

Bioscience & Bioengineering 101: BB101

# Lecture – 11

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Techniques to Study Proteins and Proteome;  
Preamble to Metabolism

# Outline

- Techniques to Study Proteins
- Contributions of Genomics & Proteomics to Biology
- Metabolism

## Q& A: A re-cap of last lecture

- Proteins - Linear polymers built of monomers (amino acids)
- Wide range of functional groups accounts for various protein function
  - reactive properties crucial for enzyme function

## Q& A: A re-cap of last lecture (2)

- Gel filtration column composed of porous beads
  - made from polyacrylamide, dextran or agarose
- Size exclusion chromatography
  - proteins are separated according to their size
  - small molecules (including salt): retained longer

## Q& A: A re-cap of last lecture (3)

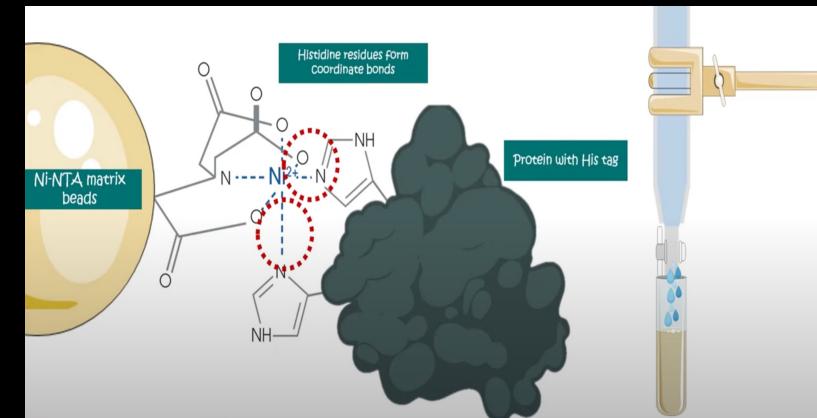
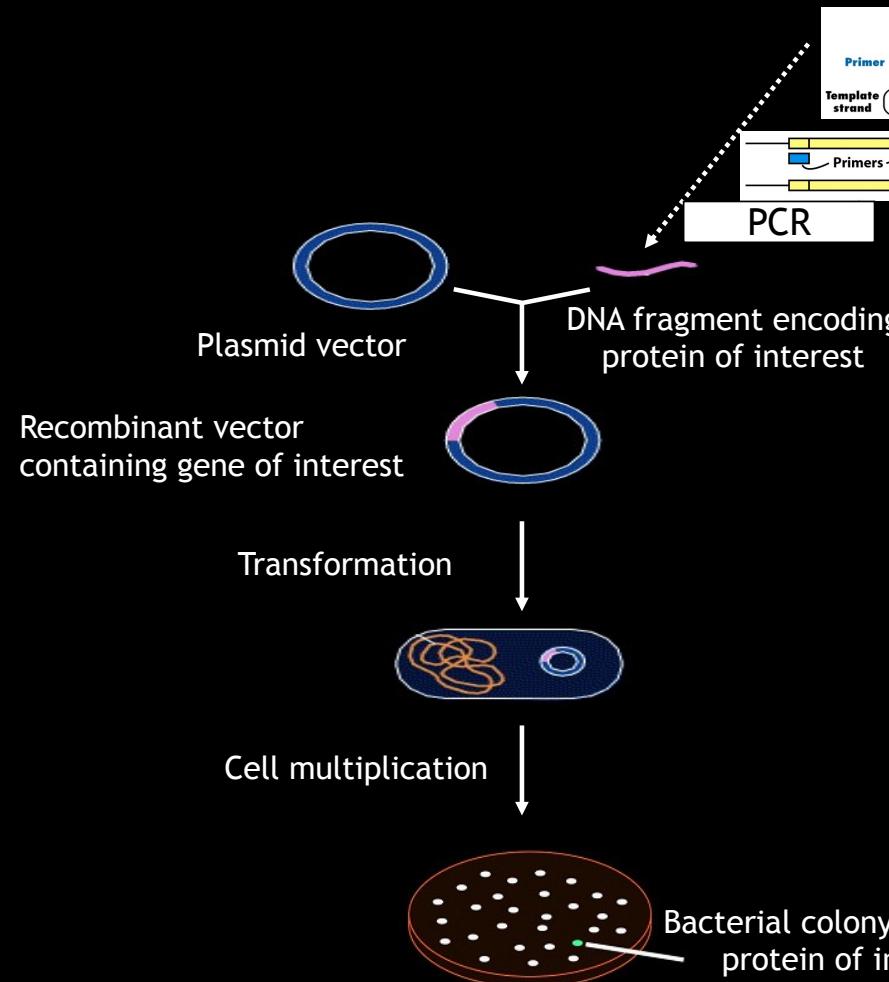
- Proteins separated based on charge difference
  - Proteins with overall negative charges will interact with positive charges or vice versa
  - varying amounts of positive/negative amino acids
  - pH influences net charge on proteins

## Q& A: A re-cap of last lecture (3)

- Based on affinity of protein to other molecules
  - substrates, products, cofactors, antibodies, metal
  - Matrix beads are chemically coupled to ligand
  - Protein binds through a specific interaction
  - all other proteins do not bind
- Protein desorbed by excess ligand in solution

# Protein expression and Purification:

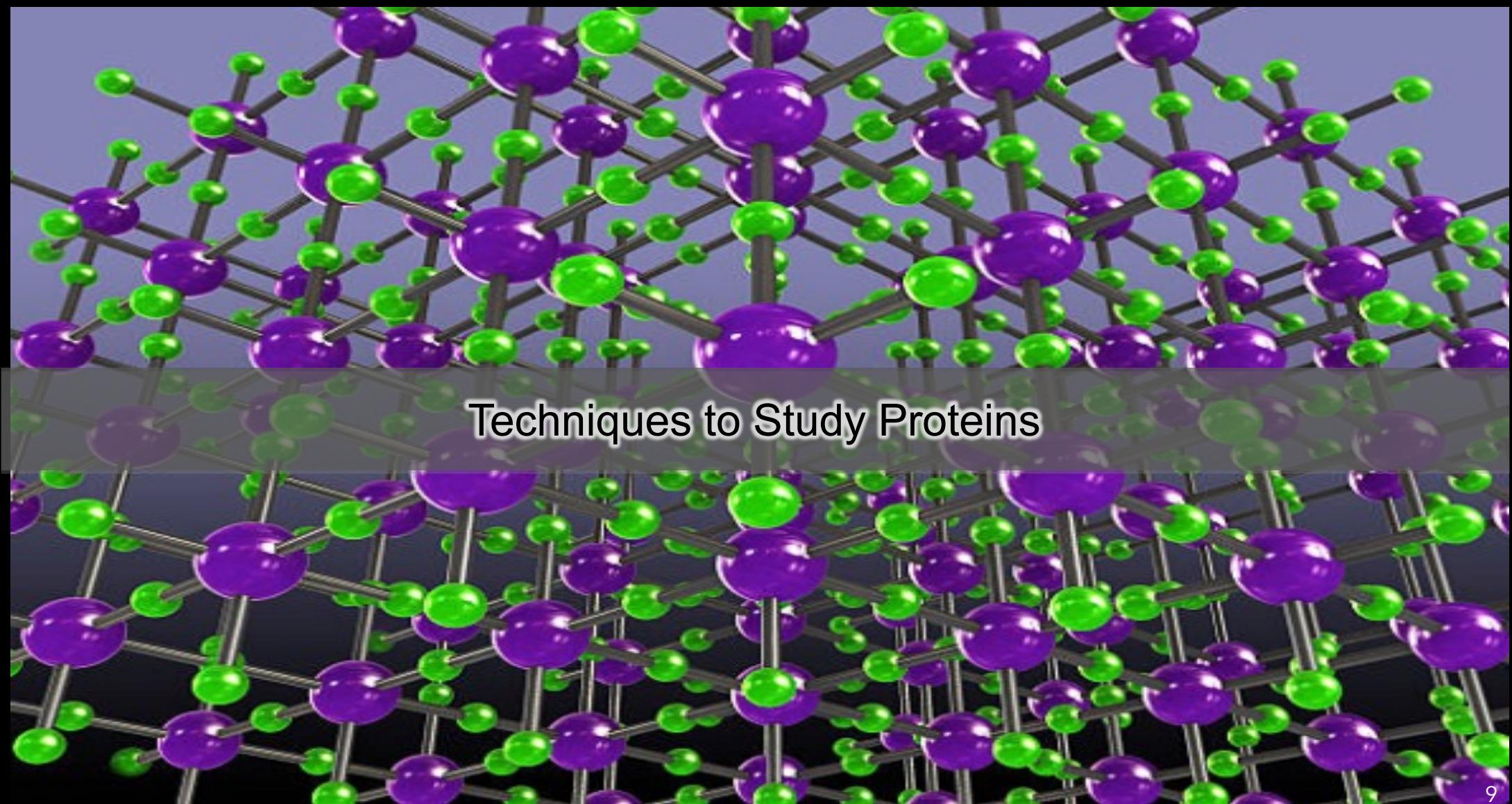
*Want to Study a Protein?*



<https://www.youtube.com/watch?v=rbcDxuc8TOw>

# Studying Protein Function

- To study the function of a particular protein, researchers could introduce different mutant forms of the gene for that protein into eukaryotic cells.
- The cells express different versions of the protein, and the resulting phenotypes provide information about the normal protein function.

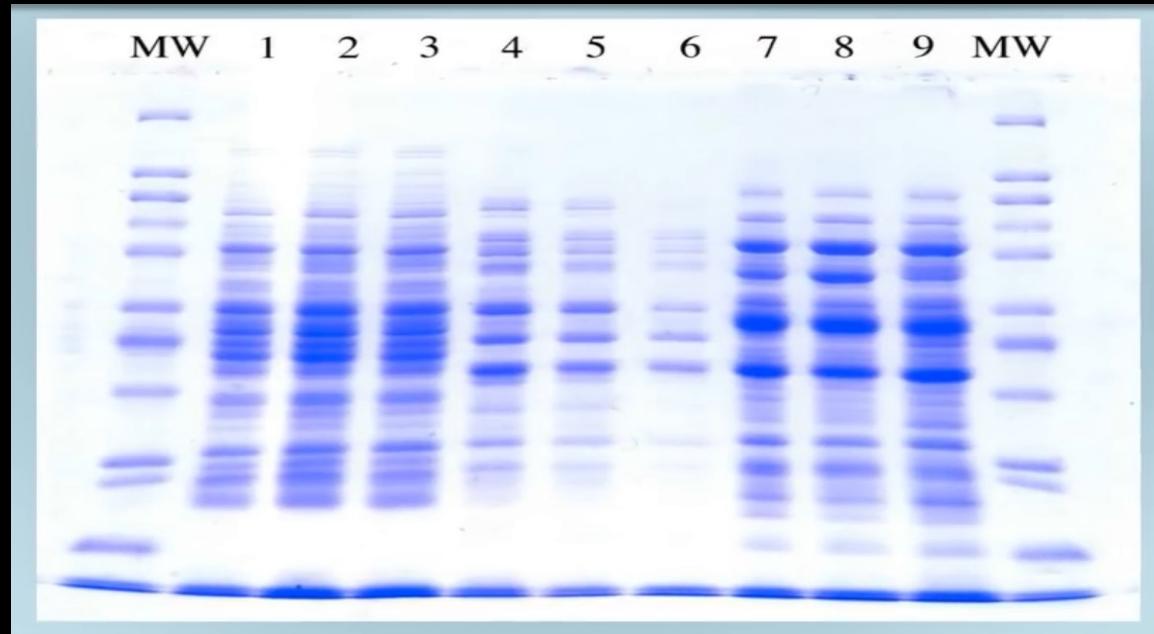


## Techniques to Study Proteins

# SDS-PAGE

- Separation based on charge-to-mass ratio and MW of protein
  - Smaller proteins migrate further distance through gel pores
- Applications
  - Subunit composition
  - Molecular weight of subunits

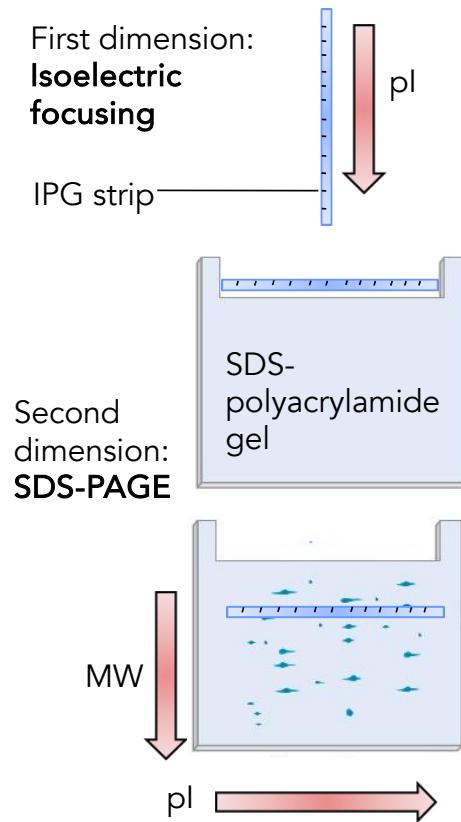
# SDS-PAGE Gel Electrophoresis



- Protein separation based on molecular weight
- Commonly used to determine MW of unknown protein by running protein of interest along with protein markers (or standards) of known MW

# Two Dimensional Electrophoresis

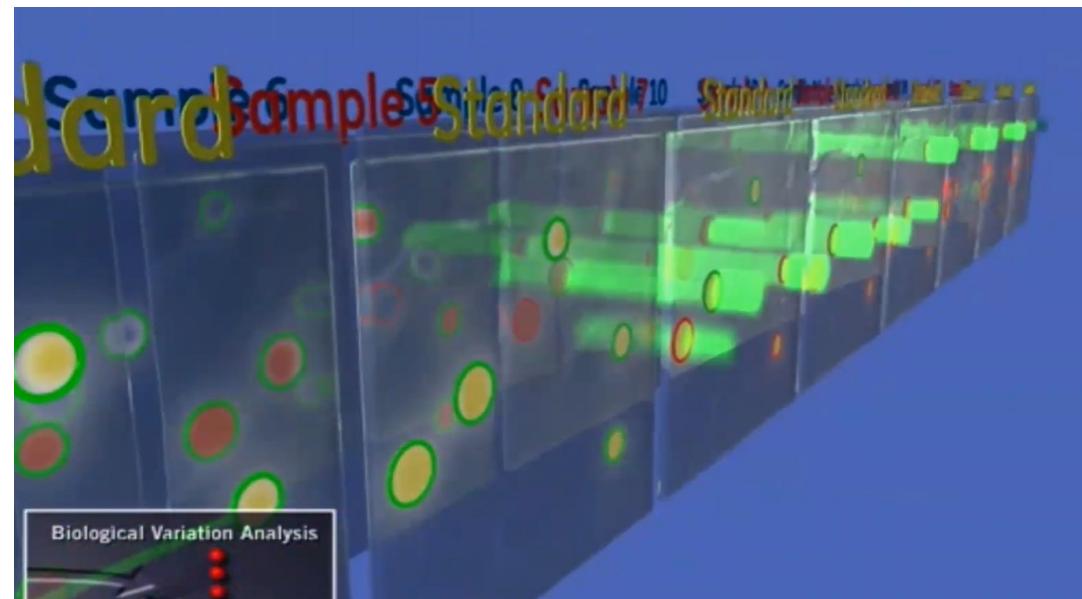
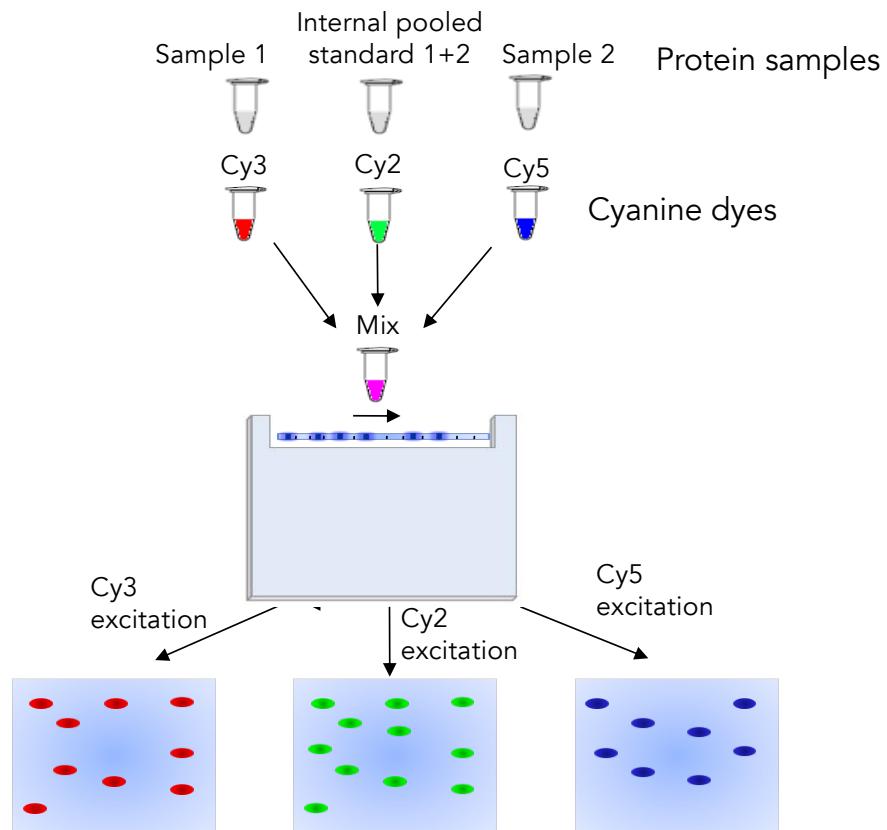
## 2-D Electrophoresis



- First dimension:
  - Separates proteins on pH gradient based on isoelectric point (pl) using isoelectric focusing
- Second dimension:
  - Following IEF, proteins are resolved according to their MW using SDS-PAGE

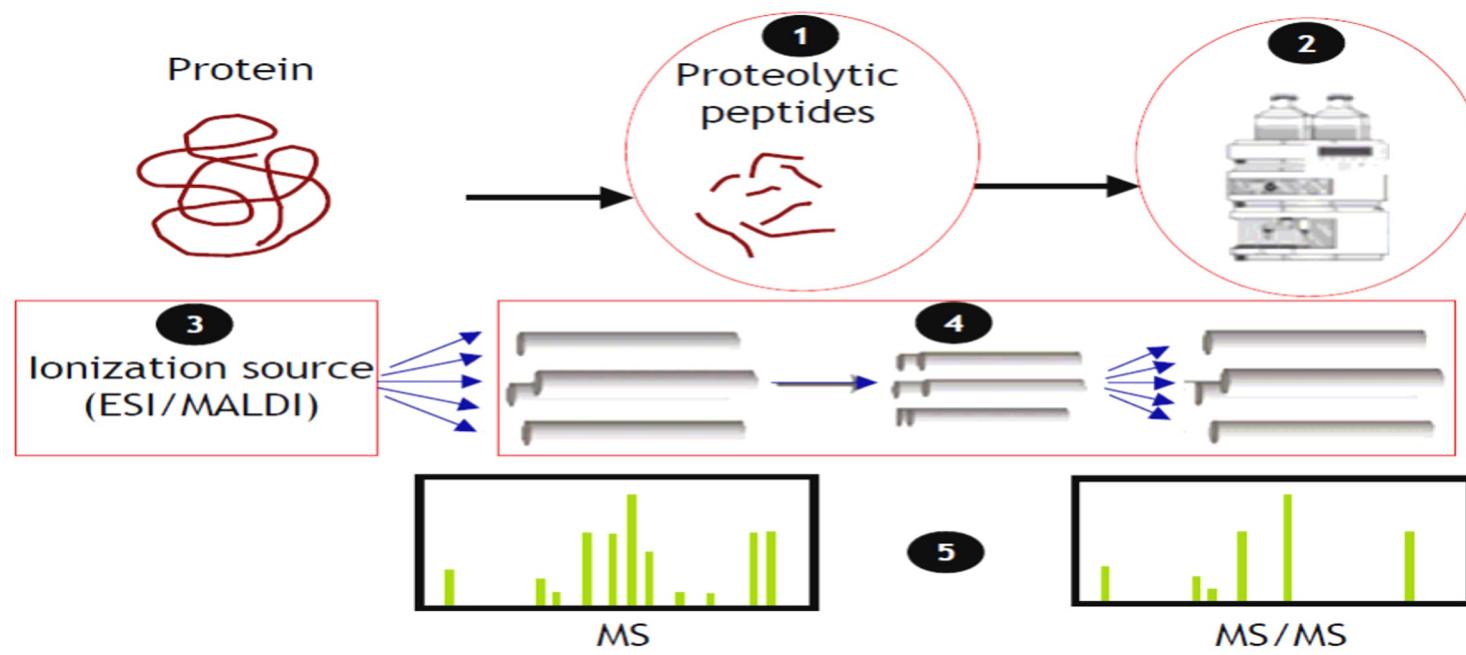
# Two Dimensional Electrophoresis for Quantitative applications

## Difference Gel Electrophoresis (DIGE)



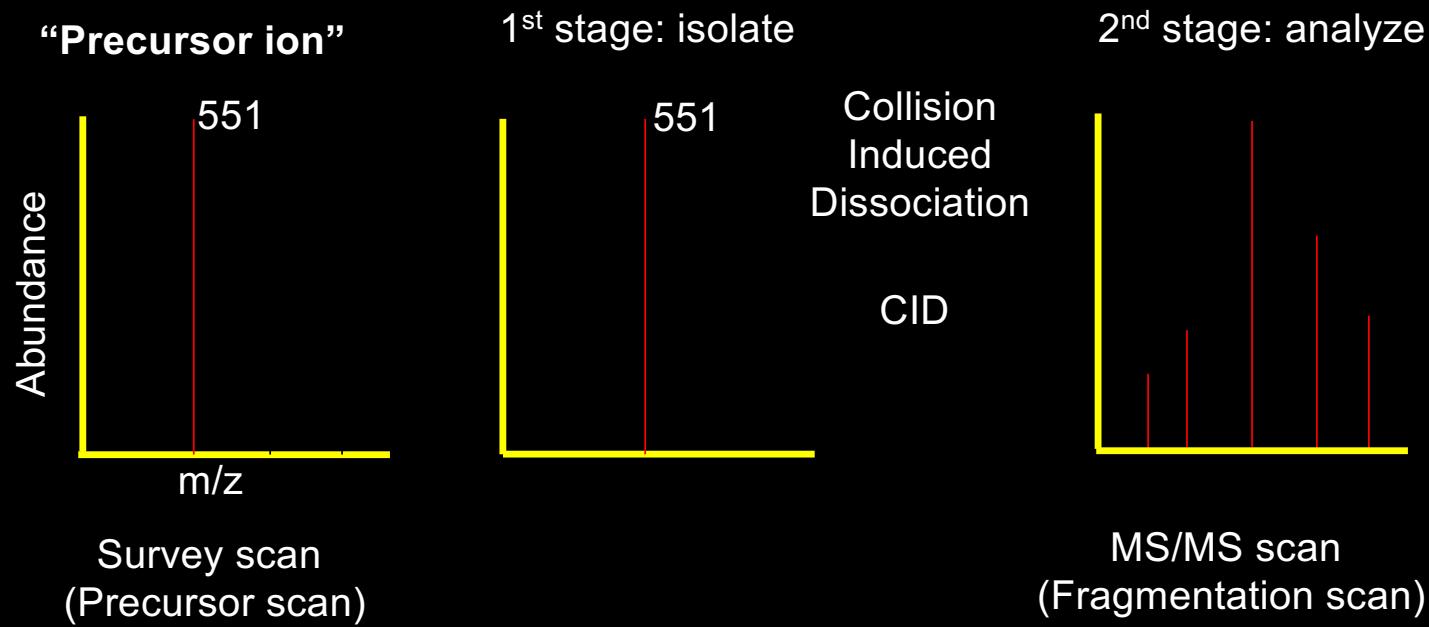
# Mass Spectrometry

Technique for protein identification and analysis by production of charged molecular species in vacuum, and their separation by magnetic and electric fields based on mass-to-charge ( $m/z$ ) ratio

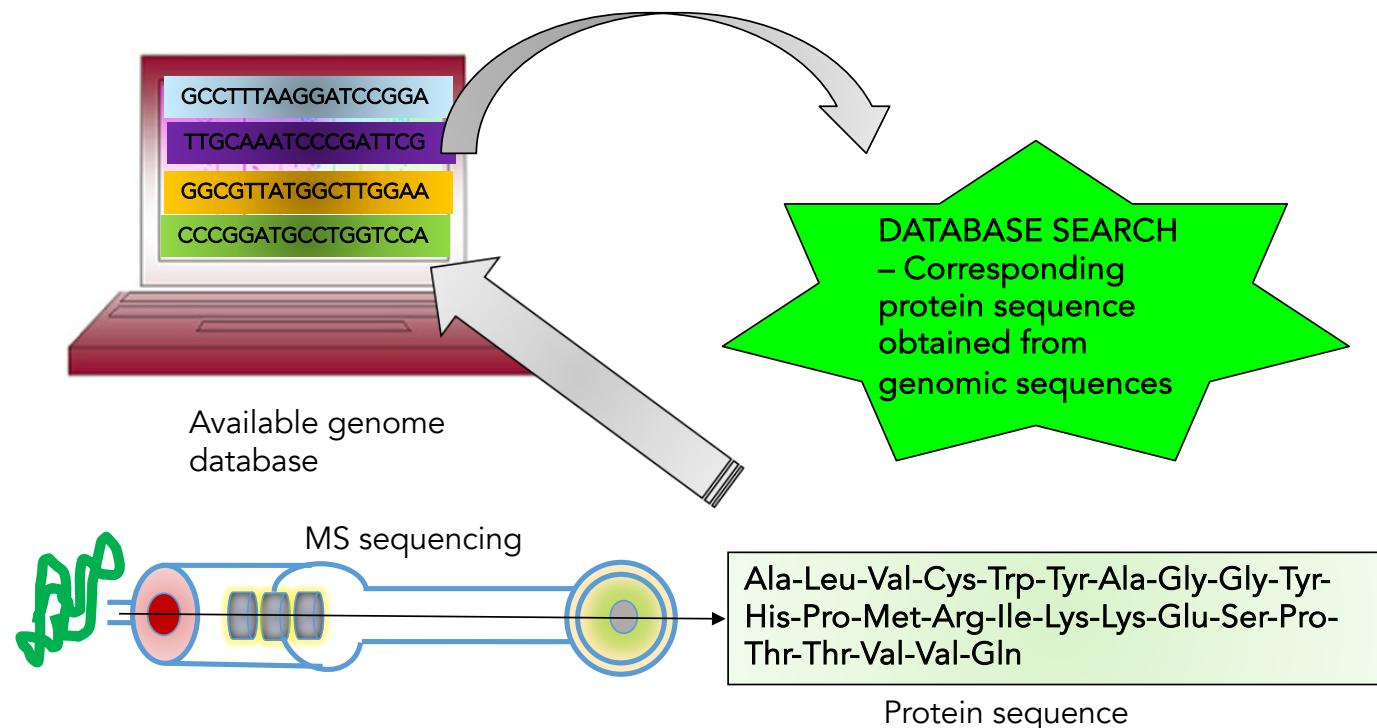


# Tandem MS or (MS/MS)

*Two consecutive stages of mass analysis to detect fragment ions*



# Completion of Genome Sequence Projects & Opportunity for proteomics

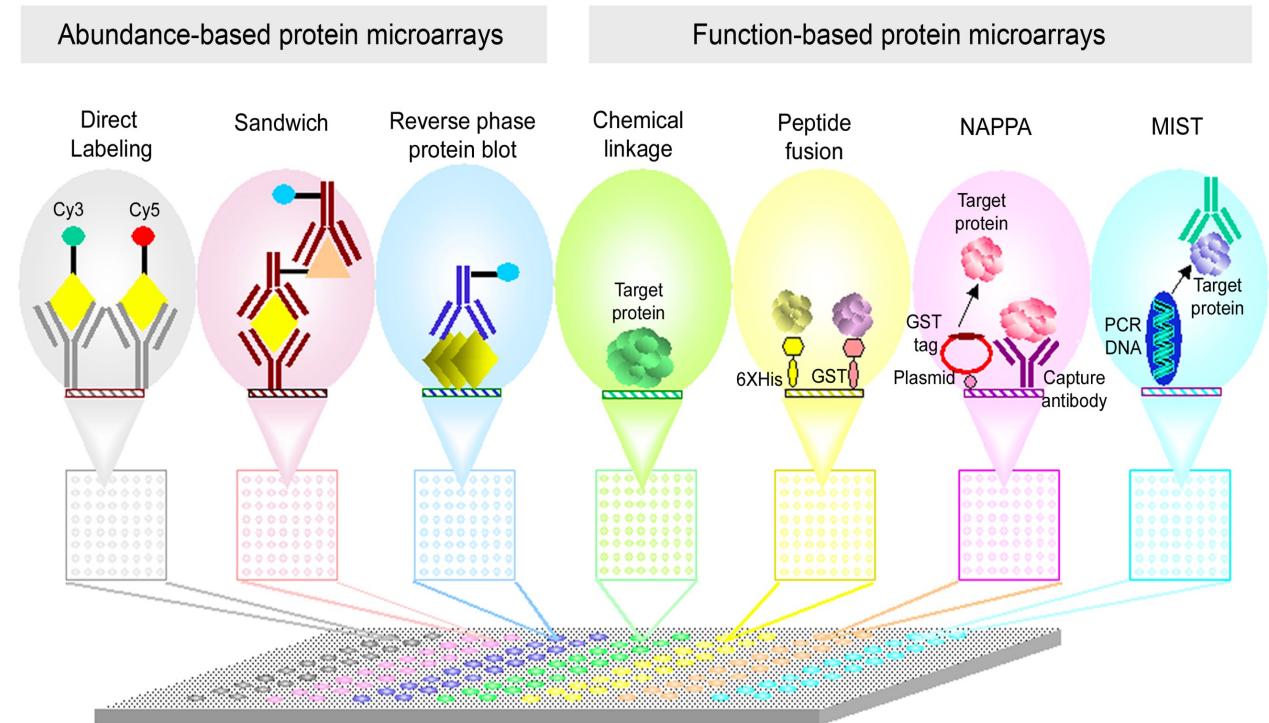


- Genome databases help in identifying gene sequence of a protein that has been sequenced by mass spectrometry.

# HT platforms for biomolecular interactions: Microarrays & SPR

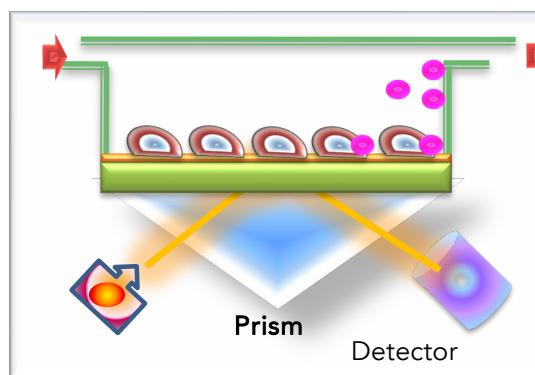
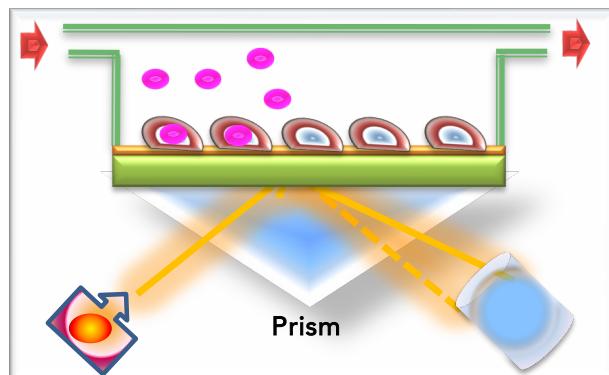
# An Overview of Microarray Platforms

- Microscopic arrays comprising thousands of discrete proteins
- High throughput platform for biomarker discovery, protein-protein interactions & functional characterization

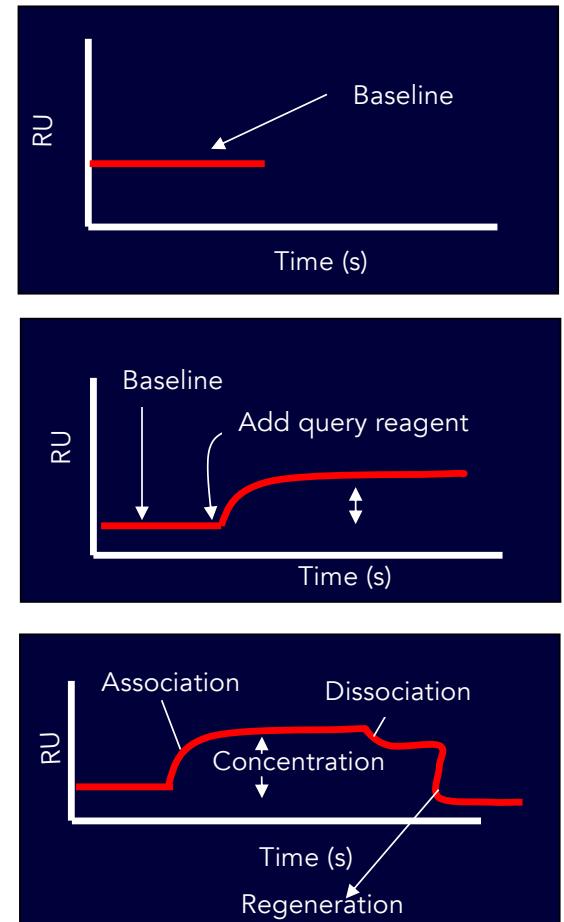


# Surface Plasmon Resonance (SPR)

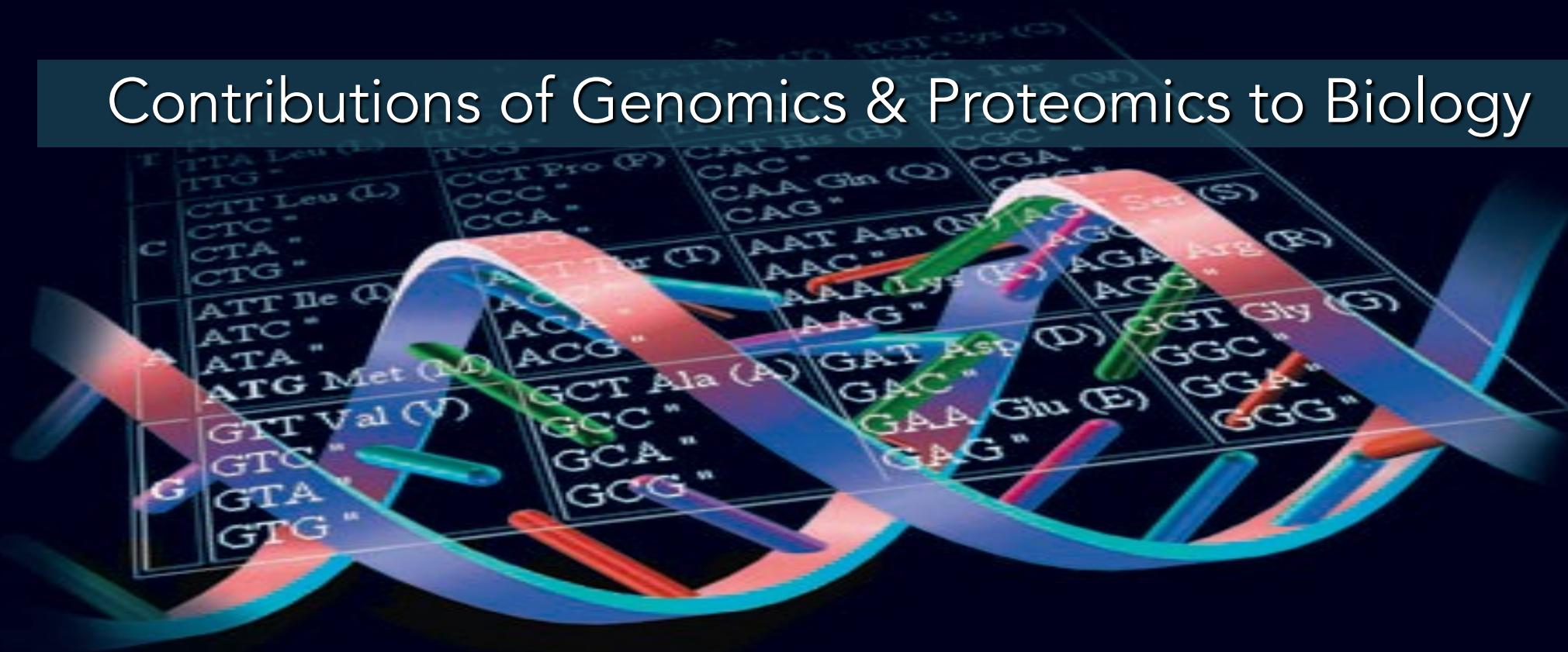
- Measures change in refractive index of medium directly in contact with sensor surface (e.g. gold)
- Medium in contact with surface is commonly an aqueous sample containing analyte “protein”



- Sensorgram - changes in SPR signal versus time



# Contributions of Genomics & Proteomics to Biology



- Proteome: set of all the proteins expressed by a genome
- Proteomics: study of proteins and their properties to provide an integrated view of cellular processes

# Genomics & Proteomics for Biological Applications



Figure 5.26<sub>21</sub>

# Genomics & Proteomics for Biological Applications

## Species Interactions



Figure 5.26<sub>22</sub>

# Genomics & Proteomics for Biological Applications



Figure 5.26<sub>23</sub>

# Genomics & Proteomics for Biological Applications

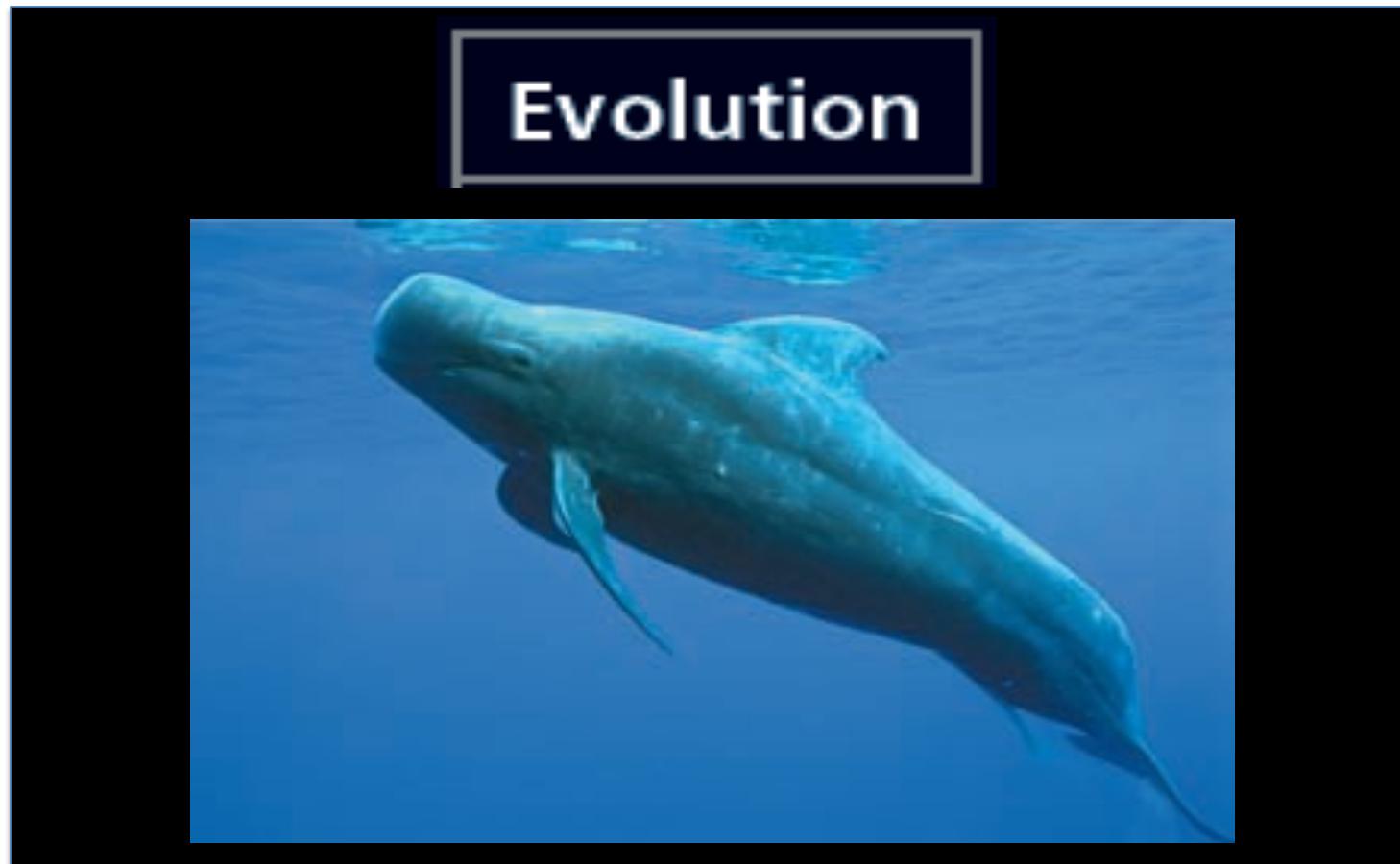


Figure 5.26<sub>24</sub>

# Genomics & Proteomics for Biological Applications

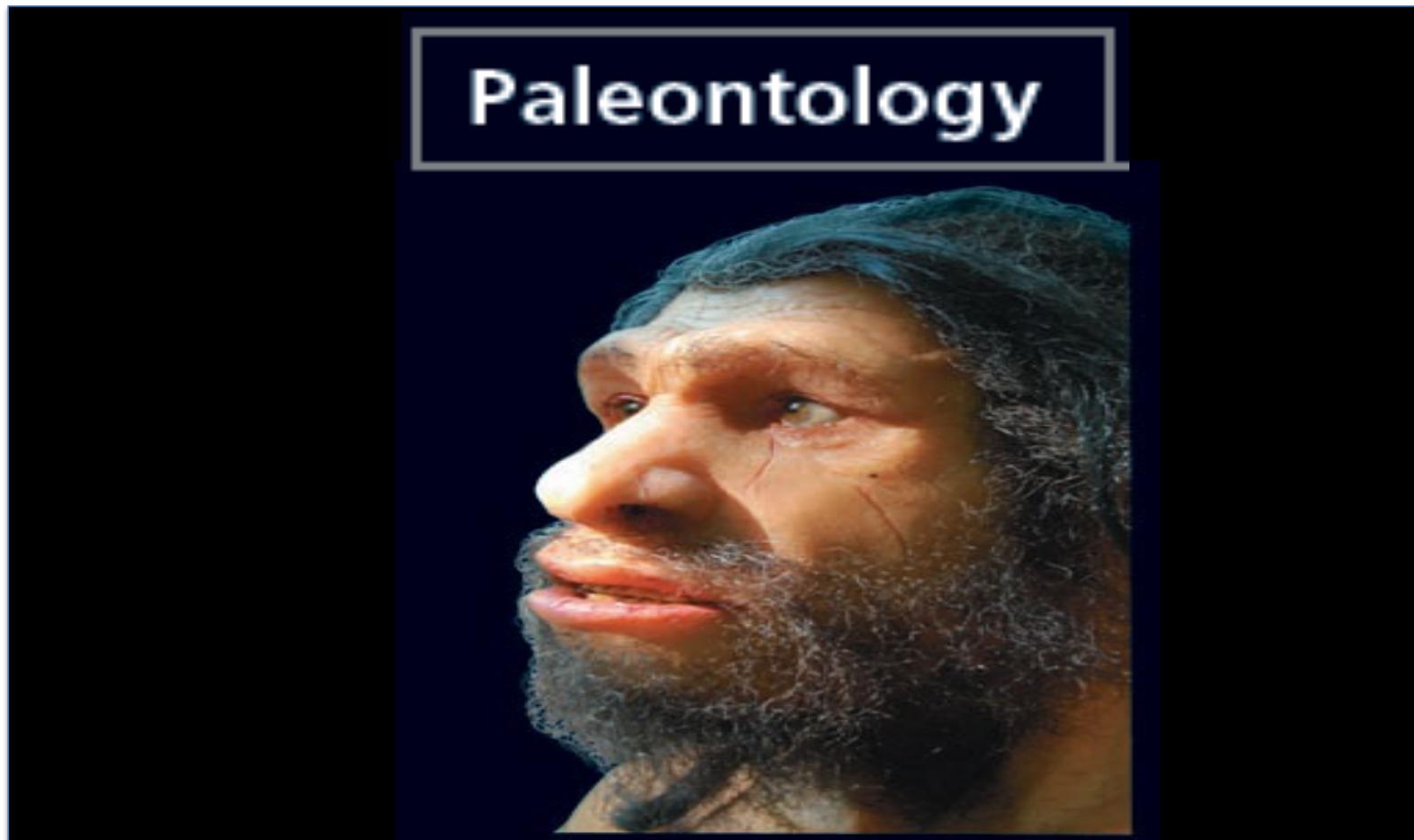


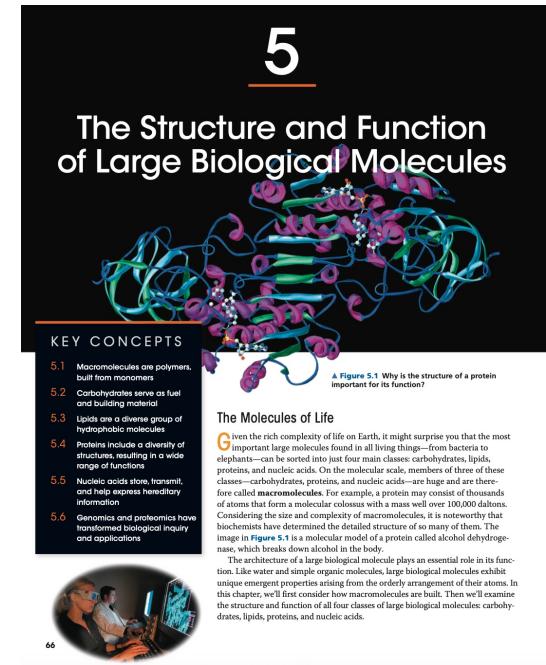
Figure 5.26<sub>25</sub>

# Summary

- Molecular medicine is progressing beyond classical genetics to genomics and proteomics
- Advent of novel technologies to study proteins has facilitated study of dynamic proteome
- Understanding physiological processes using OMICS technologies may have significant impact for functional biology & translational research

# References

- Campbell Biology - Reece, Urry, Cain, Wasserman, Minorsky, Jackson  
10th Edition, Pearson
- Wilmut, I., et al., Nature, 385: 264-267 (1997)
- *Acknowledgment*
  - Cover images – getty images



## Next Lecture... *Metabolism*

# Metabolic pathway chart

