CSB Techniques Workshop
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Imperial College London

Blue-Native PAGE

A tool to analyse membrane protein complexes

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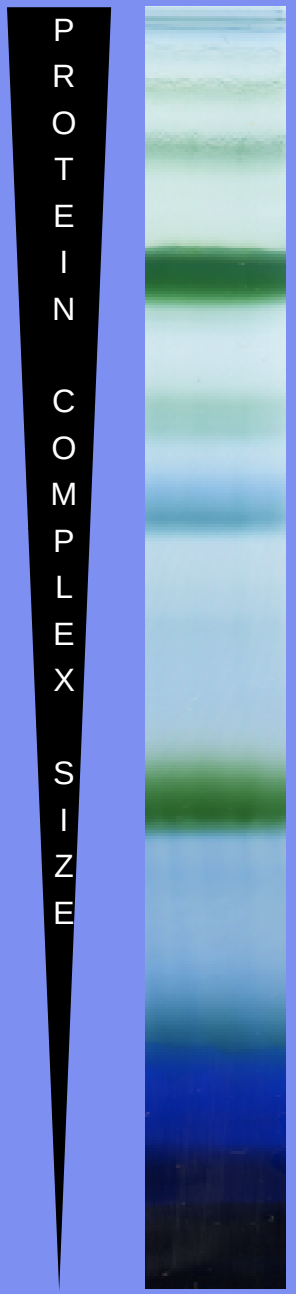
Introduction

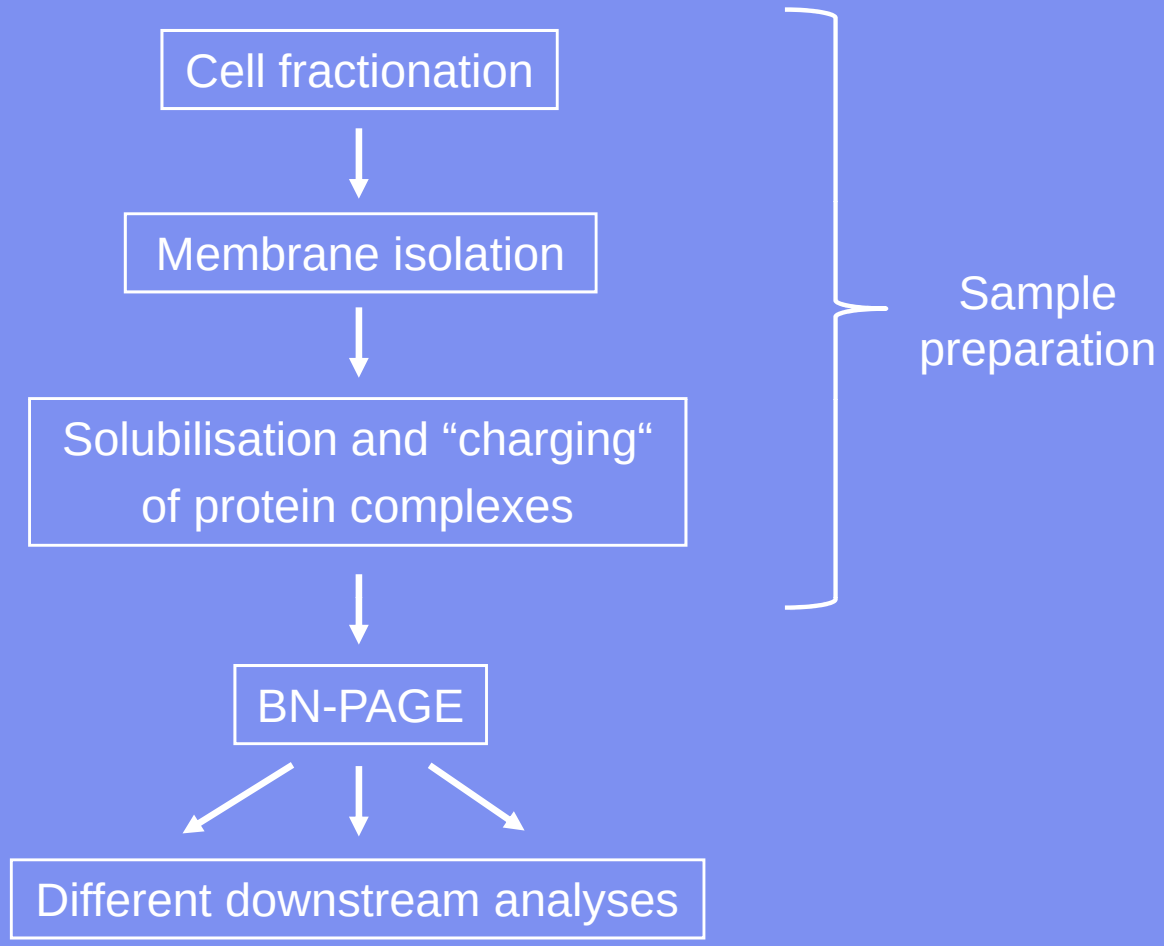
- Blue-Native (BN) PAGE was developed for the separation of mitochondrial membrane proteins and their complexes in the mass range of 10 to 10,000 kDa.

- Samples are solubilised using mild (non-ionic) detergents and protein complexes are charged with Coomassie Blue G250 prior to electrophoresis.

- BN PAGE gel strips or individual protein complex bands can be used for different downstream analyses.



Workflow for BN PAGE



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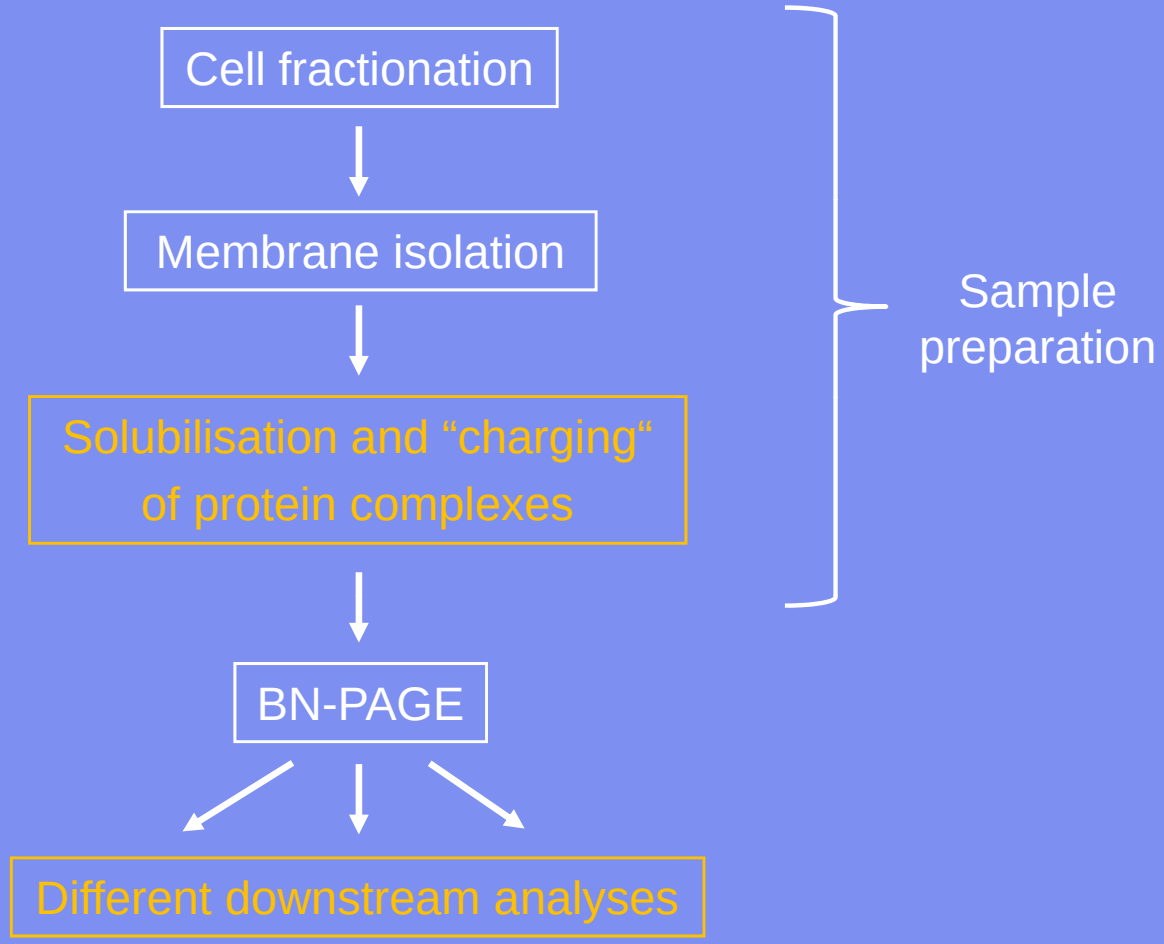
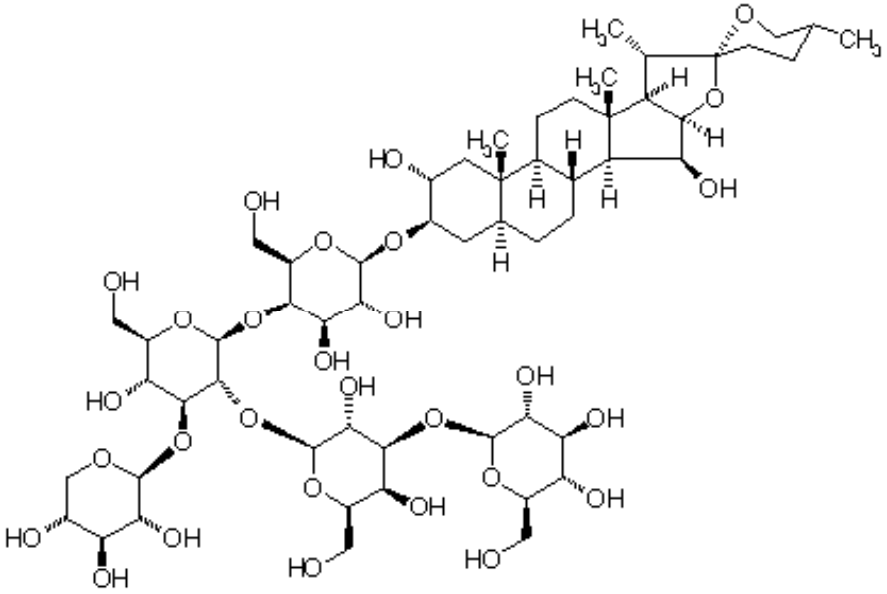


Solubilisation of protein complexes I

- Detergents used for BN PAGE -

Digitonin

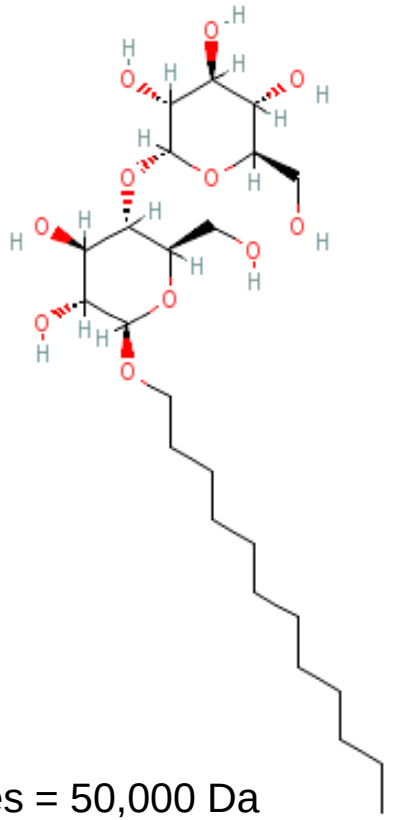
mildest detergent



- non-ionic
- MW micelles = 70,000 Da
- CMC = 0.031 %(w/v) or 0.25 mM
- $C_{56}H_{92}O_{29}$, MW = 1229.31 g/mol

Dodecyl-ß-D-maltoside (ß-DM)

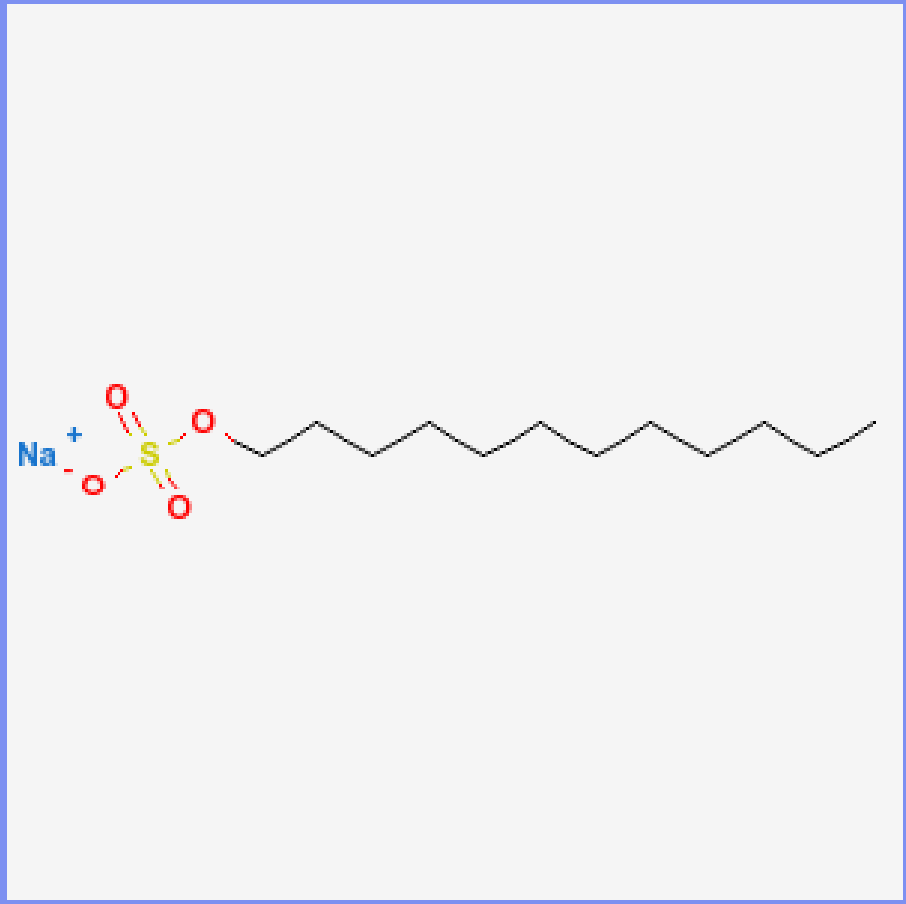
more delipidating than digitonin



- non-ionic
- MW micelles = 50,000 Da
- CMC = 0.008 %(w/v) or 0.16 mM
- $C_{24}H_{46}O_{11}$, MW = 510.62 g/mol

adapted from Reisinger and Eichacker 2006, 2007

Masking of protein charges



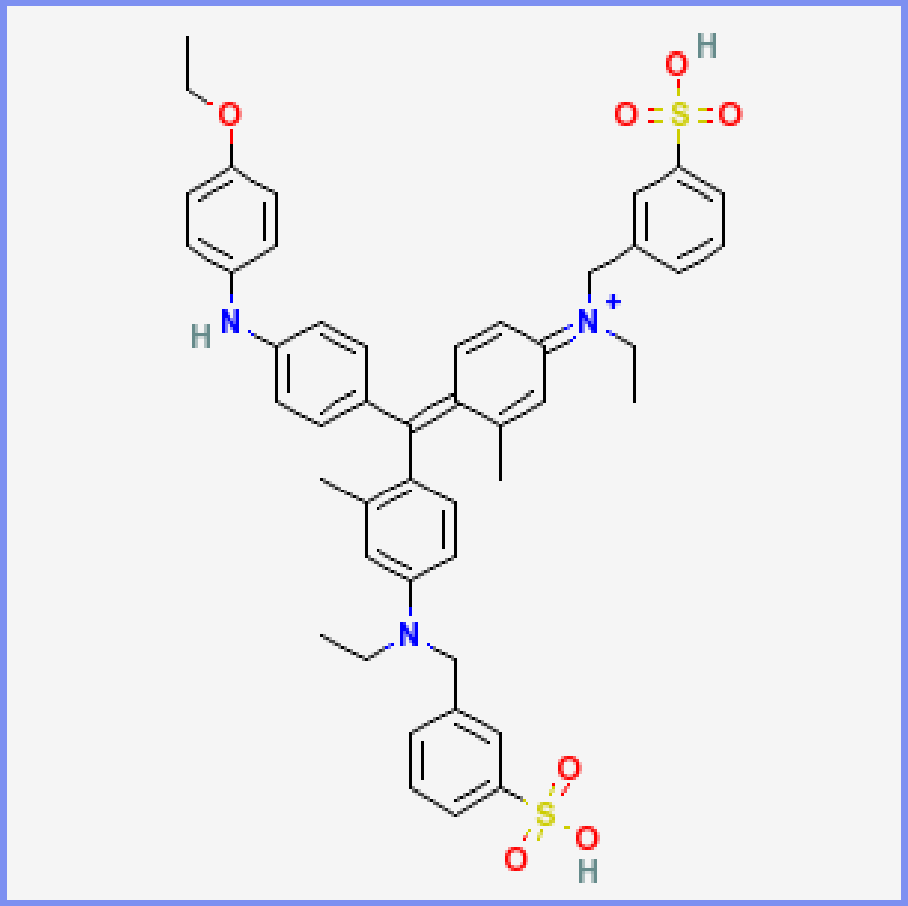
SDS

MW = 288.38 g/mol

for SDS-PAGE

adapted from Reisinger and Eichacker 2006, 2007

Masking of protein charges



Coomassie Blue G250

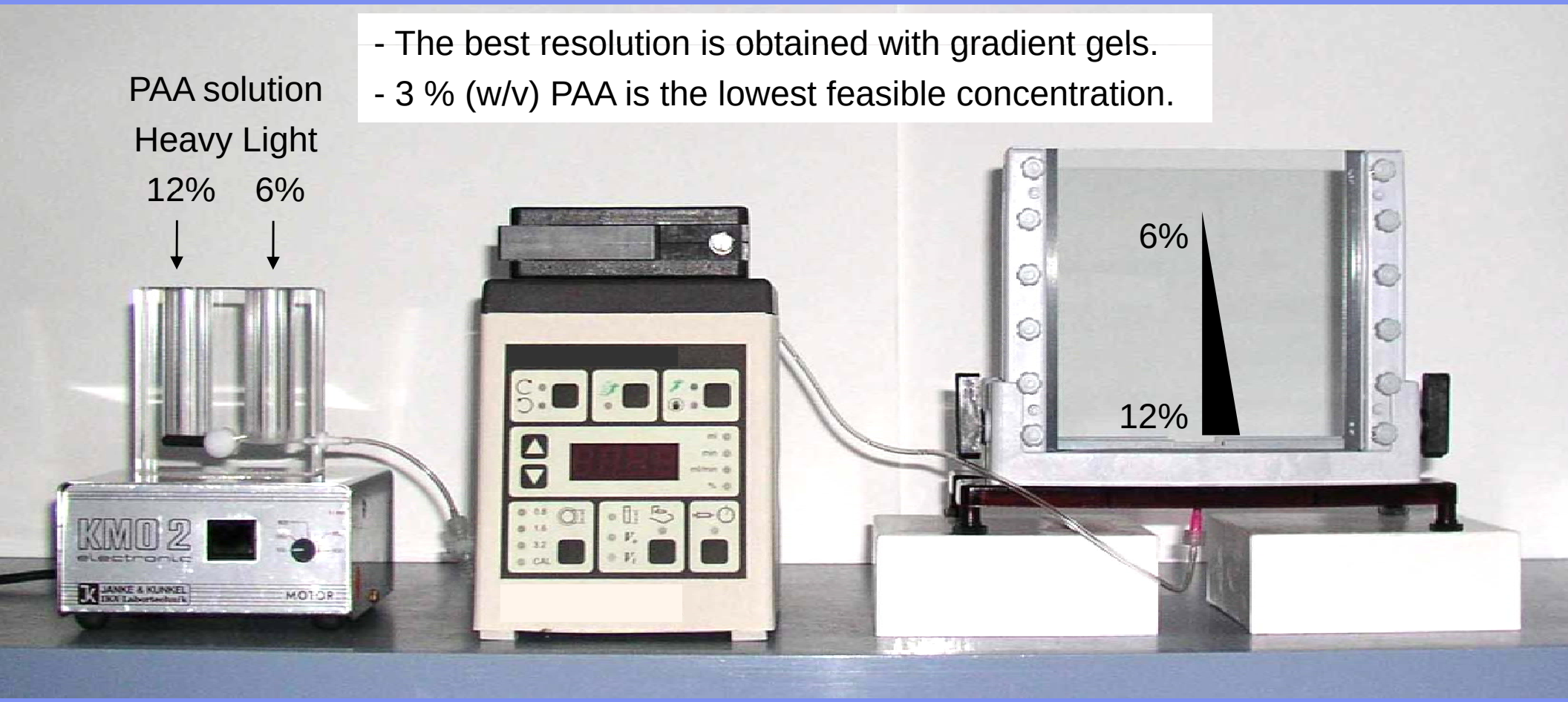
MW = 833.05 g/mol

for BN PAGE

adapted from Reisinger and Eichacker 2006, 2007

Casting a BN PAGE gradient gel

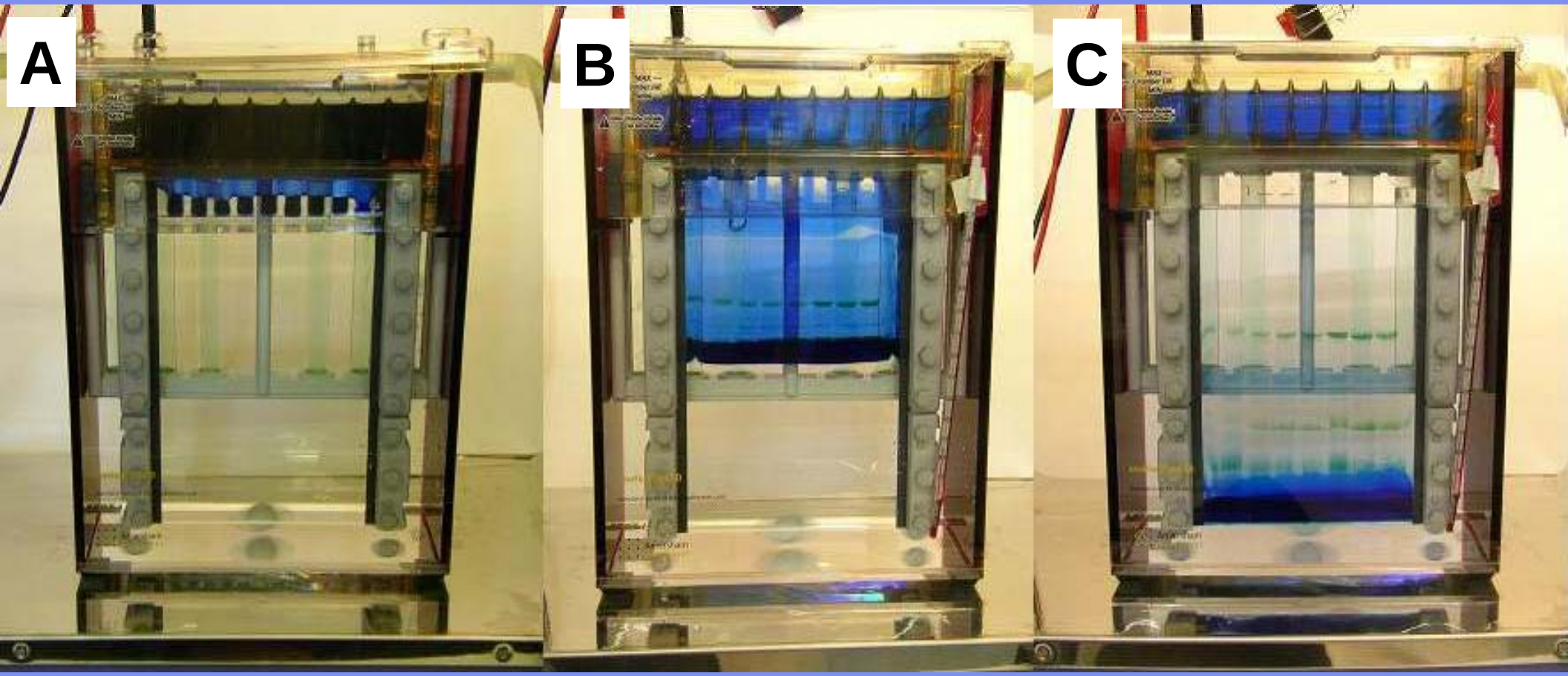
- The best resolution is obtained with gradient gels.
- 3 % (w/v) PAA is the lowest feasible concentration.



gradient mixer | peristaltic pump | gel setting apparatus

adapted from Reisinger and Eichacker 2006, 2007

A typical BN PAGE run



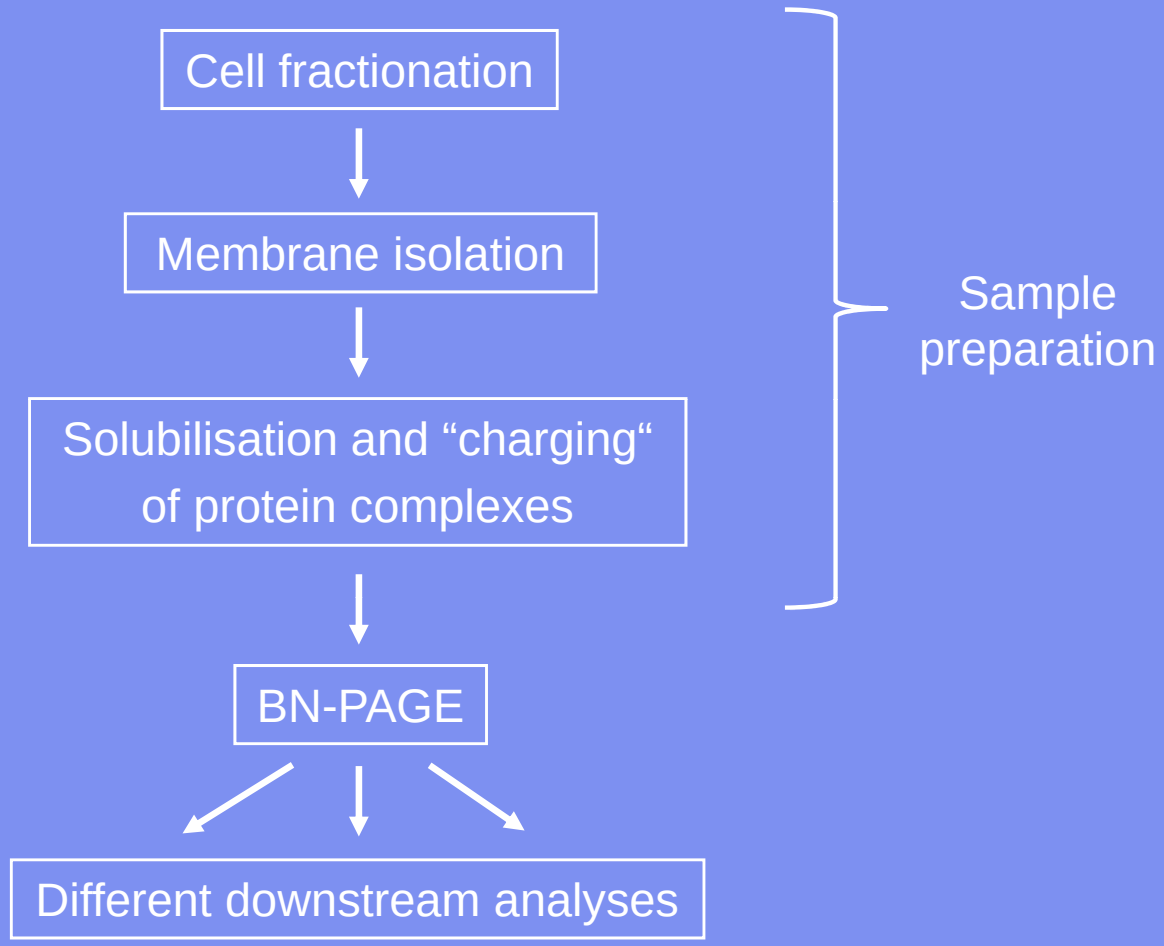
A – Sample loading

B – Buffer exchange

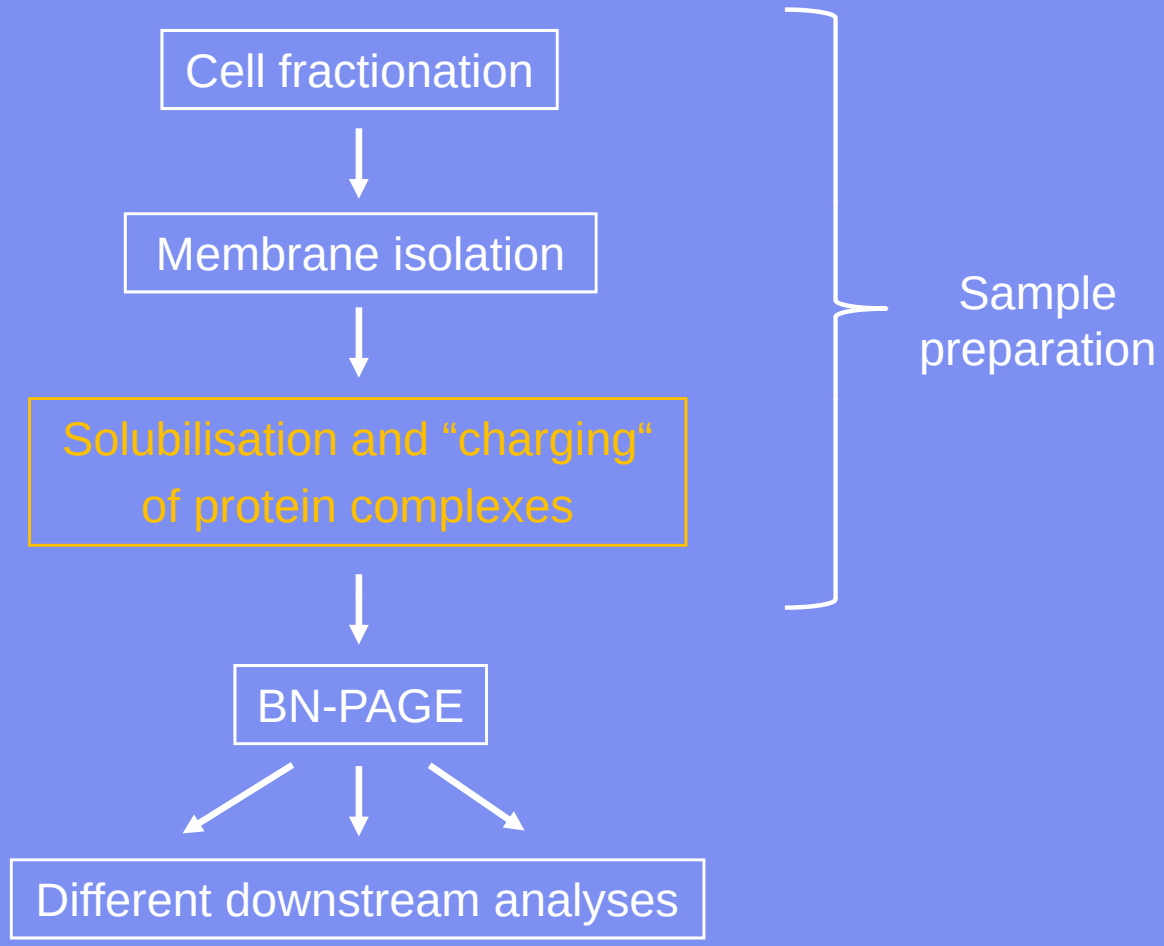
C – End of run

adapted from Reisinger and Eichacker 2006, 2007

Workflow for BN PAGE

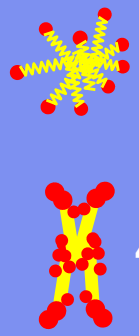


Workflow for BN PAGE



Solubilisation of protein complexes II

- Effect of detergent concentration I -



1.1 mM ß-DM

or

4.5 mM Digitonin

4.5 mM ß-DM

or

9 mM Digitonin

9 mM ß-DM

or

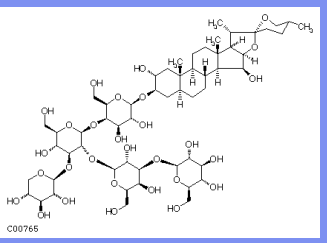
18 mM Digitonin

Concentration of detergent micelles

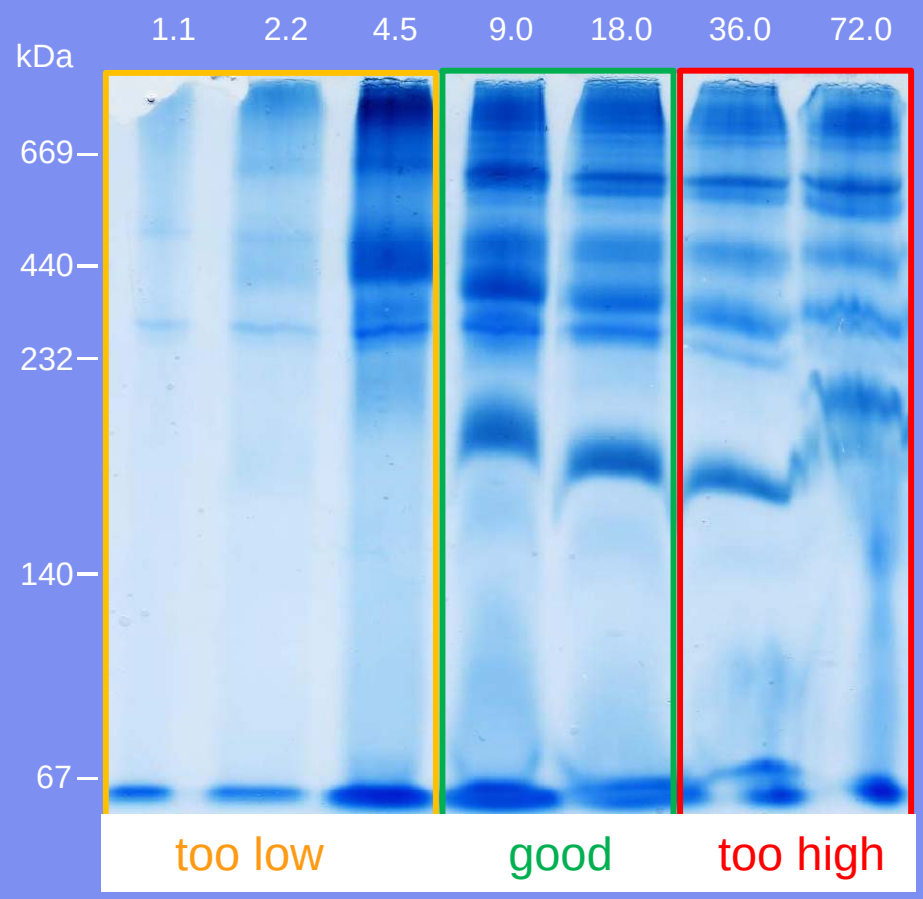
Molecular mass of protein complexes

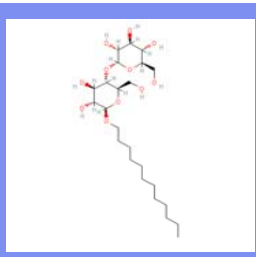
adapted from Reisinger and Eichacker 2006, 2007

Optimisation of solubilisation conditions

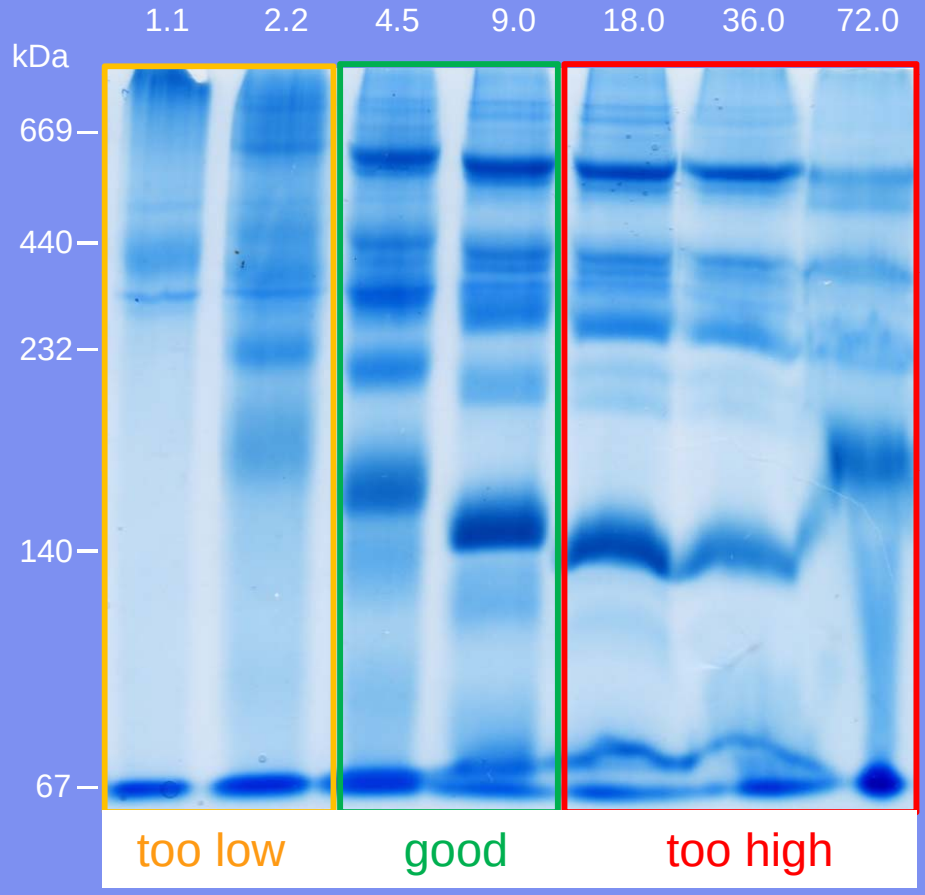


Digitonin (mmol)



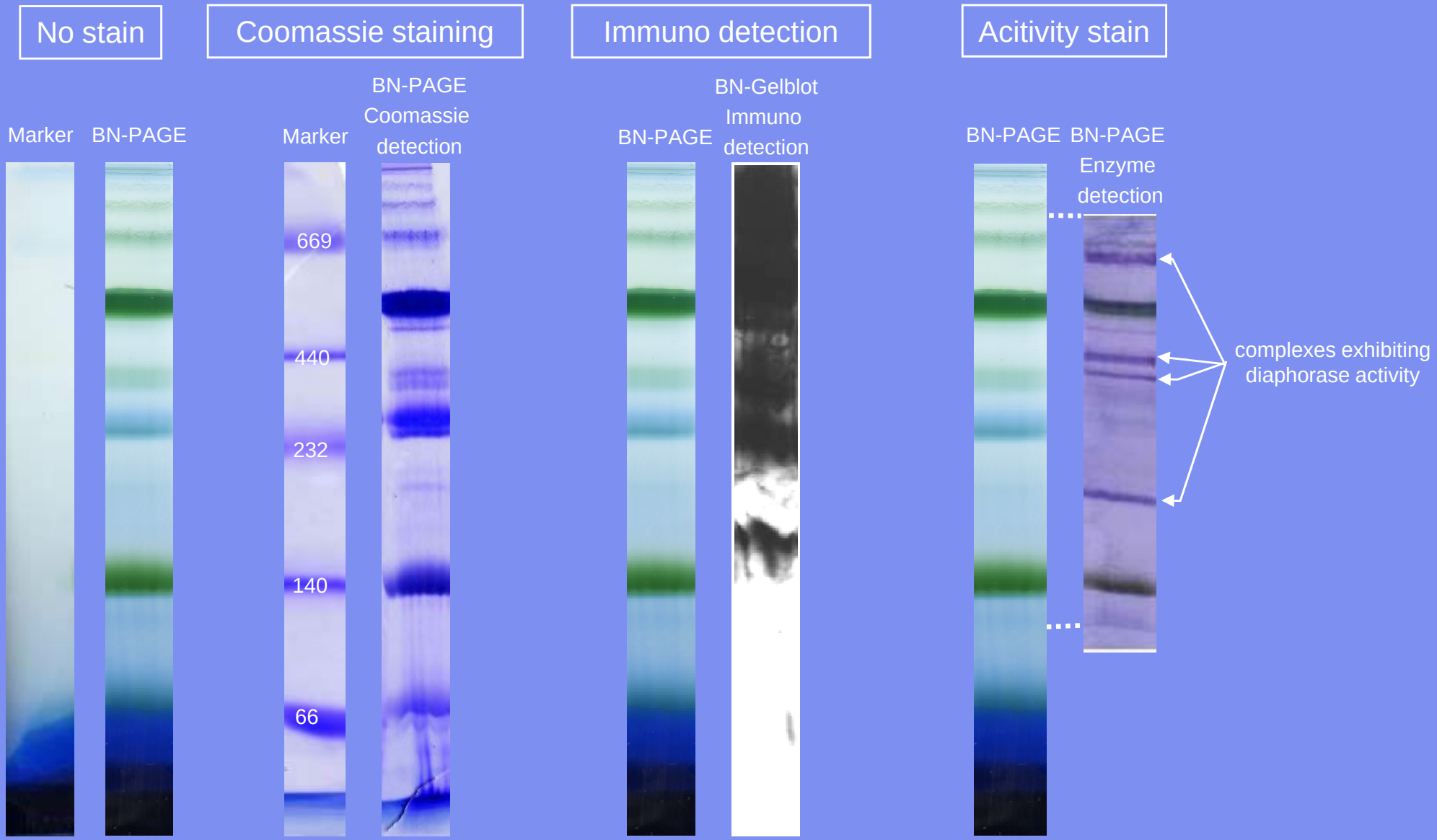


ß-DM (mmol)



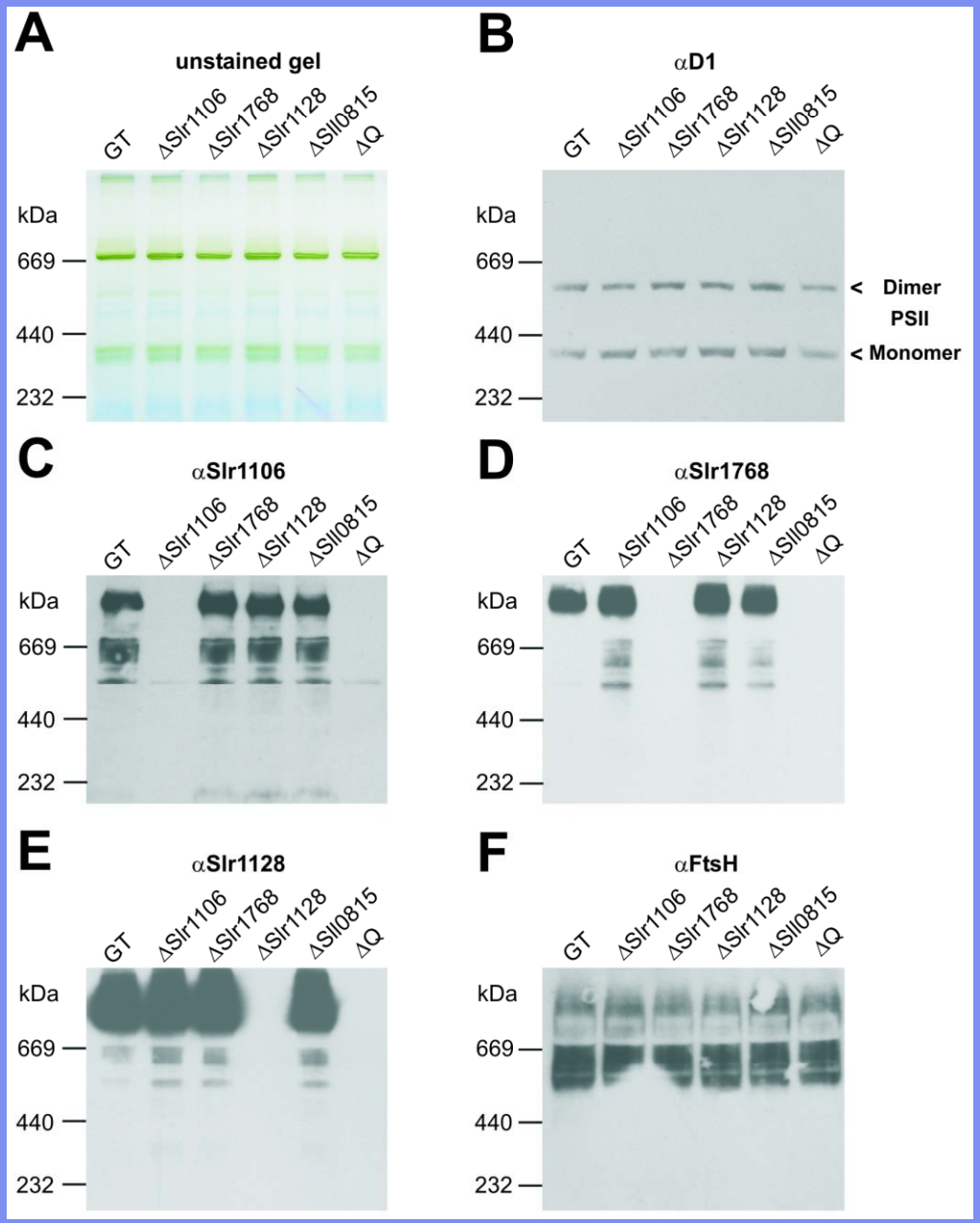
adapted from Reisinger and Eichacker 2006, 2007

Detection of protein complexes after BN PAGE



adapted from Reisinger and Eichacker 2006, 2007

Screening of multiple gene inactivation mutants using BN PAGE and immunoblotting

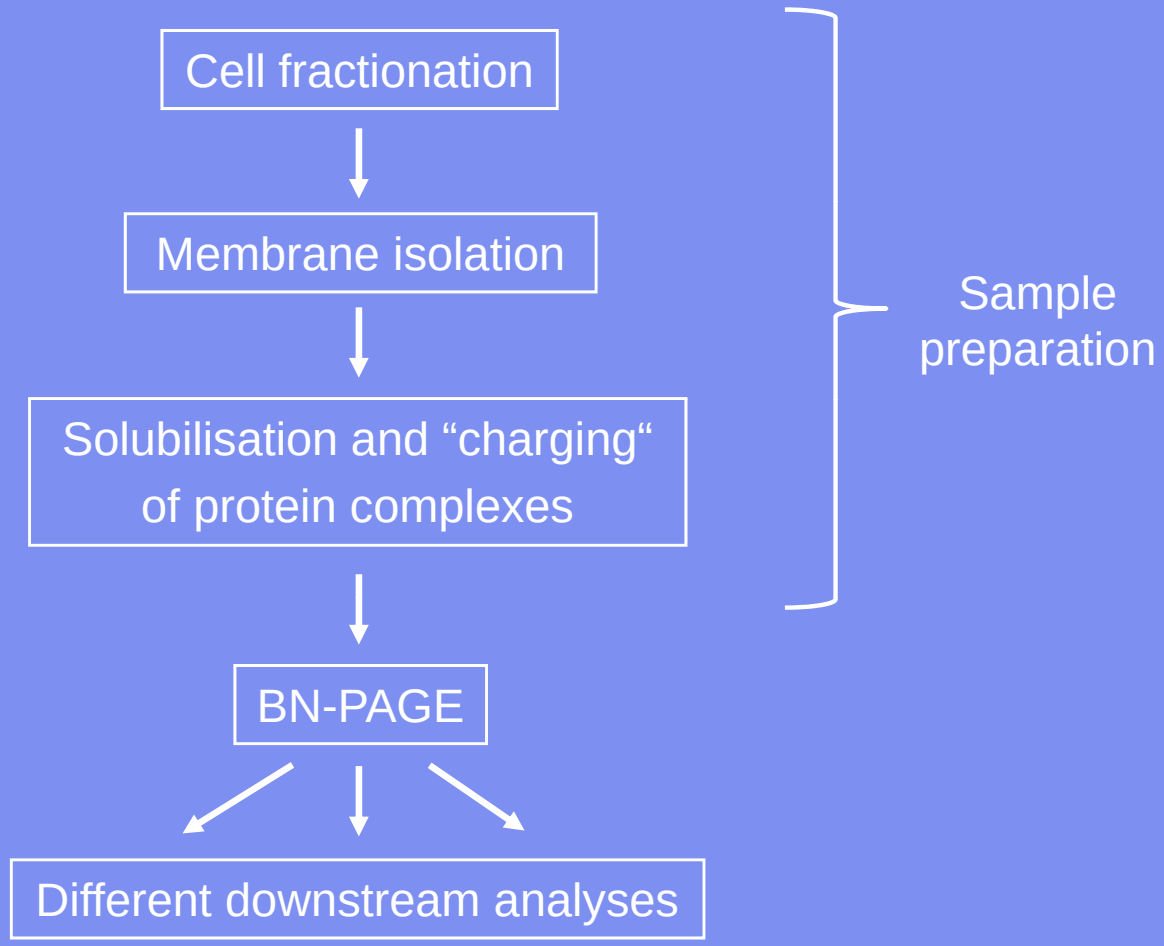


- Thylakoid extracts of multiple cyanobacterial gene inactivation mutants were separated by BN PAGE and analysed by immunoblotting with specific antibodies.

- Different protein complexes were identified as assembly or disassembly intermediates.

- The proteins were shown not to form interdependent complexes with each other.

Workflow for BN PAGE



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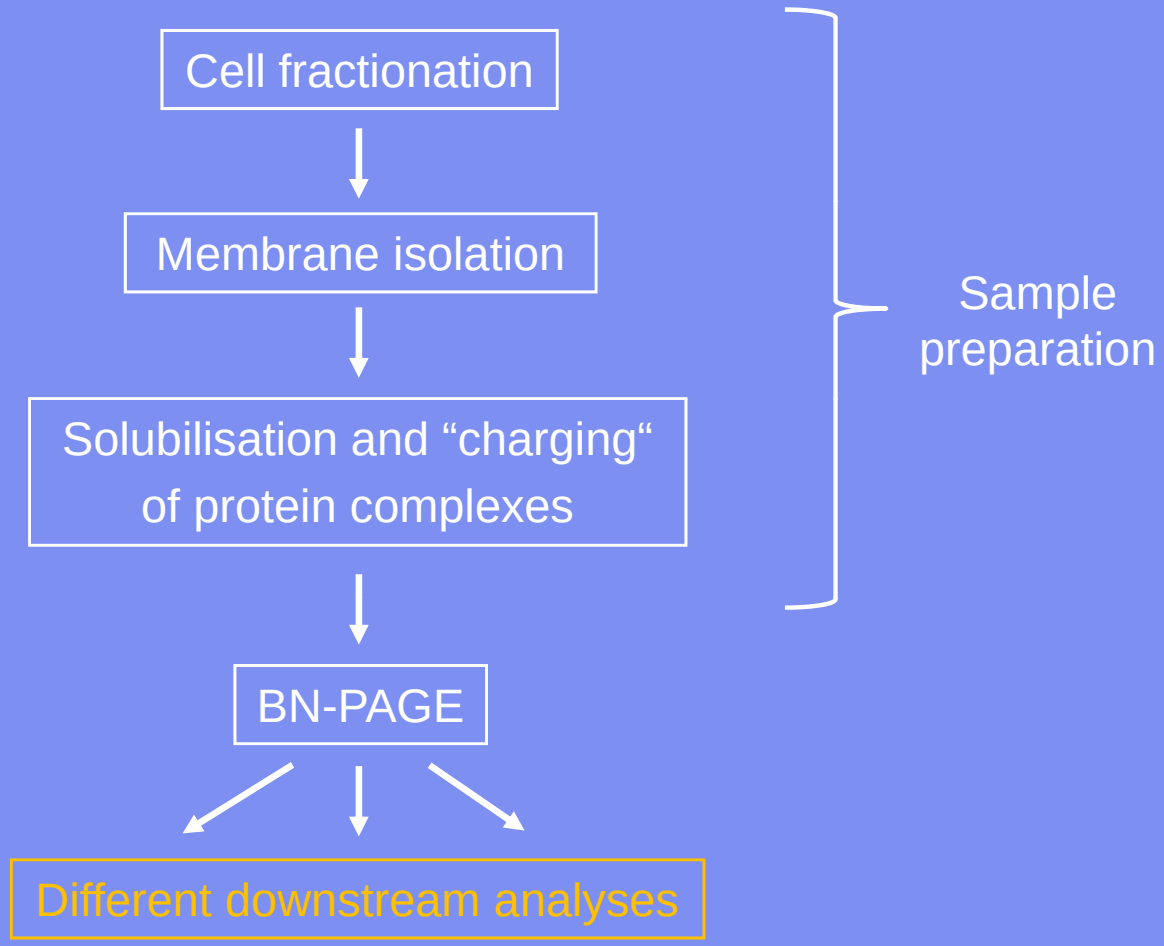

Downstream analyses after BN PAGE

- Staining of the gel (Coomassie or silver stain)
- Immunodetection of proteins in complexes or as free protein
- Activity assays

- Elution of protein complexes or proteins for
    - 2D crystallisation
    - Electron microscopy and single particle analysis
- 2D BN/SDS PAGE

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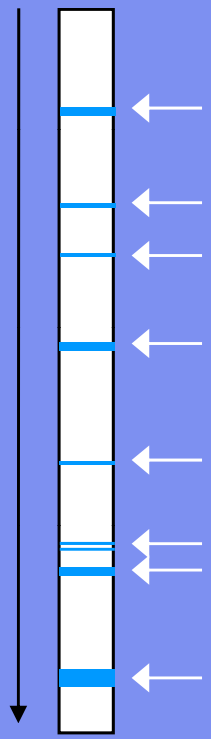
- 2D BN/SDS PAGE

BN PAGE in conjunction with SDS PAGE can give insights on protein complex composition and protein-protein interactions.

Principle of 2D BN/SDS-PAGE I

- 1st dimension -



Protein complexes

are separated

according to

their sizes using

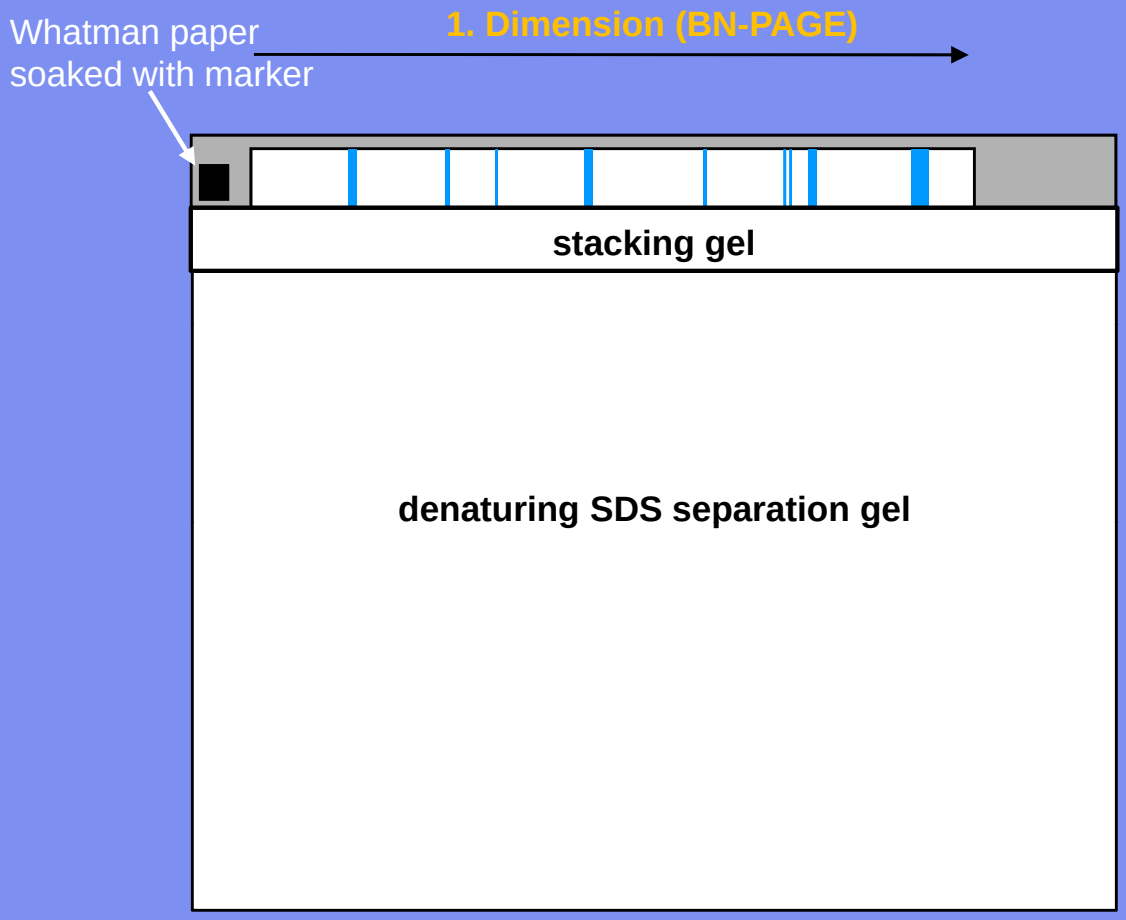
BN PAGE

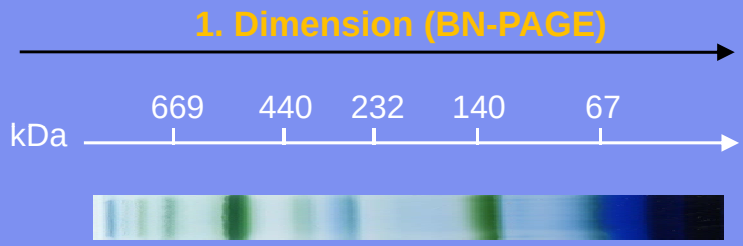
adapted from Reisinger and Eichacker 2006, 2007

Principle of 2D BN/SDS-PAGE II

- 2nd dimension -

A BN PAGE gel strip is denatured and
placed on top of a second dimension SDS PAGE gel



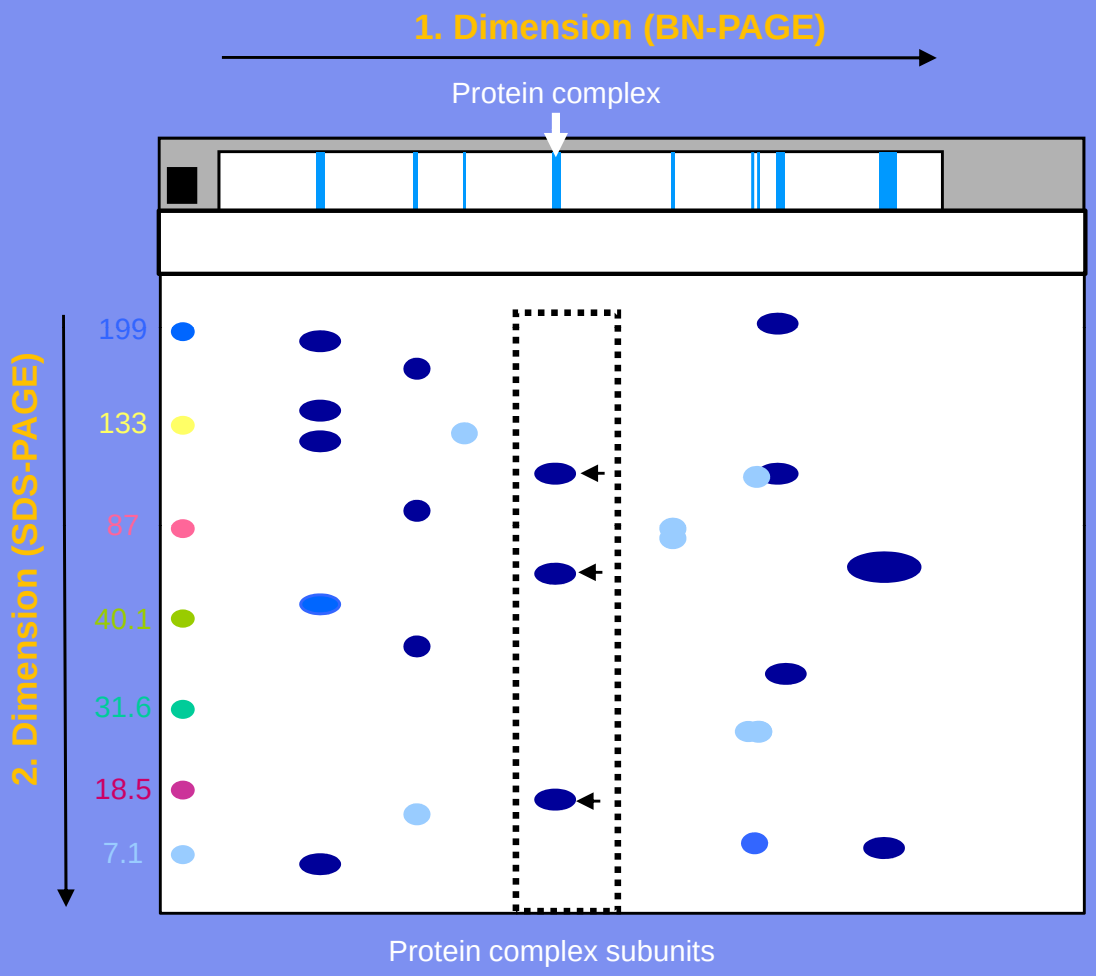


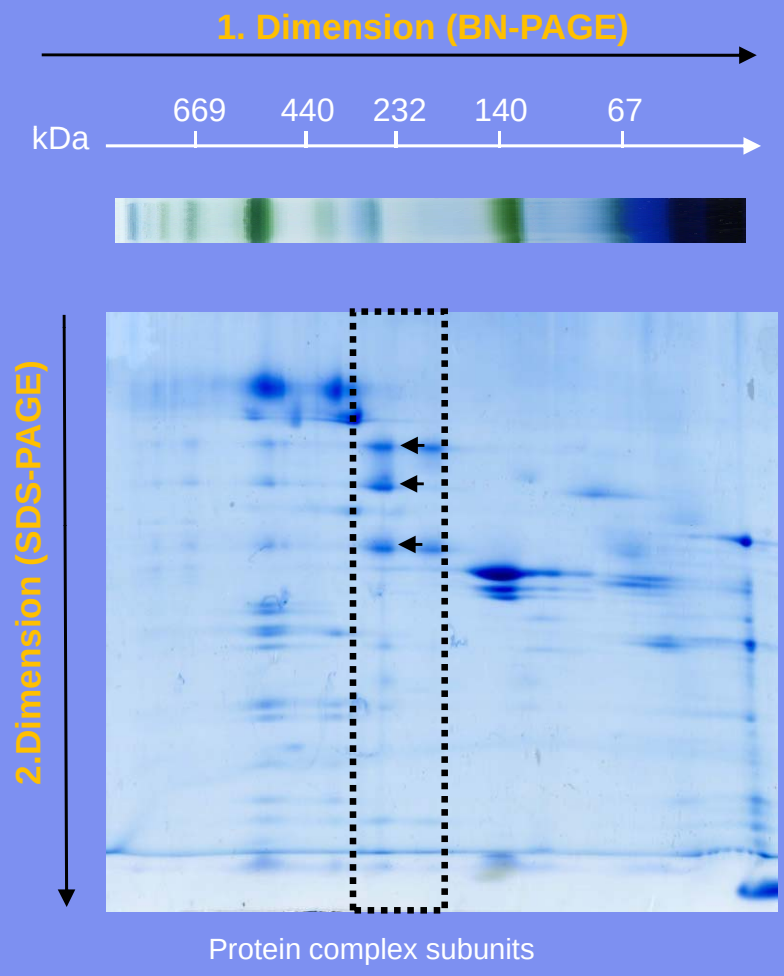
adapted from Reisinger and Eichacker 2006, 2007

Principle of 2D BN/SDS-PAGE III

- 2nd dimension -

In the second dimension the protein complexes
are separated into their individual subunits.





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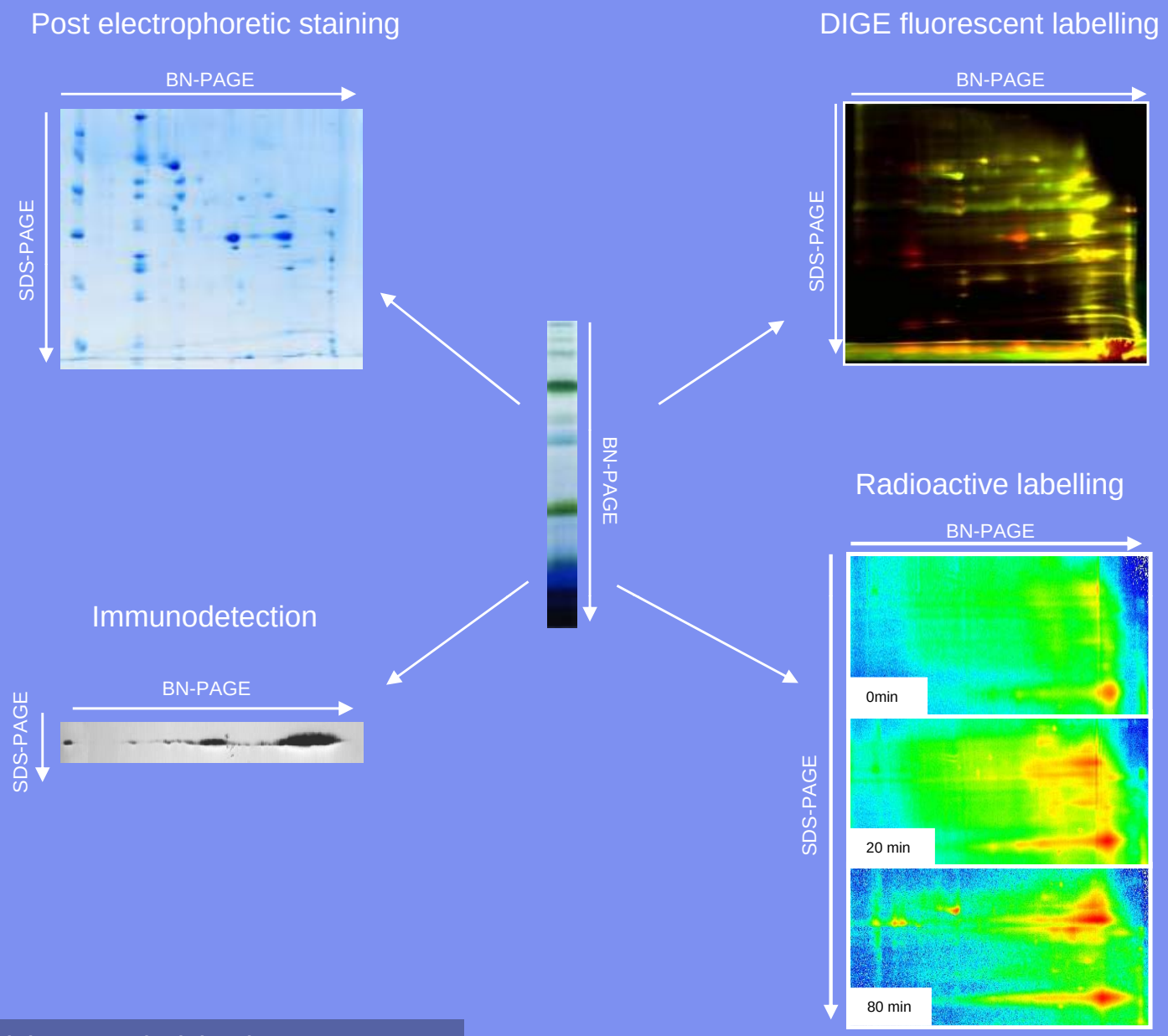
Detection methods for 2D BN-/SDS-PAGE



adapted from Reisinger and Eichacker 2006, 2007

References

Original papers:

Schägger, H., and von Jagow, G. 1991. Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. Analytical Biochemistry **199:**223-31

Schägger, H., Cramer, W. A., and von Jagow, G. 1994. Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane protein complexes by two-dimensional native electrophoresis. Analytical Biochemistry **217:**220-30

Good introductory and practical guides:

Reisinger, V., and Eichacker L. A. 2006. Analysis of membrane protein complexes by blue native PAGE. Proteomics **6 Suppl 2:**6-15

Reisinger, V., and Eichacker L. A. 2007. How to analyze protein complexes by 2D blue native SDS-PAGE. Proteomics **7 Suppl 1:**6-16

Wittig, I., Braun, H.-P., and Schägger H. 2006. Blue native PAGE. Nature Protocols **1:**418-28

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