

Two-dimensional gelelectrophoresis (2-DE):

10,000 spots in a single gel as a tool for analysis of multi-component protein mixture, and proteomes

Dr. Hanns – Rüdiger Graack MBA

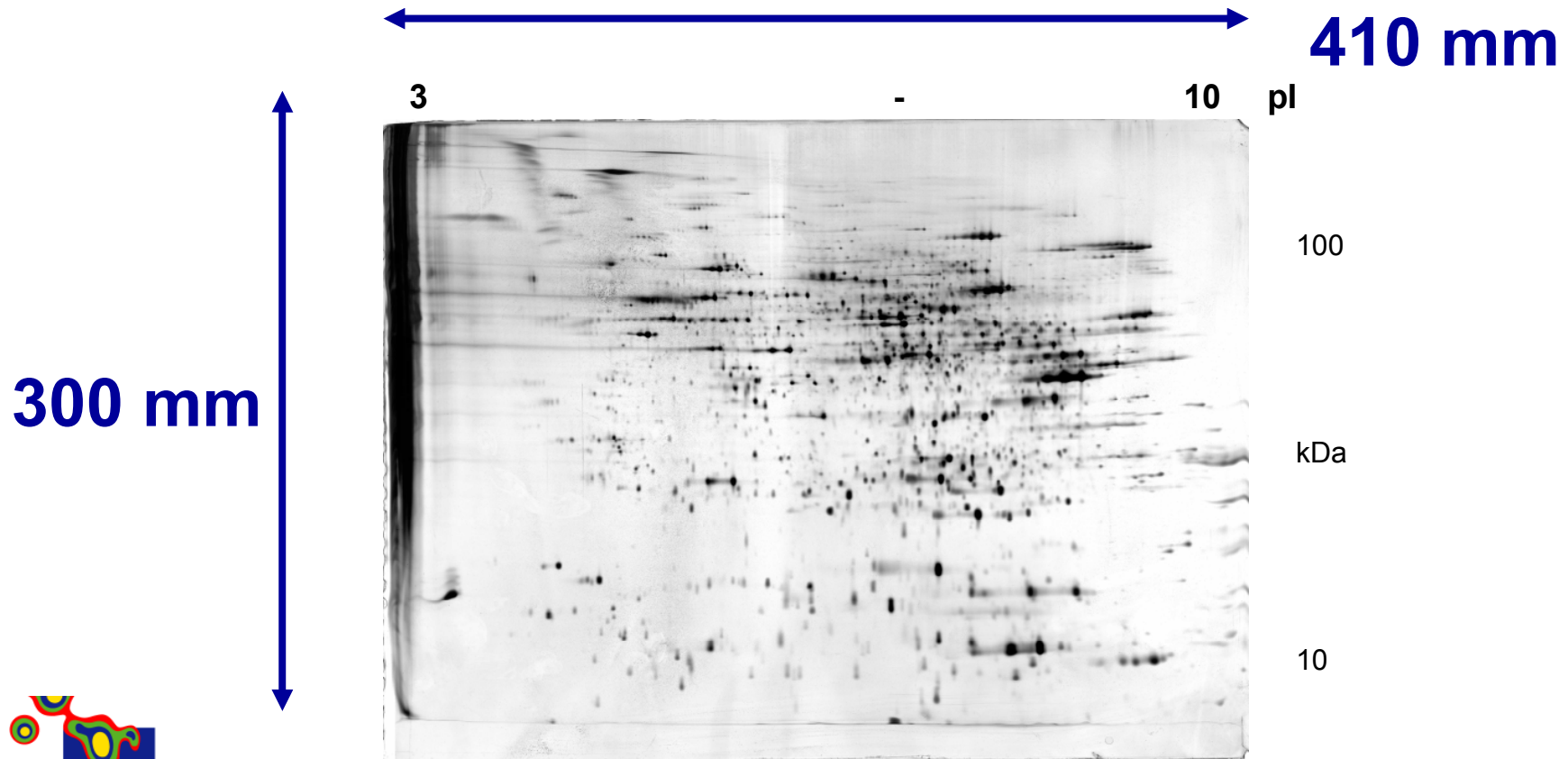
2011.5.10(火) 10:00–10:30 第61回日本電気泳動学会シンポジウム技術講演



WITA

WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules

WITA GmbH – Two dimensional gelelectrophoresis (2-DE)



WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules



神戸天然物化学株式会社

Two-dimensional gelelectrophoresis (2-DE):

**10,000 spots in a single gel as a tool
for analysis of multi-component
protein mixture, and proteomes**





WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

Hanns-Rüdiger Graack

**general manager of WITA GmbH, Berlin,
Germany**

in collaboration with KNC, Kobe



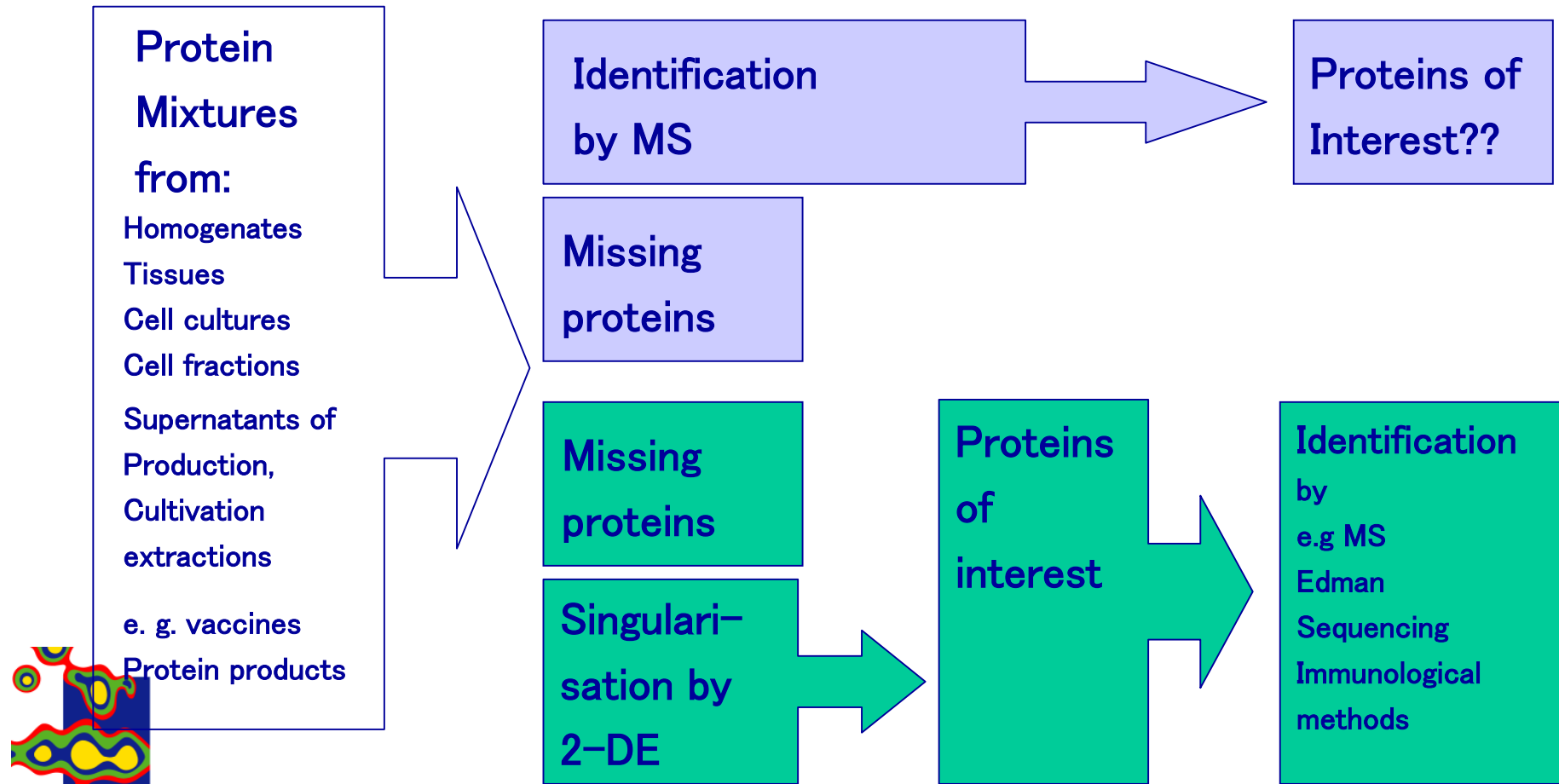
WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules



Why perform Two-dimensional gelelectrophoresis (2-DE)?

- (i) Qualitative and quantitative analysis of protein mixtures – singularisation of proteins - identification (MS, Edman, immunology)
- (ii) control of protein products - singularisation of proteins - identification (MS, Edman, immunology)

WITA GmbH – Two dimensional gelectrophoresis (2-DE)



WITA

WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules



神戸天然物化学株式会社

Singulari-
sation by
2-DE

Two different 2-DE
methods:

IPG – strips

NEPHGE

Non equilibrium pH gradient
electrophoresis

WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules

 神戸天然物化学株式会社



WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

IPG - NEPHGE what are the difference?



WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules



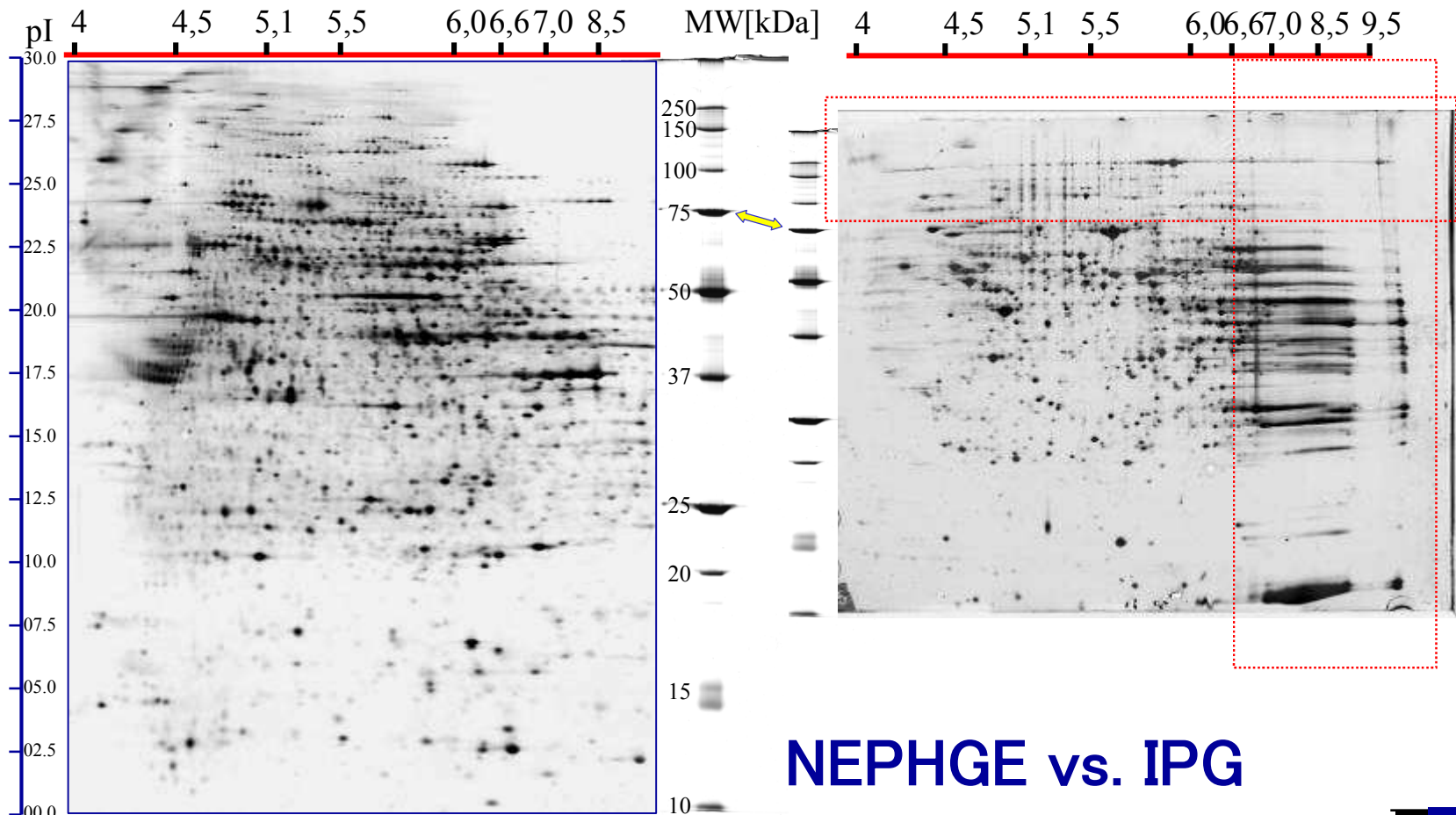
神戸天然物化学株式会社



WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

Sample:

Porcine cortex



WITA

WITA GmbH | Wittmann Institute o
Analysis of Biomolecules

NEPHGE vs. IPG

nd



神戸天然物化学株式会社

IPG

1st dimension separation according to IP of proteins. Fixed pH-gradients on pre-casted paper strips.

NEPHGE

1st dimension separation according to IP of proteins. Separation gradient within the tubing gel is forming by soluble ampholytes during electrophoresis.



IPG

In the 1st dimension due to physical limitations no separation above 24 cm in length is possible.

NEPHGE

1st dimension separation standard sizes 24 and 41 cm. Larger sizes are possible in principle.



IPG

Endpoint method. 1st dimension electrophoresis is terminated **AFTER** proteins reached their IP and point of minimal solubility: Many proteins precipitate; substantial losses of proteins from 1st to 2nd dimension of gel.

NEPHGE

NO endpoint method. 1st dimension electrophoresis is terminated **BEFORE** proteins reach their IP and point of minimal solubility: Proteins stay in solution, ready to enter 2nd dimension gel.

Staining of proteins by Coomassie, silver, fluorescent dyes

IPG **DIGE is possible**

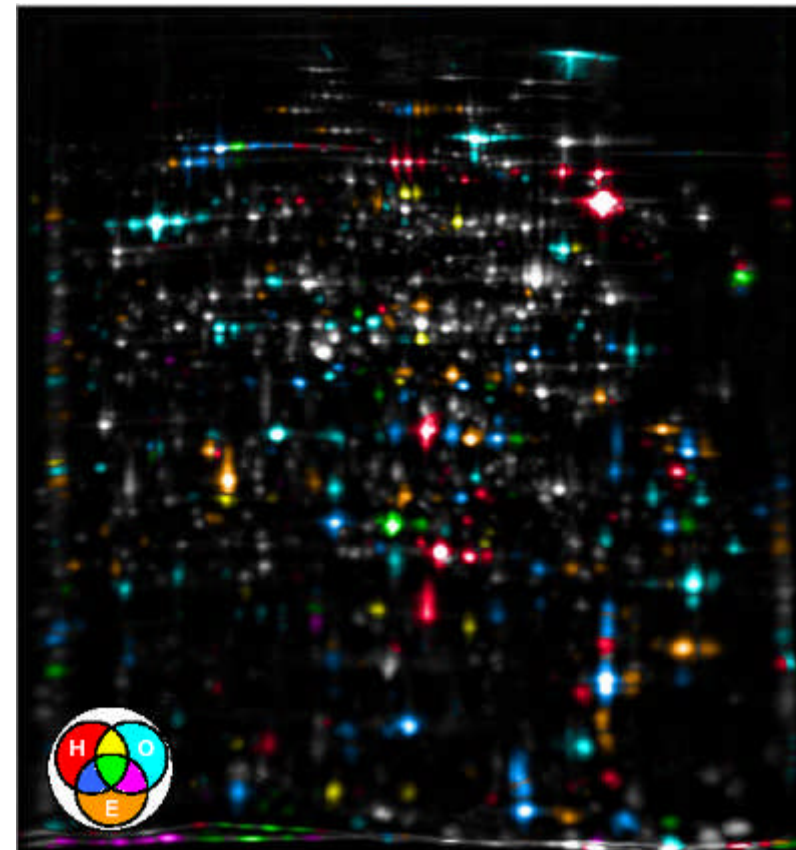
NEPHGE **DIGE is possible**

WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

pI 3 → 10

100 kDa

size ↓



10 kDa

Bacillus sp.

NEPHGE

3fold DIGE staining



WITA GmbH | Wittmann Institute of Technology and Analysis of Biomolecules

神戸天然物化学株式会社

IPG

maximal gel size 24 x 18 cm commercially available

In combination with the WITA_{VISION} 2nd dimension apparatus gel size of 24 x 30 cm possible

NEPHGE

standardized gel sizes 30 x 24 cm and 30 x 41 cm





WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

IPG No more than 2.000 spots per gel.

NEPHGE up to 10.000 individual spots per gel, suitable for MS identification of selected proteins

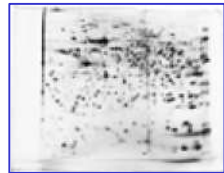


WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

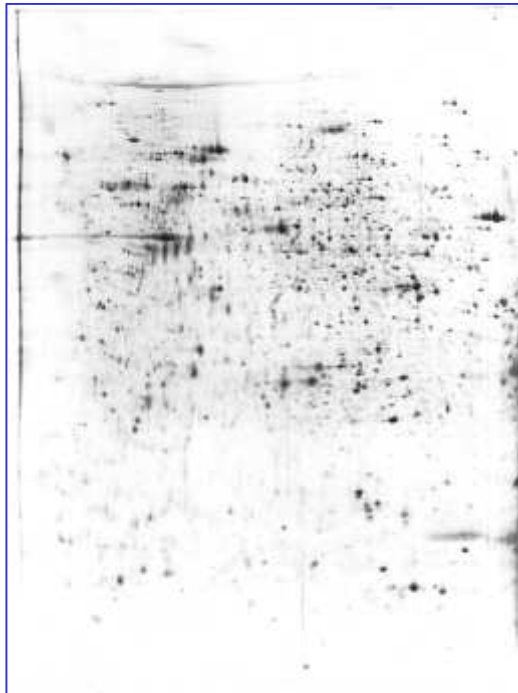
Comparison of different gel sizes for NEPHGE 2-DE

Murine Ovar

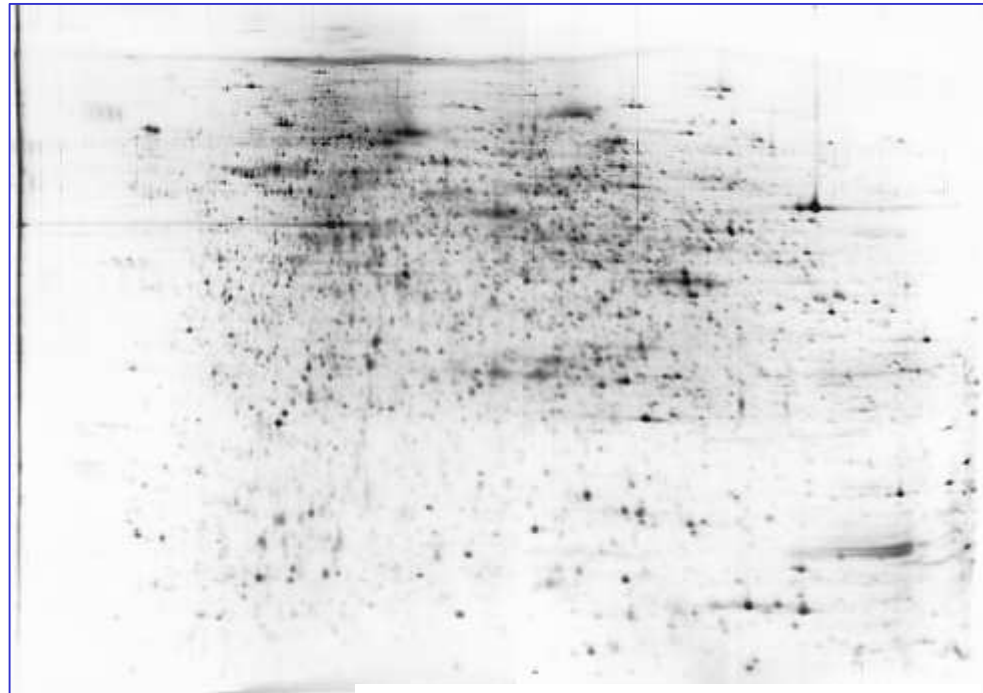
SGT 8 x 7 cm



LGT 24 x 30 cm



GGT 41 x 30 cm



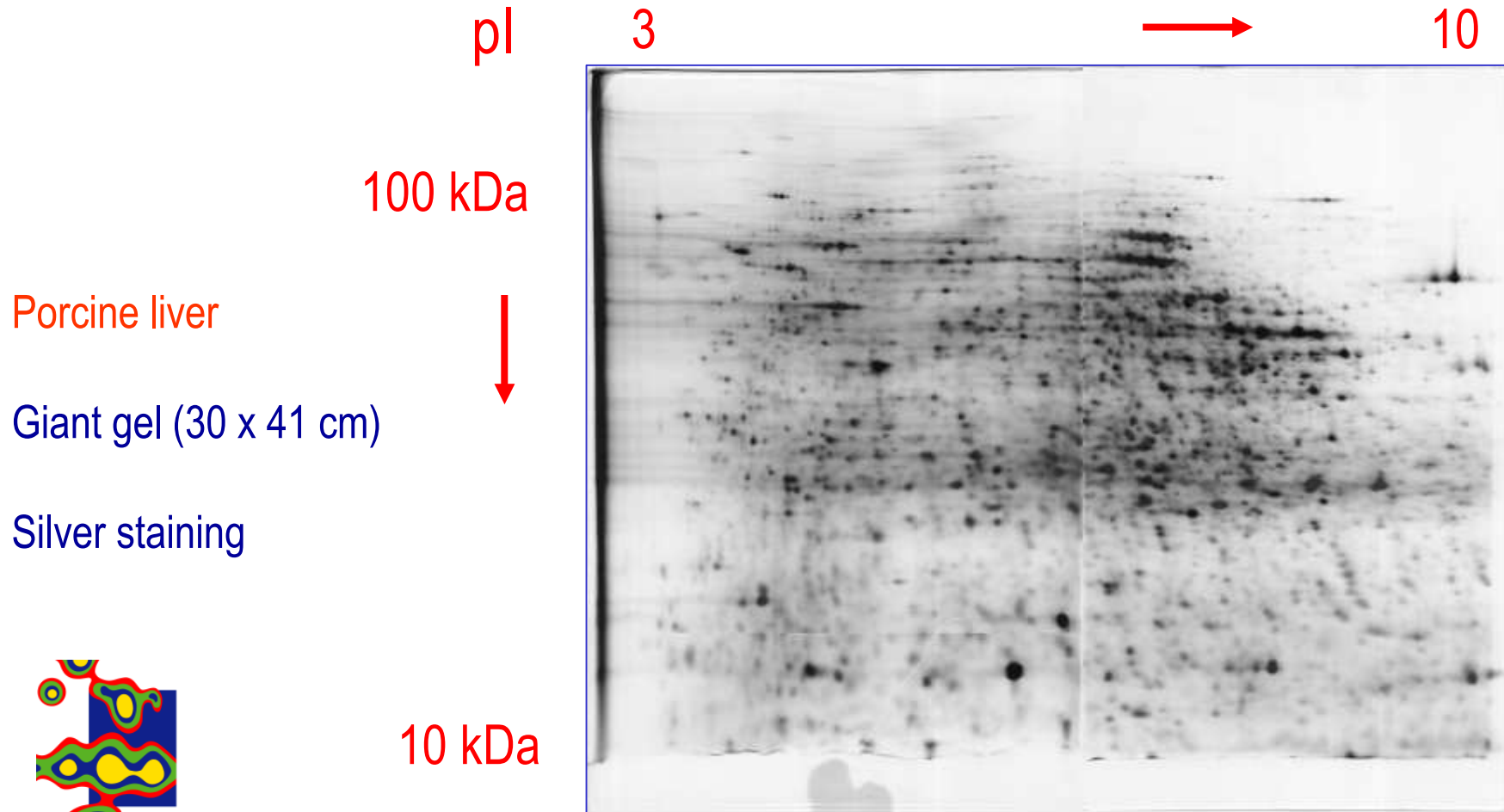
WITA

WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules



神戸天然物化学株式会社

WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

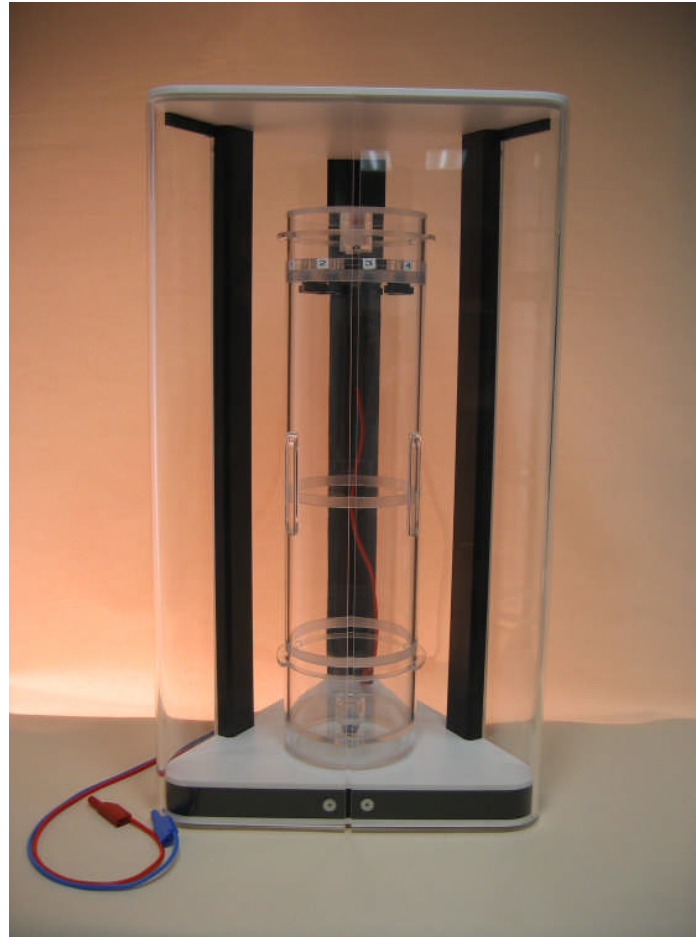


WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules

神戸天然物化学株式会社

WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

WITA VISION



WITA_g 1D

1-DE chamber

Safety cover

Gel casting stand

Gel transfer modules



WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules

 神戸天然物化学株式会社

WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

Advantages of WITA_{VISION} 1-DE chamber

- Processing of 10 simultaneous 1-D gels
- Security cover with electric protection
- Storage of processed and re-buffered 1-D gels on the gel transfer modules at $-80\text{ }^{\circ}\text{C}$



WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

WITA_{VISION}

WITA_i2D

2-DE “Pentapack”
Chamber

Either

225 x 300 mm 2D

Or

410 x 300 mm 2D



WITA

WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules

 神戸天然物化学株式会社



Advantages of WITA_{VISION} 2-DE chamber



- modular construction: processing of up to 5 2-D gels per “5pack” simultaneously
- reduction of buffer consumption to less than 0.5 L per gel
- reduction of running time to less than 4 h per gel
- simple handling: gels are casted with in the 2-D chamber and remain manually untouched up to the end of electrophoresis



WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

WITAvision is a complete set of equipment and chemicals for the performance of the best 2-DE ever possible:



+



=



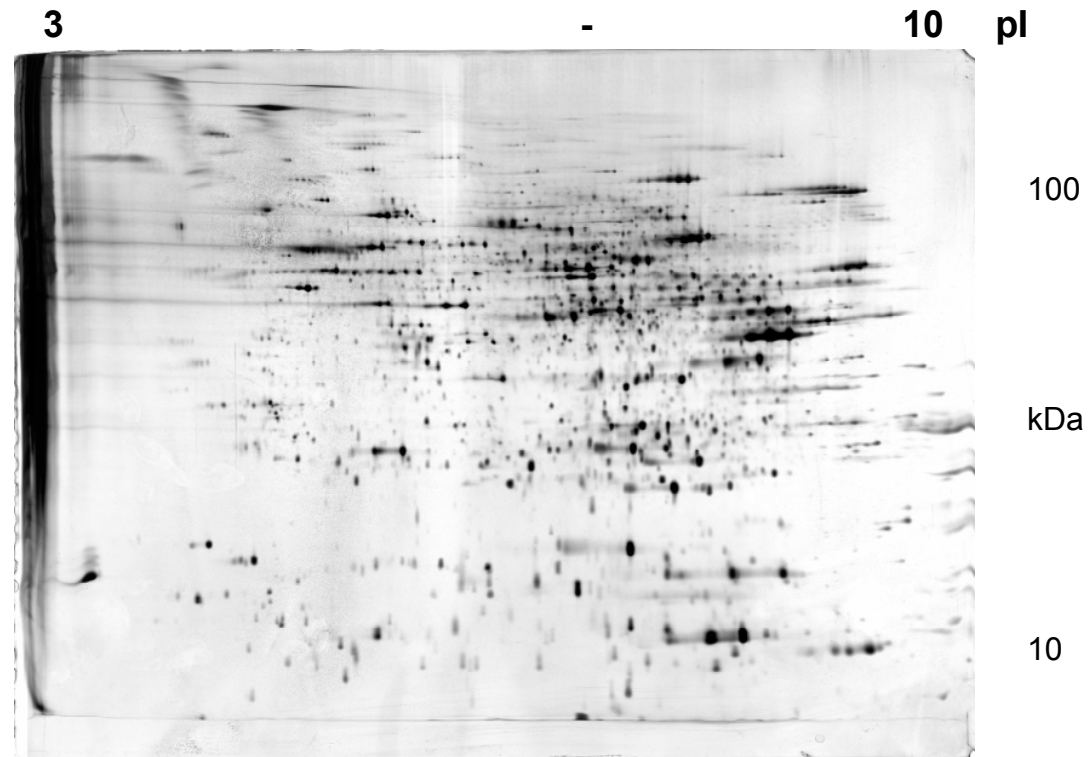
WITA

WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules



神戸天然物化学株式会社

WITA GmbH – Two dimensional gelelectrophoresis (2-DE)



results in the highest resolution

WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules

 神戸天然物化学株式会社



Crucial points for optimal high resolution 2-DE

- Strict concept for the proteomic project
- well trained staff
- Standardisation of sample collection and sample preparation procedure
- Standardisation of the 2-DE procedures
- Standardisation of the chemicals used by batchwise preparation
- optimal equipment for 1-DE and 2-DE
- MS compatible staining and analysis of 2-DE patterns for further analysis of interesting proteins



How to perform NEPHGE 2-DE?:

- prepare sample(s)
- perform 1st dimensional tubing gels
- perform 2nd dimension 2D-PAGE
- stain 2D gel(s)
- scan 2D gel(s) (410 x 300 mm fluorescent gels are suitable for Taifun scanners, e. g.)
- analyse spot pattern electronical
- identify protein(s) of interest by MS, Edman, immunologically





WITA GmbH – Two dimensional gelelectrophoresis (2-DE)

**Thank you for your
attention**

WITA GmbH, KNC Laboratories Co., Ltd.

Teltow, Germany / Kobe, Japan



WITA GmbH | Wittmann Institute of Technology and
Analysis of Biomolecules



神戸天然物化学株式会社