Technische Universität Braunschweig

Wätzig et al.
Institut für Medizinische und Pharmazeutische Chemie

# Protein Analysis by CE: Successes and Challenges

Hermann Wätzig[1]; Imke Oltmann-Norden[1]; Mona Mozafari[1]; Hassan A. Alhazmi[1, 2]; Markus Nachbar[1]; Matthias Stein[1]; Rebecca Wiesner[1]; Holger Zagst[1]; Christin Scheller[1]; Julia Kahle[1]

[1]*Institute of Medicinal and Pharmaceutical Chemistry, University of Braunschweig, Germany*

[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, Jazan University, 45142 Jazan, Saudi Arabia*

Correspondence: h.waetzig@tu-braunschweig.de

# A very beautiful protein

# but by far not the only one!

http://www.fitness4mma.de/ern-nahrungsmittel.php
http://www.protein-shake.ch/WelchesProtein/PflanzlichesProtein.aspx
http://proteineeiweiss.de/was-sind-proteine
http://www2.klett.de/sixcms/list.php?page=lehrwerk_extra&titelfamilie=&extra=MarkInline%20Biologie%20Oberstufe&modul=inhaltsammlung&inhalt=klett71prod_1.c.844549.de&kapitel=844584

# Success story SDS-PAGE and CE-SDS

A. Guttman, J. Nolan, Analytical Biochemistry 221, 285-289 (1994)

Rustandi, R. R., Washabaugh, M. W., Wang, Y.,
*Electrophoresis* 2008, *29*, 3612–3620.

Lacher, N. A., Roberts, R. K., He, Y., Cargill, H., Kearns, K. M., Holovics, H., Ruesch, M. N., *J. Sep. Sci.*, 2010, *33*, 218–227.

Nunnaly, B., Park, S. S., Patel, K., Hong, M., Zhang, X., Wang, S. X., Rener, B., Reed-Bogan, A., Salas-Solano, O., Lau, W., Girard, M., Carnegie, H., Garcia-Canas, V., Cheng, K. C., Zeng, M., Ruesch, M., Frazier, R., Jocheim, C., Natarajan, K., Jessop, K., Saeed, M., Moffatt, F., Madren, S., Thiam, S., Altria, K., *Chromatographia* 2007, *66*, 955–961.

Cari Sänger-van de Griend, CE-SDS method development, validation, and best practice—An overview,
Electrophoresis 2019, DOI 10.1002/elps.201900094

# Outline

**Proteins: classification**

antibodies and enzymes, collagen, IDPs, viruses, etc.
adsorption
selectivity; buffers, CE-MS; 2-DE

**Case study: collagen**

**Case study: AtHIRD11,** an intrinsically disordered protein (IDP)

**Protein size characterization**

**Preliminary conclusions and outlook**

# Protein classification

Clustering/Classification/Mapping by:

Sequence alignment; clustering

# Sequence alignment

- **1G1Q : P-Selectin lectin/ EGF domains**
- **4C16 : E-Selectin lectin, EGF-like and two SCR domains**

# Protein classification

Clustering/Classification/Mapping by:

Sequence alignment; clustering

# Protein classification

Clustering/Classification/Mapping by:

Sequence alignment; clustering

**Domains**

https://en.wikipedia.org/wiki/Protein_domain and examples given therein
Nir Ben-Tal, Rachel Kolodny, Isr. J. Chem. 2014, 54, 1286 – 1292
**InterPro:** Alex Mitchell et al., *Nucleic Acids Research, 2015, Vol. 43, Database issue D213–D221, doi: 10.1093/nar/gku1243*
***HMMER:*** Robert D. Finn et al., *Nucleic Acids Research, 2015, Vol. 43, Web Server issue, published online 05 May 2015, doi: 10.1093/nar/gkv397*

# Proteins: similar domains, similar selectivity?

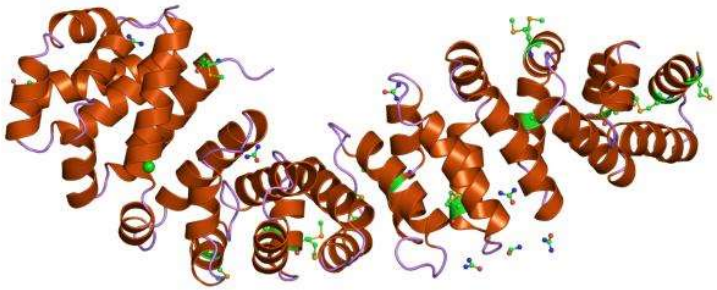
Overview of protein structures (http://absoluteantibody.com; 2015)

# Proteins: similar domains, similar selectivity?

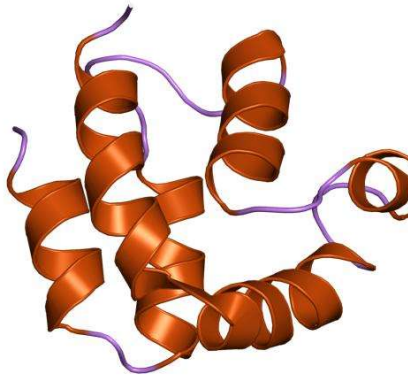
Overview of protein structures (http://absoluteantibody.com; 2015, 2019)

# Proteins: similar domains, similar selectivity?

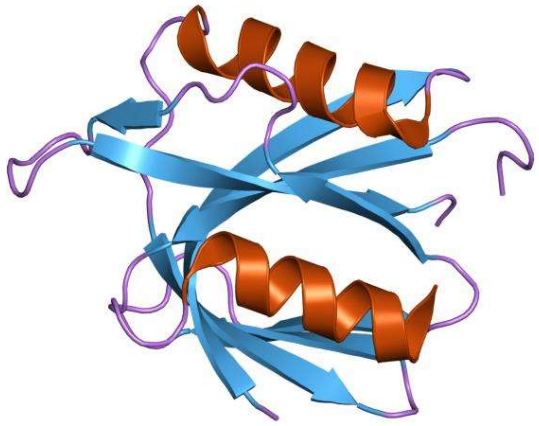
https://en.wikipedia.org/wiki/Protein_domain, examples:



Armadillo repeats



Death effector domain (DED)

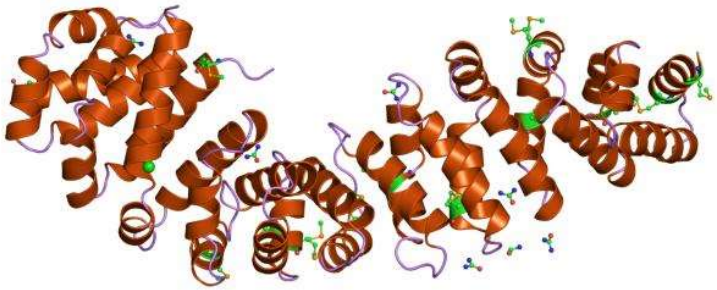


Phosphotyrosine-binding domain (PTB)

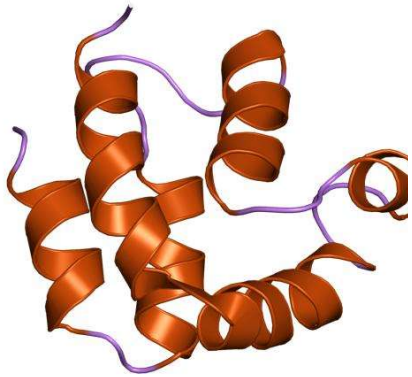
By Jawahar Swaminathan and MSD staff at the European Bioinformatics Institute - http://www.ebi.ac.uk/pdbe-srv/view/images/entry/3bct600.png, displayed on http://www.ebi.ac.uk/pdbe-srv/view/entry/3bct/summary, Public Domain, https://commons.wikimedia.org/w/index.php?curid=5937207

# But: there are properties which cannot be explained by domains only: e.g. adsorption/aggregation

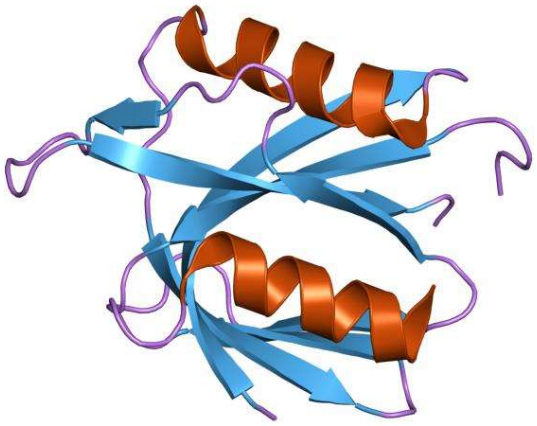
https://en.wikipedia.org/wiki/Protein_domain, examples:



Armadillo repeats



Death effector domain (DED)



Phosphotyrosine-binding domain (PTB)

By Jawahar Swaminathan and MSD staff at the European Bioinformatics Institute - http://www.ebi.ac.uk/pdbe-srv/view/images/entry/3bct600.png, displayed on http://www.ebi.ac.uk/pdbe-srv/view/entry/3bct/summary, Public Domain, https://commons.wikimedia.org/w/index.php?curid=5937207

# But: there are properties which cannot be explained by domains only: e.g. adsorption/aggregation

Agrawal NJ, Kumar S, Wang X et al. (2011) J Pharm Sci 100(12): 5081–5095. doi: 10.1002/jps.22705.

# Protein classification

Clustering/Classification/Mapping by:

Sequence alignment; clustering

**Domains**

https://en.wikipedia.org/wiki/Protein_domain and examples given therein
Nir Ben-Tal, Rachel Kolodny, Isr. J. Chem. 2014, 54, 1286 – 1292
**InterPro:** Alex Mitchell et al., *Nucleic Acids Research, 2015, Vol. 43, Database issue D213–D221, doi: 10.1093/nar/gku1243*
***HMMER:*** Robert D. Finn et al., *Nucleic Acids Research, 2015, Vol. 43, Web Server issue, published online 05 May 2015, doi: 10.1093/nar/gkv397*

# Protein classification

Clustering/Classification/Mapping by:

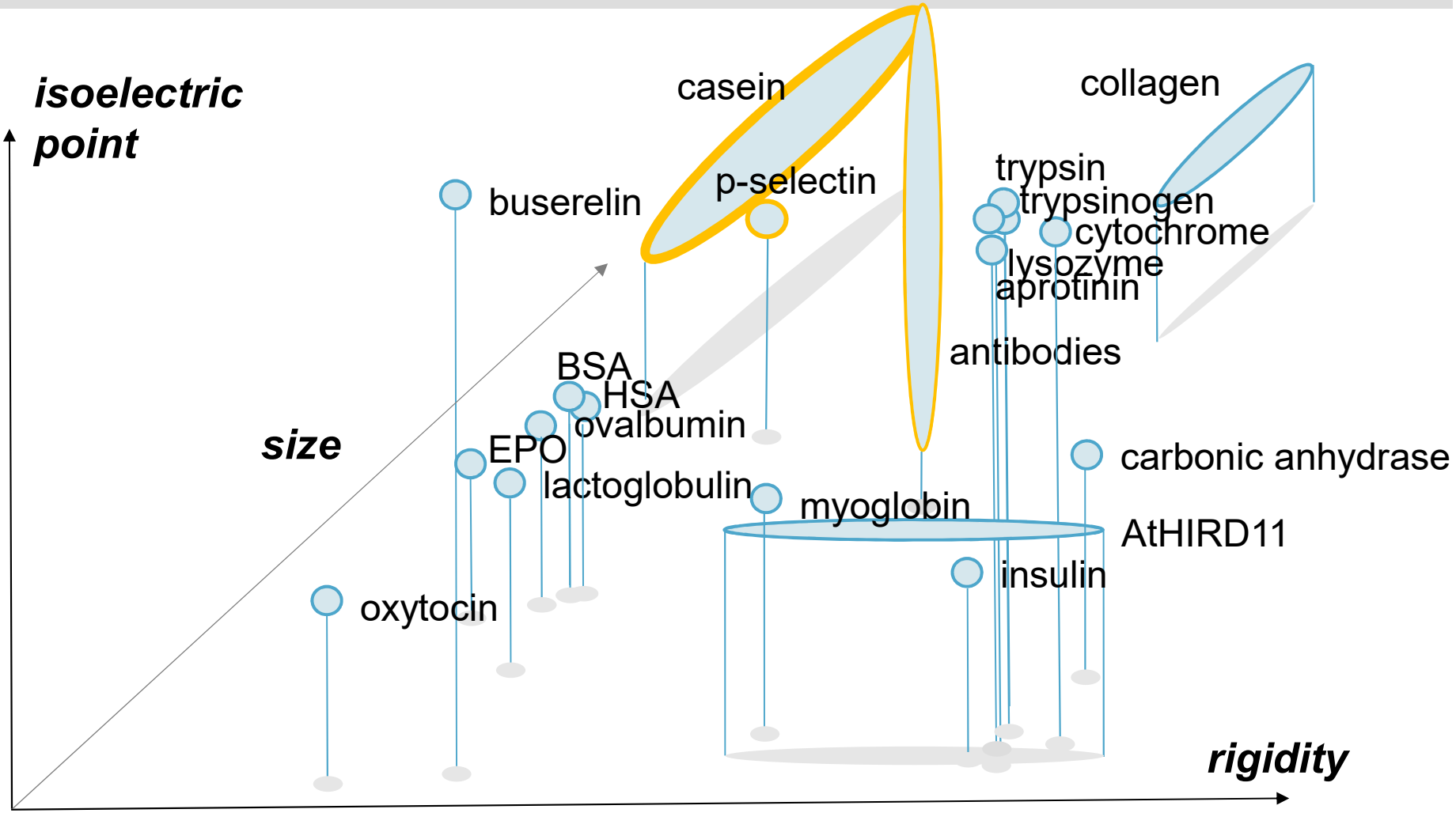
Sequence alignment; clustering

**Domains**

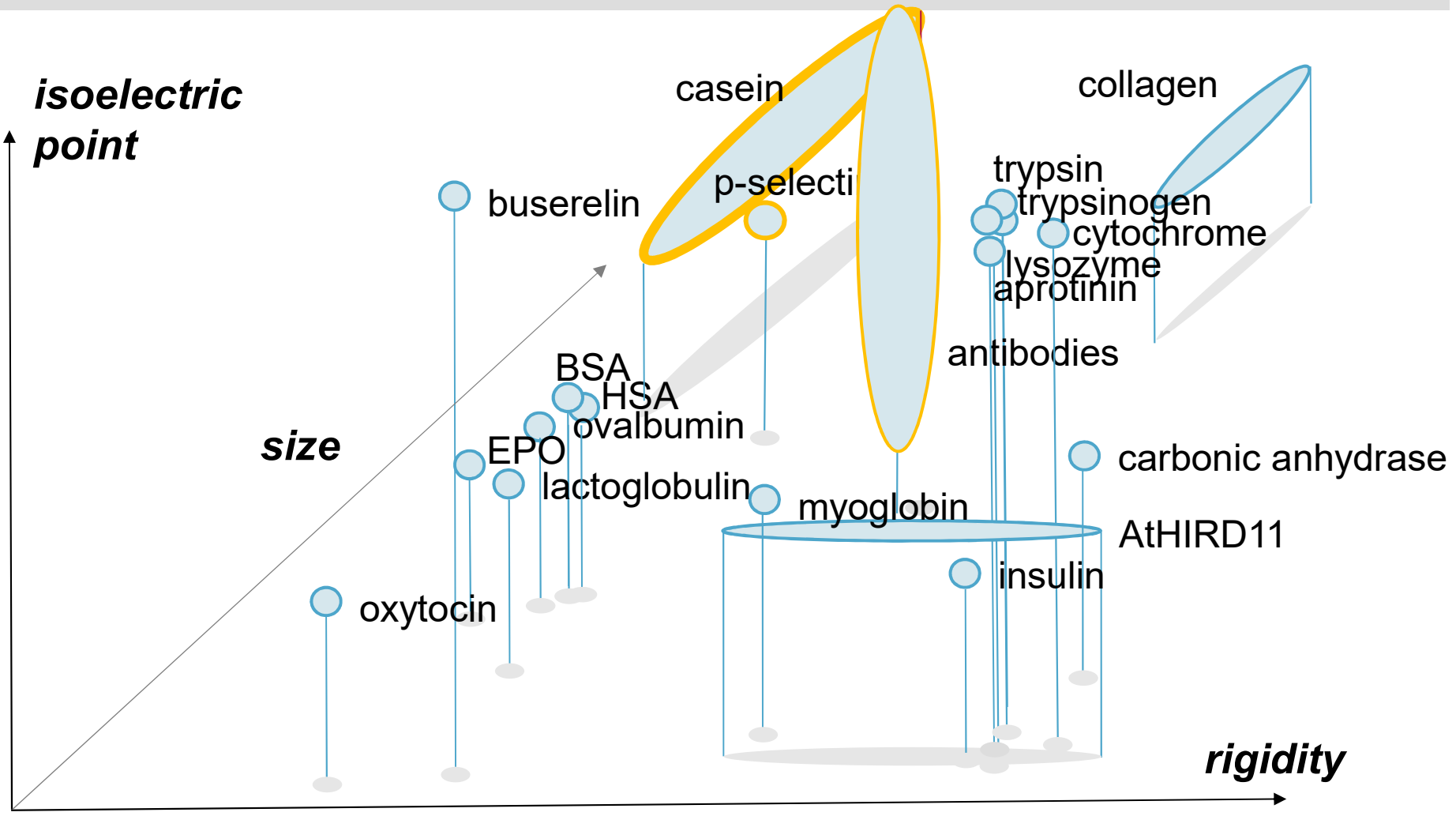
https://en.wikipedia.org/wiki/Protein_domain and examples given therein
Nir Ben-Tal, Rachel Kolodny, Isr. J. Chem. 2014, 54, 1286 – 1292
**InterPro:** Alex Mitchell et al., *Nucleic Acids Research, 2015, Vol. 43, Database issue D213–D221, doi: 10.1093/nar/gku1243*
***HMMER:*** Robert D. Finn et al., *Nucleic Acids Research, 2015, Vol. 43, Web Server issue, published online 05 May 2015, doi: 10.1093/nar/gkv397*

**Consider Function/Physicochemical Properties?**

# Physicochemical Properties of Proteins



# Physicochemical Properties of Proteins

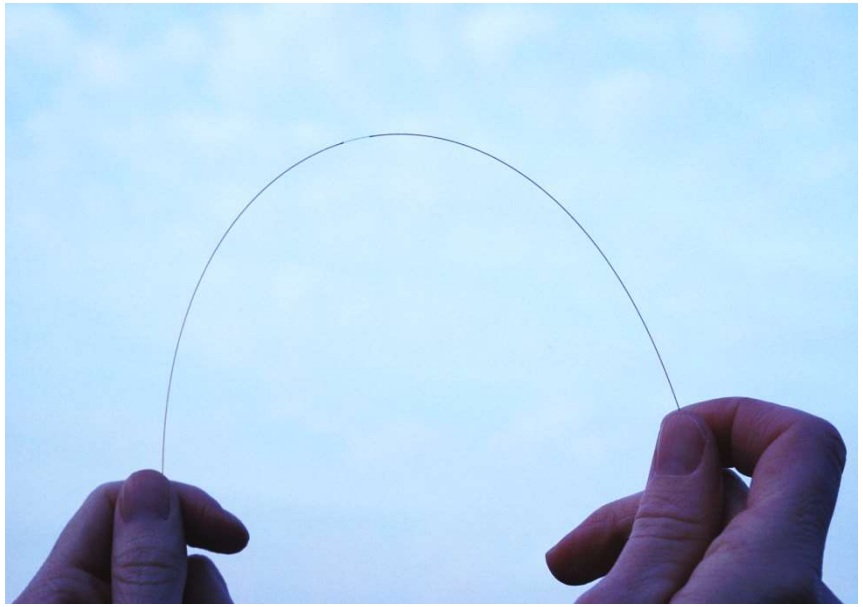


# Capillary Electrophoresis (CE): Capillaries

material

- amorphous fused silica ($SiO_2$)
- outer polyimide coating

  ➔ very flexible
- inner coating possible



typical diameters

- inner: 20-100 µm
- outer: 150-375 µm

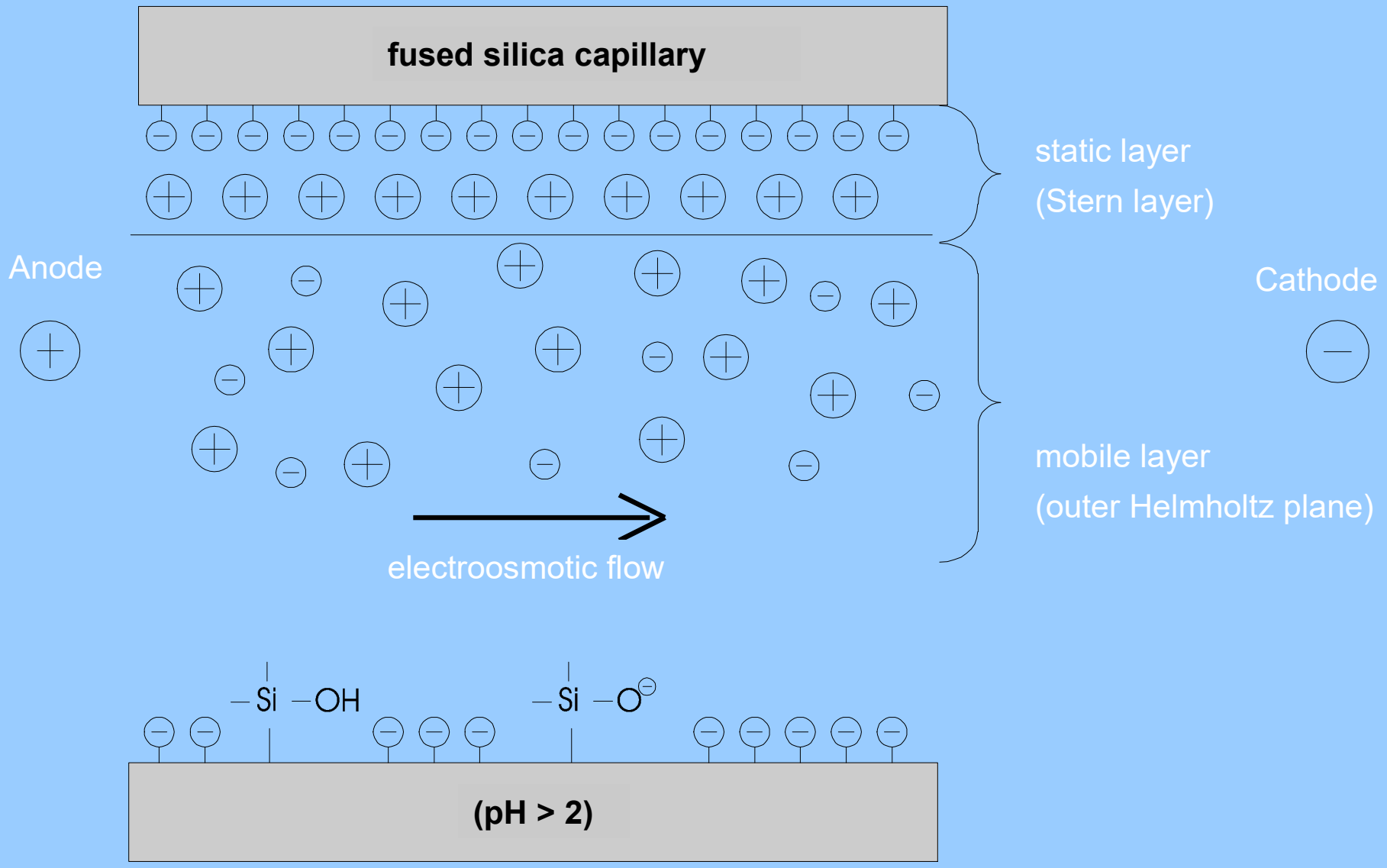
lengths

- many possibilities
- mostly 30-100 cm

# Capillary Electrophoresis (CE): Capillaries

from: Landers, J. P.: Handbook of Capillary Electrophoresis

# The ElectroOsmotic Flow (EOF)



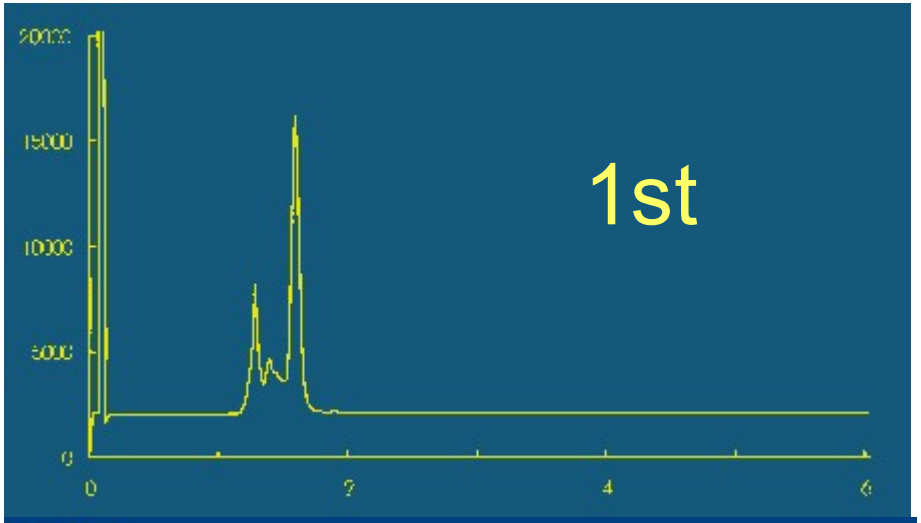
# Challenge: Protein adsorption

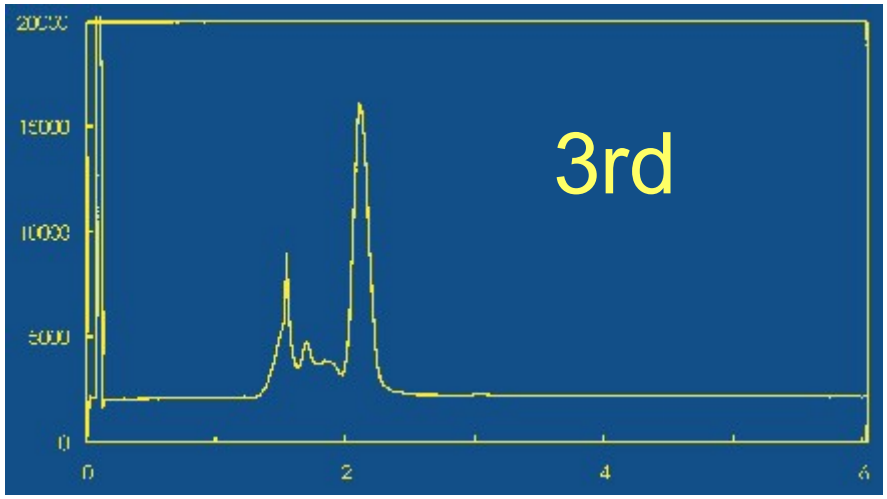
S. Kaupp, R. Steffen,
H. Wätzig
J. Chromatogr. A 744,
93-101 (1996)

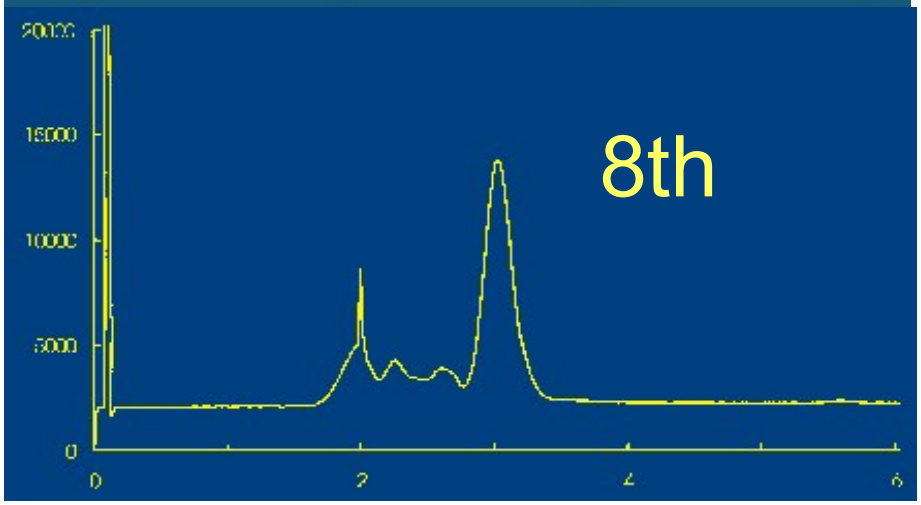
H. Wätzig, S. Kaupp,
M. Graf
Trends Anal. Chem.,
22(10), 588-604 (2003)

# Separation of plasma proteins without regenerating the capillary:

## 1st, 3rd and 8th run



# Rinsing: sucessful approaches 1997


200 mM SDS, high pH (e.g. pH 10, borate buffer), organic solvent (e.g. isopropanol)

sodium hydroxide

enzymes

hydrofluoric acid (HF)

# Precision of protein analysis by LPA-coated capillaries and 2M HCl, 3M HCl and phosphoric acid 85% (w/w) rinsing

| Protein | Concentration | pH | Rinsing Reagent | Number of Runs | tmig of EOF marker | μEOF app | |
|---|---|---|---|---|---|---|---|
| | | | | | mean ± SD [min] | mean ± SD [$10^{-8}m^2V^{-1}s^{-1}$] | RSD% |
| ß-lactoglobulin | 175 μM | 5.5 | 2M HCl | 60 | 9.82 ± 0.527 | 2.45 ± 0.129 | **5.27** |
| ß-lactoglobulin | 175 μM | 5.5 | 3M HCl | 60 | 10.51 ± 0.372 | 2.29 ± 0.079 | 3.43 |
| ß-lactoglobulin | 175 μM | 5.5 | $H_3PO_4$ 85% (w/w) | 60 | 10.77 ± 0.263 | 2.23 ± 0.054 | 2.44 |

| Protein | Concentration | pH | Rinsing Reagent | Number of Runs | tmig of EOF marker | μEOF app | |
|---|---|---|---|---|---|---|---|
| | | | | | mean ± SD [min] | mean ± SD [$10^{-8}m^2V^{-1}s^{-1}$] | RSD% |
| ß-casein | 35 μM | 3.5 | 2M HCl | 30 | 8.26 ± 0.42 | 2.91 ± 0.152 | **5.2** |
| ß-casein | 35 μM | 3.5 | $H_3PO_4$ 85% (w/w) | 30 | 8.28 ± 0.167 | 2.90 ± 0.06 | 2.08 |

A. Suratman, H. Wätzig, Electrophoresis 2007, 28, 2324-2328

# Precision of protein analysis by LPA-coated capillaries and 2M HCl, 3M HCl and phosphoric acid 85% (w/w) rinsing

| Protein | Concentration | pH | Rinsing Reagent | Number of Runs | tmig of EOF marker | | |
|---|---|---|---|---|---|---|---|
| | | | | | mean ± SD [min] | mean [$10^{-8}m^2$ | |
| ß-lactoglobulin | 175 µM | 5.5 | 2M HCl | 60 | 9.82 ± 0.527 | 2.45 ± 0.129 | **5.27** |
| ß-lactoglobulin | 175 µM | 5.5 | 3M HCl | 60 | 10.51 ± 0.372 | 2.29 ± 0.079 | 3.43 |
| ß-lactoglobulin | 175 µM | 5.5 | $H_3PO_4$ 85% (w/w) | 60 | 10.77 ± 0.263 | 2.23 ± 0.054 | 2.44 |

| Protein | Concentration | pH | Rinsing Reagent | Number of Runs | tmig of EOF marker | µEOF app | |
|---|---|---|---|---|---|---|---|
| | | | | | mean ± SD [min] | mean ± SD [$10^{-8}m^2V^{-1}s^{-1}$] | RSD% |
| ß-casein | 35 µM | 3.5 | 2M HCl | 30 | 8.26 ± 0.42 | 2.91 ± 0.152 | **5.2** |
| ß-casein | 35 µM | 3.5 | $H_3PO_4$ 85% (w/w) | 30 | 8.28 ± 0.167 | 2.90 ± 0.06 | 2.08 |

A. Suratman, H. Wätzig, Electrophoresis 2007, 28, 2324-2328

# Proteins: Rinsing: systematic approaches

**Application of Affinity Capillary Electrophoresis for Charge Heterogeneity Profiling of Biopharmaceuticals**

Hutanu et al. Andrei Hutanu*, Steffen Kiessig, Andrea Bathke, Rolf Ketterer, Sonja Riner, Jan Olaf Stracke, Markus Wild, Bernd Moritz. Electrophoresis 2019, accepted 13 SEP 2019

New approaches;
Lit. cited: [7-13, 26-31]
Outlined strategy

New reagents: guanidine hydrochloride, urea

# Rinsing: sucessful approaches today


200 mM SDS, high pH (e.g. pH 10, borate buffer), organic solvent (e.g. isopropanol)

sodium hydroxide

hydrochloric acid (e.g. 2 M)

phosphoric acid (85% )

guanidine hydrochloride (GDnCl)

urea

# Capillary coatings

**permanent coatings, e.g.**

linear polyacrylamide (LPA)
fluorocarbon

**dynamic coatings, e.g.**

(poly/oligo)amines
polyethylene oxide (PEO)
Polyvinyl alcohol (PVA)
etc.

L. Hajba, A. Guttman, Trends Anal. Chem. 2017, doi: 10.1016/j.trac.2017.02.013.

# Proteins and coatings: systematic approaches

A.-K. Schuler, O. Prucker, J. Rühe
*Macromol. Chem. Phys. 2016*
*DOI: 0.1002/macp.201600065*

Oswald Prucker, Thomas Brandstetter, and Jürgen Rühe, Surface-attached hydrogel coatings via C,H-insertion crosslinking for biomedical and bioanalytical applications (Review)
Biointerphases 13, 010801 (2018);
doi: 10.1116/1.4999786

# Selectivity in CE

Strategies for method development and validation in CE - related to pharmaceutical and biological applications
Hermann Wätzig, Matthias Degenhardt, Annette Kunkel
Electrophoresis 19, 2695-2752 (1998)

# Selectivity in CE

Strategies for method development and validation in CE - related to pharmaceutical and biological applications
Hermann Wätzig, Matthias Degenhardt, Annette Kunkel
Electrophoresis 19, 2695-2752 (**1998**)

=> needs an update!

# **Selectivity in CE**; H.Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19, 2695-2752 (1998), supplement to: Table 4. Buffer additives to enhance selectivity in CE, *ion pairing reagents*

| | |
|---|---|
| cetyltrimethylammonium bromide (CTAB) | [191][1)] |
| 2-(N-Cyclohexylamino)ethanesulfonic acid (CHES) | [192, 193] |
| N-dodecyl-N,N-dimethyl-3-amino-1-propanesulfonate (DAPS) | [194] |
| dodecyltrimethylammonium chloride | [195, 196] |
| hexadecyltrimethylammonium bromide | [197] |
| hexadimethrine-bromide (polybrene) | [102, 195, 198, 199] |
| poly(diallyldimethyl)ammonium chloride (PDDAC) | [102, 195] |
| carboxylic acids (acetate, lactate, tartrate, hydroxyisobutyrate) | [102, 200] |
| carboxylated cyclodextrins | [201][2)] |
| hexane sulfonic acid | [102, 202] |
| perchlorate | [102, 190] |
| sodium n-alkyl sulphonates | [102, 203] |
| Tetrabutylammonium bromide (TBA) | Boyce and Haddad 2003 |
| Hexamethonium bromide | Boyce and Haddad 2003 |
| Diammonium hydrogen phosphate | Boyce and Haddad 2003 |
| PDADMA | Boyce and Haddad 2003 |
| Polyethyleneimine (PEI) | Boyce and Haddad 2003 |
| Camphorsulfonate | Fillet et al. 2003 |

Christin Scheller

Boyce, Mary C.; Haddad, Paul R. (2003): *Electrophoresis* 24 (12-13), S. 2013–2022.
Fillet, Marianne; Servais, Anne-Catherine; Crommen, Jacques (2003): *Electrophoresis* 24 (10), pp. 1499–1507.

# **Selectivity in CE**; H.Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19, 2695-2752 (1998), supplement to: Table 4. Buffer additives to enhance selectivity in CE, ***ion pairing reagents***

| | |
|---|---|
| cetyltrimethylammonium bromide (CTAB) | [191][1)] |
| 2-(N-Cyclohexylamino)ethanesulfonic acid (CHES) | [192, 193] |
| N-dodecyl-N,N-dimethyl-3-amino-1-propanesulfonate (DAPS) | [194] |
| dodecyltrimethylammonium chloride | [195, 196] |
| hexadecyltrimethylammonium bromide | [197] |
| hexadimethrine-bromide (polybrene) | [102, 195, 198, 199] |
| poly(diallyldimethyl)ammonium chloride (PDDAC) | [102, 195] |
| carboxylic acids (acetate, lactate, tartrate, hydroxyisobutyrate) | [102, 200] |
| carboxylated cyclodextrins | [201][2)] |
| hexane sulfonic acid | [102, 202] |
| perchlorate | [102, 190] |
| sodium n-alkyl sulphonates | [102, 203] |
| Tetrabutylammonium bromide (TBA) | Boyce and Haddad 2003 |
| Hexamethonium bromide | Boyce and Haddad 2003 |
| Diammonium hydrogen phosphate | Boyce and Haddad 2003 |
| PDADMA | Boyce and Haddad 2003 |
| Polyethyleneimine (PEI) | Boyce and Haddad 2003 |
| Camphorsulfonate | Fillet et al. 2003 |

Christin Scheller

Boyce, Mary C.; Haddad, Paul R. (2003): *Electrophoresis* 24 (12-13), S. 2013–2022.
Fillet, Marianne; Servais, Anne-Catherine; Crommen, Jacques (2003): *Electrophoresis* 24 (10), pp. 1499–1507.

## **Selectivity in CE**; H.Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19, 2695-2752 (1998), supplement to: Table 4. Buffer additives to enhance selectivity in CE, **surfactants**

| | |
|---|---|
| several chiral surfactants | [53] |
| Triton X-100 | [204] |
| sodium deoxycholate | [204] |
| CTAB | [204] |
| SDS | [204] |
| Sodium tetradecyl sulfate (STS) | Fillet et al. 2003 |
| Sodium hexadecyl sulfate (SHS) | Fillet et al. 2003 |
| Dodecyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide (TTAB) | Fritz et a. 2002 |
| Polyoxyethylene sulfate (Brij-S) | Pirogov und Shpigun 2003 |

Christin Scheller


Fillet, Marianne; Servais, Anne-Catherine; Crommen, Jacques (2003): *Electrophoresis* 24 (10), pp. 1499–1507.

Fritz, James S.; Breadmore, Michael C.; Hilder, Emily F.; Haddad, Paul R. (2002): *Journal of Chromatography A* 942 (1-2), S. 11–32.

Pirogov, Andrei V.; Shpigun, Oleg A. (2003): *Electrophoresis* 24 (12/13), pp. 2099–2105.

## **Selectivity in CE**; H.Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19, 2695-2752 (1998), supplement to: Table 4. Buffer additives to enhance selectivity in CE, **complexing reagents**

| | |
|---|---|
| poly(ethylene glycol) (PEG 400, 4000, 20000) | [205 - 207] |
| EDTA | [208, 209] |
| crown ethers | [210] |
| heavy metal ions ($Cu^{2+}$, $Zn^{2+}$, $Ca^{2+}$) | [211] |
| $Cu^{2+}$ | [212 - 214] |
| $Cu^{+}$, $Ag^{+}$ | [215][3)] |
| $Ca^{2+}$ | [208, 215[3)], 216] |
| $Pb^{2+}$ | [215] |
| $Zn^{2+}$ | [196, 208, 213, 216, 217] |
| α, β, γ-CD's | [218 - 221, 222[2)]] |
| Borate | [20, 78, 223, 224, 225[4)], 226, 227[4)],228 - 230] |
| PVP | Pirogov und Shpigun 2003 |
| $MoO_4^{2-}$ or $WO_4^{2-}$ | Široká et al. 2011 |

Christin Scheller


Pirogov, Andrei V.; Shpigun, Oleg A. (2003): *Electrophoresis* 24 (12/13), pp. 2099–2105.

Široká, Jitka; Jáč, Pavel; Polášek, Miroslav (2011): *TrAC Trends in Analytical Chemistry* 30 (1), S. 142–152.

# Comprehensive platform for protein-metal ion interactions

- Generic methods for all relevant metal species
- including reference values for a set of standard proteins

AlHazmi et al., J. Pharm. Biomed. Anal., 2015, 107, 311–317.

Dr. Hassan AlHazmi

## **Selectivity in CE**; H.Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19, 2695-2752 (1998), supplement to: Table 4. Buffer additives to enhance selectivity in CE, **proteins**

| | |
|---|---|
| bovine serum albumine | [231[2)], 232] |
| human serum albumine | [233][5)] |
| $\alpha_1$-glycoprotein, ovomucoid, avidin, conalbumin | [232] |
| β-lactoglobulin | Lloyd et al. 1997 |
| Casein | Lloyd et al. 1997 |
| Cellobiohydrolase | Lloyd et al. 1997 |
| Human serum transferrin | Lloyd et al. 1997 |
| Riboflavin binding protein | Lloyd et al. 1997 |

Christin Scheller

Lloyd, David K.; Aubry, Anne-Françoise; Lorenzi, Ersilia de (1997): Selectivity in capillary electrophoresis: the use of proteins. In: *Journal of Chromatography A* 792 (1-2), pp. 349–369.

**Selectivity in CE**; H.Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19, 2695-2752 (1998), supplement to: Table 4. Buffer additives to enhance selectivity in CE, miscellaneous

| | |
|---|---|
| urea | [174, 234 - 237] |
| ethidinium bromide | [238, 239[6)] ] |
| DMSO | [220] |
| 2,10-ionene | Pirogov und Shpigun 2003 |

Christin Scheller

Pirogov, Andrei V.; Shpigun, Oleg A. (2003): Application of water-soluble polymers as modifiers in electrophoretic analysis of phenols. *Electrophoresis* 24 (1213), pp. 2099–2105.

## **Selectivity in CE**; H.Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19, 2695-2752 (1998), supplement to: Table 6. Chiral selectors, cyclodextrins

| | |
|---|---|
| β-CD | [48, 51] |
| hydroxyethyl- and -propyl-β-CD | [48, 51, 278 - 281] |
| heptakis-2,6–di-O-methyl-β-CD | [48, 51] |
| heptakis-2,3,6–tri-O-methyl-β-CD | [48] |
| other β-CD derivates | [48] |
| β-CD polymer | [48, 282] |
| γ-CDs | [30, 48, 51, 283, 284] |
| hydroxypropyl-γ-CD | [48] |
| heptakis-2,6–di-O-methyl-γ-CD | [48] |
| α-CDs | [30, 48, 51] |
| heptakis-2,3,6–tri-O-methyl-α-CD | [48] |
| hydroxypropyl-α-CD | [48] |
| ODMS- γ-CD | Fillet et al. 2003 |

Christin Scheller

Fillet, Marianne; Servais, Anne-Catherine; Crommen, Jacques (2003): *Electrophoresis* 24 (10), pp. 1499–1507.

## **Selectivity in CE**; H.Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19, 2695-2752 (1998), supplement to: Table 6. Chiral selectors, noncyclic saccharides

| | |
|---|---|
| non-cyclic oligosaccharides | [293, 294] |
| maltodextrin oligosaccarides | [295][1)] |
| heparin | [296, 297] |
| carboxymethyl amylose sodium salt | [298] |
| methyl- and hydroxypropyl-cellulose | [298] |
| amyloses, laminaran, pullulan | [298] |
| Dextran sulfate | Boer et al. 1999 |

Christin Scheller

Boer, Theo de; Zeeuw, Rokus A. de; Jong, Gerhardus J. de; Ensing, Kees (1999): Selectivity in capillary electrokinetic separations. *Electrophoresis* 20 (15-16), pp. 2989–3010.

## **Selectivity in CE**; H.Wätzig, M. Degenhardt, A. Kunkel, Electrophoresis 19, 2695-2752 (1998), supplement to: Table 6. Chiral selectors, miscellaneous

| | |
|---|---|
| Quinine[1)] | [155] |
| 10 new semi-synthetic surfactants | [52] |
| 18-crown-6 tetracarboxylic acid | [48[2)], 306] |
| Camphorsulfonates | Boer et al. 1999 |
| Calixarenes; (p-sulfonic calix[4]-arene) | Boer et al. 1999 |
| Ergot alkaloids | Boer et al. 1999 |
| Quinidine, other cinchonia alkaloids and derivates | Fillet et al. 2003 |
| Tert-butyl-carbamoylquinine | Fillet et al. 2003 |
| (-)-2,3:4,6-di-O-isopropylidene-2-keto-L-gulonic acid (DIKGA) | Fillet et al. 2003 |

Christin Scheller

Boer, Theo de; Zeeuw, Rokus A. de; Jong, Gerhardus J. de; Ensing, Kees (1999): *Electrophoresis* 20 (15-16), pp. 2989–3010.

Fillet, Marianne; Servais, Anne-Catherine; Crommen, Jacques (2003): *Electrophoresis* 24 (10), pp. 1499–1507.

# Selectivity: a few remarks about CE-MS

Ivan Mikšík, J. Sep. Sci. 2019 (42), 385-397; DOI: 10.1002/jssc.201800817

=> sample pretreatment and interfacing remain main issues

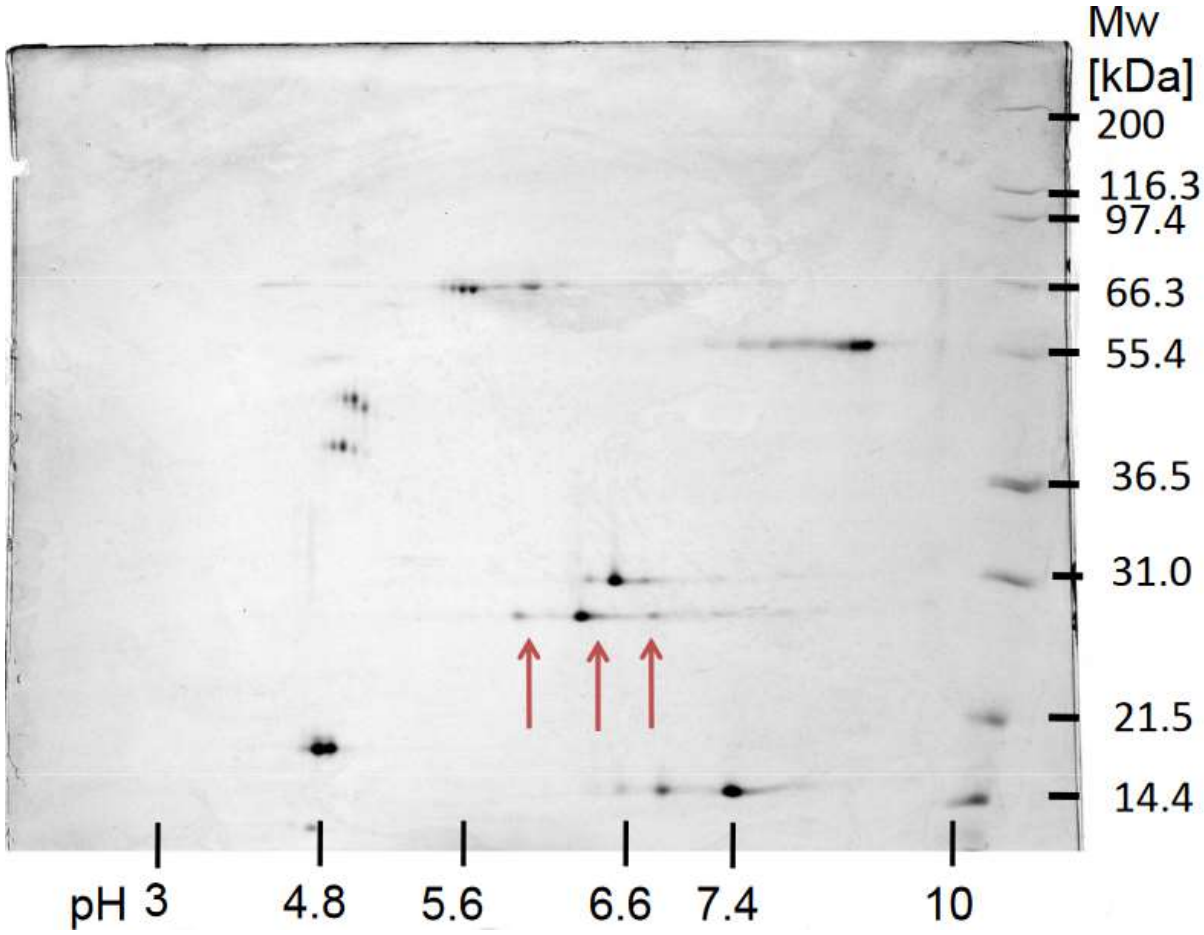
The Neusüß Interface, K. Jooß, et al.,
Electrophoresis 2019 (40), 1061-65

B. Rudisch, T. Melzer, H. G. Graf, C. Huhn,
ITP 2019, 1-4 SEP, Toulouse

Recent advantages: David D. Y. Chen,
ITP 2019

# Selectivity: two-dimensional electrophoresis (2-DE)

# Selectivity: two-dimensional electrophoresis (2-DE)

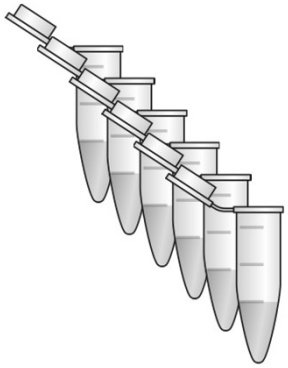



X. Deng, et al., Electrophoresis 2012, 33,263-269

www.tu-braunschweig.de/Medien-DB/pharmchem/supporting_information-5.pdf

# Selectivity: two-dimensional electrophoresis (2-DE)

HPLC-IEX



MCE-SDS

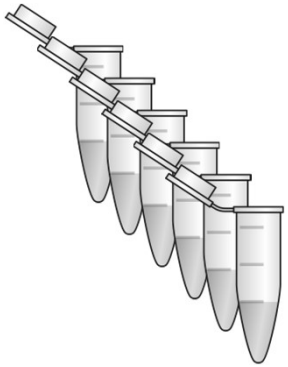
3. SIZE BASED SEPARATION

1. CHARGE BASED SEPARATION

2. FRACTION COLLECTION

# 2D separation: principle

HPLC-IEX



MCE-SDS

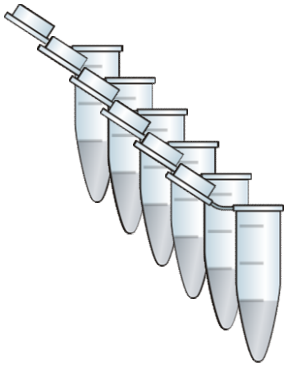
3. SIZE BASED SEPARATION

1. CHARGE BASED SEPARATION

2. FRACTION COLLECTION

# 2D separation: principle

45 minutes
HPLC-IEX



MCE-SDS

3. SIZE BASED SEPARATION

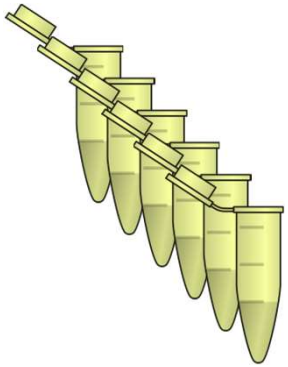
1. CHARGE BASED SEPARATION

2. FRACTION COLLECTION

# 2D separation: principle

45 minutes
HPLC-IEX

variable
amount



MCE-SDS

3. SIZE BASED SEPARATION

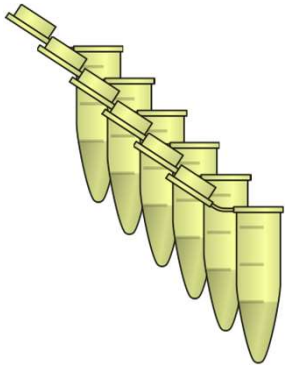
1. CHARGE BASED SEPARATION

2. FRACTION COLLECTION

# 2D separation: principle

45 minutes
HPLC-IEX

variable
amount



45 seconds
MCE-SDS

3. SIZE BASED SEPARATION

1. CHARGE BASED SEPARATION

2. FRACTION COLLECTION

# Conventional vs. Microchip-CE-SDS (MCE-SDS)

- Significantly shorter separation channel


- Significantly shorter analysis time

# Patent 18700637.4 - 1020

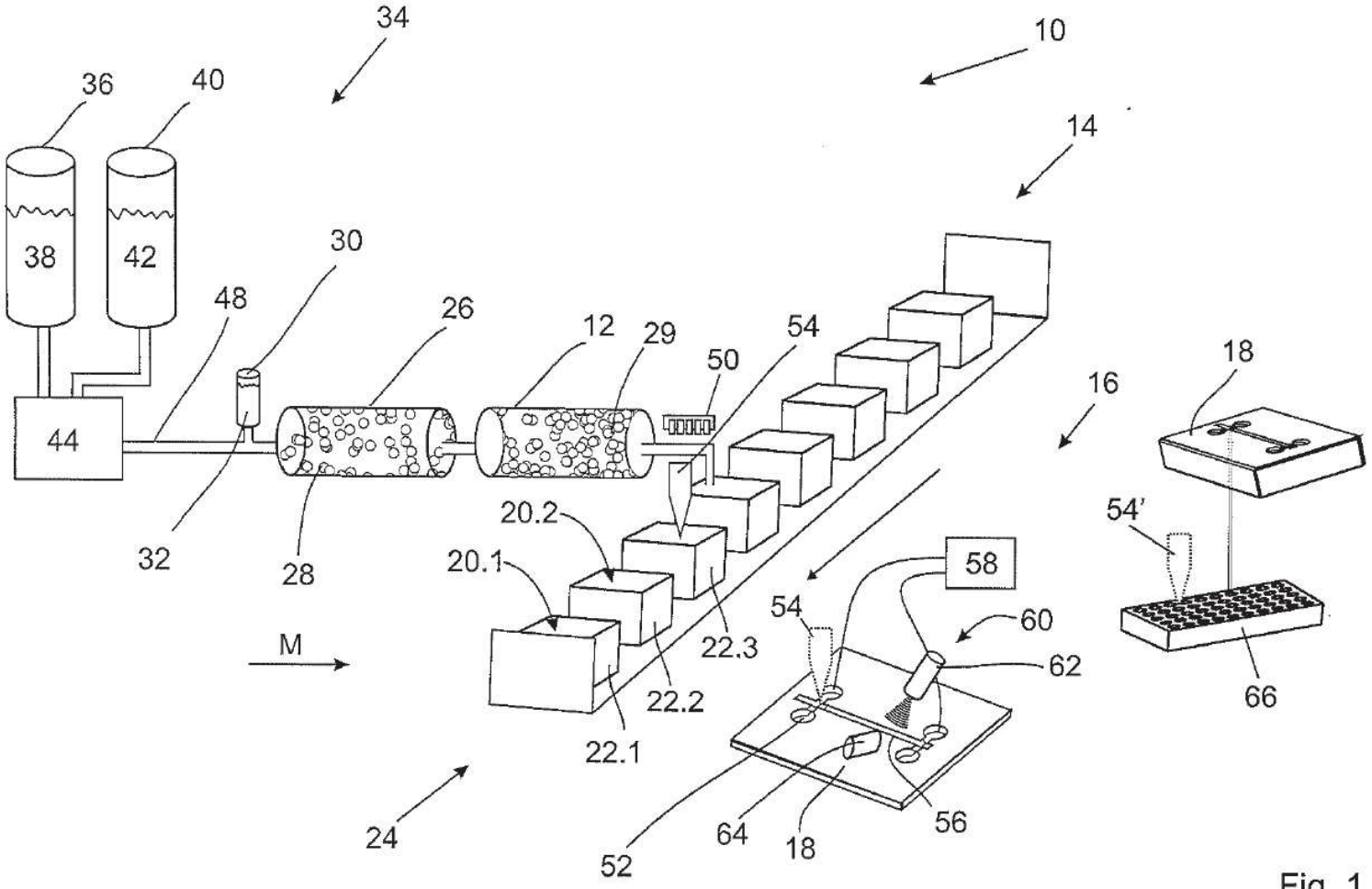



Fig. 1



Technische Universität Braunschweig

Wätzig et al.
Institut für Medizinische und Pharmazeutische Chemie

# Patent 18700637.4 - 1020

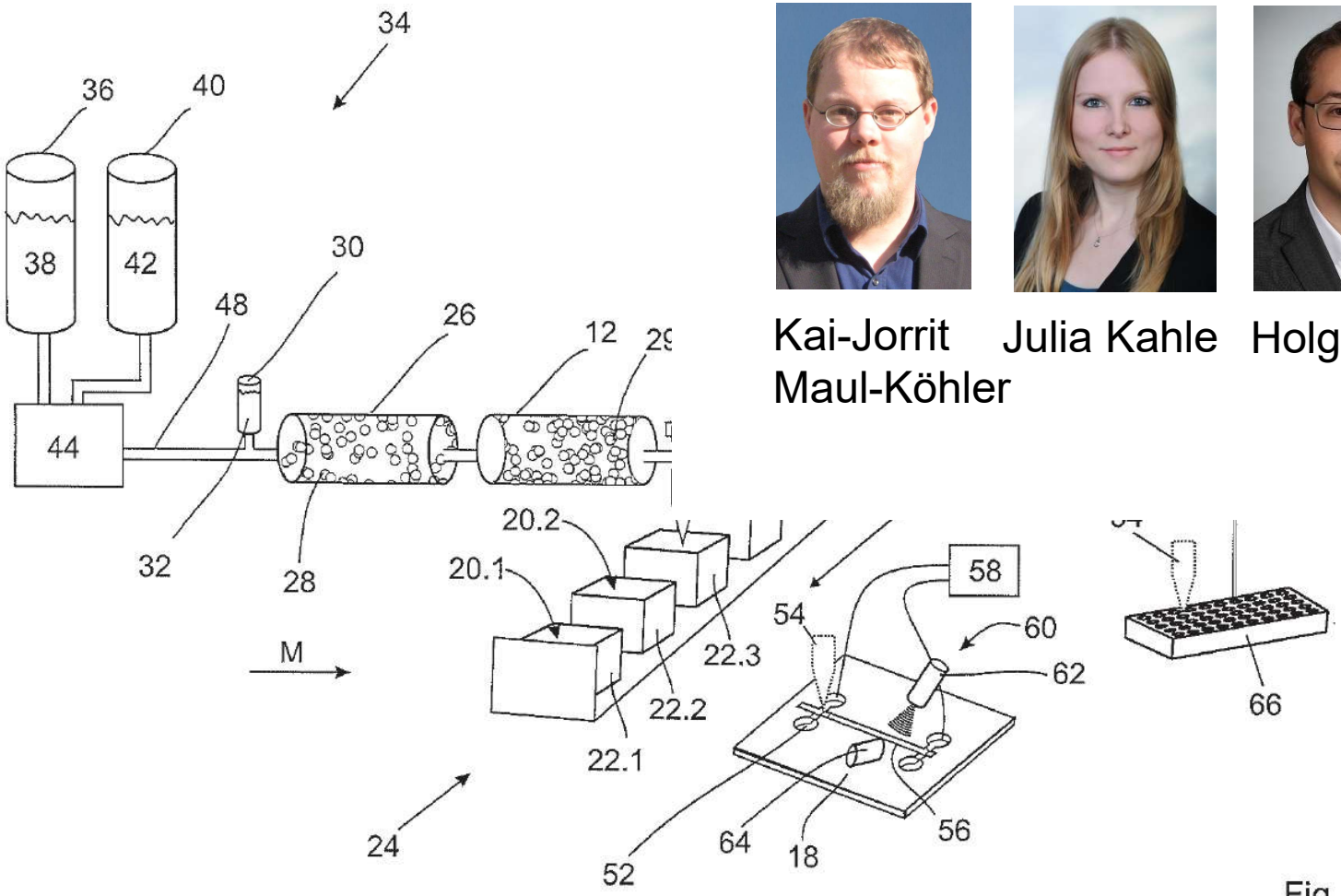



Fig. 1



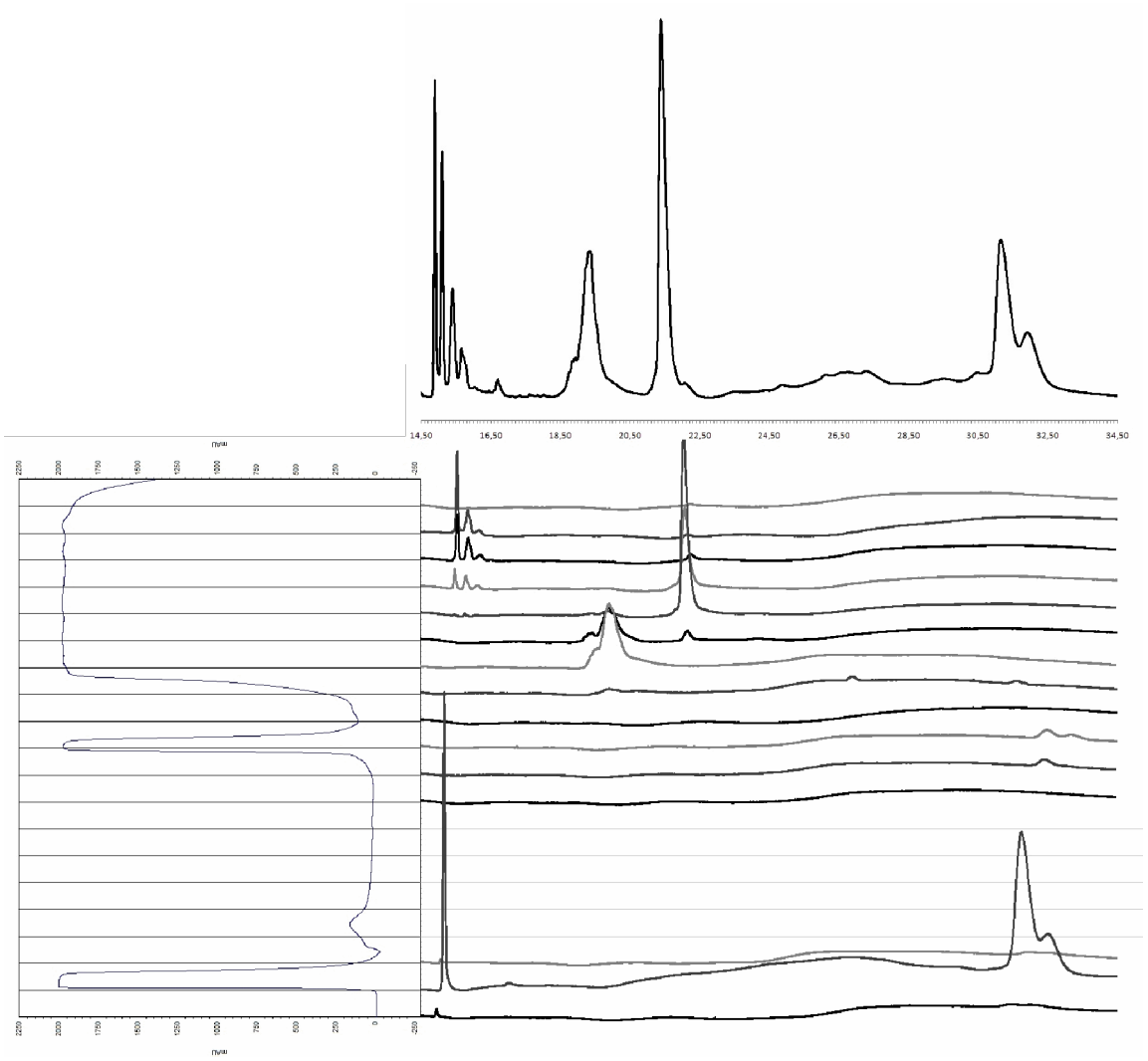
Kai-Jorrit Maul-Köhler



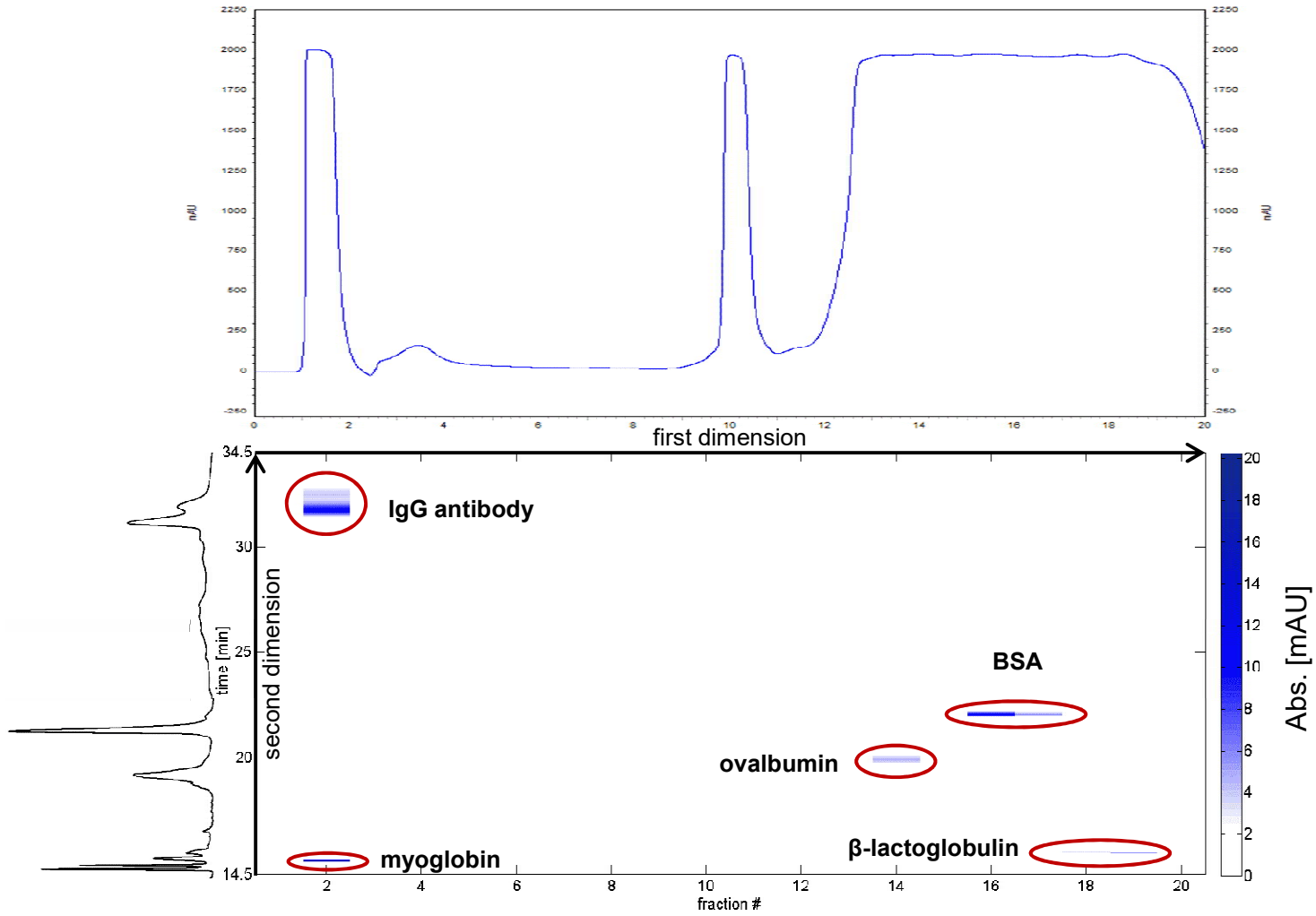
Julia Kahle

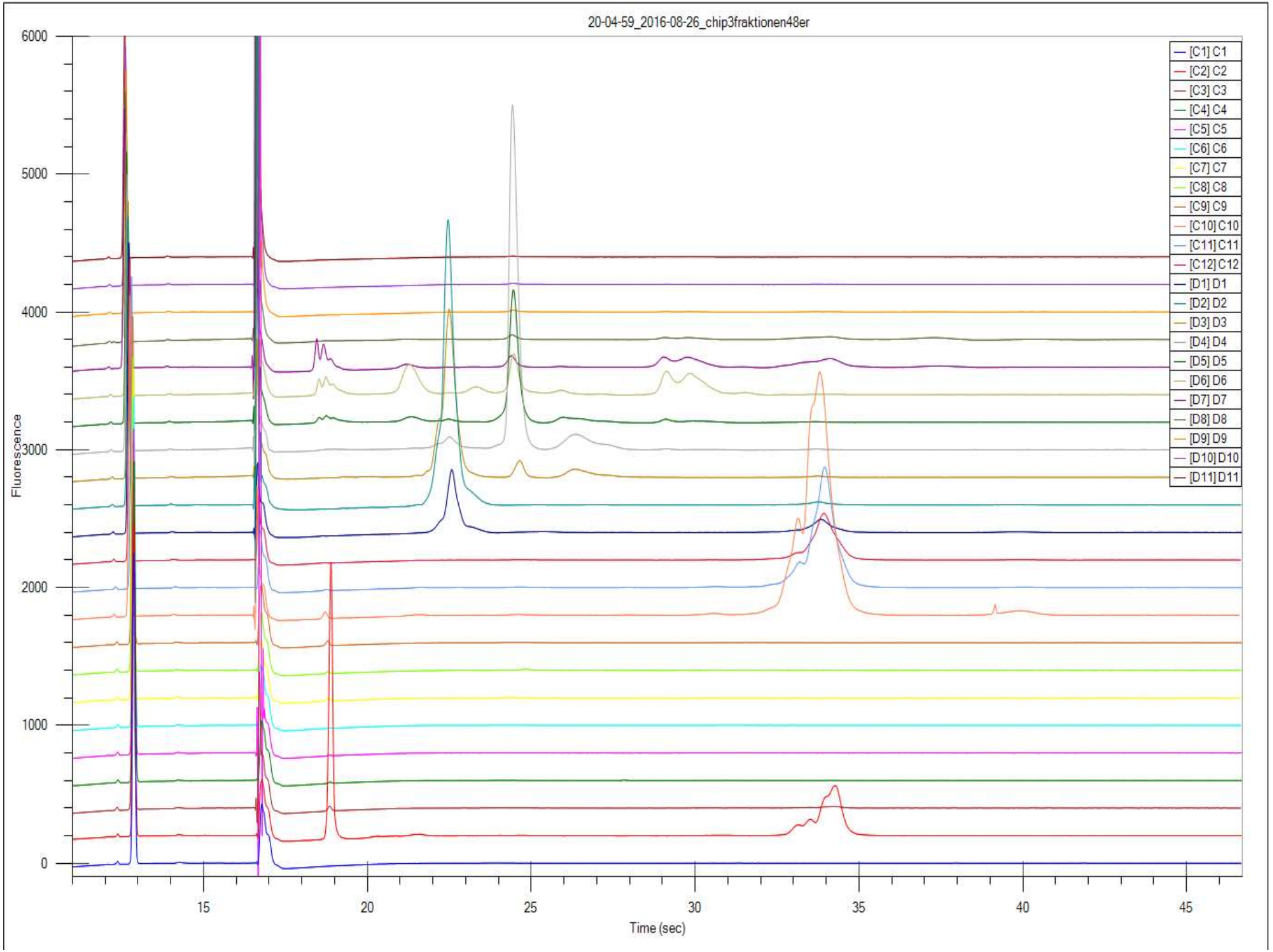
Holger Zagst

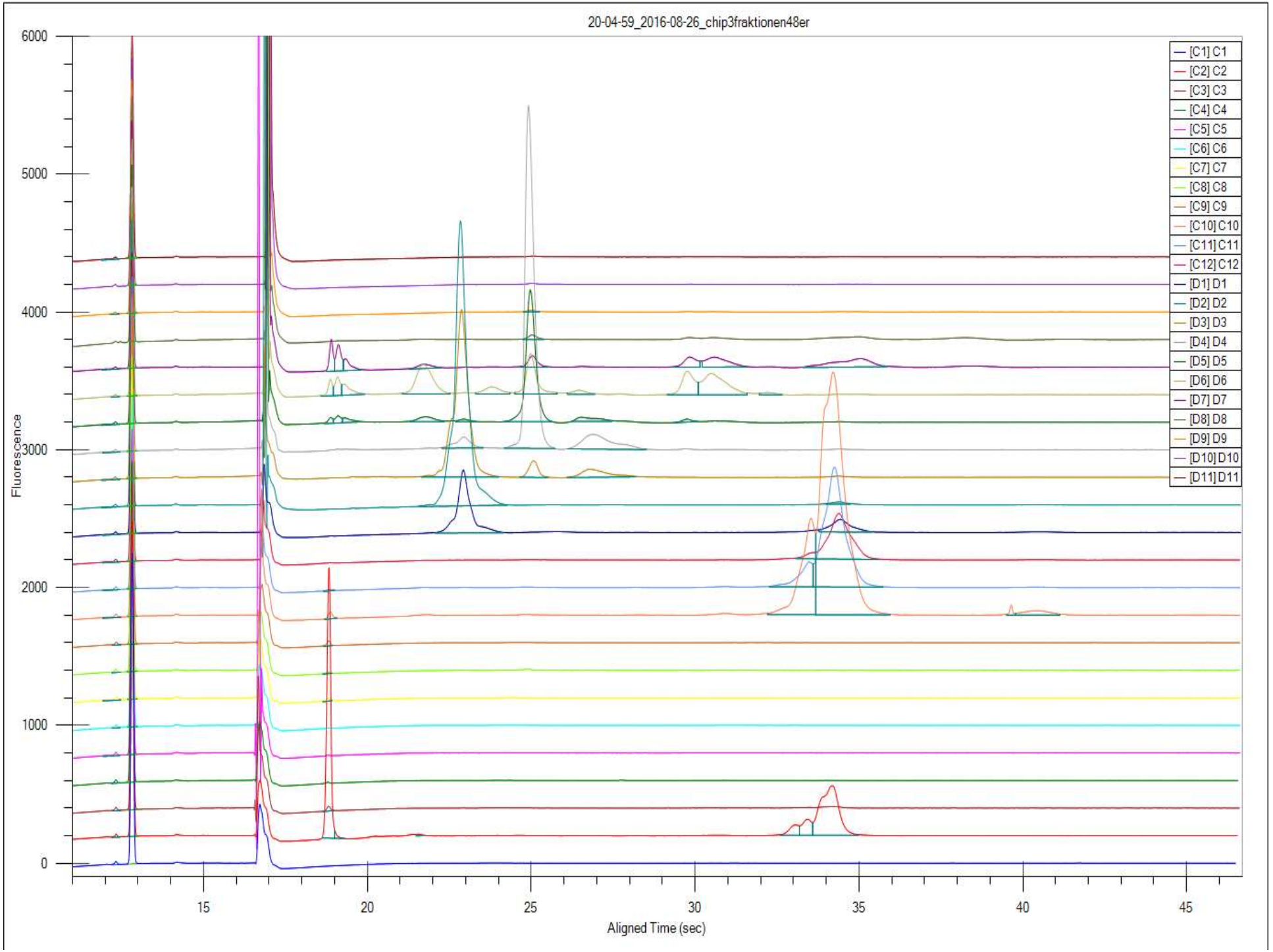
# 2D-separation of a mixture of model proteins



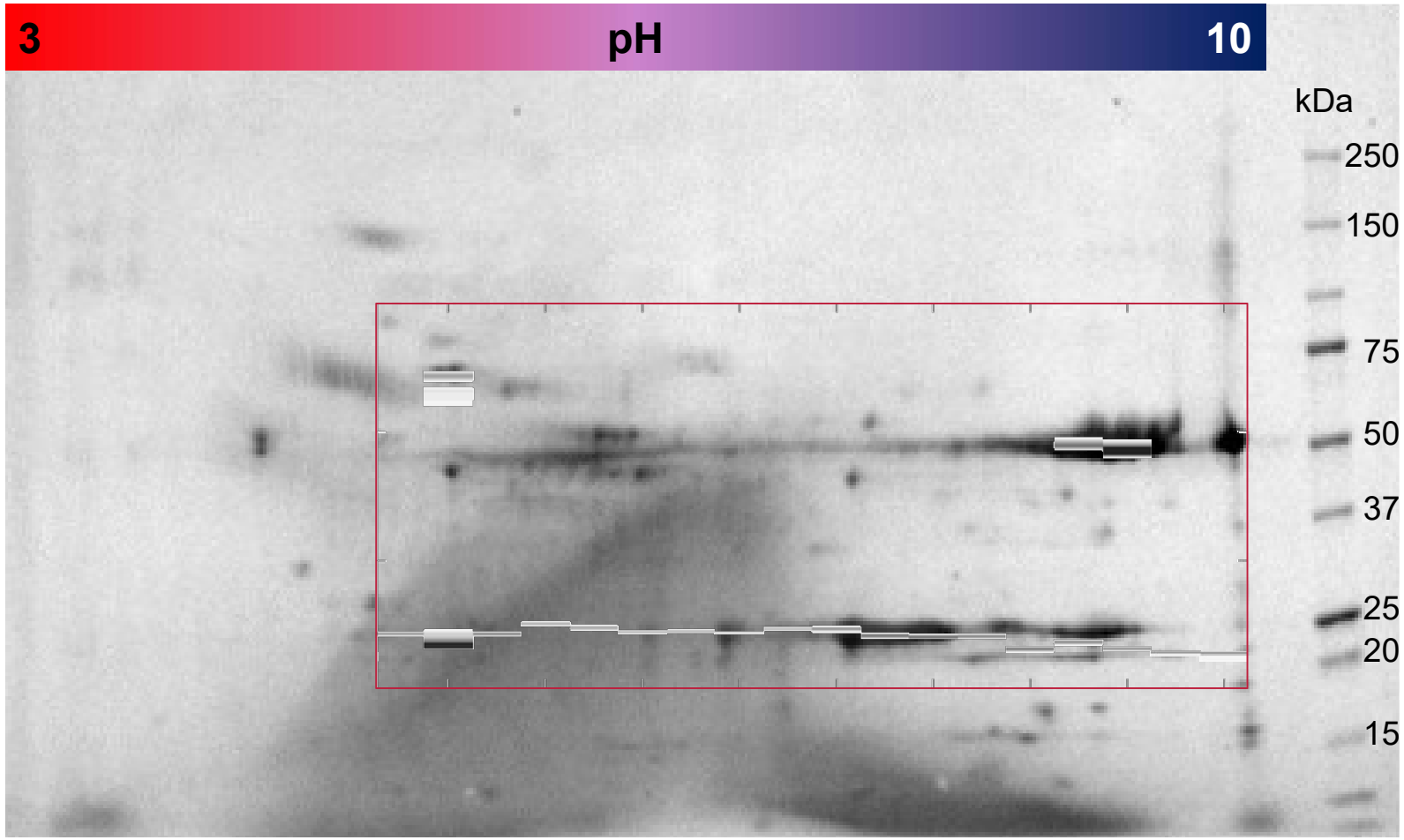
# 2D-separation of a mixture of model proteins



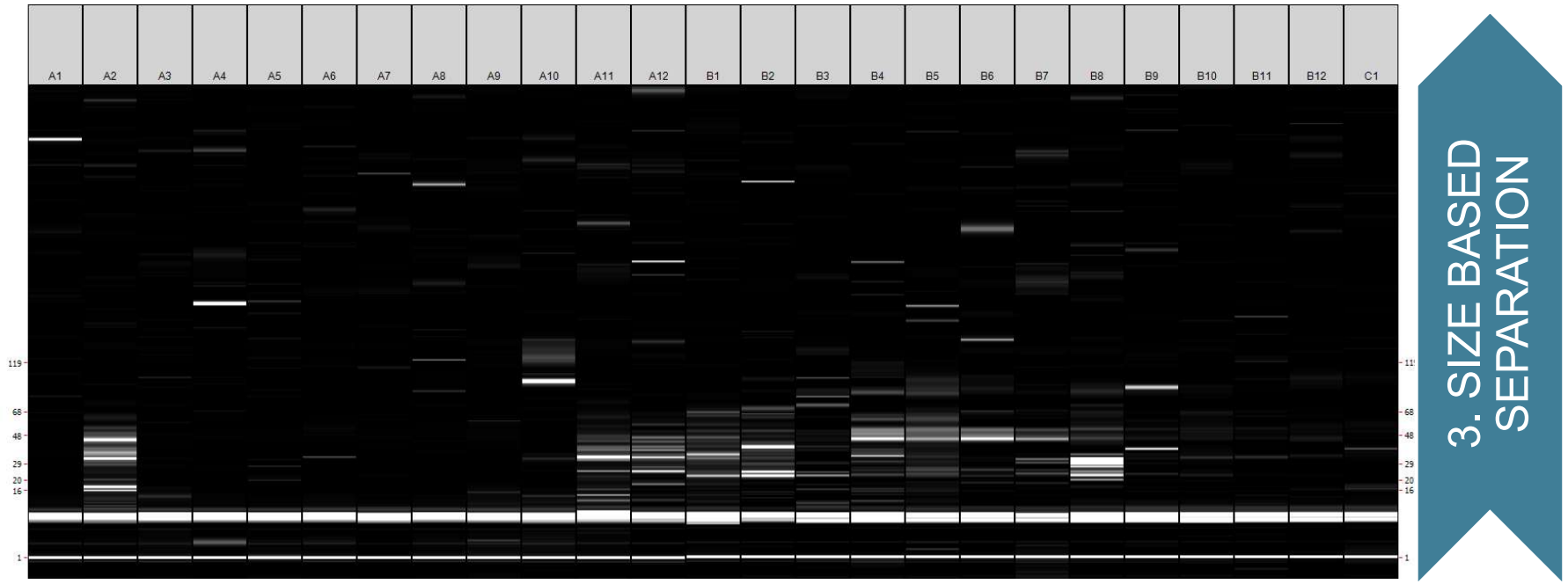

20-04-59_2016-08-26_chip3fraktionen48er
Fluorescence
Time (sec)
6000
5000
4000
3000
2000
1000
0
15
20
25
30
35
40
45
[C1] C1
[C2] C2
[C3] C3
[C4] C4
[C5] C5
[C6] C6
[C7] C7
[C8] C8
[C9] C9
[C10] C10
[C11] C11
[C12] C12
[D1] D1
[D2] D2
[D3] D3
[D4] D4
[D5] D5
[D6] D6
[D7] D7
[D8] D8
[D9] D9
[D10] D10
[D11] D11


20-04-59_2016-08-26_chip3fraktionen48er
Fluorescence
6000
5000
4000
3000
2000
1000
0
15
20
25
30
35
40
45
Aligned Time (sec)
[C1] C1
[C2] C2
[C3] C3
[C4] C4
[C5] C5
[C6] C6
[C7] C7
[C8] C8
[C9] C9
[C10] C10
[C11] C11
[C12] C12
[D1] D1
[D2] D2
[D3] D3
[D4] D4
[D5] D5
[D6] D6
[D7] D7
[D8] D8
[D9] D9
[D10] D10
[D11] D11

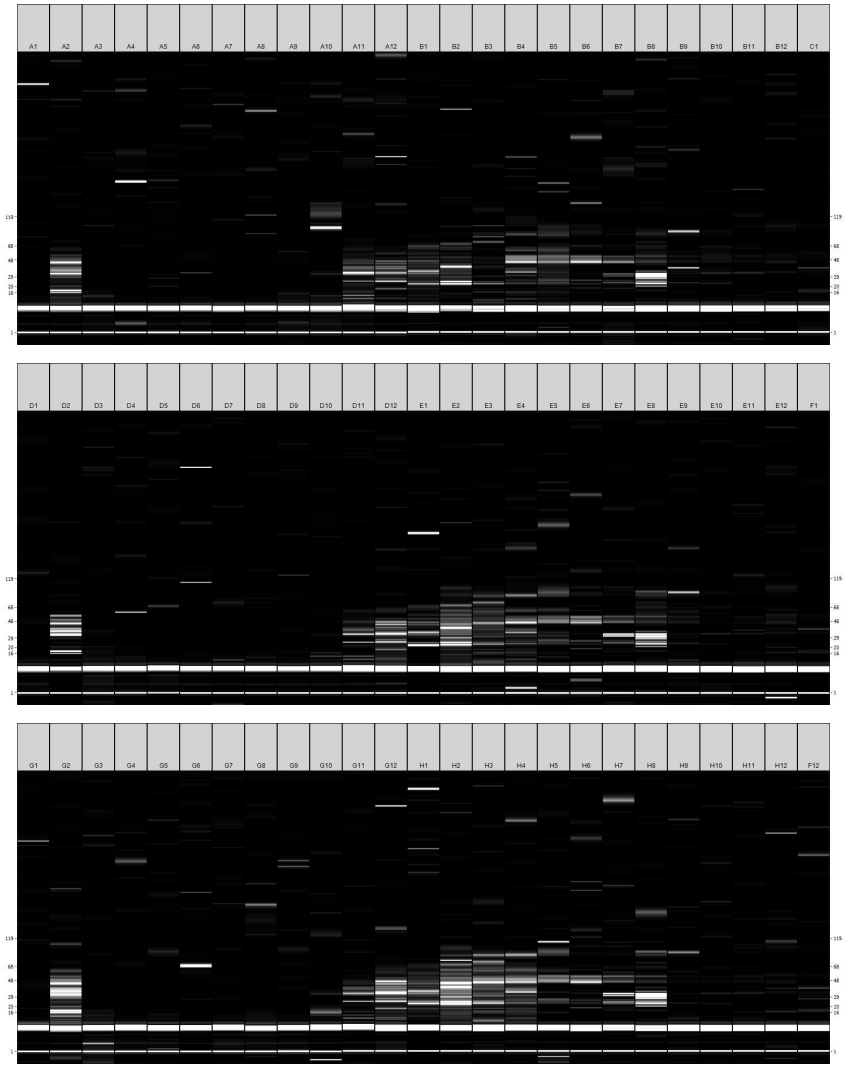
# Process analysis – superimposed 2D separations



# 2D separation of Sf9 cytosol lysate



# Sf9 cytosol lysate: 3 HPLC separations followed by MCE

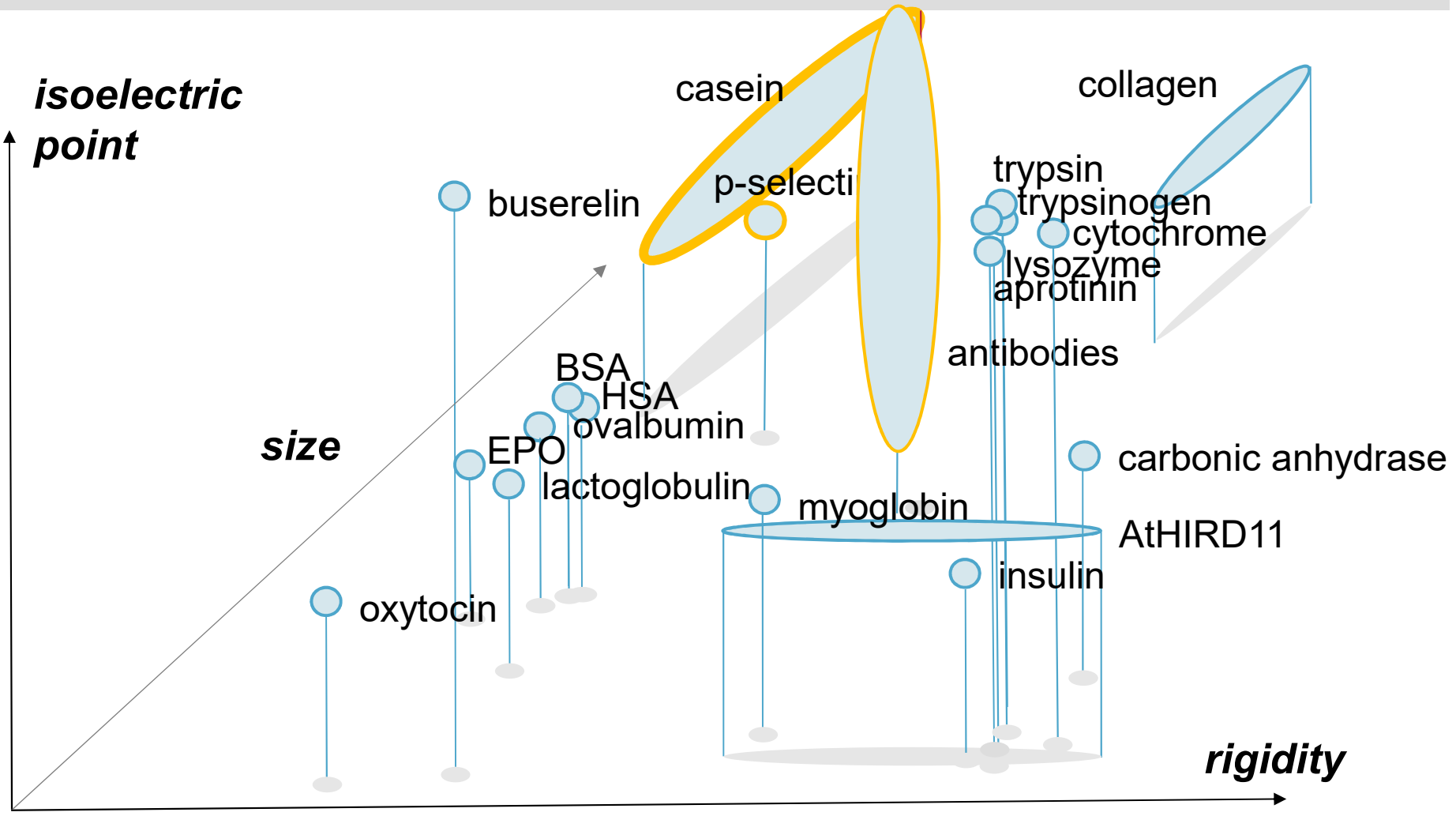




Technische Universität Braunschweig

Wätzig et al.
Institut für Medizinische und Pharmazeutische Chemie

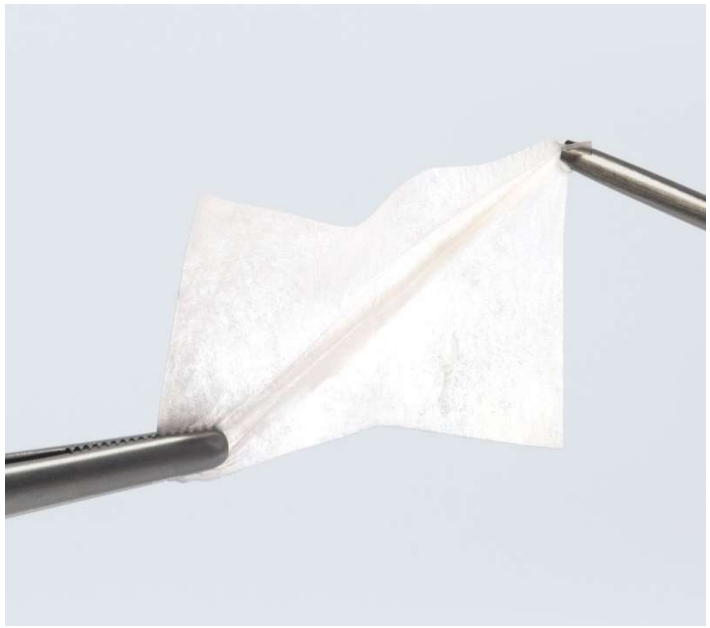
# Physicochemical Properties of Proteins



# Collagen: main constituent of connective tissues

- skin
- bones
- tendons
- etc.

Human skin, from Klafubra, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=5409304

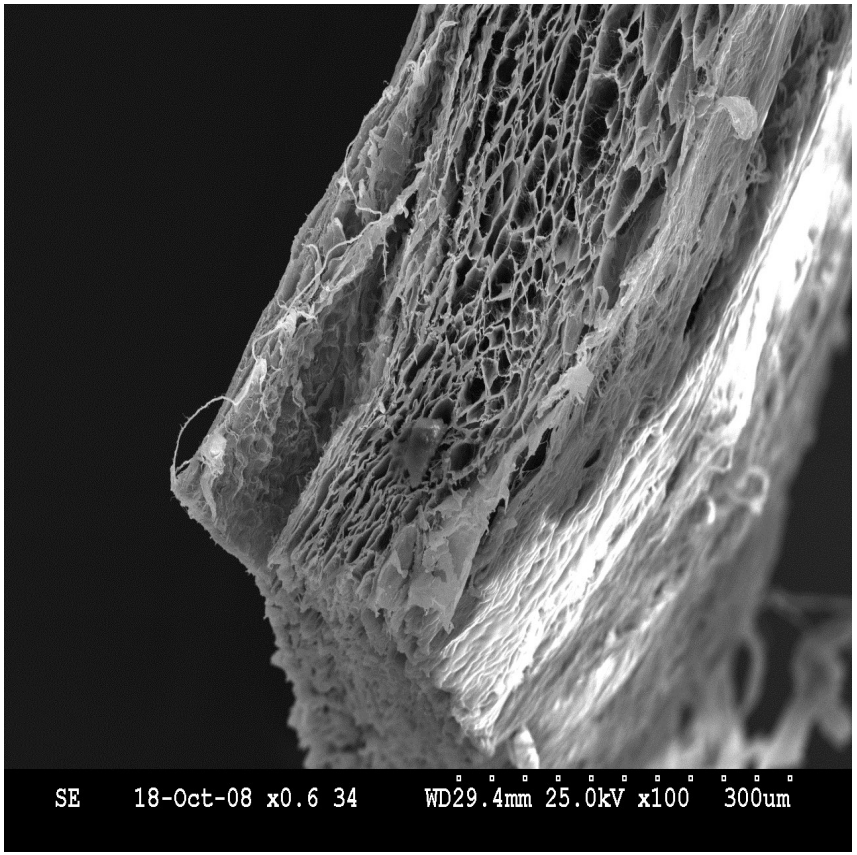


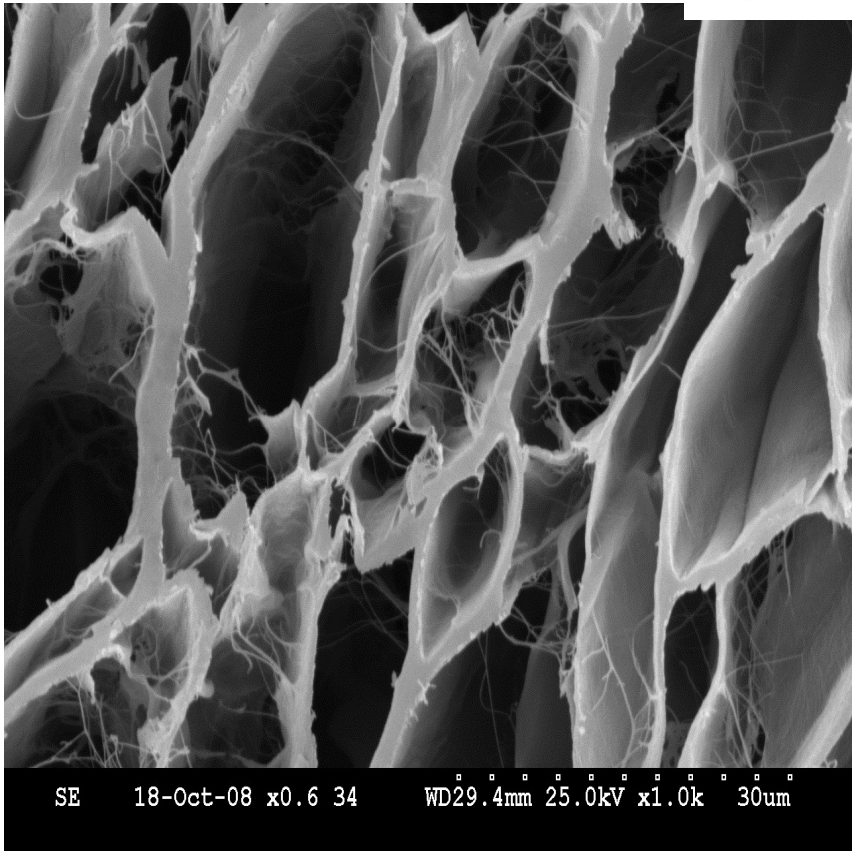
# Collagen-Structure: Triple-Helix

Dr. Imke Oltmann-Norden

Source: M.D. Shoulders and R.T. Raines „Collagen Structure and Stability“ Annu. Rev. Biochem. 2009; 78:929-958

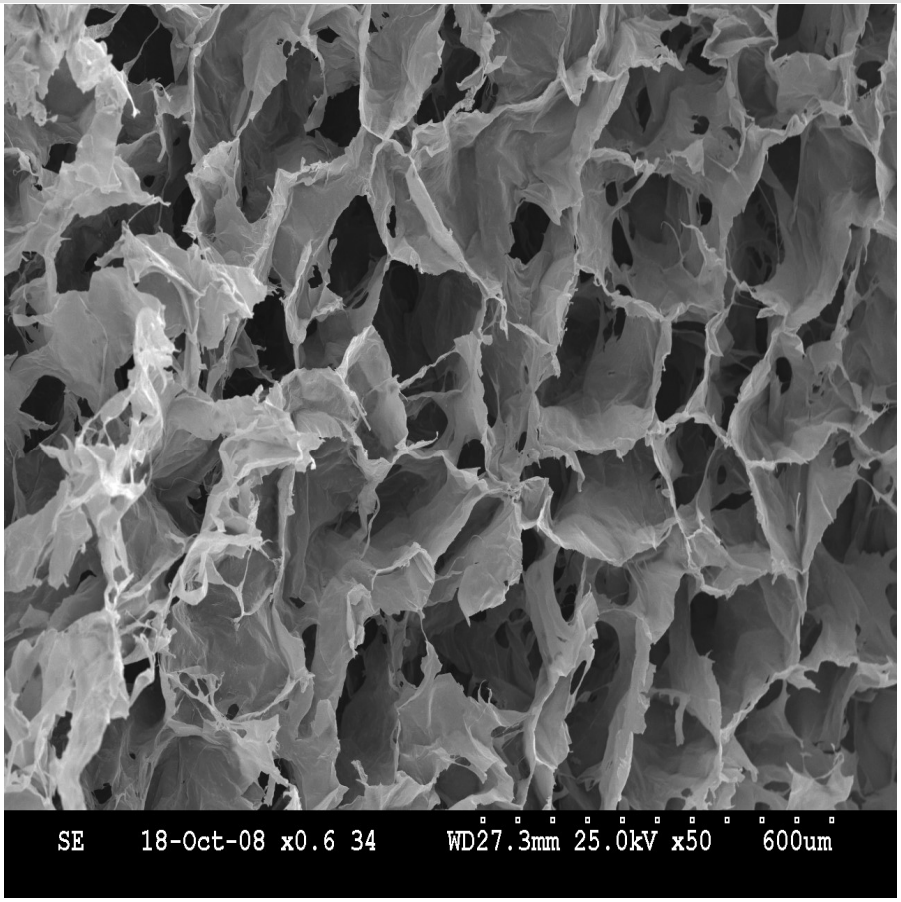
# Collagen: fleece, magnified

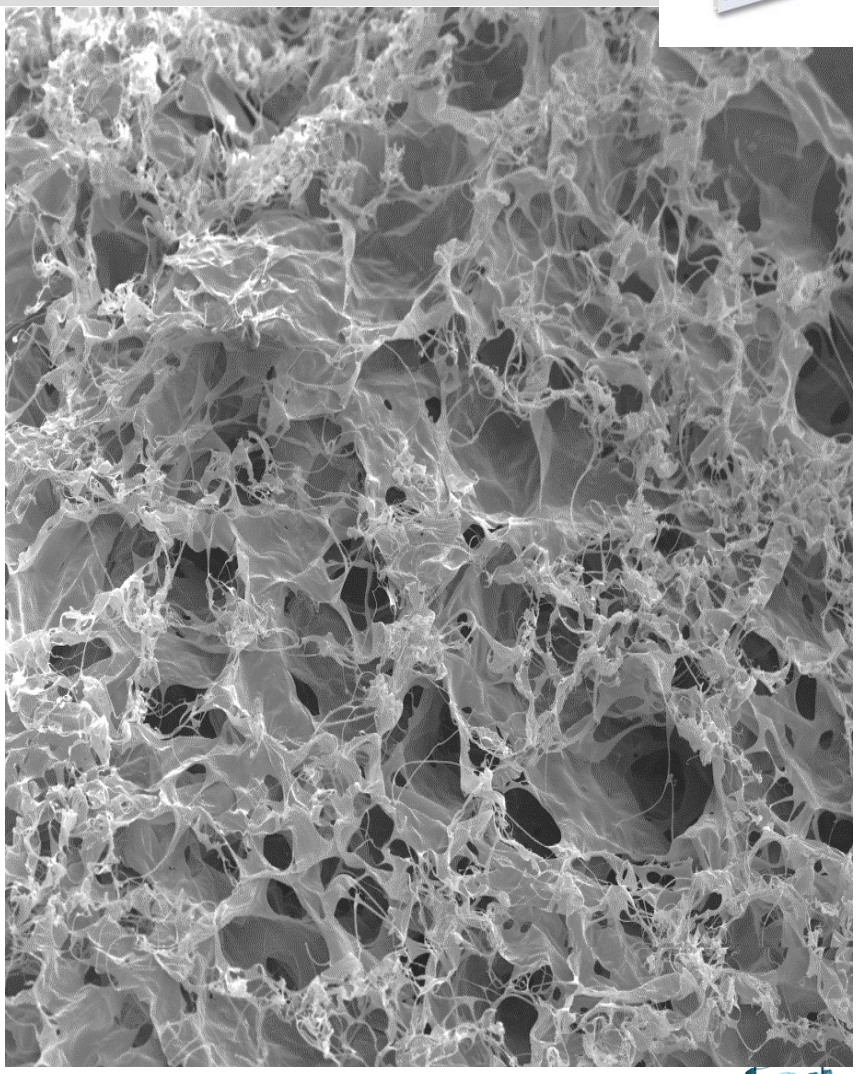



with friendly permission from Mike Barbeck,
botiss biomaterials GmbH, botiss.com

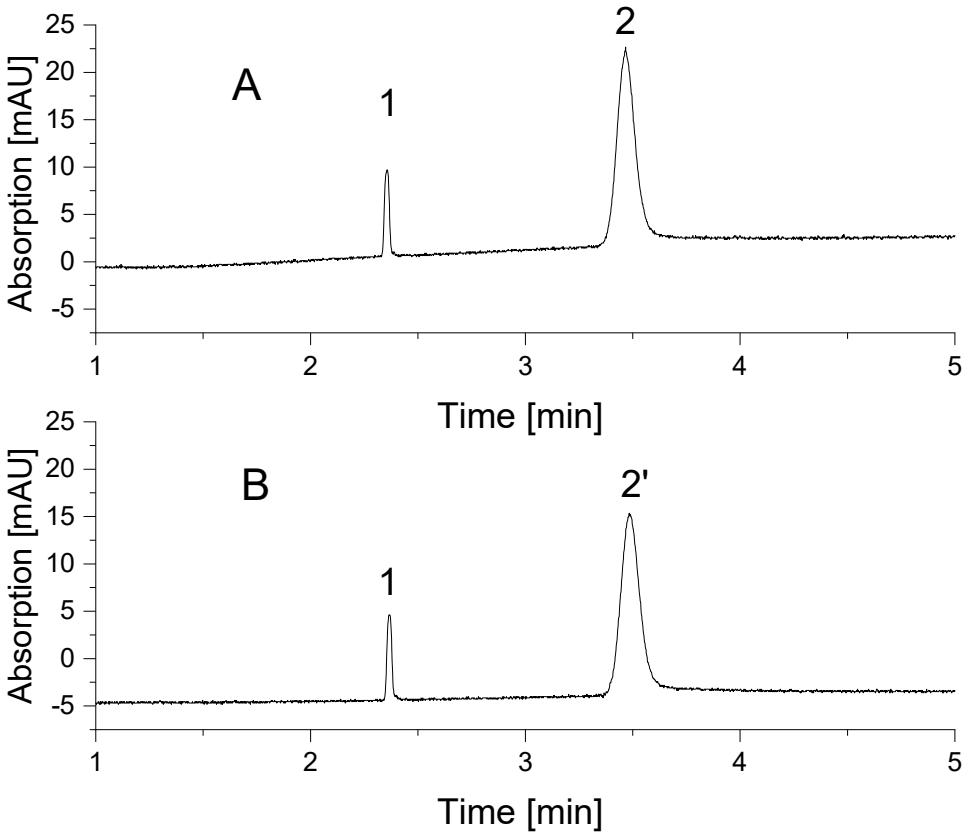
# Collagen: fleece and implants, magnified



with friendly permission from Mike Barbeck, botiss biomaterials GmbH, botiss.com

# CZE: rat tail collagen solved in acetic acid

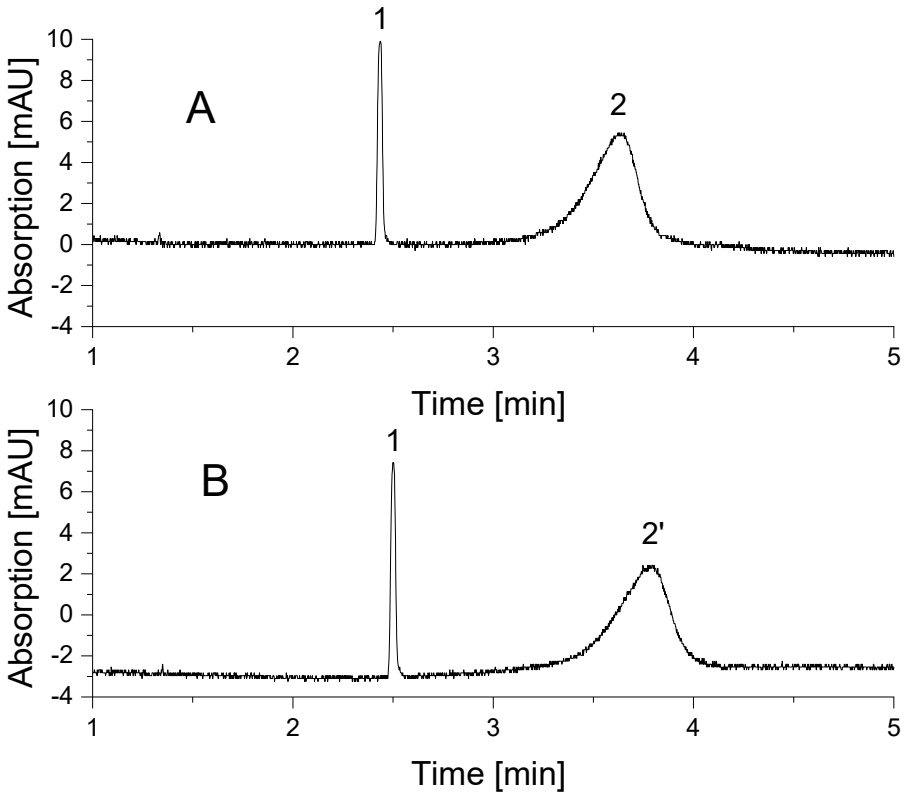



Mais Olabi

A: filtrated rat tail collagen
B: unfiltrated rat tail collagen
- 1: EOF marker acetanilide
- 2: collagen

Solution process: in 50 mM acetic acid pH 3, stirring with ice cooling (12 h)

# CZE: fleece collagen solved in acetic acid

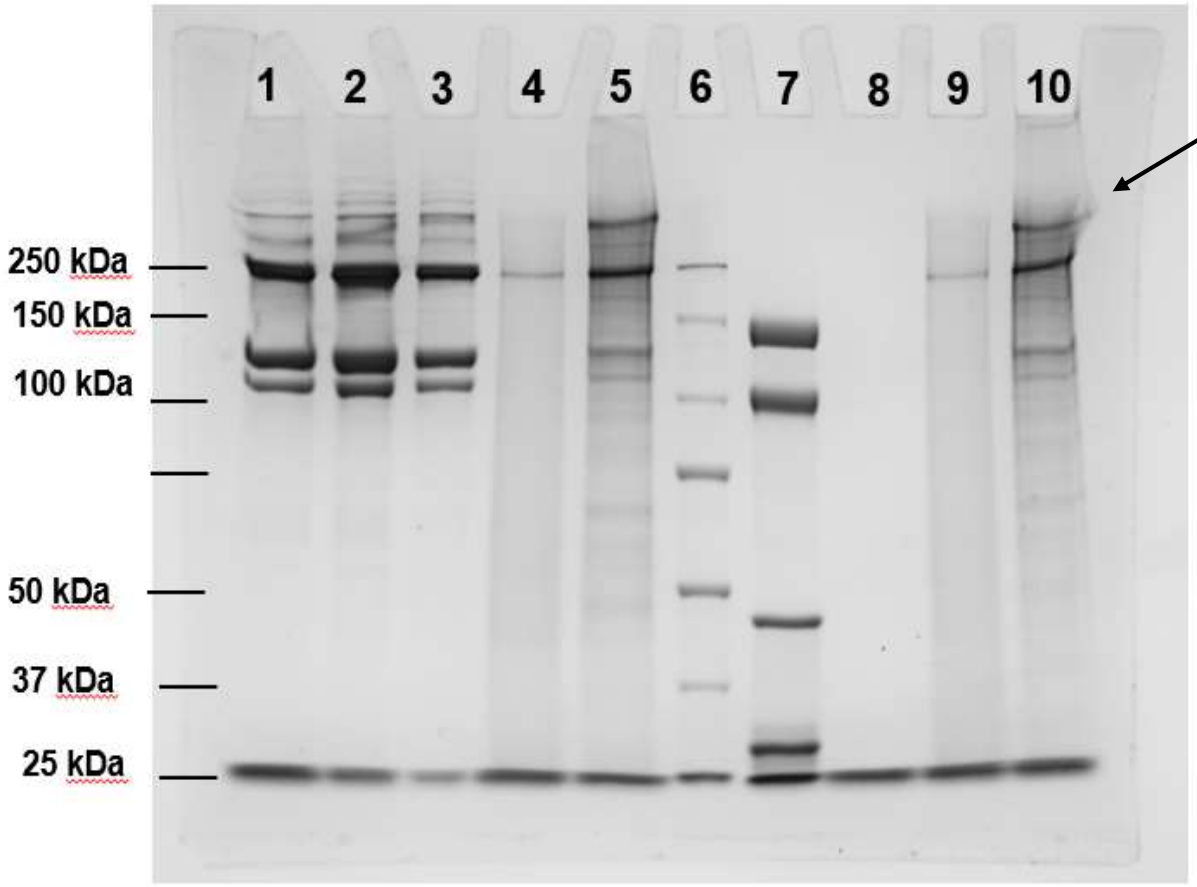



Mais Olabi

A: filtrated fleece collagen
B: unfiltrated fleece collagen
1: EOF marker Acetanilid
2: fleece collagen

Solution process: 50 mM acetic acid pH 3, sonication (12 h, without temperature control)

# Dissolved fleece collagen samples in 7 % SDS-PAGE

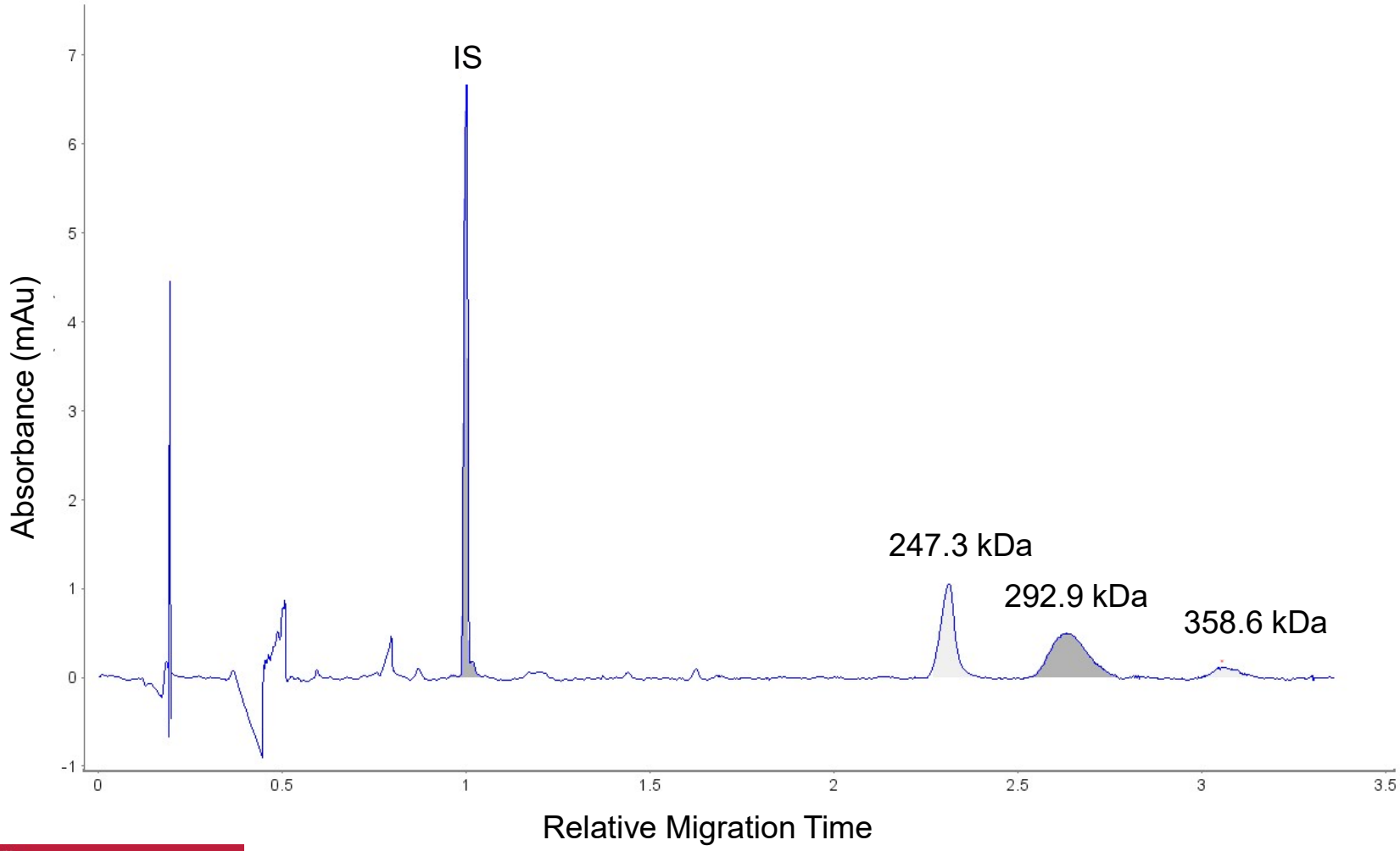



Beta-chain

lanes 1-3, 8: rat tail collagen
lanes 4, 9: fleece collagen reference material
lanes 5, 10: gelatine
lanes 6, 7: ladders

lanes 1-5: samples @ pH 3
lanes 8-10: samples @ pH 8

# Collagen: CE-SDS



# Selectivity, other proteins: (brief) IDP story

# Example Intrinsically Disordered Protein (IDP):

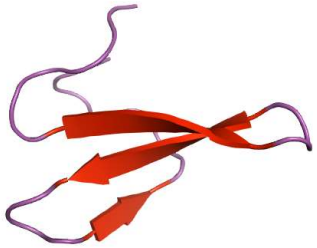
Thylakoid soluble phosphoprotein TSP9[1]

*Song J, Lee MS, Carlberg I, Vener AV, Markley JL (December 2006). Biochemistry. **45** (51): 15633–43.*

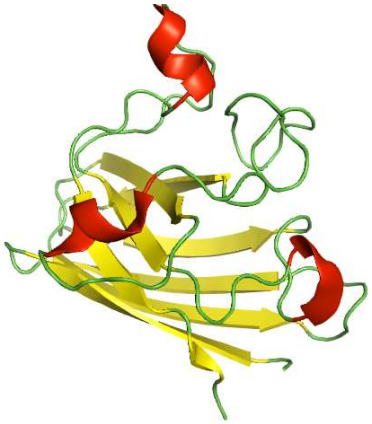
By Jawahar Swaminathan and MSD staff at the European Bioinformatics Institute - http://www.ebi.ac.uk/pdbe-srv/view/images/entry/2fft600.png, https://commons.wikimedia.org/w/index.php?curid=5877319

# Selectivity, other proteins: (brief) IDP story

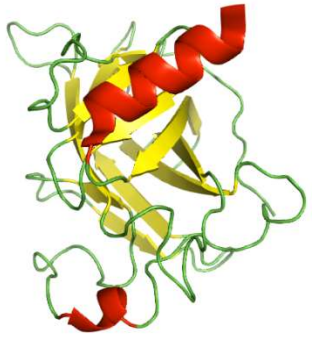
human α-synuclein
(PDB ID: 1XQ8)



human Tau protein
(PDB ID: 2N4R)



human SOD1
(PDB ID: 2V0A)



human p53 protein
(PDB ID: 1UOL)

Shaji, Divya, Intrinsically disordered proteins (idps) in human diseases: a review. *Int. Res. J. Pharm. 2018, 9 (11)*

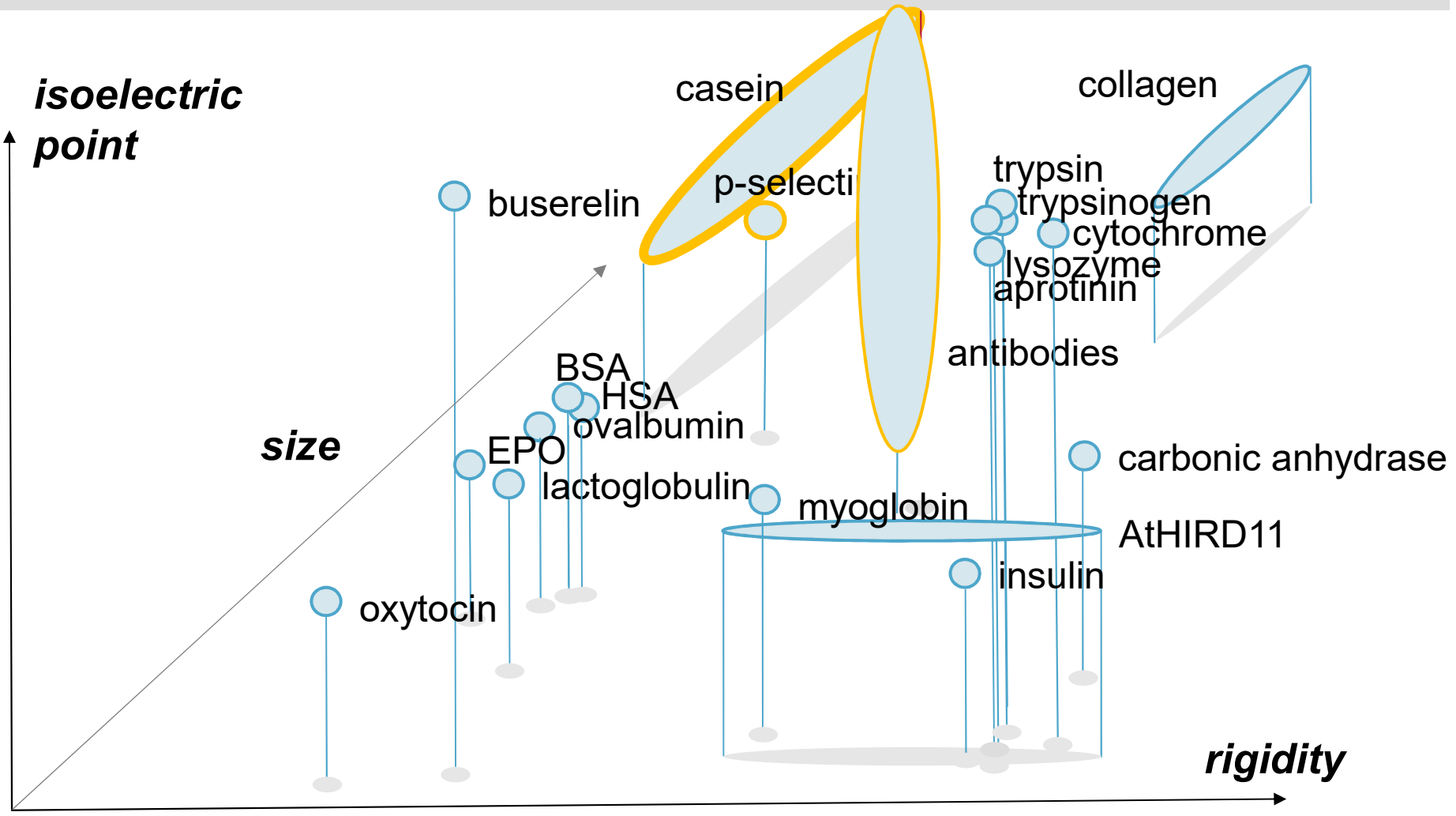
# Example Intrinsically Disordered Protein (IDP):

Thylakoid soluble phosphoprotein TSP9[1]

*Song J, Lee MS, Carlberg I, Vener AV, Markley JL (December 2006). Biochemistry. **45** (51): 15633–43.*

By Jawahar Swaminathan and MSD staff at the European Bioinformatics Institute - http://www.ebi.ac.uk/pdbe-srv/view/images/entry/2fft600.png, https://commons.wikimedia.org/w/index.php?curid=5877319

# Physicochemical Properties of Proteins



# Antibody domains VL and VH: order?

Overview of protein structures (http://absoluteantibody.com; 2015, 2019)

# Antibody domains VL and VH: order?

Overview of protein structures (http://absoluteantibody.com; 2019)

# Antibody domains VL and VH: order?

Stability ≤ 25 °C:

Infliximab: 6 months

Omalizumab,
Secukinumab: 4 hours

V. Stahl, Deutsche Apothekerzeitung 28/2019

Overview of protein structures (http://absoluteantibody.com; 2019)

# Intrinsically disordered proteins: ensembles of conformers

**Comparison of the energy landscape of a folded native protein (A) and an intrinsically disordered protein (B)*. x-y-axes: possible* conformations, z-axis: Gibbs´s Free Energy (Free Enthalpy); Fig 34 in:**

Howton, T.C., Zhan, Y.A., Sun,Y. & Mukhtar, M.S. Intrinsically disordered proteins: controlled chaos 8 or random walk. Int J Plant Sci 6: 61 52-57 (2015). doi:10.4081/pb.2015.6191

# AtHIRD11, an example intrinsically disordered protein

AtHIRD11 with 7 $Cu^{2+}$ ions bound

CZE of AtHIRD11 in Tris buffer 7.4, 30 mM, λ = 200 nm

M. Nachbar, et al., Journal of Plant Physiology 2017, 216, 219–228

Markus Nachbar, PhD thesis, TU Braunschweig 2017
https://publikationsserver.tu-braunschweig.de/receive/dbbs_mods_00065295

# Selectivity, other proteins: viruses

Partly quite flexible on the outside, IDP-like (!)

*Jim Baggen1,2, Hendrik Jan Thibaut1,2, Jeroen R. P. M. Strating1 and Frank J. M. van Kuppeveld1**
Nature Reviews | Microbiology Reviews
volume 16 | june 2018 | 369-381
https://doi.org/10.1038/s41579-018-0005-4

# Success story SDS-PAGE and CE-SDS

A. Guttman, J. Nolan, Analytical Biochemistry 221, 285-289 (1994)

Rustandi, R. R., Washabaugh, M. W., Wang, Y.,
*Electrophoresis* 2008, *29*, 3612–3620.

Lacher, N. A., Roberts, R. K., He, Y., Cargill, H., Kearns, K. M., Holovics, H., Ruesch, M. N., *J. Sep. Sci.*, 2010, *33*, 218–227.

Nunnaly, B., Park, S. S., Patel, K., Hong, M., Zhang, X., Wang, S. X., Rener, B., Reed-Bogan, A., Salas-Solano, O., Lau, W., Girard, M., Carnegie, H., Garcia-Canas, V., Cheng, K. C., Zeng, M., Ruesch, M., Frazier, R., Jocheim, C., Natarajan, K., Jessop, K., Saeed, M., Moffatt, F., Madren, S., Thiam, S., Altria, K., *Chromatographia* 2007, *66*, 955–961.

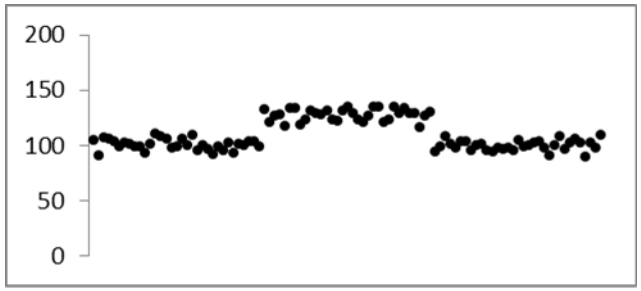
Cari Sänger-van de Griend, CE-SDS method development, validation, and best practice—An overview,
Electrophoresis 2019, DOI 10.1002/elps.201900094

# Precision and possible insight

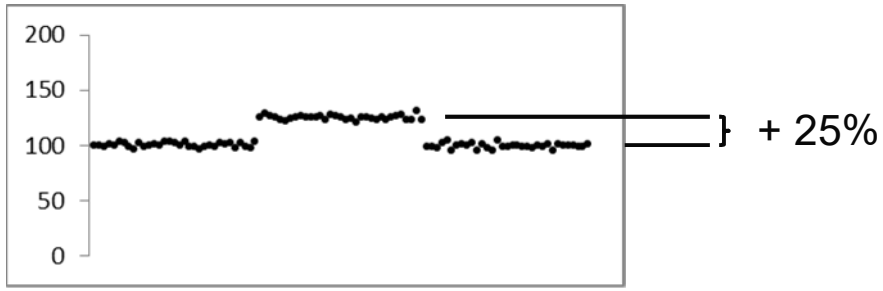
RSD% = 20%


RSD% = 10%


RSD% = 5%

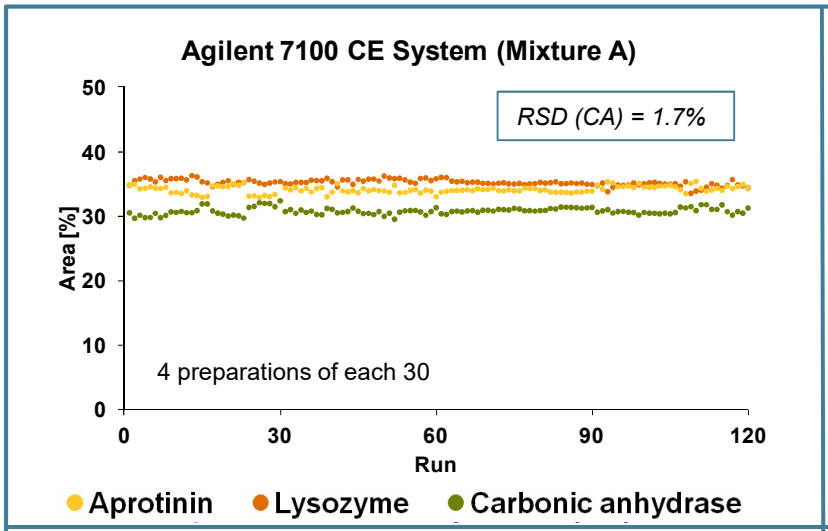


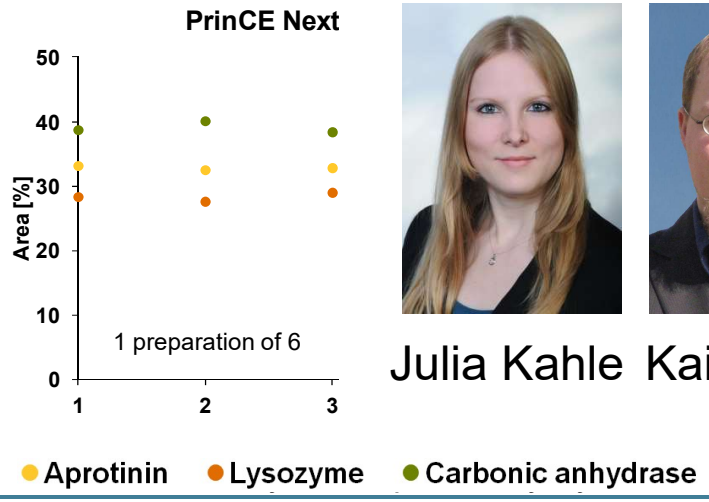

RSD% = 2%



# CE-SDS – Intermediate precision of %peak areas

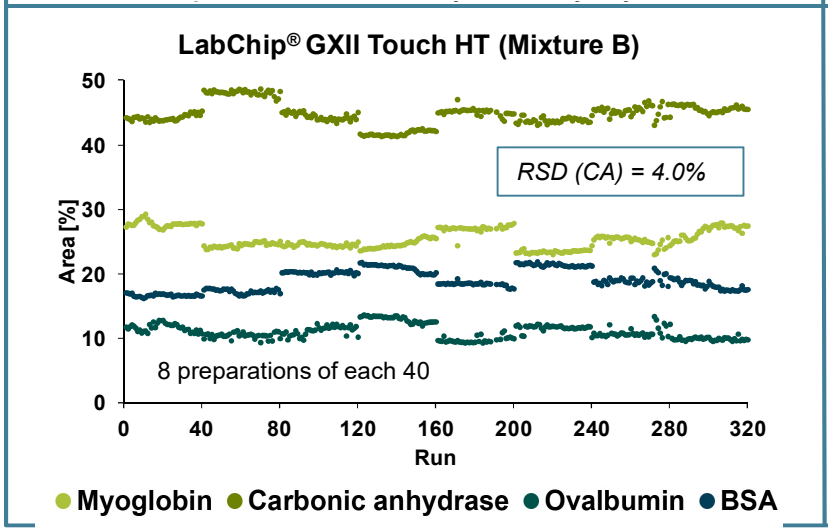
Kahle, J., Maul, K. J., Wätzig, H., *Electrophoresis 2018, 39, 311–325.*

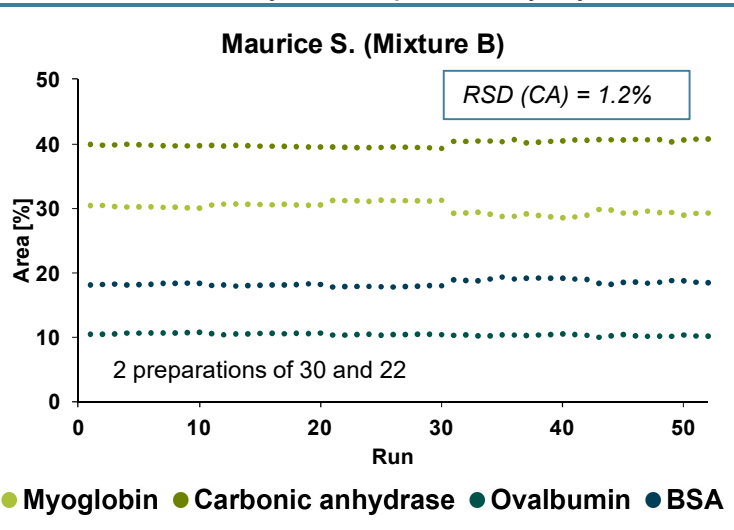


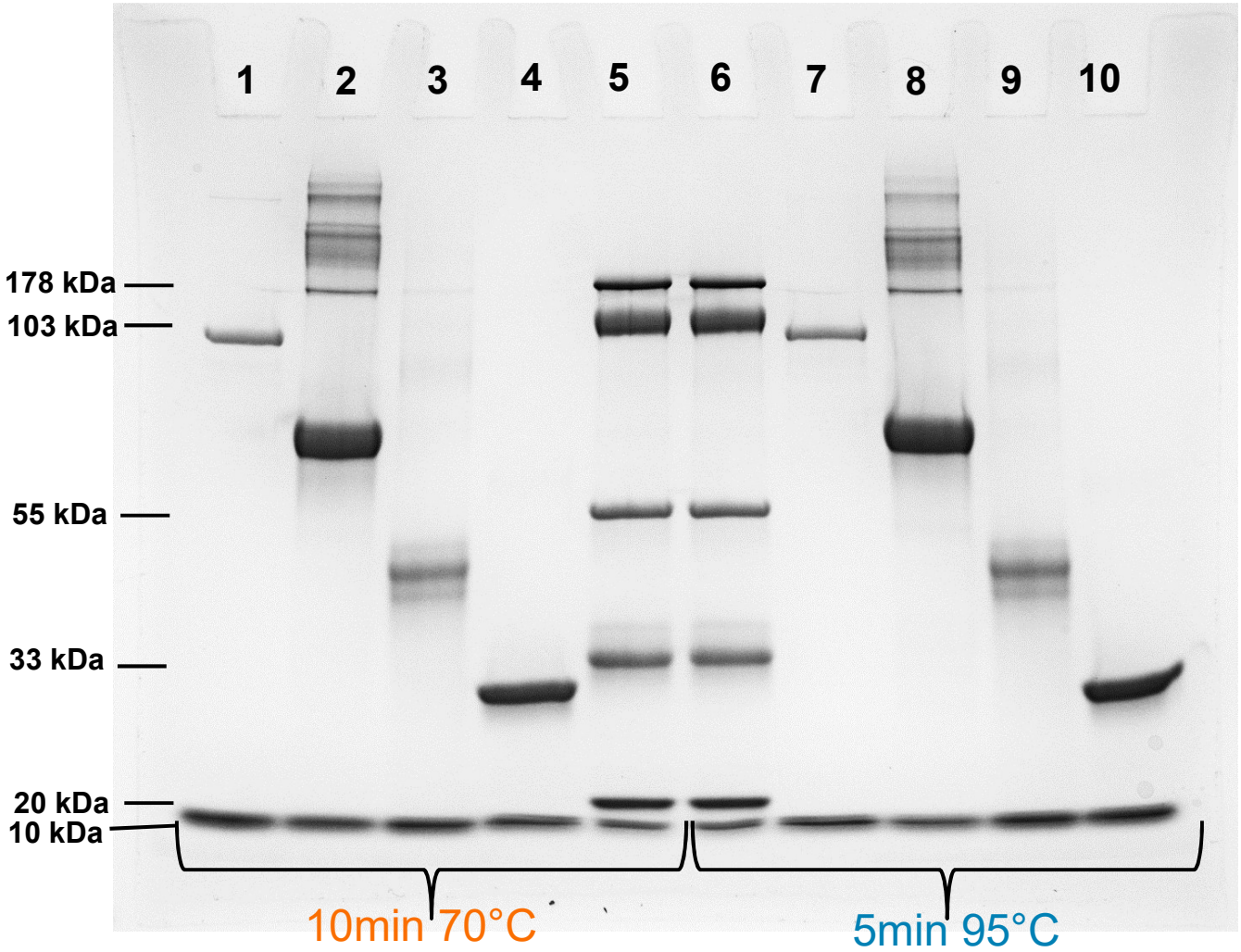



Julia Kahle Kai-Jorrit Maul



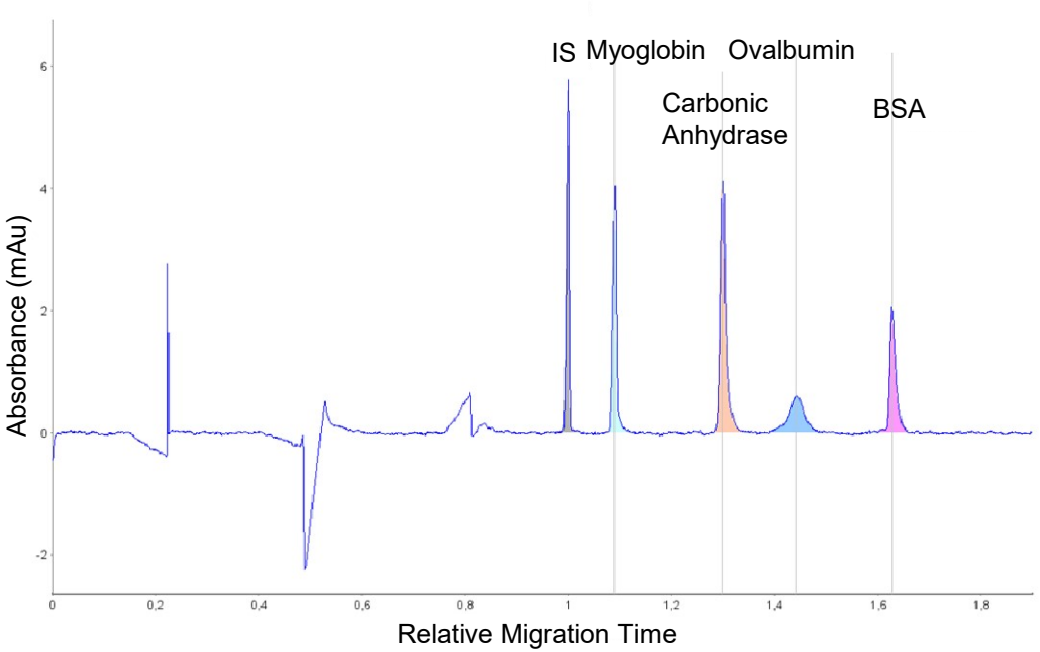



# Success of CE-SDS: transfer to other proteins




1. Phosphorylase B
2. BSA
3. Ovalbumin
4. Carbonic anhydrase
5. Ladder
6. Ladder
7. Phosphorylase B
8. BSA
9. Ovalbumin
10. Carbonic anhydrase

# Method: CE-SDS

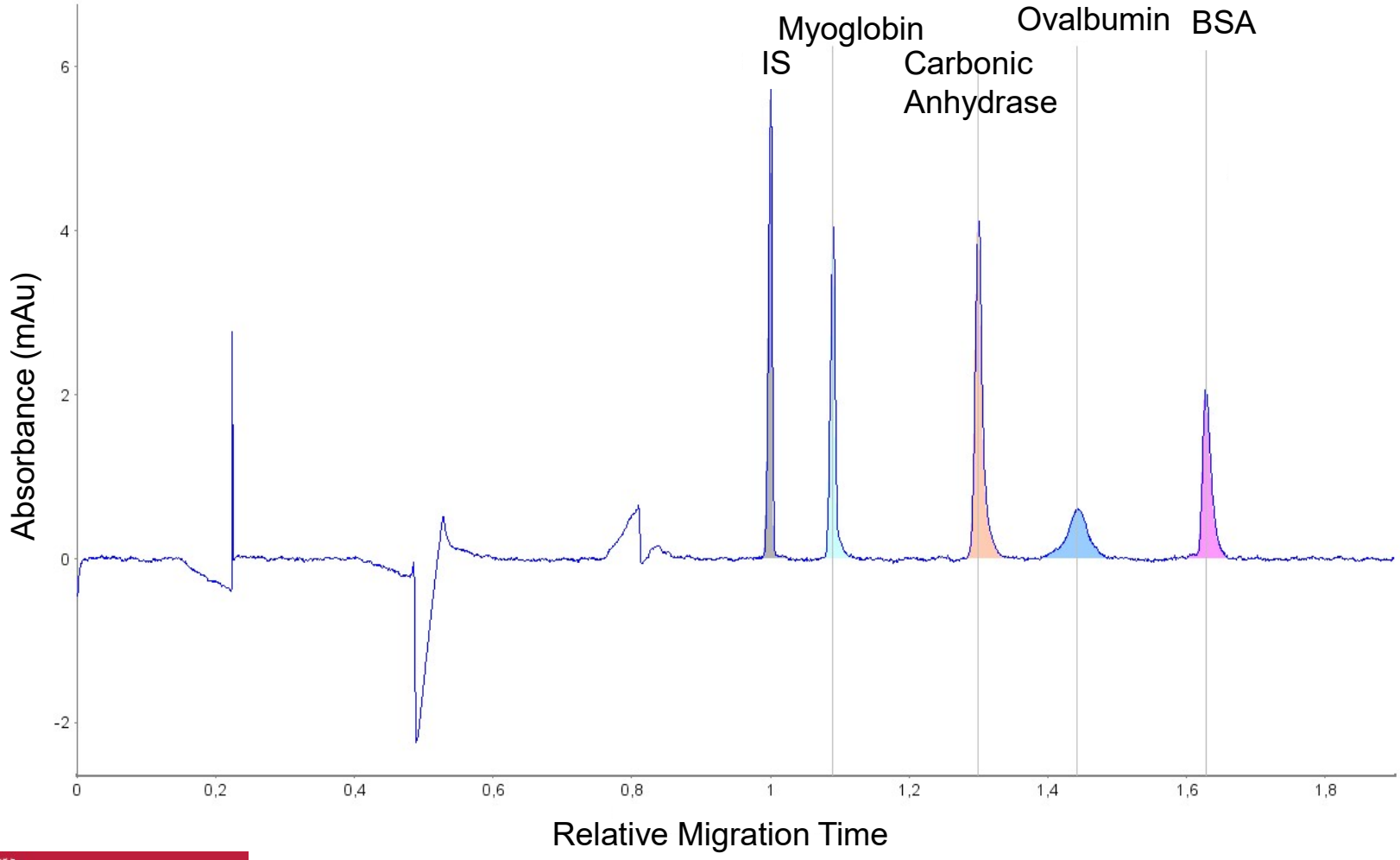




**Instrumentation:**
**Maurice (ProteinSimple)**

**Maurice Ladder:** 10-270 kDa
**Sample preparation**:
1 mg/ml stock solution in
100 mM Tris & 1% SDS (pH=8)
→ 1:1 Dilution in Maurice 1x SB
→ 70°C 10 min, 710 mM β-ME

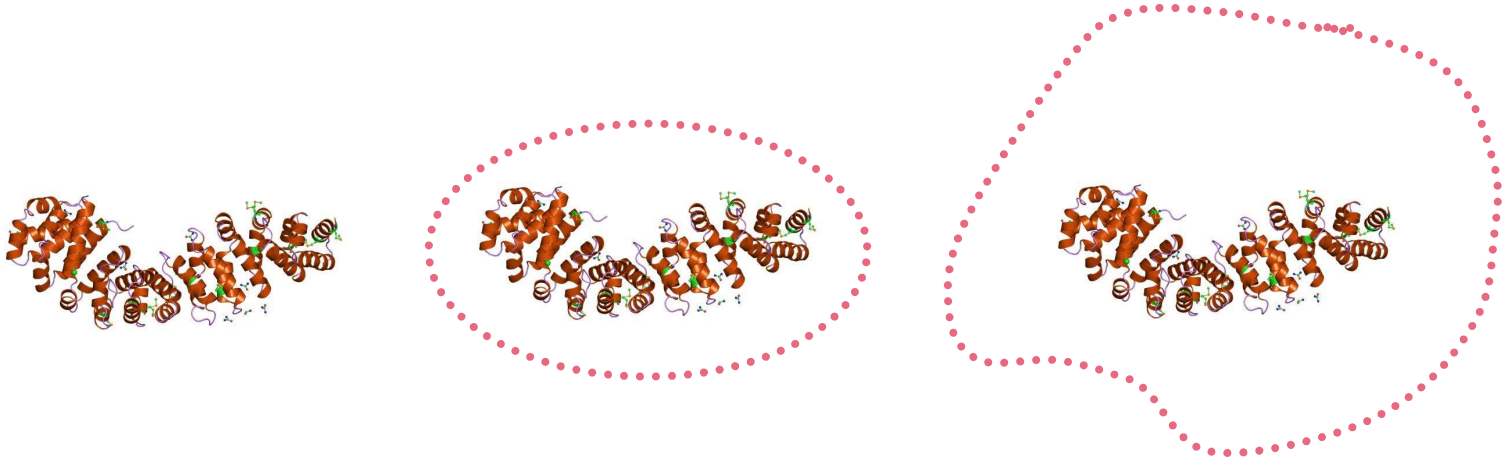
**SST:** IgG reduced
**Sample injection:** 20 sec, 4600 V
**Separation time:** 25/35 min, 5750 V
**Detection wavelength:** 220 nm
**Data evaluation**: IS = 10 kDa (RMT)

# Success of CE-SDS: transfer to other proteins

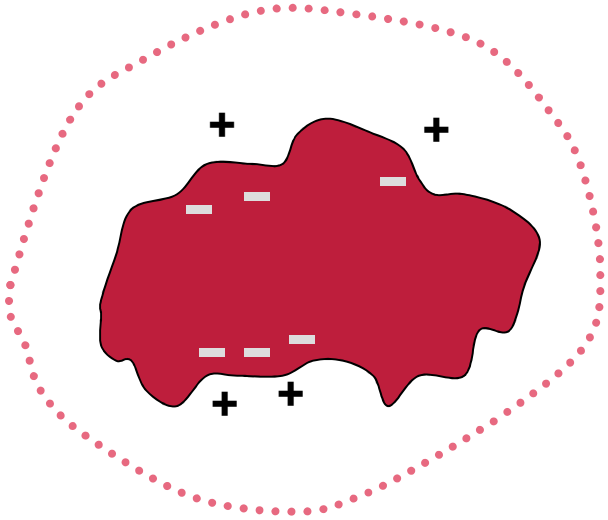


# A. Guttman, J. Nolan, Analytical Biochemistry 221, 285-289 (1994)

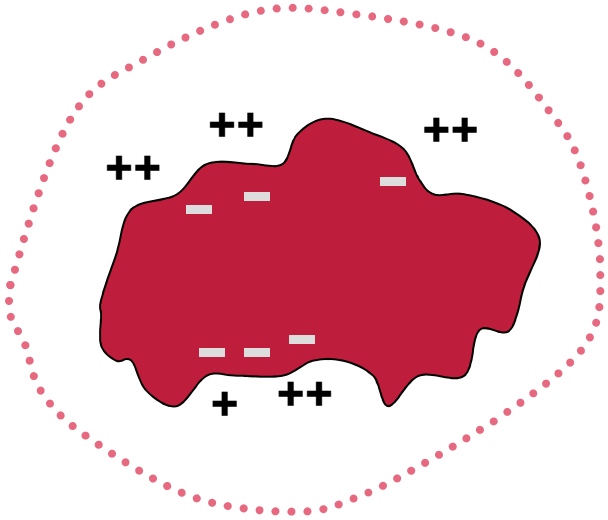
# What is size?



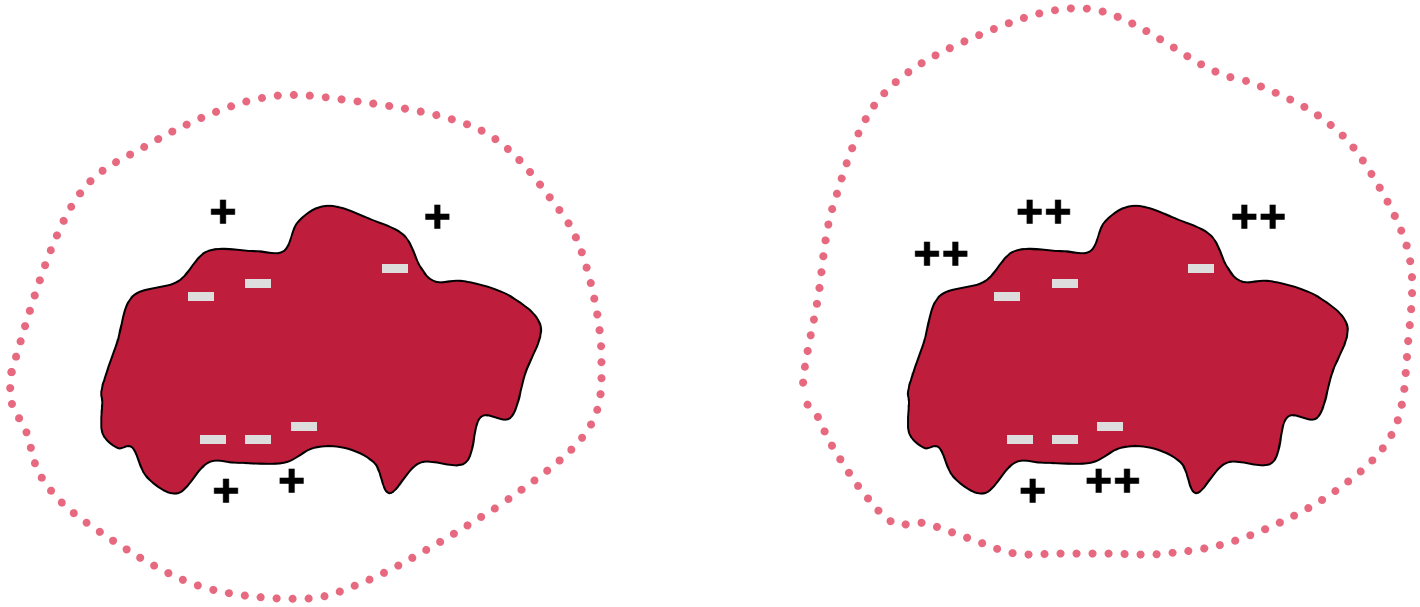
# What is size?



# What is size?



# What is size?



S. Redweik, C. Cianciulli, M. Hara, Y. Xu, H. Wätzig, Electrophoresis, 2013, 34, 1812–1819.

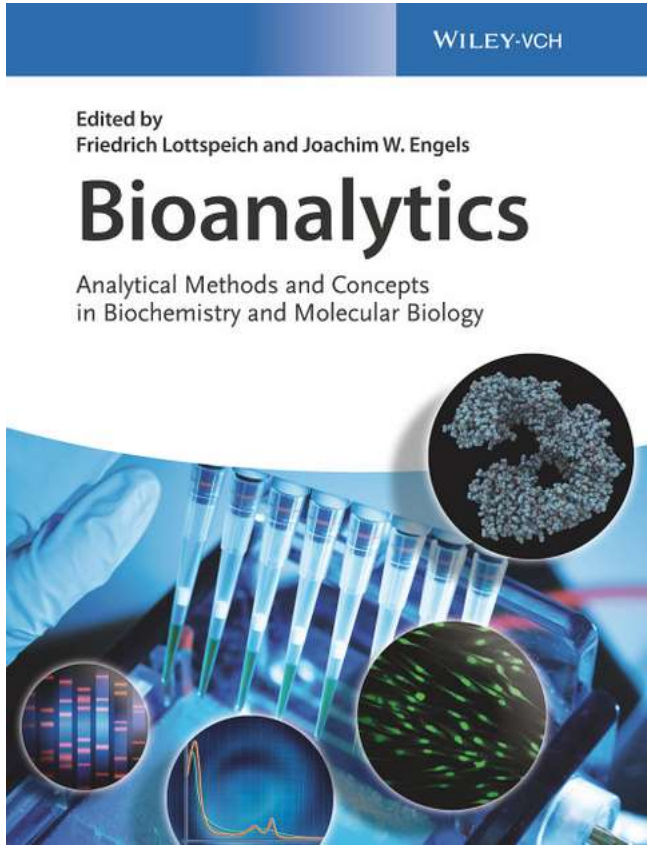
# Examples for different sizes of one protein: BSA

| method | parameter adjustment | protein concen-tration | measuring condition | size (nm) | source |
|---|---|---|---|---|---|
| Atomic force microscopy | tapping mode, resonance frequenz ca. 10 kHz, scan rate 1 Hz | 5 µg/ml | aqueous solution, pH 4,5 (near IEP) | max. height: 1.4 | S. Demanèche et al., 2009 |
| Dynamic light scattering | 514 nm, scattering angle 90°, parallel mode | 1 mg/ml | pH 7, 24°C | radius: 3.8 | A. Adel et al., 2008 |
| Dynamic light scattering | 488 nm, scattering angle 90° | 0,02 mg/ml | phosphate buffer pH 7,2, ionic strength 0,02 | radius: 2,7±0,1 | R. E. Tanner et al., 1982 |
| Small angle neutron scattering | mean wavelength: 6Å, scattering angle 90° | 0,01 mg/ml | $D_2O$, acetate buffer pH 5,4, ionic strength of 0,5 M NaCl, 30°C | prolate ellipsoid: a=7,02±0,51, b=2,22±0,08 | S. Chodankar et al., 2008 |

# Size measurements: overall variability (SDS-PAGE and CE-SDS)

1 g protein binds 1 to 1.4 g SDS
(+- 20% is normal variability)

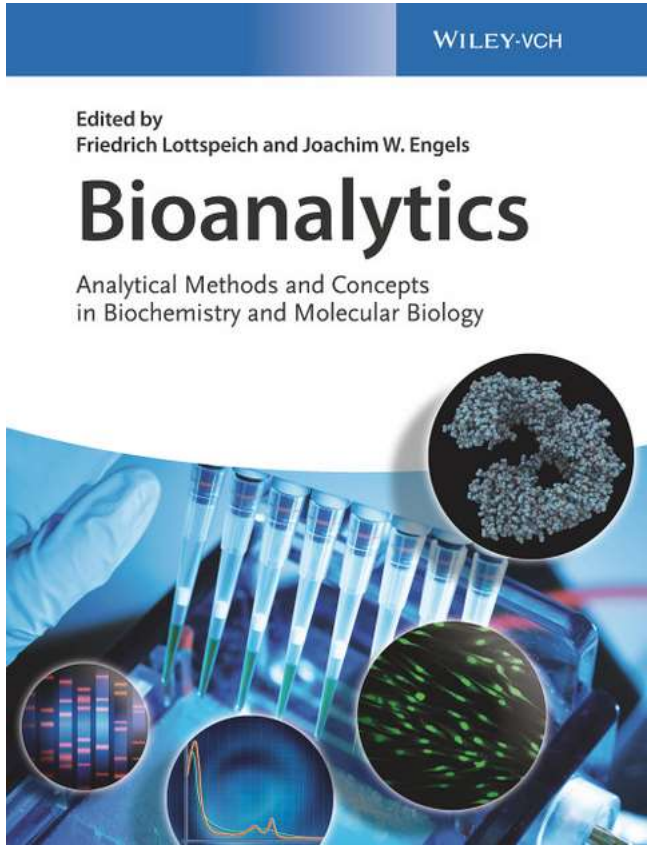
Friedrich Lottspeich, Joachim Engels: *Bioanalytics*, Wiley 2018



# Size measurements: overall variability (SDS-PAGE and CE-SDS)

1 g protein binds 1 to 1.4 g SDS
(+- 20% is normal variability)

Friedrich Lottspeich, Joachim Engels: *Bioanalytics*, Wiley 2018




**Mechanistic models for size-based separations:**

Heller, C., *J. Chromatogr. A* 1995, *698*, 19–31.
Guttman, A., *Electrophoresis* 1996, *17*, 1333–1341.

# A. Guttman, J. Nolan, Analytical Biochemistry 221, 285-289 (1994)

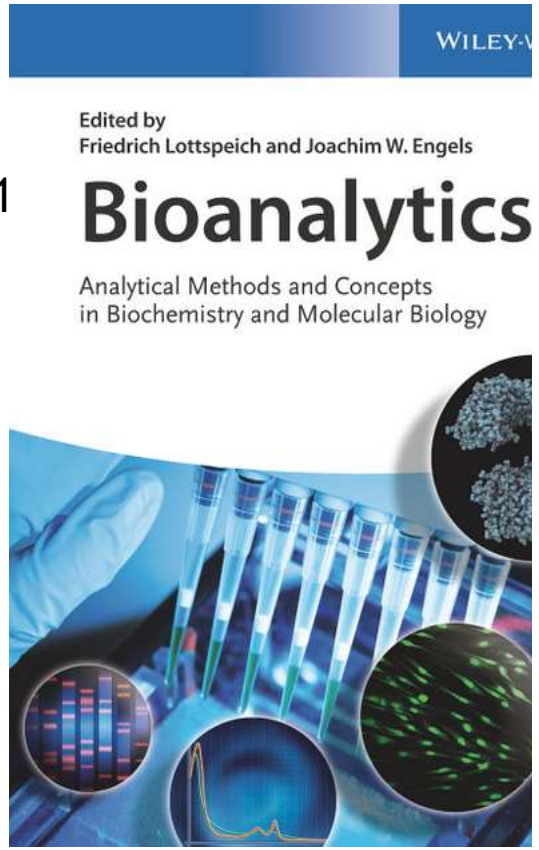
# Size measurements: overall variability (SDS-PAGE and CE-SDS)

Friedrich Lottspeich,  Joachim Engels: *Bioanalytics*, Wiley 201

1 g protein binds 1 to 1.4 g SDS (+- 20% is normal variability)

Few proteins show stronger deviations; reasons?

Sample pre-treatment?
Sample buffer or running buffer, pH?
Additional interactions to the gel matrix?
Influence of Glycosylation?
Ladder?
Calculation?



## SDS-PAGE: Buffer compositions

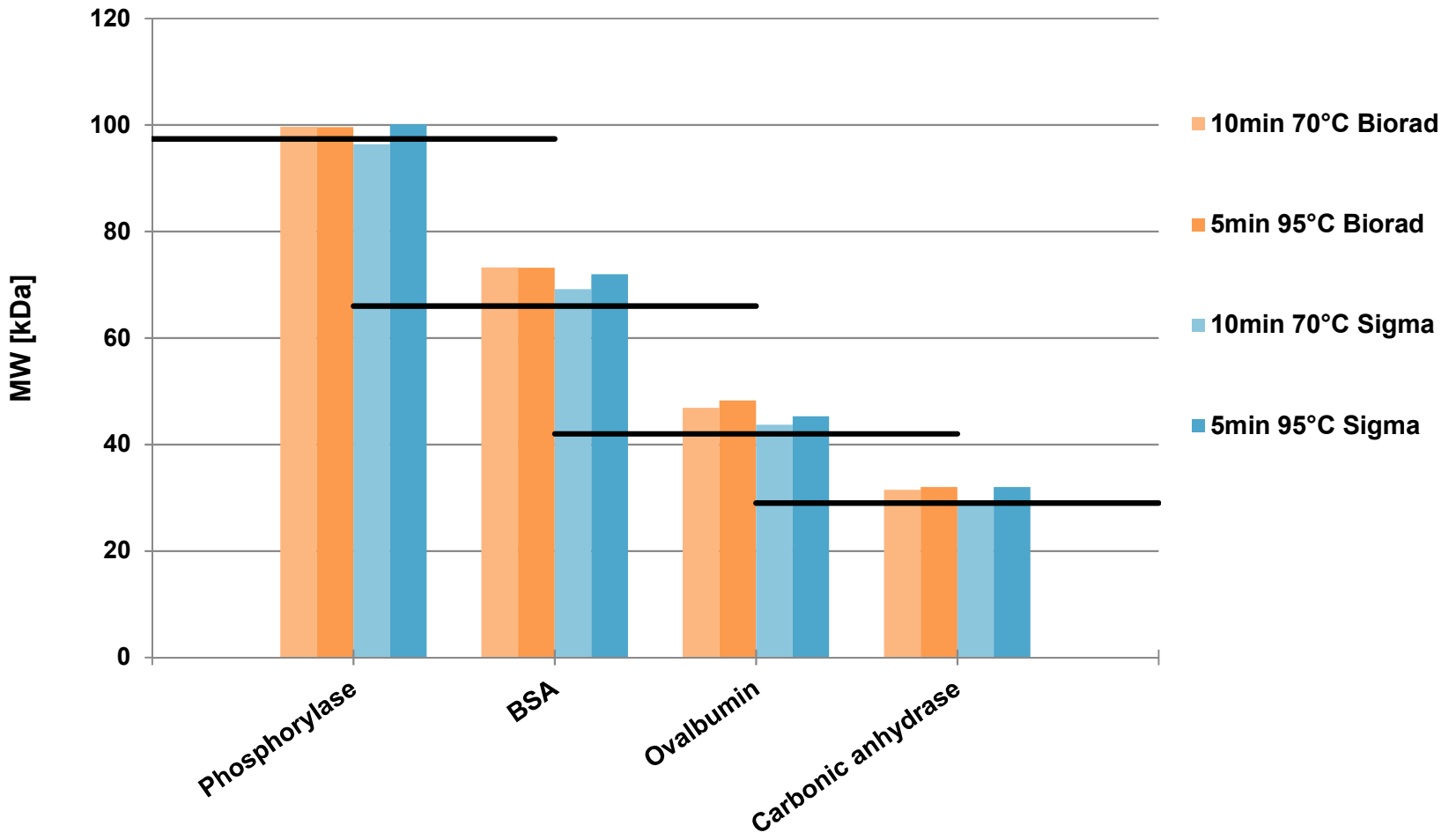
| | Original Laemmli * | Biorad * | Sigma * |
|---|---|---|---|
| Tris-HCl [mmol/l] | 62.5 | 32.9 | 62.5 |
| Glycerol [%] | 10.0 | 13.15 | 10.0 |
| SDS [%] | 2.0 | 1.05 | 2.0 |
| Bromophenolblue [%] | 0.001 | 0.005 | 0.002 |
| β-mercaptoethanol [%] | 5.0 | 5.0 | 5.0 |
| pH | 6.8 | 6.8 | 6.8 |

* all data refer to the final concentration in the sample

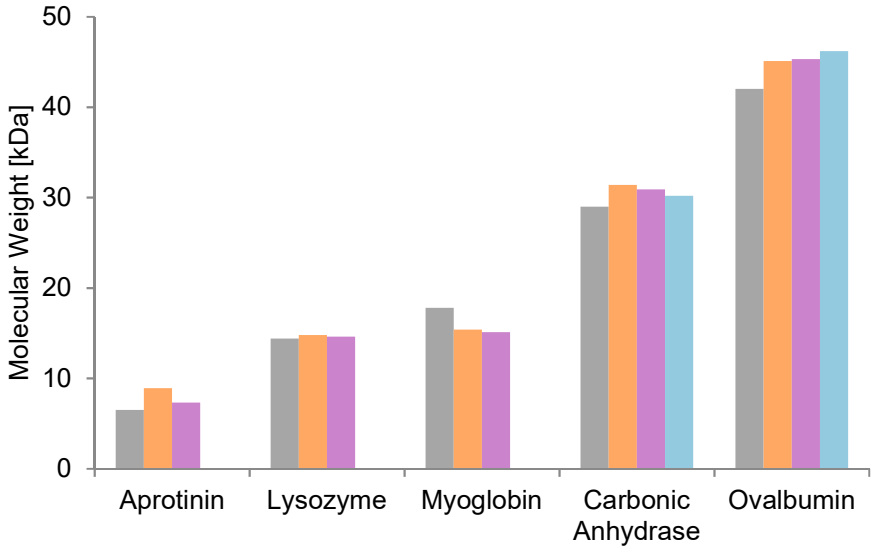
# SDS-PAGE:
# Comparison of sample buffers

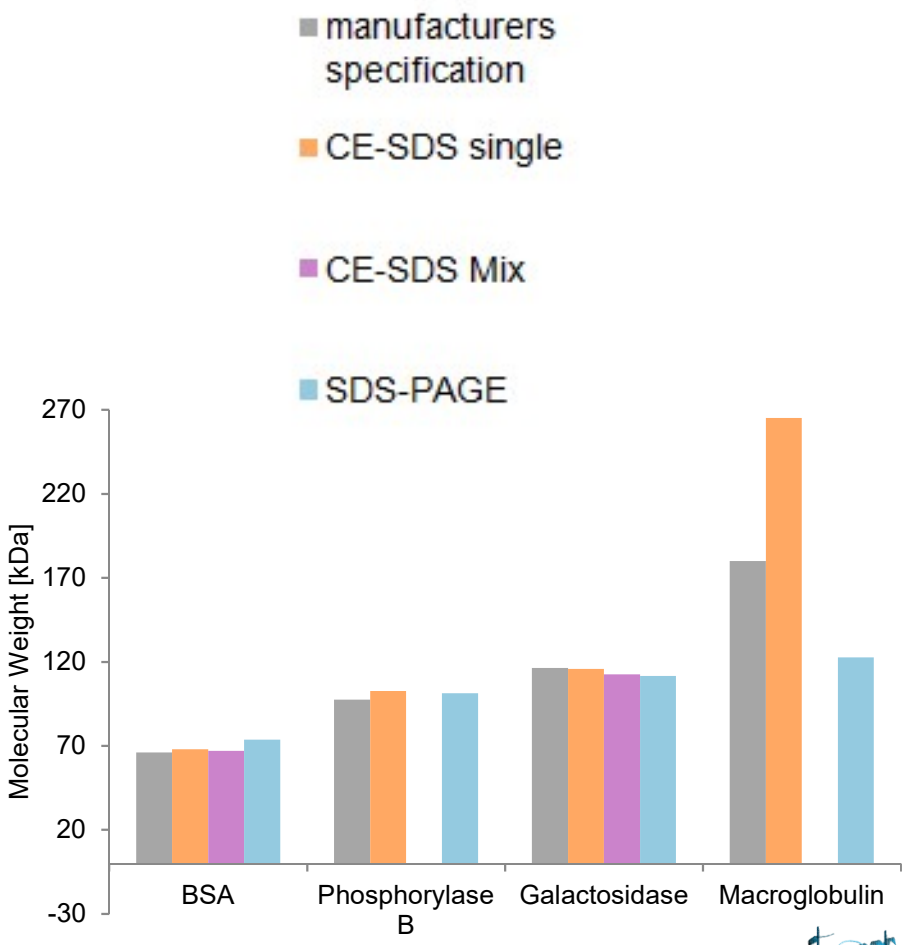
| | MW of proteins [kDa] | | | |
|---|---|---|---|---|
| | **Phosphorylase** | **BSA** | **Ovalbumin** | **Carbonic anhydrase** |
| manufacturers specification | 97.40 | 66.00 | 42.00 | 29.00 |
| 10min 70°C Biorad | 99.68 | 73.26 | 46.88 | 31.45 |
| 5min 95°C Biorad | 99.61 | 73.19 | 48.25 | 32.00 |
| 10min 70°C Sigma | 96.40 | 69.17 | 43.68 | 28.65 |
| 5min 95°C Sigma | 100.12 | 71.99 | 45.31 | 32.00 |

# SDS-PAGE:
# Comparison of sample buffers



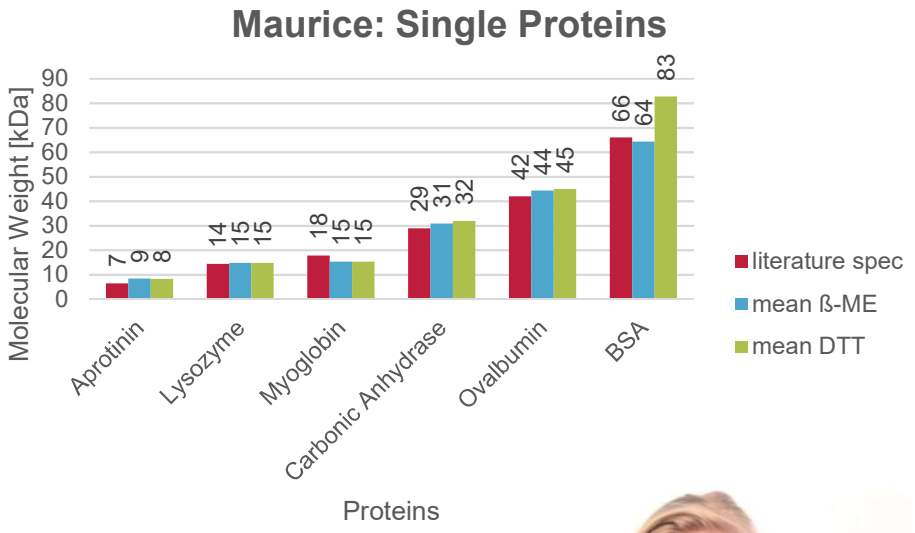
# Overview: previous results



# Maurice: ß-ME vs. DTT

Maurice
70°C 10 min
710 mM ß-ME
10 mM DTT
Single Proteins: 0.5 mg/ml
Mix A+B: 0.1 mg/ml




Rebecca Wiesner

Technische Universität Braunschweig

Wätzig et al.
Institut für Medizinische und Pharmazeutische Chemie

# CE-SDS vs. SDS-PAGE
## A comparative study of molecular mass determination methods

Rebecca Wiesner, Christin Scheller, Holger Zagst, Hermann Wätzig,
Imke Oltmann-Norden

CE Pharm Young Scientist Session, September 30th 2019

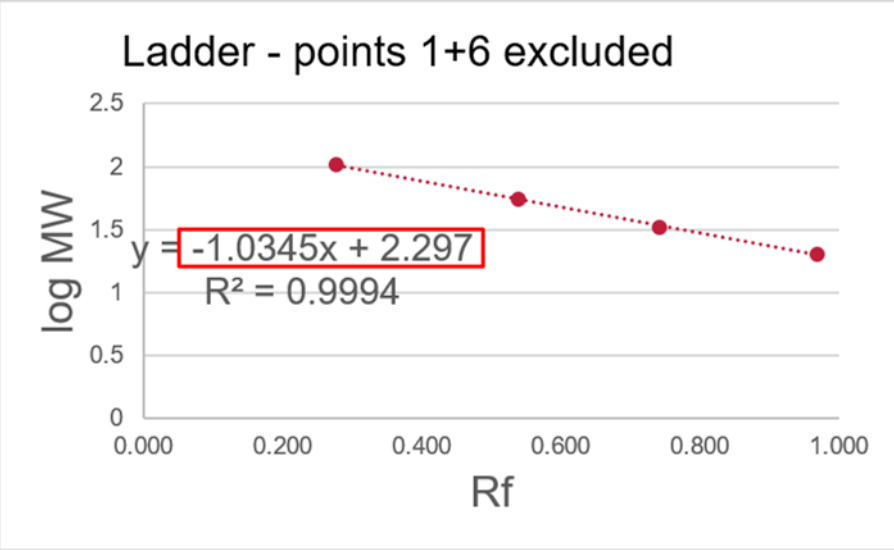
# SDS-PAGE and CE-SDS: preliminary conclusions (part 1)

- Sample pre-treatment, sample buffer or running buffer:
  only minor influences on apparent molecular mass

# SDS-PAGE - data evaluation

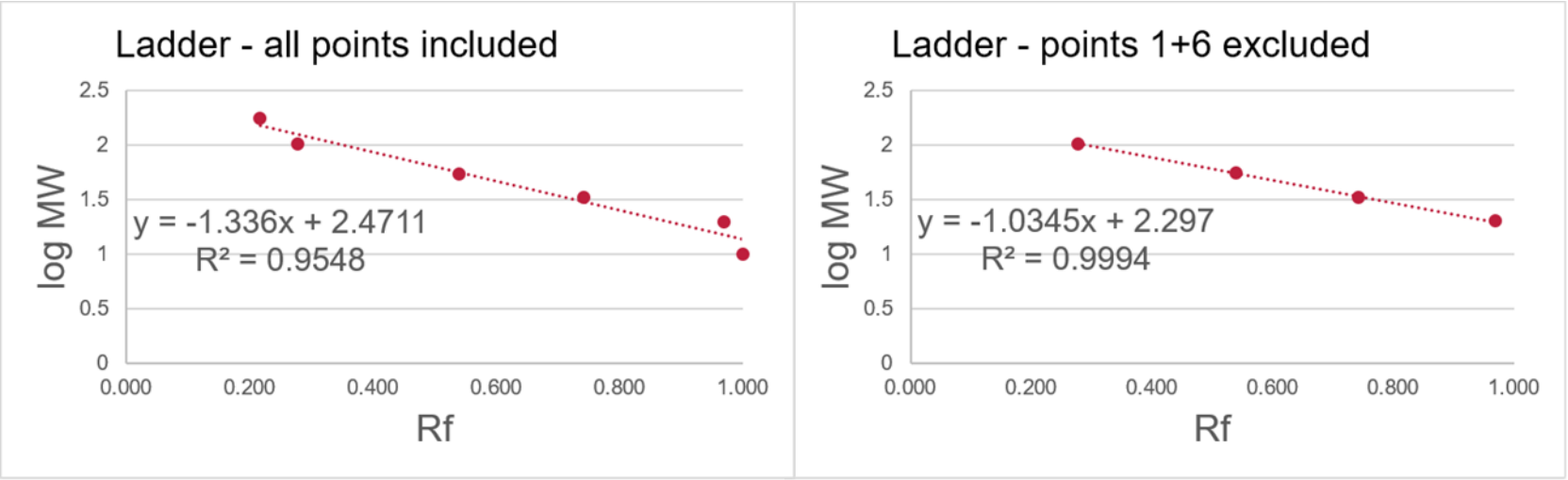
$$y = mx + b$$
$$y = \log MW$$
$$x = R_f \text{ of the protein band}$$

$$\Leftrightarrow MW = 10^y$$



# SDS-PAGE - data evaluation

## Linear regression



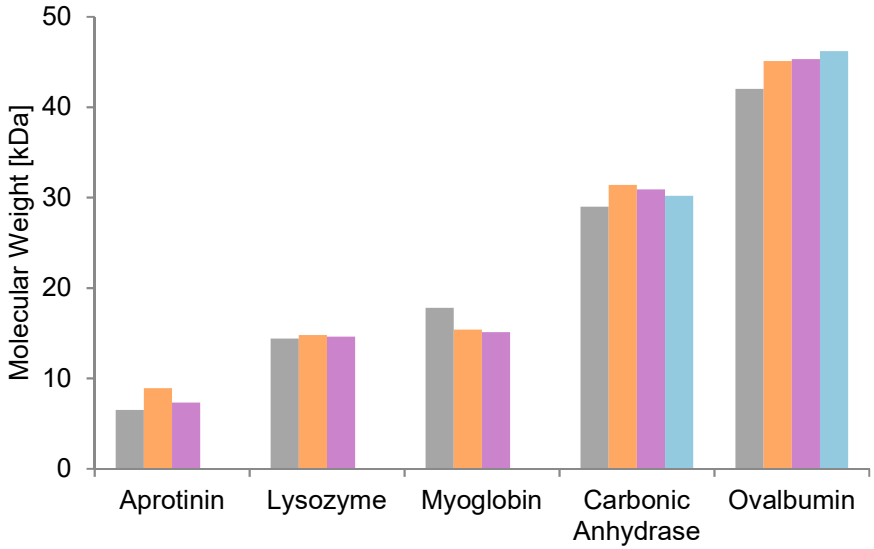
## One interesting finding

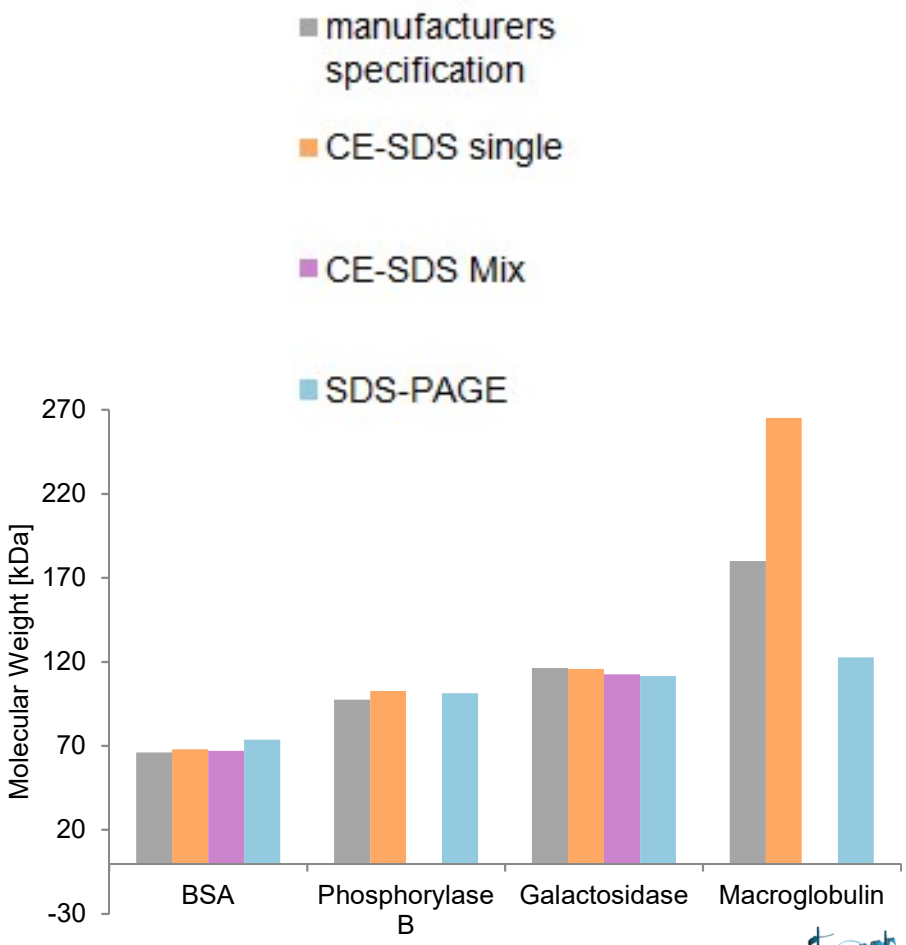
[2] A. Guttman, J. Nolan, Analytical Biochemistry 221, 285-289 (1994)

| | CE-SDS | | | SDS-PAGE | | |
|---|---|---|---|---|---|---|
| | Guttman et al. | Wätzig-Maurice | Sigma [1] | Guttman et al. | Wätzig | Sigma [1] |
| Lysozyme | 15.9 | 14.8 | 14.3 | < 29 | n/a | 14.3 |
| Myoglobin | 17.9 | 15.4 | 17.2 | < 29 | n/a | 17.2 |
| Carbonic Anhydrase | 29.7 | 31.6 | 29 | 28.8 | 28.5 | 29 |
| Ovalbumin | 45.5 | 45.3 | 45 | 42.8 | 44.9 | 45 |
| BSA | 71.1 | 69.8 | 66 | 62.5 | 71 | 66 |
| Phosphorylase B | 89.7 | 103.8 | 97.4 | 81.6 | 97.3 | 97.4 |
| ß-Galactosidase | 116.2 | 117.4 | 116 | 106.7 | 106 | 116 |
| α-Macroglobulin | 188.8 | 257.3 | 180 | 178 | 117.3 | 180 |

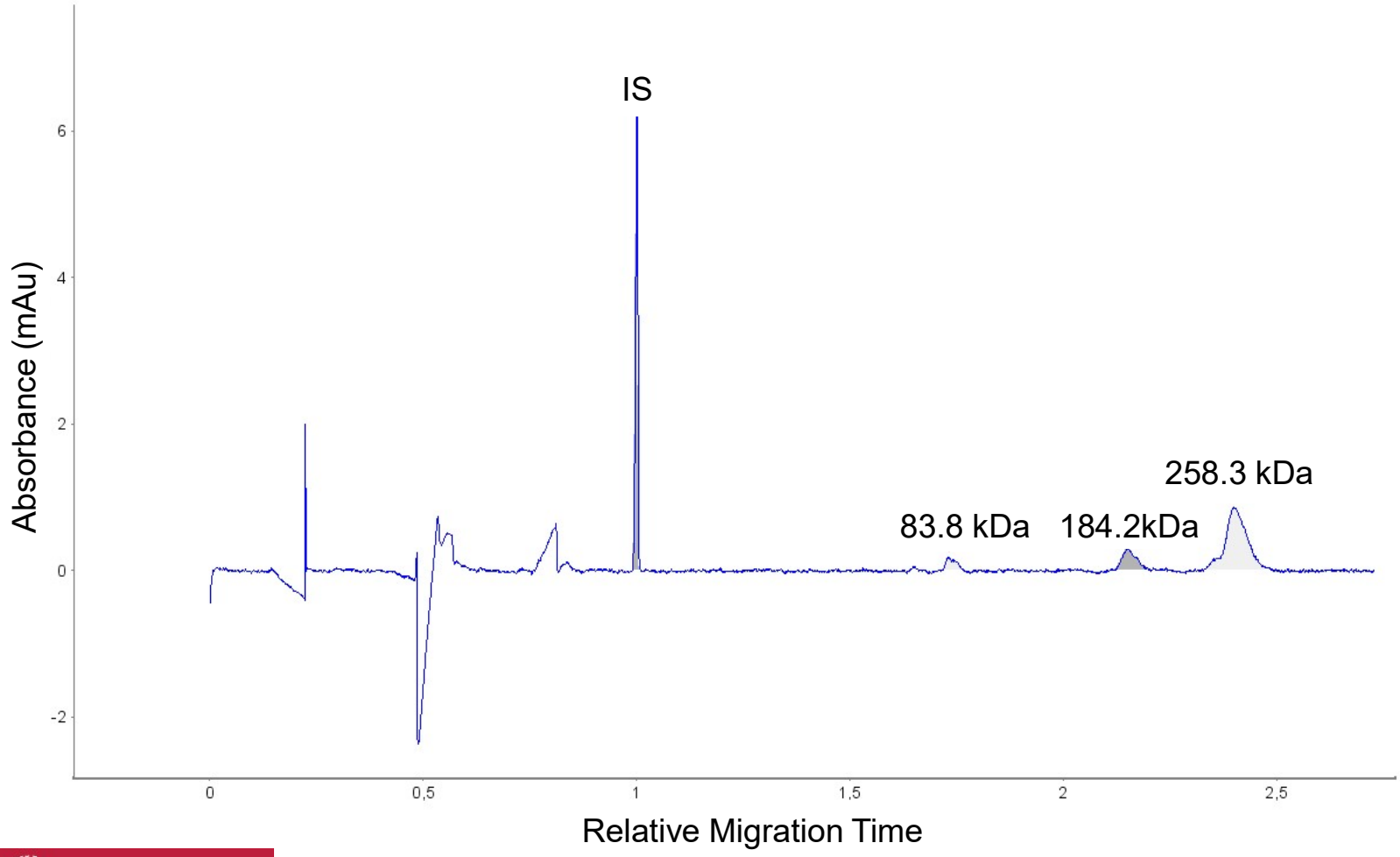
[1] Sigma Catalog (1994) Sigma Chemical Co.

# Overview: previous results



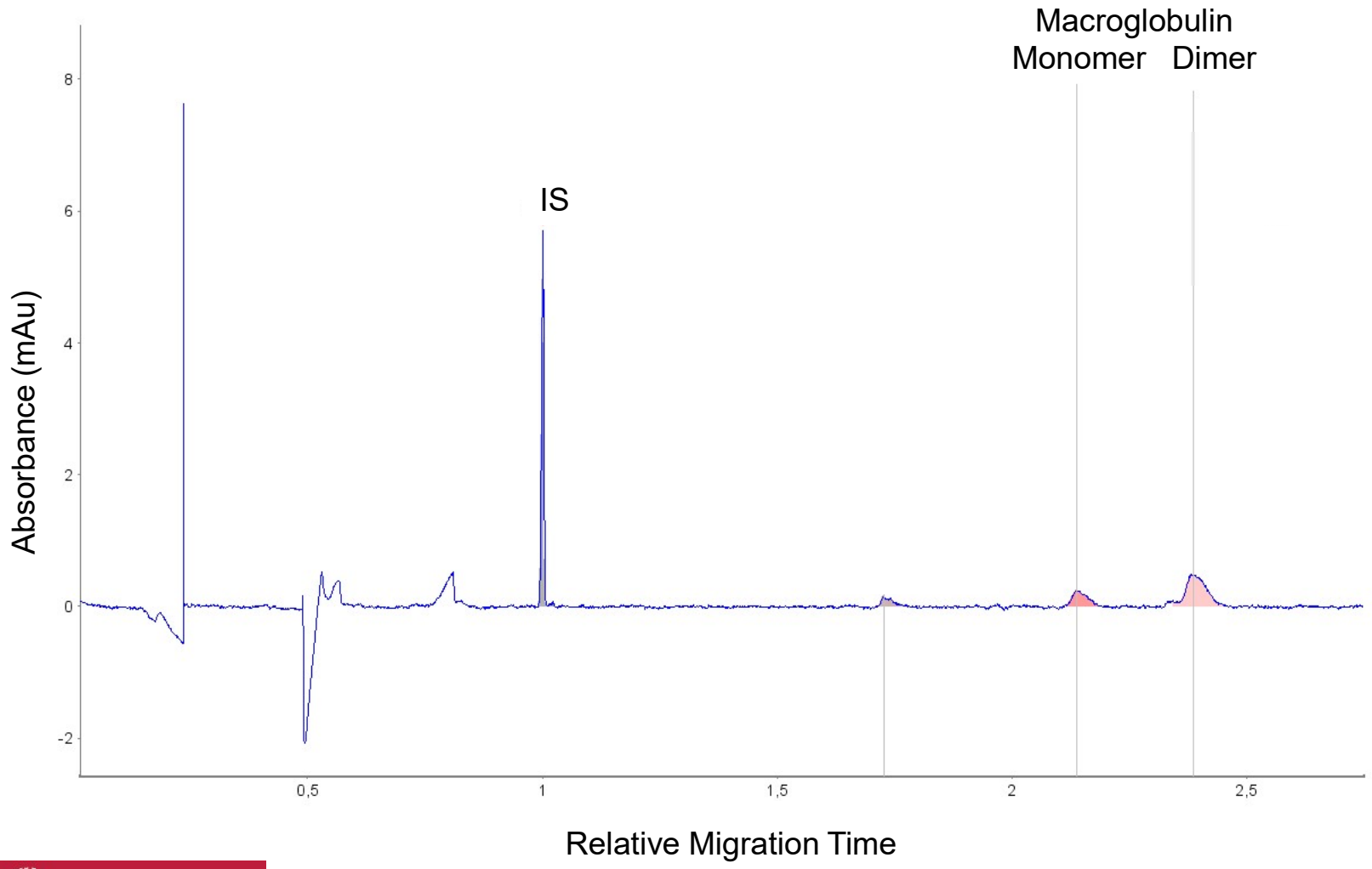
# Maurice: Macroglobulin



# α-Macroglobulin: a homotetramer

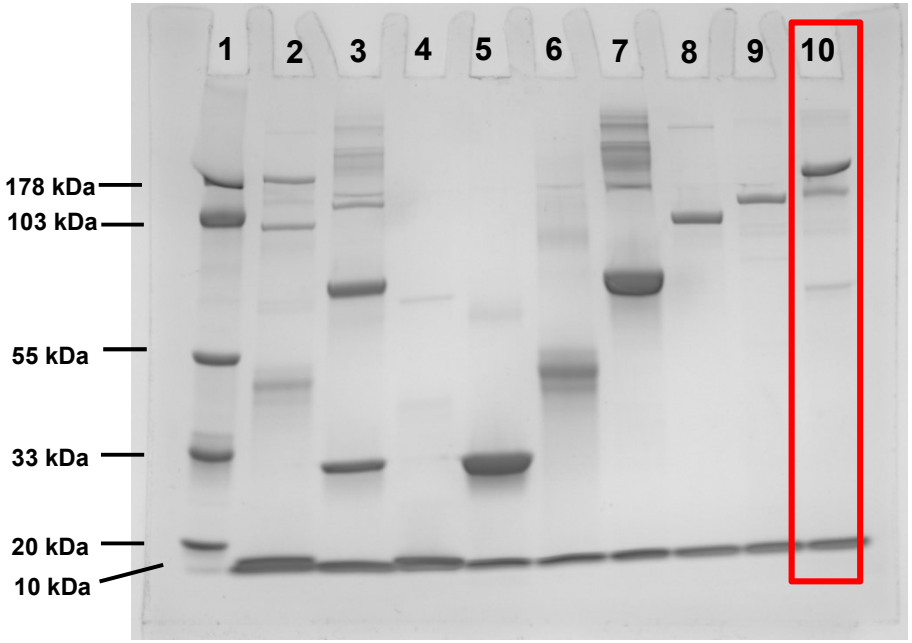
A. R. Wyatt et al., PLOS ONE | DOI:10.1371/journal.pone.0130036 , 2015

# Maurice: Macroglobulin



# SDS-PAGE: Macroglobulin

SDS-PAGE – 10 min 70°C (10% gel)

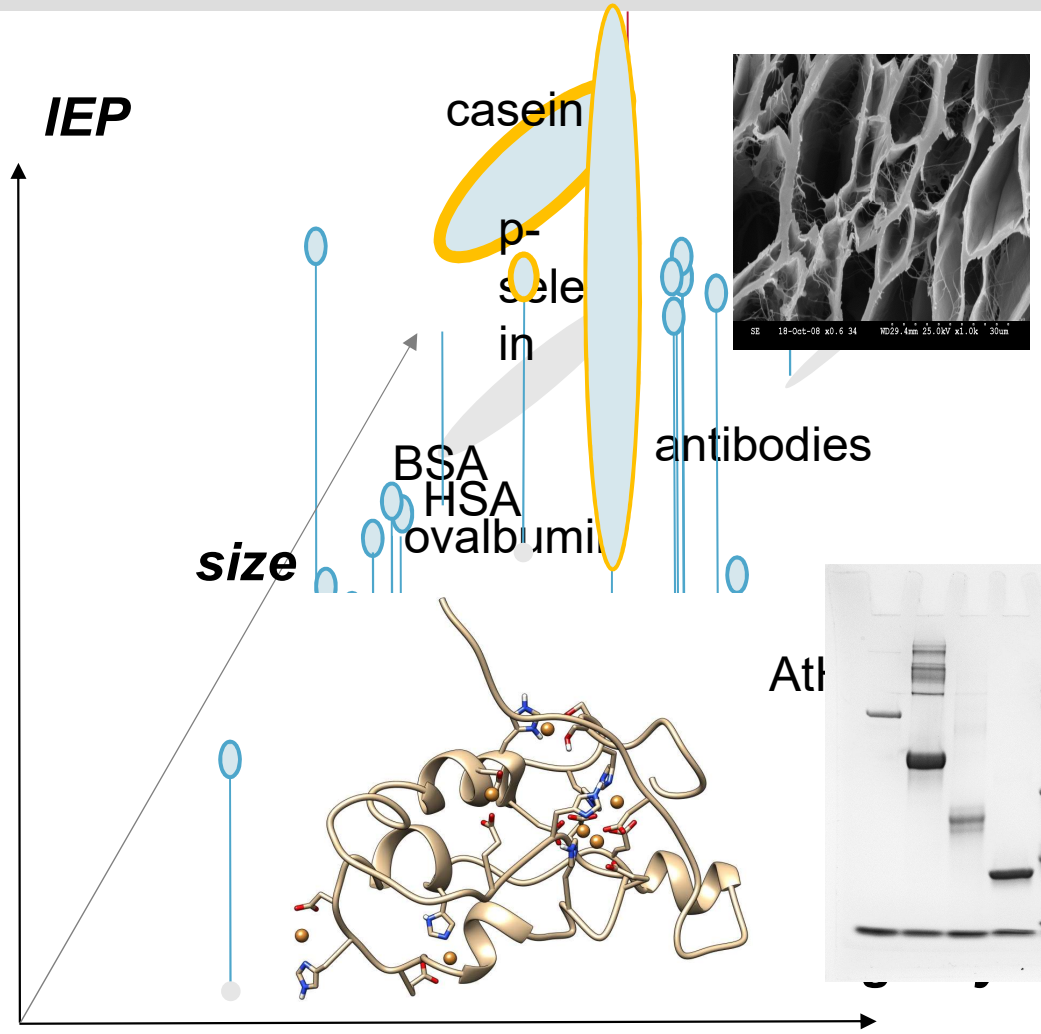



1. Ladder
2. Mixture D
3. Mixture E
4. Myoglobin
5. Carbonic anhydrase
6. Ovalbumin
7. BSA
8. Phosphorylase
9. Galactosidase
10. Macroglobulin

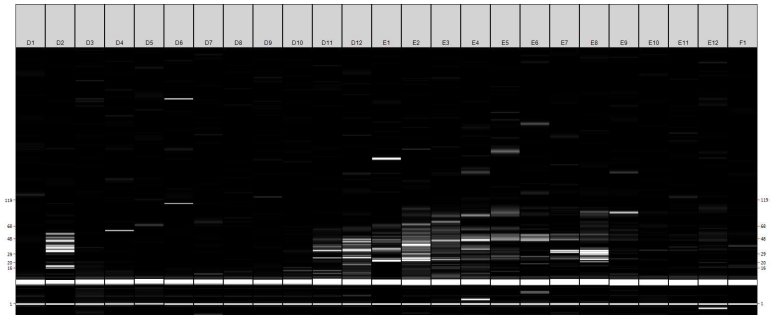
# SDS-PAGE and CE-SDS: preliminary conclusions

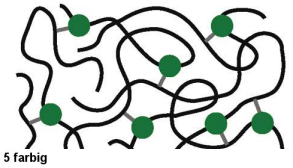
- Sample pre-treatment, sample buffer or running buffer:
  only minor influences on apparent molecular mass
- Ladders and calculation algorithms make a difference
- Limited suitable size range
- Typically no major discrepancies
- Sometimes observed discrepancies are possibly only apparent
  (particular properties of a few proteins)

# Summary and Conclusions

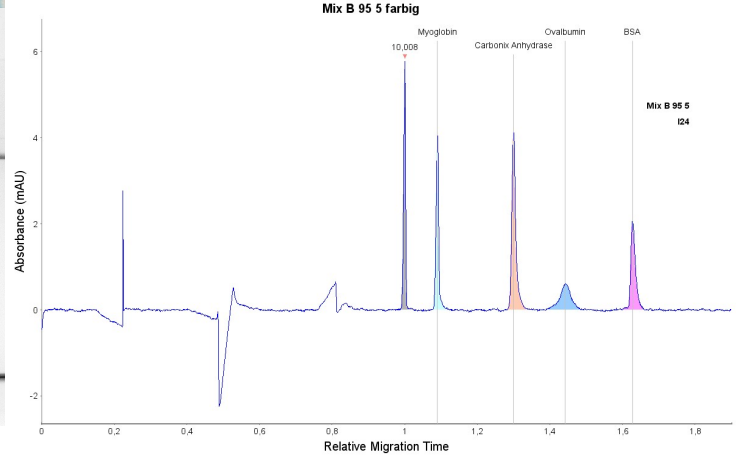






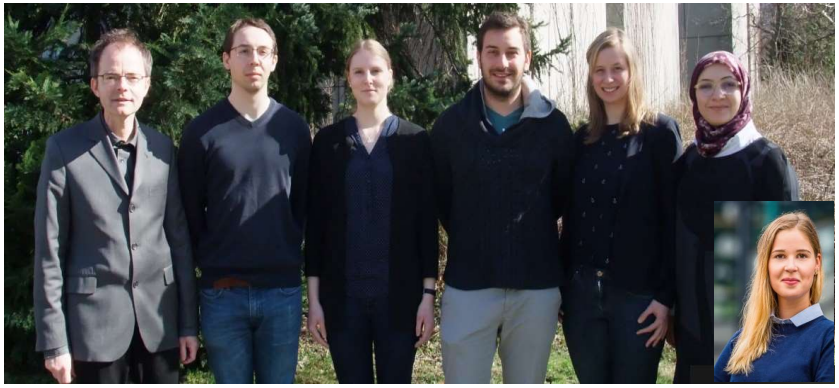


# Thank you very much!

**Hermann Wätzig, Holger Zagst, Julia Kahle, Matthias Stein, Rebecca Wiesner, Mais Olabi; and Imke Oltmann-Norden, Kai-Jorrit Maul-Köhler, Christin Scheller**

# Thank you very much!

**Hermann Wätzig, Holger Zagst, Julia Kahle, Matthias Stein, Rebecca Wiesner, Mais Olabi; and Imke Oltmann-Norden, Kai-Jorrit Maul-Köhler, Christin Scheller**



botiss biomaterials

proteinsimple® a bio-techne brand

PerkinElmer® For the Better

ZIM Zentrales Innovationsprogramm Mittelstand

ZF4544401 AJ8

# Outline

**Proteins: classification**

antibodies and enzymes, collagen, IDPs, viruses, etc.
adsorption
selectivity; buffers, CE-MS; 2-DE

**Case study: collagen**

**Case study: AtHIRD11,** an intrinsically disordered protein (IDP)

**Protein size characterization**

**Preliminary conclusions and outlook**