

Capillary Electrophoresis in biotechnology – protein analysis and affinity studies.



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ANALYTICAL EQUIPMENT



Capillary electrophoresis system CAPEL

- **Pharmacological industry**
medicines formulations,
pharmacokinetics
- **Environmental analysis**
pesticides, inorganic ions
- **Food industry**
preservatives, dyes, target compounds,
etc.
- **Forensic studies**
drugs of abuse, explosives, lethal
markers, etc.
- **Chemical industry**
- **Biotechnological research**
peptides, proteins, DNA / RNA,
affinity studies,
immunoanalyses



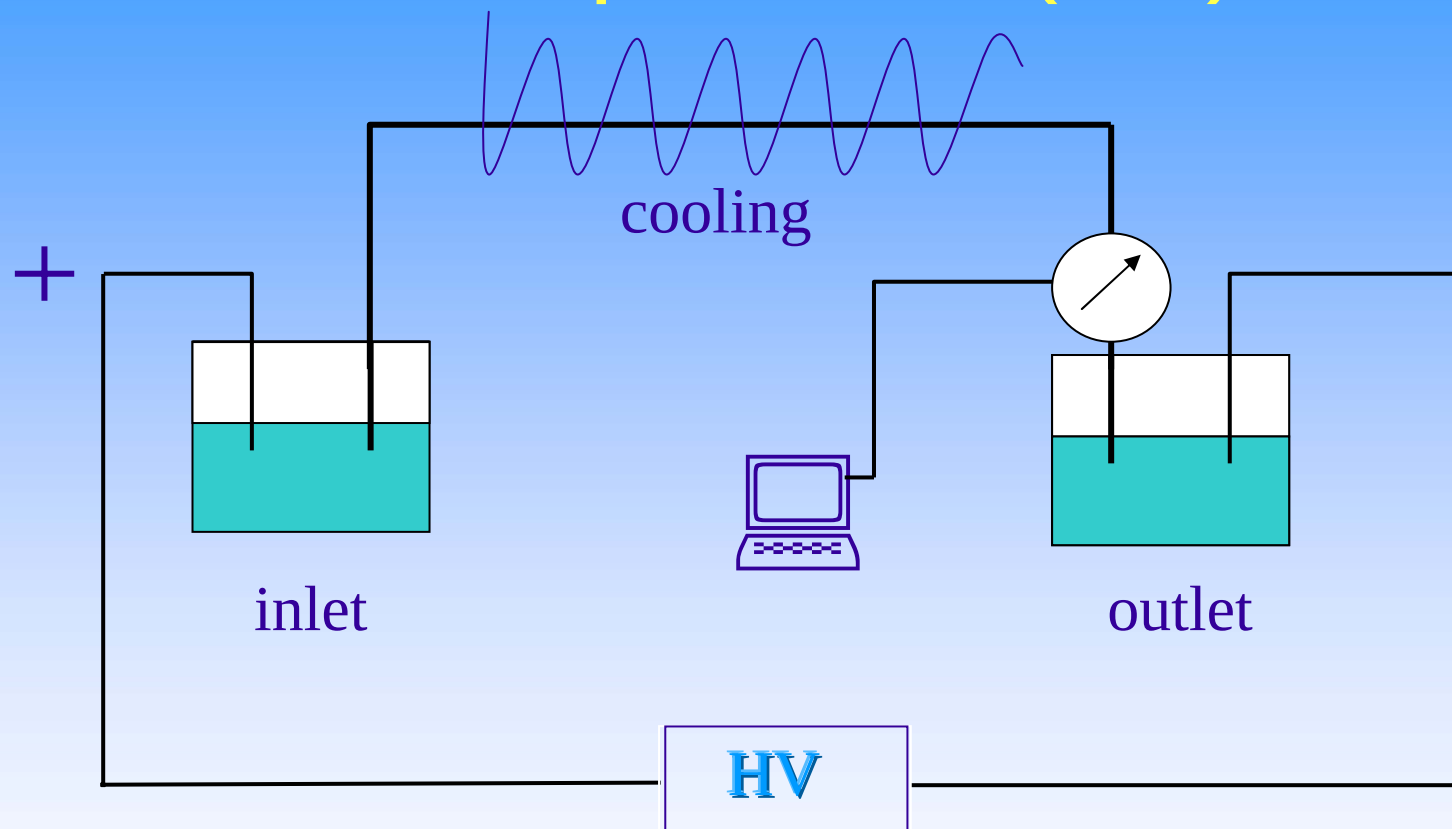
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ISO 9001:2000
Reg. №: Q - 04.04.18

General scheme of capillary electrophoresis (CE)



Separation is due to the differences in charge-to-mass ratio (e/m)

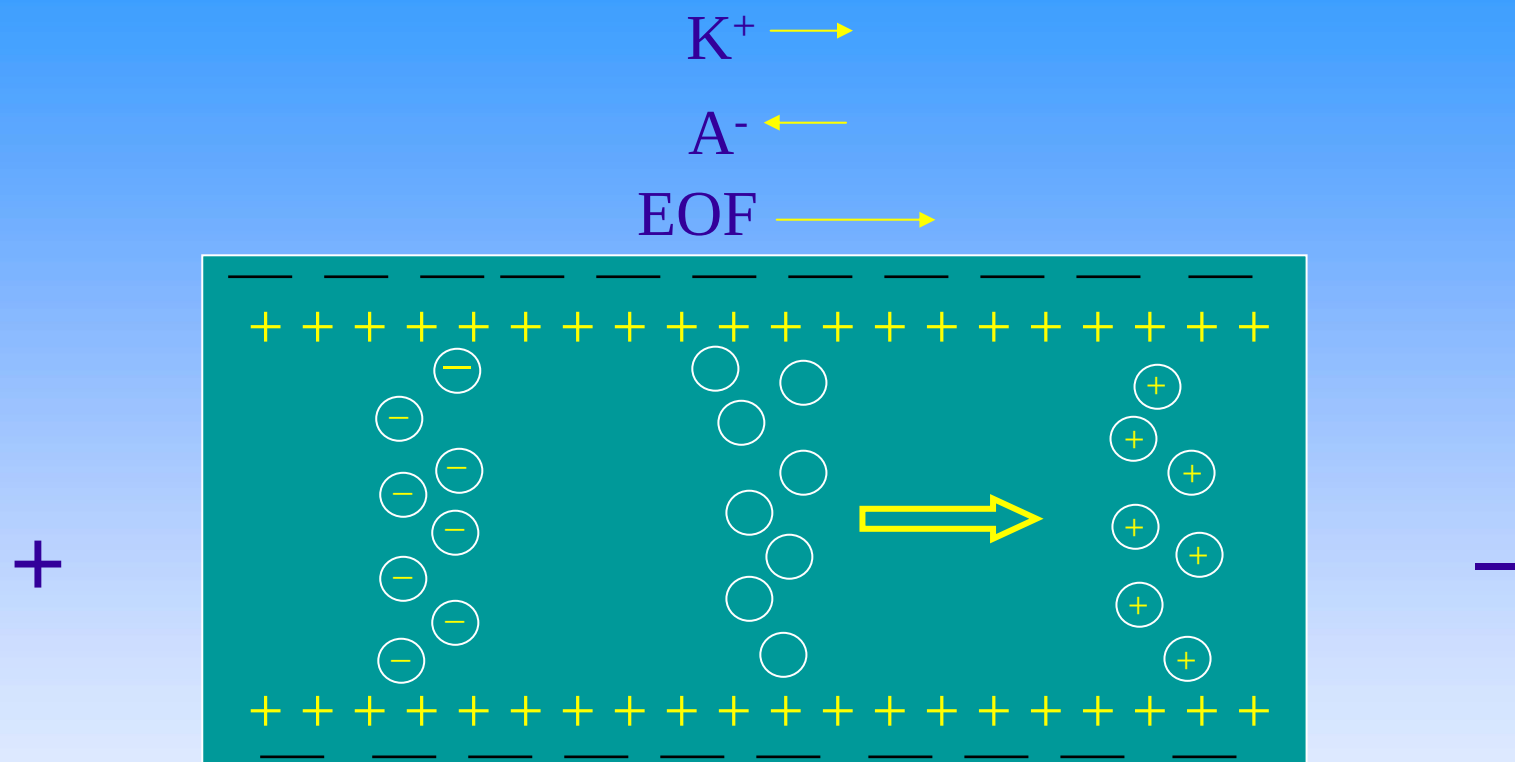


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Electroosmotic flow (EOF)



pH > 3.0

Both anions and cations can be analysed in one run



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Features, which make any CE device a “commercial CE system”

- Precisely controlled pressure injection
- *Thermostatic* cooling of the capillary
- Highly reproducible and stable voltage
- Optical scheme, which is independent from the surrounding light



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Features, favorable for bioanalytical studies

- ✓ Reagents are free in solution
- ✓ Physiological conditions of interaction
- ✓ Absence of sieving material and secondary labelling
- ✓ Short analysis time



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How CE can be used for protein analysis

➤ „Just“ separation

➤ Affinity studies



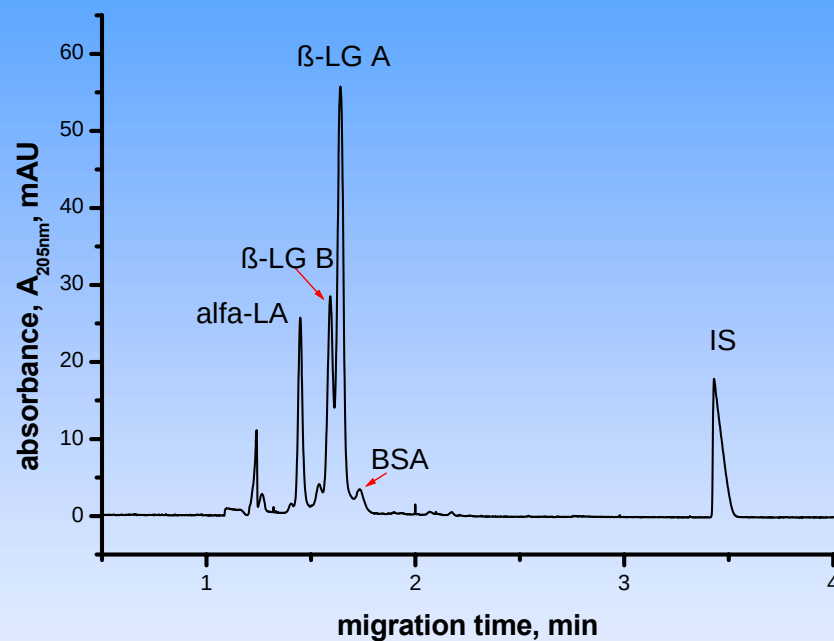
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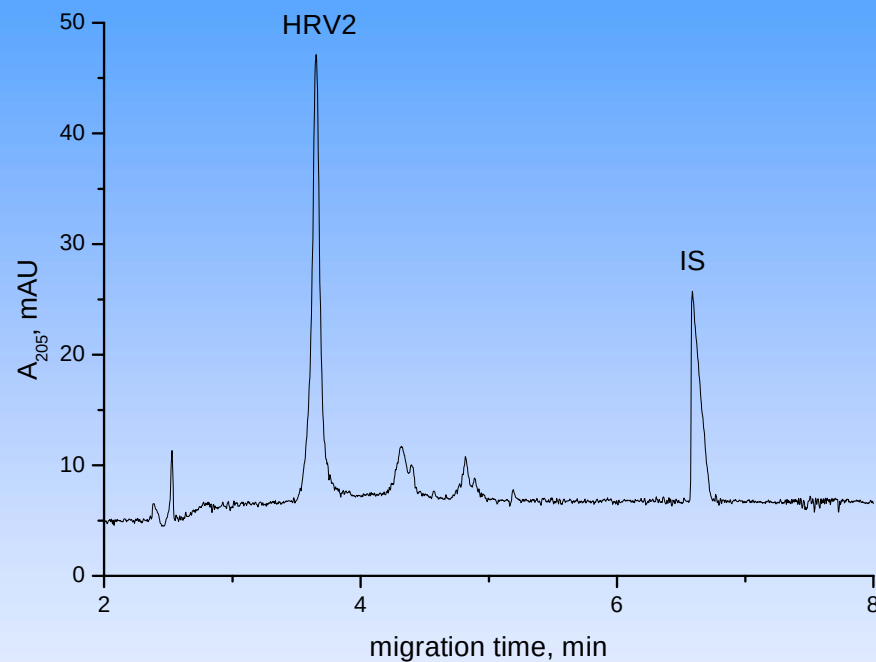


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Protein separation



Analysis of milk whey proteins



Analysis of human rhinovirus



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Instrumental features, favorable for protein analysis

- ✓ Flexibility in temperature settings
- ✓ Option to choose any desired wavelength
- ✓ High pressure mode to rinse the capillary



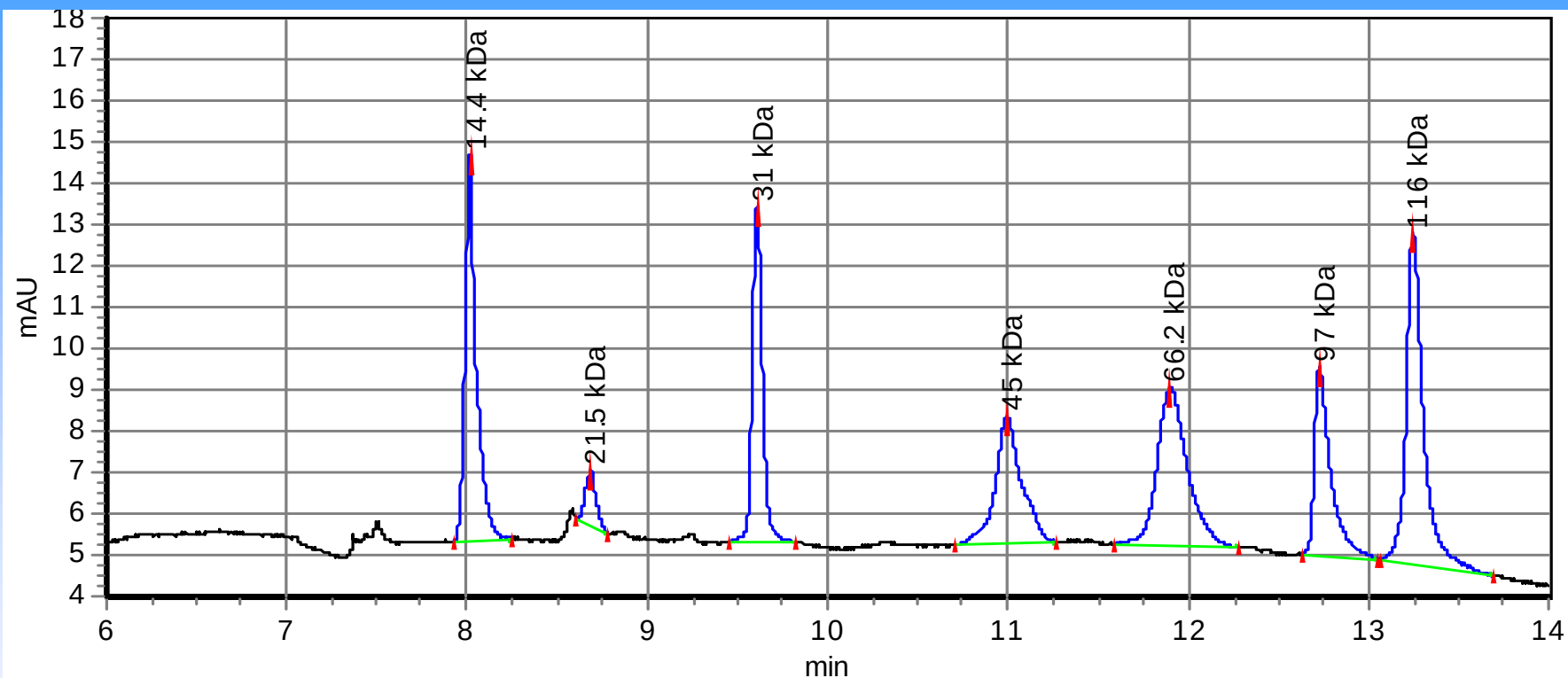
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Protein separation by capillary gel-electrophoresis



Proteins are separated based on their molecular weights



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Instrumental features, favorable for protein analysis

- ✓ Two independent autosamplers – from multiple injections to fractions collection
- ✓ Extended range of low pressure settings for the very complex injections



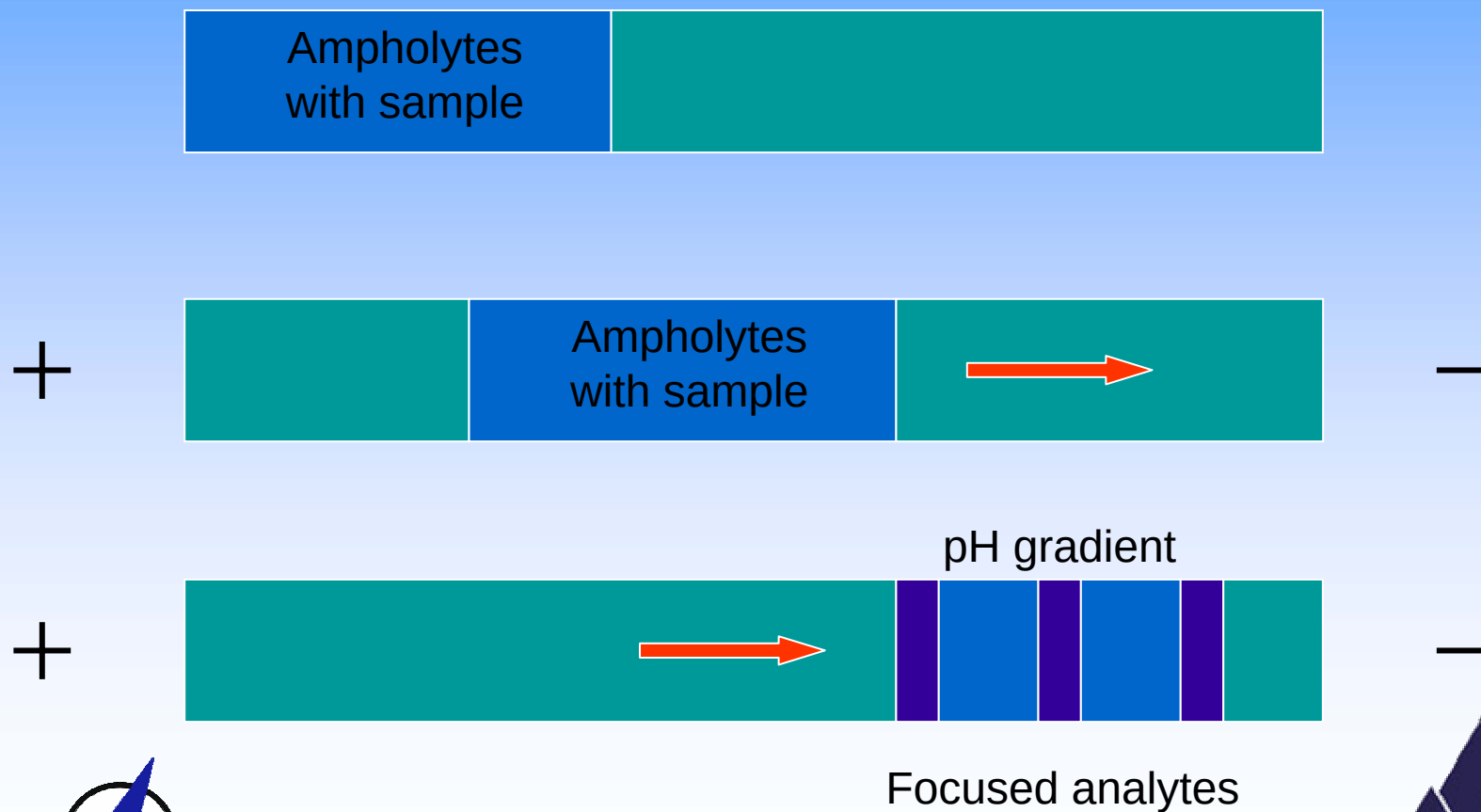
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Capillary isoelectric focusing

Sample : zwitterionic compounds like peptides or proteins with different isoelectric points (pI 's)



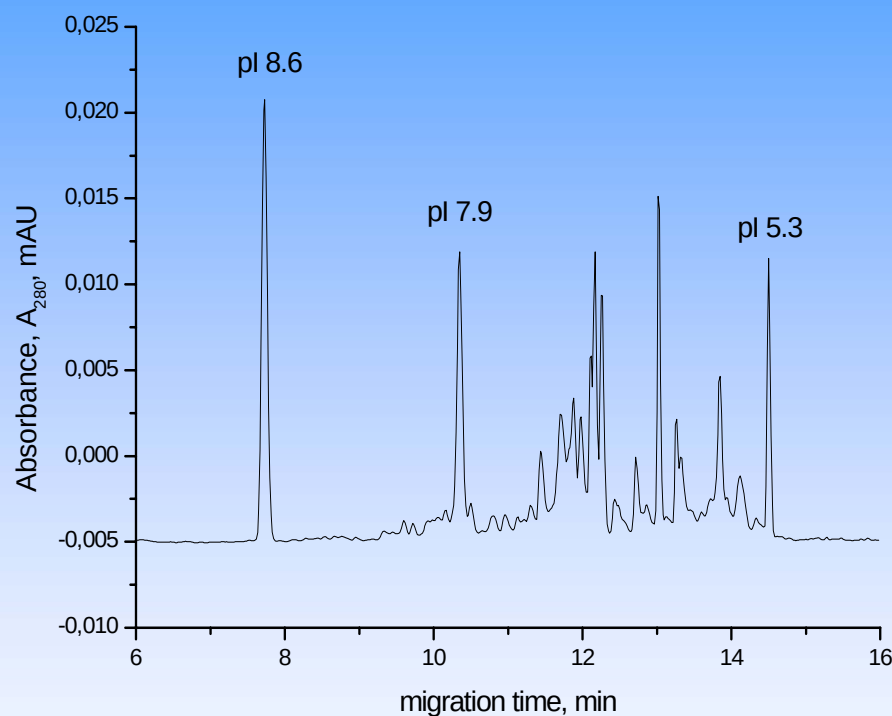
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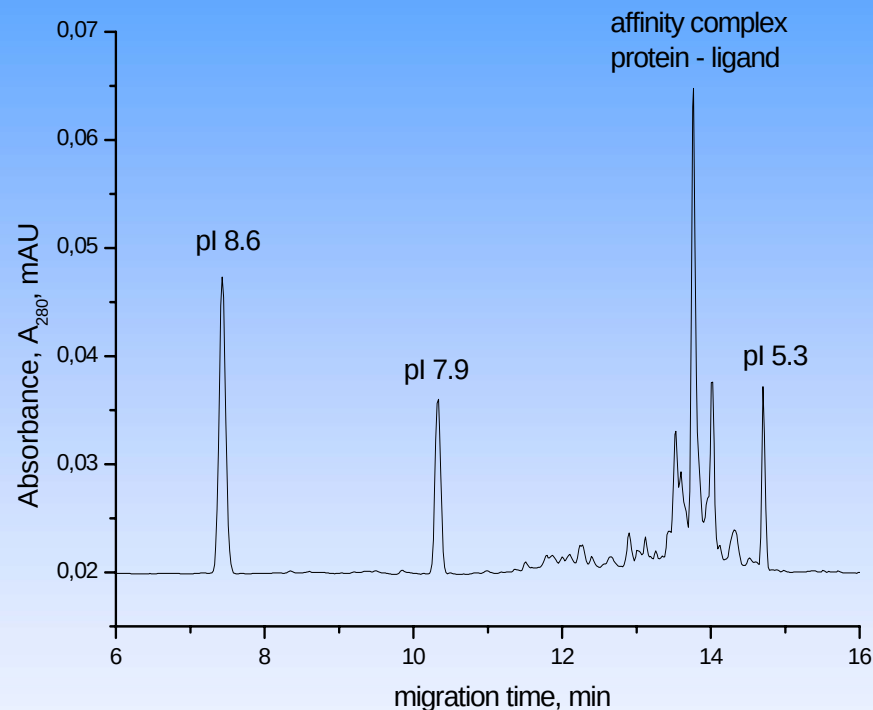


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Capillary IEF of protein and protein – ligand affinity complex



Sample: actinavidin



Sample: actinavidin
saturated with its ligand
biotin



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Affinity studies

- **Which interactions can be studied:**
 - antigen/antibody, receptor/ligand, drug/target protein, virus/neutralising antibodies, etc.
- **What can be determined:**
 - the amount of one of the reagent in a non-direct mode
 - affinity constants
 - stoichiometry
- **General affinity CE (ACE) approaches:**
 - in-column affinity interaction with one of the reagent dissolved in BGE
 - pre-column incubation of the reactants



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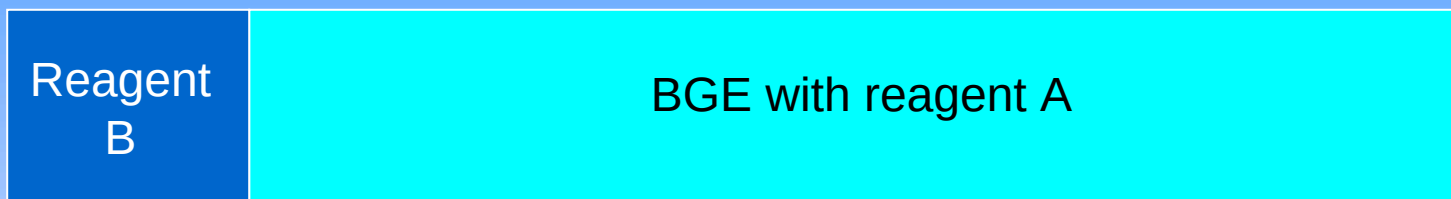


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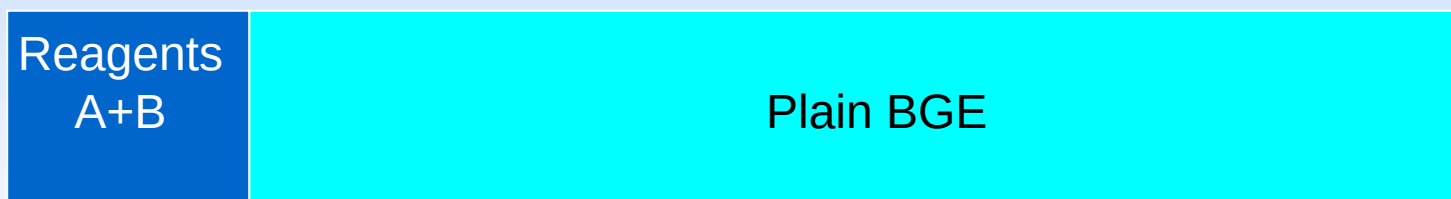
Two general ACE approaches



- in-column affinity reaction for low affinities



- off-column reaction prior to CE for high affinities



Affinity interactions affect migration time

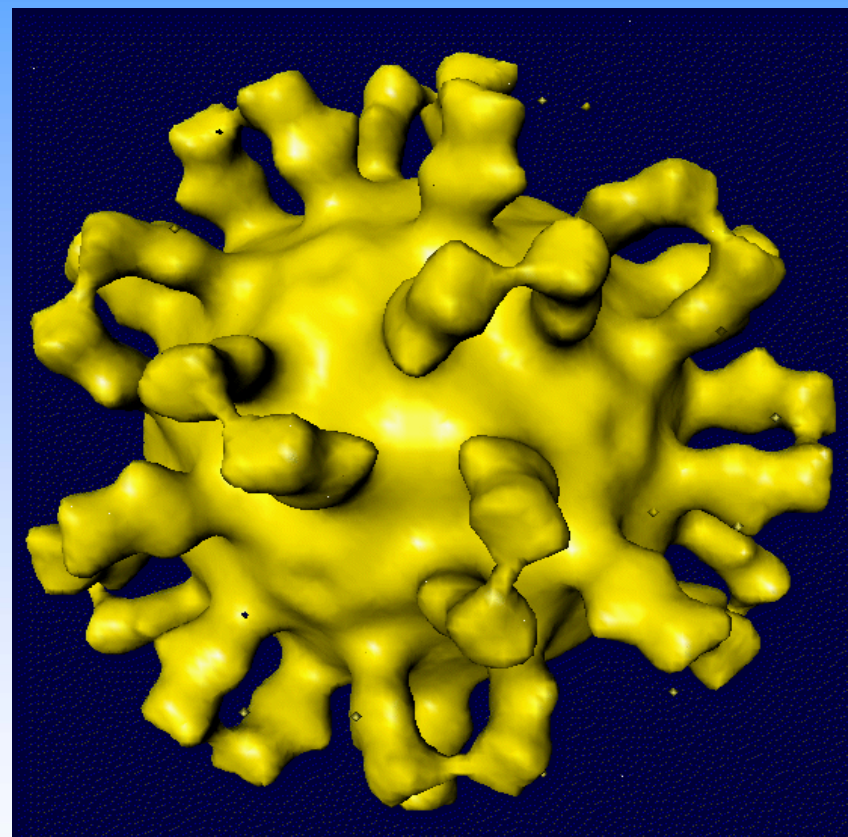
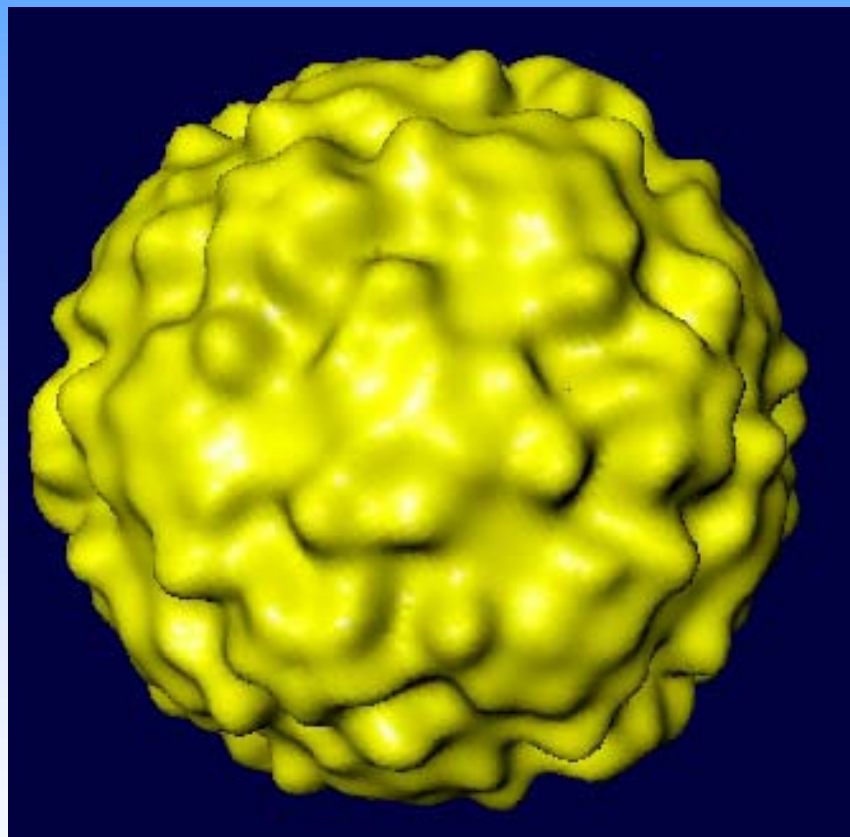


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Structure of HRV2 complexed to 8F5 IgG



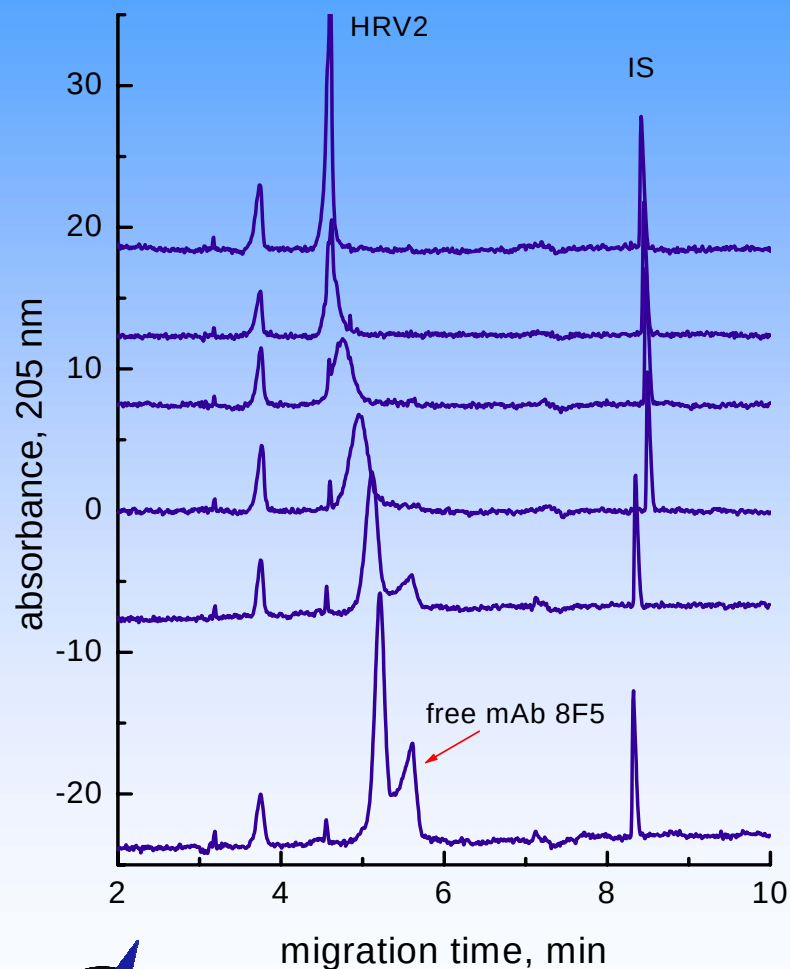
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Complex formation mAb–HRV2



Amount of antibodies

0 μM

0.06 μM

0.21 μM

0.62 μM

1.53 μM

3.06 μM



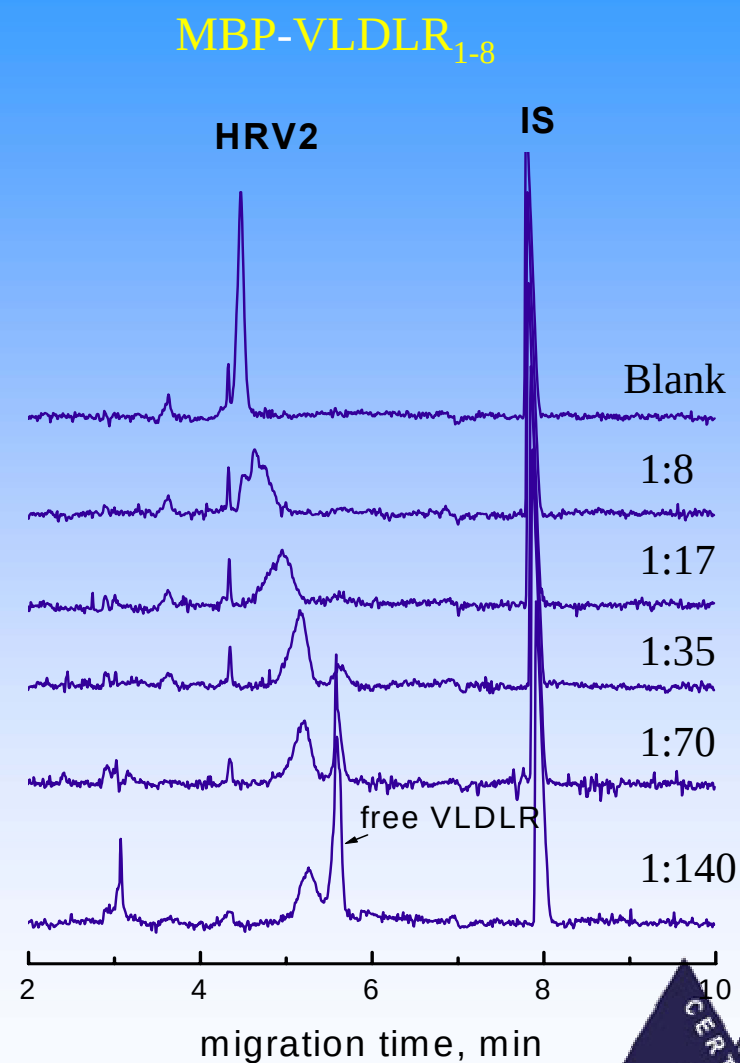
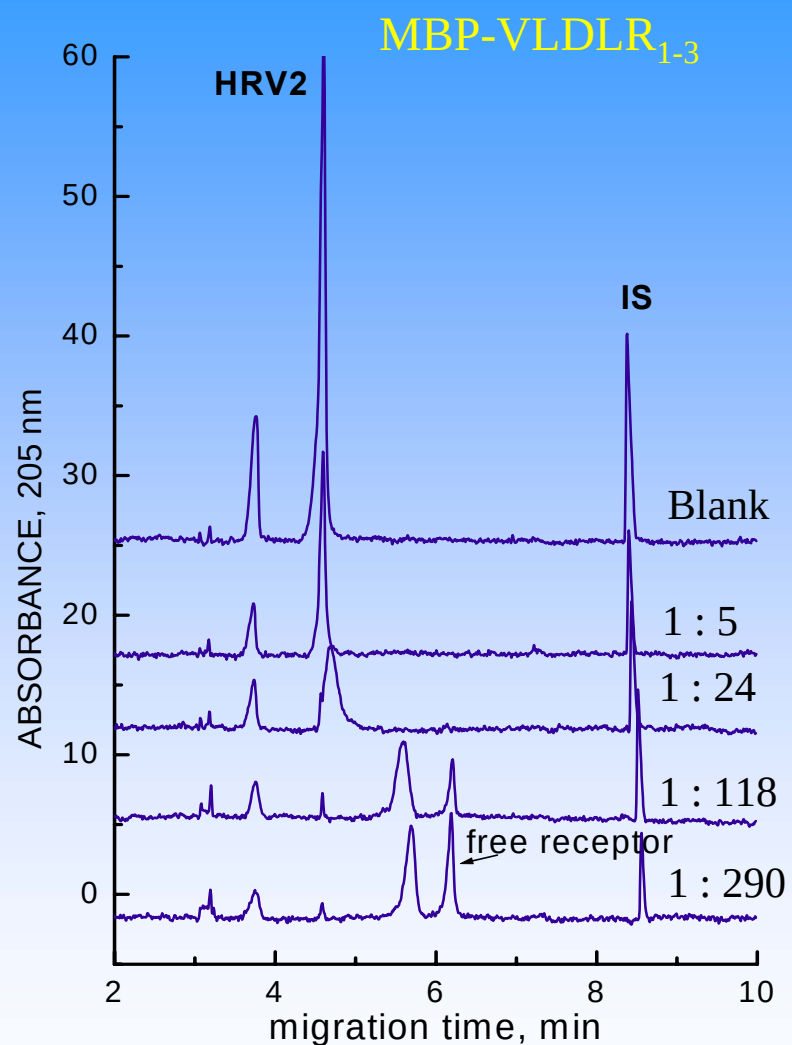
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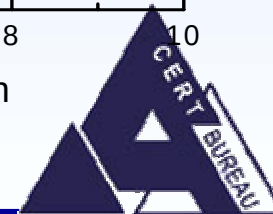
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Complex formation HRV2-Receptor



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Instrumental features, favorable for protein analysis

- ✓ Very easy to change capillary cassette or even a capillary for the multiple protein applications
- ✓ Flexibility in programming complex analyses



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Programming complex analyses

Program. C:\Documents and Settings\Vadim Okun\My Documents\Capel data\Capel data\standard.prg

Operation	Parameters	Cycle	Left	Right	Time	Pressure	Voltage	Wavelength	Temp.	Status
☒ Rinse			2	1	1		0			
☒ Rinse			3	1	1		0			
☒ Injection			5	2	10	50	0			
☒ Analysis			4	2	0,5	0,60	25,25	205,270		
Cycle	☐									
Left	4									
Right	2									
File	I\test2.mdf ...									
Time unit	min									
Invert	☐									
All time	10									
☐ Stage	1				0	0	25	205		Done
Start time	0									
Pressure	0									
Voltage	25									
Wavelength	205									
☐ Stage	2				5	60	25	270		
Start time	5									
Pressure	60									
Voltage	25									
Wavelength	270									

Operations

- Temperature
- Wavelength
- Rinse
- Injection
- Sweeping
- Analysis Stage
- Auto sampler
- Cycle end
- Pause
- Delete
- Clear
- Save
- Load
- Show all
- Collapse all
- To end

Run Close



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Capillary electrophoresis

Important economic considerations

- Very low sample consumption
normally 2 – 10 nL
- Minimum sample volume is very low
normally 50 µL
- Very low reagents consumption
normally 2 – 3 mL per day
- **Very low analysis cost**



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Conclusion

Capillary
Electrophoresis
System Capel-105M.



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