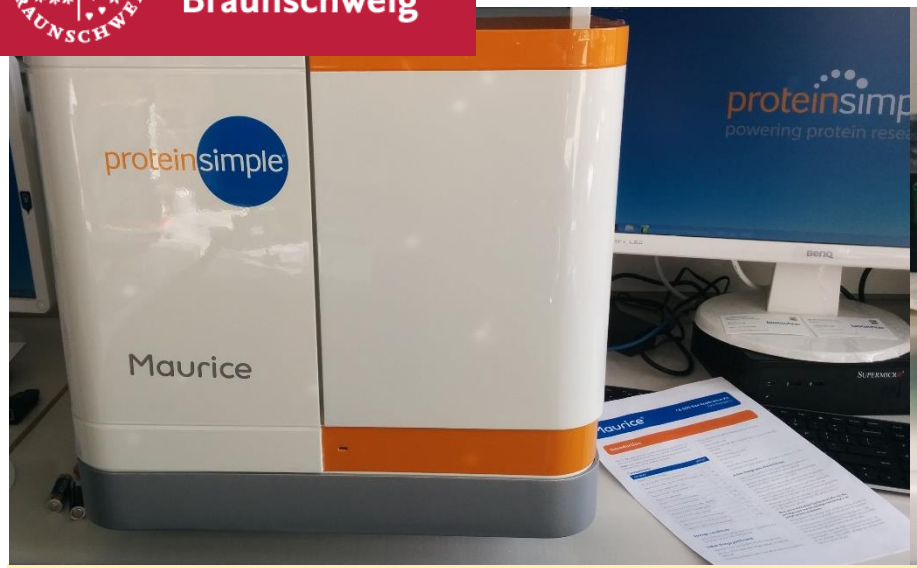
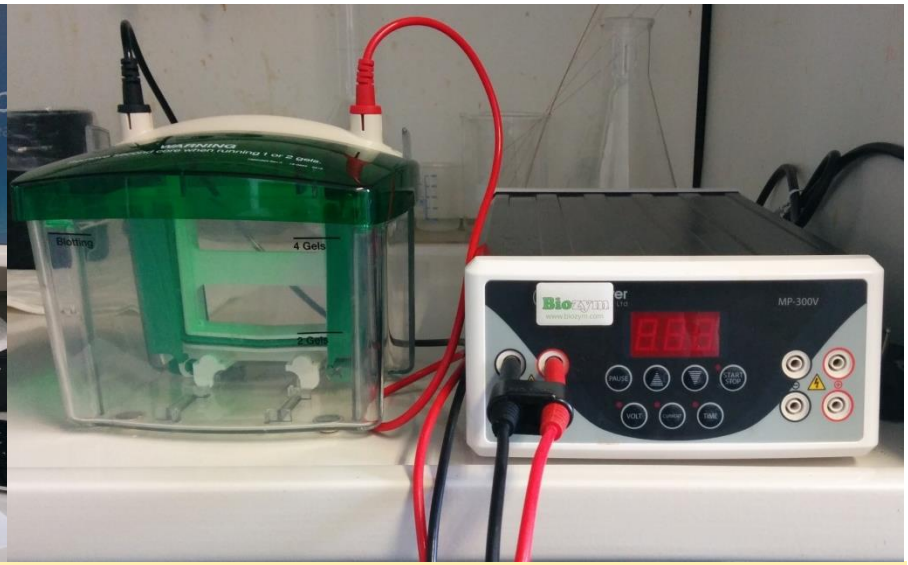
Technische Universität Braunschweig

Wätzig et al.
Institute of Medicinal and Pharmaceutical Chemistry





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

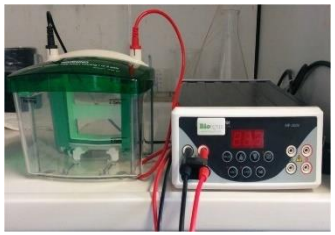
Rebecca Wiesner, Christin Scheller, Holger Zagst, Hermann Wätzig, Imke Oltmann-Norden

CE Pharm Young Scientist Session, September 30th 2019

Outline

1. Introduction

- CE-SDS vs. SDS-PAGE vs. Simple Western




- Experimental Design

2. Results

- Temperature conditions: 70°C 10 min vs. 95°C 5 min
- Reducing agents: TCEP vs. DTT vs. ß-ME
- Precision

3. Conclusion

Introduction

- Approximately 20% size differences between CE-SDS and SDS-PAGE (Friedrich Lottspeich, Joachim Engels: *Bioanalytics*, Wiley 2018)

- SDS PAGE is commonly used, but it is
  - time consuming (10 hours SDS-PAGE vs. 6 hours CE-SDS) and
  - requires many individual manual steps (e.g. washing and staining).
- CE-SDS is superior due to
  - direct quantification of proteins,
  - higher resolution,
  - and mostly automated user-friendly devices.

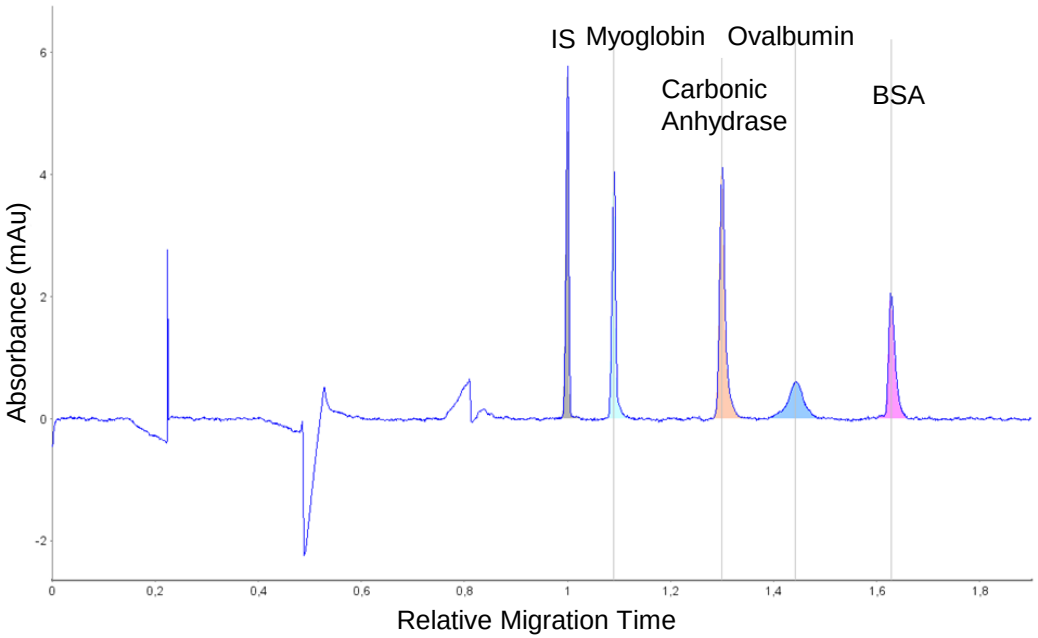
Introduction

Model proteins

- Aprotinin (6.5 kDa)
- Lysozyme (14.4 kDa)
- Myoglobin (17.8 kDa)
- Carbonic Anhydrase (29 kDa)
- Ovalbumin (42 kDa)
- BSA (66 kDa)
- Phosphorylase B (97.4 kDa)
- β-Galactosidase (116.25 kDa)
- α -Macroglobulin (180 kDa)

Size range:
7-180 kDa

Method: CE-SDS

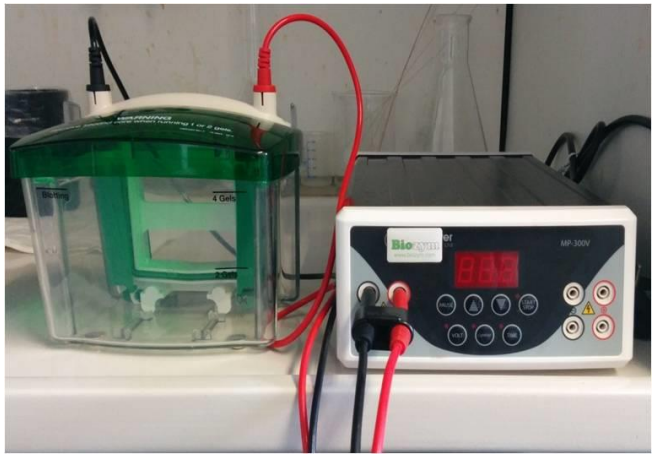


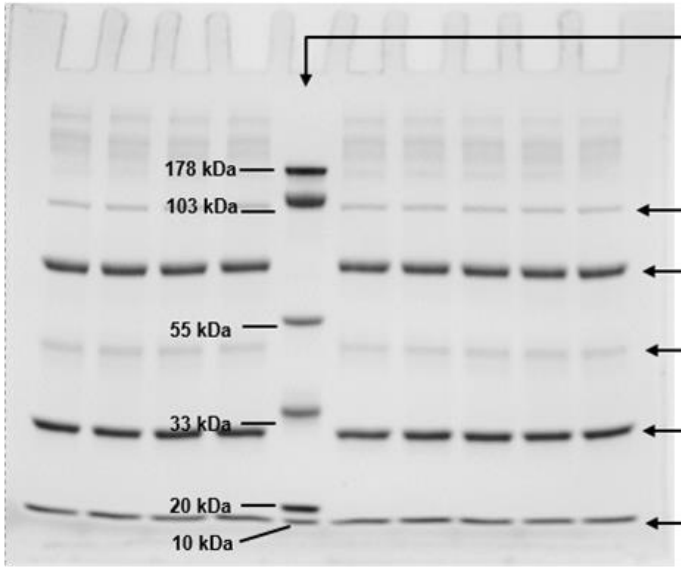
Instrumentation:
Maurice (ProteinSimple)

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)

Method: SDS-PAGE



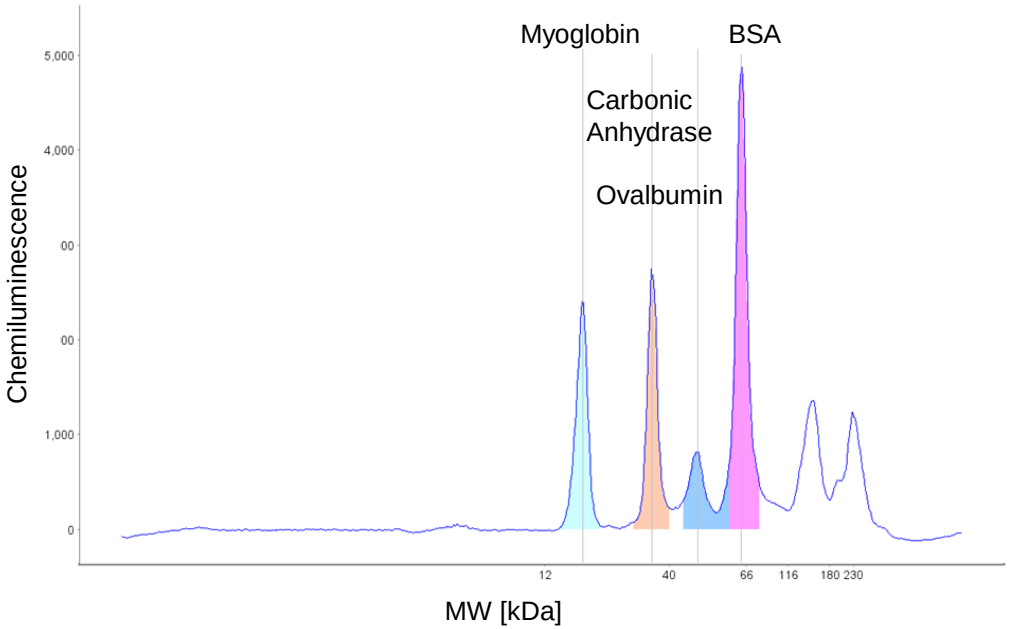


Instrumentation:
SDS-PAGE (Biorad)

Maurice Ladder: 10-270 kDa
Sample preparation:
1 mg/ml stock solution in
100 mM Tris & 1% SDS (pH=8)
→ 1:1 Dilution in Laemmli SB
→ 95°C 5 min, 710 mM β-ME

Running buffer: 25 mM Tris, 192 mM Glycine, 0.1% SDS, pH 8.3 (Biorad)
Run time: 35-40 min; 50 mA
Detection: Coomassie Blue Staining
Data Evaluation: Image J; IS = 10 kDa (RMT)
→ **Linear range (10% gels): 20-100 kDa**

Method: Simple Western



Instrumentation:
WES (ProteinSimple)

Total Protein Assay
WES Ladder:12-230 kDa
Sample preparation:
1 mg/ml stock solution in
100 mM Tris & 1% SDS (pH=8)
→ 0.2 mg/ml in 0.1 x SB (WES)
→ 95°C 5 min, 200 mM DTT

SST: Hela Lysate
Runtime: 3 h
Detection: Chemiluminescence
Data evaluation: 3 fluorescence marker

Method: Simple Western

https://www.proteinsimple.com/

Experimental Design

- **Measuring triplicates in total**

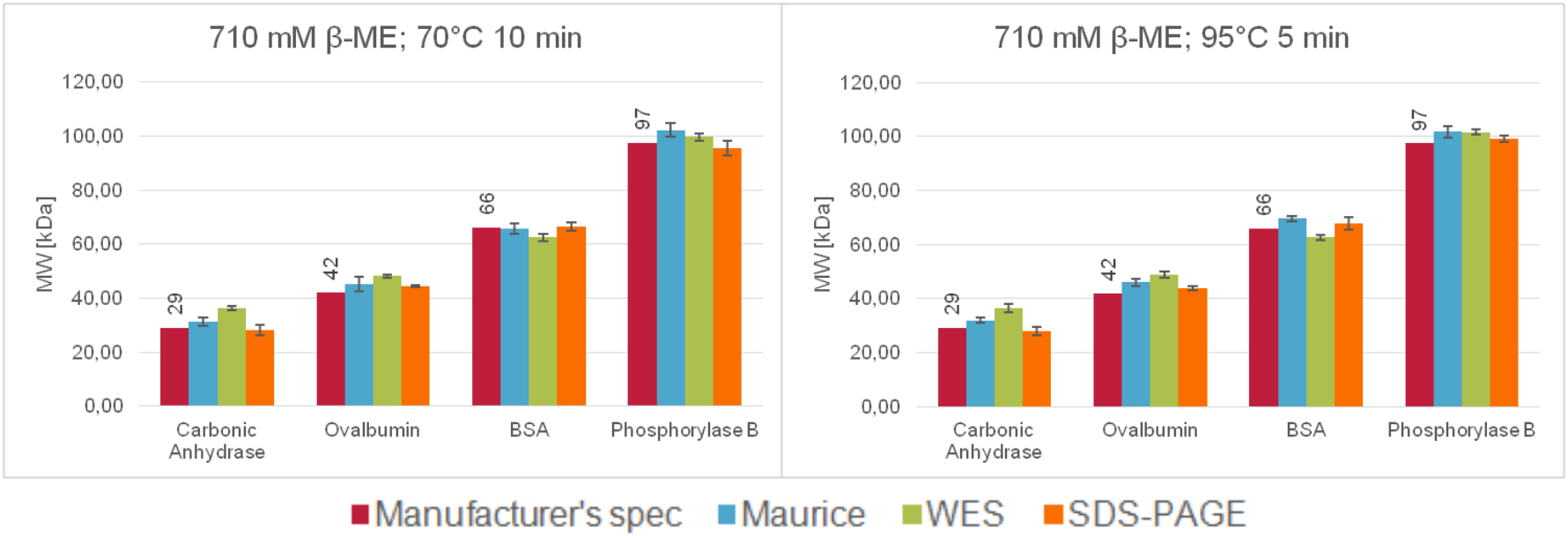
Maurice	**WES**	**SDS-PAGE**
3 injections/sample 0.5 mg/ml protein	2 capillaries/sample 0.2 mg/ml protein	1 sample 0.5 mg/ml protein

- **Model proteins:** Aprotinin, Lysozyme, Myoglobin, Carbonic Anhydrase, Ovalbumin, BSA, Phosphorylase B, β-Galactosidase and α-Macroglobulin

- **Reducing conditions:**
  - 70°C 10 min and 95°C 5 min
  - Reducing agents: β-Mercaptoethanol (β-ME), DTT and TCEP

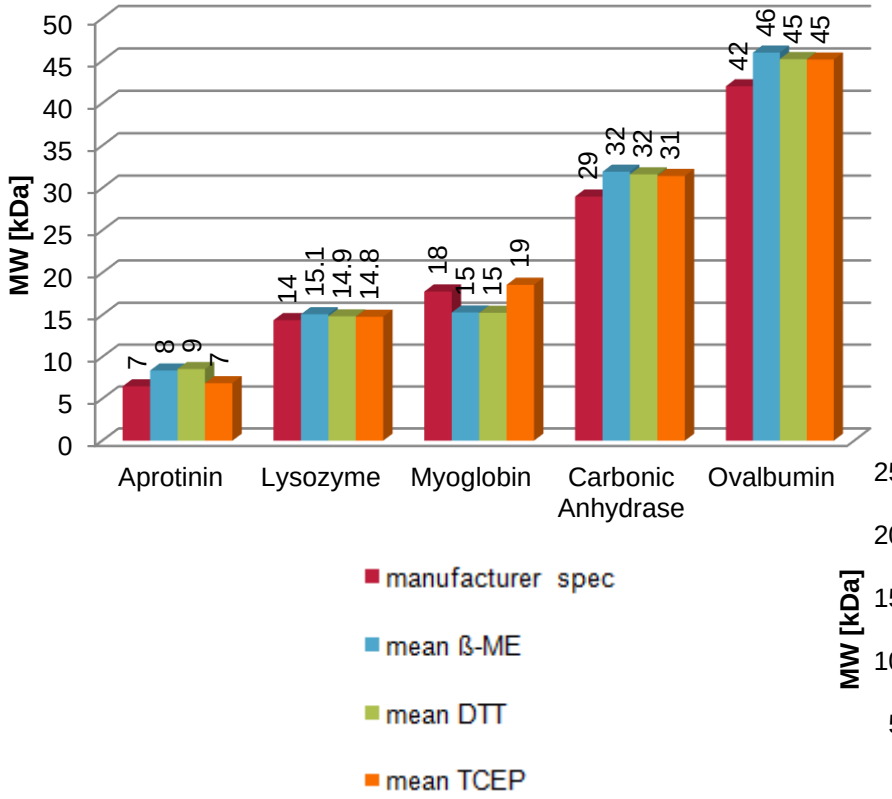
- **Precision**: n = 10, evaluated for Carbonic Anhydrase, Ovalbumin, BSA and Phosphorylase B

Results: Temperature

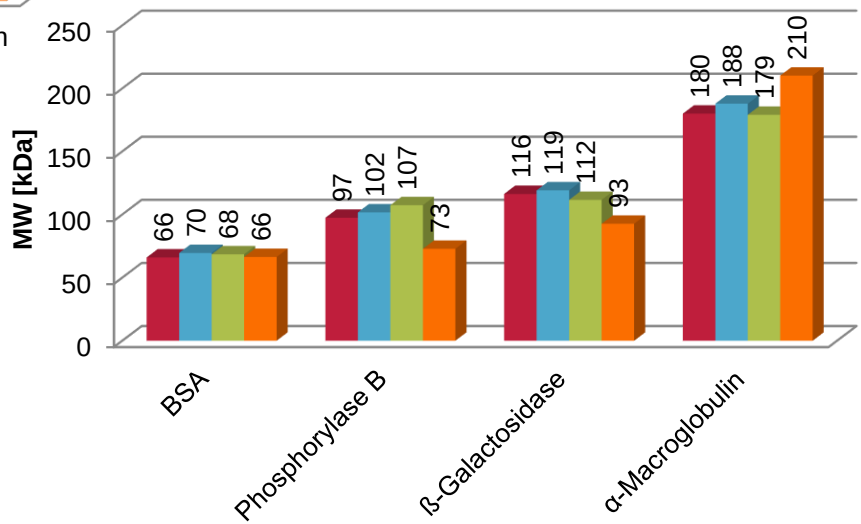


Comparison of CE-SDS, SDS-PAGE and Simple Western:
70°C 10 min vs. 95°C 5 min
0.5 mg/ml CE-SDS and SDS-PAGE, 0.2 mg/ml in WES

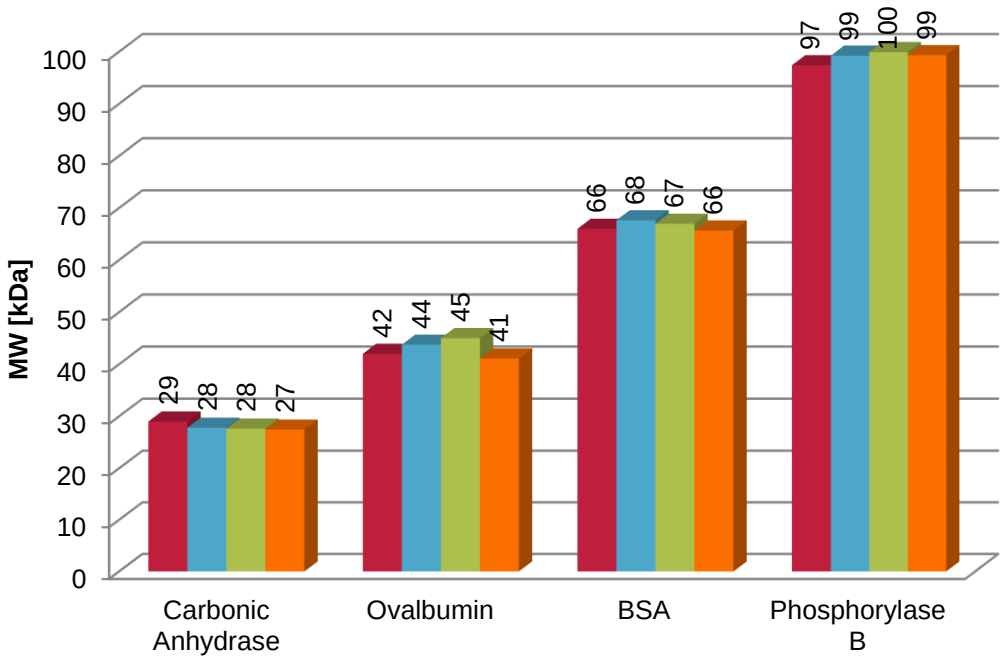
Results: Reducing agents Maurice



Maurice
95°C 5min
710 mM ß-ME
20 mM DTT
10 mM TCEP
Single Proteins: 0.5 mg/ml

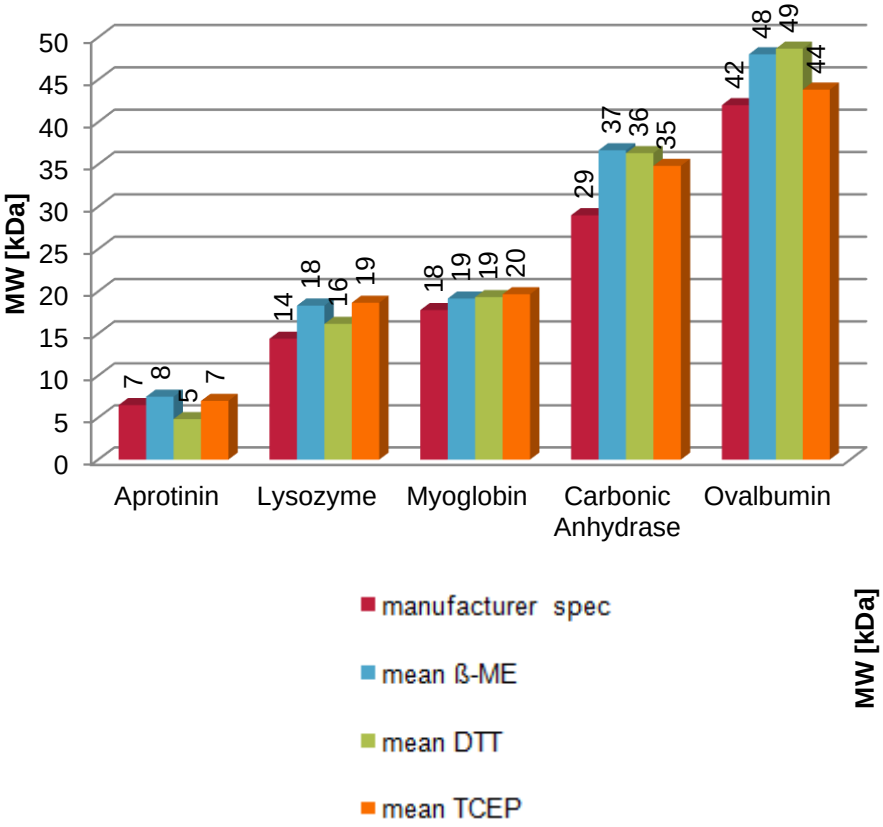


Results: Reducing agents SDS-PAGE

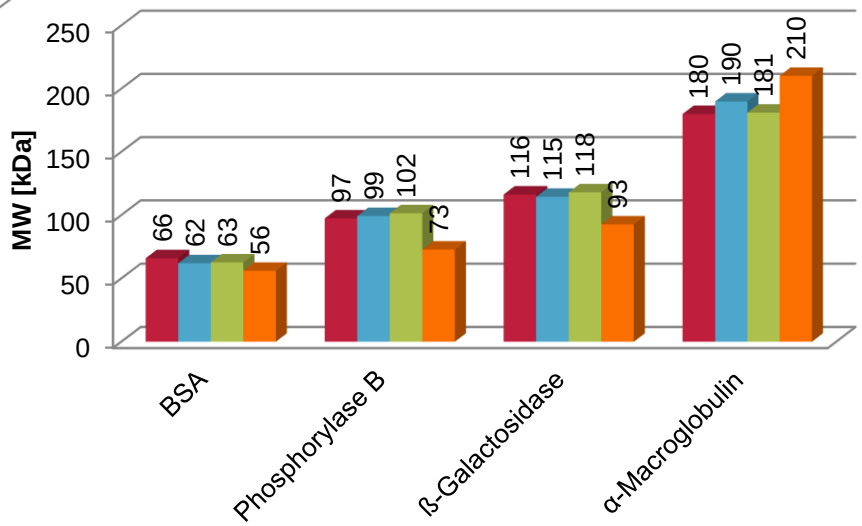


SDS-PAGE
95°C 5min
710 mM ß-ME
20 mM DTT
10 mM TCEP
Single Proteins: 0.5 mg/ml

Results: Reducing agents Simple Western



Simple Western
95°C 5 min
200 mM ß-ME
200 mM DTT
0.5 mM TCEP
Single Proteins: 0.2 mg/ml



Results: Precision

Mean	Manufacturer's specification	Maurice	SDS-PAGE	WES
Carbonic Anhydrase [kDa]	29	31.99±0.17	28.08±0.28	36.80±0.26
Ovalbumin [kDa]	42	45.41±0.33	44.46±0.47	48.90±0.66
BSA [kDa]	66	67.57±1.30	68.31±1.26	62.65±0.39
Phosphorylase B [kDa]	97.4	102.64±1.55	98.45±1.06	101.30±0.37

RSD%	Maurice	SDS-PAGE	WES
Carbonic Anhydrase [kDa]	0.88%	1.61%	1.15%
Ovalbumin [kDa]	1.18%	1.71%	2.20%
BSA [kDa]	3.11%	2.99%	1.00%
Phosphorylase B [kDa]	2.43%	1.74%	0.58%

Sample preparation

n=10

CE-SDS and SDS-PAGE
95°C 5min
710 mM ß-ME
Single Proteins:
0.5 mg/ml

WES
95°C 5min
200 mM DTT
Single Proteins:
0.2 mg/ml

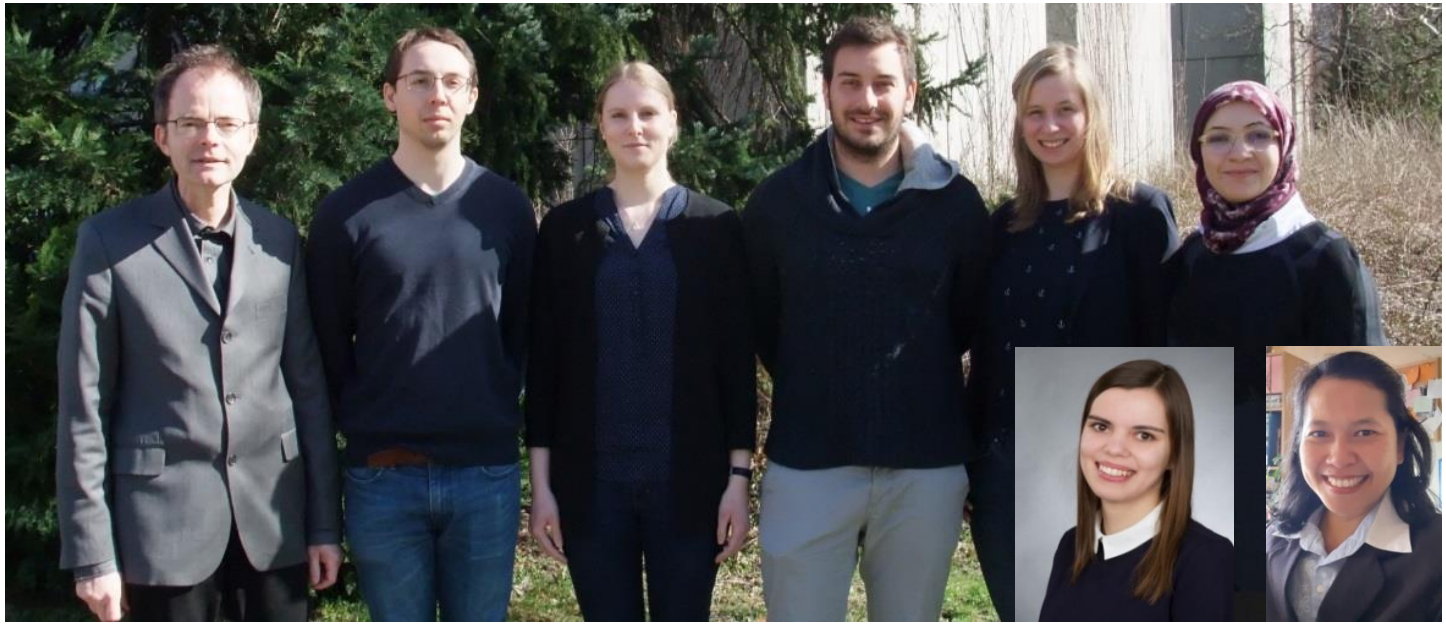
Take home messages

- Only minor differences can be explained by sample preparation.
- The linear range of SDS-PAGE (10% gels) includes 20-100 kDa, compared to CE-SDS with 10-270 kDa.
- CE-SDS and SDS-PAGE show similiar precisions in the range of 1-3 % RSD.
- CE-SDS is superior due to the direct quantification of proteins and the higher resolution. It is mostly automated inclusive user-friendly devices and kits.

Thank you to

GENDERED CONFIGURATIONS
OF HUMANS AND MACHINES
Interdisciplinary Analyses of Technology

Hermann Wätzig, Holger Zagst, Julia Kahle, Matthias Stein, Rebecca Wiesner, Mais Olabi, Anne Bormann, Ratih Ratih, Imke Oltmann-Norden and Christin Scheller



Thank you to

- Thanks to **Protein Simple** for providing their instruments and in particular Susanne Dörks, Udo Burger and Chris Heger from Protein Simple for the tremendous support.

- Special thanks to the CASSS committee for the Student Travel Grant for the possibility to participate in this amazing conference

- I would like to thank the state lower saxony for providing my fellowship „Gendered Configurations of Humans and Machines (KoMMa.G)“

Niedersächsisches Ministerium
für Wissenschaft und Kultur

CASSS
SHARING SCIENCE SOLUTIONS

proteinsimple
a bio-techne brand

KoMMa.G

GENDERED CONFIGURATIONS
OF HUMANS AND MACHINES
Interdisciplinary Analyses of Technology

Gendered Configurations of Humans and Machines (KoMMa.G)

Interdisciplinary Analyses of Technology

The program is organized by the Technische Universität Braunschweig and the Ostfalia University of Applied Sciences, in cooperation with the Braunschweig University of Art. 15 Principal Investigators from eight institutions have drawn up a program for up to 15 doctoral students. A number of associated researchers will cooperate, and up to five doctoral students may be associated to the program, if external financing can be obtained.

Scholarships offered: 15 Georg-Lichtenberg-grants for PhD students (1.500 EUR per month for up to 3 years), funded by the Ministry of Science and Education, Lower Saxony

Starting on: January 1st, 2017

Head of program: Prof. Dr.-Ing. Corinna Bath (Maria-Goeppert-Mayer chair for Gender, Technology and Mobility) and Prof. Dr. Bettina Wahrig (Department for the History of Science and Pharmacy).

Participating Institutions: TU Braunschweig: Institute for Flight Guidance, Department for the History of Science and Pharmacy, Institute for Communications Technology, Institute of Philosophy, Institute of Psychology, Institute of Steel Structures, Institute for Engineering Design, Ostfalia University of Applied Sciences: Faculties of Computer Science/IT, Social Affairs, Electrical Engineering and Mechanical Engineering and Braunschweig University of Art Institute of Media Studies.

https://www.tu-braunschweig.de/kommag