

# TECHNIQUES IN PROTEOMICS

*A review to proteomics common methods*

# Definitions

- **Proteome:** The set of all expressed proteins in a cell, tissue, or organism.
- **Proteomics:** A science that focuses on the study of proteins (their roles, their structures, their localization, their interactions, and other factors).
- SO → there should be methods to understand these items!



# Proteomics techniques

- **Molecular techniques**
- **Separation techniques**
- **Protein Identification techniques**
- **Protein Structure techniques**



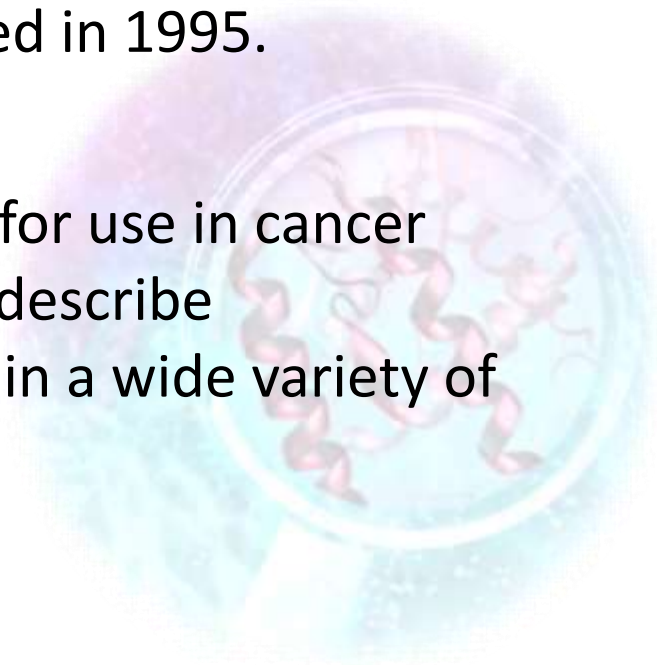
# Molecular techniques

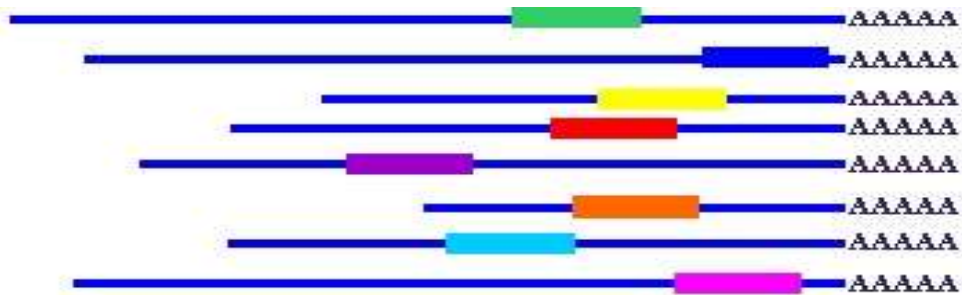
- I. DNA Microarrays or Gene Chips
- II. Differential Display\*
- III. Northern/Southern Blotting
- IV. RNAi (small RNA interference)
- V. Serial Analysis of Gene Expression (SAGE)\*
- VI. Yeast two-hybrid analysis



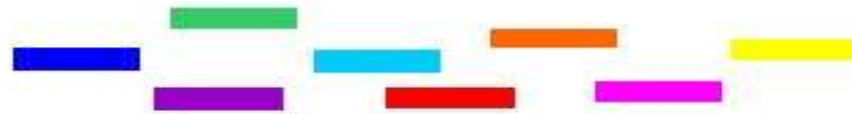
# SAGE

- a technique used to produce a snapshot of the messenger RNA population in a sample of interest in the form of small tags that correspond to fragments of those transcripts, developed by Dr. Victor Velculescu at the Oncology Center of Johns Hopkins University and published in 1995.
- Although SAGE was originally conceived for use in cancer studies, it has been successfully used to describe the transcriptome of other diseases and in a wide variety of organisms.





Isolate SAGE tags



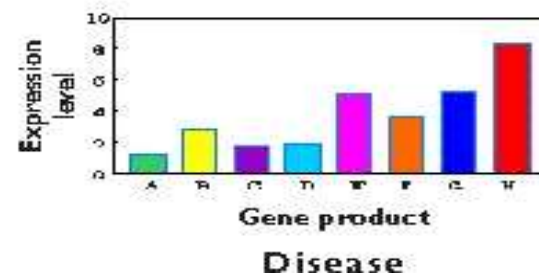
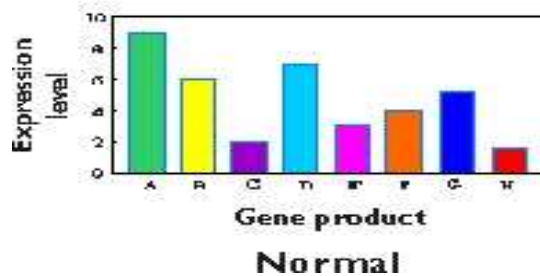
Link tags together

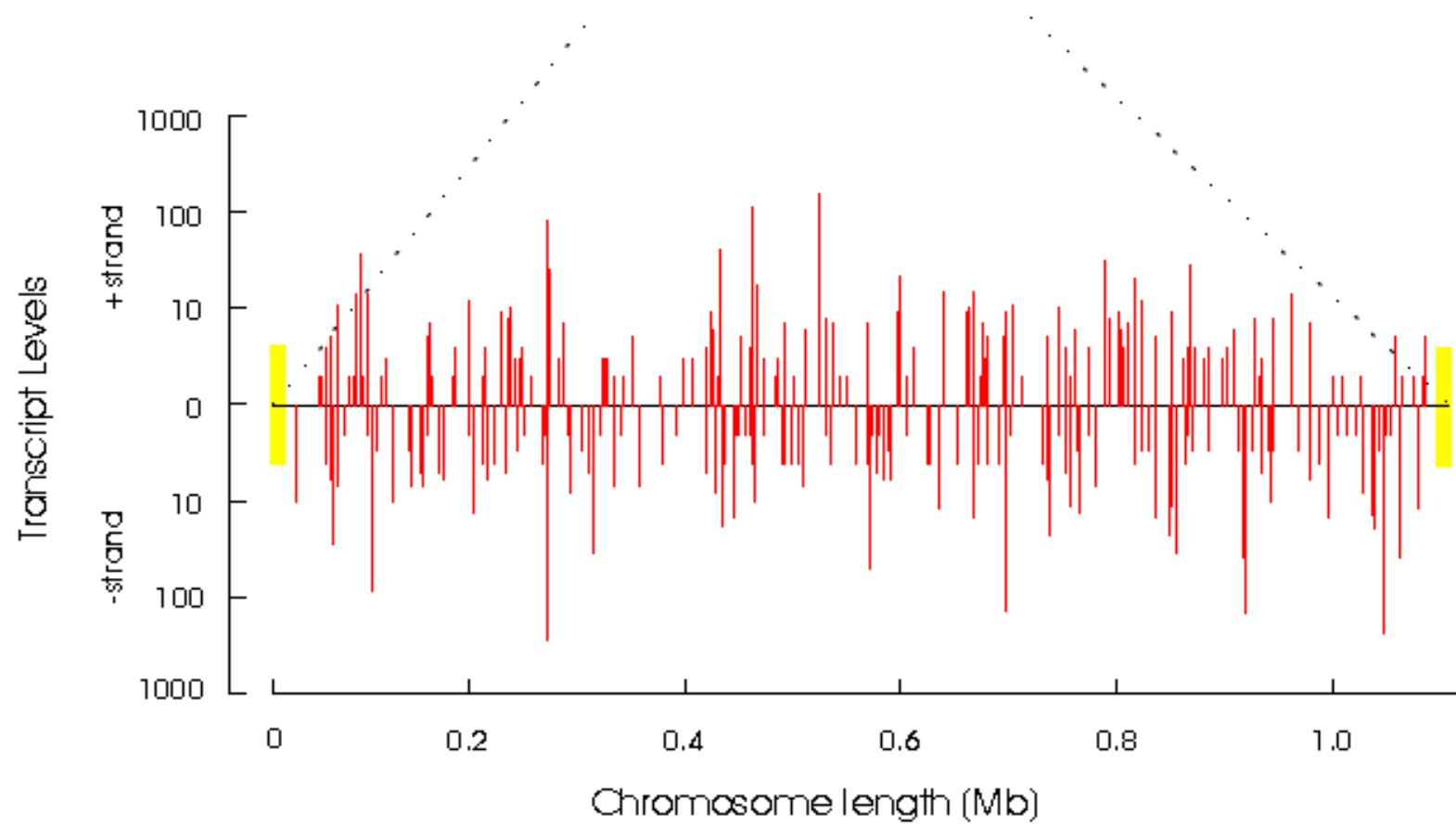


Sequence linked tags



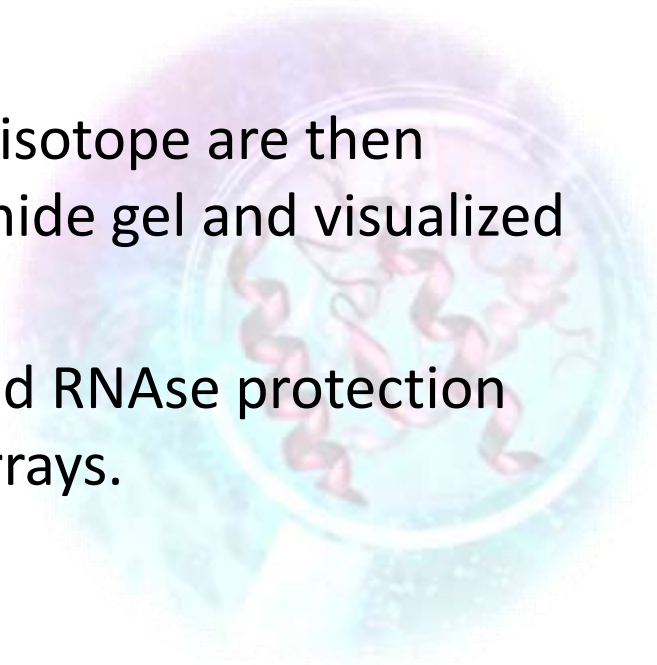
Quantitate tags and determine patterns of gene expression





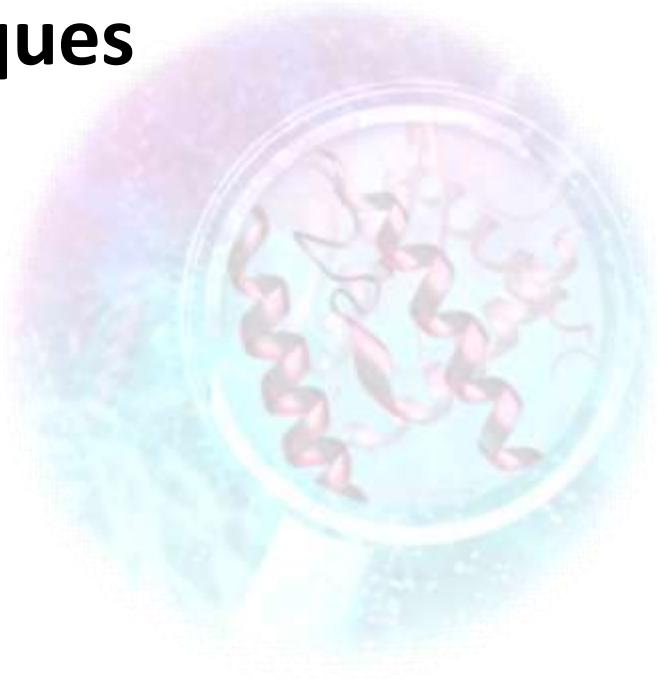
# Differential Display (DD-PCR)

- Since its invention in the early 1990s, differential display has become one of the most commonly used techniques for identifying differentially expressed genes at the mRNA level.
- The essence of the method is to amplify messenger RNA 3' termini using a pair of anchored oligo-dT primer and a short primer with an arbitrary sequence.
- The amplified cDNAs labeled with radioisotope are then distributed on a denaturing polyacrylamide gel and visualized by autoradiography.
- In the mid-2000s, differential display and RNase protection assay were superseded by DNA Microarrays.



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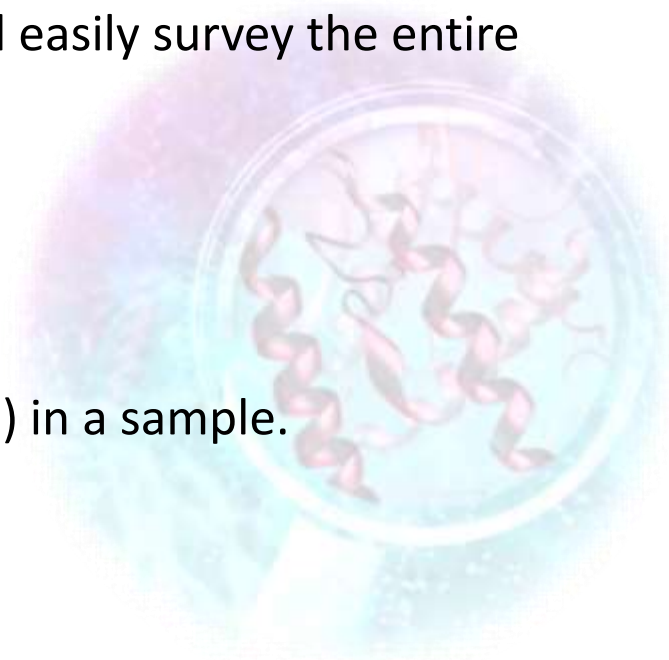
# Separation techniques

- I. 1D Slab Gel Electrophoresis
- II. 2D Gel Electrophoresis (SDS-PAGE/IEF)
- III. Capillary Electrophoresis
- IV. Chromatography (HPLC ,SEC, IEC, RP, Affinity, etc.)
- V. Protein Chips (Protein microarray)\*

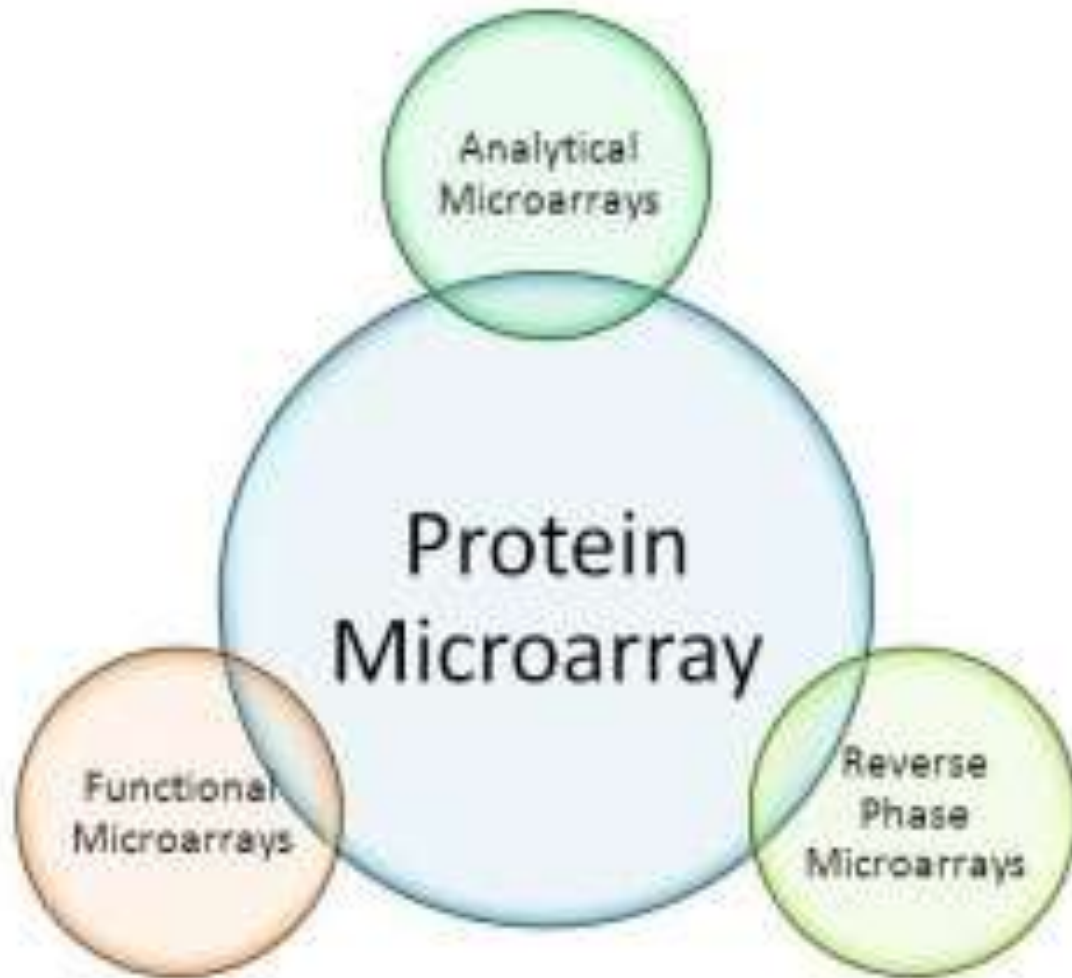


# Protein array(protein chip)

- They are modeled after DNA microarrays, in 2000 at Harvard University
- The success of DNA microarrays in large-scale genomic experiments inspired researchers to develop similar technology to enable large-scale, high-throughput proteomic experiments.
- Protein chips enable researchers to quickly and easily survey the entire proteome of a cell within an organism.
- **Applications include:**
  - identifying biomarkers for diseases,
  - investigating protein-protein interactions,
  - testing for the presence of a protein(i.e. Ab) in a sample.

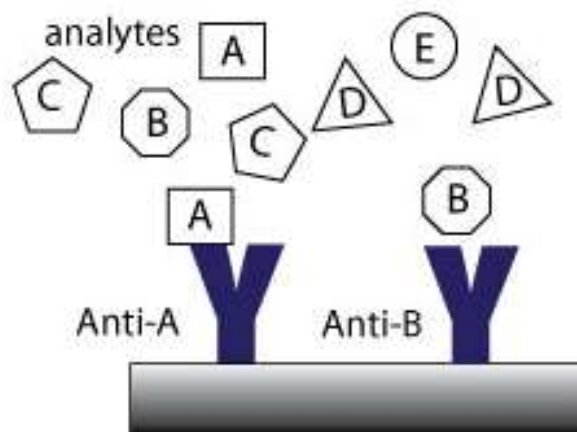


# Types of protein arrays



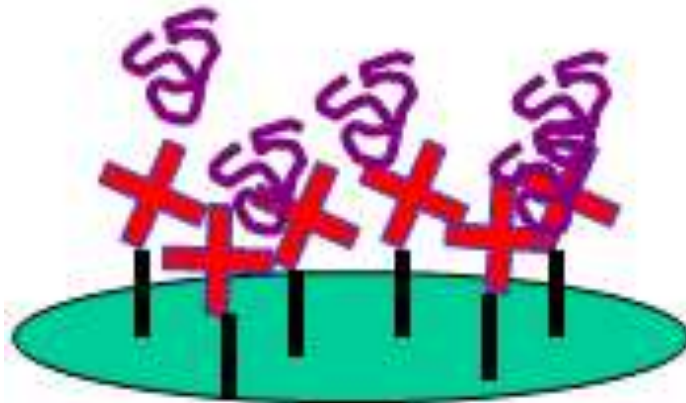
# 1. Analytical microarrays (capture arrays)

- Used to understand:
  - expression levels,
  - binding affinities and specificities,
  - response of the cells to a particular factor,
  - identification and profiling of diseased tissues.



## 2. Functional protein microarrays (target protein arrays)

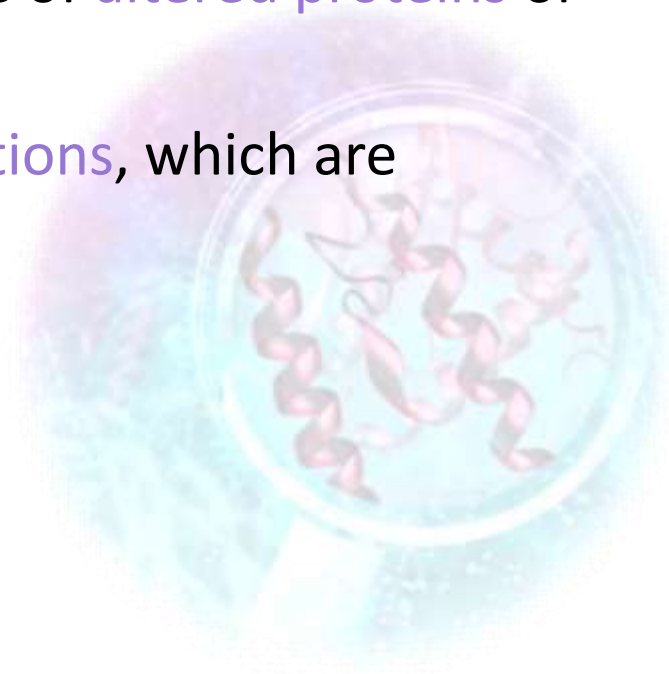
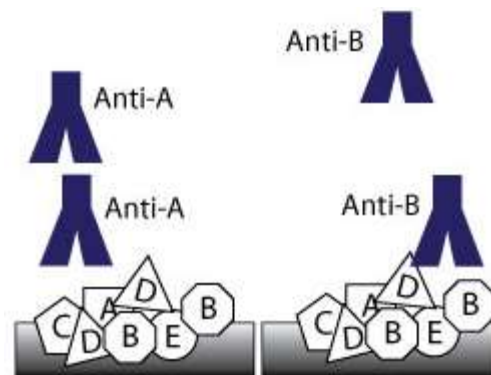
- Immobilised purified proteins are used to:
  - identify protein-protein/DNA/RNA/PL/SM,
  - assay enzymatic activity.
- They differ from analytical arrays in that they contain full-length **functional proteins**.



### 3. Reverse phase protein microarray (RPPA)

- involve complex samples, such as tissue lysates probed with antibodies against the target protein of interest.
- These antibodies are typically detected with chemiluminescent, fluorescent or colorimetric assays.
- Used to: determination of the presence of **altered proteins** or other agents as a result of disease.

Specifically, **post-translational modifications**, which are typically altered as a result of disease.



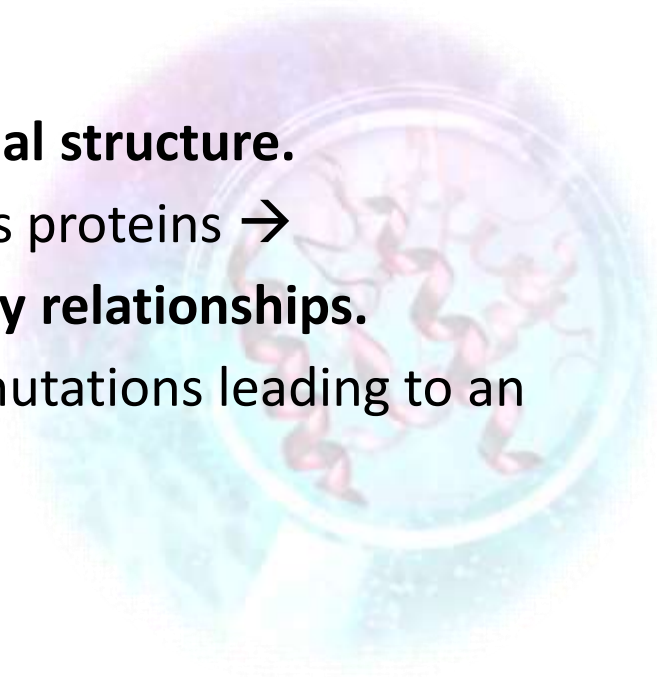
# Proteomics techniques

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# Protein Identification techniques

- I. Edman sequencing
  - II. Microsequencing
  - III. Mass spectroscopy
- Sequencing done for:
    1. Protein's amino acid → **three-dimensional structure.**
    2. Sequence comparisons among analogous proteins → **protein function and reveal evolutionary relationships.**
    3. Many inherited diseases are caused by mutations leading to an amino acid change in a protein.



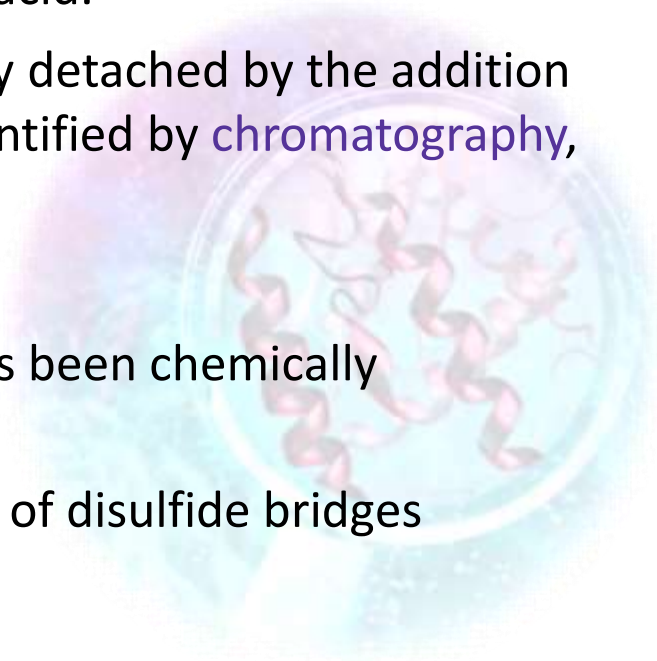
# Edman sequencing

- **Mechanism:**

- The peptide to be sequenced (50 aa) is adsorbed onto a solid surface - one common is **glass fibre coated with polybrene, a cationic polymer**.
- The Edman reagent, **phenylisothiocyanate (PITC)** , is added to the adsorbed peptide, together with a mildly basic buffer solution. This reacts with the amine group of the N-terminal amino acid.
- The terminal amino acid can then be selectively detached by the addition of **anhydrous acid**. It can be washed off and identified by **chromatography**, and the cycle can be repeated.

- **Limitations:**

- it will not work if the N-terminal amino acid has been chemically modified.
- separate procedure to determine the positions of disulfide bridges needed.

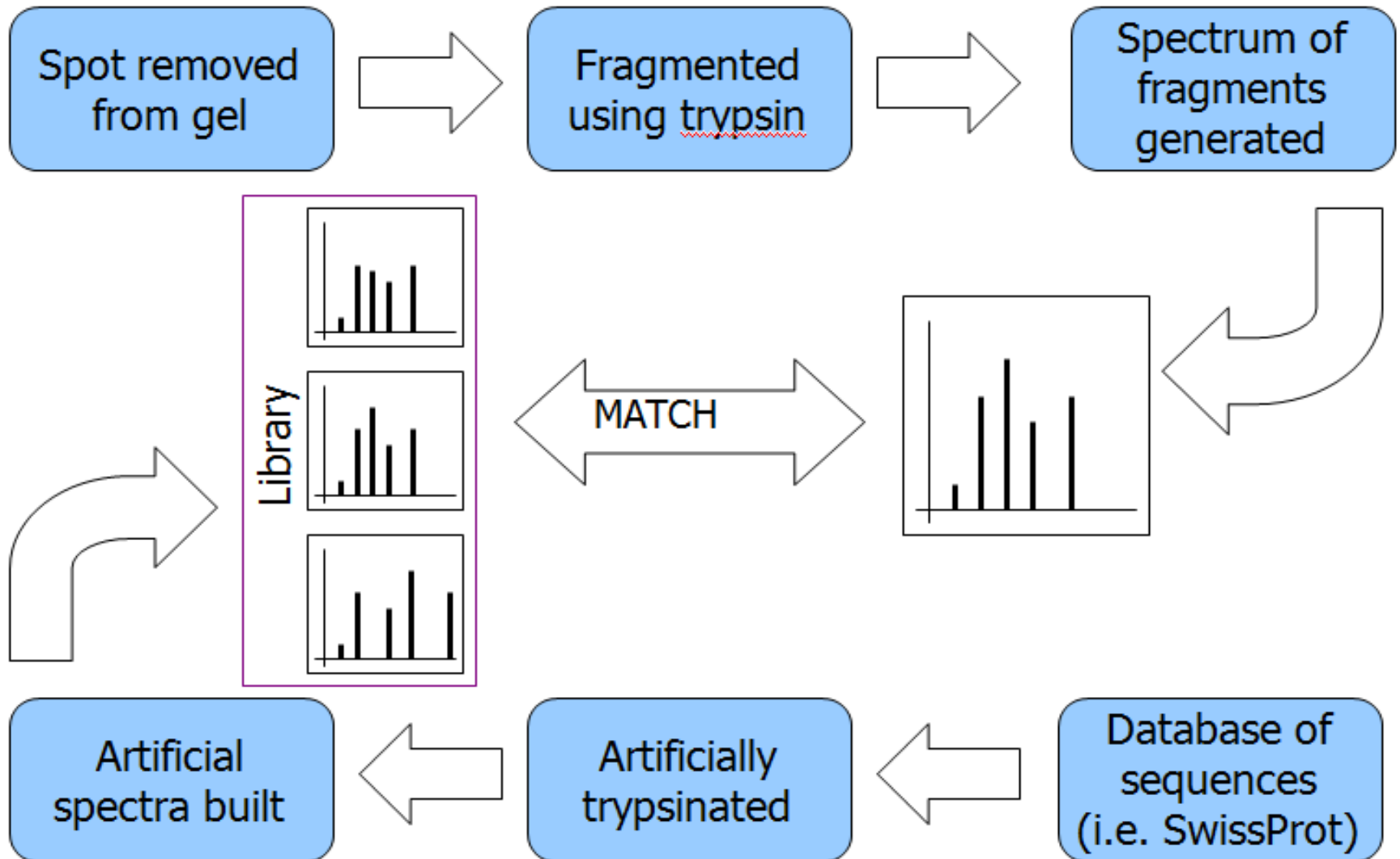


# Microsequencing

- Automated process of edman reaction.
- (A Beckman-Coulter Porton LF3000G protein sequencer.)



# Mass spectrometry



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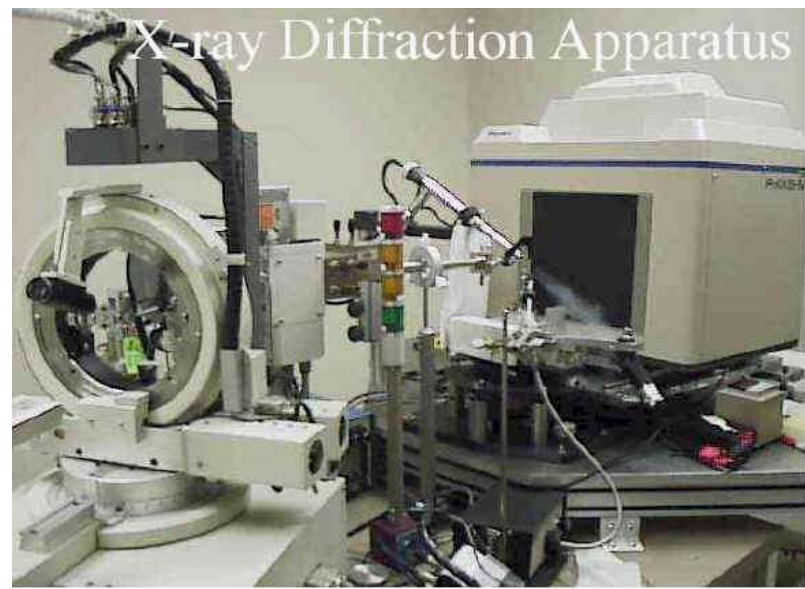


# Protein Structure techniques

- I. NMR(Nuclear magnetic resonance spectroscopy)
- II. X-ray crystallography
- III. Computational prediction



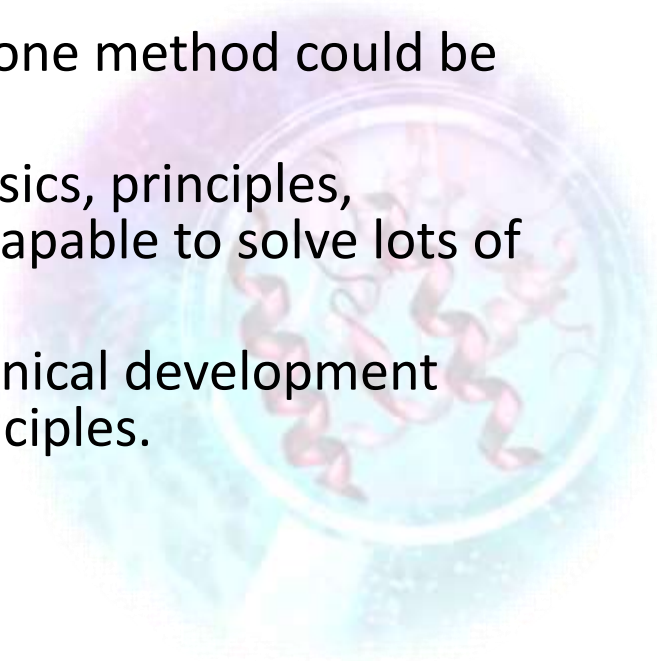
NMR



X-ray crystallography

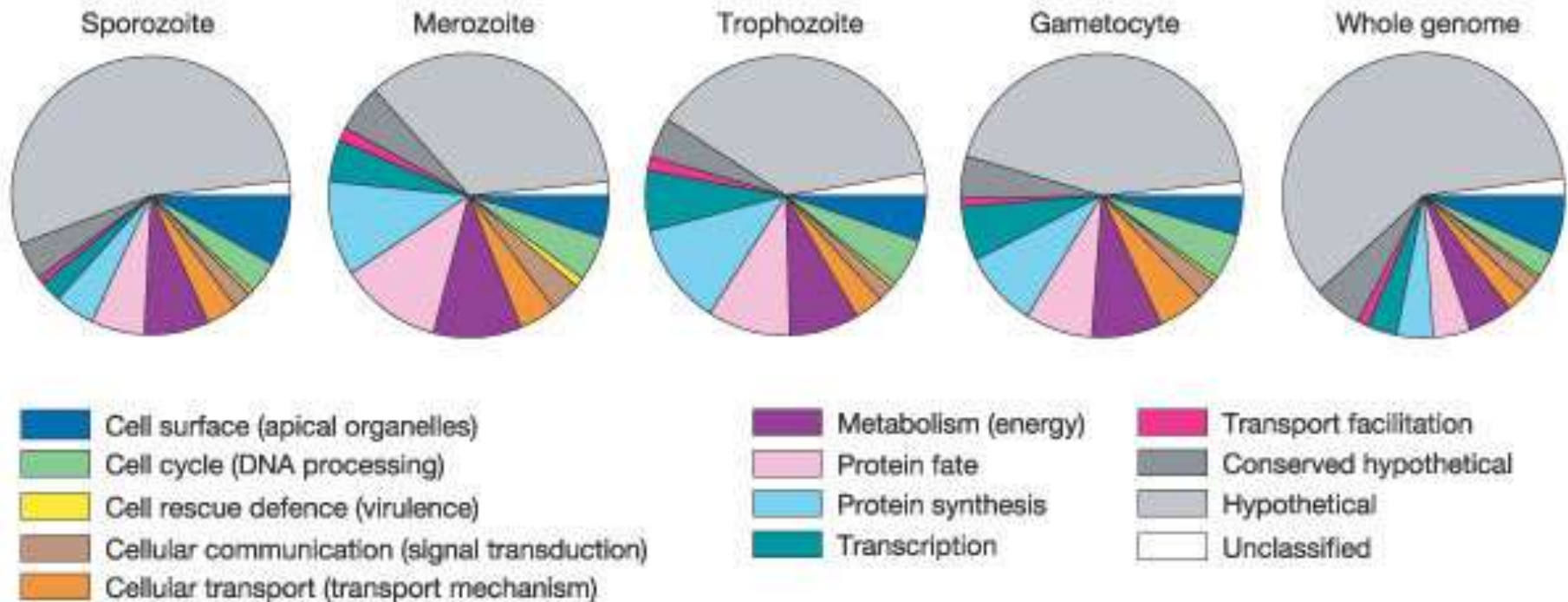
# NMR and X-ray crystallography help

- the two methods are very basic and critical in the field of protein structure determination.
- The strengths and weaknesses of one of the two methods fortunately supplement the holes and gaps in the other method to make it possible that different kind of important data for a structural question can be answered with the parallel or supplemental application of the two methods.
- There may be several instances when only one method could be used.
- If the two methodologies had the same basics, principles, strengths and weaknesses we may be not capable to solve lots of structural problems.
- We should thank this possibility to the technical development which could catch different biophysical principles.



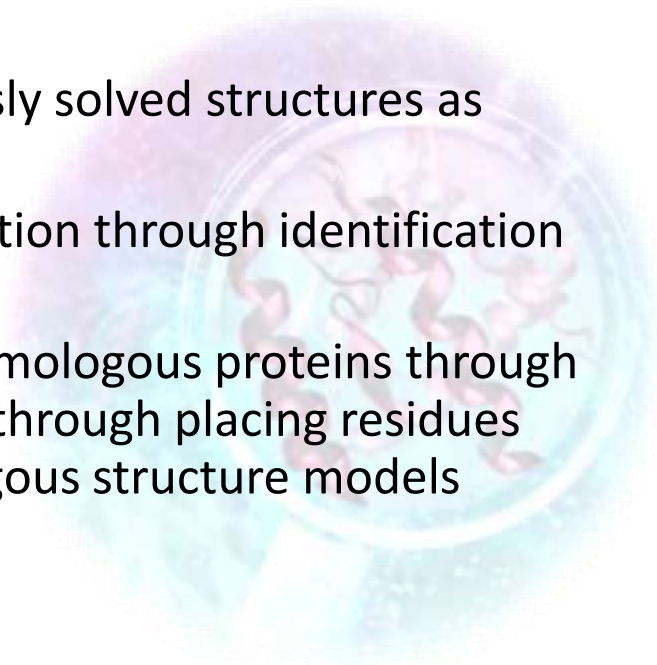
# An example:

## Analysis of *Plasmodium falciparum* (malaria parasite) proteome

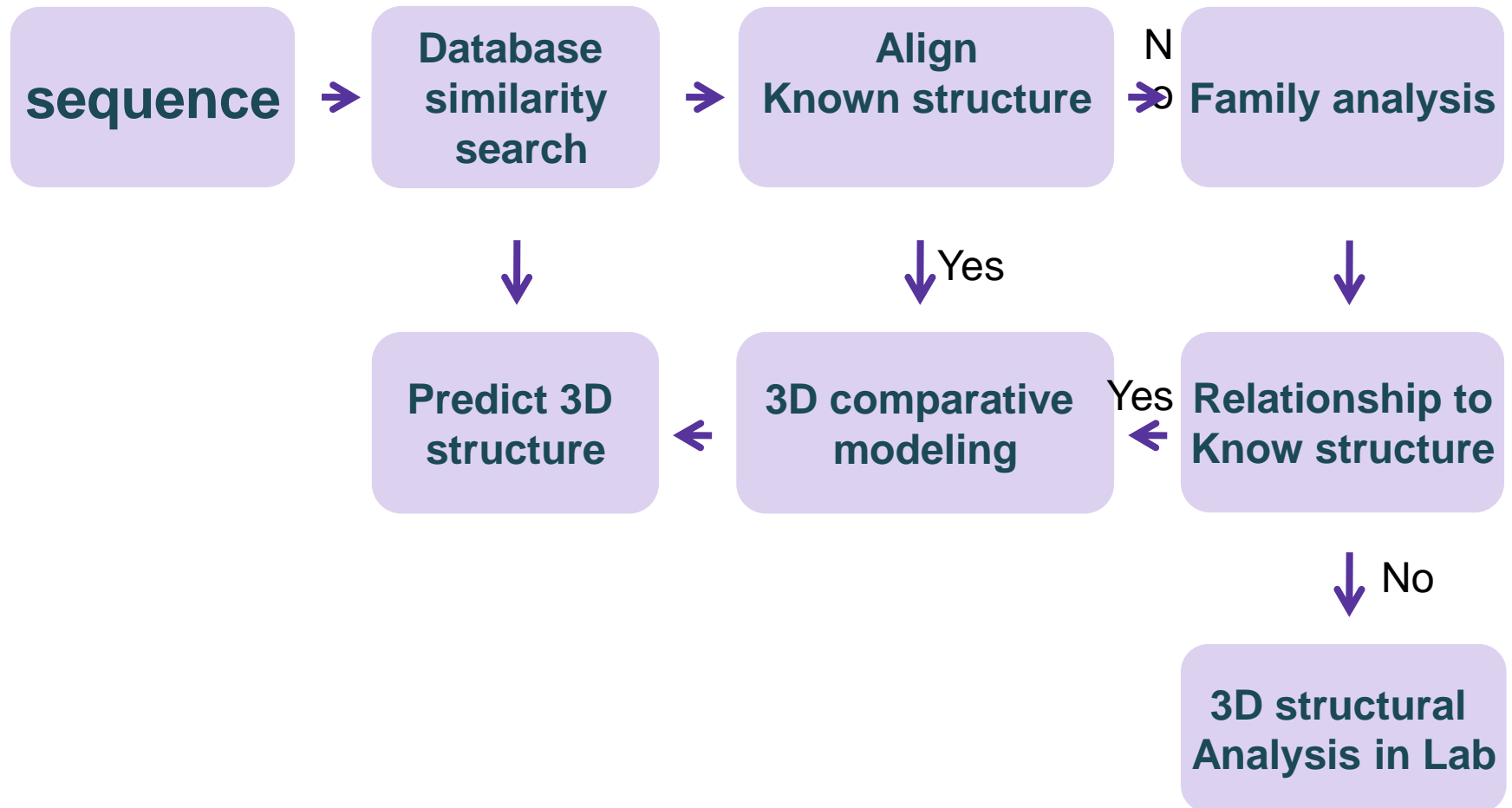


# Computational prediction

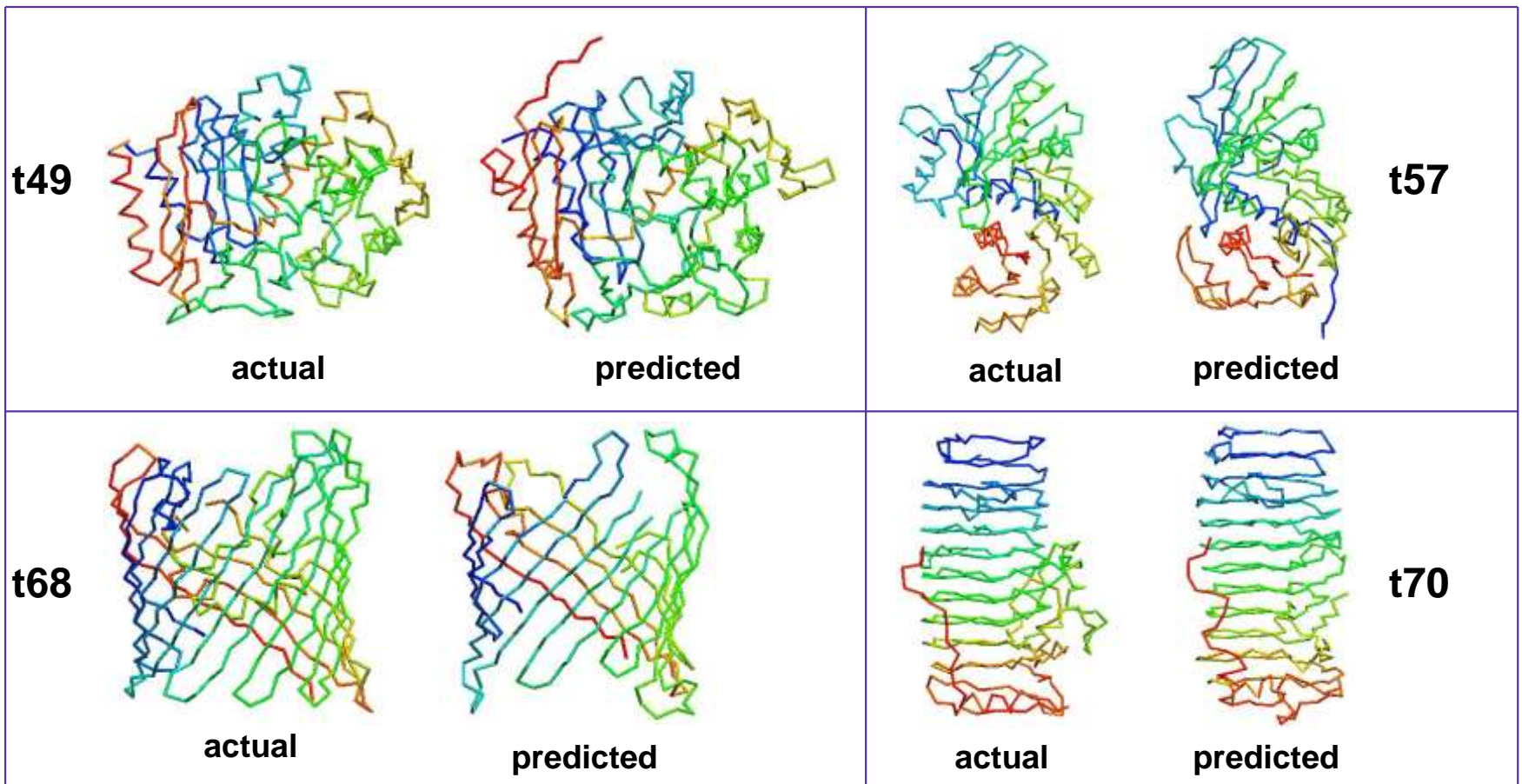
- in theory, a protein structure can be solved computationally as it folds into a 3D structure to minimize its potential energy.
- ***ab initio/de novo* folding methods:** not practical (yet) due to its high computational complexity. These procedures tend to require vast computational resources, and have thus only been carried out for tiny proteins
- **Comparative modeling methods:** uses previously solved structures as starting points, or templates.
  - a. **Protein threading** – make structure prediction through identification of “good” sequence-structure fit
  - b. **Homology modeling** – identification of homologous proteins through sequence alignment; structure prediction through placing residues into “corresponding” positions of homologous structure models



# Procedure of Prediction

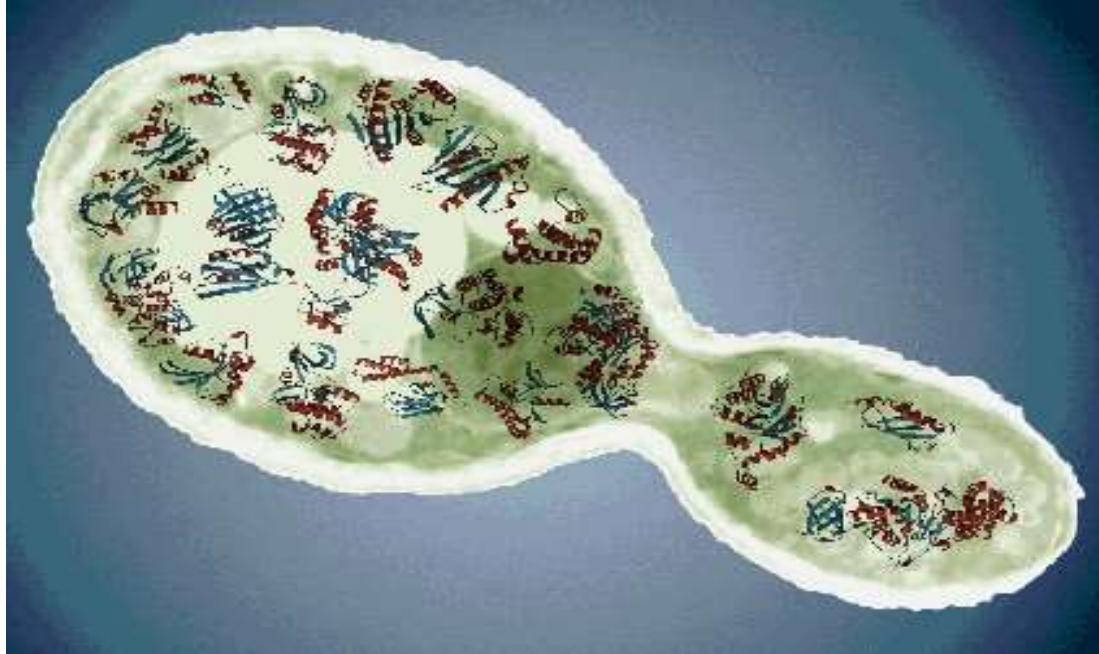


# PROSPECT Predictions



# The goal

“The ultimate goal of systems biology is the integration of data from these observations into models that might, eventually, represent and simulate the physiology of the cell.”



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**THANK YOU**