









Motion of charged molecules in an electric field.

Polyacrylamide gel provides a porous matrix

□ (PAGE – Polyacrylamide Gel Electrophoresis)

Sample is stained with Comassie blue to make it visible in the gel.

Sample placed in wells on the gel.





























Isoelectric Focusing

- The *isoelectric point* is the pH at which the net charge of the protein molecule is neutral.
- Different proteins have different isoelectric points.
- Isoelectric point is found by drawing the sample through a stable pH gradient.
- The range of the gradient determines the resolution of the separation.





2D-PAGE Analysis Software

- 2D-PAGE technology has been in use for over 20 years, and potentially provides a vast amount of information about a protein sample.
- However, due to difficulties with data analysis, it remains only partially exploited.



Current state of software

- Correct identification and alignment of spots from the two gels has generally been a process with a lot of manual intervention - hence very slow.
- The processing power available with today's PCs means that automated analysis is starting to become possible.
- One vendor claims a throughput of 4 gel pairs per hour can be compared and annotated by an experienced user of their package.

Automated gel matching

- Gel matching, or "registration", is the process of aligning two images to compensate for warp.
- Some packages still require the user to identify corresponding spots to help with gel matching.
- The Z3 program from Compugen has a fullyautomated gel matching algorithm:
 - □ define set of small, unique rectangles.
 - □ compute optimal local transformations for rectangles.
 - □ Interpolate to make smooth global transformation.
- Note that this makes use of spot shape, streaks, smears and background structure, which other programs discard.



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