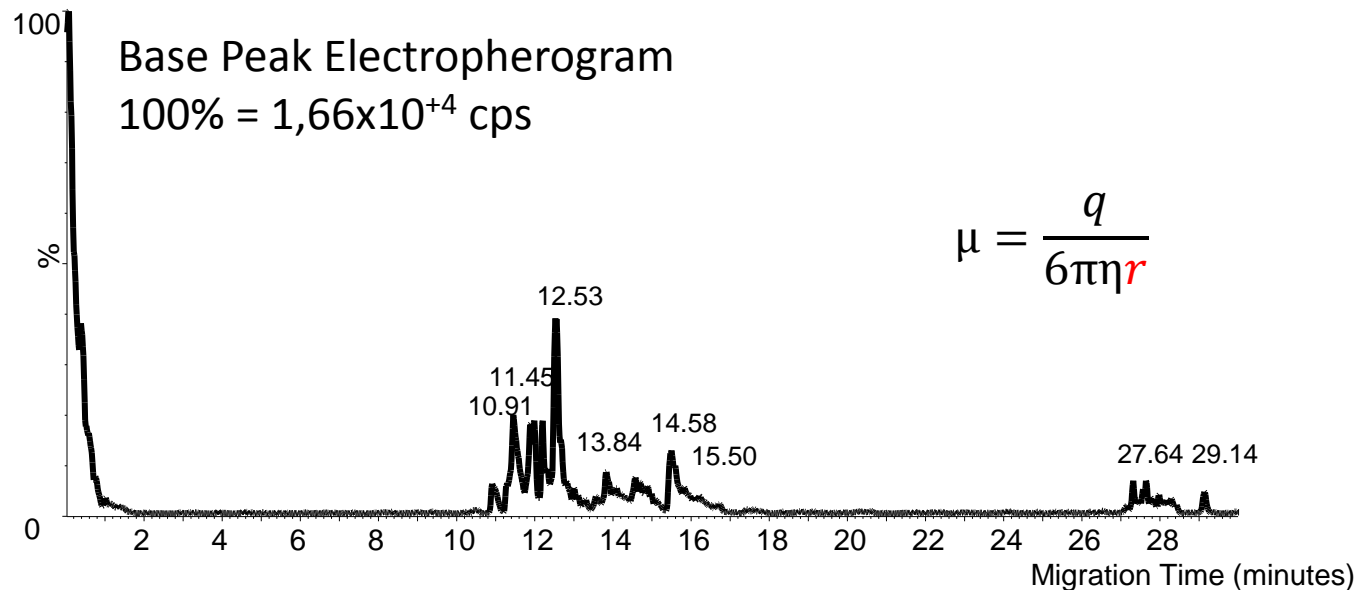
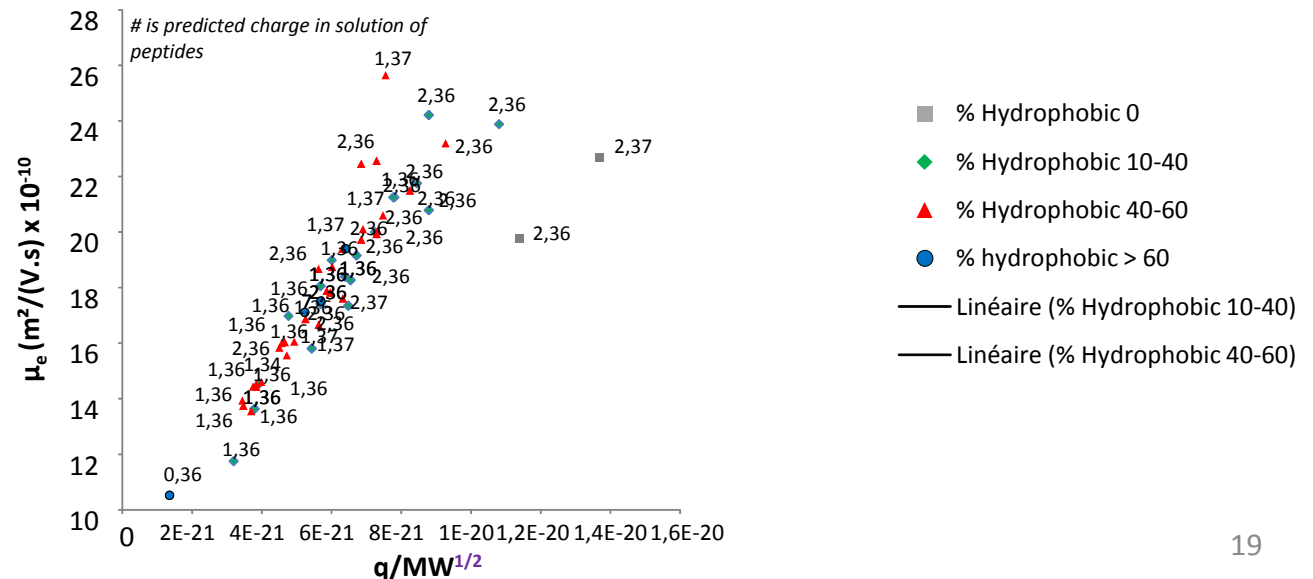# Mobility / surface comparison in solution
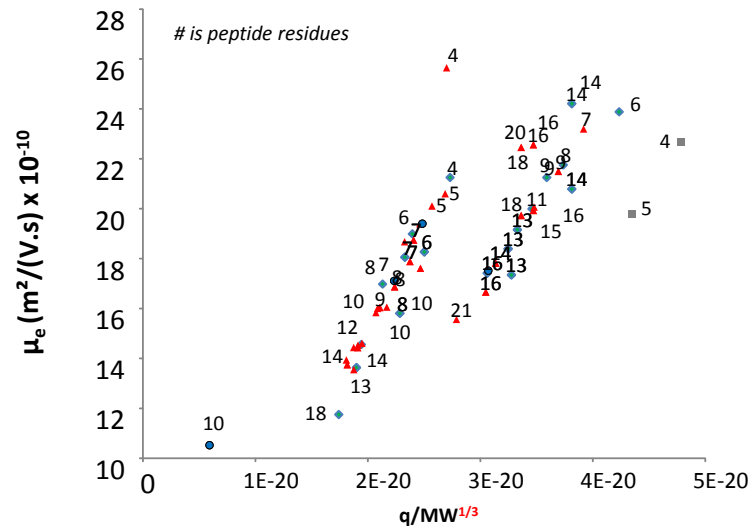
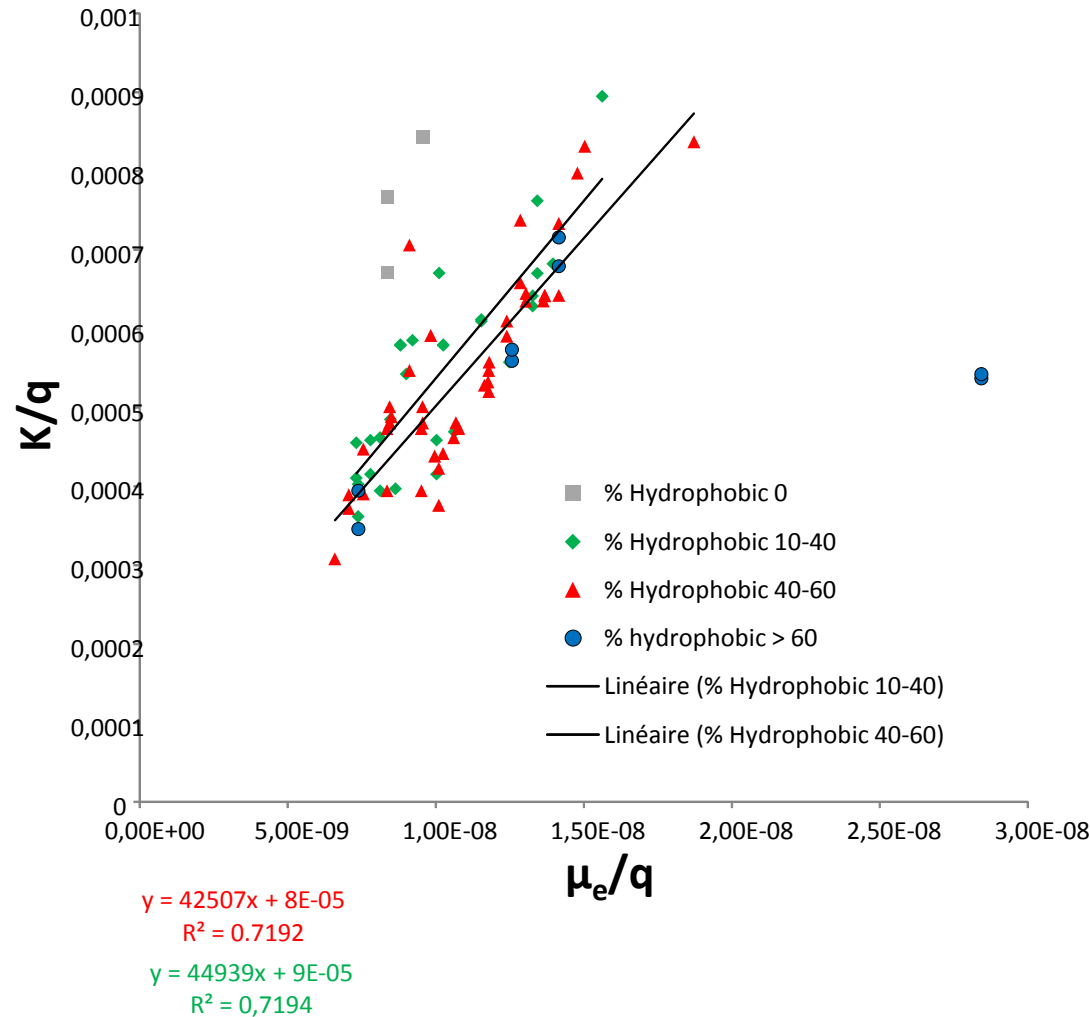Radii surface correlation according to Stoke' laws

Sphere's surface correlation according to Offord' laws

Gyration radii surface correlation according to Rickard *et al.* Anal. Biochem. (1991) **197**, 197-201

Mobility CE / Mobility IM comparison

Correlation between the mobility in solution (denaturing condition) and gas phase mobilities (CCS converted to mobility coefficient K) exists but not for all peptides !

Memory effect limited ?

Conclusion

For polymers with no constraint:

- Spheric shape in gas phase seems to be the rules, excepted if Coulomb repulsion prevent it

For polymers with steric hindrance:

- Same conclusion

For peptides (denatured):

- In solution, the spherical shape is a good approximation for most of the BSA peptides (denatured)
- In gas phase: spherical shapes seem to be the rules for most of the studied peptides WITH exceptions

Investigation in progress to determine the other major parameters driving the shape in gas phase

Special thanks to
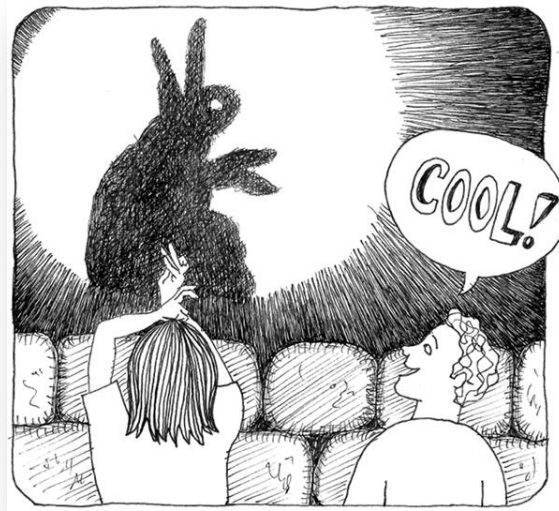
- Christopher Kune and Cédric Delvaux (CE-MS), Dr Denis Morsa, Jean Haler,
- FNRS, Walloon Region, Liege University for funding and support, Analis SA (R&D) for sharing the CE hardware,

And you for attention.