PROTEIN SEPARATION

Protein separation

- The most frequently used technology for separation and isolation of proteins is polyacrylamide gel electrophoresis (PAGE).
- Introduced in 1970, it is the most efficient technique to solve complex mixtures of proteins.

- Two PAGE types:
  - Monodimensional (SDS-PAGE)
  - Bidimensional (2D-PAGE)

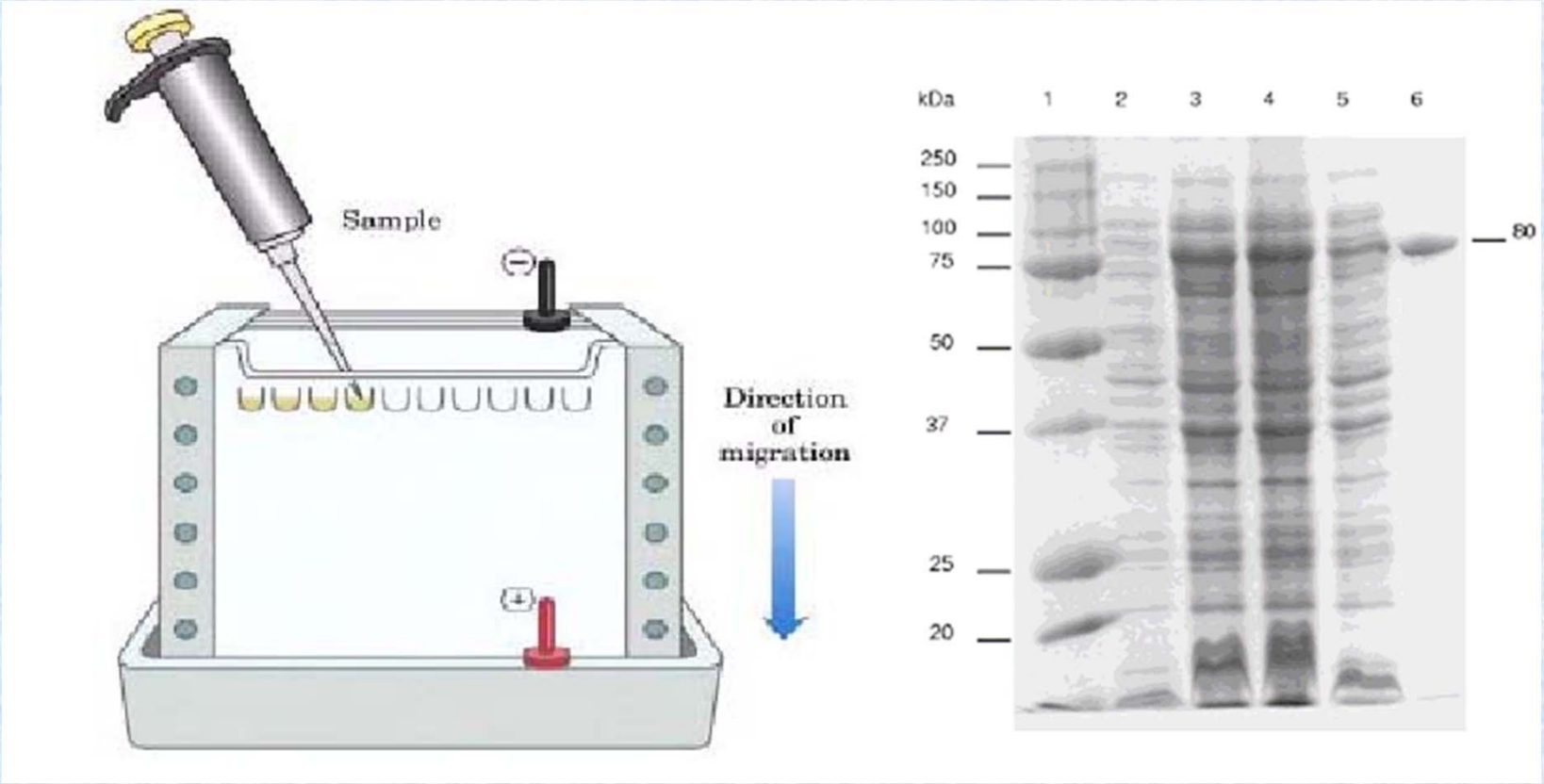
Protein separation – SDS-PAGE

- For many proteomics applications, SDS-PAGE is the method of selection to solve protein mixtures.

- Proteins are separated according to their mass and as they are solubilized in sodium dodecyl sulphate (SDS), and there are no solubilization problems.

- Is it a simple, recurrent technique, and allows the separation of proteins of 10-300 kDa.

Protein separation – SDS-PAGE



- One of the most common applications of SDS-PAGE is the characterization of proteins after any kind of previous purification.

Protein separation – 2D-PAGE

- It nowadays constitutes the most efficient method for separation of very complex mixtures of proteins: it allows to separate thousands of proteins in just one experiment.

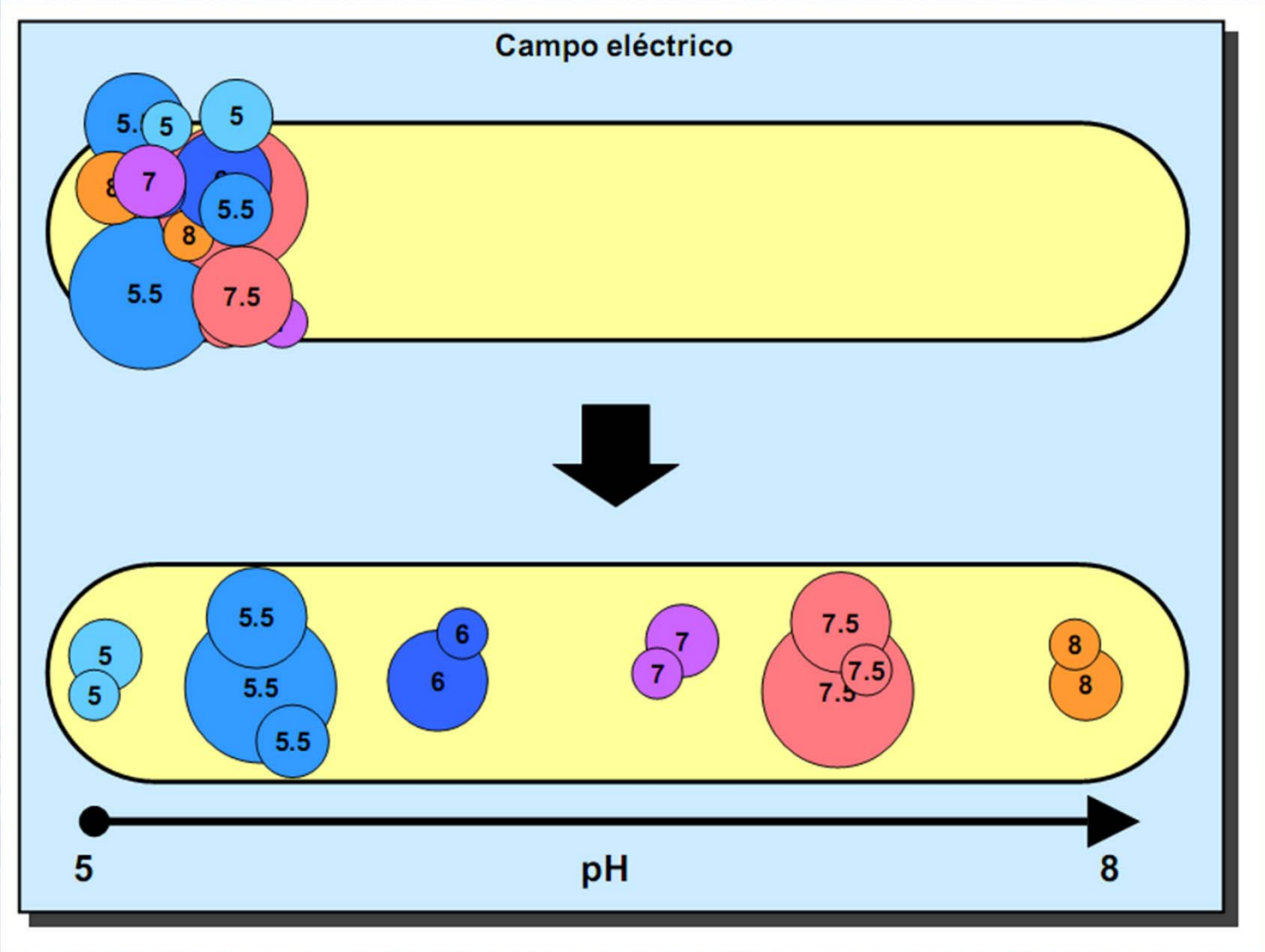
- It is based on protein separation in terms of net charge, followed by protein separation in terms of molecular mass.

- This technique's high resolution is due to the fact that the two separations are based on different parameters.

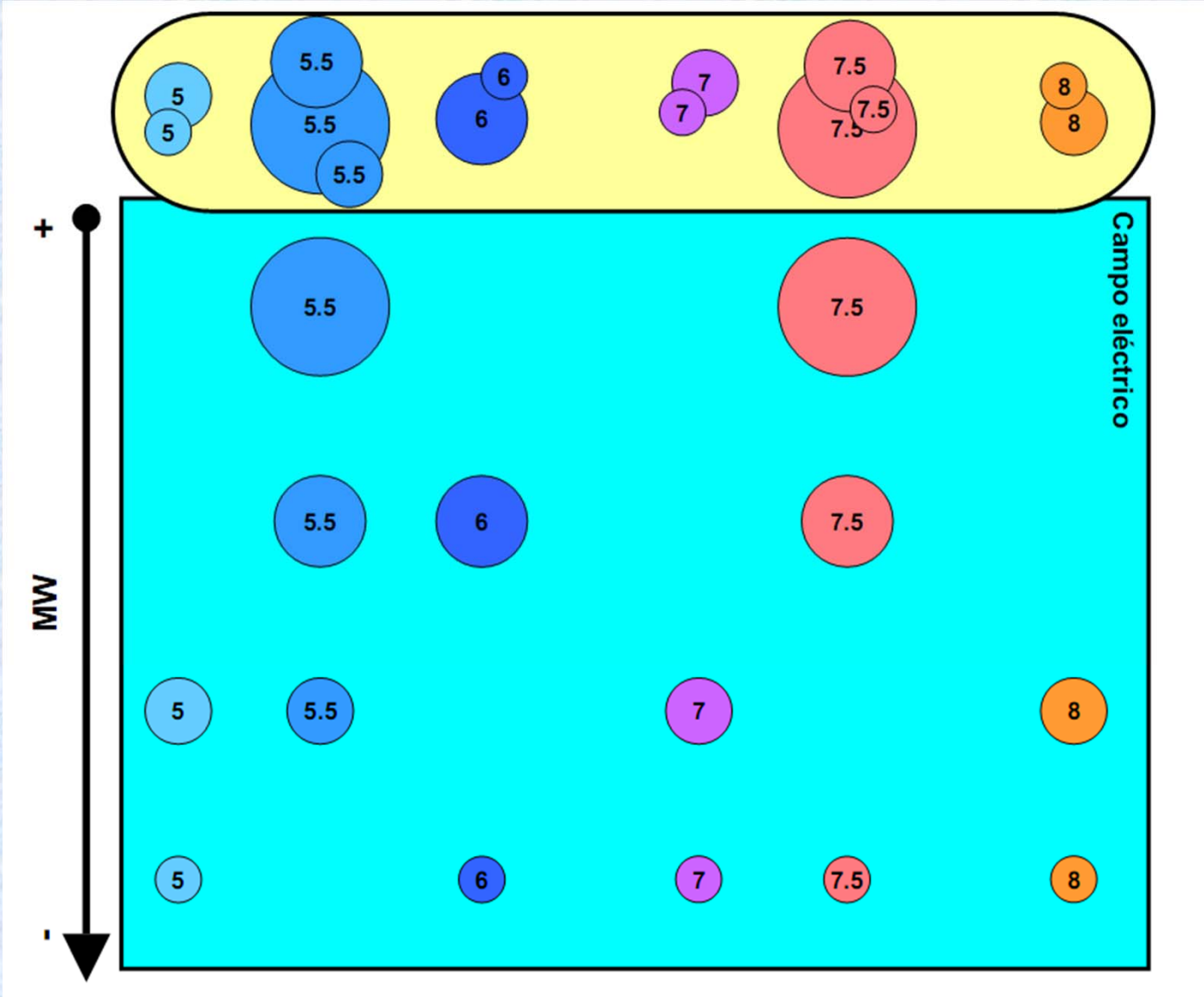
Protein separation – 2D-PAGE

- The separation of the first dimension is done by means of isoelectric focusing.
- By isoelectric focusing proteins are separated in a pH gradient until they reach a position in which their charge is 0 (isoelectric point).
- In the second dimension, proteins are separated by means of SDS-PAGE.
- It allows the separation of the 4,000-5,000 proteins in a cell.

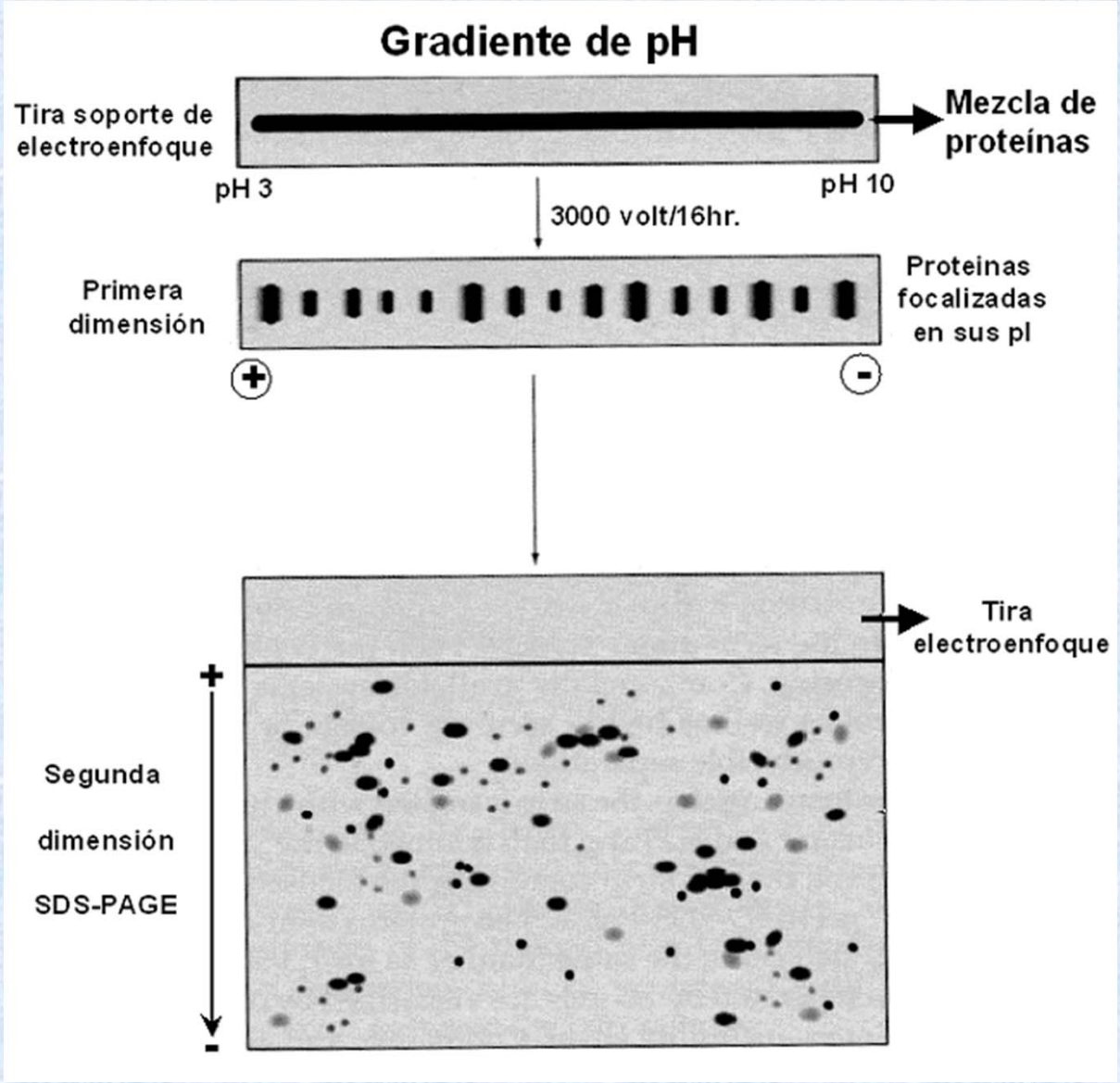
2D-PAGE: pH gradient



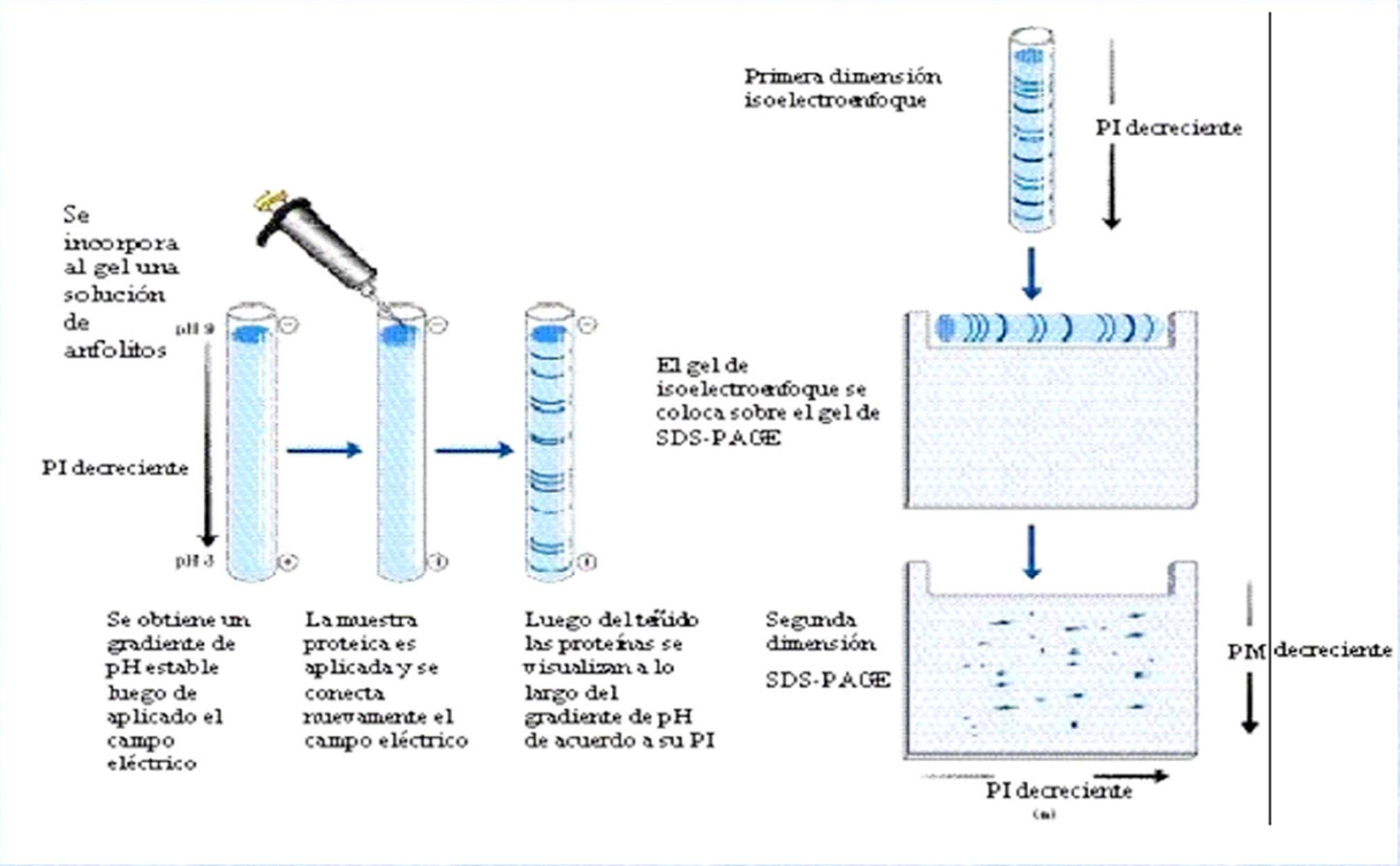
2D-PAGE: SDS PAGE



2D PAGE


Gradiente de pH
Tira soporte de electroenfoque
Mezcla de proteínas
pH 3
pH 10
3000 volt/16hr.
Primera dimensión
Proteinas focalizadas en sus pl
+
-
Tira electroenfoque
Segunda dimensión SDS-PAGE
+
-

Protein separation – 2D-PAGE



Protein separation – 2D-PAGE

- The key innovation has been the development of gels with an immovilized pH gradient (IPG)

- In IPG gels, the pH gradient is generated by immobilines, and it is co-polymerized with the acrylamide matrix.

- Elimination of instability problems of pH gradient and low charge capacity associated to pH gradients prepared with carrier ampholytes.

Protein separation – 2D-PAGE

- IPG gels allow to:
  - Improve resolution
  - Improve capacity
  - Increase the amount of charged proteins
- Reproducibility obtained with IPG gels allows the comparison of protein maps among different labs.
- It facilitates information interchange.

Protein separation - Detection

- Traditional techniques for protein detection:
  - Radioactive labeling
  - Coomassie Blue staining
  - Silver staining (high sensibility)
- New techniques:
  - Surface silver staining
  - Fluorescent staining and labeling (Sypro-Ruby, Cy3, Cy5...)
- These new techniques allow the subsequent analysis of samples using mass spectrometry.

Protein separation – 2D-PAGE Applications

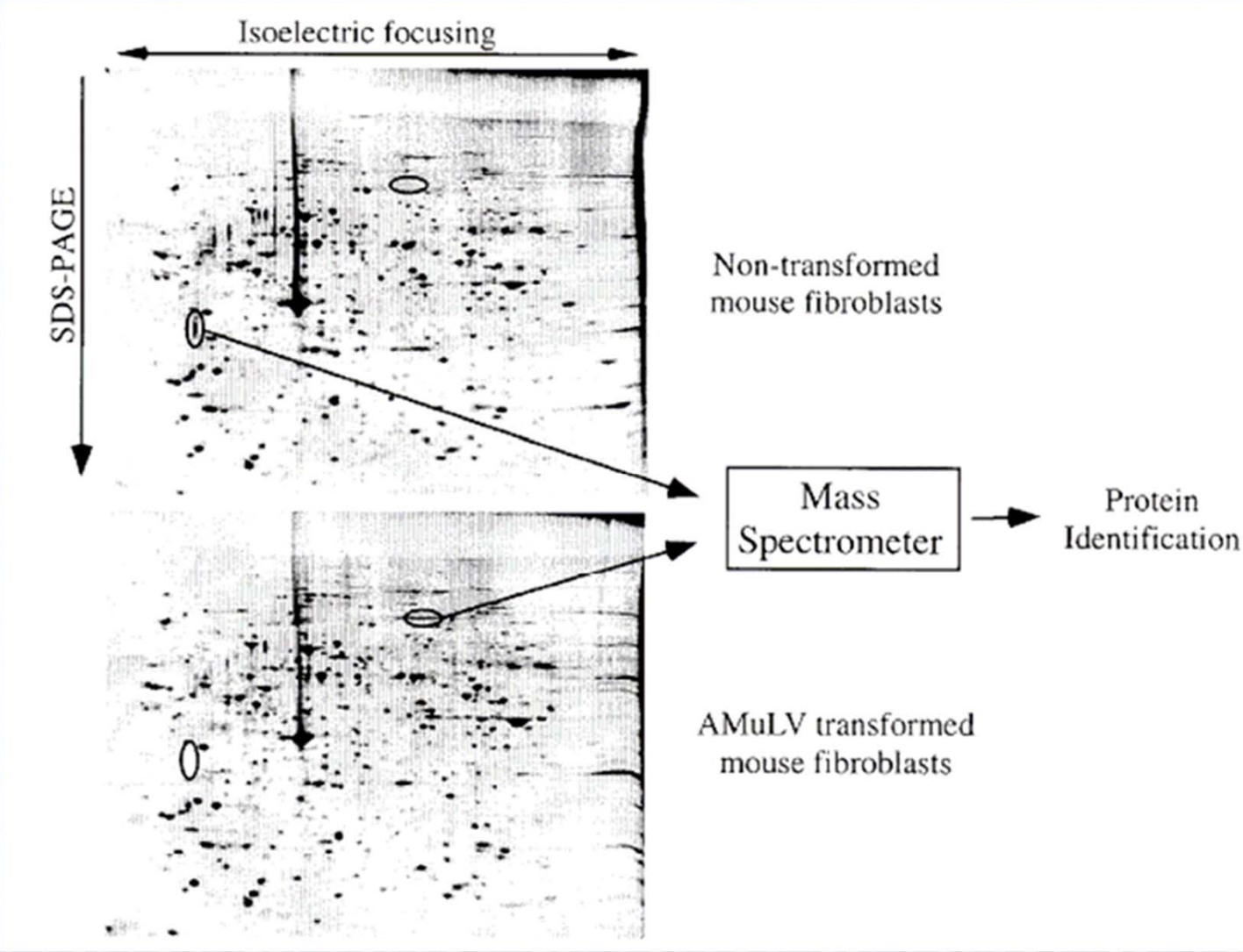
- The main application is Expression Proteomics.
- Protein expression of two samples could be compared quantitative and qualitatively.
- The appearance or disappearance of spots gives information about the differential expression of proteins.
- Spot intensity allows to know expression levels.

Protein separation – 2D-PAGE Applications

- To carry out Expression Proteomics studies, there could be used:
  - Complete organisms
  - Cellular lines
  - Biological fluids

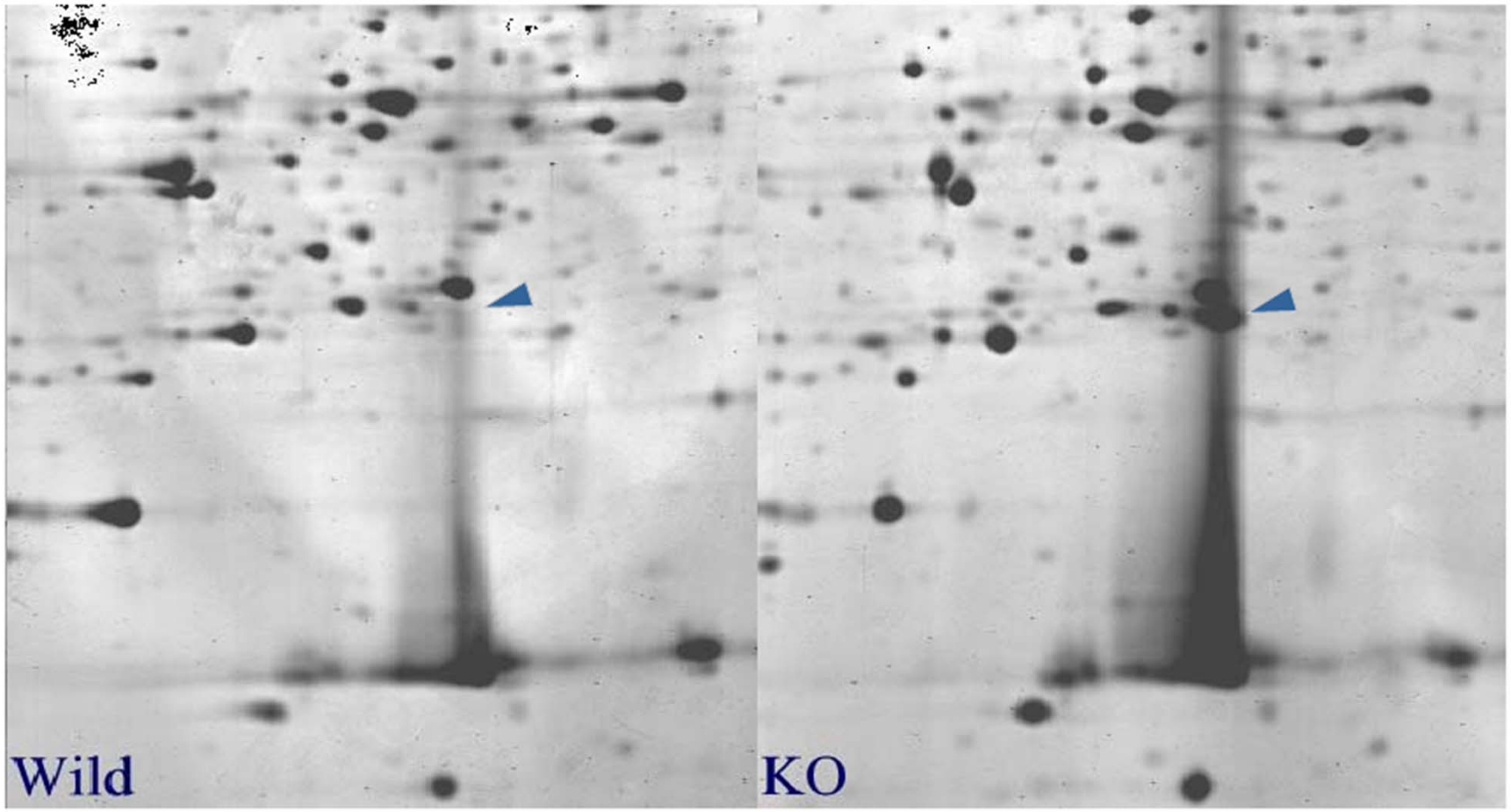
- Comparison of normal tissues with ill tissues (cancer, heart diseases).
- Comparison of cells treated with drugs or different stimuli.

Protein separation – 2D-PAGE Applications



Abelson murine Leukemia Virus (AMuLV)

Protein separation – 2D-PAGE Applications



Pseudomonas syringae Secretome

Protein separation – 2D-PAGE Applications

- Other important application is protein cell mapping in:
  - Microorganisms
  - Cellular organelles
  - Protein complexes
- It can also be used for the characterization of proteins in subproteomes, obtained by means of some technique of proteome purification.

Protein separation – 2D-PAGE Limitations

- Very complex technique: it requires a long time (2 days) and it is difficult to automate.
- Analysis of a unique sample with 2D-PAGE gel.
- Limited by the number and type of proteins to solve:
  - Very big or hydrophobic proteins do not enter the gel during the first dimension.
  - Very acid (pIs < pH= 3) or very basic proteins (pIs> pH= 10) are not very well solved.
- Limited detection of rarely abundant proteins.