Complementary Methods for Structural Information

Structure is the skeleton, complementary methods give it muscle and motion

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SR-71

Space Shuttle

B52

Hot air balloon

Hang-glider

Biplane

Spitfire

Unidentified

Stealth fighter

Sleek,
very mobile?

Only flies
vertically?

Excretes numerous droppings

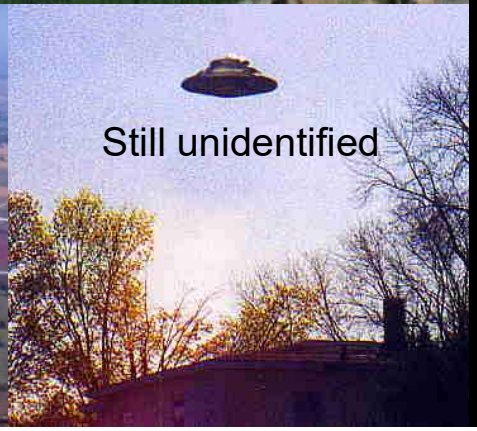
Needs others for
reproduction

Symbiotic relationship?

Two wings, must fly
really high?

Nothing there,
false observation?

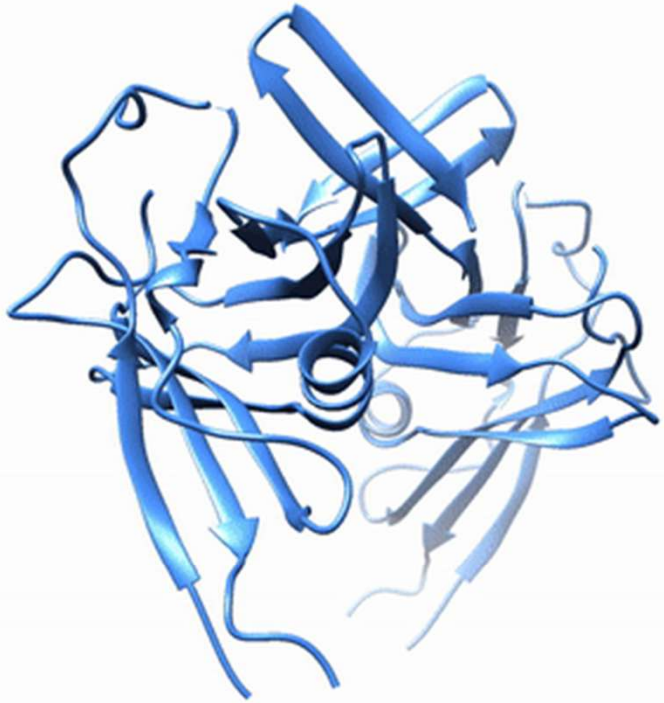
False eyes to scare predators



Still unidentified

Introduction: Why Complementary Methods

- Structural biology is not just about shape, it's about function, flexibility, and context.
- These methods are often used to validate, guide, or expand core structure results
- This provides mechanism information.



Categories of Complementary Techniques

- Sequence-Based and Predictive Tools (Computational)
- Experimental Biophysical Probes
- Other X-ray probes
- Structural Validation and Assembly Tools
- Integrative and Hybrid Modeling

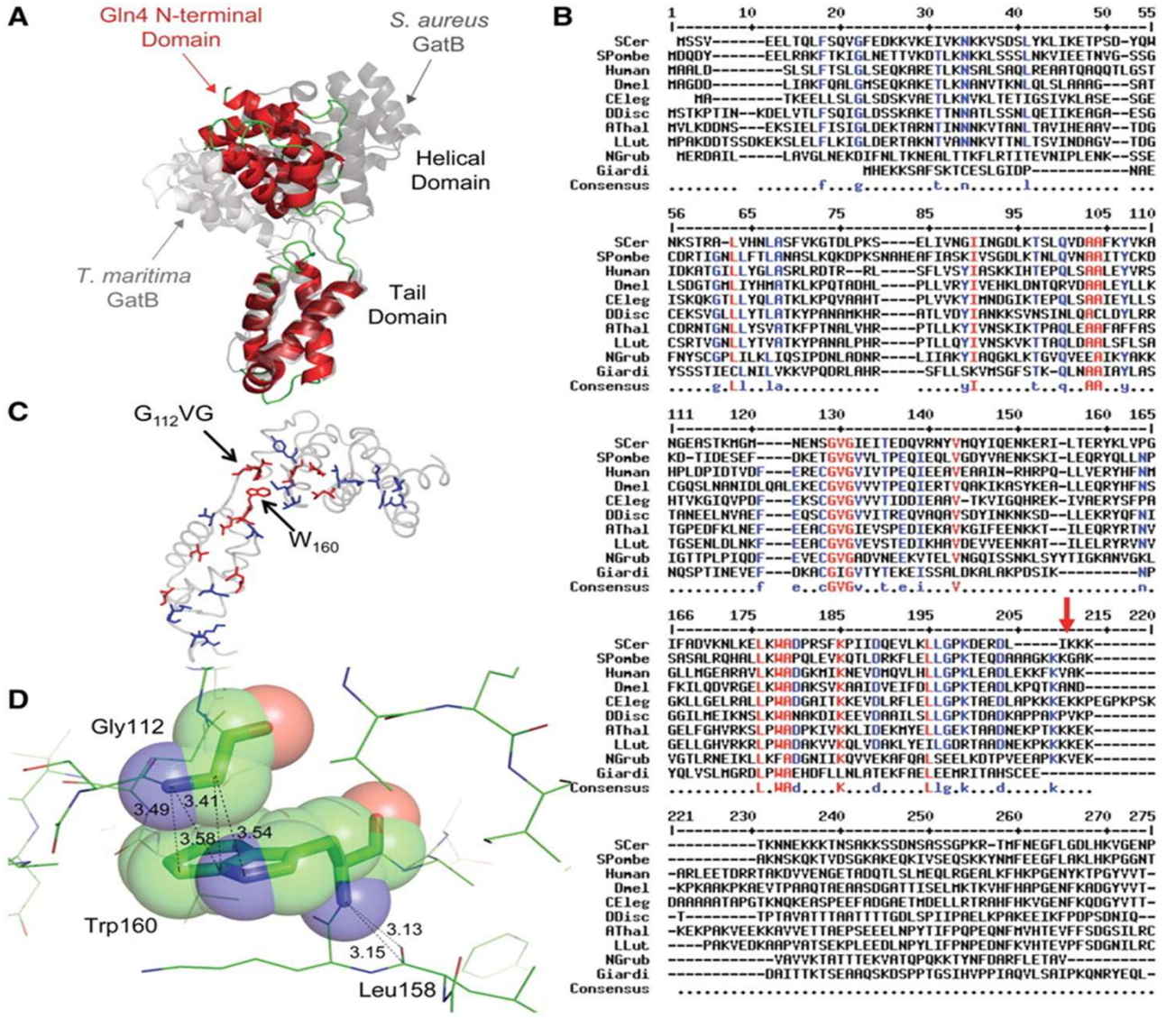
Sequence-Based and Predictive Tools (Computational)

- Sequence Alignment / Conservation:
  - Highlight conserved motifs; informs likely structural or functional importance.
- Disorder Prediction Tools: e.g., IUPred, PONDR:
  - explains missing density, IDPs.
- AlphaFold / RoseTTAFold:
  - Predict structures where experiments fail; discuss confidence metrics (pLDDT), limitations.
- Co-evolution / Contact Prediction:
  - Use in guiding modeling and validating assemblies.

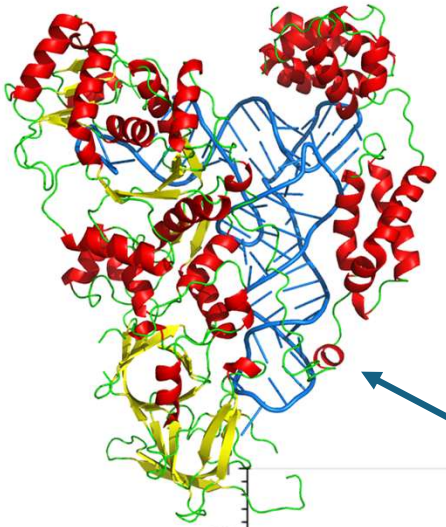
Sequence alignment

The linker between the two domains in Gln4(1–187) likely behaves as a hinge, is highly conserved and is and is important for tRNA binding.

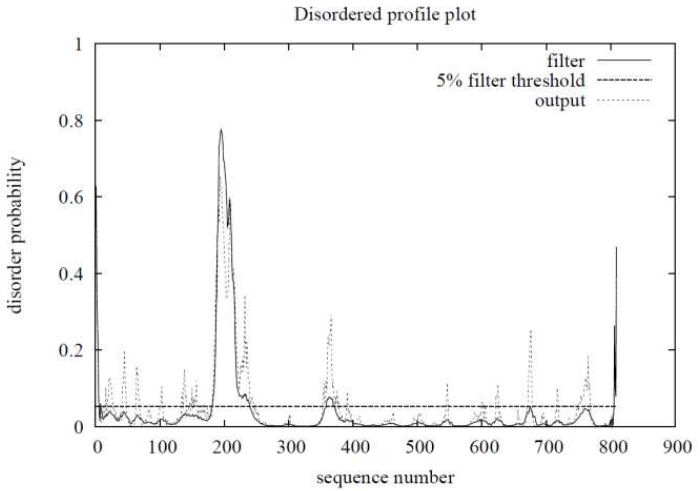
Grant, Snell, et al. Structural conservation of an ancient tRNA sensor in eukaryotic glutaminyl-tRNA synthetase, Nucleic Acids Research, Volume 40, Issue 8, 1 April 2012, Pages 3723–3731

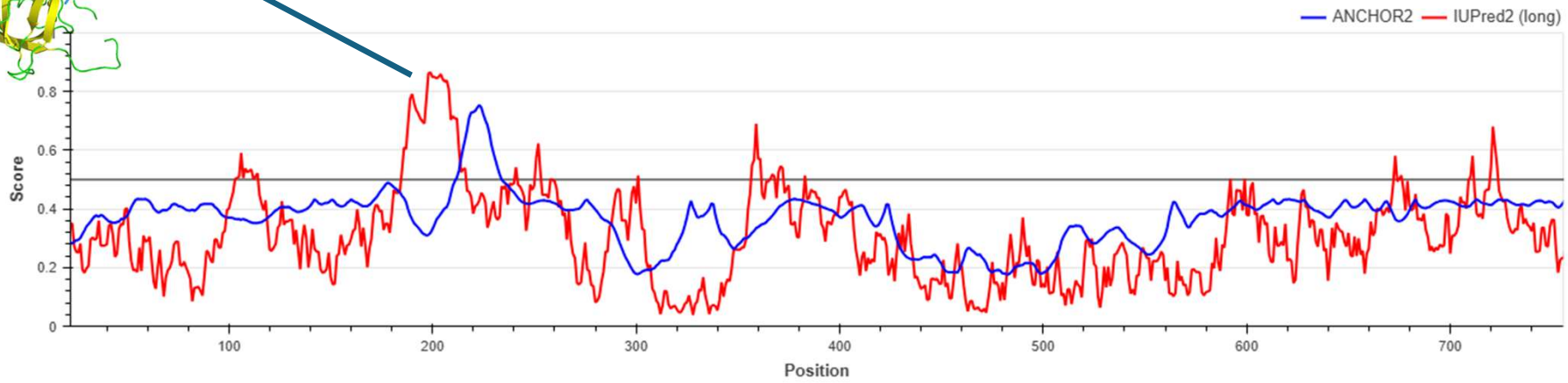


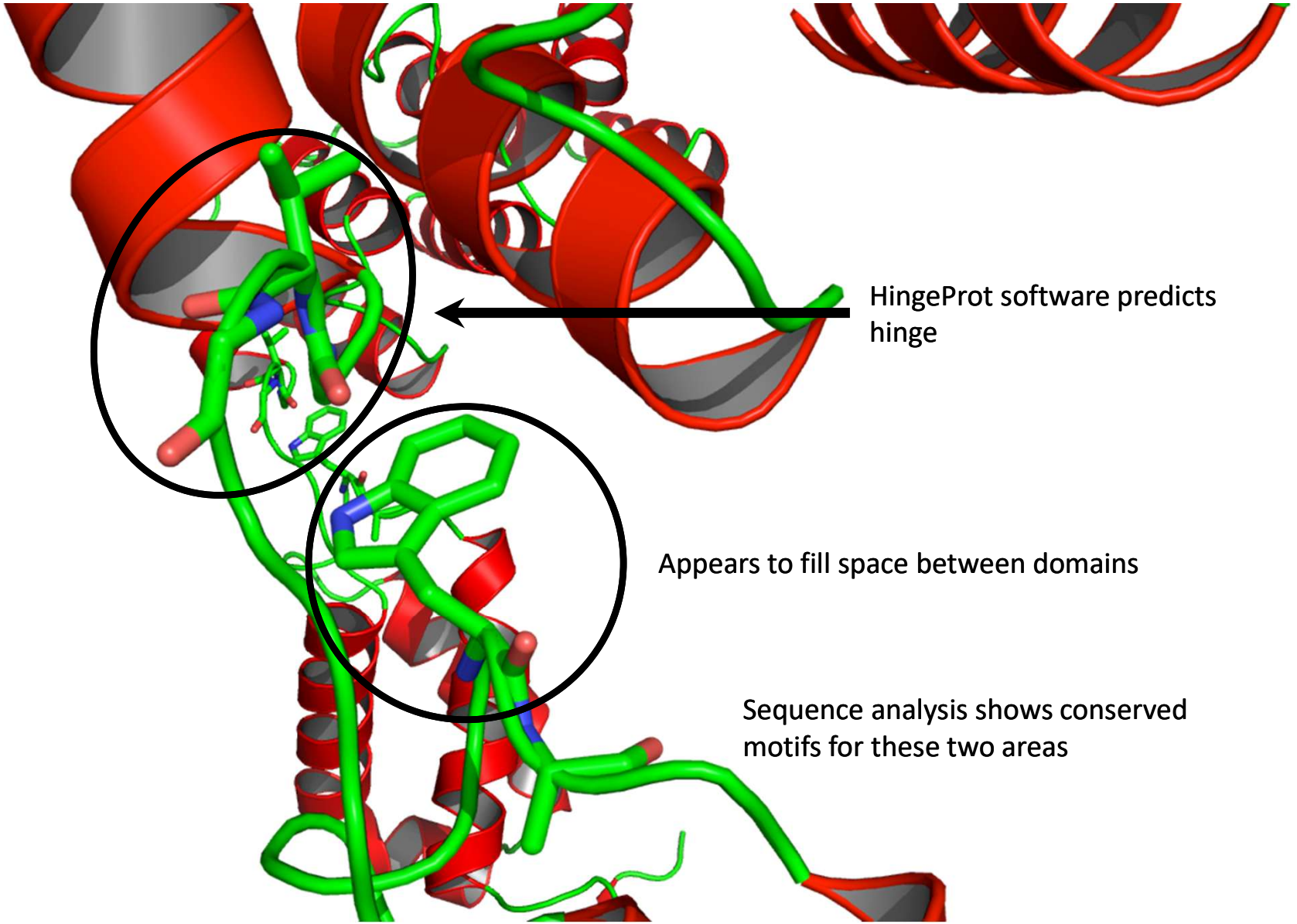
Disorder prediction



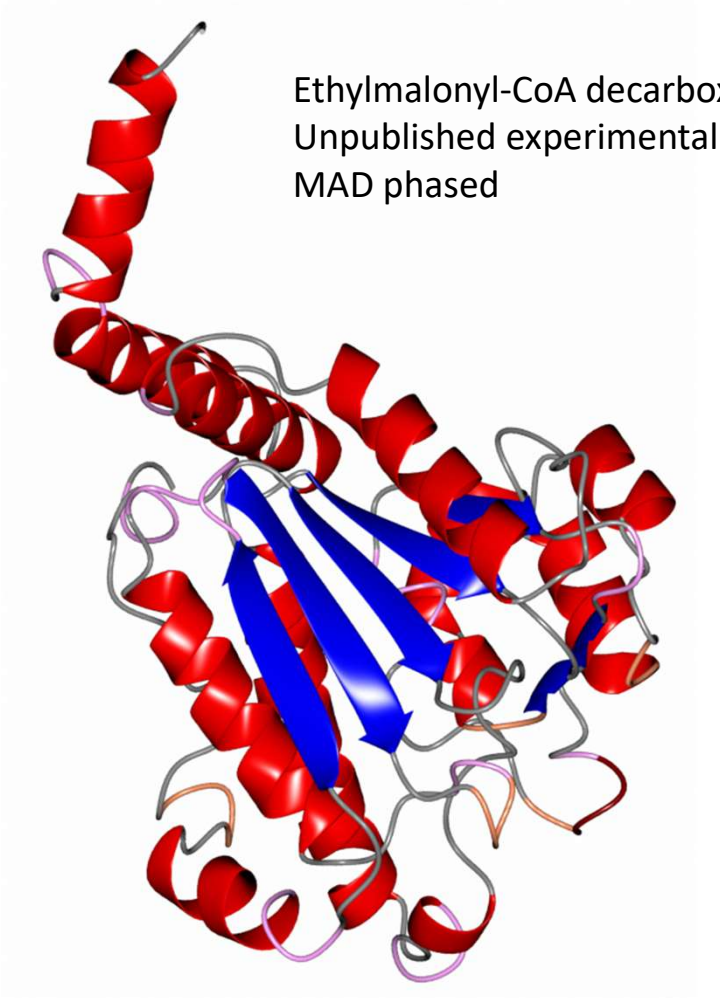
Sequence based
Many servers – new ones almost monthly
Very useful to indicate potential sites for truncation if trying crystallization



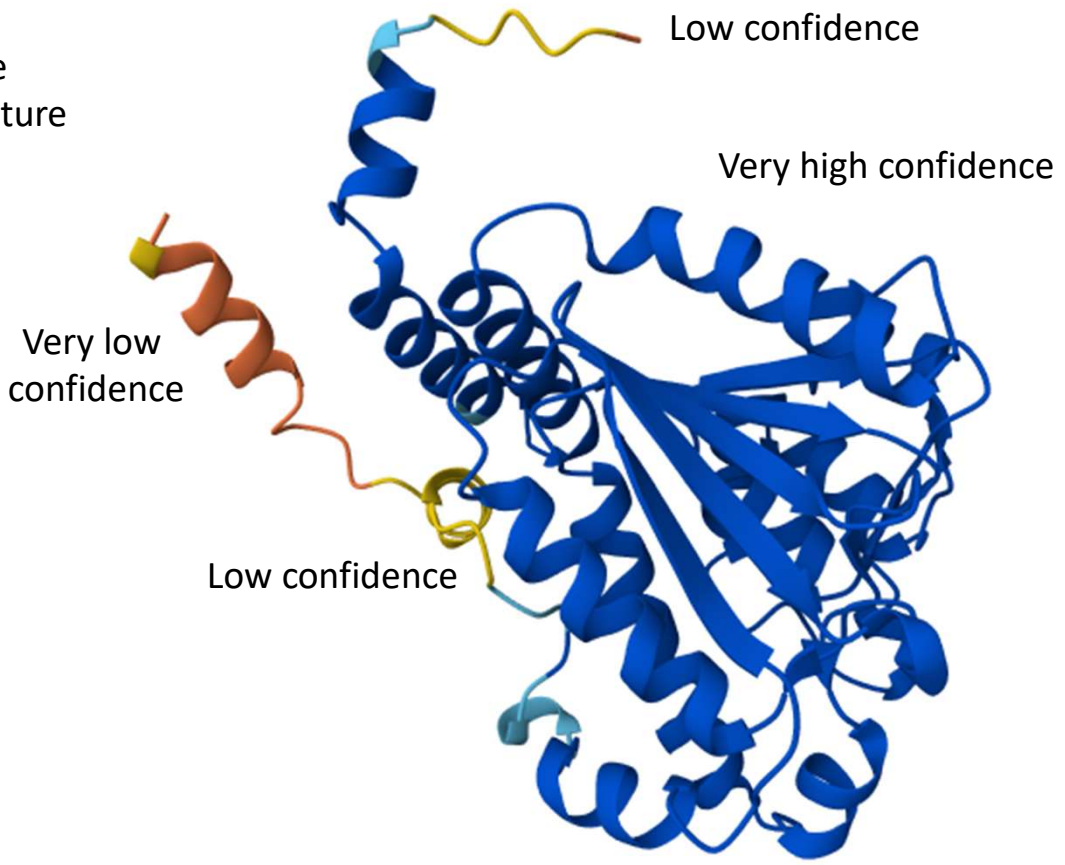



HingeProt software predicts hinge
Appears to fill space between domains
Sequence analysis shows conserved motifs for these two areas

Computational modelling - Alphafold



Ethylmalonyl-CoA decarboxylase
Unpublished experimental structure
MAD phased



Alphafold model

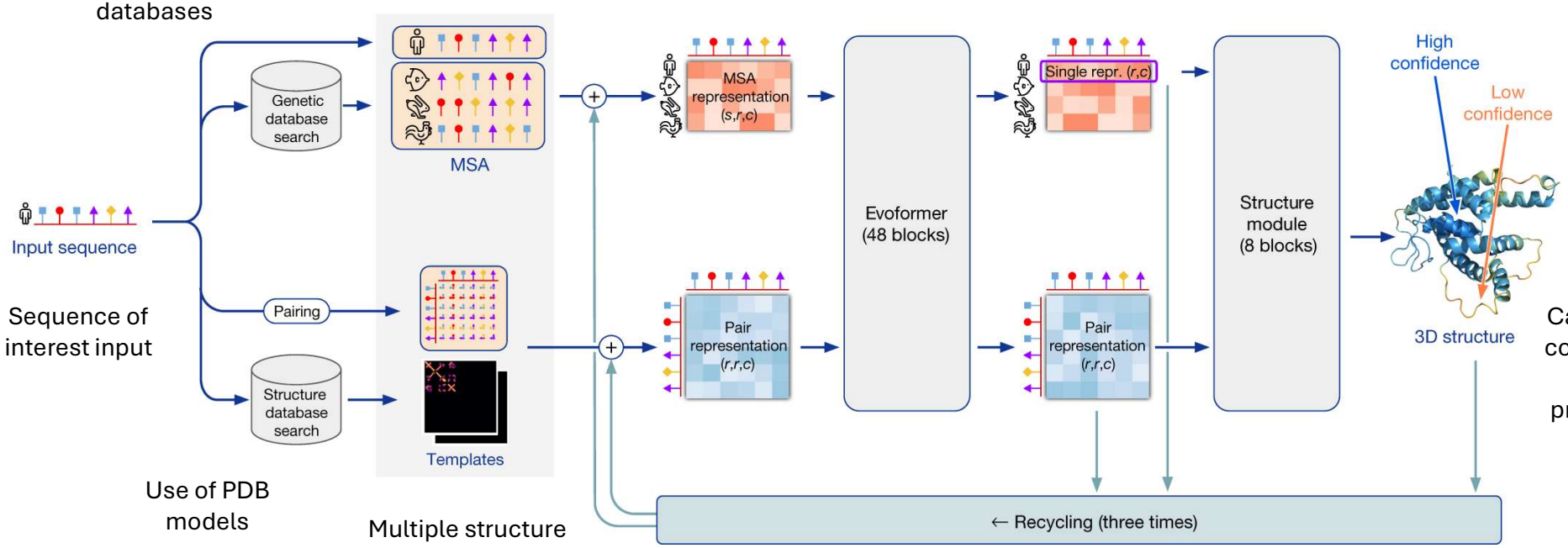
How AlphaFold 2 works

Use of sequence databases

Multiple sequence alignment

Combines sequence alignment and structural pairs and identifies the 'driving' information – *i.e.* what parts of the data it should pay attention to

Tests pathways to produce a hypothetical protein structure, enforcing geometrical restraints, then using a relaxation step for stereo-chemical constraints



Sequence of interest input

Use of PDB models

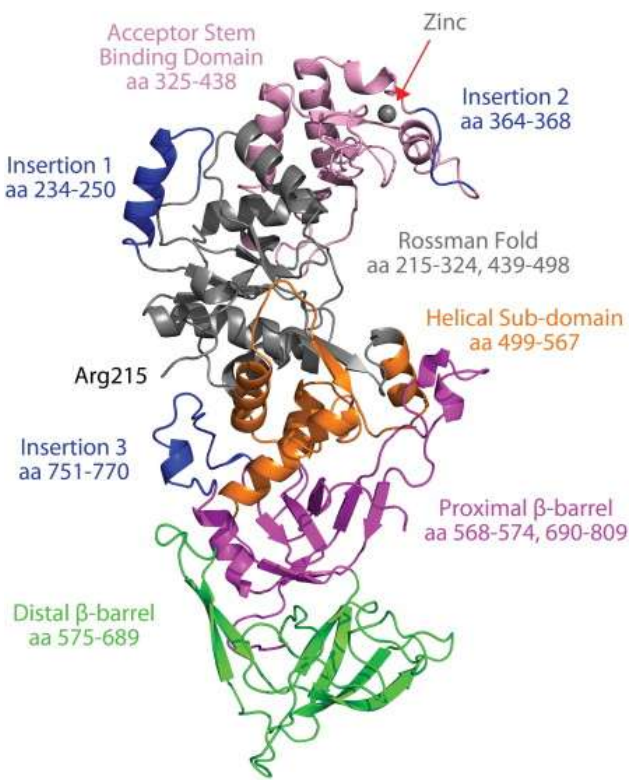
Multiple structure study to determine what pairs of amino acids may align

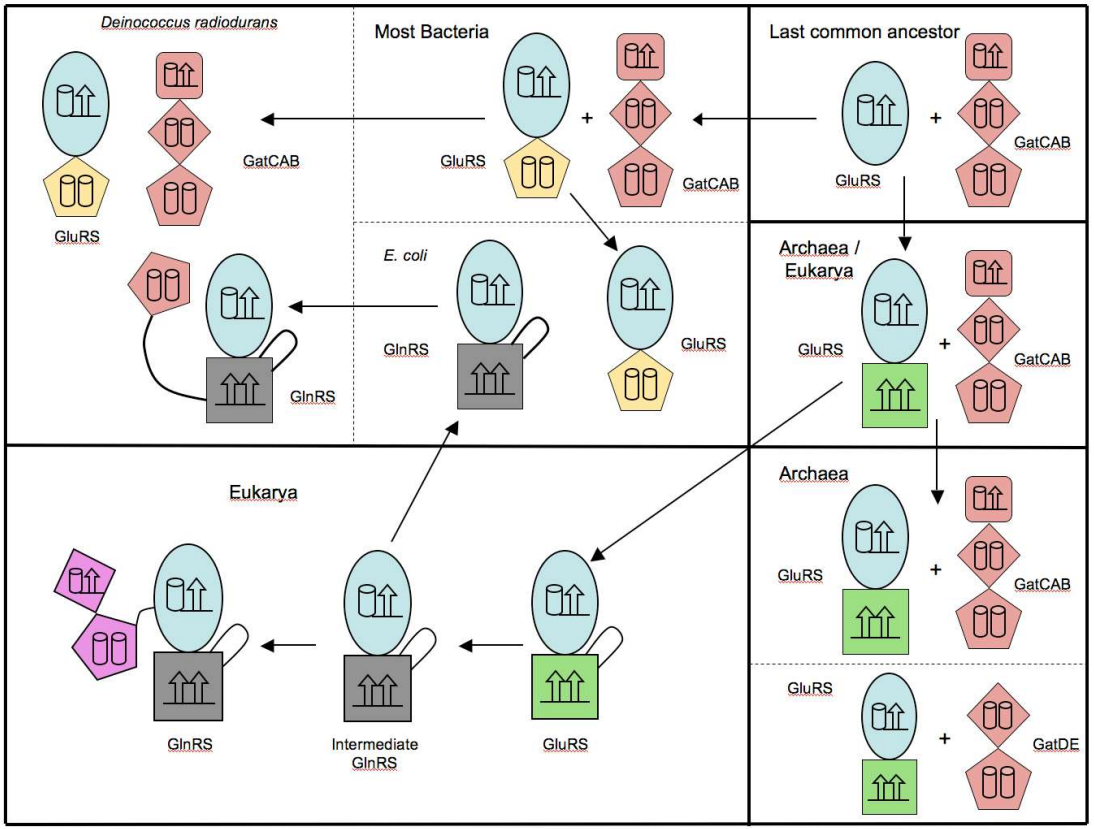
Calculates confidence in the prediction

Repeats the process three times to refine the attention and improve the prediction

Jumper, J., et al. Nature 596, 583–589 (2021)

Co-evolution, contact prediction



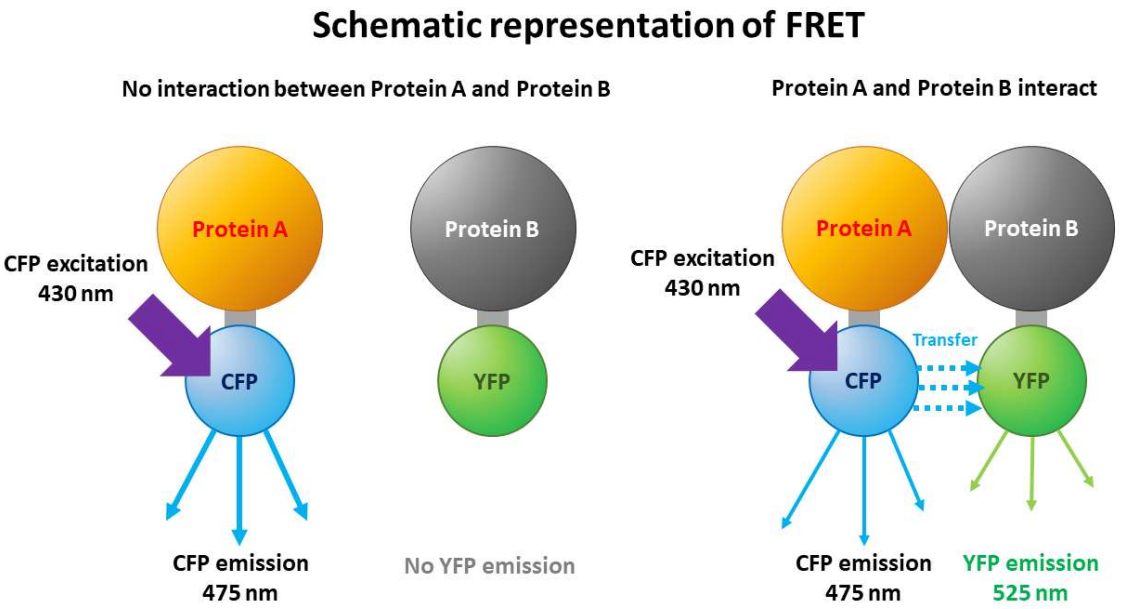


Experimental Biophysical Probes

- FRET (Förster Resonance Energy Transfer): Detect nanoscale conformational changes or interactions.
- Hydrogen-Deuterium Exchange (HDX-MS): Dynamics, solvent accessibility, protein-ligand interactions.
- Crosslinking-MS: Spatial constraints: used in integrative modeling.
- Analytical Ultracentrifugation / DLS / SEC-MALS: Oligomeric state, hydrodynamic radius. 
- Other experimental measurements.

FRET (Förster resonance energy transfer)

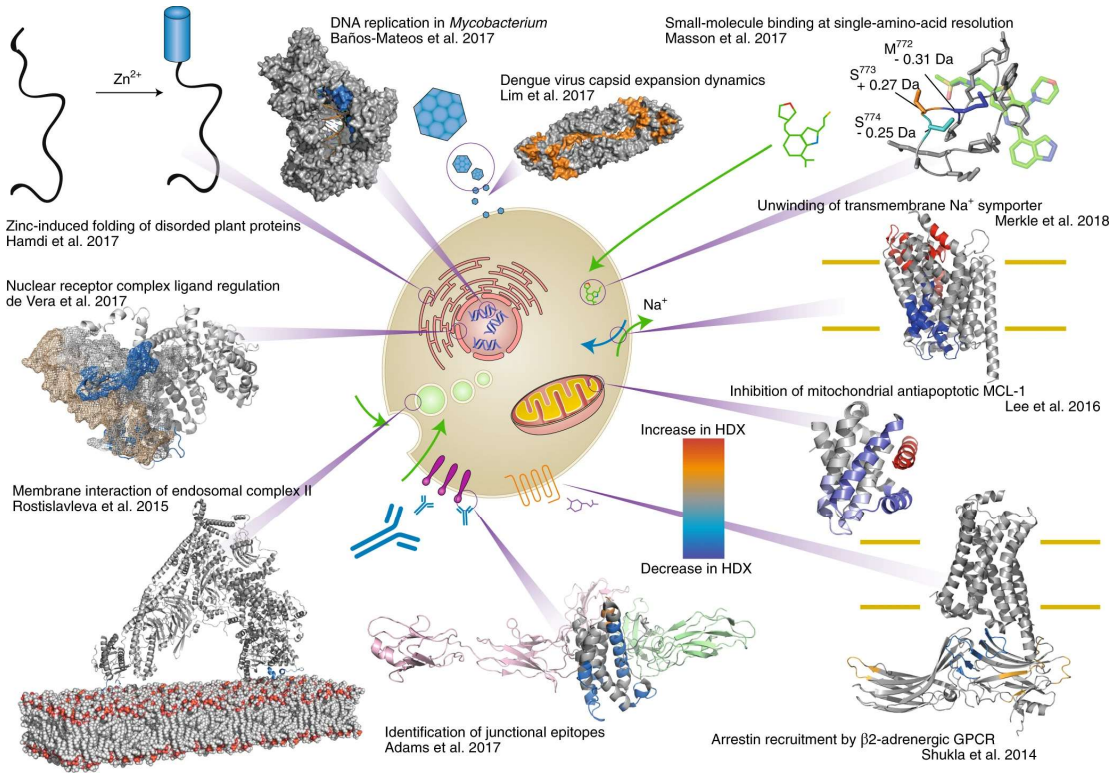
- FRET is based on the fact that a donor dye (e.g. CFP) in an excited state can transfer a part of its energy to an acceptor molecule like YFP.
- The technology involves fusion of donor and acceptor fluorescent proteins (or parts of the same protein) to molecules of interest.
- Co-expression of fusion constructs in living cells enables their interaction to be studied in real time in a quantitative manner.
- The emission from the acceptor can be detected as soon as both dyes are in proximity, e.g. when interaction of two proteins has taken place.



From https://www.berthold.com/en-us/bioanalytics/knowledge/glossary/fluorescence-resonance-energy-transfer-fret/

HD Exchange

- HDX-MS measures changes in mass associated with the isotopic exchange between amide hydrogens of the protein backbone and its surrounding solvent.
- The rate of this exchange is dependent on the folded state of the protein and its dynamics (particularly the stability of hydrogen bonding networks) and the intrinsic chemical properties of the underlying amino acid sequence.
- HDX-MS can be used to examine conformations of individual proteins or large protein complexes, locate protein sites directly or indirectly involved in binding, probe for allosteric effects, monitor the folding dynamics of protein domains, examine intrinsic disorder and provide insights into protein–membrane interaction

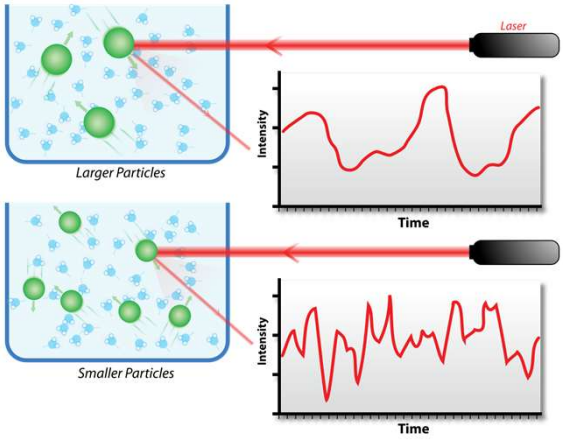


Masson, G.R., Burke, J.E., Ahn, N.G. et al. Recommendations for performing, interpreting and reporting hydrogen deuterium exchange mass spectrometry (HDX-MS) experiments. Nat Methods 16, 595–602 (2019)

Light scattering

Dynamic light scattering (DLS)

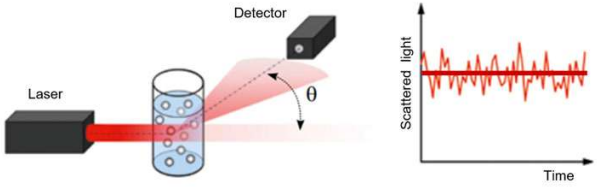
- Used to characterize protein size, determine aggregation, and study protein-ligand interaction
- Recorded over time



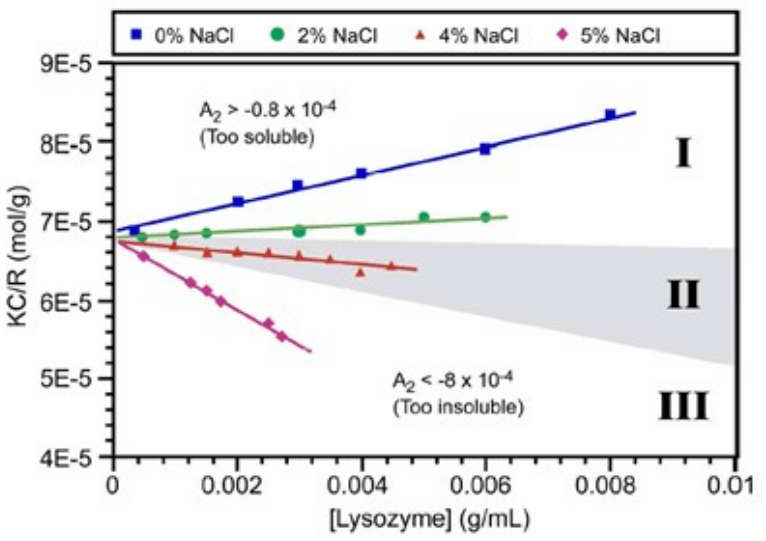
By Mike Jones - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=10502233

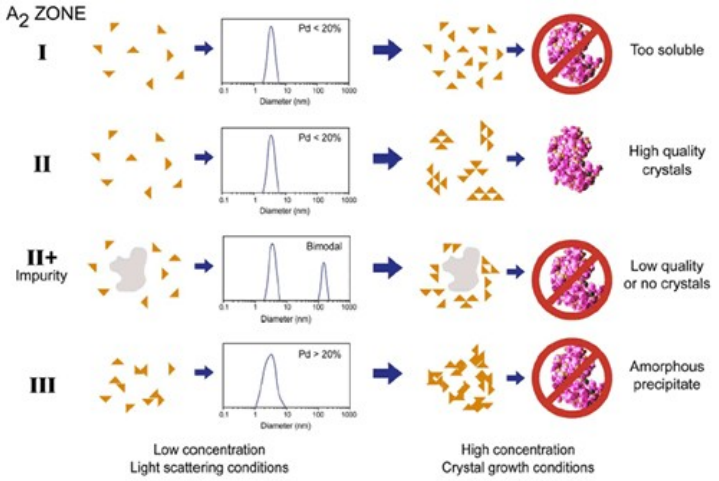
Static light scattering (SLS)

- Used to characterize molecular weight and radius of gyration
- Recorded over different angles or concentrations

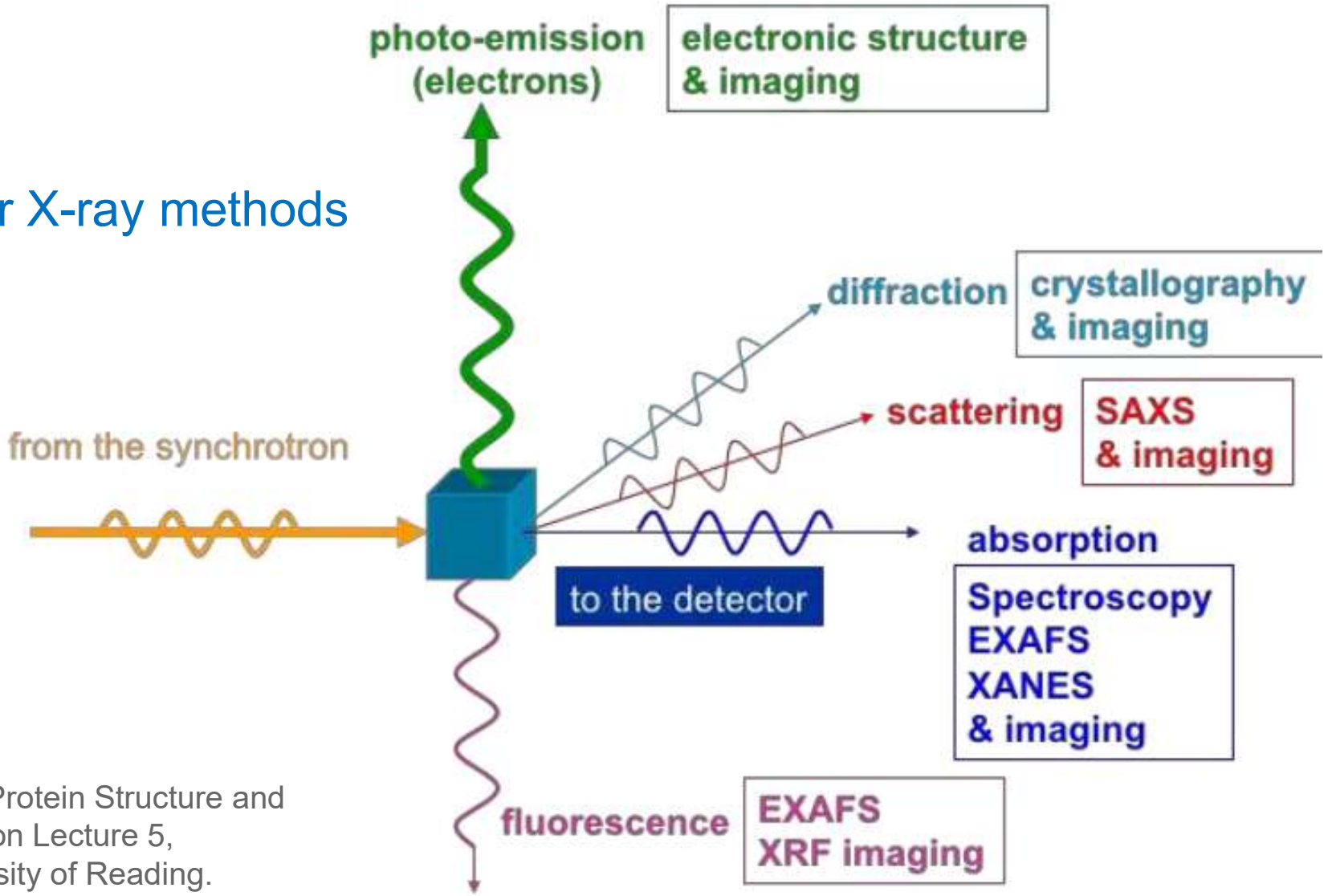


https://wiki.anton-paar.com/en/molecular-mass-measurement-using-static-light-scattering/



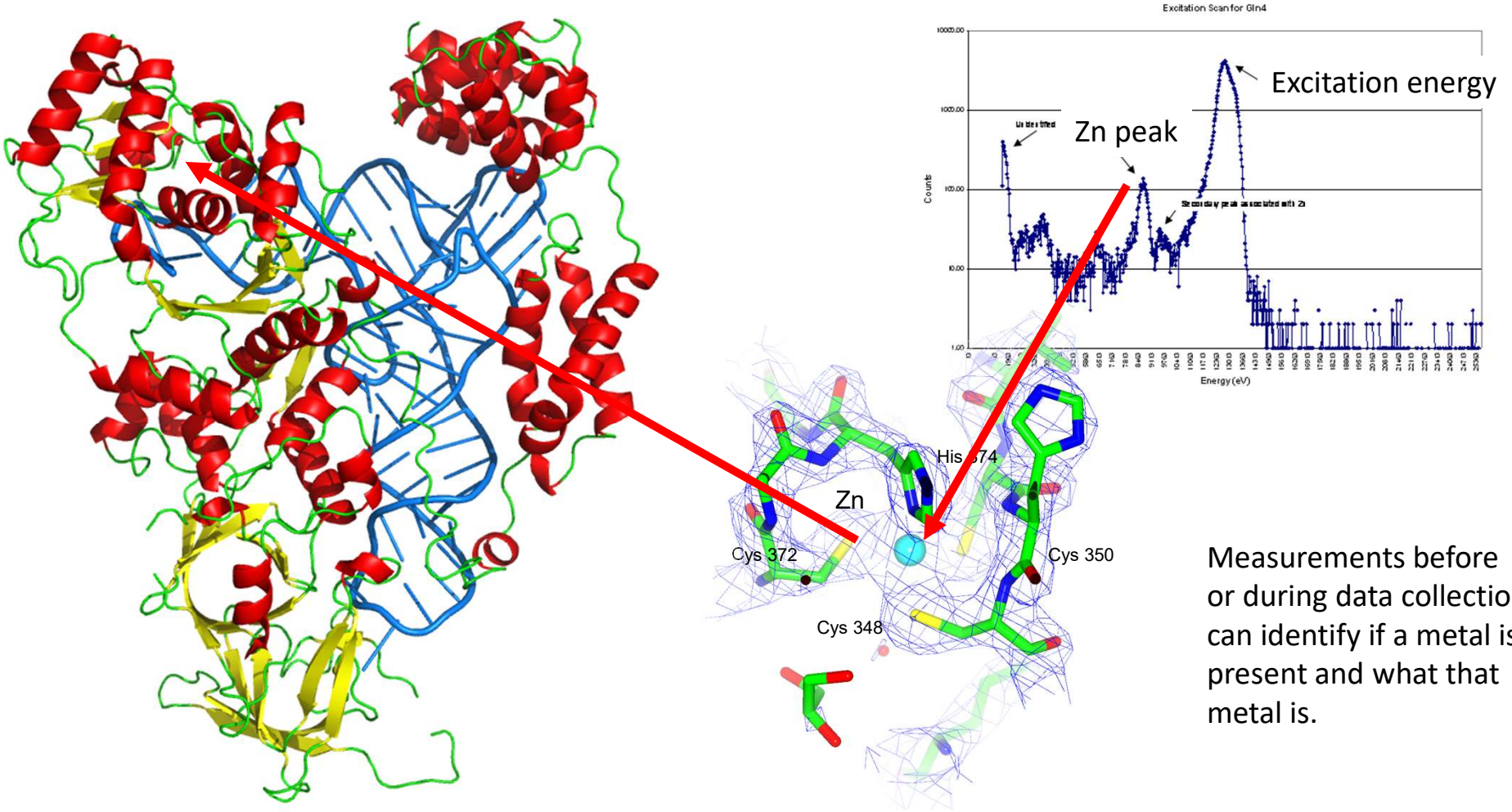


Other X-ray methods

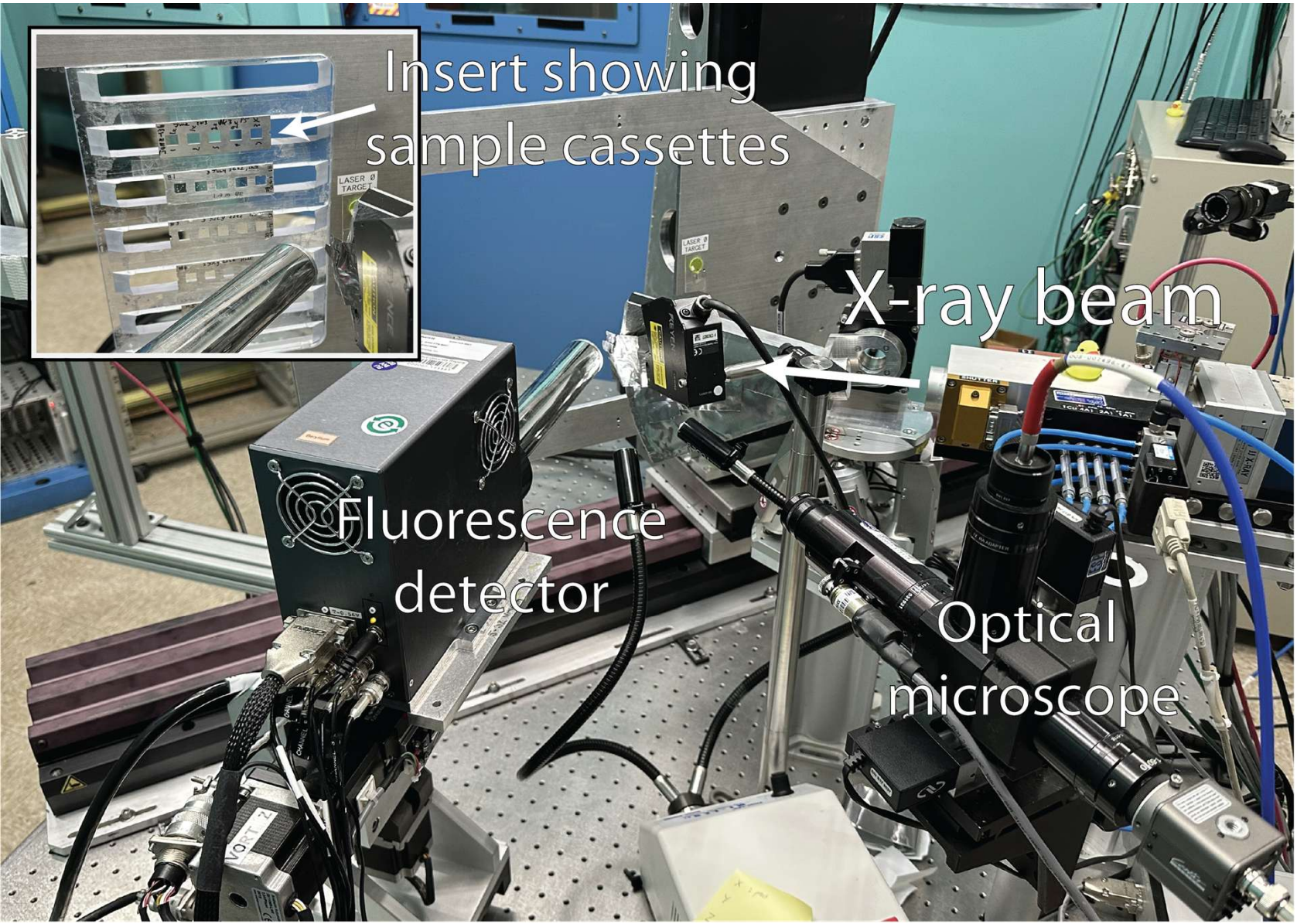


From Protein Structure and Function Lecture 5, University of Reading.

A good experiment can identify the metal



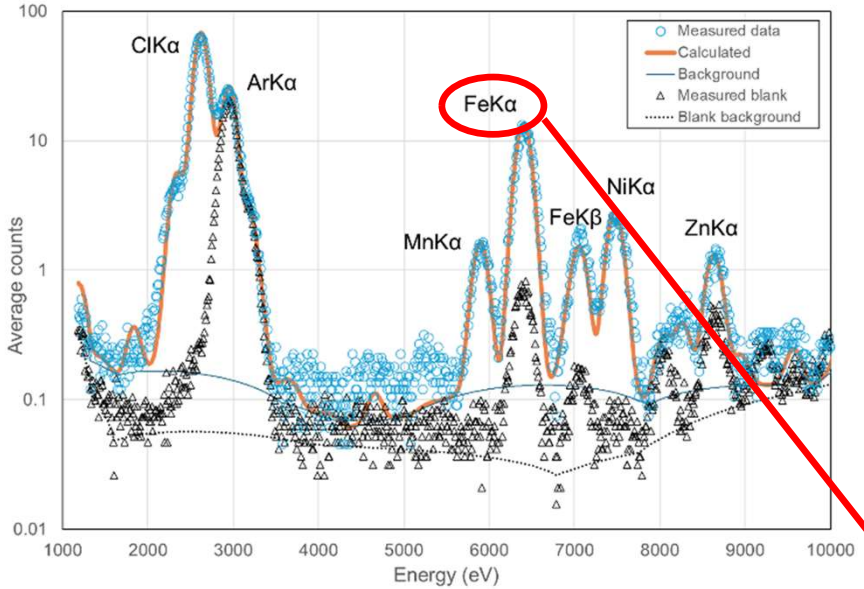
Measurements before or during data collection can identify if a metal is present and what that metal is.



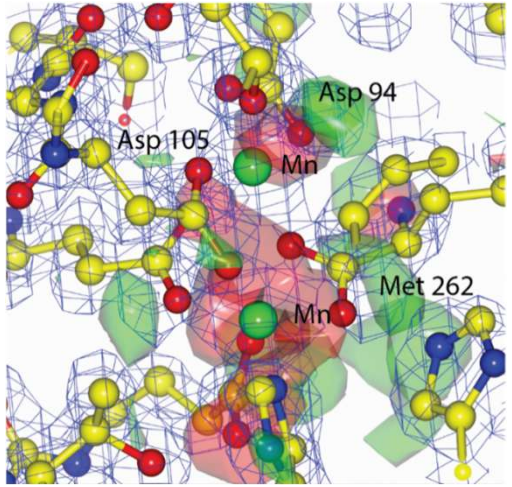
Beamline 7-2

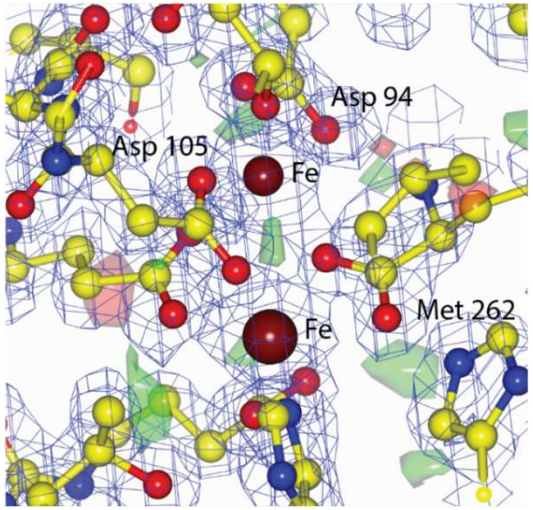
Stanford
Synchrotron
Radiation
Lightsource

Used for
studying
artwork,
manuscripts,
fossils, and now
…
proteins



The Fe is clearly indicated in the XRFS data (and in the PIXE dada)





The R and Rfree of the original model were 0.170 and 0.203 with an RSZD of -5.7

The re-refined models gave an R and Rfree of 0.158 and 0.196 for and the new RSZD was -3.5.

EXAFS (Extended X-ray Absorption Fine Structure) and XANES (X-ray Absorption Near Edge Structure)

EXAFS: Local atomic environment and structure.

- Determines the distances between the absorbing atom and its neighbors, the number of neighbors, and the type of neighbors.
- Data analysis: Involves Fourier transformation of the modulated X-ray absorption data to obtain radial distribution functions.
- Advantages: Can be used to study disordered and amorphous materials but may not provide information on the exact geometry of the coordination complex.

XANES: Electronic structure, oxidation state, and coordination chemistry.

- Information: Reveals the formal oxidation state, coordination geometry (e.g., octahedral, tetrahedral), and d-band occupancy of the absorbing atom.
- Data analysis: Involves fitting the XANES spectrum to theoretical models to extract information about the electronic structure.
- Advantages: Can be used to study the local environment of a specific atom within a larger structure but may not provide as detailed structural information as EXAFS, particularly in disordered systems.

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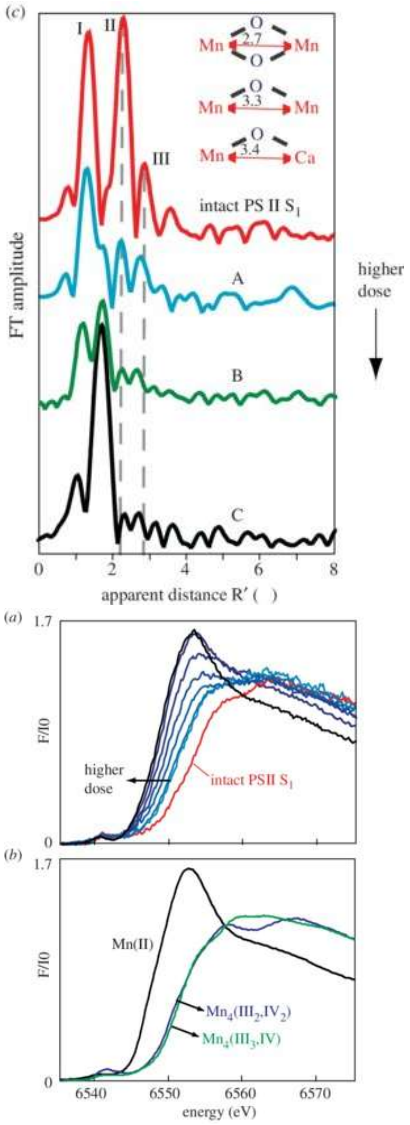
XANES primarily provides information about the electronic structure and coordination chemistry, while EXAFS focuses on the local atomic structure.



Mn EXFAS AND XANES of single crystals of PSII Thermosynechococcus elongatus as a function of X-ray dose.

the EXAFS spectra show that the three Fourier peaks characteristic of Mn-bridging-oxo, Mn-terminal and Mn-Mn/Ca interactions (dashed vertical line) are replaced by one Fourier peak characteristic of a Mn(II) environment.

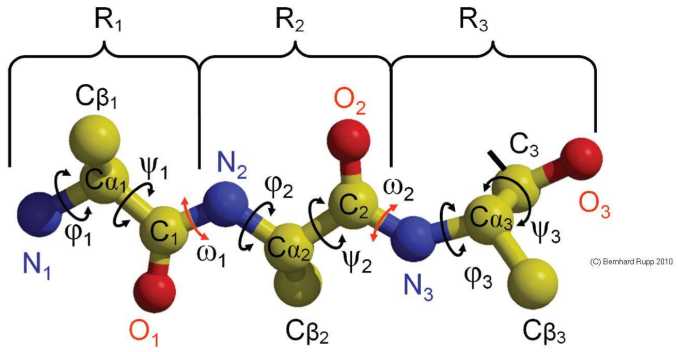
Mn in PSII normally present as Mn 4 (III 2 , IV 2) is reduced to Mn(II) as seen by the changes in XANES spectra. Mn models: the Mn XANES from Mn complexes in formal oxidation states II, III and IV.

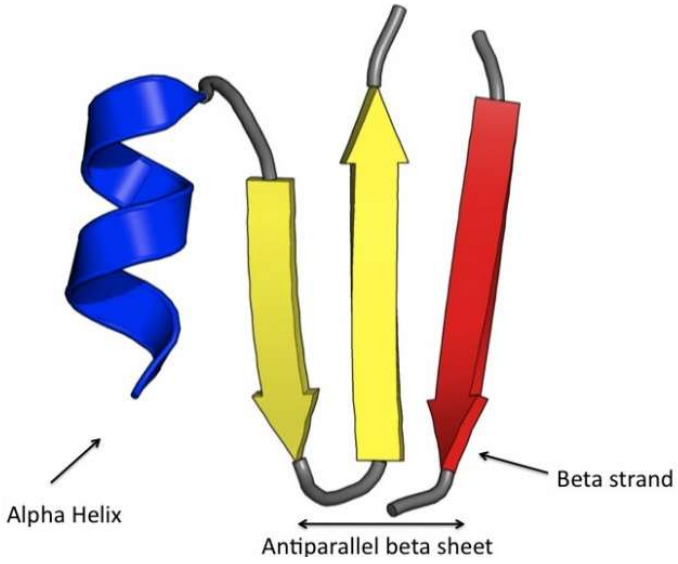
From Jan Kern

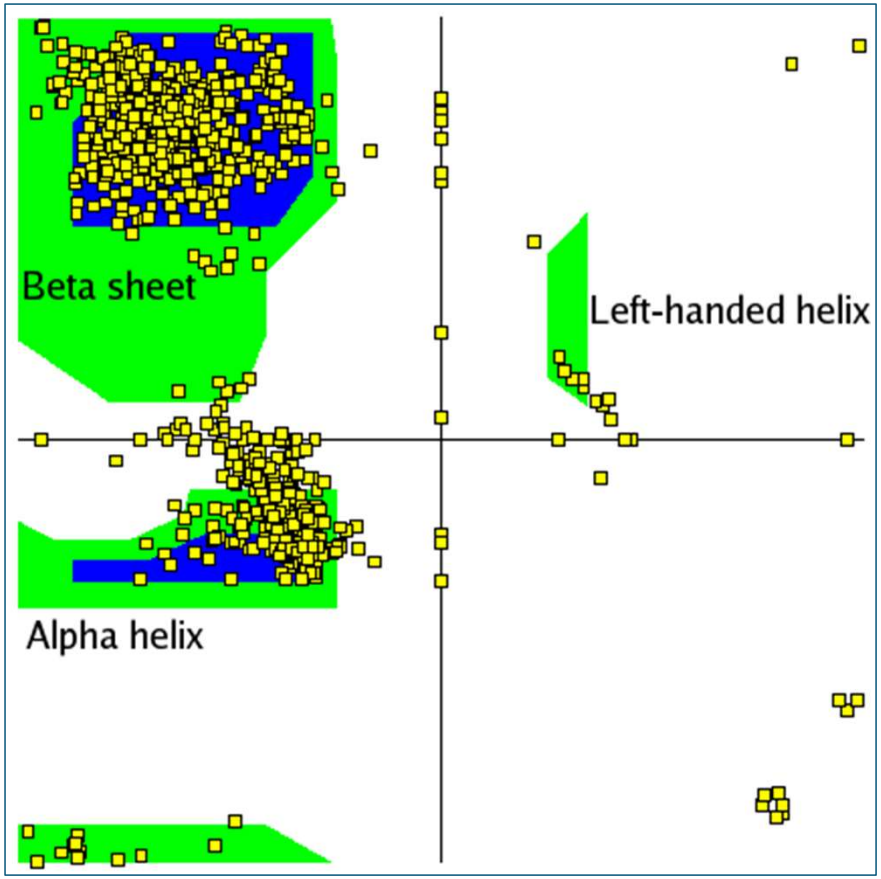
Structural Validation and Assembly Tools

- **Computational validation:**
  - Ramachandran plot, geometric expectations.
- **Native and Top-Down Mass Spectrometry**:
  - Subunit composition, heterogeneity, PTMs.
- **Biochemical Assays (mutagenesis, footprinting, cleavage)**:
  - Validate functional regions; probe active sites.
- **Light Scattering (DLS, SEC-MALS)**:
  - Identify aggregation or complex formation.

Structural expectations

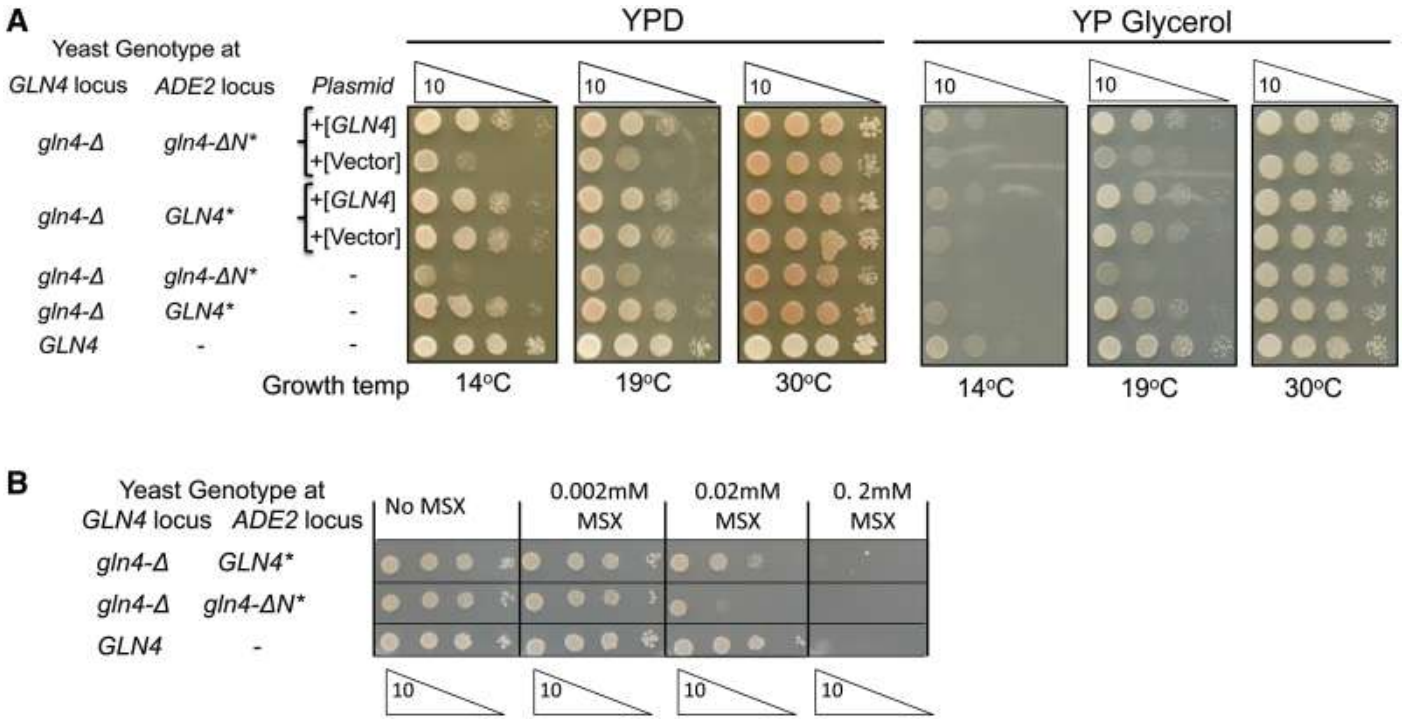






From Bernhard Rupp and EMBL-EBI

Biochemical assays



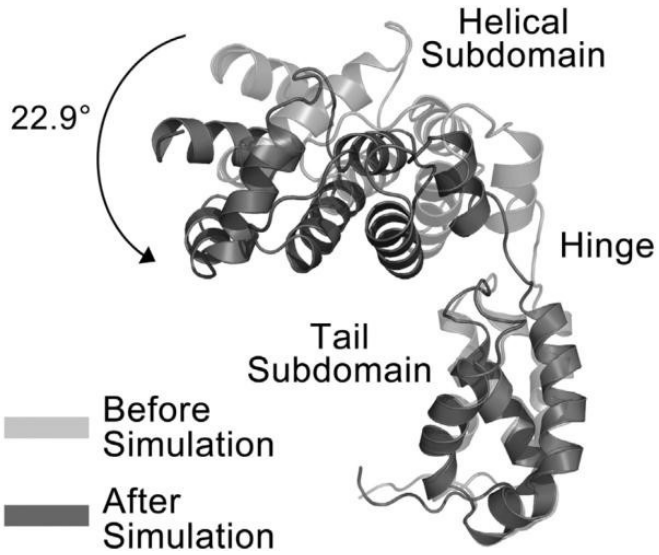
Deletion of the N-terminal domain of *GLN4* impairs function. (**A**) Mutants bearing a *gln4* mutation in which amino acids 2–210 are deleted are defective in growth at low temperature on YP media containing glucose or glycerol as a carbon source. Serial dilutions of strains with either wild-type *GLN4* or *gln4(*211–809) (marked *gln4-ΔN**) integrated at the *ade2* locus in the *gln4-ΔKanR* mutant were grown as indicated. Indicated strains carry CEN plasmids either with or without *GLN4*. (**B**) Mutants bearing a *gln4* mutation in which amino acids 2–210 are deleted are sensitive to the glutamine synthase inhibitor L-methionine sulfoximine (MSX).

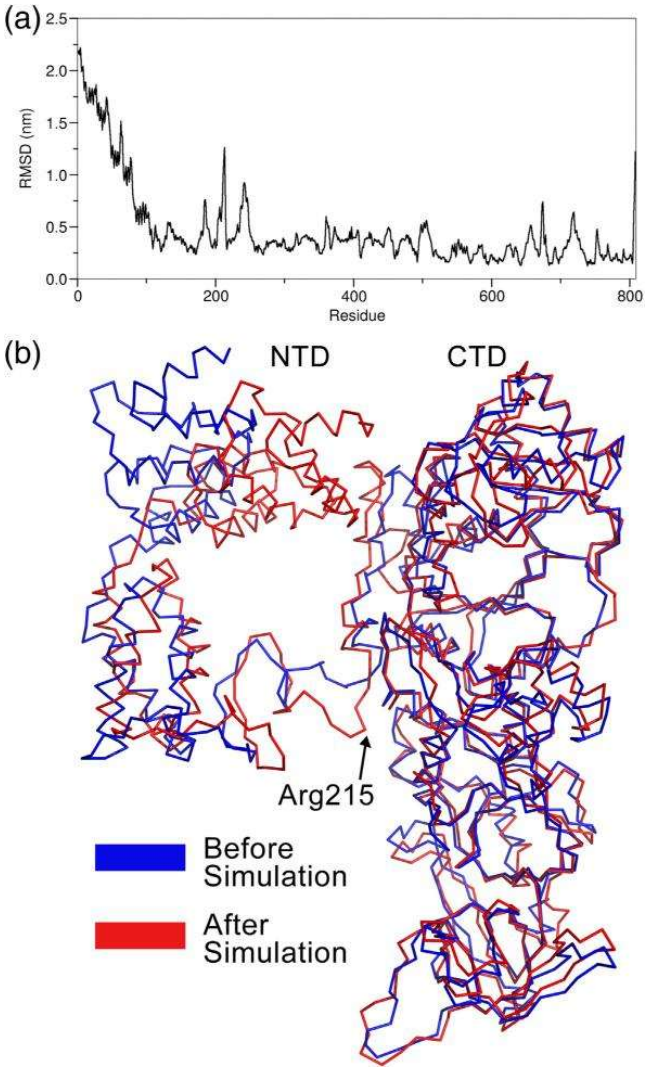
Integrative and Hybrid Modeling

- Docking and Molecular Dynamics:
  - Fit models into cryo-EM maps or SAXS envelopes; simulate motion.
- Integrative Modeling Platform (IMP):
  - Combine EM, XL-MS, FRET into a unified structure.
- AI Tools for Binding Site Prediction / Pocket Mapping

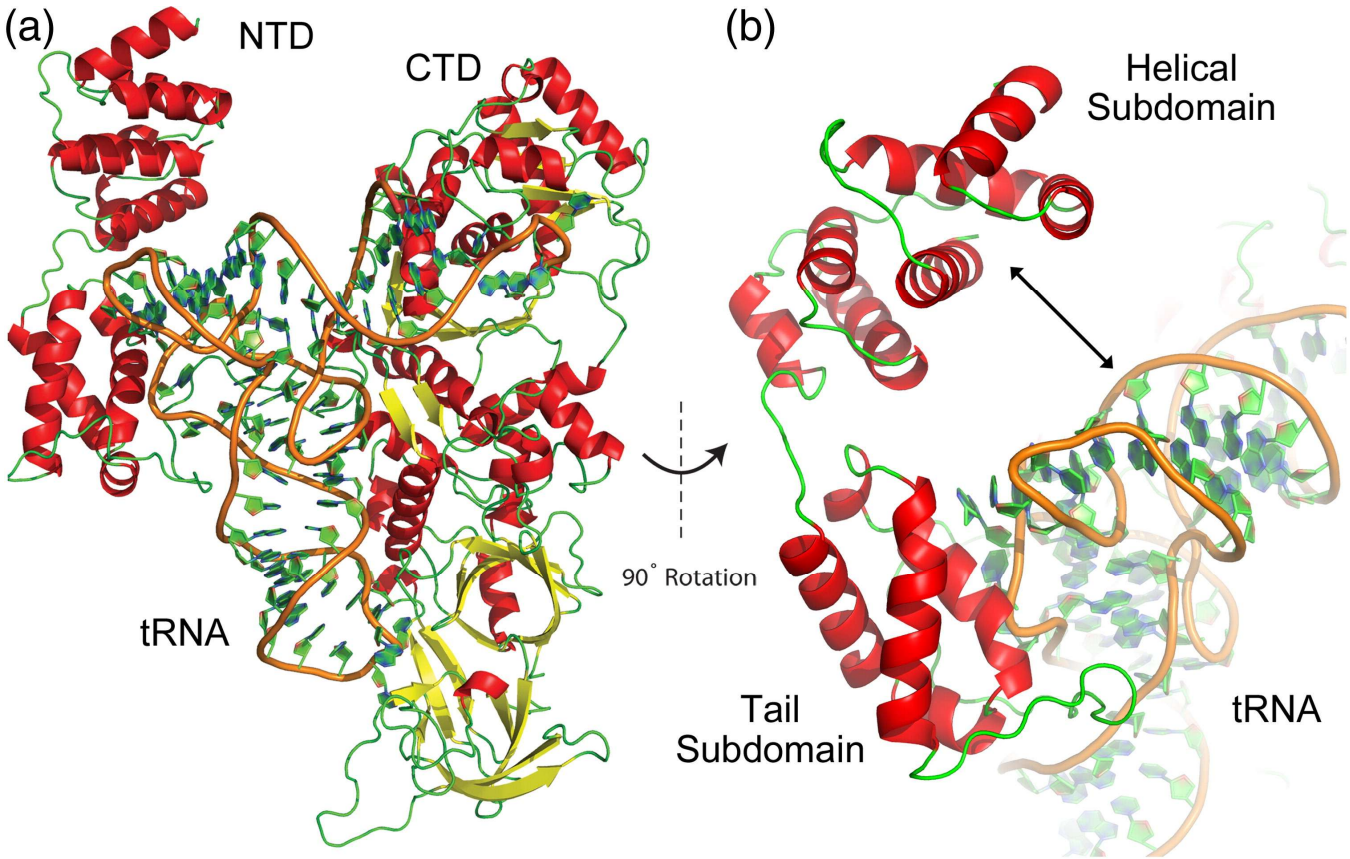
Molecular dynamics

Can provide information on potential motion that is mechanistically important



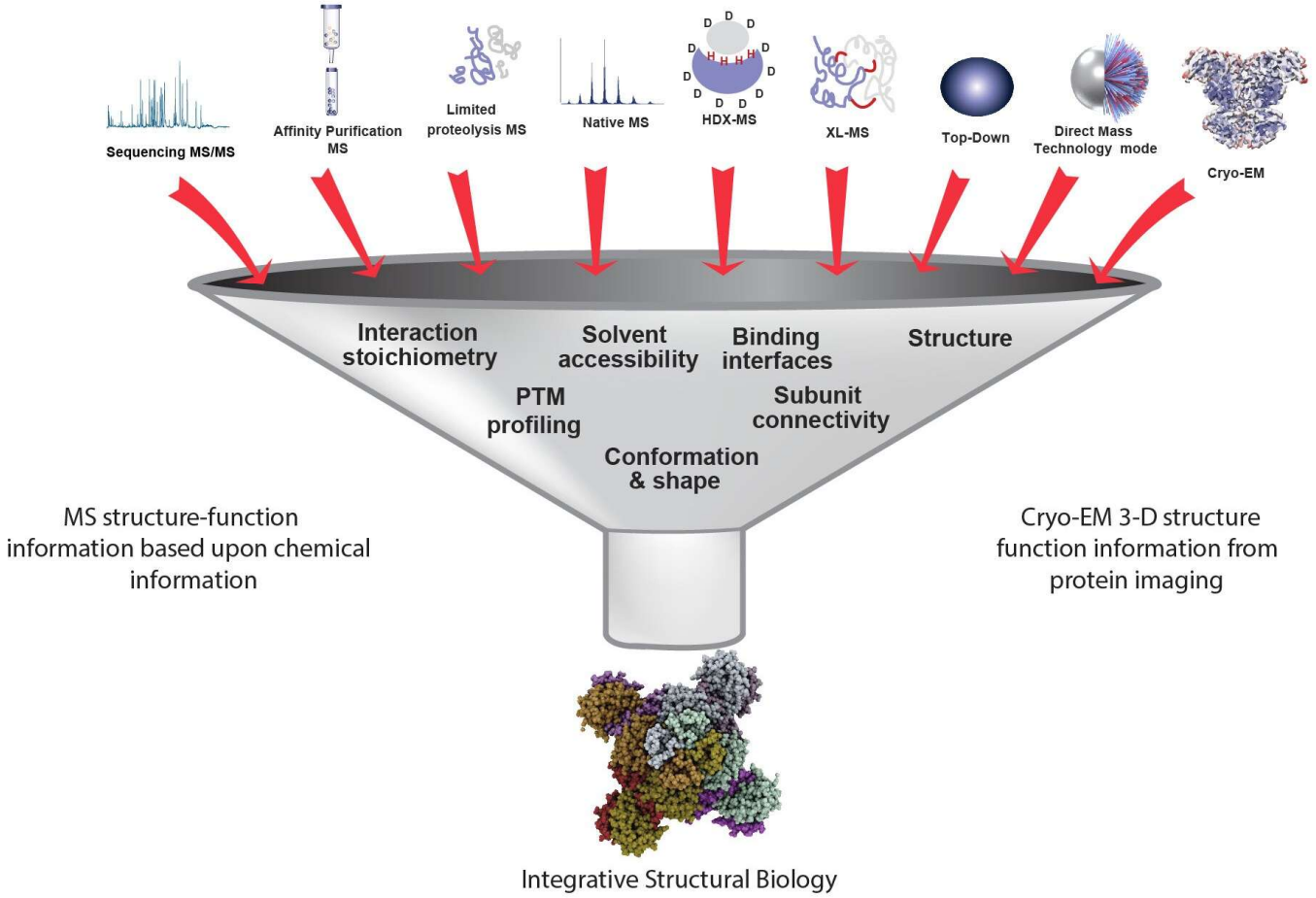


Mechanism


(a)
NTD
CTD
tRNA
90° Rotation
(b)
Helical
Subdomain
Tail
Subdomain
tRNA

Integration

- Many techniques combine to produce a complete picture.
- Each has limitations, but combined, those limitations are overcome



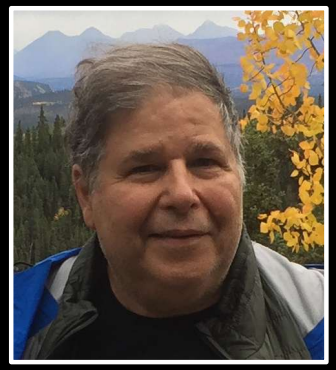
Choosing the right tool

- Want to know conformation? Use SAXS or FRET.
- Want to know stoichiometry? Use MS or SEC-MALS.
- Want to model flexibility? Use MD or HDX.

Thanks to an enormous number of people







BioXFEL

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Hauptman-Woodward Research Institute

SSRL

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DEPARTMENT OF ENERGY · UNITED STATES OF AMERICA

DEFENSE THREAT REDUCTION AGENCY · UNITED STATES OF AMERICA

Questions?

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