Discontinuous native protein gel electrophoresis

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Niepmann and Zheng, Electrophoresis (2006)

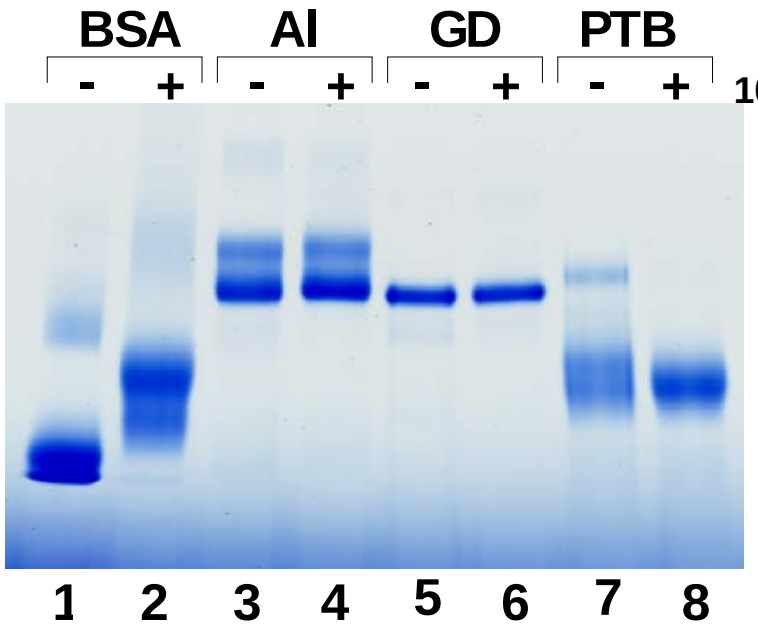
Summary

We have developed a discontinuous native protein gel electrophoresis system that allows the separation of even basic proteins according to their size, oligomeric state and shape. This gel system combines the addition of negative charges to the proteins by Serva Blue G with a discontinuous buffer system and gradient gels. As in SDS-PAGE, chloride constitutes the high mobility anion in the gel and anode buffer. However, for sample focusing this system employs histidine instead of glycine as slow dipolar ion following from the cathode buffer to improve migration of basic proteins. In addition, proteins run into gel pores corresponding to their size and shape in the gradient gel.

In this presentation, we

1. show some example gels with oligomeric proteins
2. explain the principle of function of the gel system (in comparison to the well-known Laemmli system)
3. show calibration curves
4. detail the unusual migration behavior of BSA under reducing vs. non-reducing conditions.

Discontinuous native protein gel electrophoresis

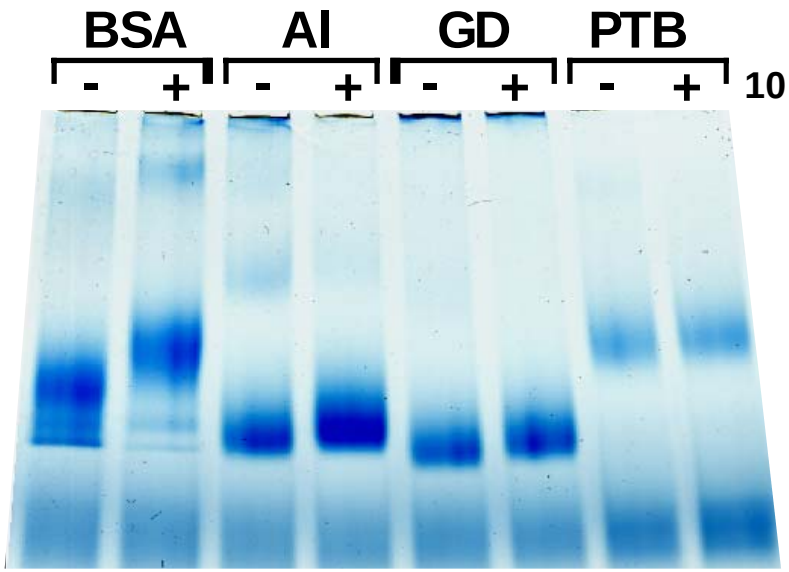


Protein:	MW of monomer	Oligomeric status	total MW
BSA	69	1	69
Aldolase	39	4	157
GAPDH	36	4	143
PTB	59	?	?

Niepmann and Zheng, Electrophoresis (2006)

Discontinuous native protein gel electrophoresis

Native gel run after dissociation of protein subunits at low pH *(pH = 3)*



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Niepmann and Zheng, Electrophoresis (2006)

How does it work?

reminder:

Functional principle of the

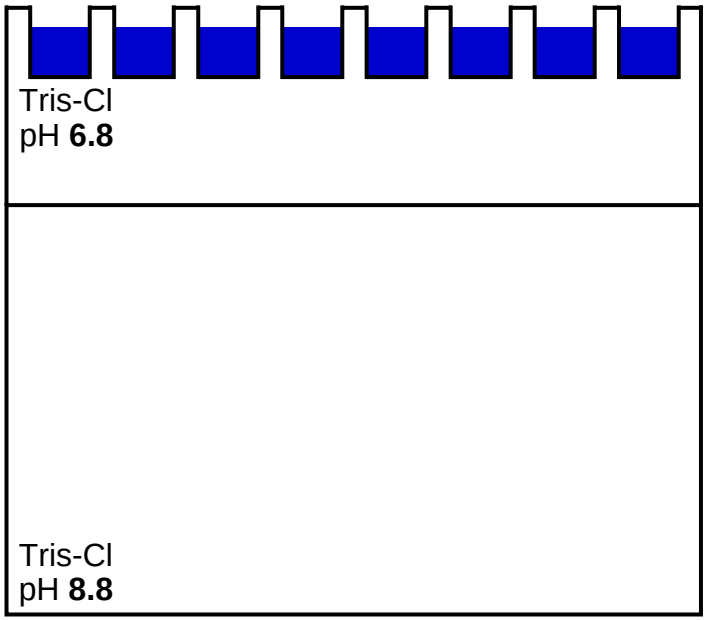
Laemmli SDS-PAGE

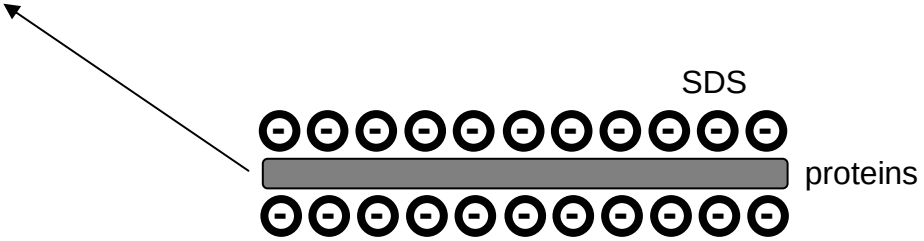
system

Laemmli UK, Nature (1970)

Laemmli gel:

Cathode buffer:
25 mM **Tris**, 192 mM **glycine**, 0.1% SDS, pH 8.3





Proteins are denatured by SDS:

- **SDS confers negative charges to the protein at a more or less uniform charge/mass ratio**
- **separation of the unfolded proteins in the molecular sieve is achieved only by the size of denatured proteins**

Anode buffer:
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Laemmli UK, Nature (1970)

Laemmli gel:

Cathode buffer:
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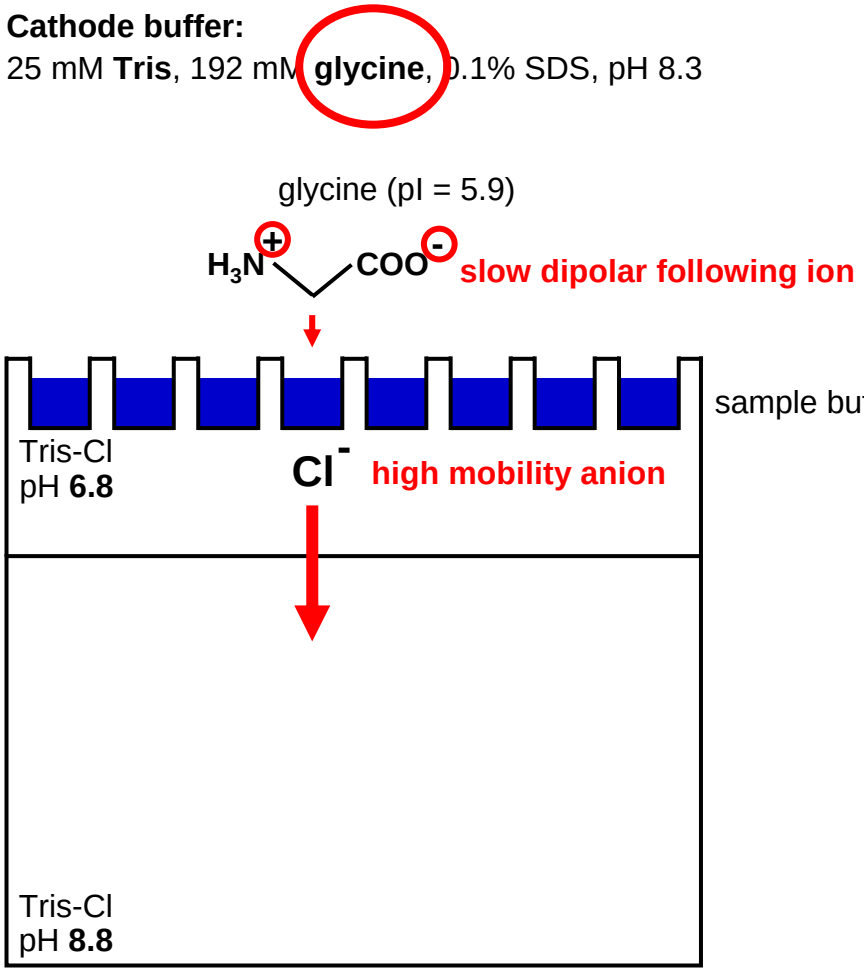

Discontinous buffer system

→ **focusing of protein sample prior to entry into separation gel**

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Laemmli UK, Nature (1970)

Laemmli gel:

Cathode buffer:
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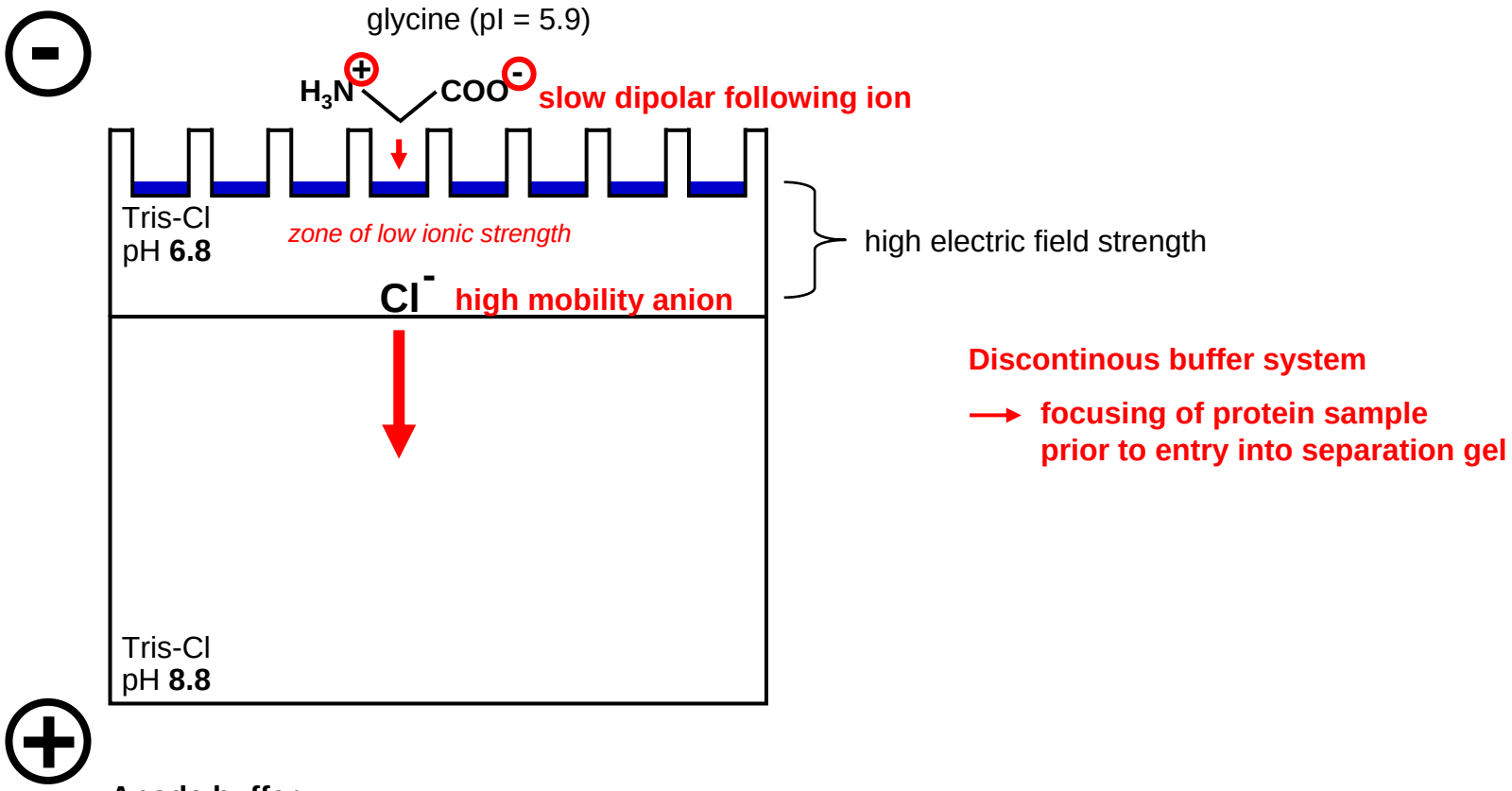

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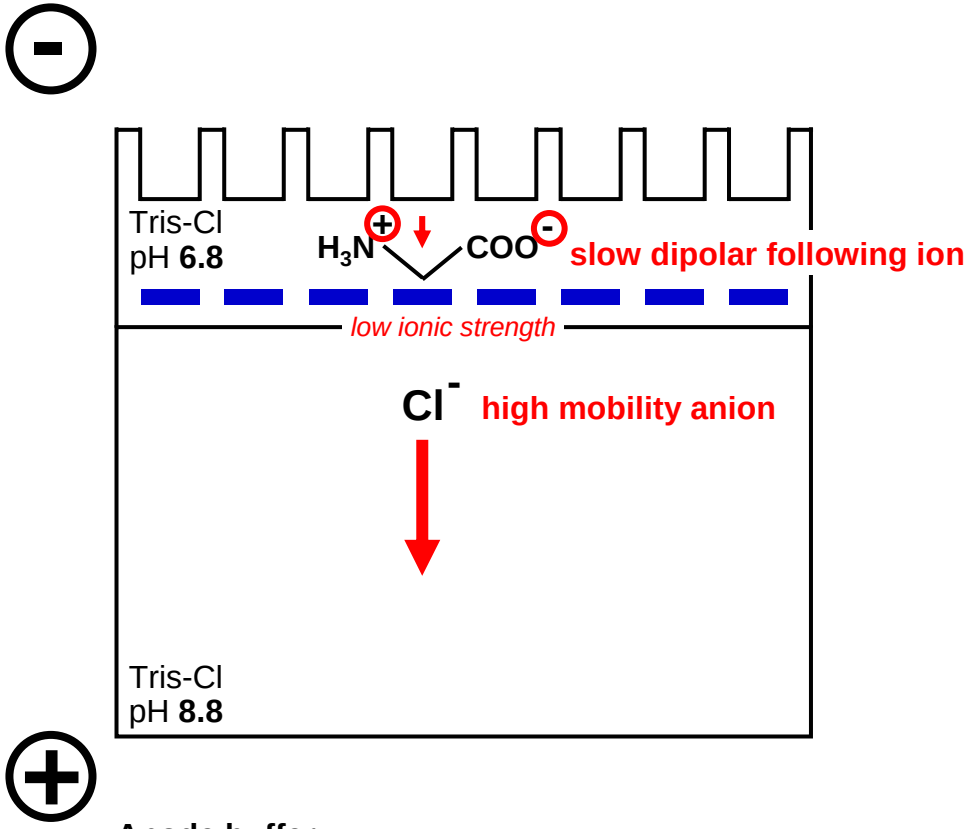

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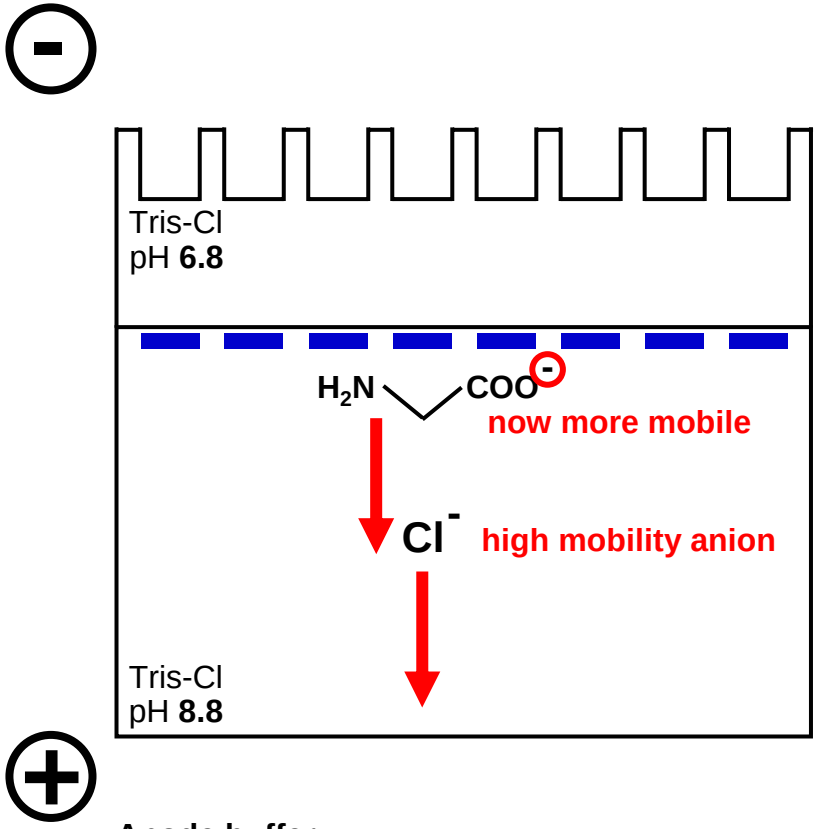

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Laemmli UK, Nature (1970)

Functional principle of the

Discontinuous native protein gel electrophoresis

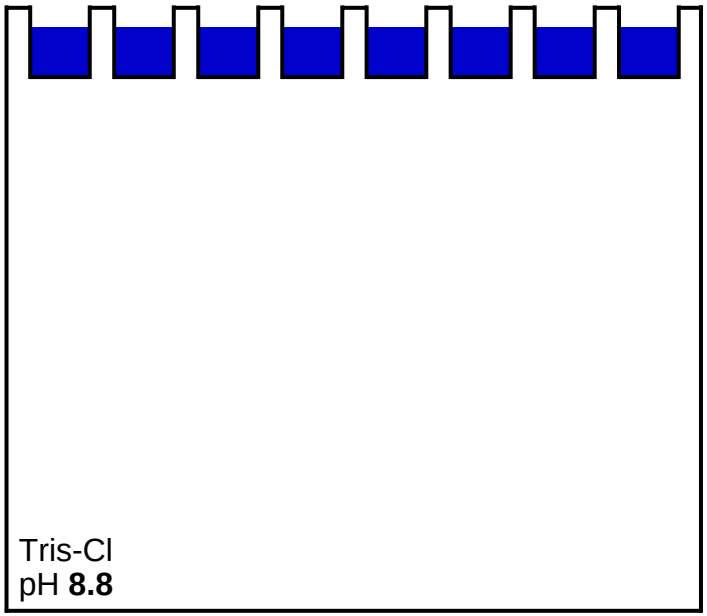
Niepmann and Zheng, Electrophoresis (2006)

Discontinuous native protein gel electrophoresis

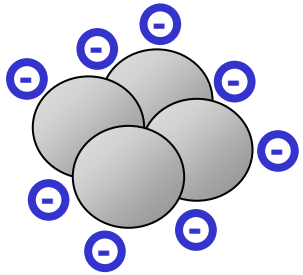
Cathode buffer: 100 mM **histidine**, adjusted with Tris base to pH **8.0**, 0.002 % Serva Blue G

Technical note:

*Mix the Blue G to the upper buffer tank carefully only **after** sample loading (otherwise you will not easily see which slots are already loaded)!*

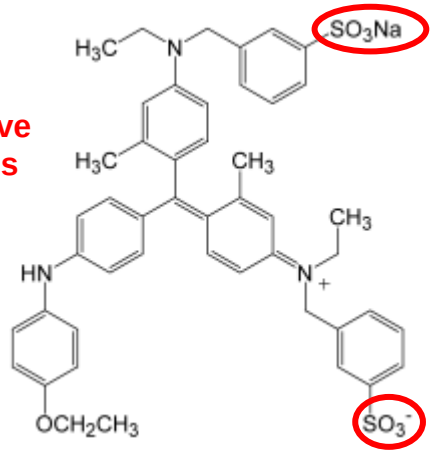


sample buffer: Tris-Cl pH **8.0**, 0.5 % Serva Blue G



native
oligomeric protein
with attached **Blue G**

→ **Blue G confers negative charges to the proteins without denaturing or dissociating them.**

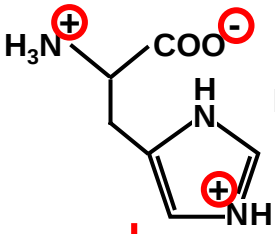


Anode buffer:
100 mM **Tris-Cl**, pH **8.8**

Niepmann and Zheng, Electrophoresis (2006)

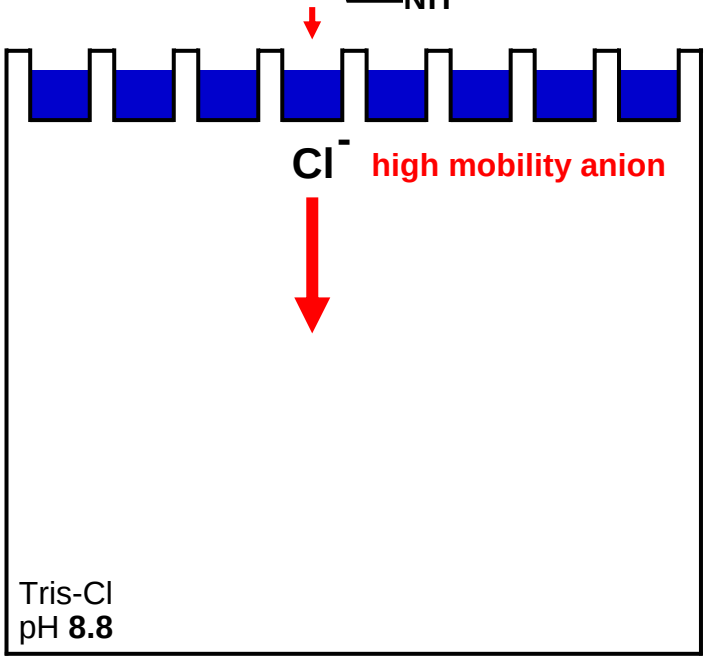
Discontinuous native protein gel electrophoresis

Cathode buffer: 100 mM **histidine**, adjusted with Tris base to pH **8.0**, 0.002 % Serva Blue G



histidine (pI = **7.6**) **(allows migration also of basic proteins)**

slow dipolar following ion



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100 mM **Tris-Cl**, pH **8.8**

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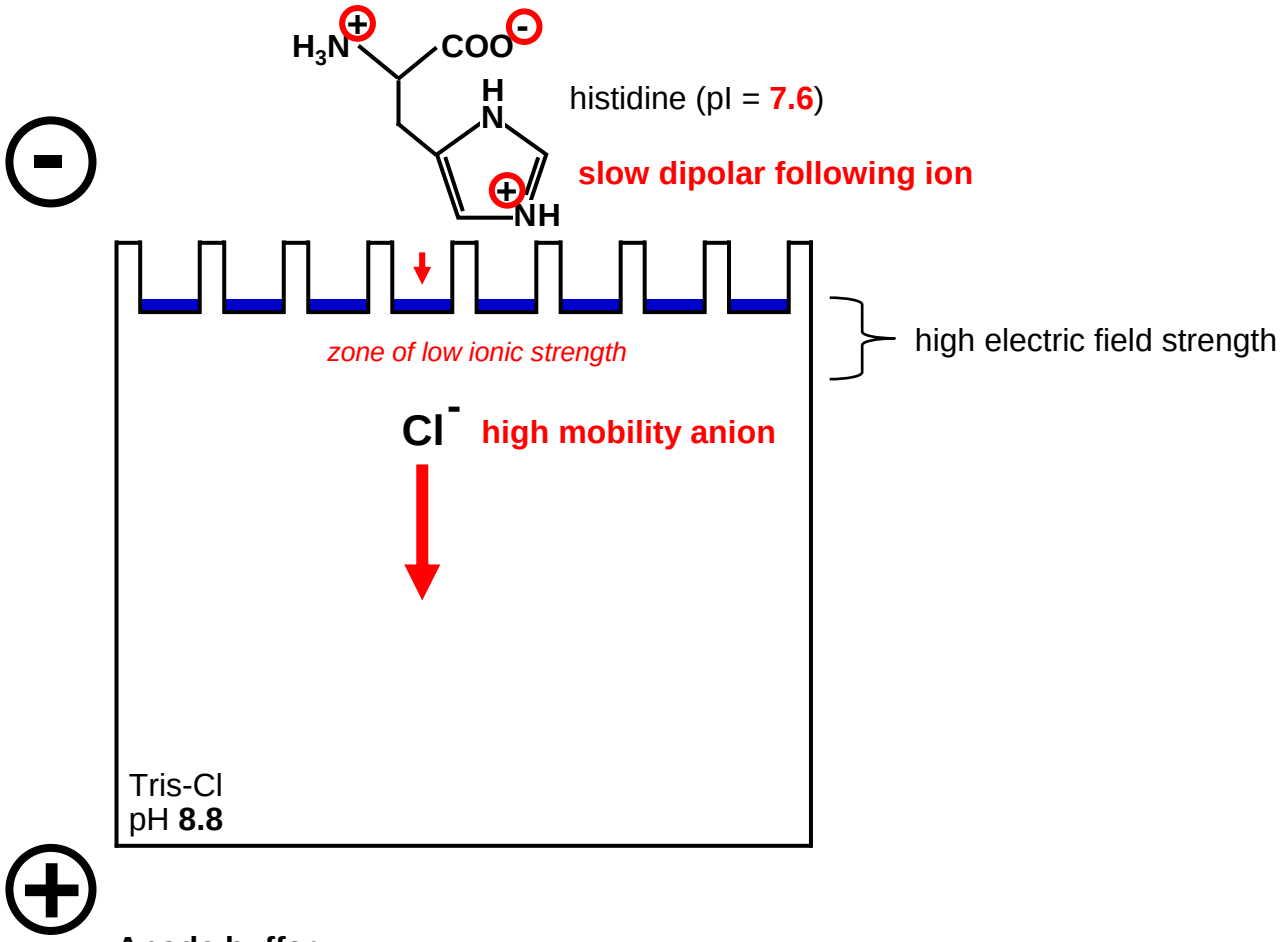

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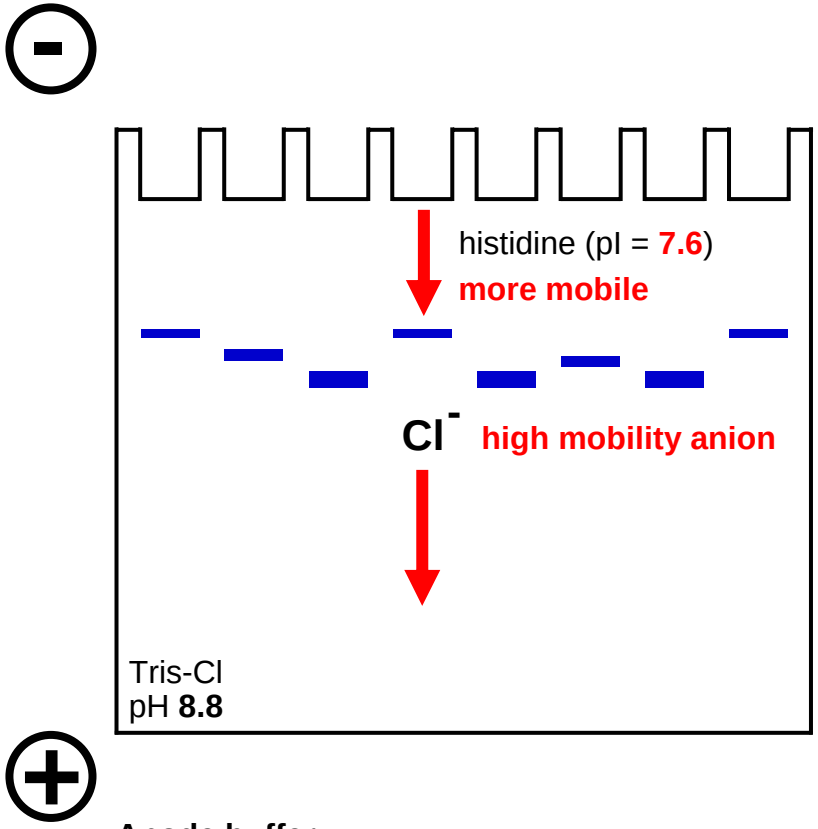

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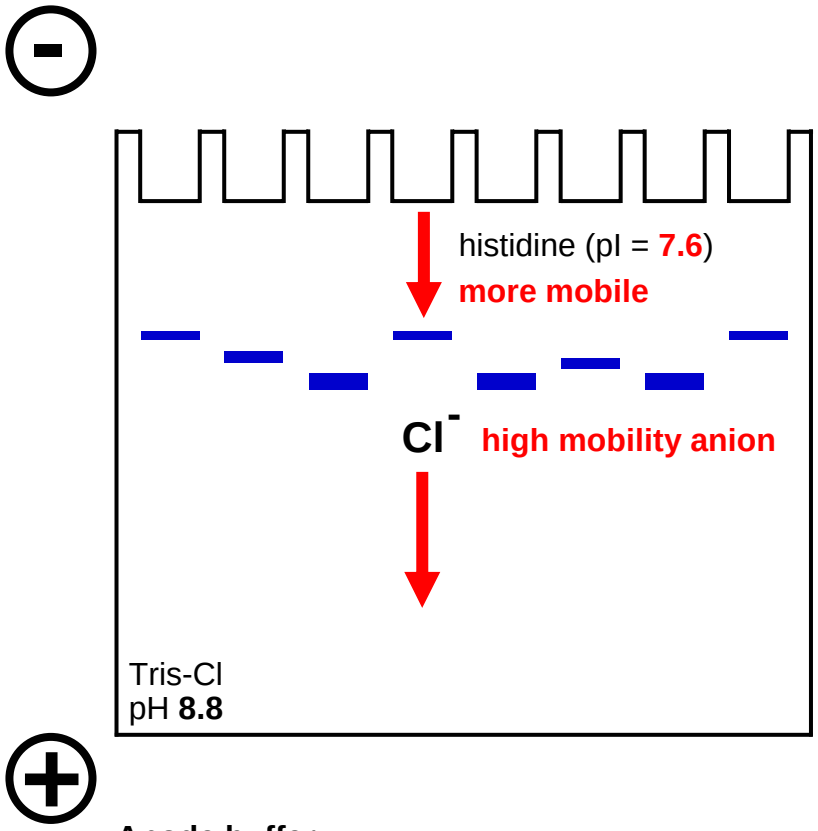
Niepmann and Zheng, Electrophoresis (2006)

Discontinuous native protein gel electrophoresis

Cathode buffer: 100 mM **histidine**, adjusted with Tris base to pH **8.0**

Technical note:

*Change cathode buffer with Blue G to buffer **without** Blue G after half of run time to allow most of the unbound Blue G to leave the gel.*



Anode buffer:
100 mM **Tris-Cl**, pH **8.8**

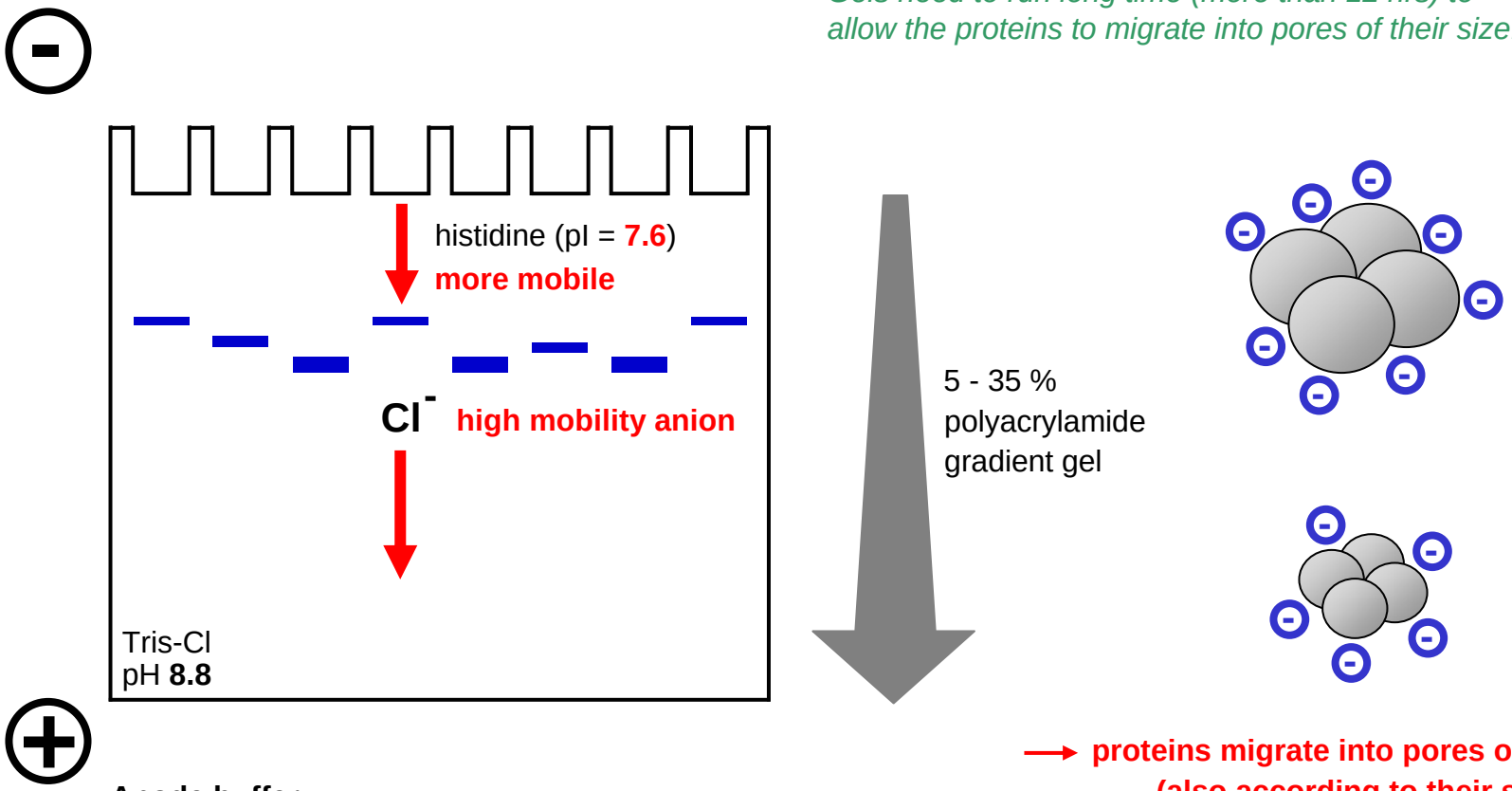
Niepmann and Zheng, Electrophoresis (2006)

Discontinuous native protein gel electrophoresis

Cathode buffer: 100 mM **histidine**, adjusted with Tris base to pH **8.0**, 0.002 % Serva Blue G

Technical note:

Gels need to run long time (more than 12 hrs) to allow the proteins to migrate into pores of their size.



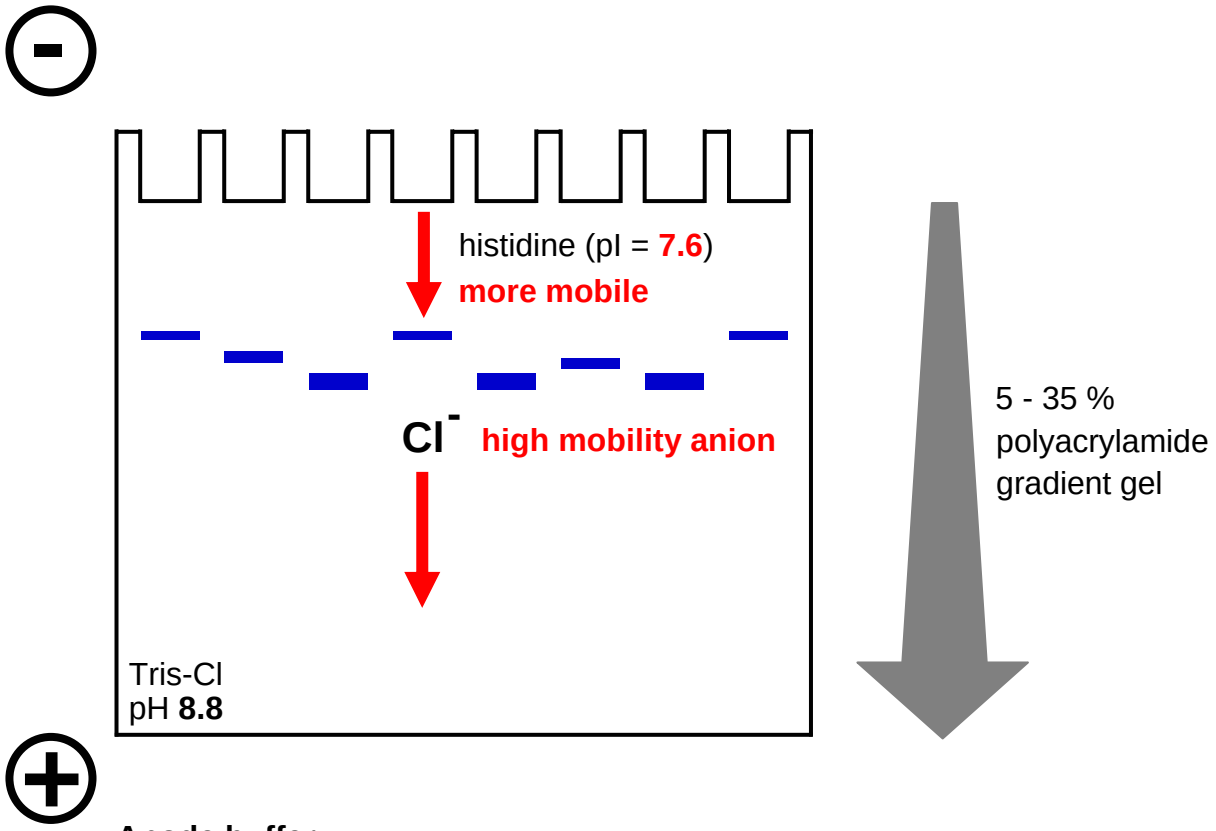
Anode buffer:
100 mM **Tris-Cl**, pH **8.8**

→ **proteins migrate into pores of their size (also according to their shape) in the molecular sieve and then get stuck**

Niepmann and Zheng, Electrophoresis (2006)

Discontinuous native protein gel electrophoresis

Cathode buffer: 100 mM **histidine**, adjusted with Tris base to pH **8.0**, 0.002 % Serva Blue G



Anode buffer:
100 mM **Tris-Cl**, pH **8.8**

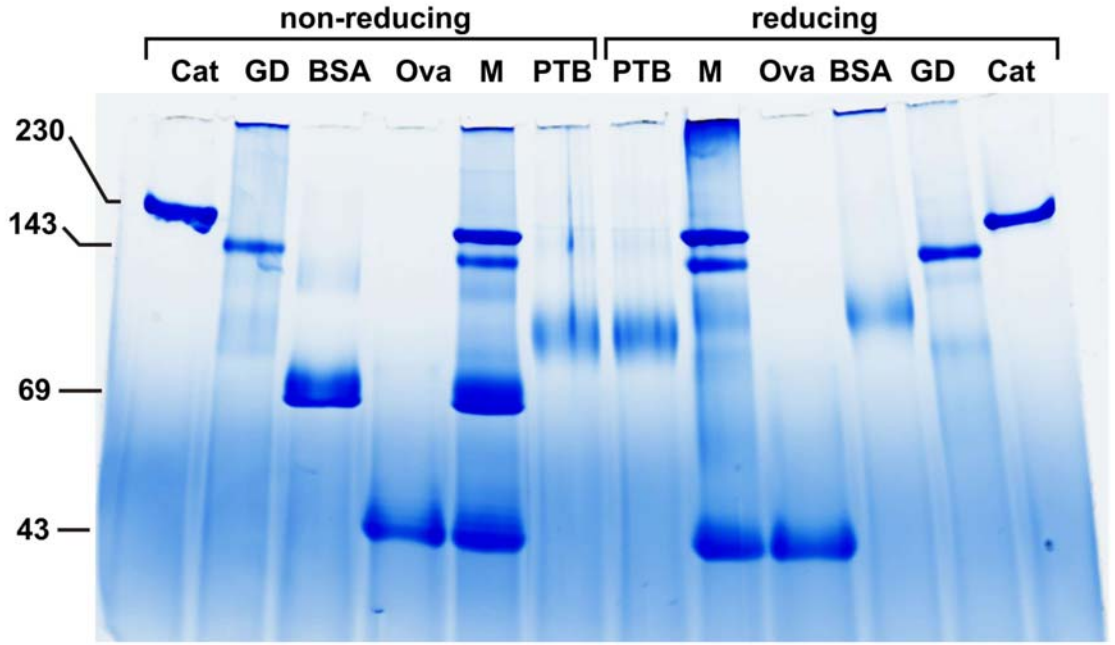
Technical note:

*You can easily cast **gradient gels** of sufficient quality without a gradient mixer.*

Use a wide-pore glas pipet. First suck in the light acrylamide solution (e.g., 5%), then suck in the heavy solution (e.g., 35%), then carefully suck in 2 to 3 air bubbles which slightly mix the solutions when they ascend, and then release the solution slowly between the assembled gel plates.

Niepmann and Zheng, Electrophoresis (2006)

Calibration curves



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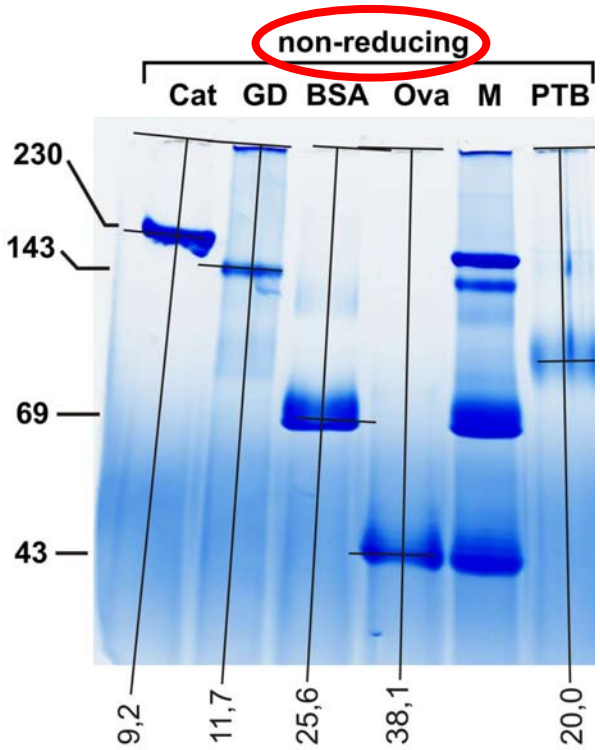

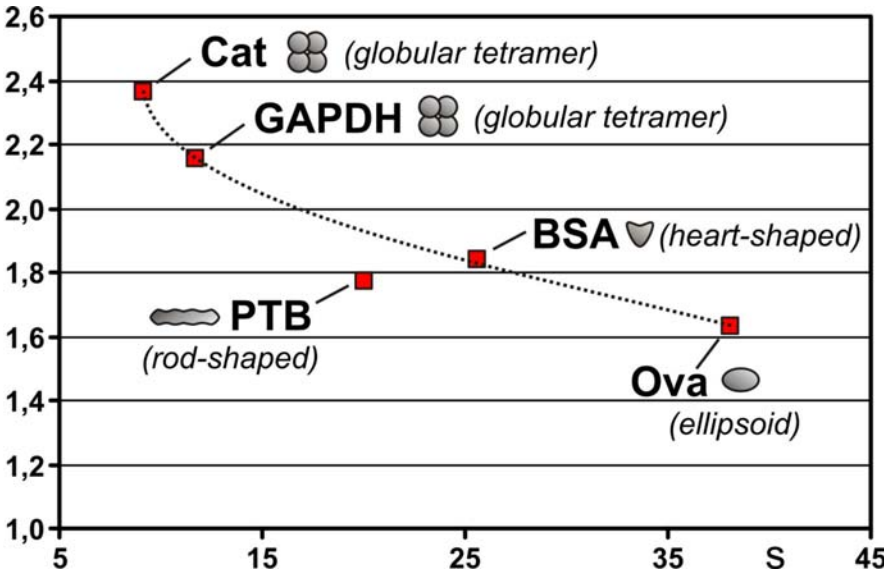

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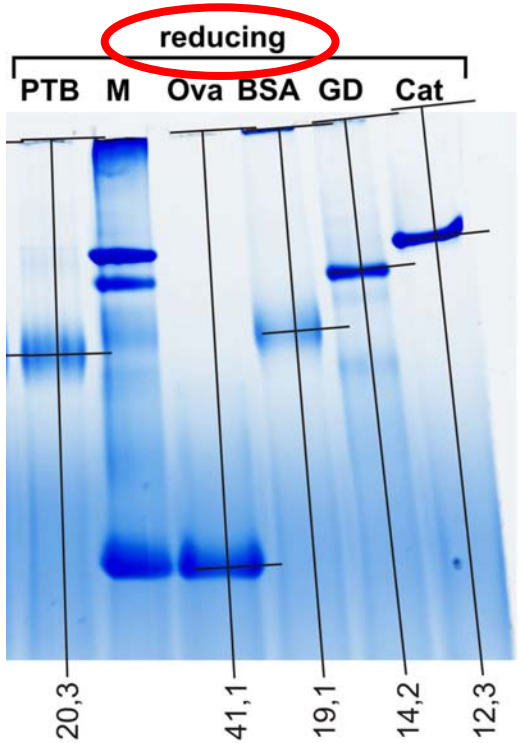
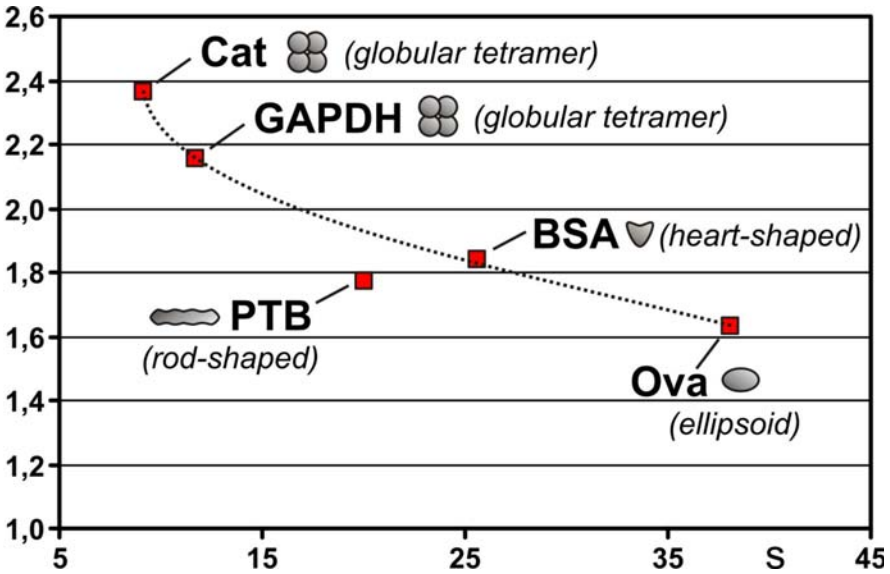
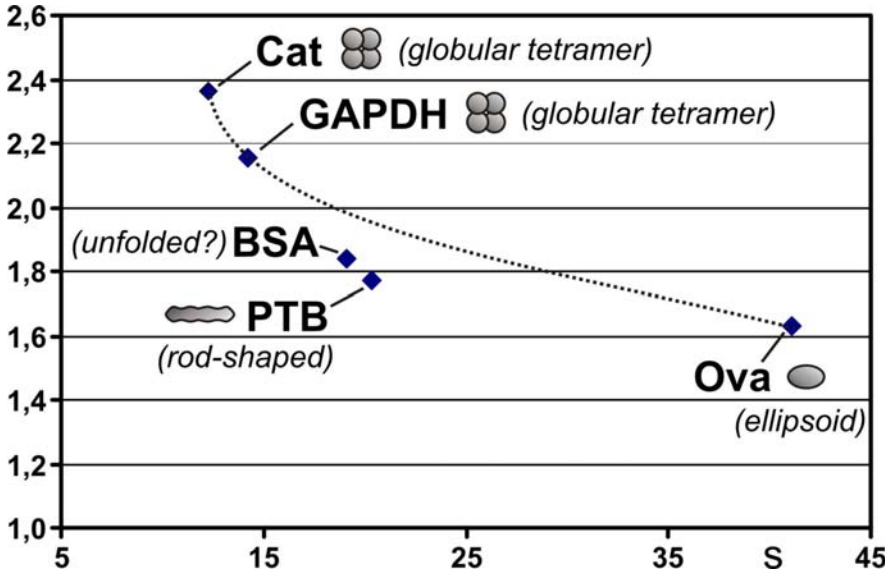
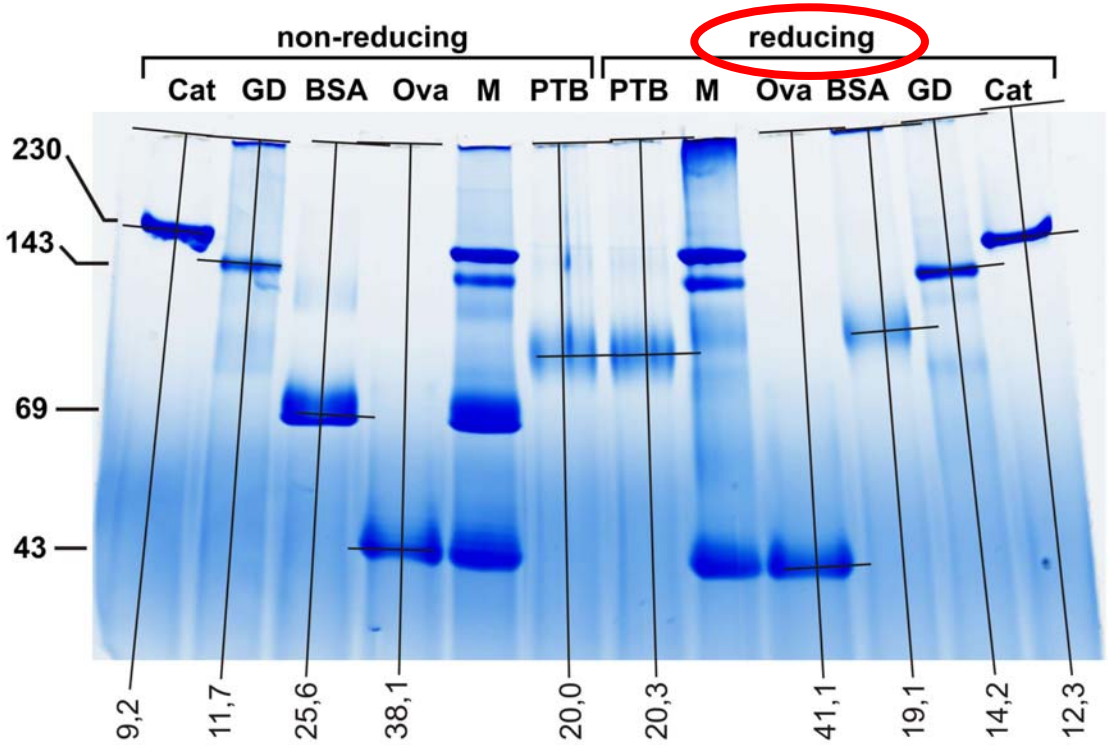
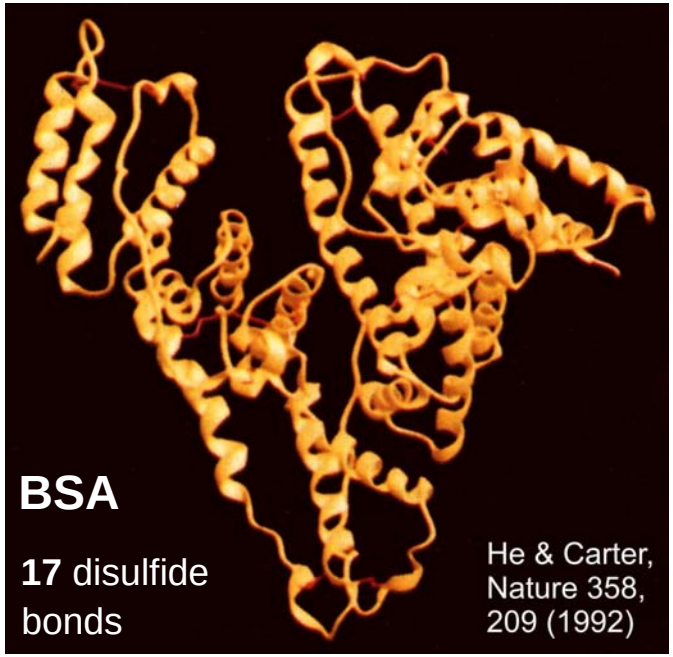
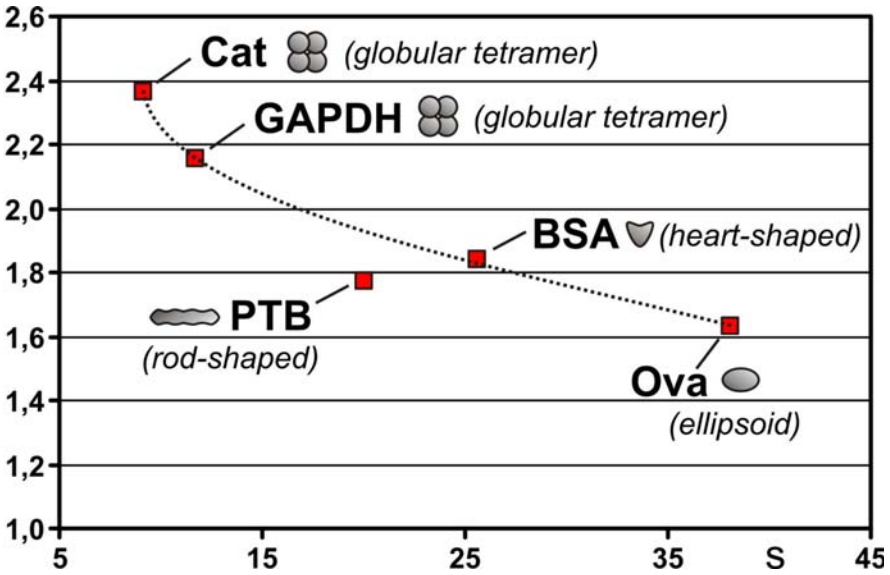


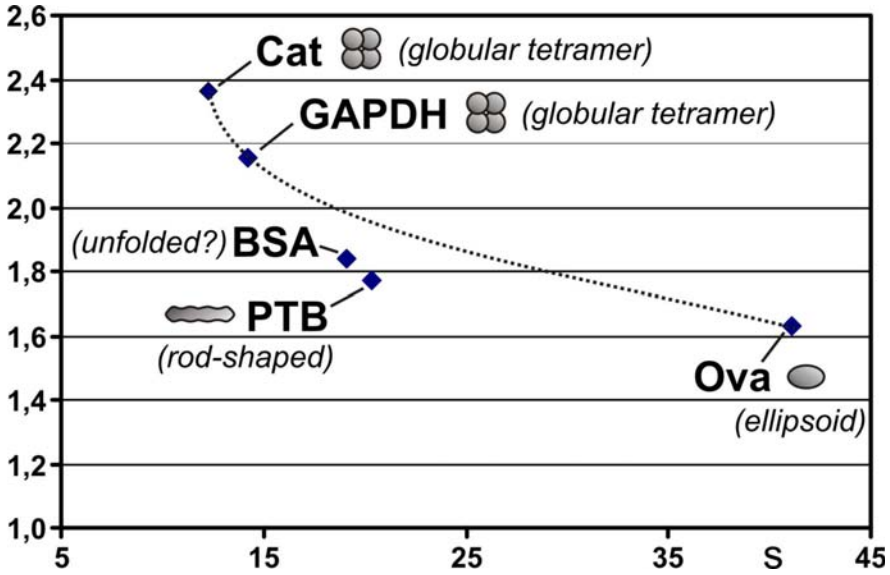





non-reducing
reducing
Cat GD BSA Ova M PTB
PTB M Ova BSA GD Cat
230
143
69
43
9,2
11,7
25,6
38,1
20,0
20,3
41,1
19,1
14,2
12,3


BSA
17 disulfide bonds
He & Carter, Nature 358, 209 (1992)


2,6
2,4
2,2
2,0
1,8
1,6
1,4
1,2
1,0
Cat (globular tetramer)
GAPDH (globular tetramer)
BSA (heart-shaped)
PTB
(rod-shaped)
Ova
(ellipsoid)
5
15
25
35
S
45

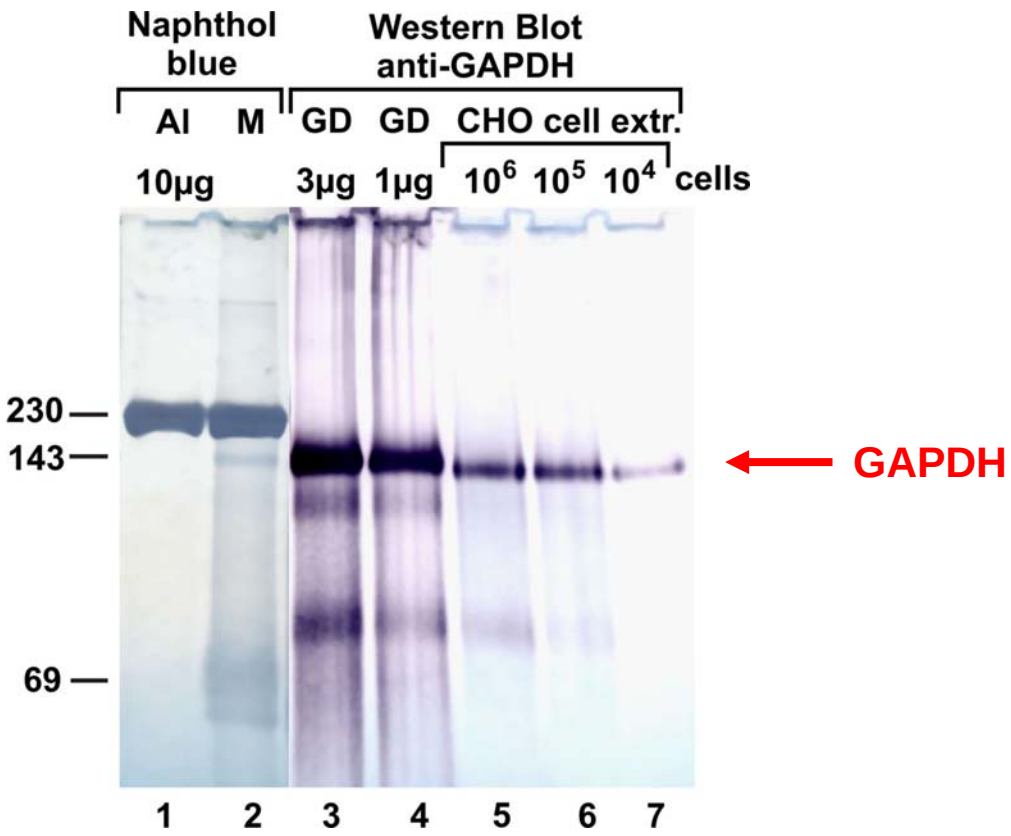

2,6
2,4
2,2
2,0
1,8
1,6
1,4
1,2
1,0
Cat (globular tetramer)
GAPDH (globular tetramer)
(unfolded?) BSA
PTB
(rod-shaped)
Ova
(ellipsoid)
5
15
25
35
S
45

Conclusions about the migration of BSA

The secondary and tertiary structure of BSA (an extracellular protein) is essentially stabilized by several disulfide bonds.

When BSA is used as a marker under reducing conditions, be aware that BSA may unfold and change its shape from globular to rod-like. Thus, it may migrate to a position in the gel which corresponds to its size as a rod-like protein, not as a globular protein.

Western blot



Technical notes:

- *use a wet blot apparatus to avoid concentration of the Blue G on the membrane*
- *try ECL light exposure system (or similar) to avoid visualization of the blue dye on your result image*

Summary

1. The new discontinuous native gel system uses Serva Blue G to confer negative charges to the proteins (leaving the proteins in their native states).

Niepmann and Zheng, Electrophoresis (2006)

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4. Proteins are separated according to their molecular sizes (including shape).

5. The oligomeric states of the proteins are preserved.

Niepmann and Zheng, Electrophoresis (2006)