Exploring 3D Molecular Structures with iCn3D

Alexa M. Salsbury, Ph.D.

NIH National Library of Medicine
National Center for Biotechnology Information

Overview

- Background
- iCn3D Fundamentals
  - Selection
  - Coloring
  - Style
- Interaction Networks
- Mutation Analysis
- iCn3D + AlphaFold
- Office hours

Background: https://www.nlm.nih.gov/ncbi/workshops/2023-03_3d-molecular-structures/background.html

Structural Biology

1952-1953- Pioneering DNA structure work by Wilkins, Franklin, Watson, & Crick.

1956-1960- Rich & Davies' structural experiments showed how information could be transferred from DNA to RNA.

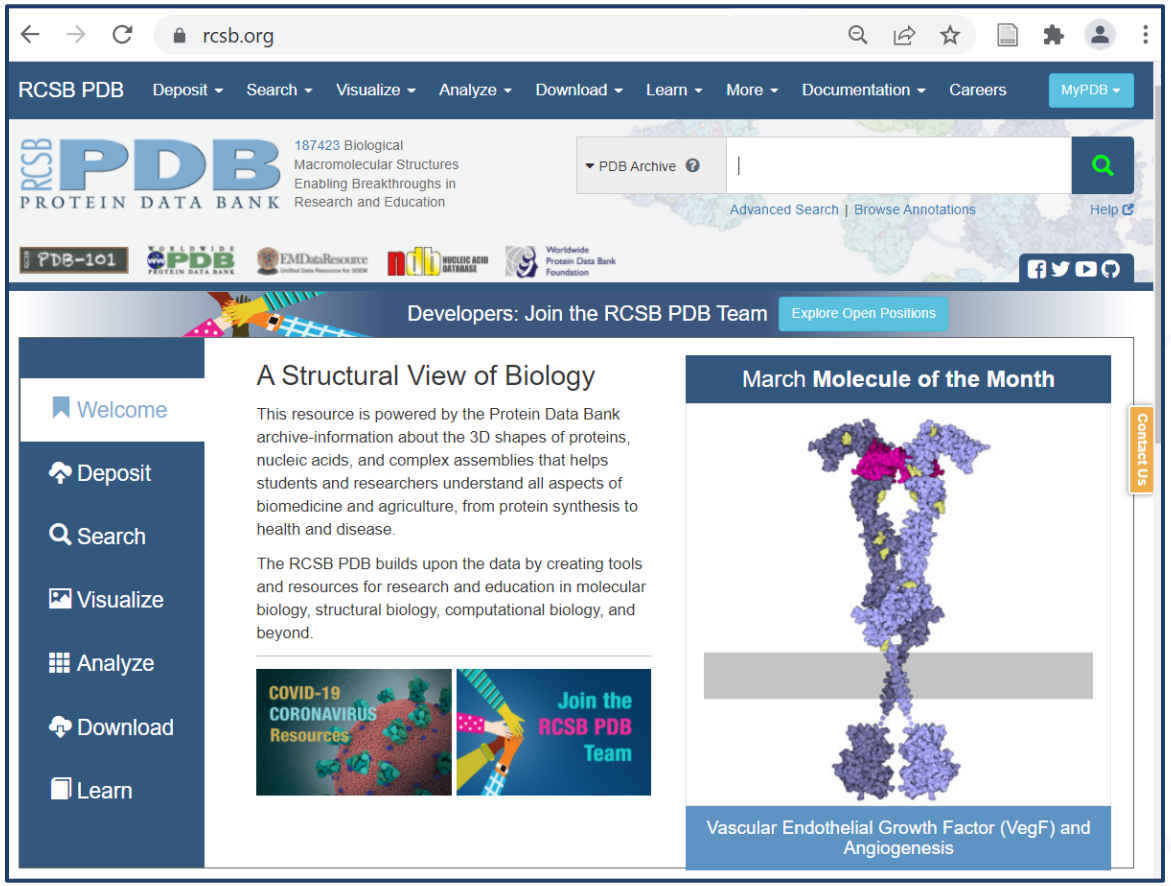
1957- The first protein with a crystal structure was solved in by Kendrew and co-workers

Now- over 175,000 structures are publicly available and structure prediction is improving!

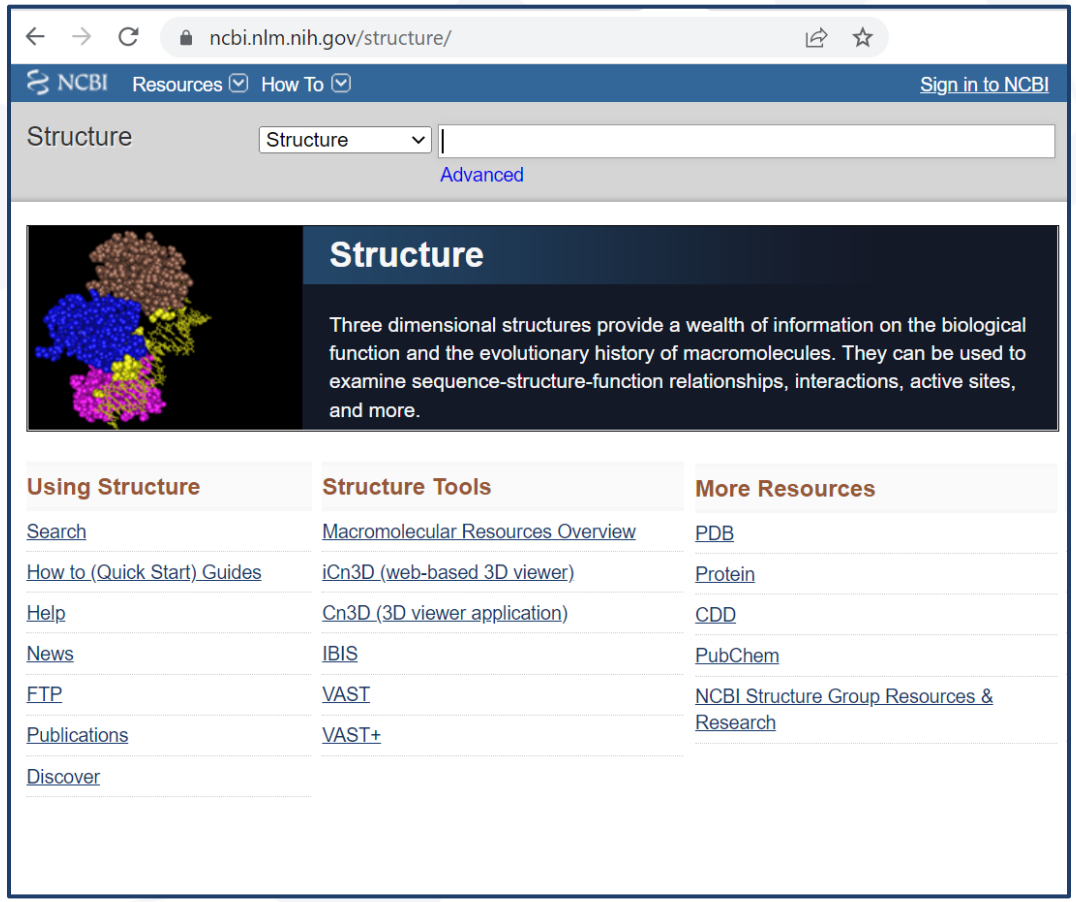
Experimental techniques

	Advantages	Disadvantages
X-ray crystallography	• Well developed • High resolution • Broad molecular weight range	• Difficult sample prep • Static crystalline state
NMR	• High resolution • 3D structure in solution • Good for dynamic study	• Difficult sample prep • High sample purity needed • Static crystalline state captured
Cryo-EM	• Simple sample prep • Structure in native state • Small sample size needed	• Lower resolution • Works best for samples with high molecular weight • Equipment can be expensive, but costs are decreasing

Where do I find experimentally determined structures?



RCSB Protein Data Bank



NCBI Structure Database

Protein Data Bank (PDB)

- New Structures are deposited daily

Each structure contains:

- 3D atomic coordinates
- Mandatory Metadata
  - Author Information
  - Primary citation
  - Experimental Data
  - Polymer sequence(s)- proteins, DNA, RNA
  - Small Chemical component structures- ligands, inhibitors, etc.

Display Files | Download Files

6LU7

The crystal structure of COVID-19 main protease in complex with an inhibitor N3

DOI: 10.2210/pdb6LU7/pdb

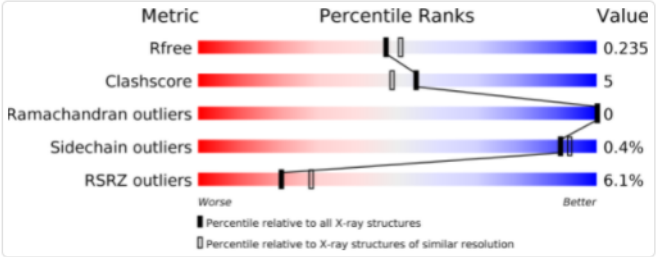
Classification: VIRAL PROTEIN
Organism(s): Severe acute respiratory syndrome coronavirus 2, synthetic construct
Expression System: Escherichia coli BL21(DE3)
Mutation(s): No

Deposited: 2020-01-26 **Released:** 2020-02-05
Deposition Author(s): Liu, X., Zhang, B., Jin, Z., Yang, H., Rao, Z.

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 2.16 Å
R-Value Free: 0.235
R-Value Work: 0.202
R-Value Observed: 0.204

wwPDB Validation | 3D Report | Full Report



Literature | Download Primary Citation

Structure of Mprofrom SARS-CoV-2 and discovery of its inhibitors.

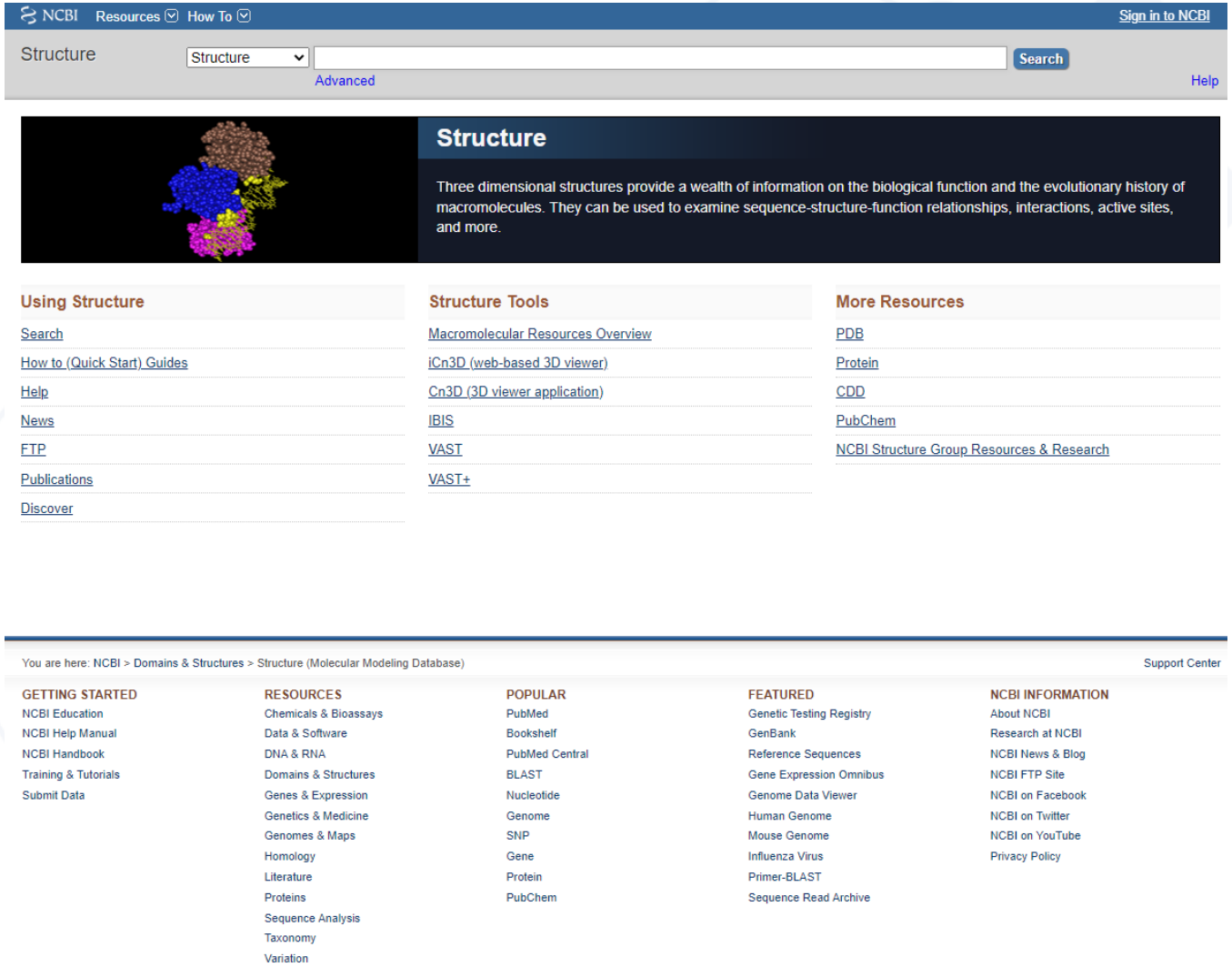
Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., Jiang, R., Yang, X., You, T., Liu, X., Yang, X., Bai, F., Liu, H., Liu, X., Guddat, L.W., Xu, W., Xiao, G., Qin, C., Shi, Z., Jiang, H., Rao, Z., Yang, H.
(2020) Nature **582**: 289-293

PubMed: 32272481 Search on PubMed
DOI: 10.1038/s41586-020-2223-y
Primary Citation of Related Structures:
7BQY, 6LU7

PubMed Abstract:
A new coronavirus, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is the aetiological agent responsible for the 2019-2020 viral pneumonia outbreak of coronavirus disease 2019 (COVID-19) [1-4]. Currently, there are no targeted therapeutic agents for the treatment of this disease, and effective treatment options remain very limited ...

NCBI's Structure Database

- Updated monthly
- Derived from PDB records
- Additional information added, including:
  - Explicit chemical graph information
  - Validation (secondary structure elements)
  - Includes taxonomy
- Connects 3D to associated literature, molecular data, chemical data, and other NCBI tools



iCn3D

- Interactive, web-based 3D structure viewer
  - No installation needed!
- Users can
  - Visualize structure in 1D, 2D, and 3D
  - View sequence and structure alignments
  - Probe perturbations
  - And more!

3D Viewer Feature Comparison

	Web-based	1D Sequence	2D Diagram	Annotation	Align	Share Link	Script	Jupyter Notebook	Virtual Reality	3D Printing
iCn3D	✓	✓	✓	✓	✓[a]	✓[b]	✓[c]	✓[d]	✓	✓
Mol*	✓	✓	Web	Web						
Aquaria	✓	✓		✓					✓	
Chimera		✓		✓					✓	✓
PyMol		✓		✓			✓			
Cn3D		✓	Web	✓	✓					

[a]: iCn3D aligns structures (PDB or AlphaFold) based on structures or sequences.
[b]: iCn3D sharable links could be a short URL or a URL containing the address of an iCn3D PNG Image
[c]: iCn3D supports command-line analysis with either Python scripts or Node.js scripts
[d]: iCn3D can also be used in Jupyter Notebook

iCn3D Features of Interest

- iCn3D aligns structures (PDB or AlphaFold) based on structures or sequences.
- iCn3D sharable links (https://structure.ncbi.nlm.nih.gov/icn3d/share.html?XCxR6fSTmXHxR3o1A)
- iCn3D supports command-line analysis with either Python scripts or Node.js scripts
- iCn3D can also be used in Jupyter Notebook (https://pypi.org/project/icn3dpy)
- 3D printing: structure.ncbi.nlm.nih.gov/icn3d/share.html?wt4TDqzhC2rhCYTD7
- Contact map: structure.ncbi.nlm.nih.gov/icn3d/share.html?rnMbe26tNsAjJLGK9
- Precalculated symmetry: structure.ncbi.nlm.nih.gov/icn3d/share.html?bGH1BfLsiGFhhTDn8
- Symmetry dynamically: structure.ncbi.nlm.nih.gov/icn3d/share.html?6NvhQ45XrnbuXyGe6
- Electron density map: structure.ncbi.nlm.nih.gov/icn3d/share.html?QpqNZ3k65ToYFvUB6
- EM map: structure.ncbi.nlm.nih.gov/icn3d/share.html?L4C4WYE85tYRiFeK7
- Transmembrane protein: structure.ncbi.nlm.nih.gov/icn3d/share.html?jMN16mJyR9STUx6E6
- Solvent Accessible Area: structure.ncbi.nlm.nih.gov/icn3d/share.html?xKSyfd1umbKstGh29

iCn3D Fundamentals Demo

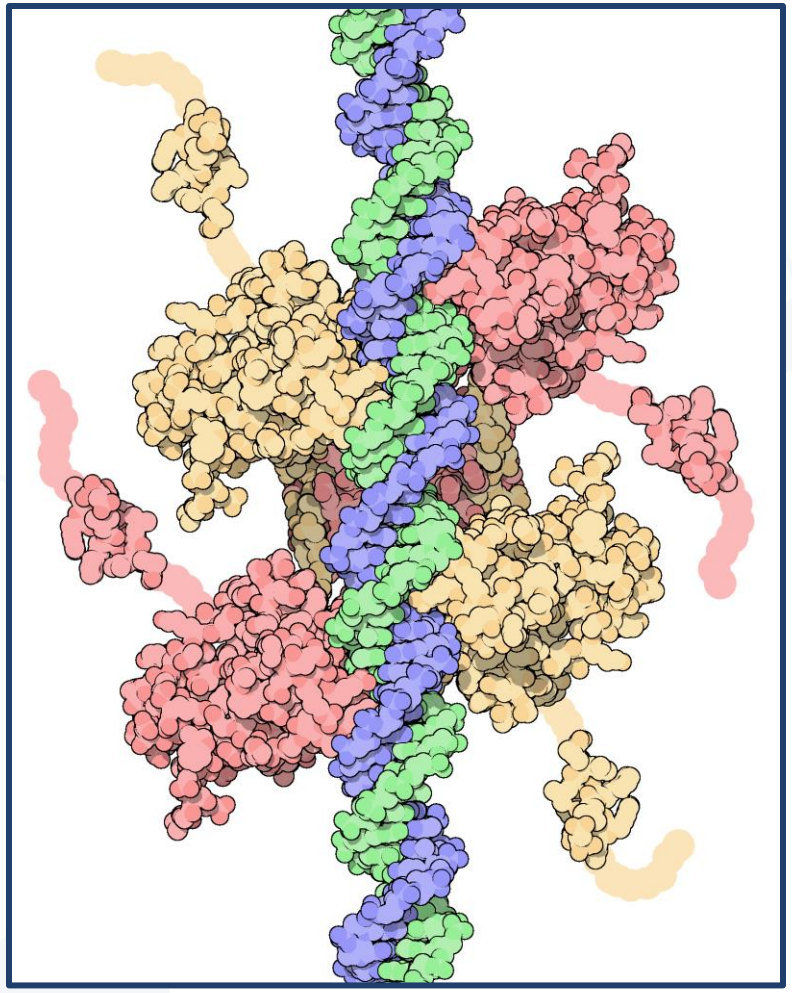
https://www.nlm.nih.gov/ncbi/workshops/2023-03_3d-molecular-structures/iCn3D_Fundamentals.html

iCn3D Mutation Analysis Demo

https://www.nlm.nih.gov/ncbi/workshops/2023-03_3d-molecular-structures/mutation_demo.html

P53 DNA-binding

- P53 binds to regulatory sites in the genome and:
  - Initiates protein production that stops cell division until damage is repaired
  - Initiates apoptosis
- Rich in + charged amino acids (Arg, His, Lys)
  - + charged amino acids commonly interact with negatively charged nucleic acid backbones
  - Usually interact at the major grove



P53 DNA- binding (from PDB)

Mutation Example 1

- Go to back to iCn3D
- What residues seem important to P53-binding domain?

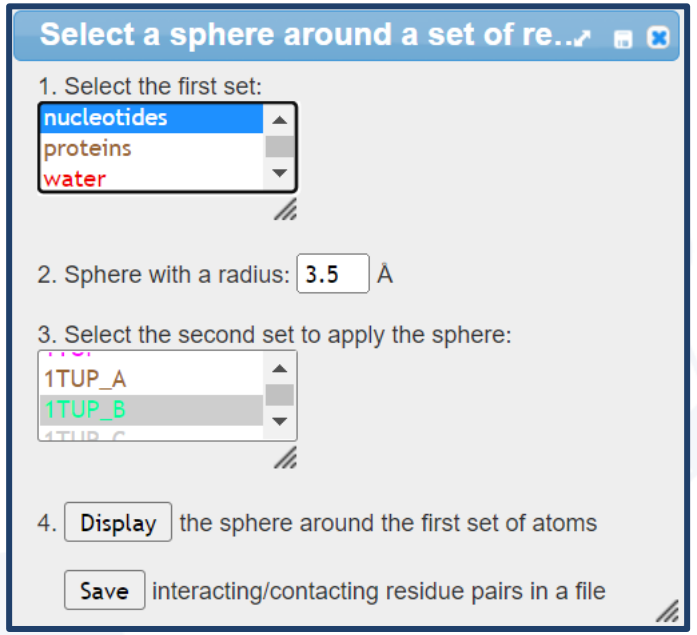
  Select > by Distance

- Take a closer look at these residues

  Style > Protein > Stick

- Lys120 points into the major groove to make bas-specific contact. How might a mutation affect interactions?

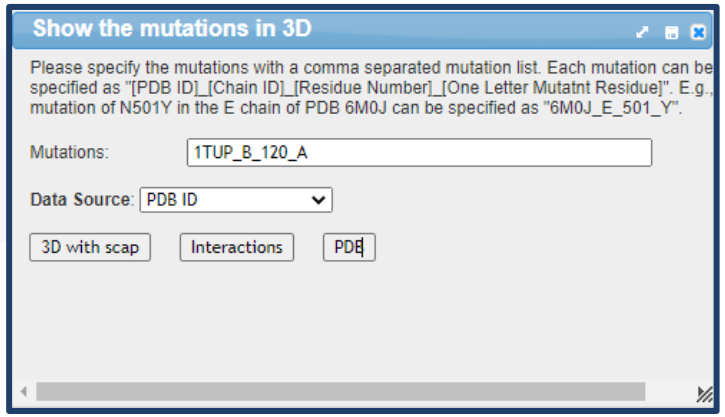
  Analysis > Mutation > 1TUP_B_120_A > Interactions



Lys120 (Lys117 in mouse) at the loop's tip points into the major groove to make a base-specific DNA contact. In contrast, here, Lys117 and the L1 loop in each subunit has moved nearly 15 Å away from the DNA and adopts some α-helical structure.

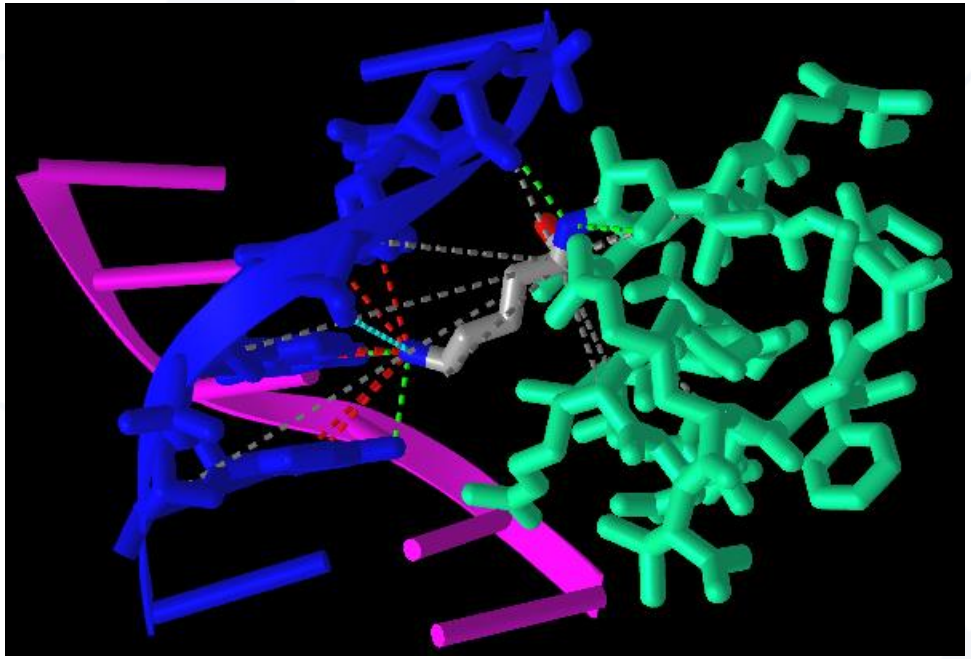
The DNA contacts made by each subunit are essentially as reported by Cho *et al.* (1994). Three minor variations, which are also observed in the other p53DBD/DNA structures, are seen in each subunit.

Oncogene

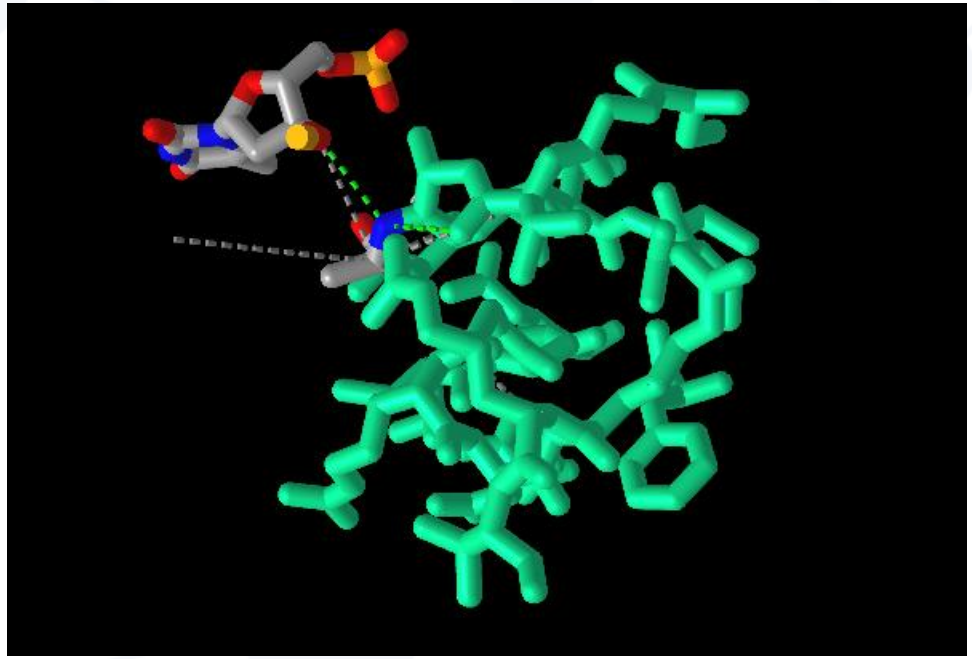


Mutation Discussion 1

- K120A mutation results in loss of interaction with the DNA groove



Wild type K120



Mutant A120

Mutation Example 2

5-minute exercise!

- Literature shows that Arg248 and Arg273 are common P53 mutations implicated in disease
- Use the Mutation analysis to understand how these mutations may affect interactions

```
1TUP_B_248_W
1TUP_B_273_H
```

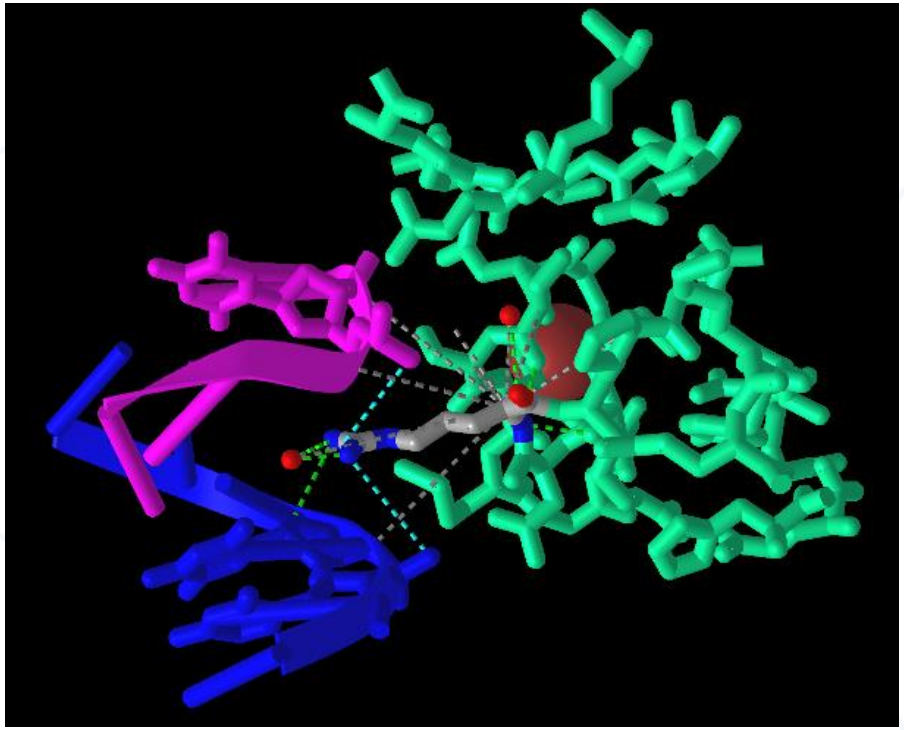
Distinct pattern of p53 phosphorylation in human tumors

Phosphorylation of mutant p53 in tumor-derived cell cultures

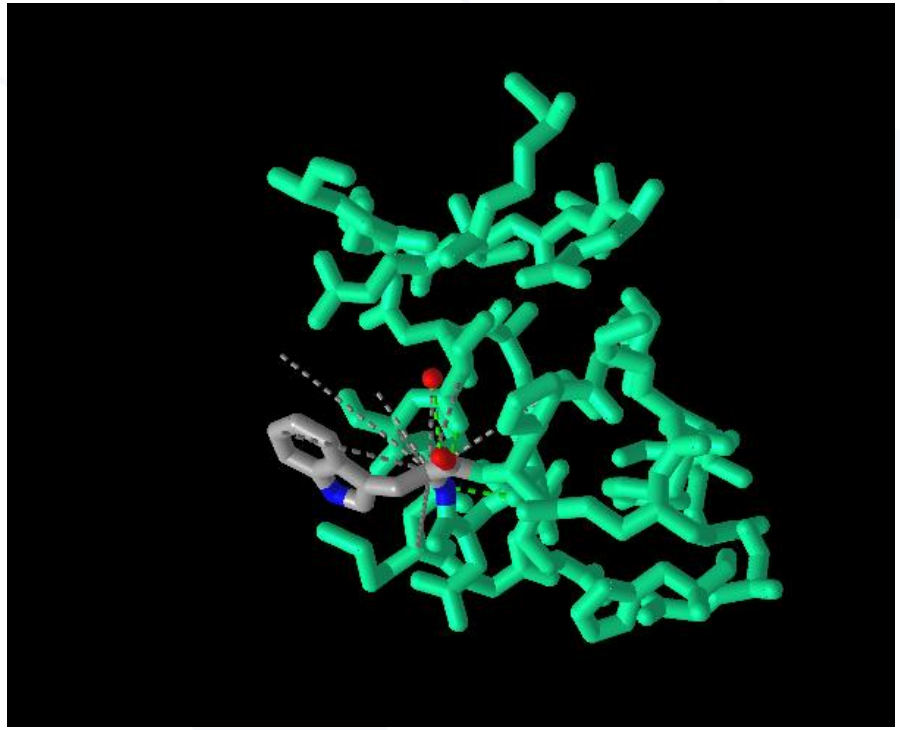
To determine the pattern of mutant p53 phosphorylation and acetylation in tumor-derived cell lines under normal growth conditions we analysed 18 cell lines with defined p53 mutations. Cell lines used for phospho-analysis were derived from seven tumor types and included a total of nine different mutations. Several tumor-derived cell lines with the same hot spot mutation (R248W or R273H) were included in this analysis to enable comparison of the phosphorylation pattern among different tumors that have the same mutation. As controls for this analysis, we used two non-transformed fibroblast cell lines (GM00038 and TIG) known to harbor wild type p53. Additional analysis has been carried out in parallel on tumor-derived tissues and cell lines that harbor wild type p53, thus allowing comparison of the phosphorylation pattern of wild type and mutant forms of p53 within the tumor environment.

Mutation Discussion 2

- R248W mutation results in loss of hydrogen and salt bridge interactions



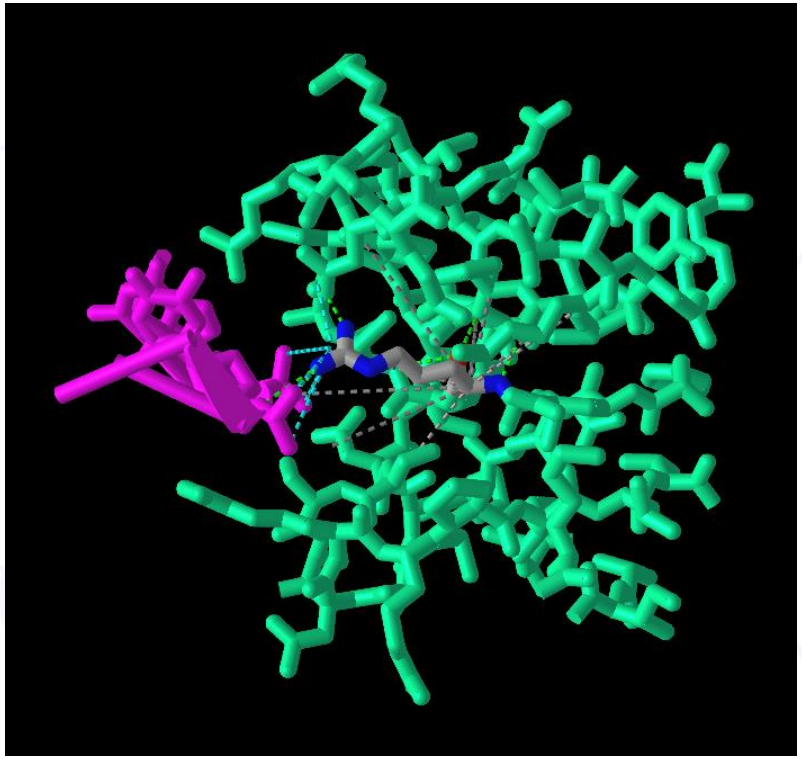
Wild type R248



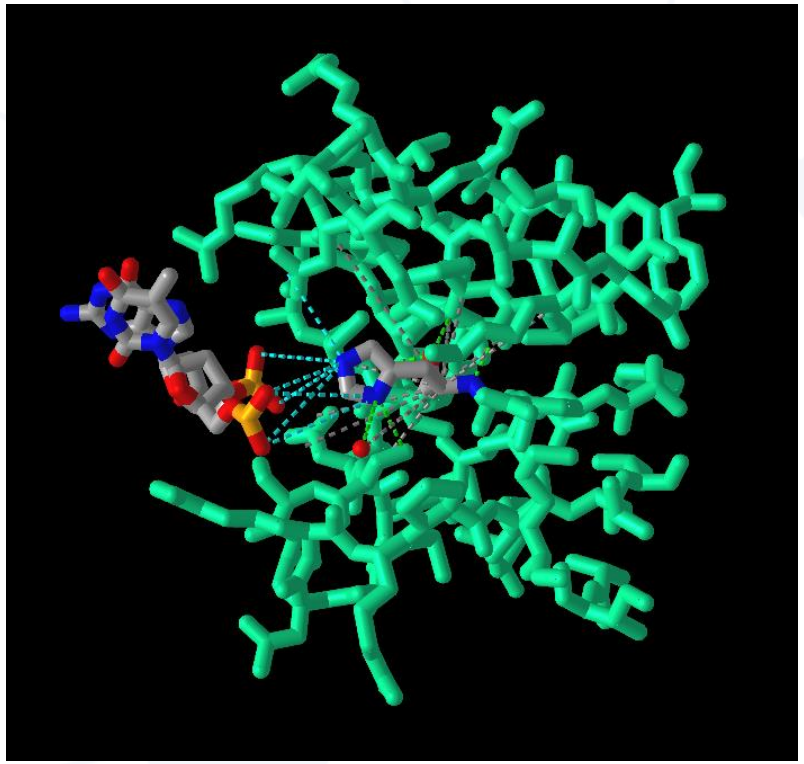
Mutant W248

Mutation Discussion 3

- R273H mutation results in loss of hydrogen bond interactions



Wild type R273



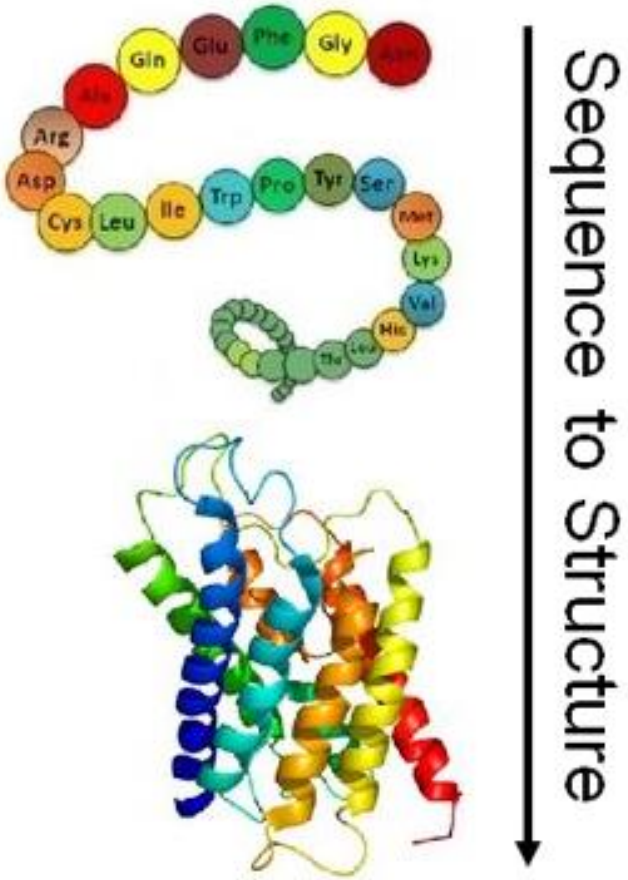
Mutant H273

iCn3D + AlphaFold Demo

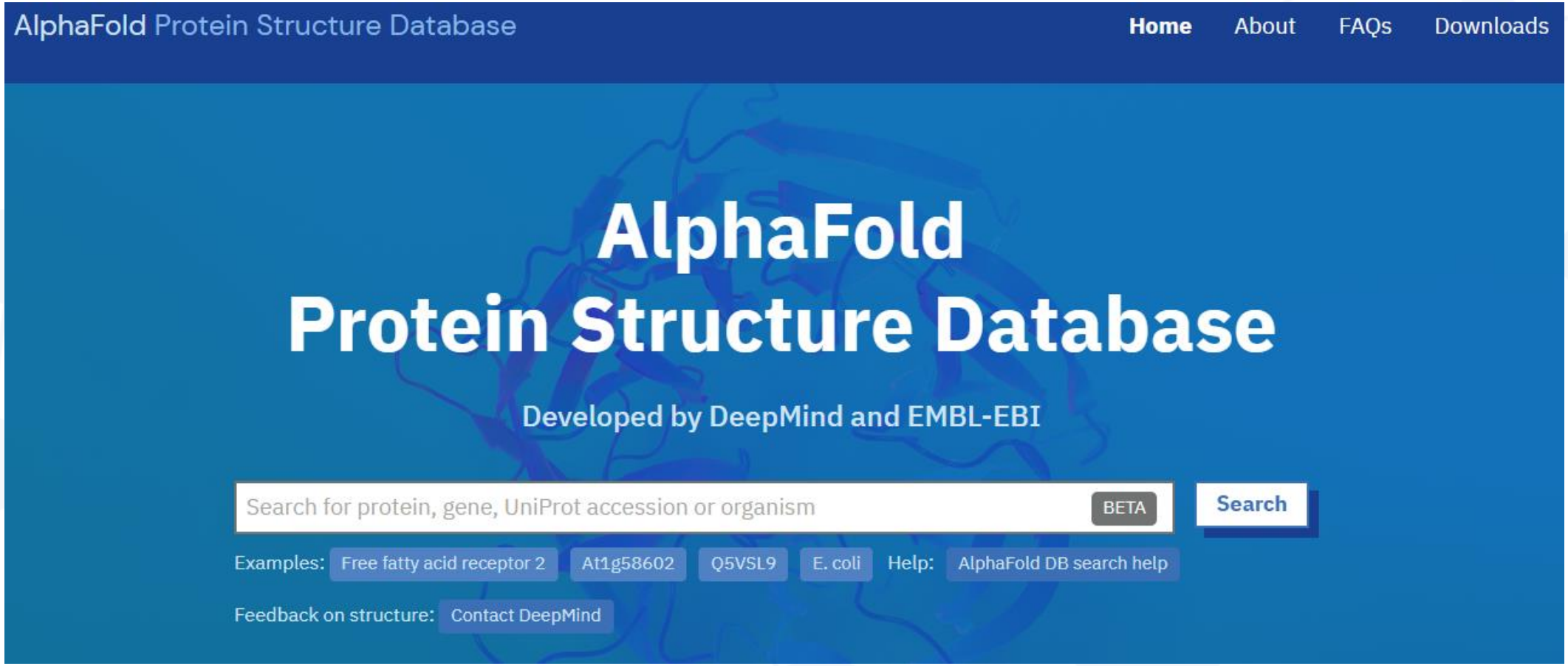
https://www.nlm.nih.gov/ncbi/workshops/2023-03_3d-molecular-structures/icn3d_alphafold.html

Structure Prediction

- When no or incomplete structure is available
- Prominent research focus of bioinformatics and theoretical chemists
  - Drug discovery
  - Biotechnology/bioengineering
- Predicted structures aren’t housed in most structural databases
  - Structure prediction websites, such as [AlphaFold](#) Protein Structure Database, are housed separately



Where do I find predicted structures?



https://alphafold.ebi.ac.uk/

Limitations of AlphaFold DB

- Only predicts monomers (AlphaFold Multimer model is published separately as open-source)
- Unreliable for IDP, it does convey the information via a low confidence score
- Not validated for mutational analysis
- Can only output one conformation of proteins with multiple conformations
- Only predicts the main peptide chain, not missing co-factors, metals, and co- and post-translational modifications

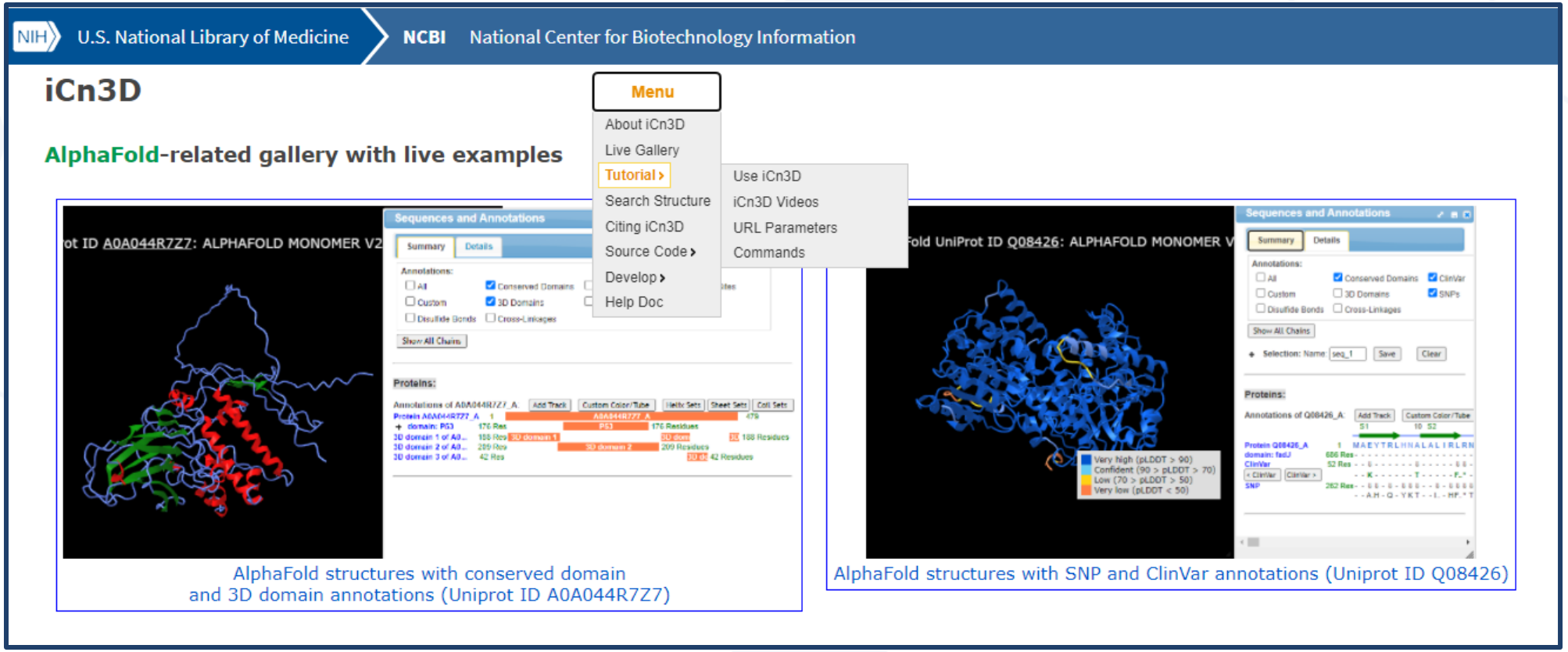
pLDDT Ranges

pLDDT- per-residue estimate of confidence on a scale from 0–100 and is used to color-code the residues of the model in the 3D structure viewer

- **pLDDT > 90** - expected to be modelled to high accuracy. These are suitable for applications requiring high accuracy (e.g. characterizing binding sites)
- **pLDDT between 70 and 90** - expected to be modelled well (generally good backbone prediction)
- **pLDDT between 50 and 70** - low confidence and should be treated with caution.
- **pLDDT < 50** - should not be interpreted

Continue learning about iCn3D

Tutorials and help documents are available here:



AlphaFold structures with conserved domain and 3D domain annotations (Uniprot ID A0A044R7Z7)

AlphaFold structures with SNP and ClinVar annotations (Uniprot ID Q08426)

Continue learning about NCBI Resources

- Join us for workshops, webinars, or codeathons!

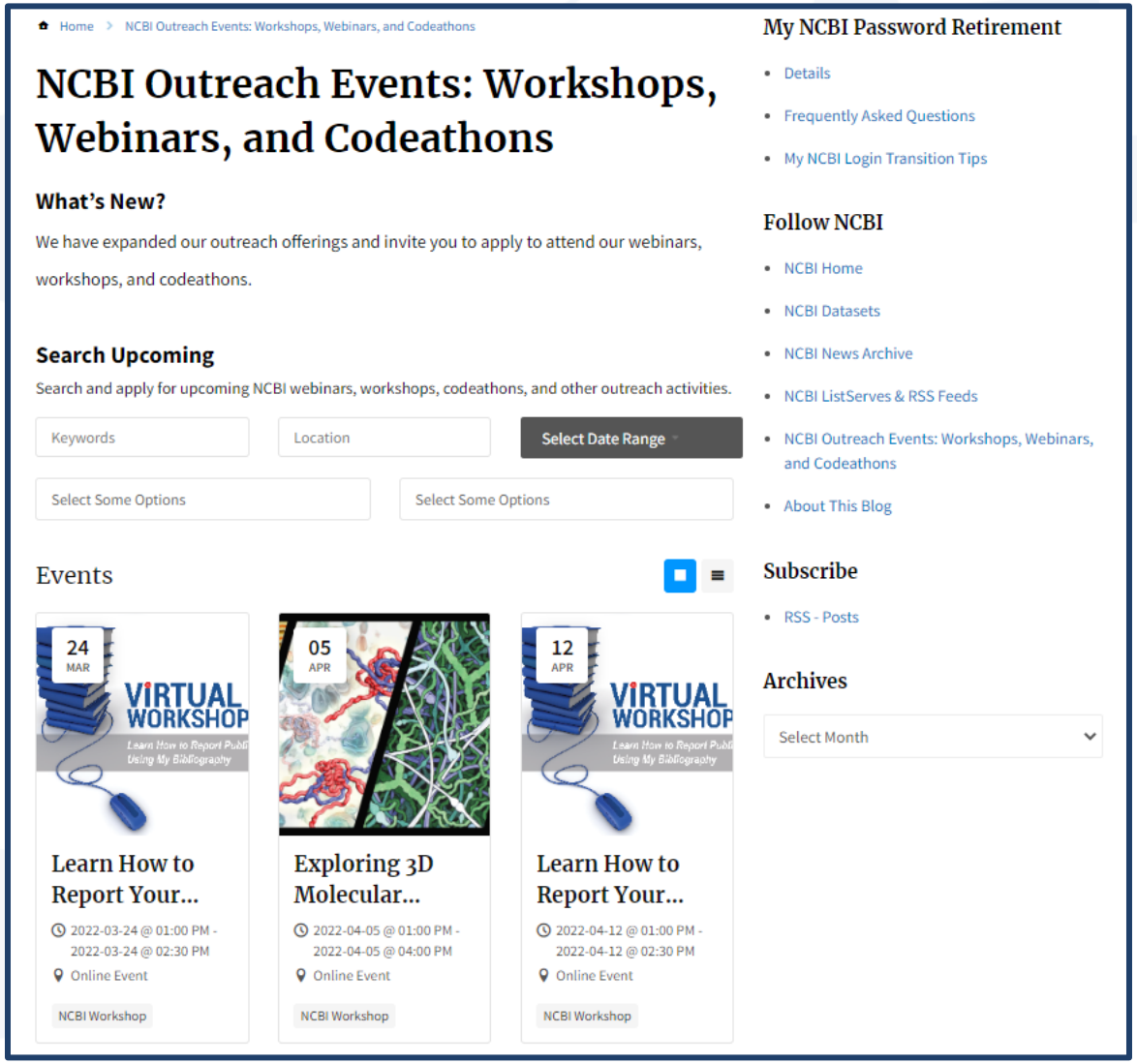
  [NCBI Insights Blog]

- Follow us on social media:

  [Twitter]

  [LinkedIn]

  [Facebook]



Questions & Discussion

Exploring 3D Molecular Structures with iCn3D Supplemental Learning Materials

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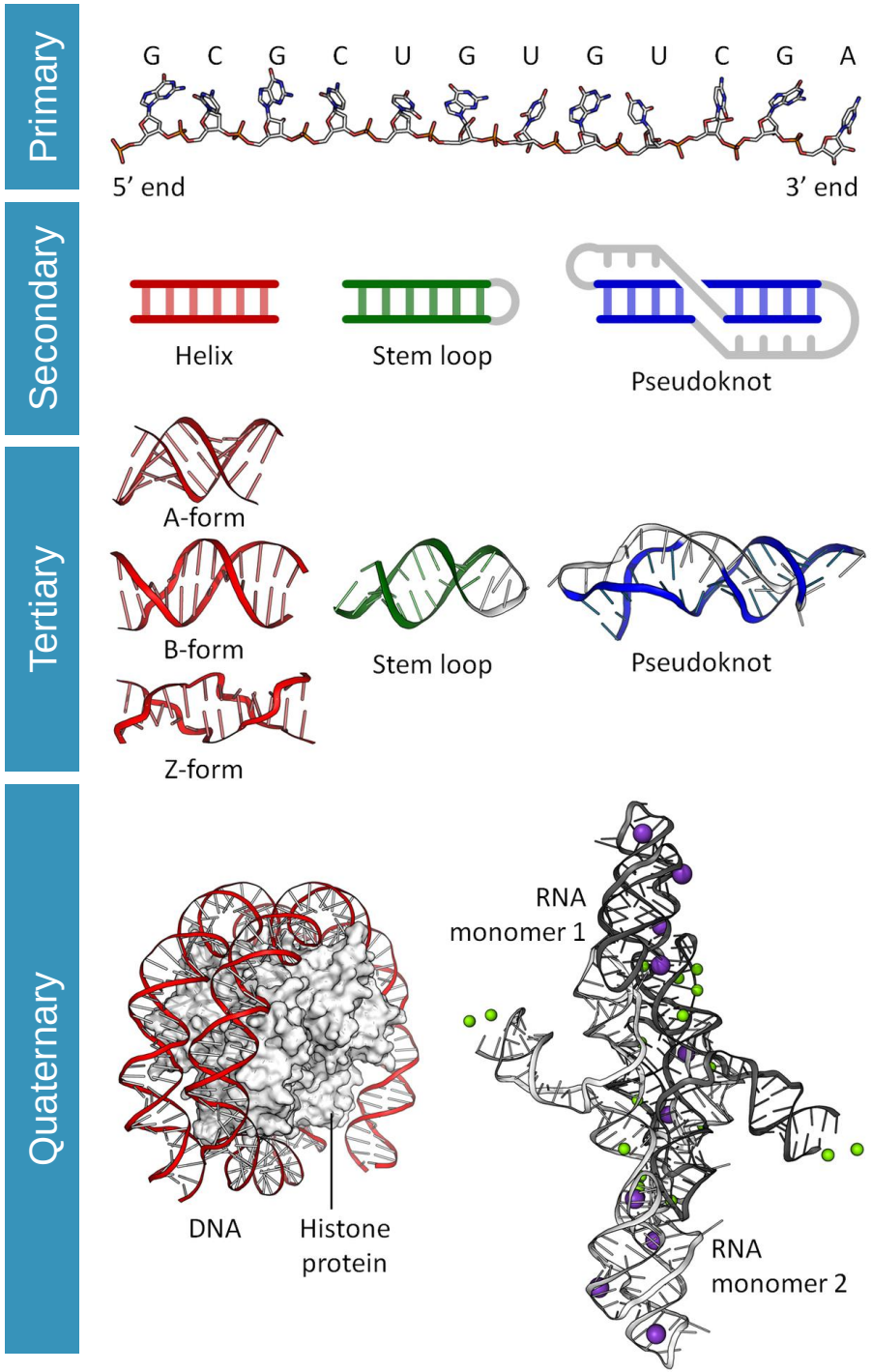
Nucleic Acid Structure

- **Primary-** sequence of nucleotides
- **Secondary-** base pairing interactions between polymers (DNA) or within a single polymer (RNA)
- **Tertiary-** 3D folding pattern
- **Quaternary-** interactions of nucleic acids with other molecules (DNA, RNA, or Protein)



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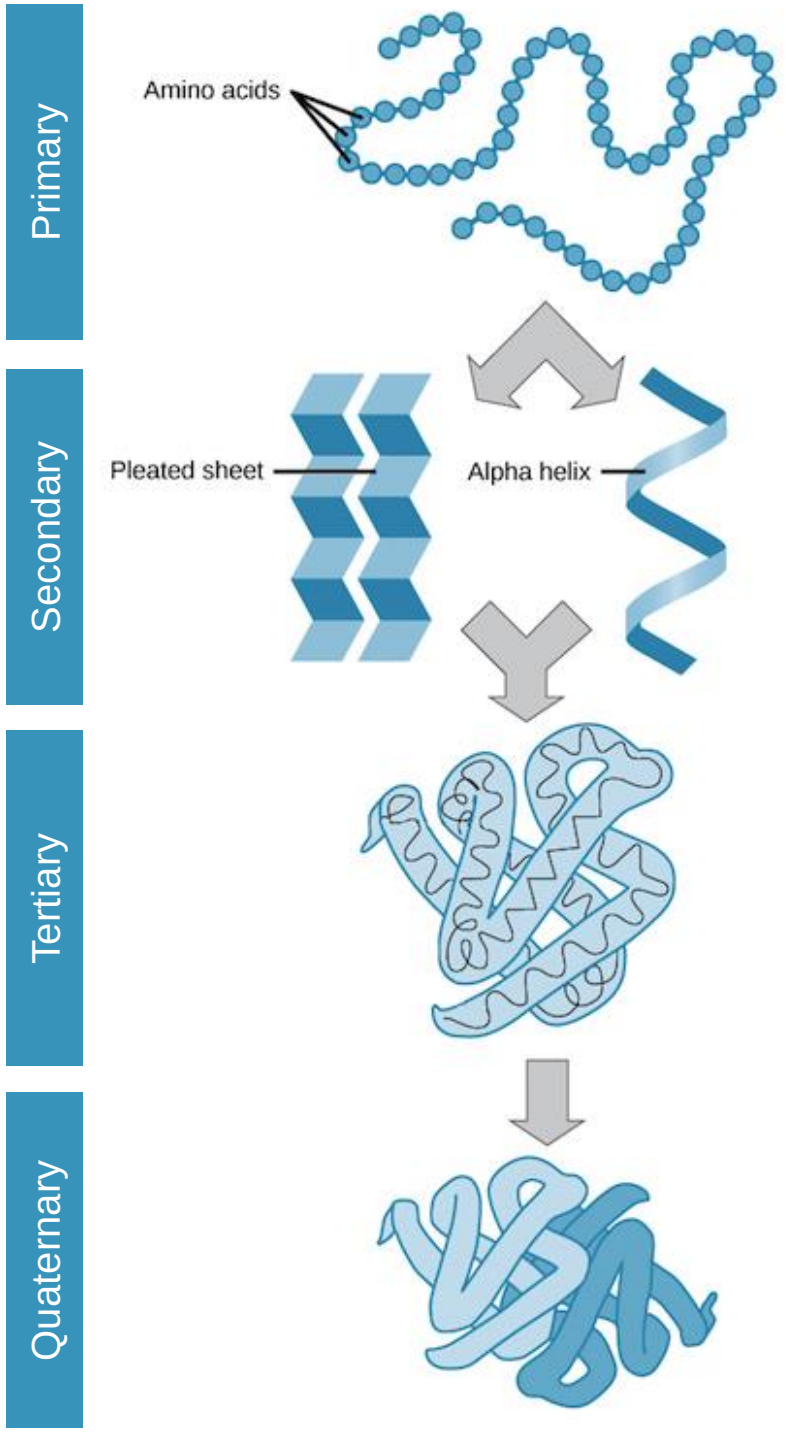
Protein Structure

- **Primary-** sequence of amino acids
- **Secondary-** hydrogen bonding of the peptide backbone that causes amino acids to fold into a repeating pattern
- **Tertiary-** 3D folding pattern of a protein due to side chain interactions
- **Quaternary-** protein consisting of more than one polypeptide



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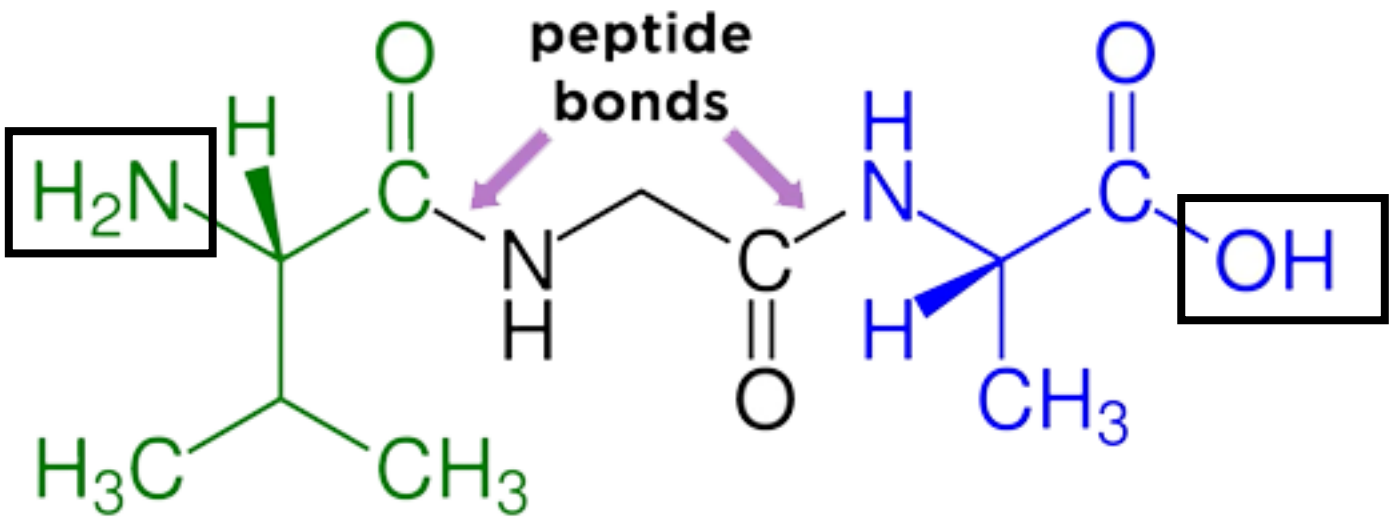
Terminology

N-terminus
(ends in amino group)

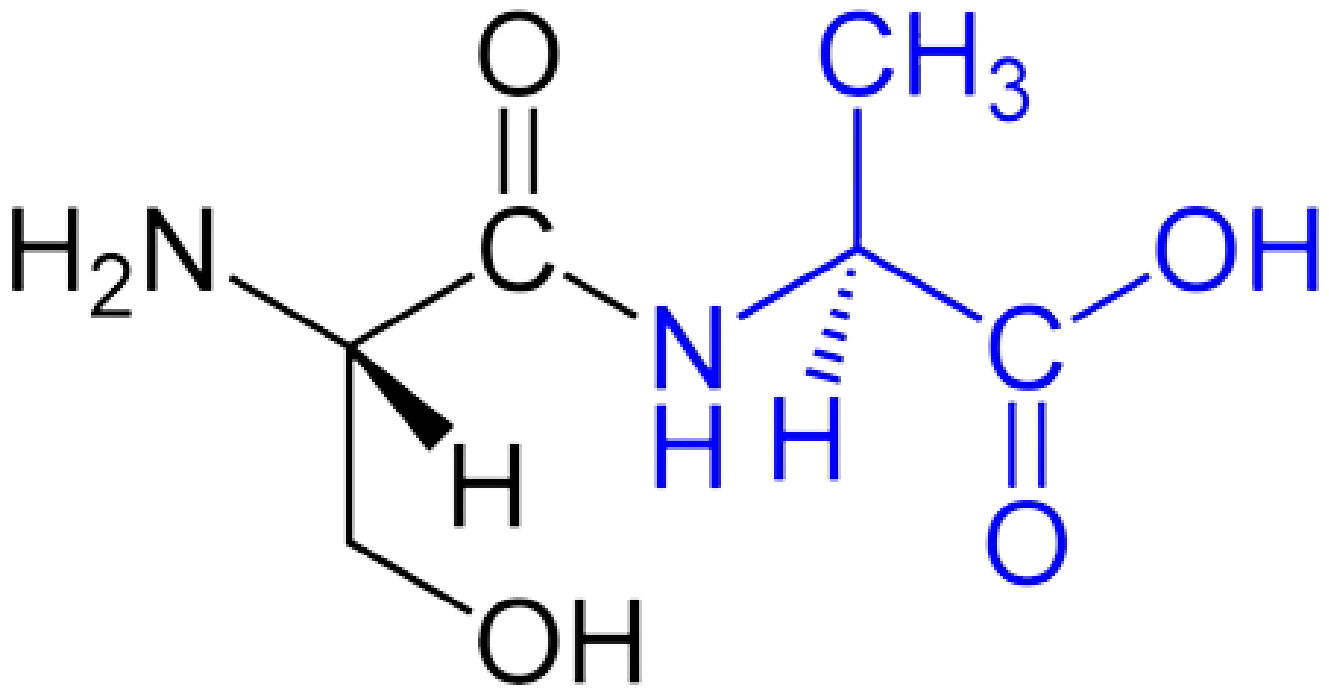
C-terminus
(ends in carboxyl group)



valine-glycine-alanine

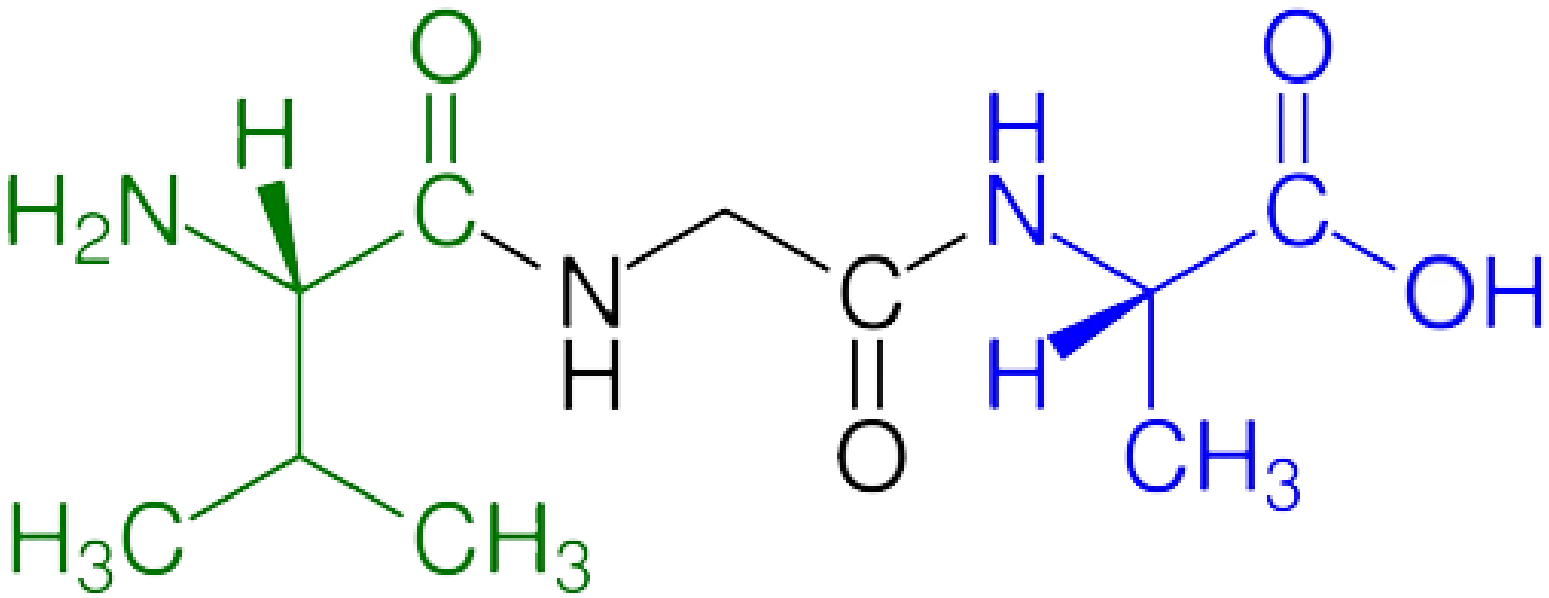
Terminology

dipeptide (2 amino acids)

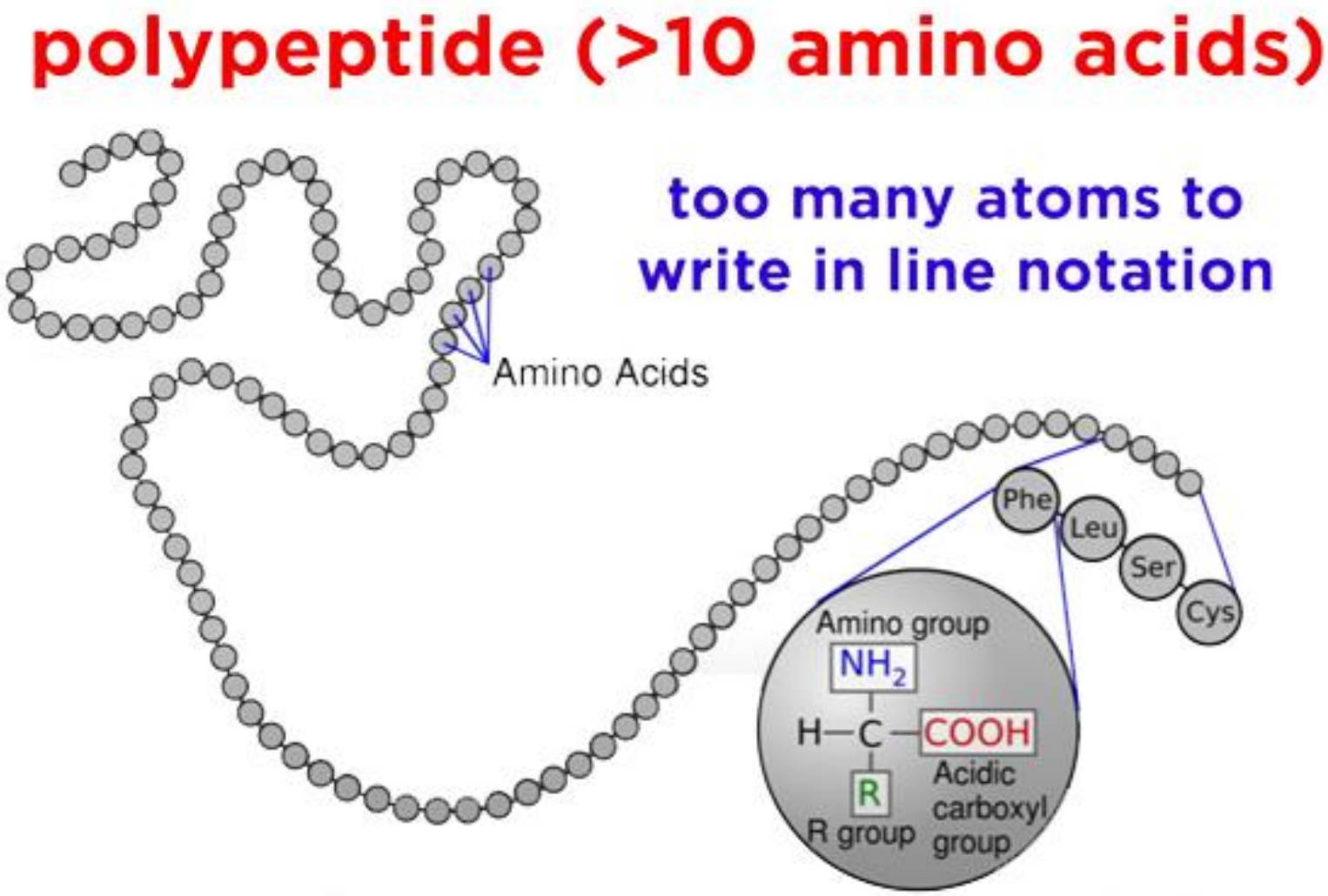


Terminology

oligopeptide (3-10 amino acids)

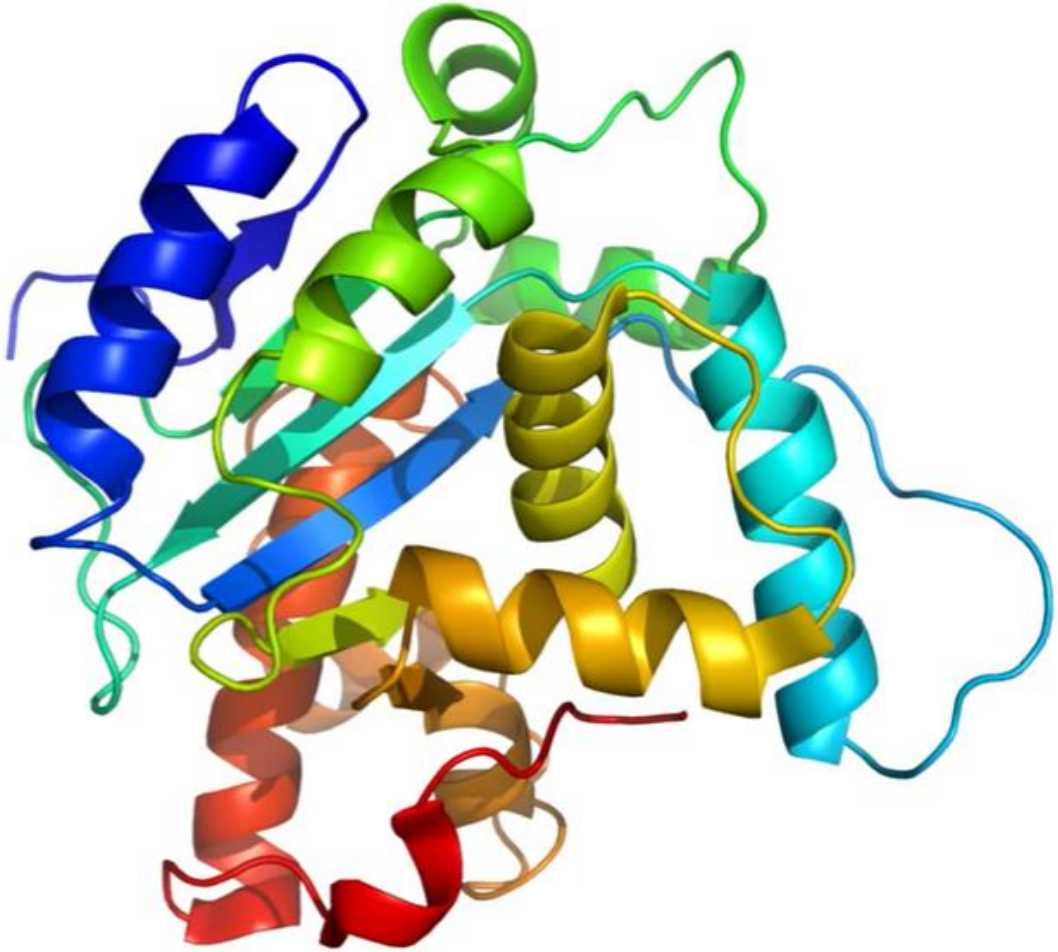


Terminology

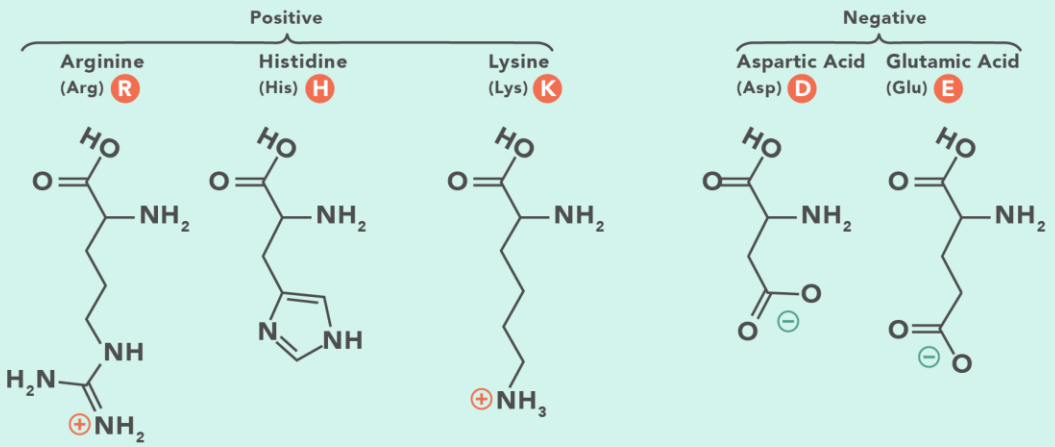


Terminology

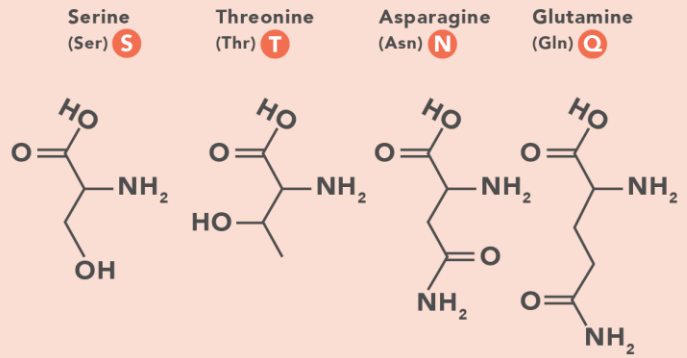
protein (generally 300-1000 amino acids)



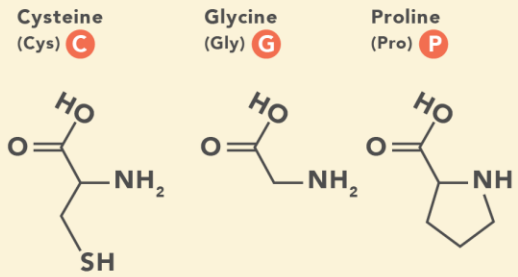
A. Amino Acids with Electrically Charged Side Chains



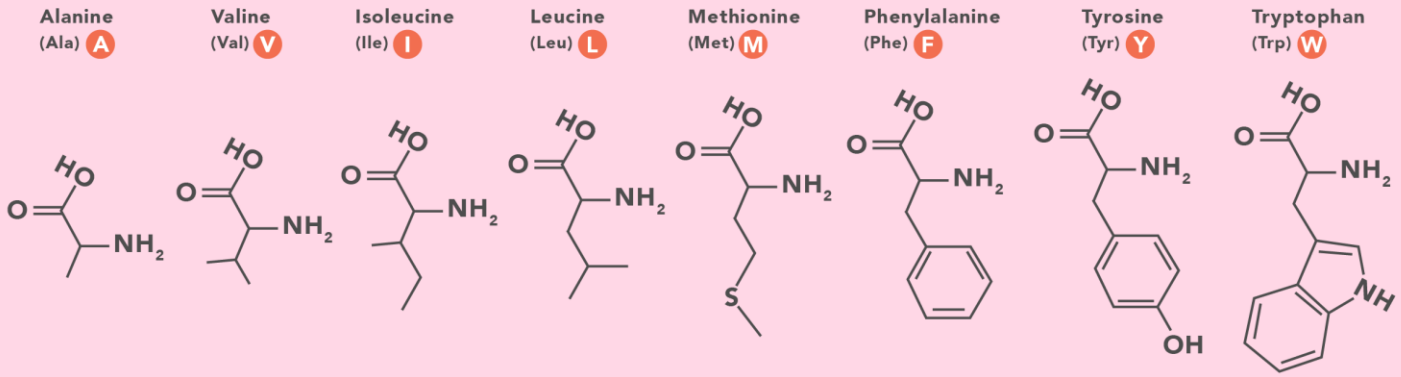
B. Amino Acids with Polar Uncharged Side Chains



C. Special Cases



D. Amino Acids with Hydrophobic Side Chains



Functional definition:

Enzymes: Accelerate biochemical reactions

Structural: Form biological structures

Transport: Carry biochemically important substances

Defense: Protect the body from foreign invaders

Structural definition:

Globular: Complex folds, irregularly shaped tertiary structures

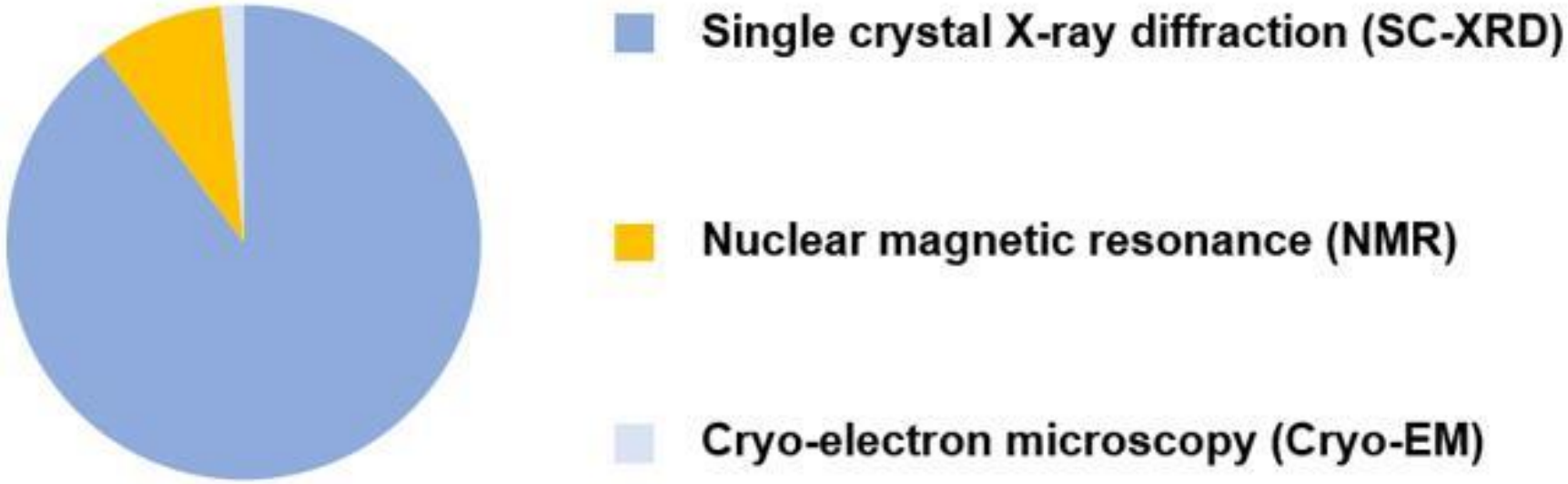
Fibrous: Extended, simple folds -- generally structural proteins

Cellular localization definition:

Membrane: In direct physical contact with a membrane; generally water insoluble.

Soluble: Water soluble; can be anywhere in the cell

Experimental techniques

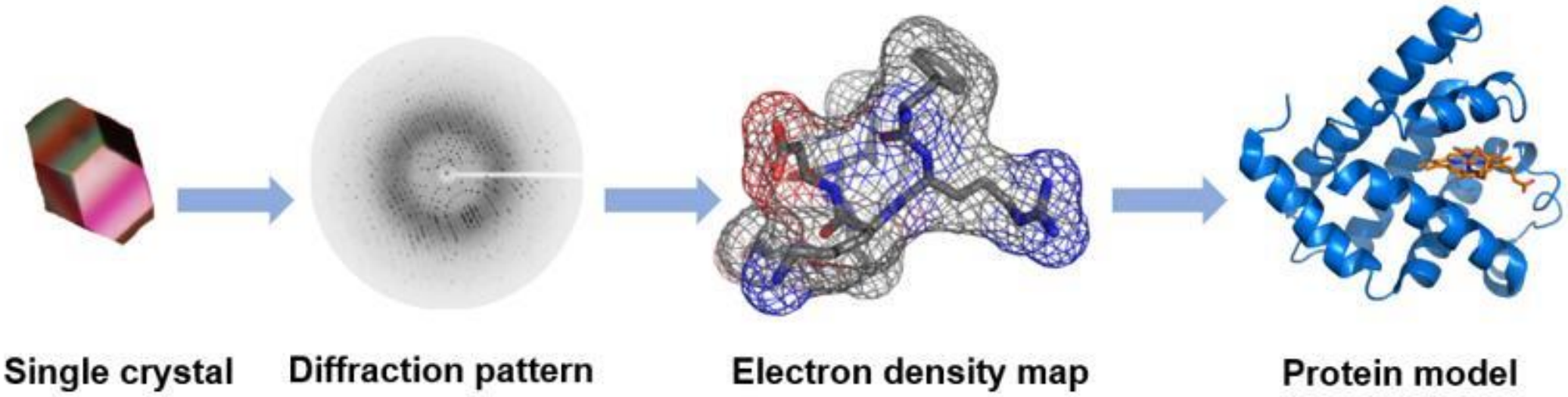


Three main research techniques for structural biology.
According to the statistics of PDB (https://www.rcsb.org/)

https://www.creative-biostructure.com/

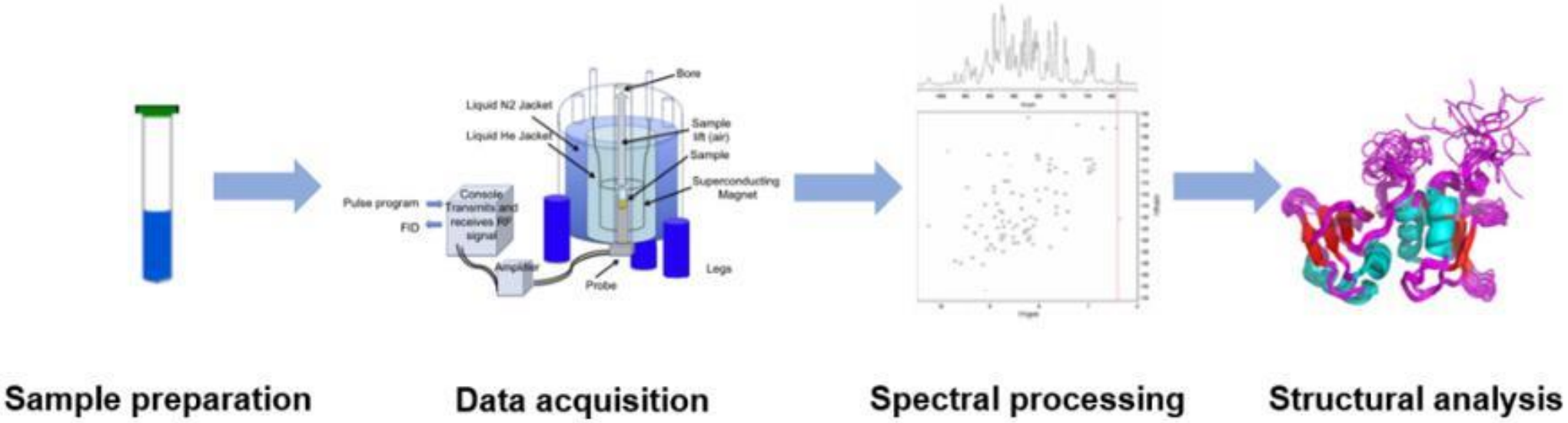
X-ray Crystallography

- Requires crystals, which can be hard to make
- Can handle very large proteins and complexes (e.g. ribosome)
- Provides a “flash picture” with little or no data about motions
- Can include packing artifacts from crystallization



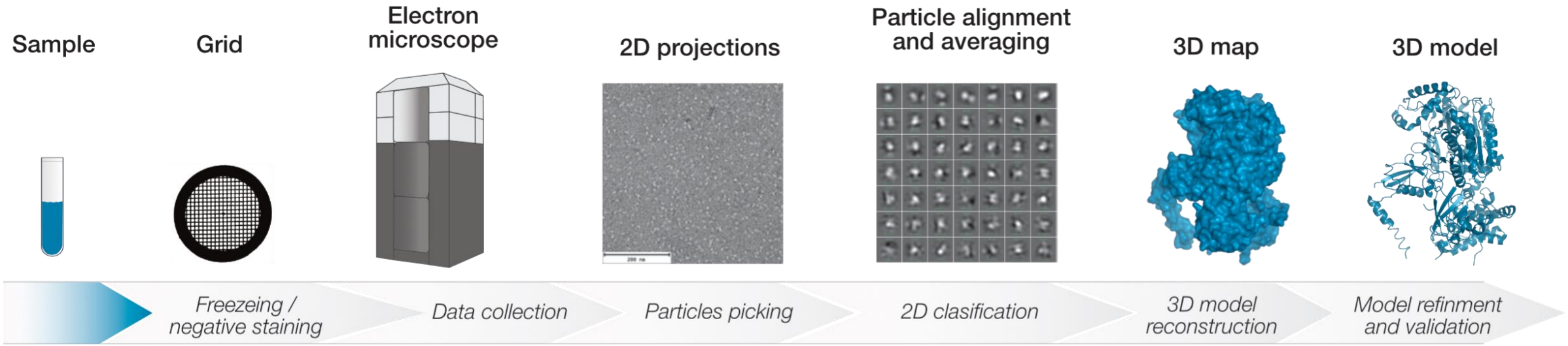
Nuclear Magnetic Resonance

- Requires highly concentrated, C13/N15-labeled protein solutions
- Limited to relatively small proteins (<30 kDa)
- Sensitive to molecular motions
- High protein concentrations may induce non-biological binding



Cryo-electron microscopy

- Requires expensive equipment
- Only small amount of sample
- Rapid freezing sample allows sample to maintain a closer-to-native state
- Useful for biomolecules with high molecular weight



NCBI Structure Database Search Tips

Entrez is a molecular biology database system that provides access to a wealth of NCBI data

- More Entrez Help is available on the NCBI website

Finding structures with Entrez

```
"term1"[field1] AND/OR/NOT "term2"[field2] AND/OR/NOT …
```

- Use field limits and Boolean operators
- Put phrases in quotes

NCBI Structure Database Search Examples

Useful Search Fields
Organism Ex. “Homo sapiens”[orgn]
Experimental Method Ex. “NMR”[exp]
Chemical Name “zinc”[chemical name]
PDB Description Ex. “Tumor Suppressor p53”[title]
[Filter] Ex. “Complex DNA”[filter]

More Search Field Options

```
term1[field1] AND/OR/NOT term2[field2] AND/OR/NOT …
```

```
“Homo sapiens”[orgn] AND “X-ray diffraction”[exp]
```

Search results

Items: 1 to 20 of 47803

```
“Homo sapiens”[orgn] AND “X-ray diffraction”[exp]AND “zinc”[chemical name]
```

Search results

Items: 1 to 20 of 6092

```
“Homo sapiens”[orgn] AND “X-ray diffraction”[exp] AND “zinc”[chemical name] AND “Complex DNA”[filter]
```

Search results

Items: 1 to 20 of 288

```
1TUP
```

PDB File

```
HEADER    ISOMERASE/DNA                           04-OCT-07   2RGR
TITLE     TOPOISOMERASE IIA BOUND TO G-SEGMENT DNA
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: DNA TOPOISOMERASE 2;
COMPND   3 CHAIN: A;
COMPND   4 FRAGMENT: DNA BINDING AND CLEAVAGE DOMAIN (RESIDUES 419-
COMPND   5 1177);
COMPND   6 SYNONYM: DNA TOPOISOMERASE II;
COMPND   7 EC: 5.99.1.3;
COMPND   8 ENGINEERED: YES;
COMPND   9 MOL_ID: 2;
COMPND  10 MOLECULE: DNA;
COMPND  11 CHAIN: C;
COMPND  12 ENGINEERED: YES;
COMPND  13 MOL_ID: 3;
COMPND  14 MOLECULE: DNA;
COMPND  15 CHAIN: D;
COMPND  16 ENGINEERED: YES
SOURCE    MOL_ID: 1;
SOURCE   2 ORGANISM_SCIENTIFIC: SACCHAROMYCES CEREVISIAE;
SOURCE   3 ORGANISM_COMMON: BAKER'S YEAST;
SOURCE   4 ORGANI
SOURCE   5 GENE: 
SOURCE   6 EXPRES
SOURCE   7 EXPRES
SOURCE   8 EXPRES
SOURCE   9 EXPRES
SOURCE  10 EXPRES
SOURCE  11 EXPRES
SOURCE  12 MOL_ID
SOURCE  13 SYNTHE
SOURCE  14 MOL_ID
SOURCE  15 SYNTHE
```

```
REMARK   2
REMARK   2 RESOLUTION.    3.00 ANGSTROMS.
REMARK   3
REMARK   3 REFINEMENT.
REMARK   3   PROGRAM     : PHENIX
…
REMARK 280
REMARK 280 CRYSTAL
REMARK 280 SOLVENT CONTENT, VS   (%): 59.90
REMARK 280 MATTHEWS COEFFICIENT, VM (ANGSTROMS**3/DA): 3.07
REMARK 280
REMARK 280 CRYSTALLIZATION CONDITIONS: 12-20% PEG 1000, 100-250 MM MGCL2,
REMARK 280  100 MM SODIUM CACODYLATE, PH 7.0, VAPOR DIFFUSION, HANGING
REMARK 280  DROP, TEMPERATURE 277K
REMARK 290
```

PDB File: Data

```
ATOM      46  N    THR A 424       17.857  11.128 -95.193  1.00 57.50          N
ANISOU    46  N    THR A 424      7252   7857   6740    -72    842    107      N
ATOM      47  CA   THR A 424       16.610  11.906 -95.142  1.00 56.73          C
ANISOU    47  CA   THR A 424      7153   7780   6623    -85    799    141      C
```

```
ATOM      46  N    THR A 424       17.857  11.128 -95.193  1.00 57.50
```

```
ANISOU    49  O    THR A 424      6950   7583   6324   -111    717    158      O
ATOM      50  CB   THR A 424       16.451  12.75[illegible]6.406  1[illegible]  60.37          C
ANISOU    50  CB   THR A 424      7628   8271[illegible]  -94[illegible]20    18[illegible]      C
[illegible]  51  OG1  THR A 424       16.550  11.914 -97.557  1.00 63.33          O
AN[illegible]  OG1  THR A 424      8049   8666   7346   -104    846    16[illegible]      [illegible]
AT[illegible]  CG2  THR A 424       17.523  13.836 -96.462  1.00 61.80
AN[illegible]  CG2  THR A 424      77[illegible]  7271    -81    860    20[illegible]      C
```

Name

Atom Number

Atom Name

Residue Name

Chain ID

Residue Number

X

Y

Z

Occupancy

Temperature Factor

More about PDB Data

Computational Structural Biology

- Structure Prediction- inference of 3D structure from sequence data
- Molecular Docking- predicts the orientation of one molecule to another
- Molecular Dynamics Simulations- analyzes physical movements of atoms and molecules over time

NCBI

Computational Structural Biology

- Structure Prediction- inference of 3D structure from sequence data
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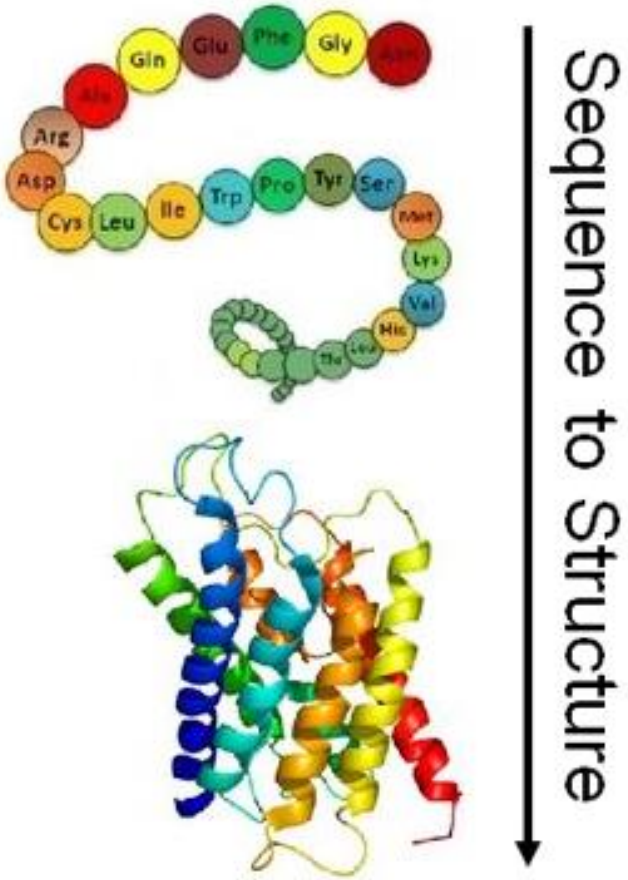
- Rely on experimental information from public databases
  - NCBI Databases and RCSB Protein Data Bank

NCBI

Structure Prediction Methods

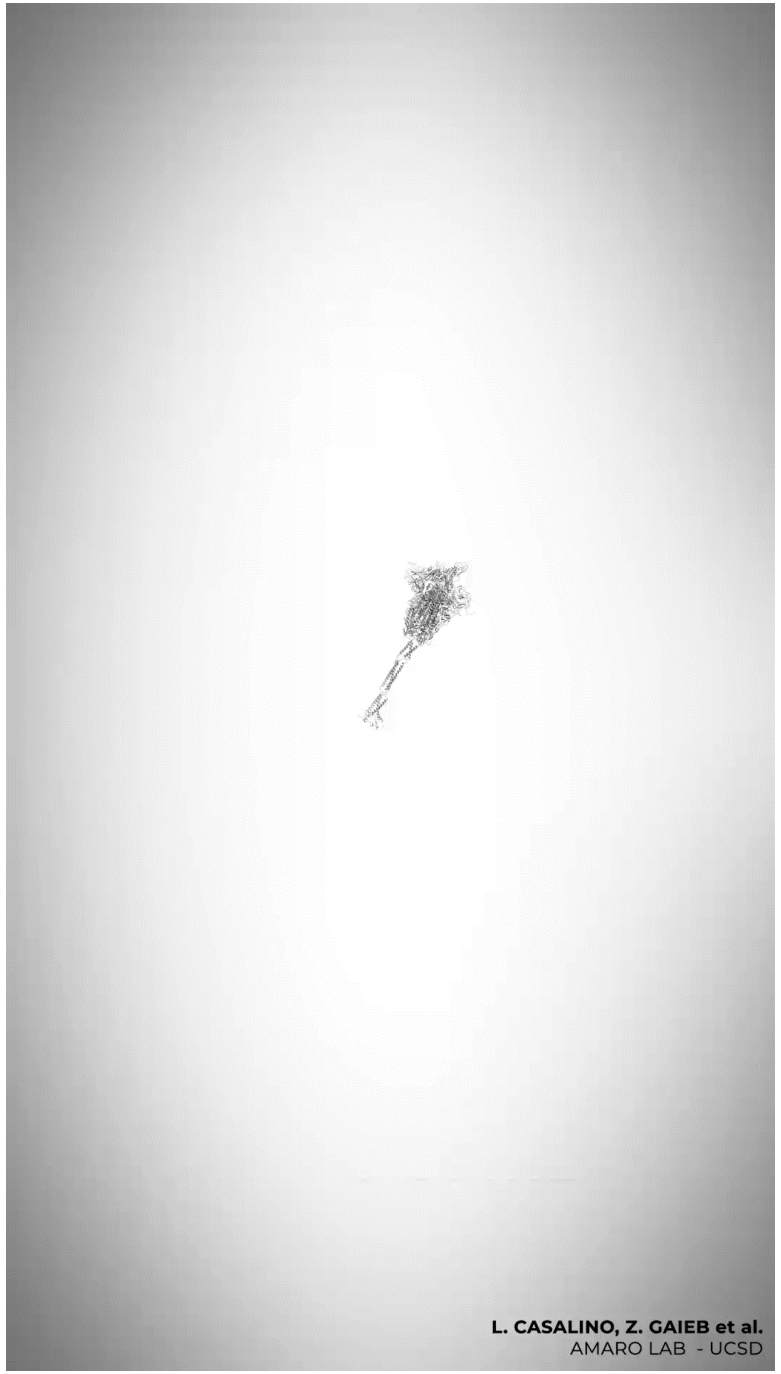
- Comparative Modeling
  - Prediction is based on amino acid sequence and structures of similar molecules available
- Fold recognition
  - Predicts folded structure by aligning a protein of unknown structure and a protein of known structure for low levels of sequence identity (<25%)
- Ab initio
  - Predicts the structure of proteins from the sequence and using molecular energy calculations (Schrodinger equation)



Structure Prediction Example

Impact on COVID-19 research

- Researchers have provided key insights into the SARS-CoV-2 proteins through structure prediction
  - Identified critical residues
  - Contextualized variant perturbations
  - Improved understanding of molecular recognition
- Spike fusion glycoprotein example
  - Challenging to characterize experimentally
  - Modeling + molecular dynamics helped researchers understand the roles of glycans on the dynamics of the protein



Casalino et al, *Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein*, PMID:33140034

Homology Modeling *vs Ab initio* Prediction

Ab initio Prediction	**Comparative Modeling**
Applicable to any sequence	Applicable to only those sequences with recognizable similarity to a template structure
Not very accurate (>4Å RMSD)	Fairly accurate (<3Å RMSD), similar to low resolution X-ray structure
Attempted for proteins of <100 residues	Not limited by size
Accuracy and applicability are limited by our understanding of the protein folding problem	Accuracy and applicability are limited by the number of known folds

Quick Background to AlphaFold

Learn more about AlphaFold here