Interference and inhibition of protein synthesis
Protein folding

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General lecture plan:

Post-translational modifications of the polypeptide chain

- covalent modifications:
- ✓ acetylation
- ✓ glycosylation,
- ✓ ubiquitination

Translation Inhibitors

- Classification and Mechanisms of Action
- Aminoglycosides. Streptomycin
- Macrolides. Erythromycin
- Fusidic Acid
- Diphtheria toxin
- Interferons

Protein folding

- The Anfinsen Dogma
- The Levinthal Paradox
- The Folding Funnel
- Intrinsically disordered proteins

Protein Structure Modeling

- RoseTTAFold
- "Foldit" ("Fold It").

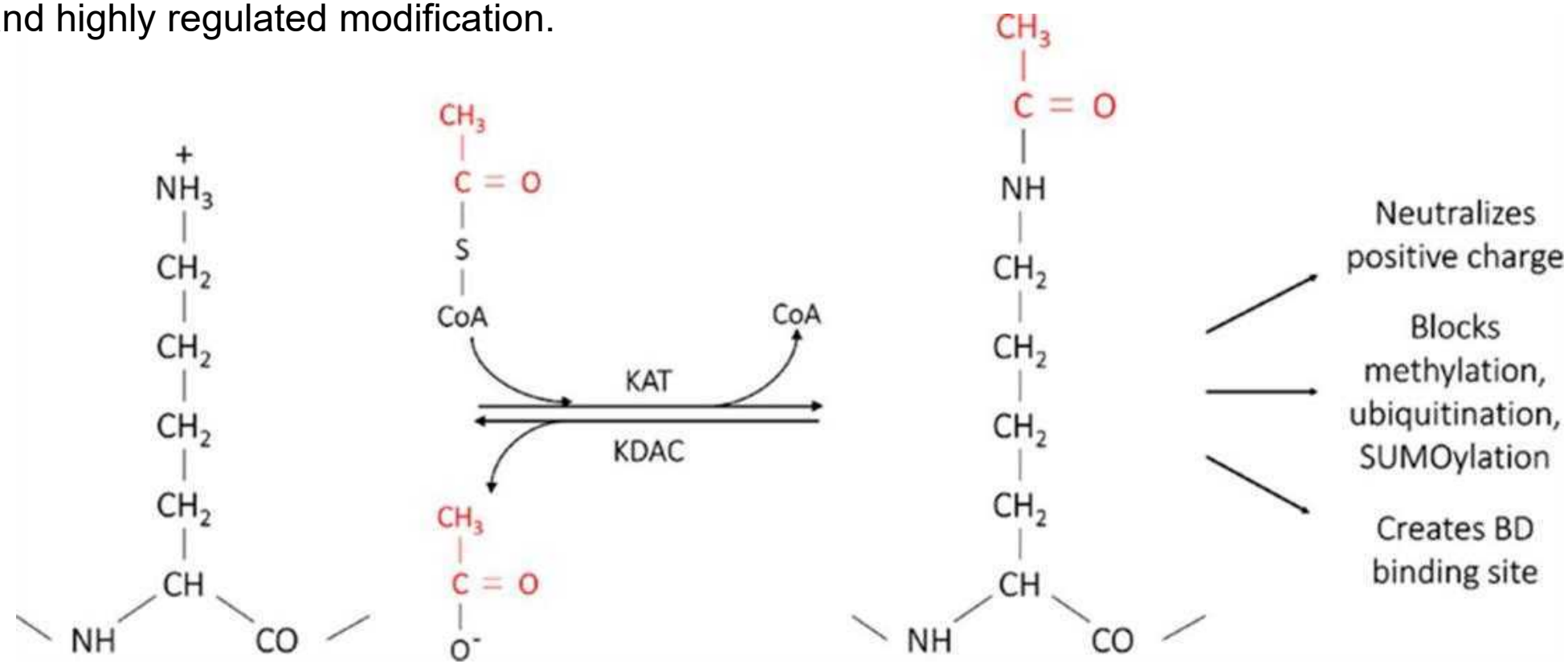
Post-translational modifications of the polypeptide chain

Covalent modifications. Acetylation

Protein acetylation is a key PTM in which an acetyl group ($CH_3CO–$) is transferred to a protein

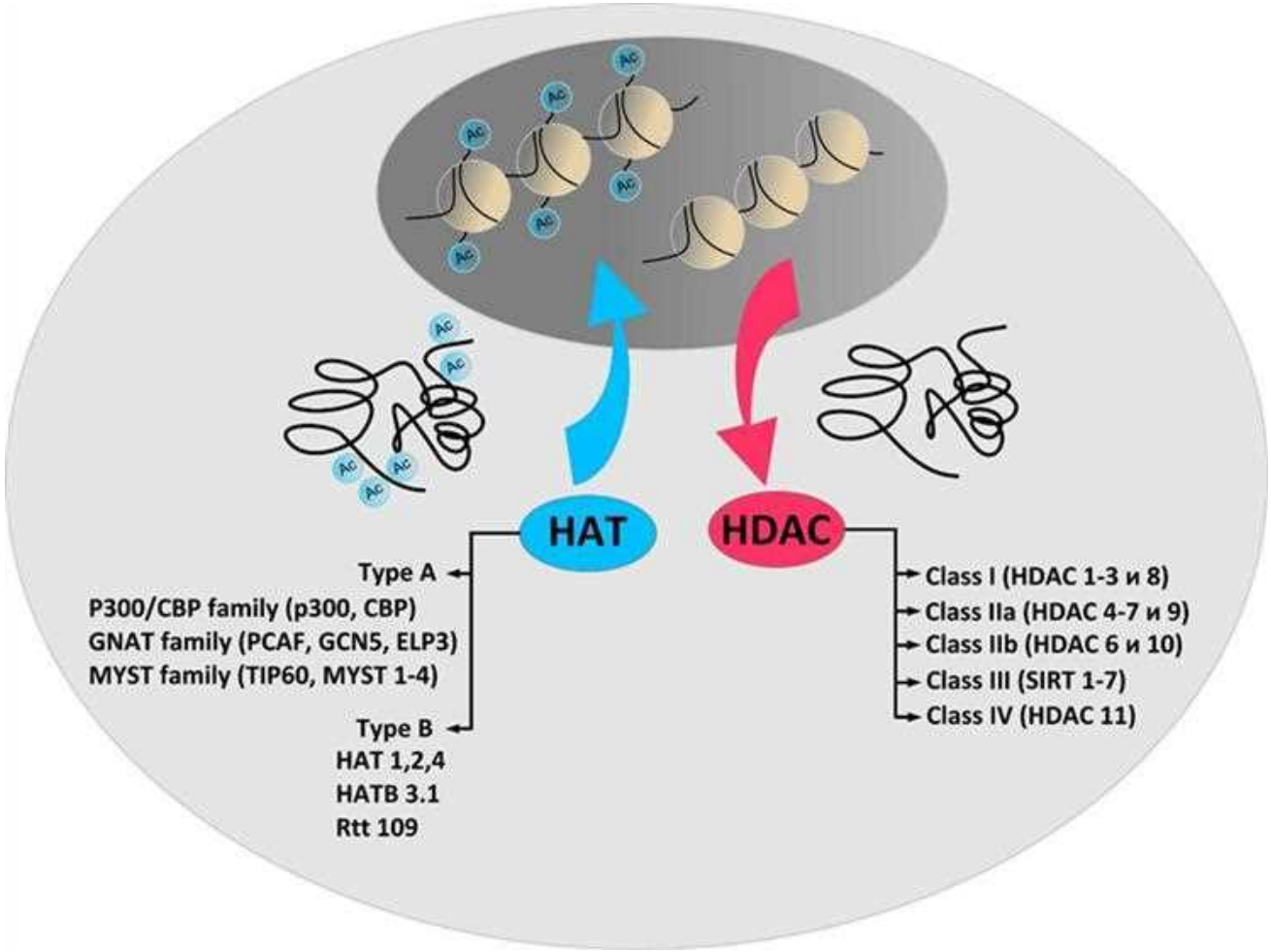
Types of Acetylation:

- N-α-acetylation: Occurs at the N-terminus of a protein shortly after synthesis. Very common; can affect protein stability.
- N-ε-acetylation: Occurs on the ε-amino group of lysine residues (K-Ac). This is a reversible and highly regulated modification.



Post-translational modifications of the polypeptide chain

Covalent modifications. Acetylation



Enzymes:

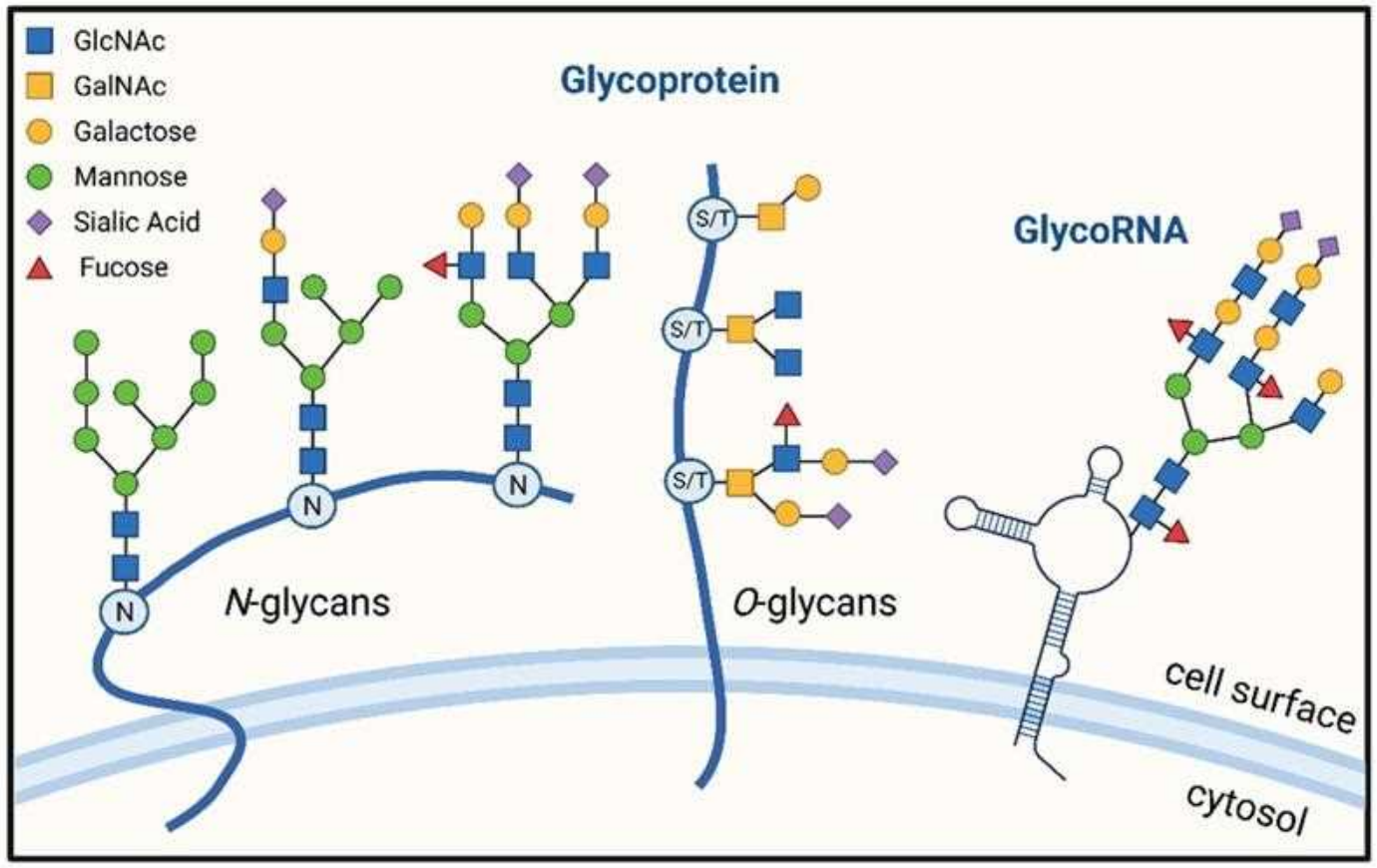
- Acetyltransferases (HATs, KATs): Add an acetyl group. The donor of the acetyl group is typically Acetyl-CoA.
- Deacetylases (HDACs, KDACs): Remove the acetyl group.

Functions and Roles:

- Regulation of Transcription: Acetylation of histones weakens their interaction with DNA, making chromatin more open and active for transcription. This is a central mechanism of epigenetic regulation.
- Charge Alteration: The addition of an acetyl group neutralizes the positive charge of lysine, which can drastically alter a protein's structure and function.
- Stability Control: Can affect the recognition of a protein by ubiquitin ligases and thus its lifespan.
- Metabolism Regulation: Acetylation of non-histone proteins (e.g., transcription factors and metabolic enzymes) coordinates the cellular response to nutrient availability (e.g., glucose).

Post-translational modifications of the polypeptide chain

Covalent modifications. Glycosylation



Glycosylation is a biochemical process involving the attachment of an oligosaccharide glycan to a nitrogen atom, most commonly the N4 nitrogen of asparagine residues.

The nature of the N-linked glycans attached to a glycoprotein is determined by the protein itself and the cell in which it is expressed. It also varies between biological species and is therefore used to recognize "self" and "non-self" proteins.

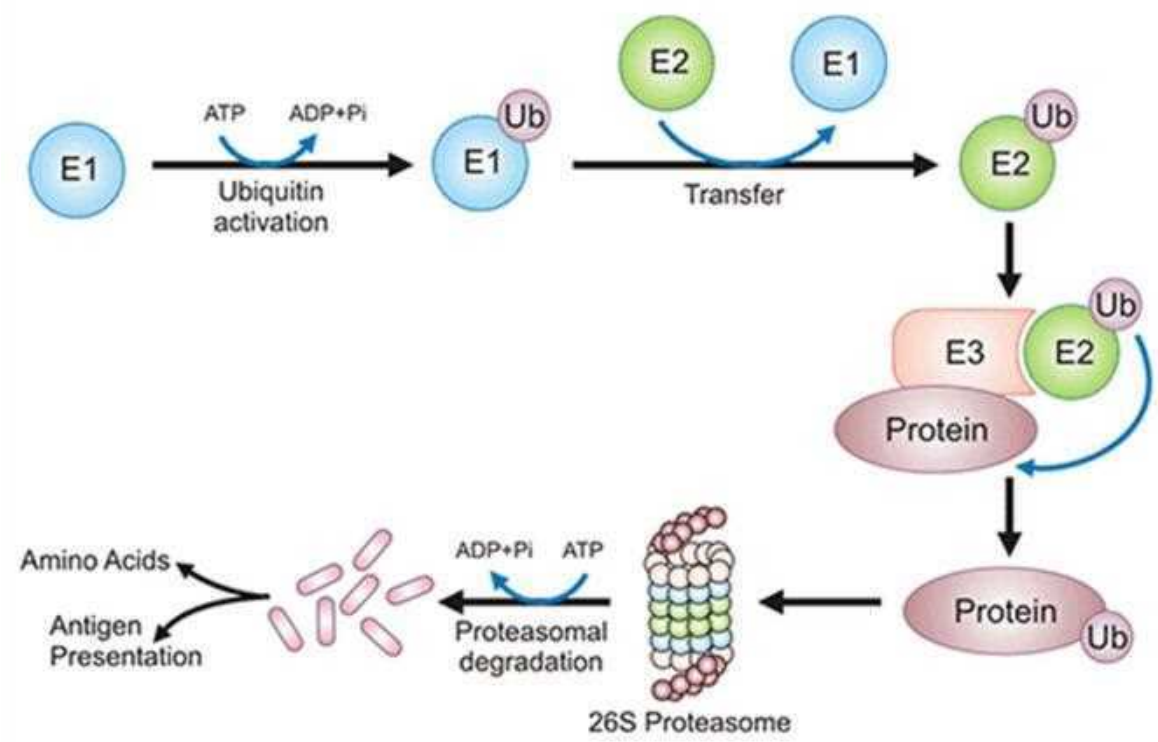
Glycoproteins include:

- antibodies (immunoglobulins);
- interferons;
- complement factors;
- membrane proteins;
- blood plasma proteins, including those that determine blood group;
- hormones;
- receptors;
- enzymes.

According to various estimates, between 50% and 70% of human proteins are glycosylated. More than 40% of proteins already used for medical purposes and those in preclinical development phases are also glycoproteins, for which functionality, efficacy, and stability are linked to the presence and pattern of glycosylation.

Post-translational modifications of the polypeptide chain

Covalent modifications. Ubiquitination



Protein ubiquitination is a crucial PTM that acts as a universal signal for targeted protein degradation by the proteasome. Essentially, it is a "death tag" for a protein.

Key Steps:

1. Activation: An E1 enzyme (ubiquitin-activating enzyme) activates the small protein ubiquitin using ATP.
2. Conjugation: The activated ubiquitin is transferred to an E2 enzyme (ubiquitin-conjugating enzyme).
3. Ligation: An E3 enzyme (ubiquitin ligase) recognizes the specific target protein and attaches ubiquitin to it. E3 ligases determine the specificity of the entire process, as each one recognizes a specific set of target proteins.

Functions:

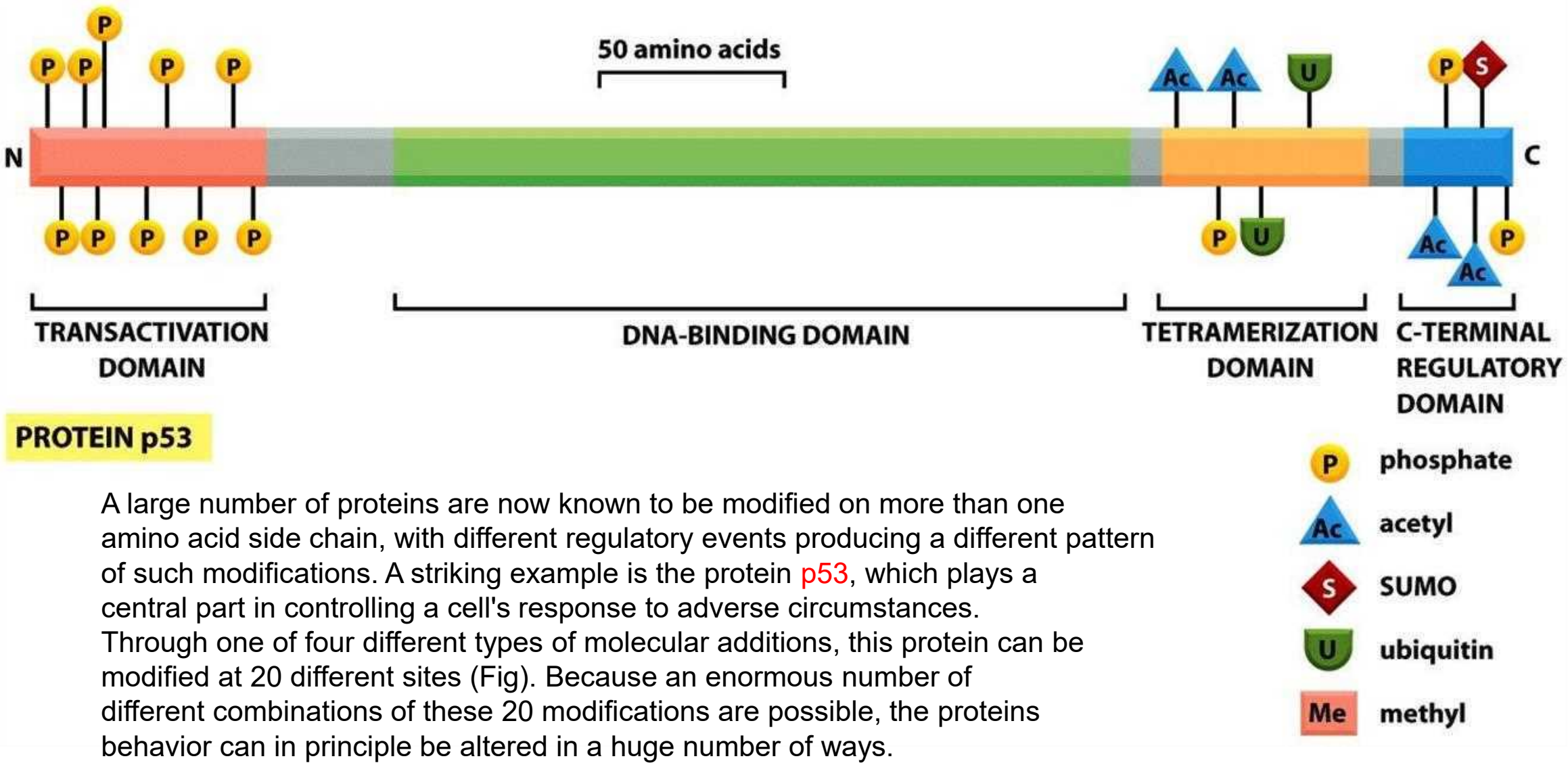
- ❑ Degradation: A chain of multiple ubiquitin molecules (a polyubiquitin chain) serves as a signal for the proteasome, which recognizes it and degrades the target protein into short peptides.
- ❑ Regulation: Ubiquitination controls the levels of key regulatory proteins involved in:
- Cell cycle
- Inflammation
- Signaling pathways
- Stress response
- DNA Repair: Marks damaged proteins for removal.

Other Signals: A single ubiquitin molecule (monoubiquitination) can regulate a protein's activity, localization, and interactions.

Macromolecular juggling by ubiquitylation enzymes:

A hypothetical transition between distinct states in the catalytic cycle of canonical E1 enzymes

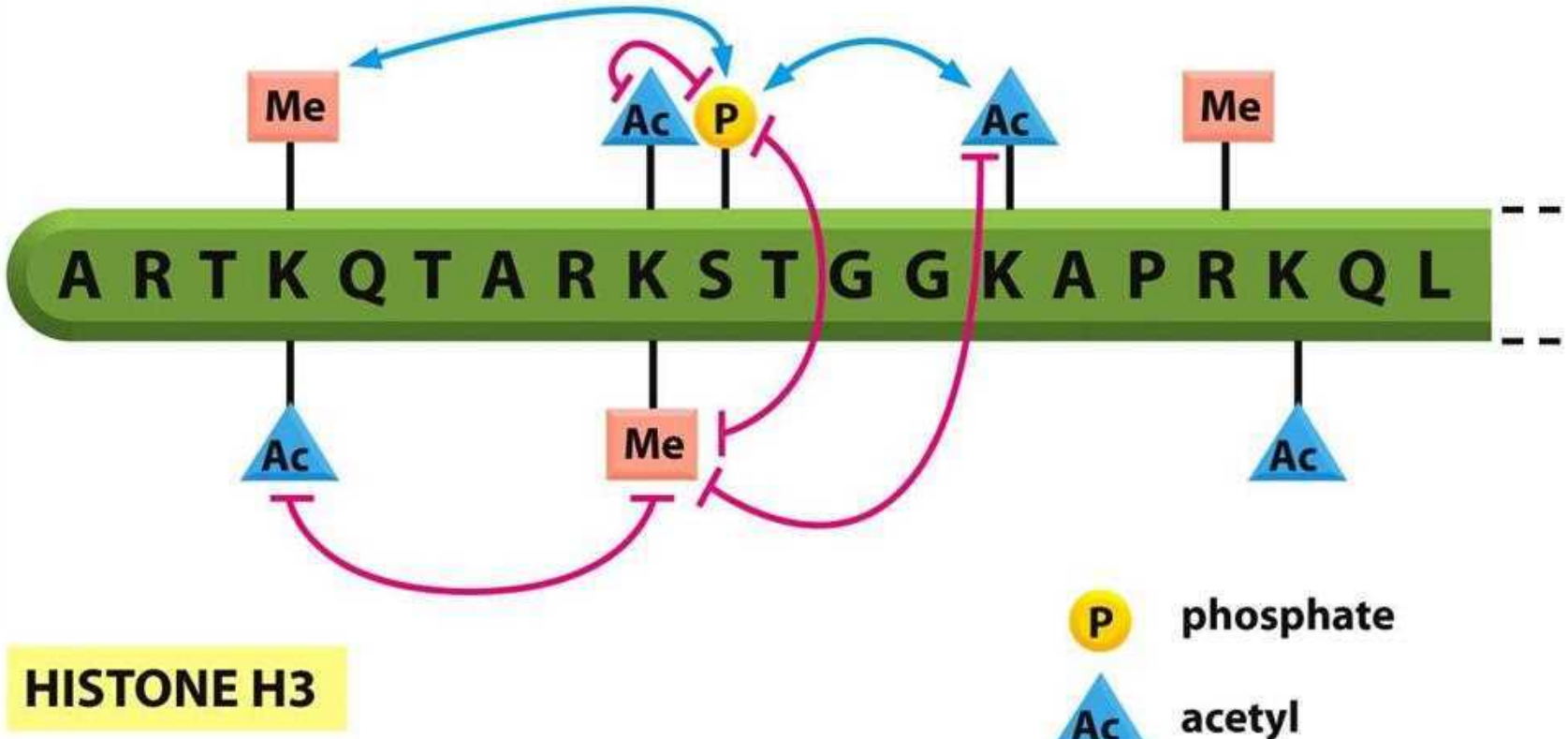
Post-translational modifications of the polypeptide chain



A large number of proteins are now known to be modified on more than one amino acid side chain, with different regulatory events producing a different pattern of such modifications. A striking example is the protein p53, which plays a central part in controlling a cell's response to adverse circumstances. Through one of four different types of molecular additions, this protein can be modified at 20 different sites (Fig). Because an enormous number of different combinations of these 20 modifications are possible, the proteins behavior can in principle be altered in a huge number of ways.

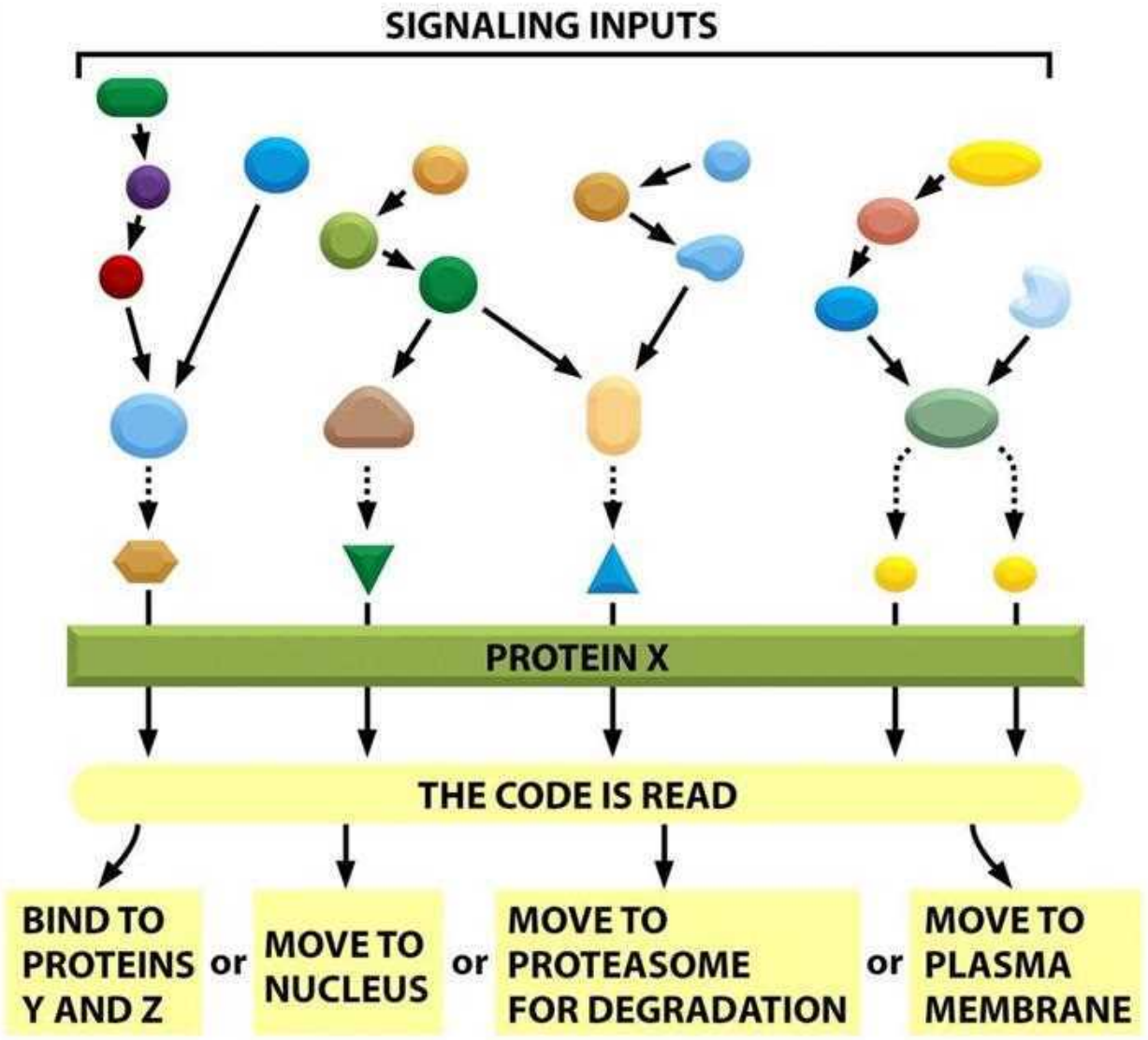
Post-translational modifications of the polypeptide chain

The pattern of modifications on a protein can determine its susceptibility to further modification, as illustrated by histone H3.



Possible modifications of the first 20 amino acids at the N-terminus of histone H3, showing not only their locations but also their activating (blue) and inhibiting (red) effects on the addition of neighboring covalent modifications. In addition to the effects shown, the acetylation and methylation of a lysine are mutually exclusive reactions.

Post-translational modifications of the polypeptide chain



Biologists have only recently begun to realize that the set of covalent modifications on each protein constitutes an important "combinatorial regulatory code." As specific modifying groups are added to or removed from a protein, this code alters the protein's behavior—changing its activity or stability, its binding partners, and its specific location within the cell. This enables the cell to respond rapidly and flexibly to changes in its condition or environment.

Diagram showing the general manner in which multisite modification are added to (and Removed from) a protein through signaling networks and how the resulting combinatorial regulatory code on the protein is read to alter its behavior in the cell.

Translation Inhibitors

Translation inhibitors are a broad group of compounds that block the process of protein synthesis on the ribosome. They disrupt various stages of translation: initiation, elongation, or termination.

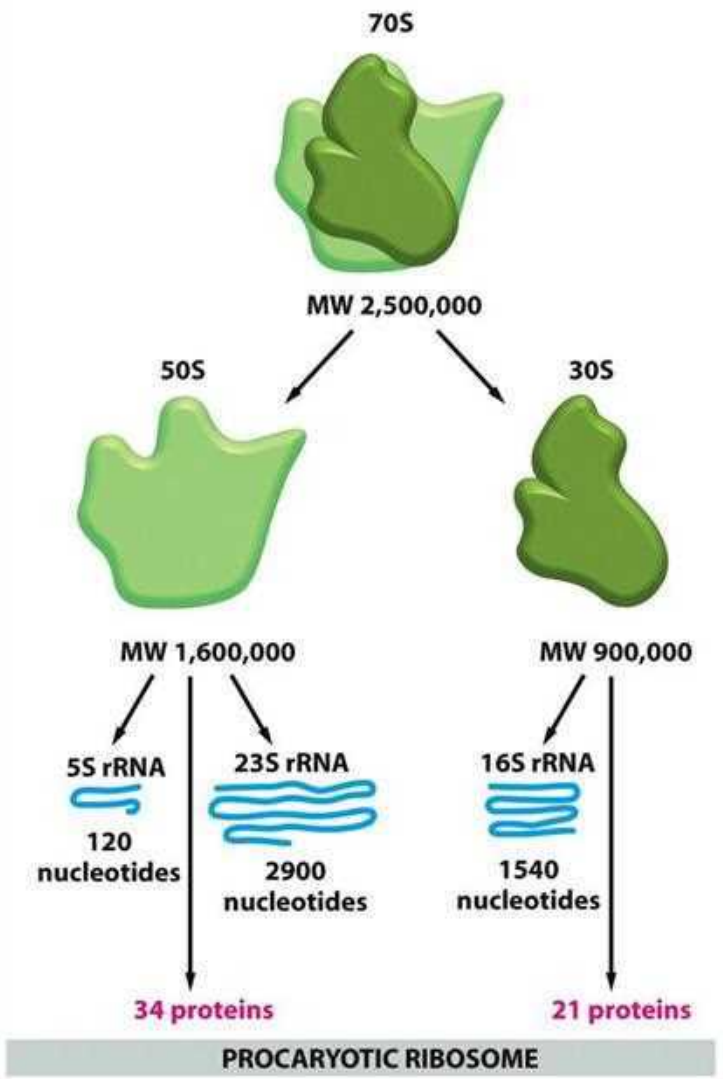
- Antibiotics
- Toxins
- Interferons

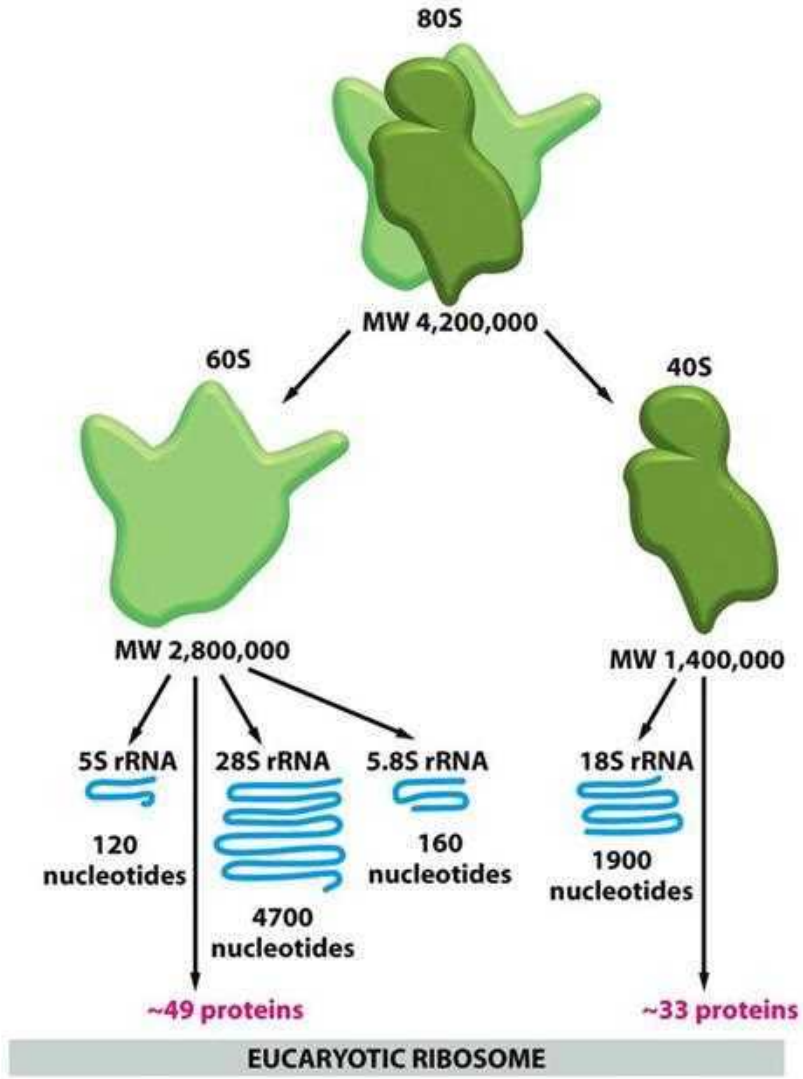
Translation Inhibitors

Therapeutic Application: The vast majority of antibiotics that target bacteria are translation inhibitors, making them crucial for modern medicine.

Selectivity: Bacterial ribosomes (70S) are structurally different from eukaryotic ribosomes (80S). This allows for the development of drugs that attack the ribosomes of pathogens while minimally affecting human ribosomes.

Fundamental Research: These compounds serve as powerful tools for studying the ribosome's mechanism and the translation process as a whole.





Translation Inhibitors

Classification and Mechanisms of Action

Translation inhibitors can be classified according to several criteria:

A. By Source of Origin:

- Antibiotics (e.g., Tetracycline, Erythromycin): Produced by microorganisms to fight competitors.
- Toxins (e.g., Ricin, Diphtheria toxin): Often produced by pathogens to damage host cells.
- Interferons: In mammalian cells, they stimulate a set of mechanisms, including the activation of enzymes (e.g., protein kinase R), which suppress translation, often by phosphorylating the initiation factor eIF2. This is part of the antiviral immune response.

B. By Target (Prokaryotes vs. Eukaryotes):

- Inhibitors of Prokaryotic Ribosomes (70S): Antibacterial drugs.
- Inhibitors of Eukaryotic Ribosomes (80S): Antifungal agents, some anti-cancer drugs, and toxins.
- Inhibitors of Organelles (mitochondria and chloroplasts), whose ribosomes resemble bacterial ones.

Translation Inhibitors

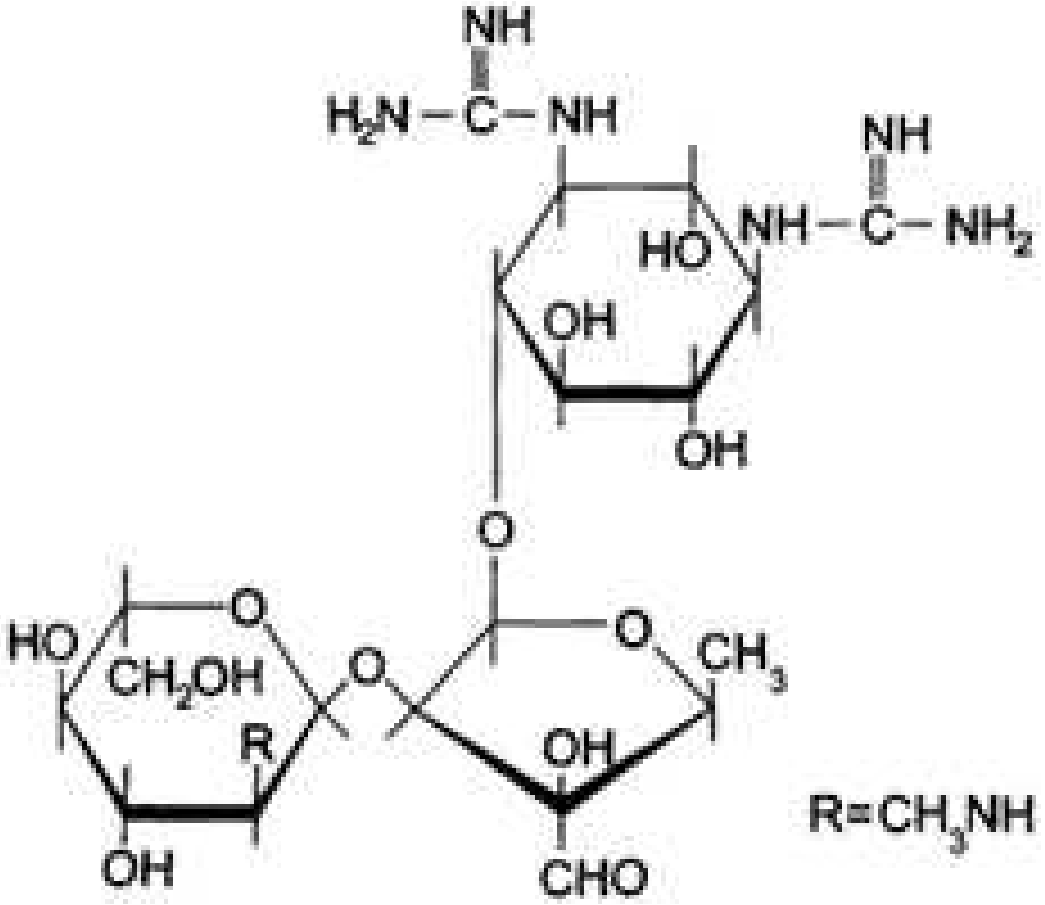
Classification and Mechanisms of Action

Target / Stage	Examples of Inhibitors	Mechanism of Action
Small Subunit (30S)	Aminoglycosides (Streptomycin, Gentamicin)	Disrupt initiation; cause misreading of mRNA.
	Tetracyclines	Block the binding of aminoacyl-tRNA to the A-site.
Large Subunit (50S)	Macrolides (Erythromycin, Azithromycin)	Block the peptide exit tunnel, preventing translocation.
	Chloramphenicol	Inhibits the peptidyl transferase reaction (formation of the peptide bond).
	Lincosamides (Clindamycin)	Bind to the peptidyl transferase center.
Elongation Factors	Fusidic Acid	Blocks the release of elongation factor EF-G (in prokaryotes) from the ribosome.
	Diphtheria Toxin (in eukaryotes)	Catalyzes the ADP-ribosylation and inactivation of elongation factor eEF2.

Translation Inhibitors

Aminoglycosides. Streptomycin

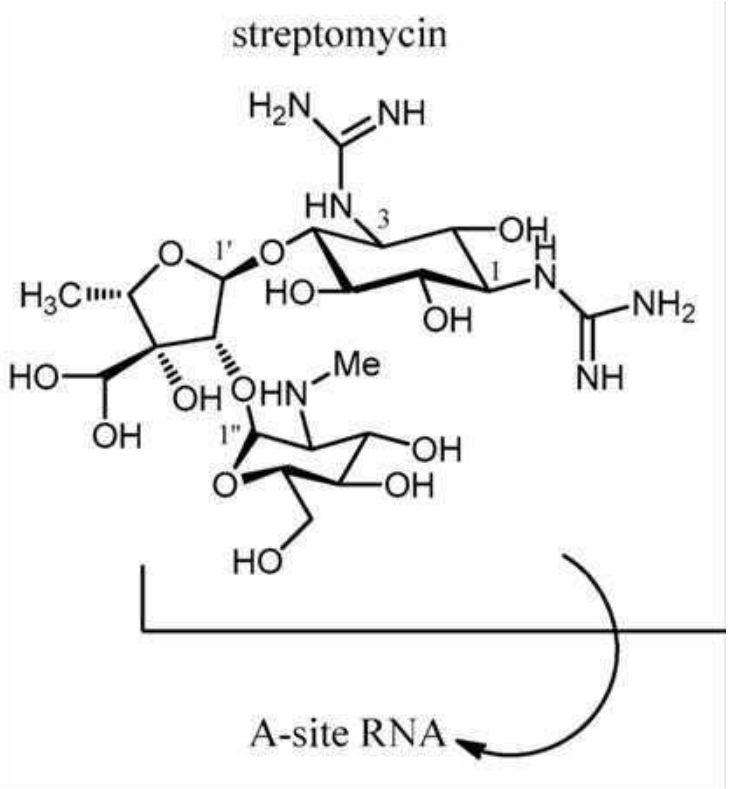
Streptomycin, one of the most well-known aminoglycosides, inhibits the initiation of protein synthesis in prokaryotes and causes misreading of the information encoded in mRNA. Streptomycin is often used to treat infectious diseases of the cardiovascular system.

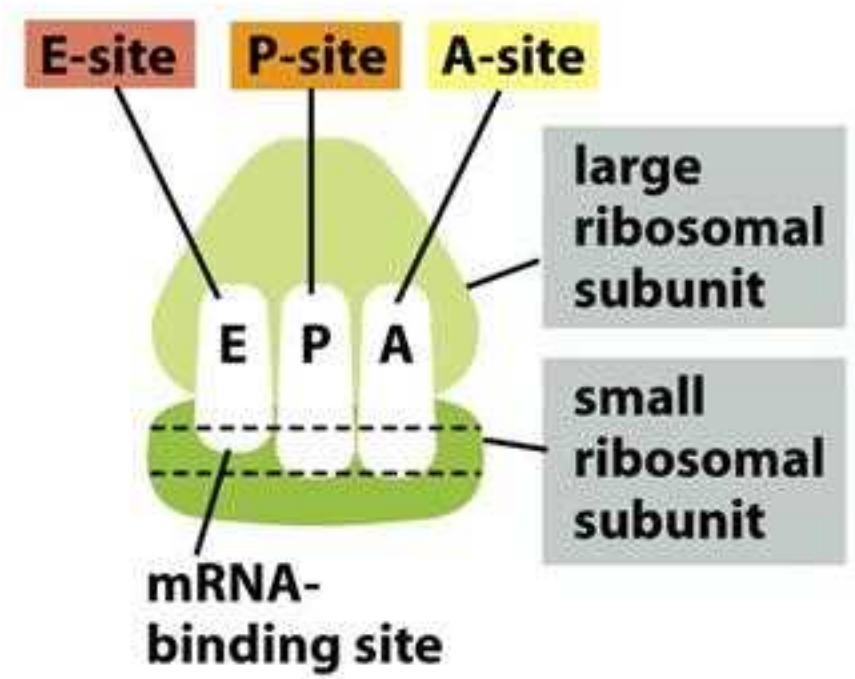


Translation Inhibitors

Mechanism of Action of Streptomycin

The mechanism of action of streptomycin is a classic and well-studied example of how aminoglycoside antibiotics suppress bacterial growth. Its action targets a key cellular process—protein synthesis.





1. Primary Target: The Bacterial 70S Ribosome
Streptomycin binds with high specificity and affinity to the small (30S) subunit of the bacterial ribosome. The precise binding site involves specific nucleotides in the 16S rRNA molecule and, critically, the ribosomal protein S12.

Translation Inhibitors

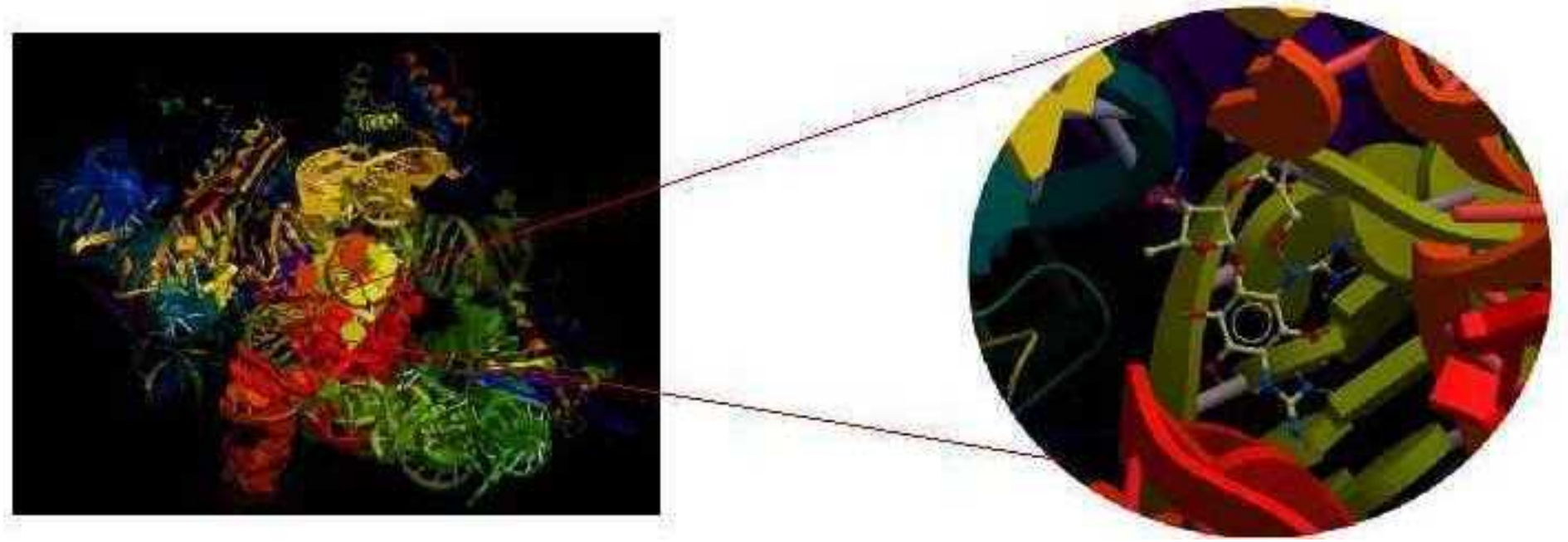
Mechanism of Action of Streptomycin

2. Molecular Consequences of Binding

After binding to the 30S subunit, streptomycin causes two main effects that disrupt translation:

Inhibition of Protein Synthesis Initiation: The antibiotic prevents the formation of a stable initiation complex (70S ribosome + mRNA + initiator fMet-tRNA). This blocks the very start of the protein synthesis process.

Induction of mRNA Misreading: This is the most characteristic effect of streptomycin. Normally, the ribosome ensures high accuracy in matching the mRNA codon with the tRNA anticodon. By binding to the 30S subunit, streptomycin alters the conformation of the A-site, where codon recognition occurs. This reduces the ribosome's ability to discriminate, causing it to accept incorrect aminoacyl-tRNAs that do not match the codon in the mRNA.



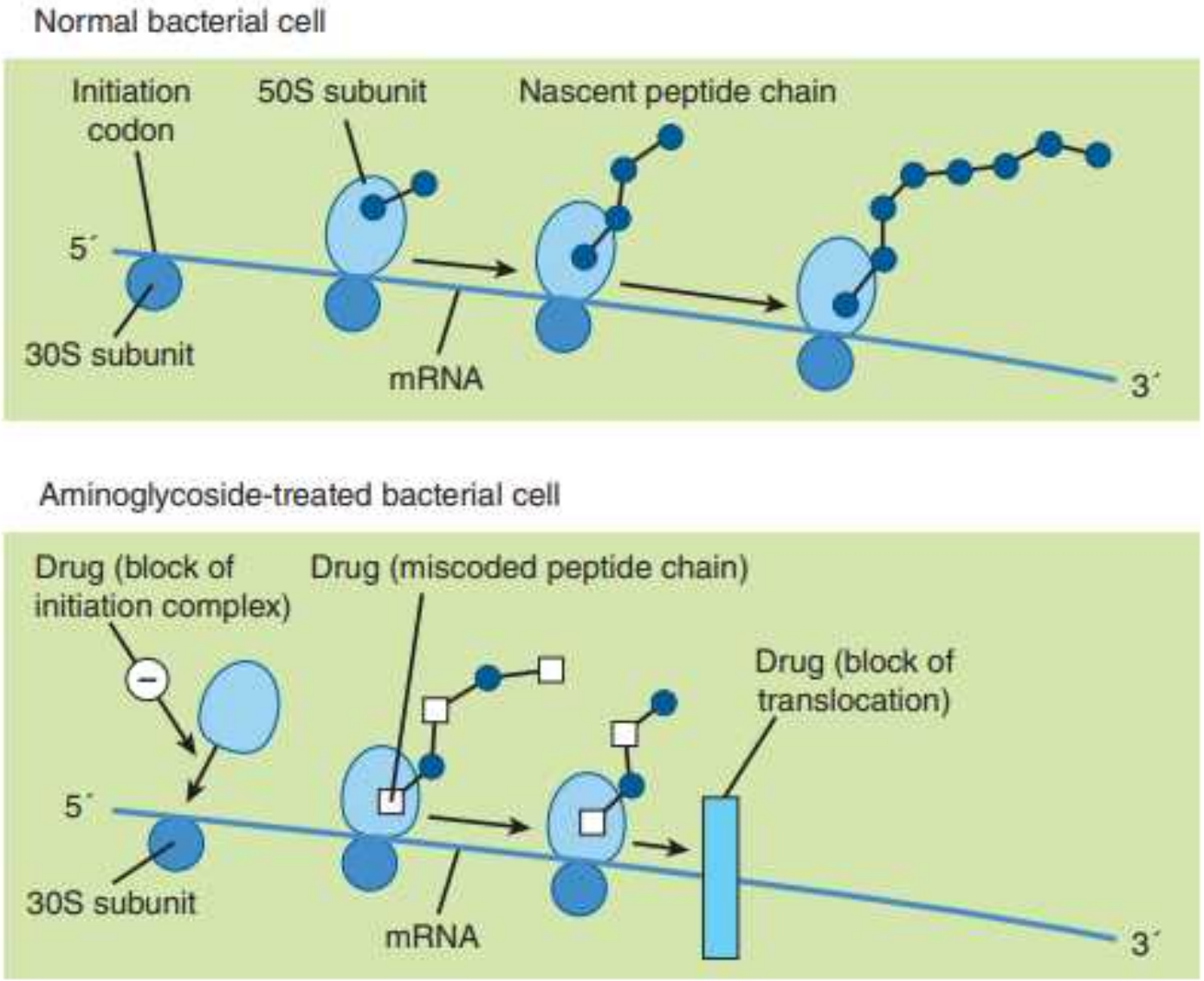
Translation Inhibitors

Aminoglycosides. Streptomycin

Final Outcome: Bactericidal Effect

Unlike bacteriostatic antibiotics, which only stop growth, streptomycin has a pronounced bactericidal action (it kills bacteria). This occurs due to a cascade of events:

- Accumulation of Defective Proteins: The incorporation of incorrect amino acids leads to the synthesis of non-functional, misfolded, or truncated polypeptides.
- Disruption of Cellular Metabolism: These defective proteins incorporate into cellular membranes, disrupting their integrity, and also disable critical enzymes.
- Cell Death: The combination of inhibited protein synthesis, production of toxic polypeptides, and membrane damage leads to the irreversible death of the bacterial cell.

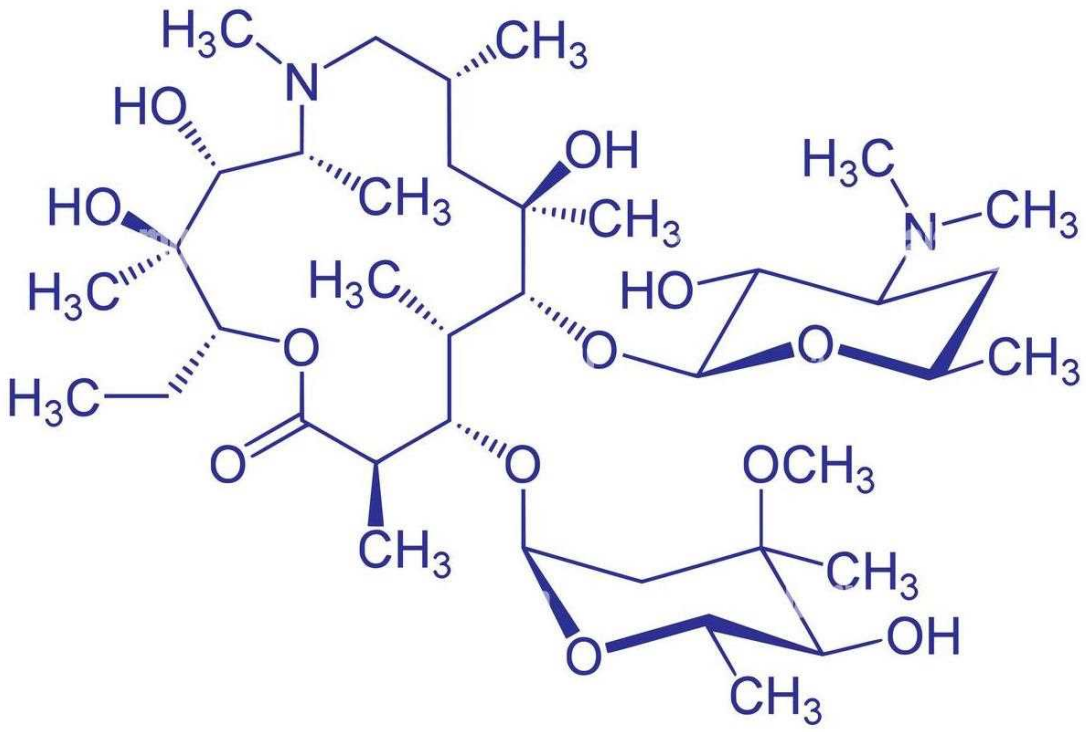


Translation Inhibitors

Macrolides. Erythromycin

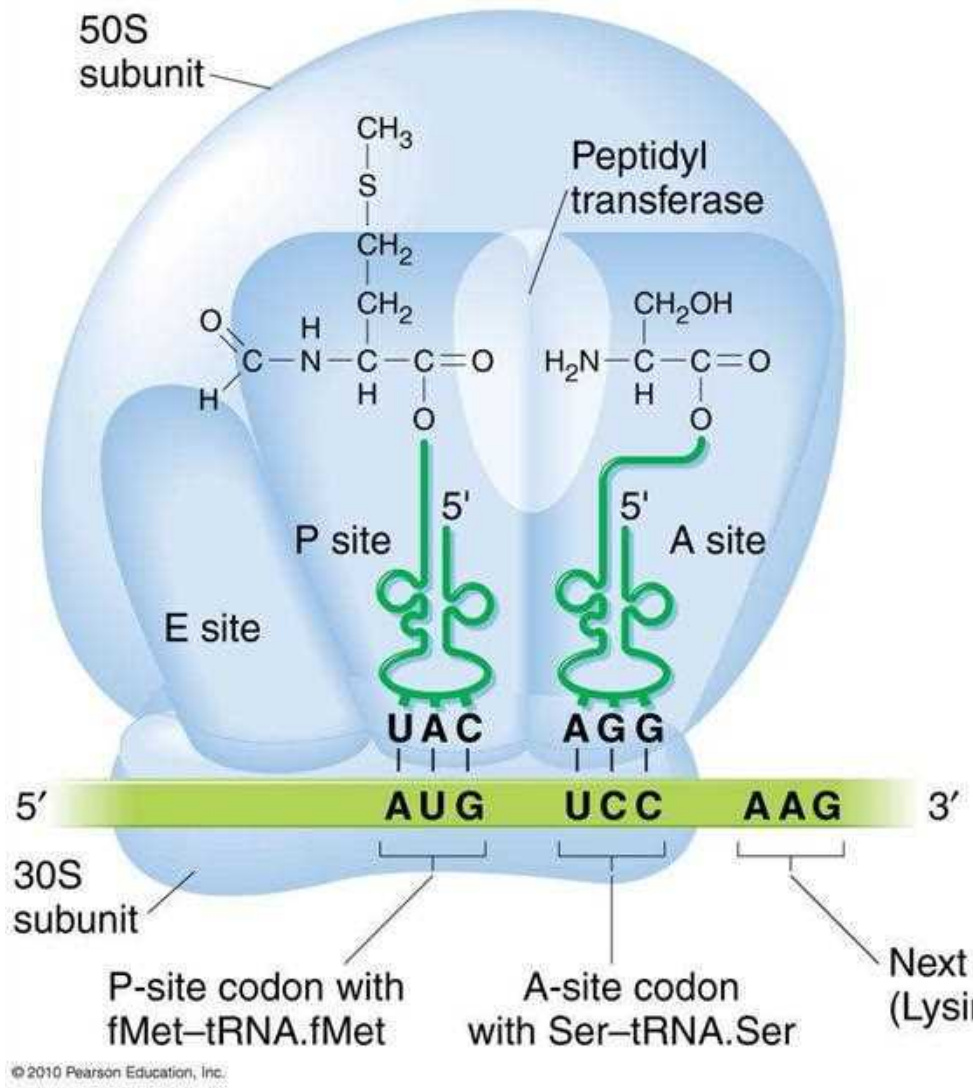
The mechanism of action of erythromycin, as a representative of macrolide antibiotics, is fundamentally different from that of streptomycin.

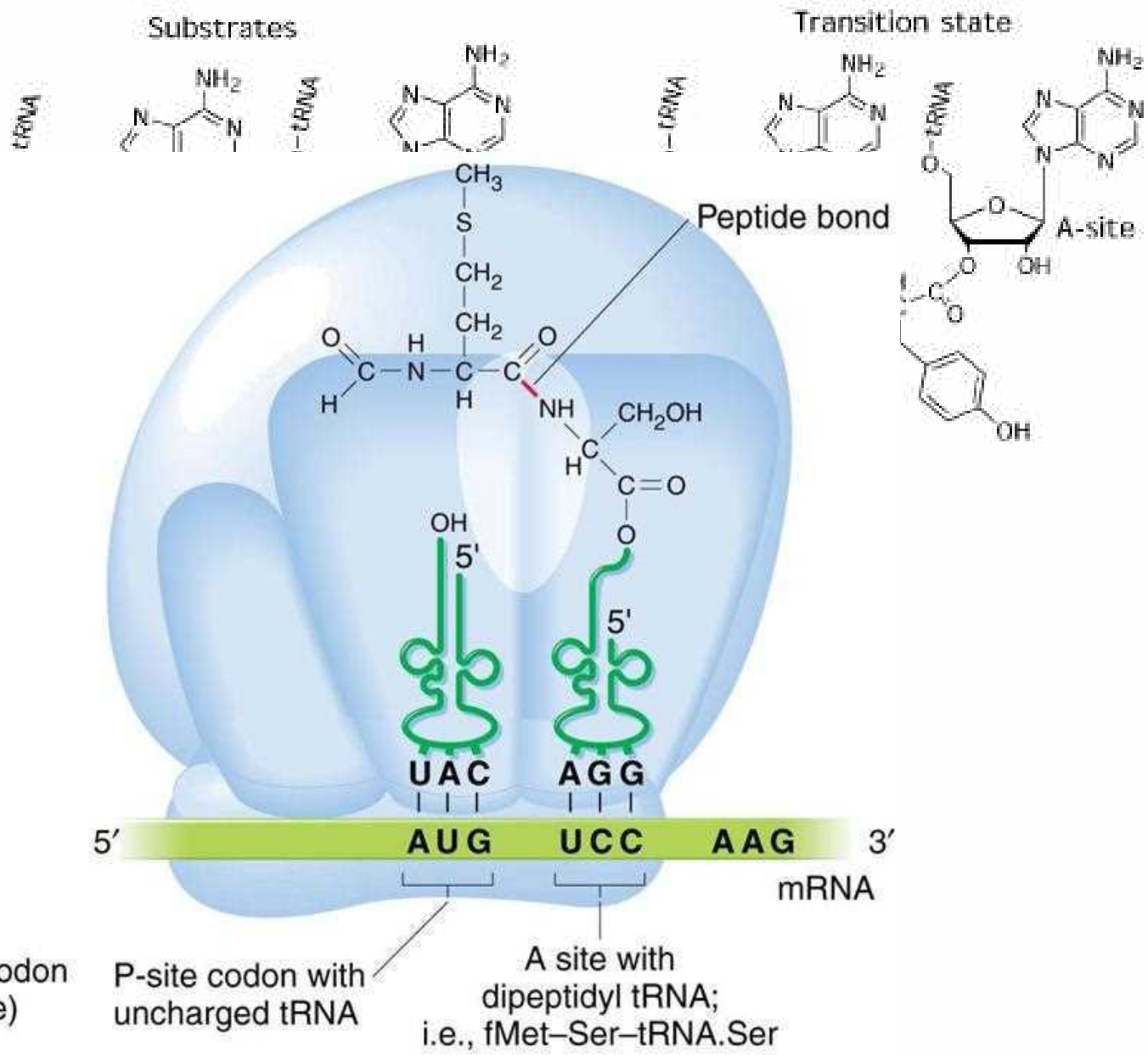
1. Primary Target: The Bacterial Ribosome (50S)
Erythromycin specifically binds to the large (50S) subunit of the bacterial ribosome. Its target is the **peptidyl transferase** center, located within a cavity known as the nascent peptide exit tunnel.



Translation Inhibitors

Macrolides. Erythromycin





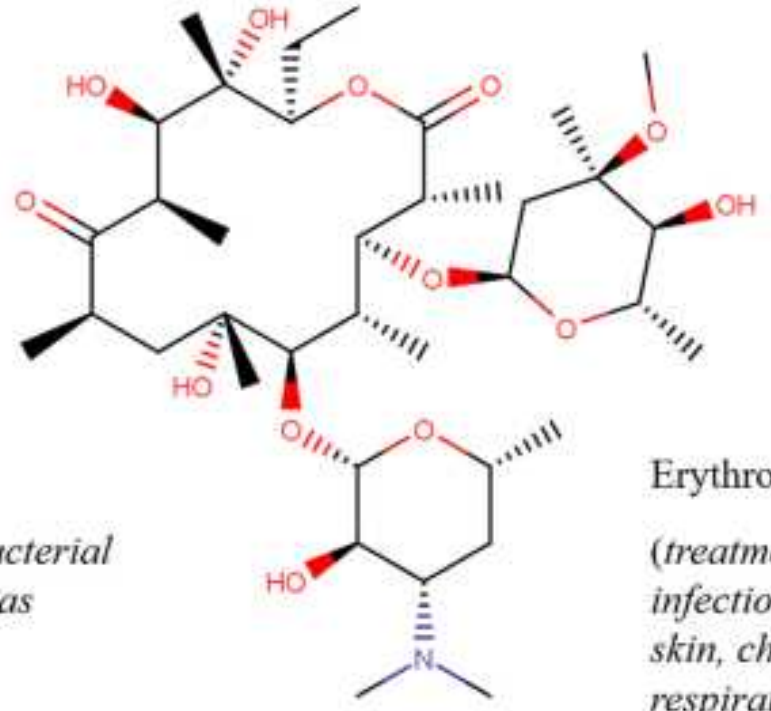
Translation Inhibitors

Macrolides. Erythromycin

At typical concentrations, erythromycin exerts primarily a bacteriostatic action—meaning it reversibly inhibits the growth and reproduction of bacteria without directly killing them. This allows the host's immune system an opportunity to clear the infection. However, at high doses or against highly susceptible organisms, it can also exhibit bactericidal activity.

Azithromycin

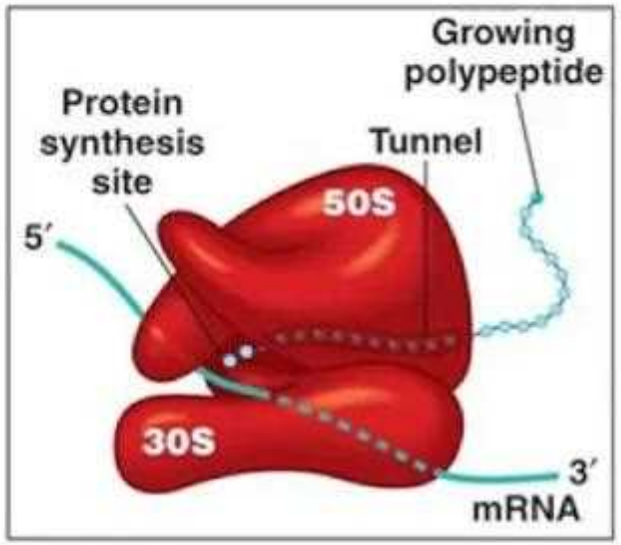
(*treatment of bacterial infections such as Bronchitis, Pneumonia*)



Erythromycin

(*treatment of bacterial infections including skin, chlamydia, respiratory*)

Aminoglycosides. Macrolides



(a) Three-dimensional detail of the protein synthesis site showing the 30S and 50S subunit portions of the 70S prokaryotic ribosome

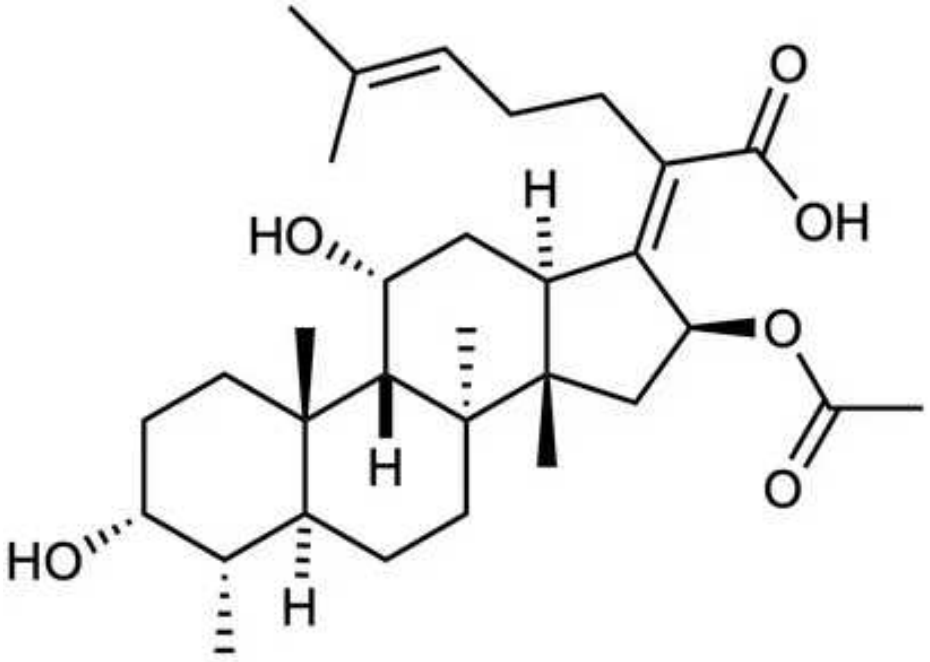
Translation Inhibitors

Fusidic Acid

The mechanism of action of fusidic acid is unique and differs from that of most other antibiotics.

1. Primary Target: Elongation Factor G (EF-G)

Fusidic acid is a steroid antibiotic that targets not the ribosome itself, but a key protein involved in protein synthesis—**Elongation Factor G (EF-G)**. EF-G plays a critical role in the process of **translocation**.



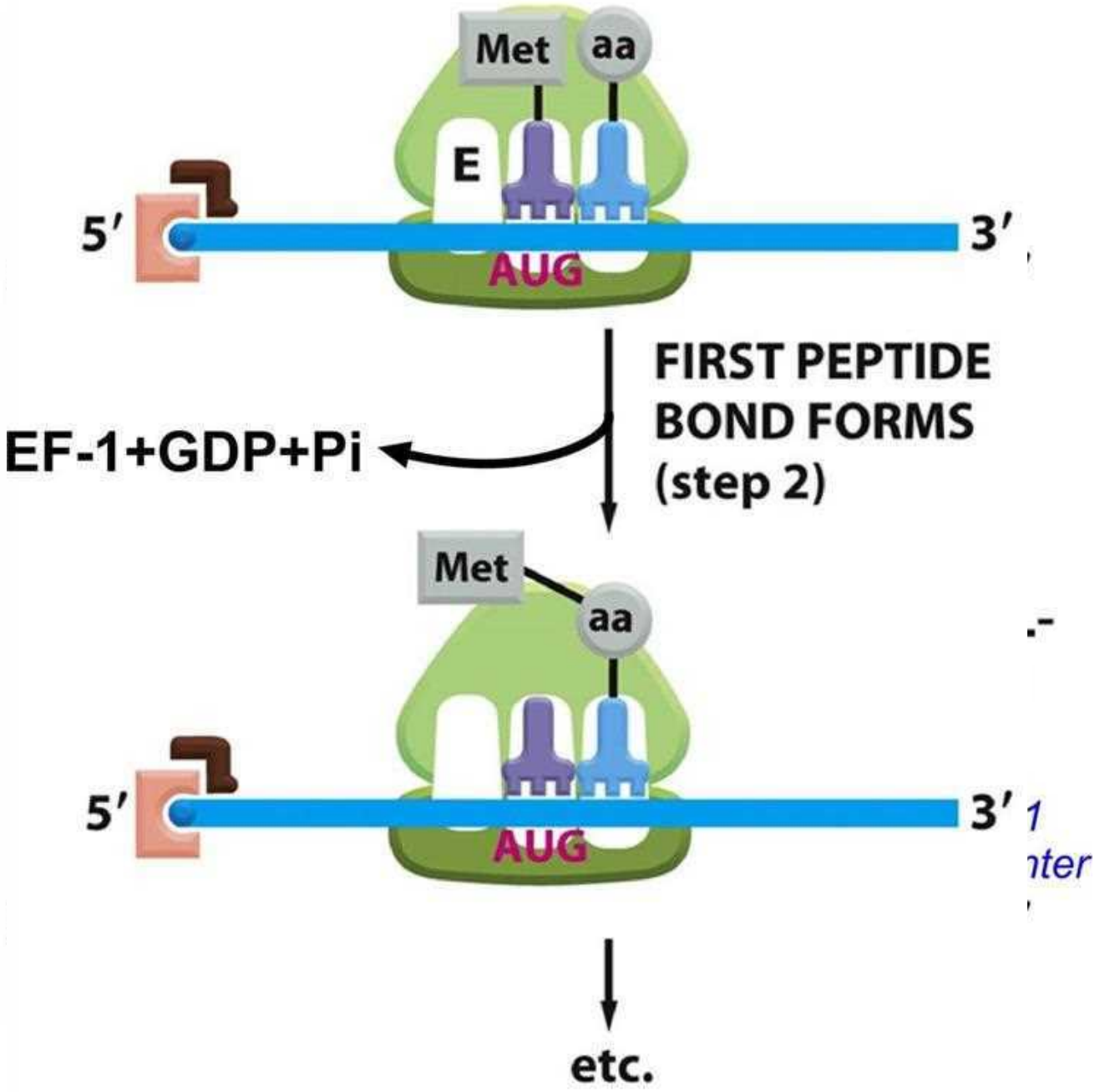
Fusidic acid

Translation Inhibitors

Fusidic Acid

Normally, the cycle of EF-G function is as follows:

1. After peptide bond formation, the ribosome has occupied A- and P-sites.
2. EF-G, bound to GTP, binds to the ribosome.
3. The hydrolysis of GTP to GDP provides the energy for a conformational change in EF-G and the actual movement of the ribosome.
4. Translocation occurs: the ribosome moves one codon along the mRNA, freeing the A-site for the next aminoacyl-tRNA.
5. EF-G in its GDP form is released from the ribosome.

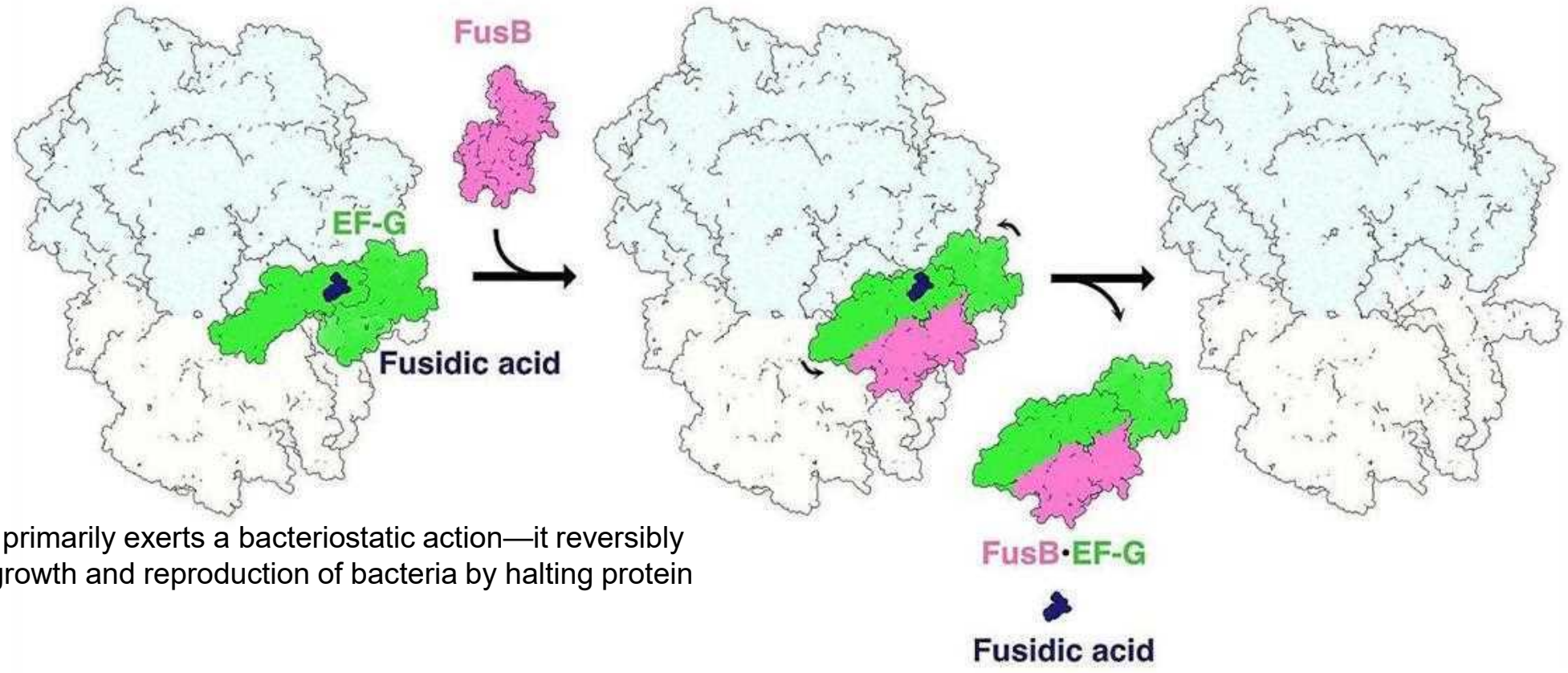


Translation Inhibitors. Fusidic Acid

5. EF-G in its GDP form is released from the ribosome

Fusidic acid disrupts precisely this final, critical step.

"Freezing" the Complex: Fusidic acid binds to the EF-G-GDP-ribosome complex after translocation has already occurred.
Blocking Release: It stabilizes this complex, preventing the release of EF-G-GDP from the ribosome.
Halting Synthesis: As a result, the ribosome becomes "blocked." The A-site remains inaccessible to the next aminoacyl-tRNA, and the process of polypeptide chain elongation cannot continue.



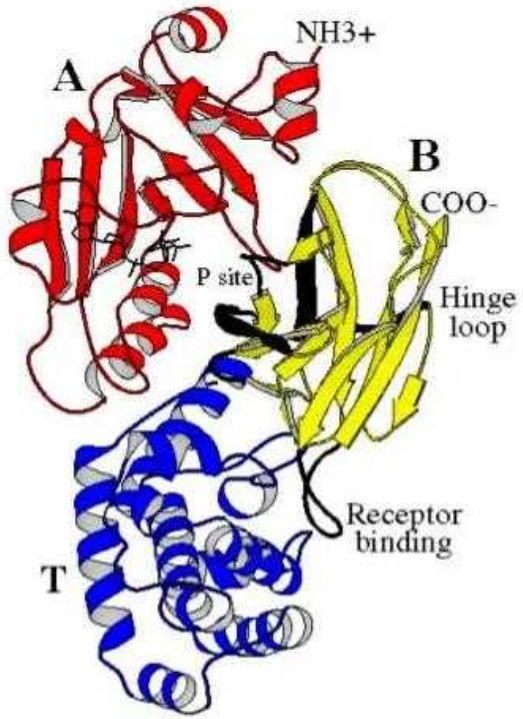
Fusidic acid primarily exerts a bacteriostatic action—it reversibly inhibits the growth and reproduction of bacteria by halting protein synthesis.

Translation Inhibitors.

Diphtheria toxin

Diphtheria toxin is a protein produced by lysogenic strains of *Corynebacterium diphtheriae* infected with a bacteriophage carrying the tox gene. It is the primary virulence factor responsible for the symptoms of diphtheria.

- Toxin of 58,342 daltons molecular weight
- Subunit B
  - 21,500 daltons
  - receptor-binding domain and translocation domain
- Subunit A
  - catalytic domain



1. Receptor Binding and Endocytosis

The toxin circulates in the blood.

The B-subunit of the toxin specifically binds to a receptor on the surface of mammalian cells—the heparin-binding EGF-like growth factor (HB-EGF).

After binding, the "toxin-receptor" complex is internalized by the cell via receptor-mediated endocytosis.

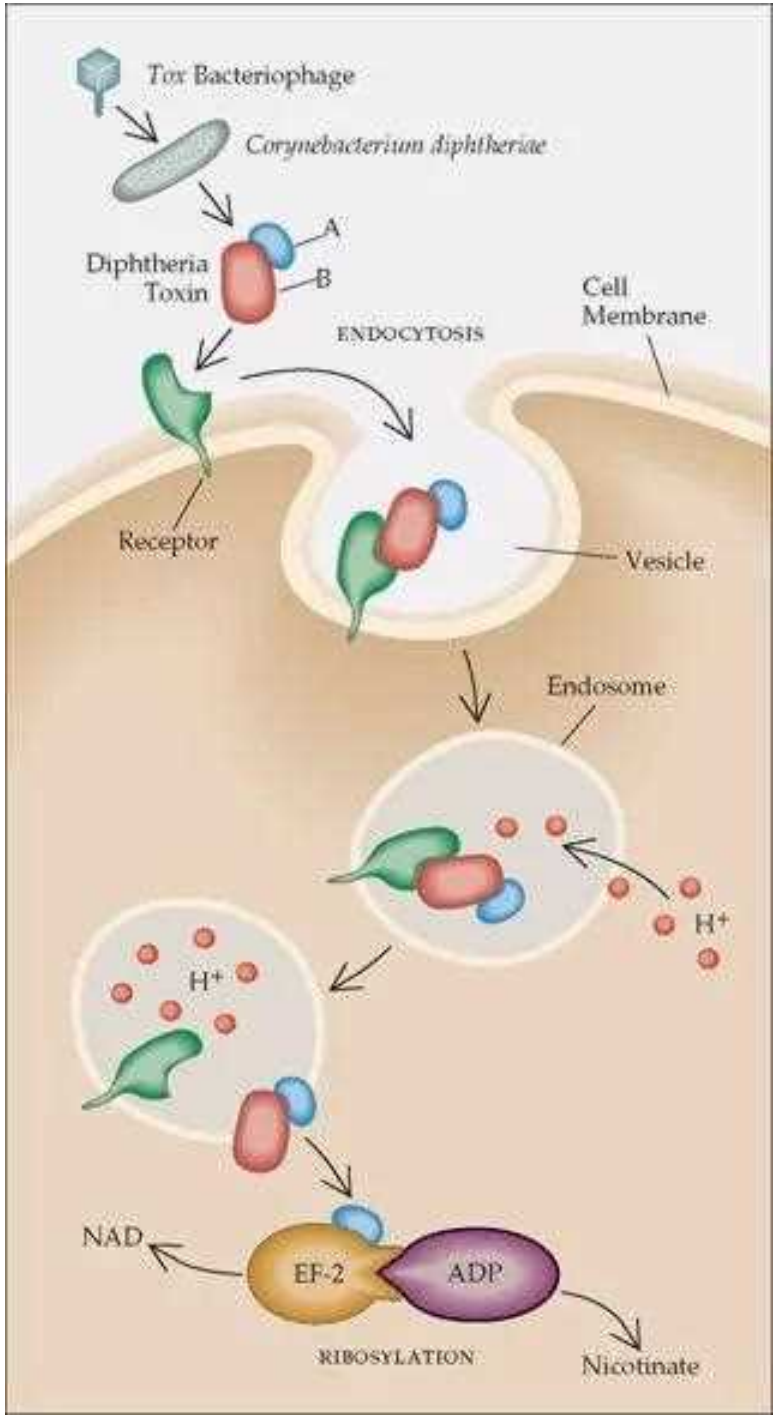
Translation Inhibitors.

Diphtheria toxin

Translocation into the Cytoplasm

Inside the endosome (an acidic vesicle), the low pH causes a conformational change in the toxin.

This change allows the T-subunit (Fragment A) to translocate across the endosomal membrane into the cell's cytoplasm.



Translation Inhibitors
Diphtheria toxin

Enzymatic Action and Inhibition of Protein Synthesis

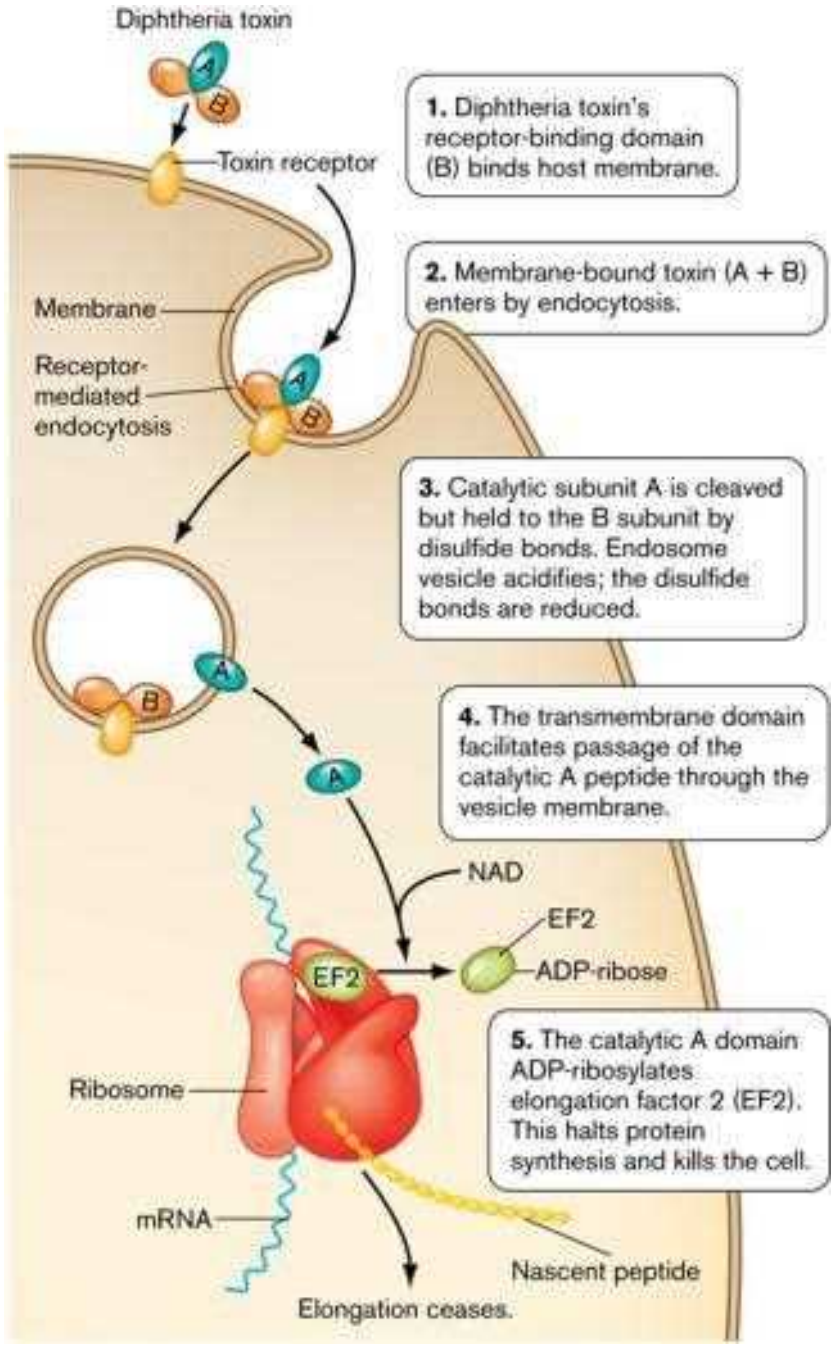
Once in the cytoplasm, the T-subunit exerts its enzymatic activity. It is an NAD-dependent ADP-ribosyltransferase.

Elongation Factor 2 (EF-2, or eEF2)—a protein absolutely essential for protein synthesis in eukaryotes.

Mechanism of Inhibition: The enzyme catalyzes the transfer of an ADP-ribose group from a nicotinamide adenine dinucleotide (NAD+) molecule to a unique modified amino acid called diphthamide within the structure of EF-2.

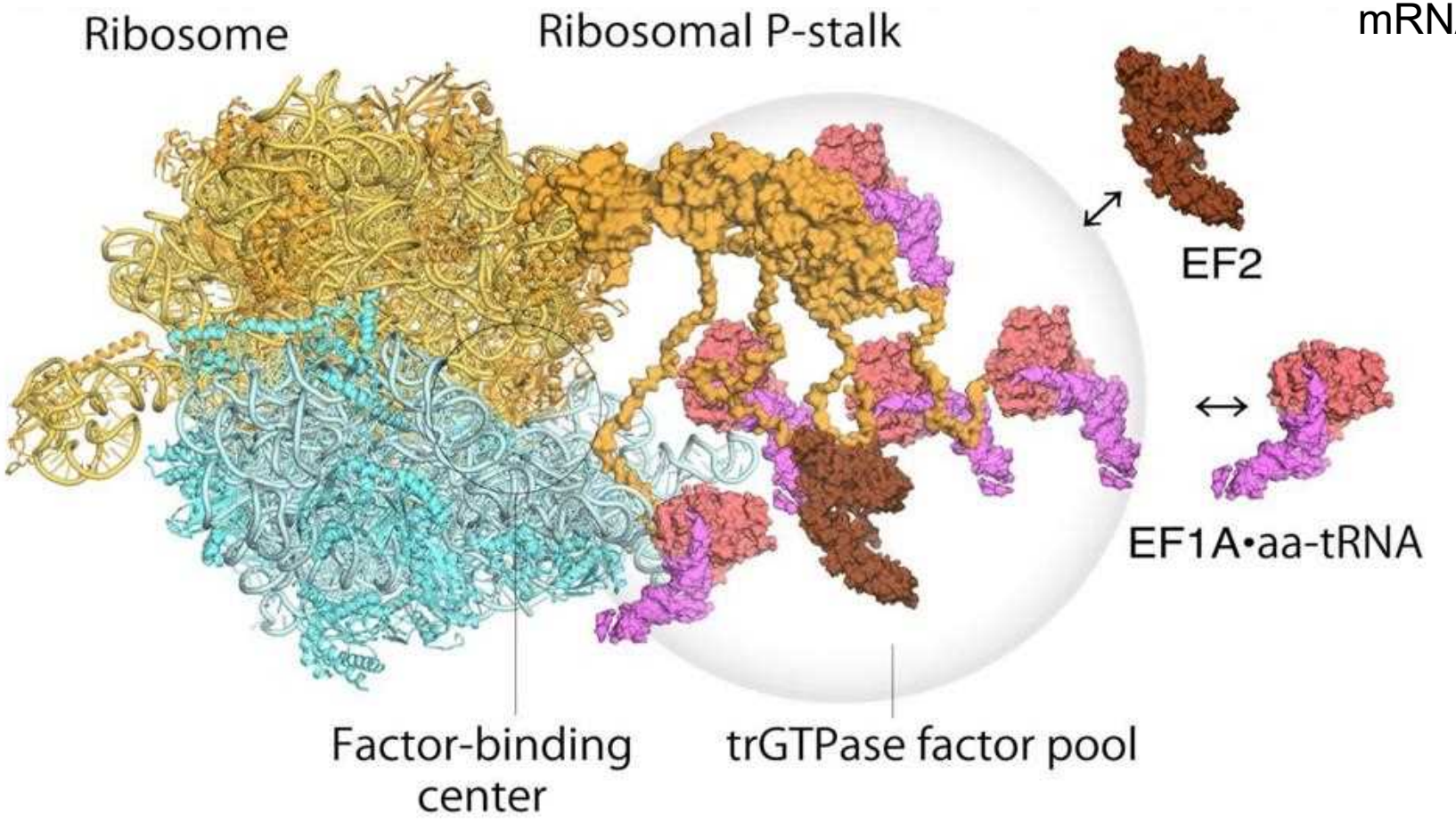
EF-2 + NAD^+ → ADP-ribosyl-EF-2 + Nicotinamide + H^+

Result: The ADP-ribosylated EF-2 is completely inactive. It cannot interact with the ribosome, and the process of translocation (the movement of the ribosome along the mRNA after peptide bond formation) halts.



Translation Inhibitors

Diphtheria toxin



The ADP-ribosylated EF-2 is completely inactive. It cannot interact with the ribosome, and the process of translocation (the movement of the ribosome along the mRNA after peptide bond formation) halts.

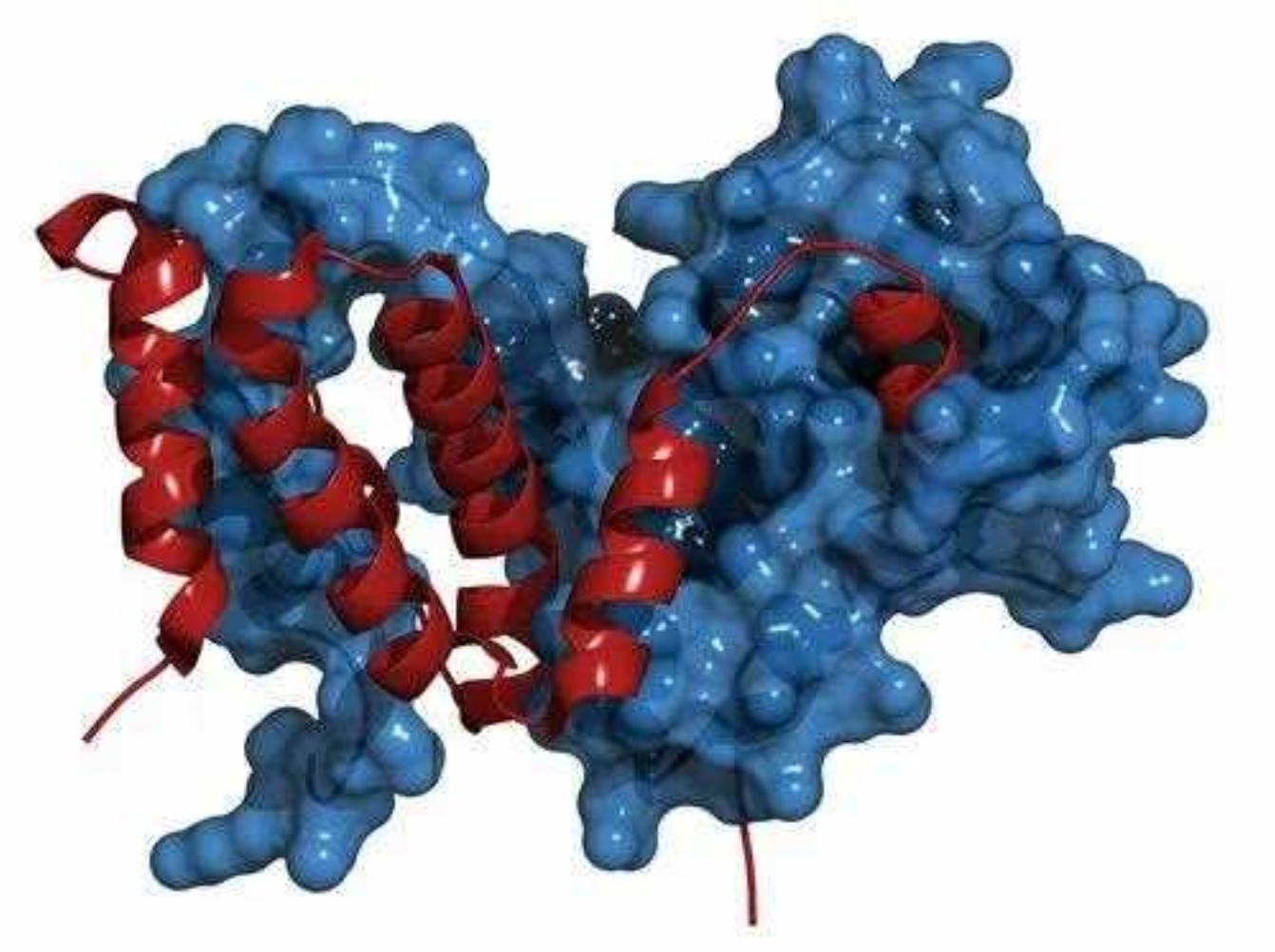
Why is the Toxin so Effective?

- High Specificity: A single Fragment A molecule can inactivate thousands of EF-2 molecules per minute (catalytic efficiency).
- Irreversible Action: The ADP-ribosylation reaction is irreversible. The cell cannot restore EF-2 function.
- Critical Target: It blocks a universal and vital process—protein synthesis.

Translation Inhibitors

Interferons

Interferons are small **glycoproteins** composed of approximately 160 amino acid residues.
They are secreted by certain vertebrate cells in response to viral infection and prevent the spread of the viral infection.
These proteins are synthesized in exceptionally small quantities: ~10^{-9} to 10^{-12} g.
Nevertheless, interferons are extremely potent, non-specific antiviral agents, exhibiting 10^{6} to 10^{9} units of antiviral activity per 1 mg of protein, which corresponds to the ability of a single interferon molecule to protect one cell from infection.

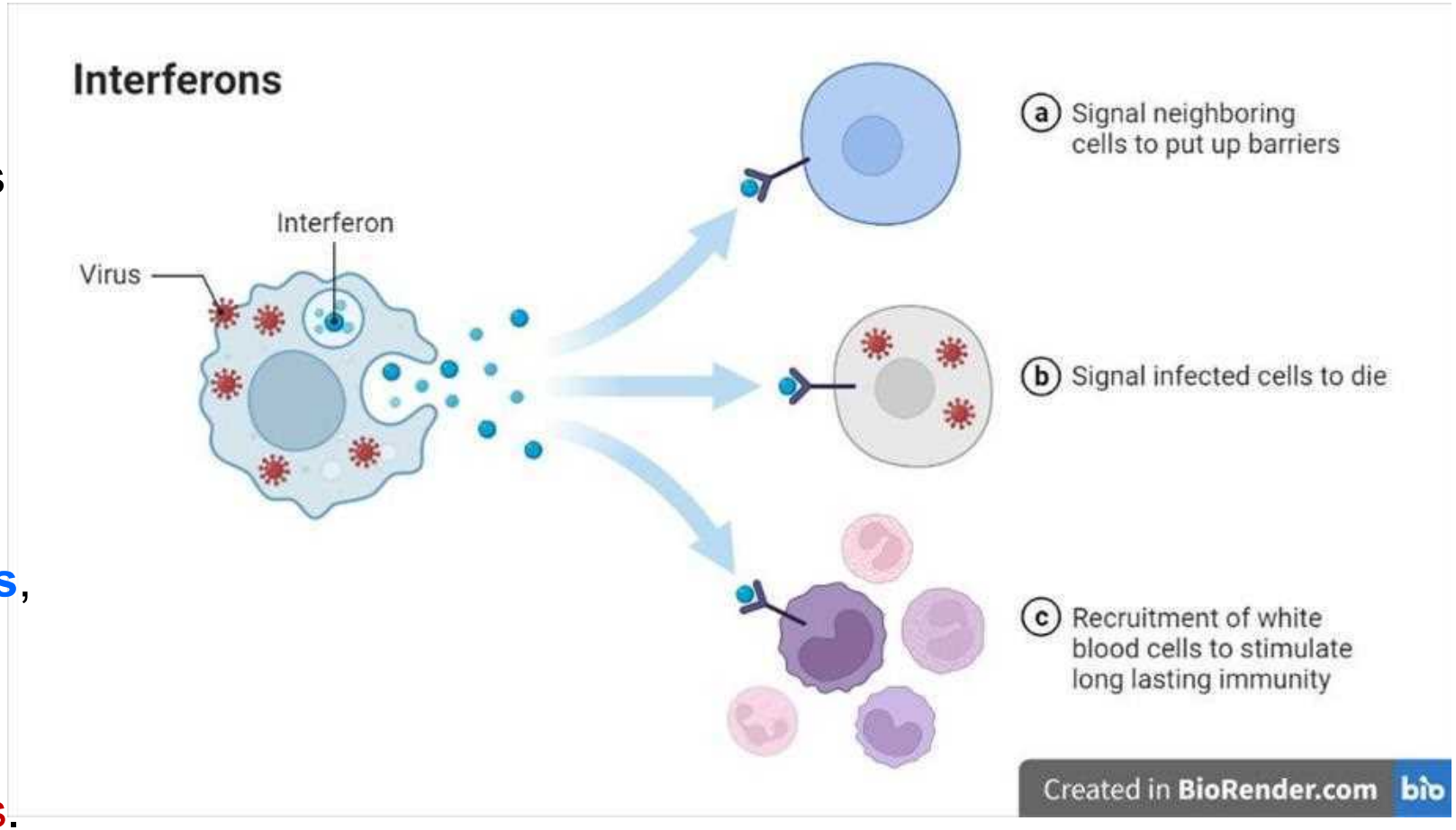


Translation Inhibitors

Interferons

Certain components of viral particles, such as double-stranded RNA, induce the synthesis of at least three types of interferons. In humans, there are:

- 14 genes encoding: **alpha-interferons**, which are produced by *B-lymphocytes and macrophages*;
- 5 genes for **beta-interferons**, produced by *fibroblasts*;
- 1 gene for **gamma-interferon**, which is expressed by T-lymphocytes.



Translation Inhibitors

Interferons

By binding to receptors on the plasma membrane of infected cells, these proteins stimulate the synthesis of enzymes capable of degrading viral mRNA and halting protein synthesis on ribosomes, thereby preventing the expression of viral genes in eukaryotic cells.

The action of interferons is based on the following mechanisms:

- Interferons inhibit the synthesis of proteins required for viral replication;
- Interferons stimulate the synthesis of the enzyme oligoadenylate synthetase, which catalyzes the production of small amounts of short 2'-5' oligoadenylates. These compounds activate ribonuclease L, an enzyme that cleaves both mRNA and rRNA;
- Interferons stimulate the synthesis of a specific protein kinase (PKR) that phosphorylates, and thereby inactivates, the translation initiation factor eIF-2:

$$\textbf{eIF-2 + ATP} \rightarrow \textbf{eIF-2-OPO}_3\textbf{H}_2 \textbf{ + ADP}$$

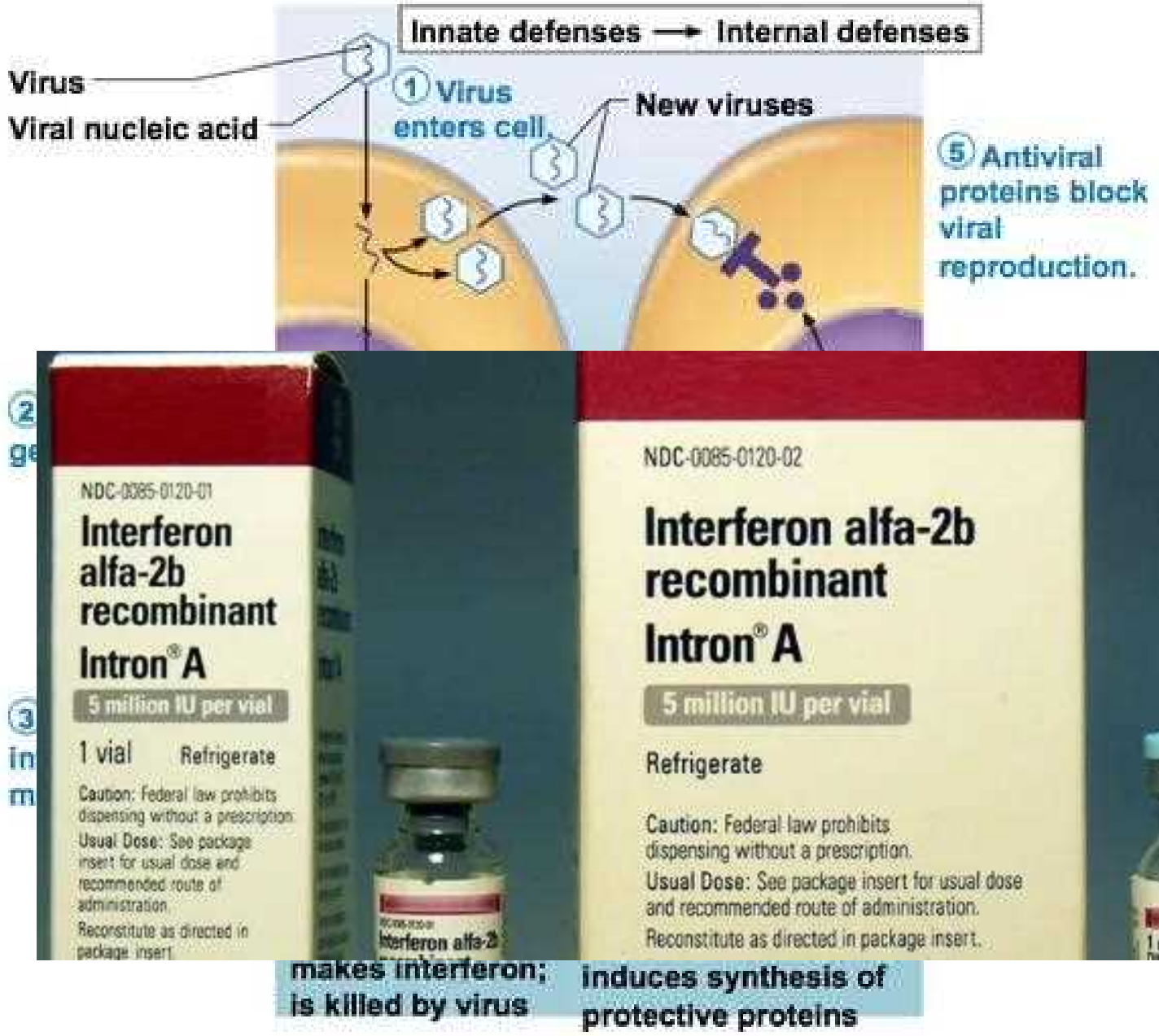
As a result, the synthesis of all proteins in the infected cells ceases. The infected cells subsequently die, but this process also halts viral replication.

Translation Inhibitors

Interferons

As a result, the synthesis of all proteins in the infected cells ceases. The infected cells subsequently die, but this process also halts viral replication.

Currently, interferons are produced using recombinant DNA technology and are widely used to treat viral diseases such as influenza, poliomyelitis, chickenpox, herpes, viral hepatitis, as well as certain types of malignant neoplasms.

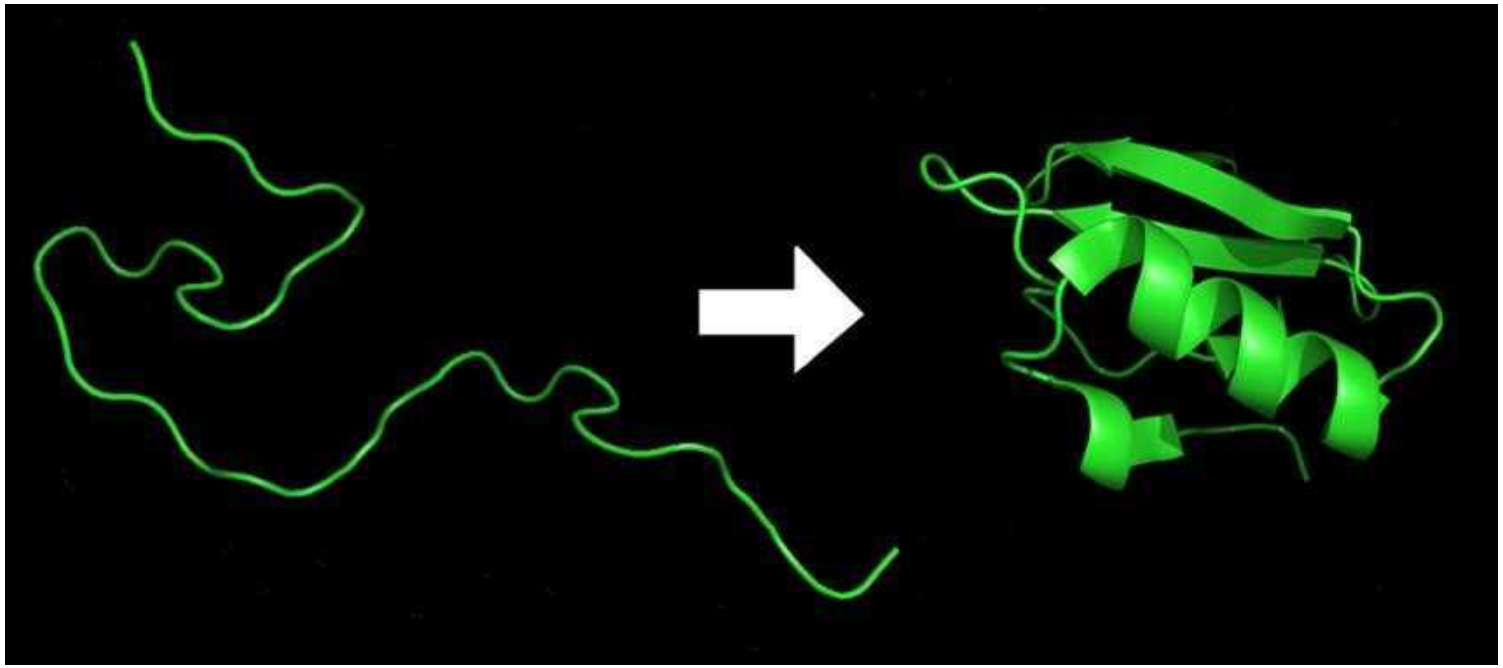


Protein folding

After translation, the polypeptide chain must fold into a specific spatial conformation.

What secondary structures of proteins do you know?

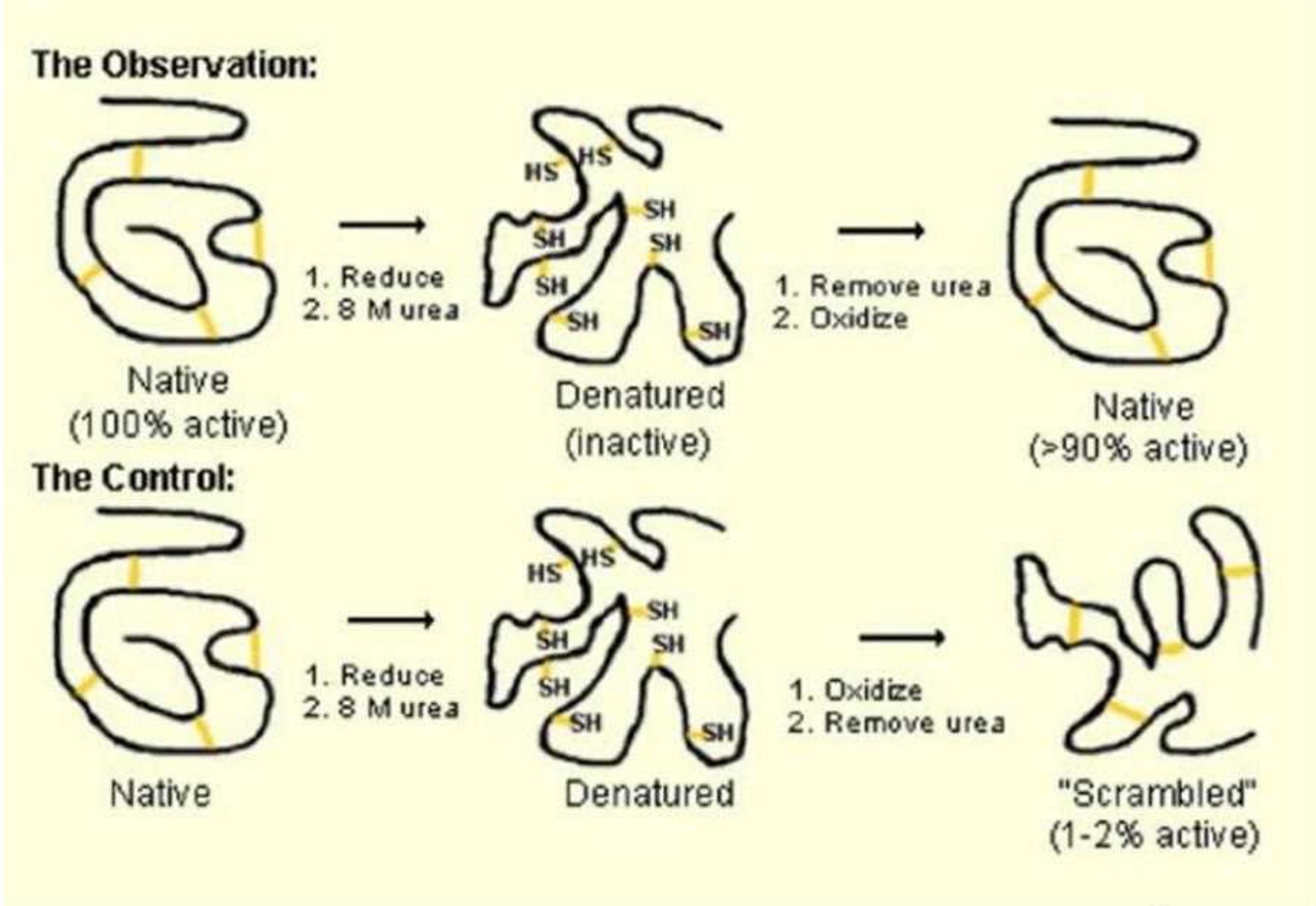
Protein folding is the physical process by which a polypeptide chain, after being synthesized by the ribosome, transitions from an unstable, random coil into a more ordered, three-dimensional structure. This structure enables the protein to become biologically functional or active.



The Anfinsen Dogma

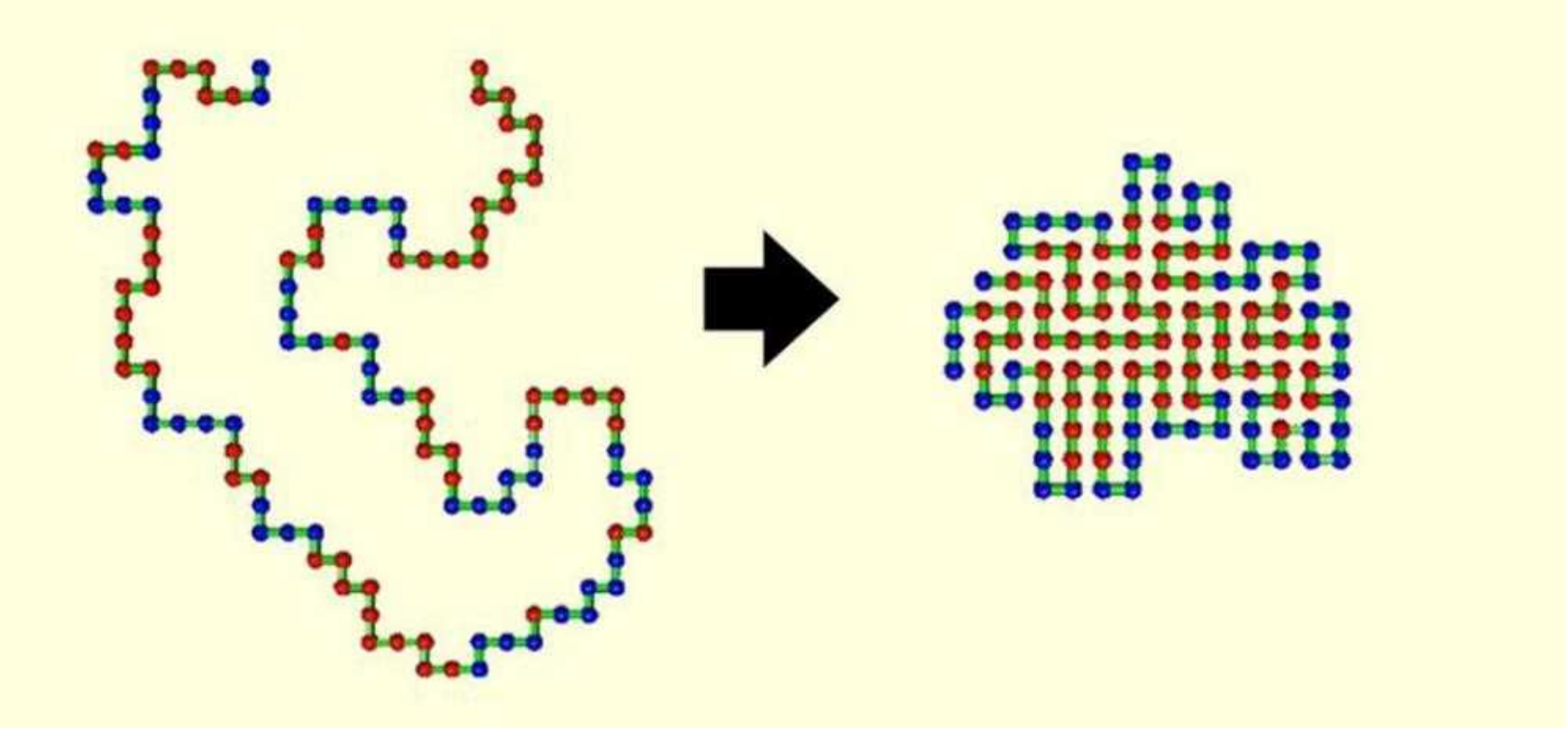
In the 1960s, Christian B. Anfinsen conducted a series of in vitro experiments that led him to the "thermodynamic hypothesis": as he stated a decade later in his Nobel Prize lecture in 1972,
"the native conformation is determined by the totality of interatomic interactions and hence by the amino acid sequence, in a given environment."

Anfinsen's dogma has some exceptions: *some proteins have multiple stable conformations, and some proteins require the assistance of chaperones to fold correctly.*

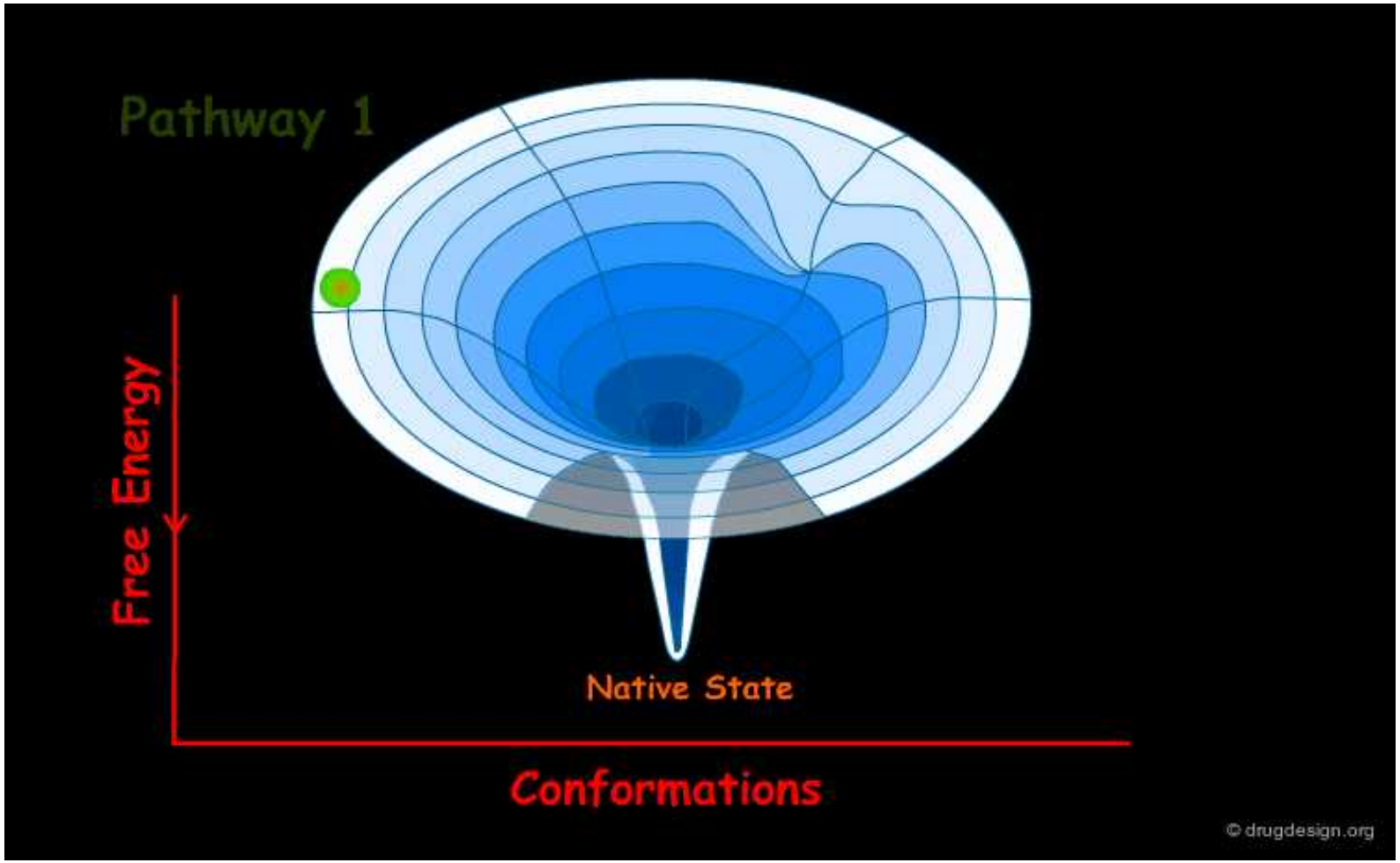


The Levinthal Paradox

For a protein chain of 100 residues, the number of possible conformations is approximately **10^{100}**. A complete search of all these conformations would take about 10^{80} years, assuming one conformational transition takes approximately **10^{-13} seconds**. Thus, the crux of the problem is that this question cannot be resolved experimentally, as one would have to wait ~**10^{80} years**.

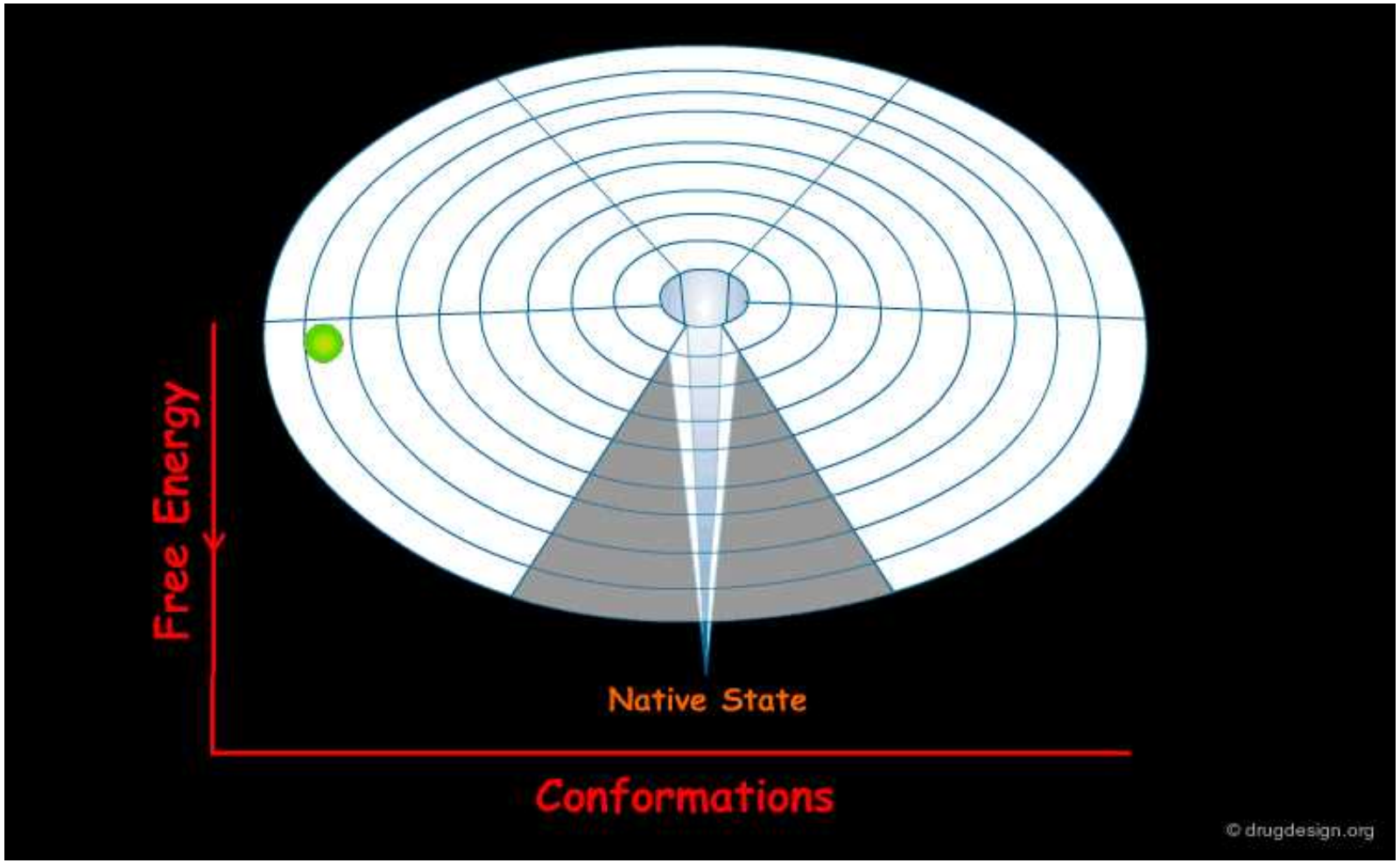


The Folding Funnel

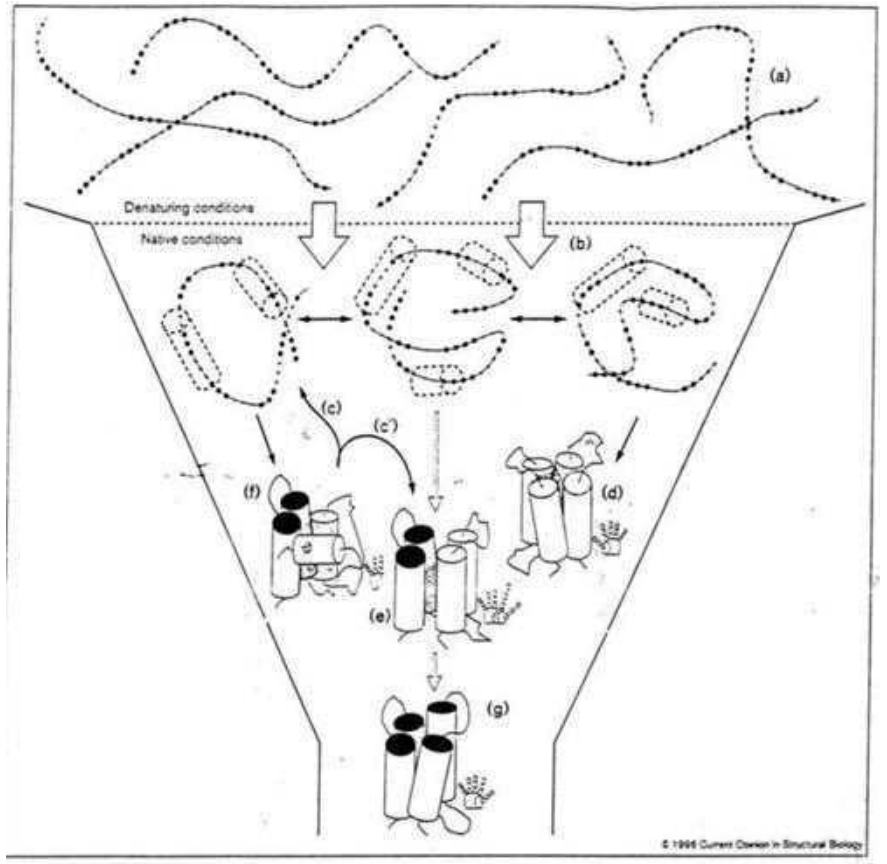


A modern perspective on protein folding suggests that there is no single unique **folding pathway.** Instead, a large ensemble of unfolded structures follows a multidimensional folding funnel towards the native state.

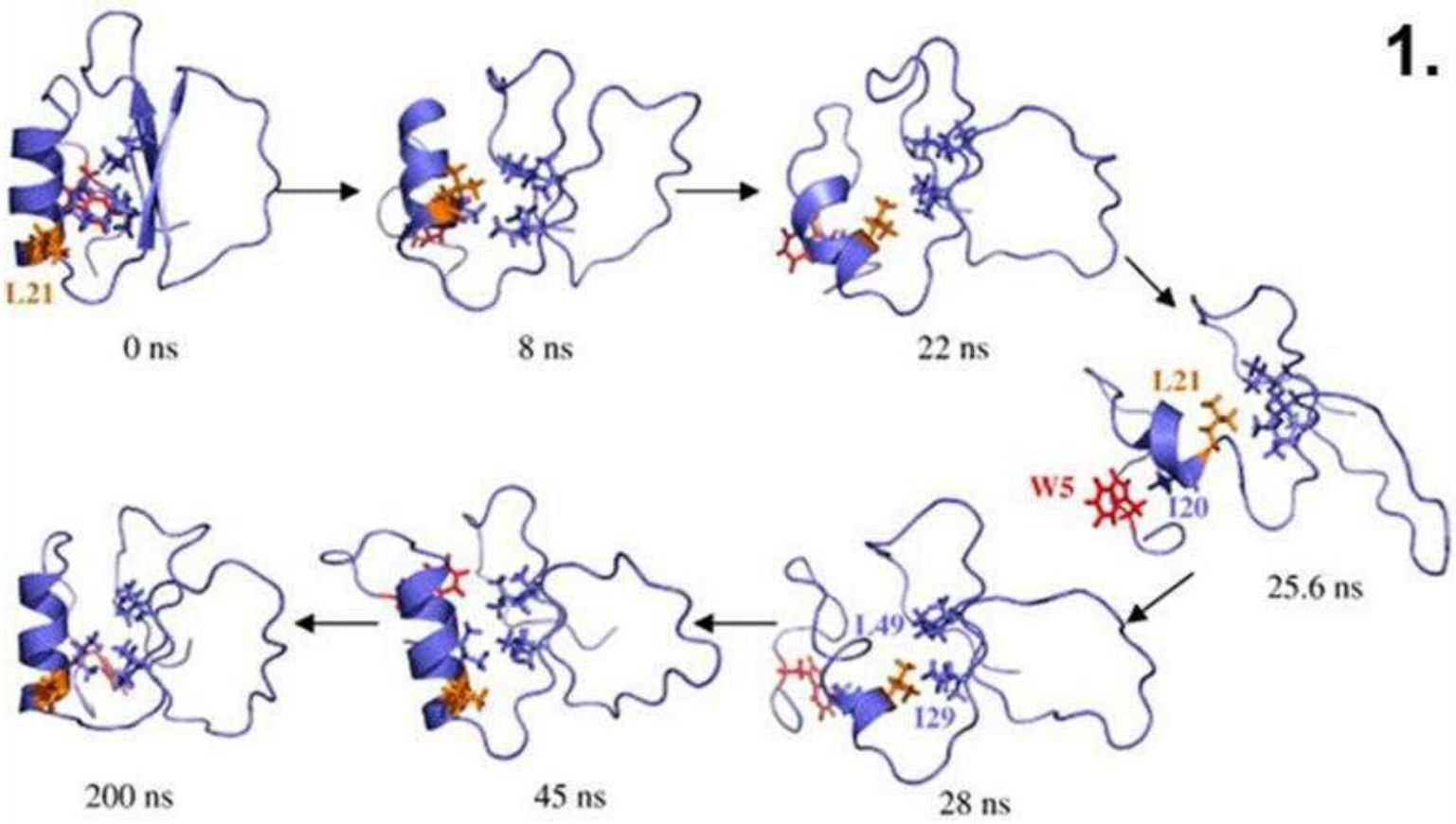
The Folding Funnel

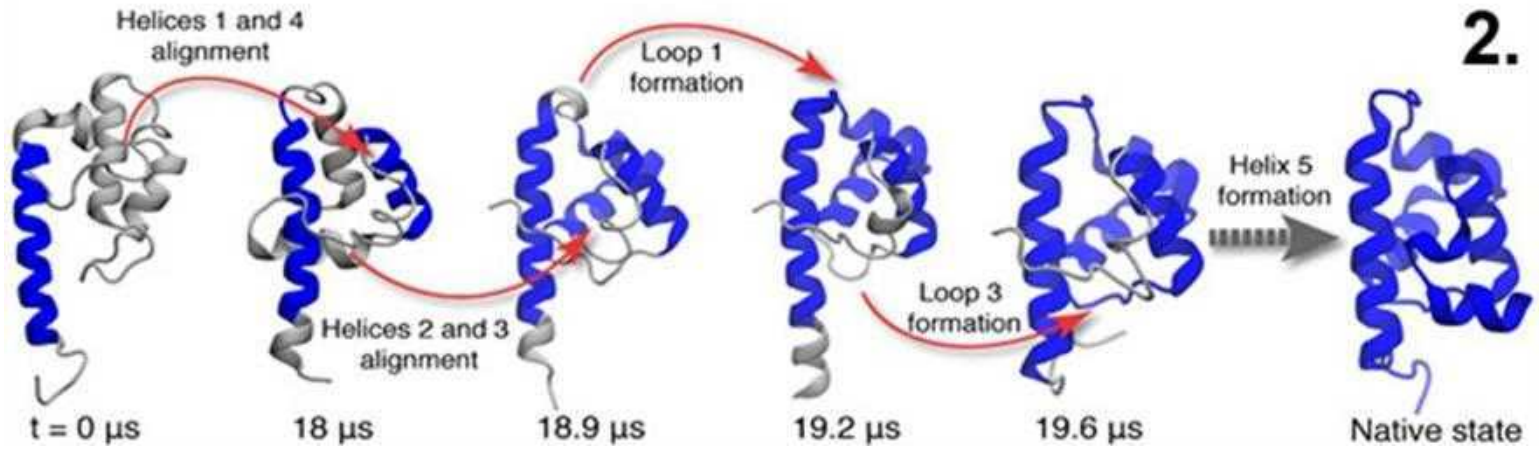


Progressing down the funnel is accompanied by an increase in **native-like structural** elements as the protein folds.



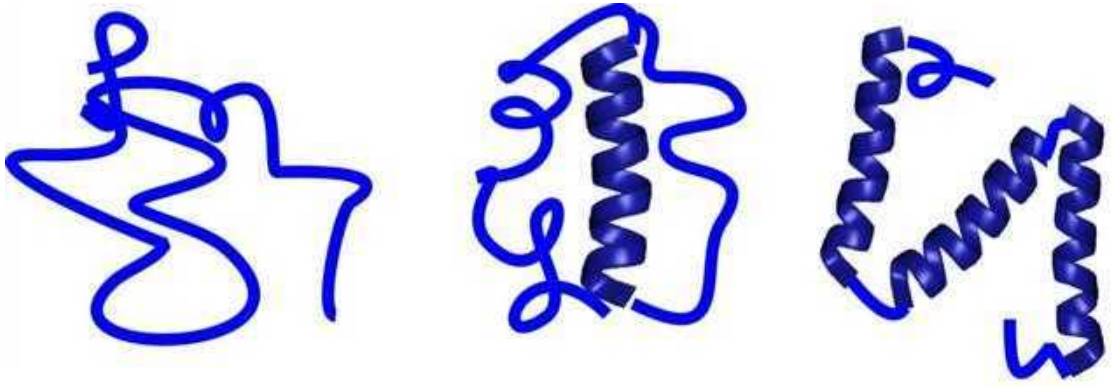
Protein folding





Examples of events occurring during protein folding on the nanosecond (top) and microsecond (bottom) timescales.

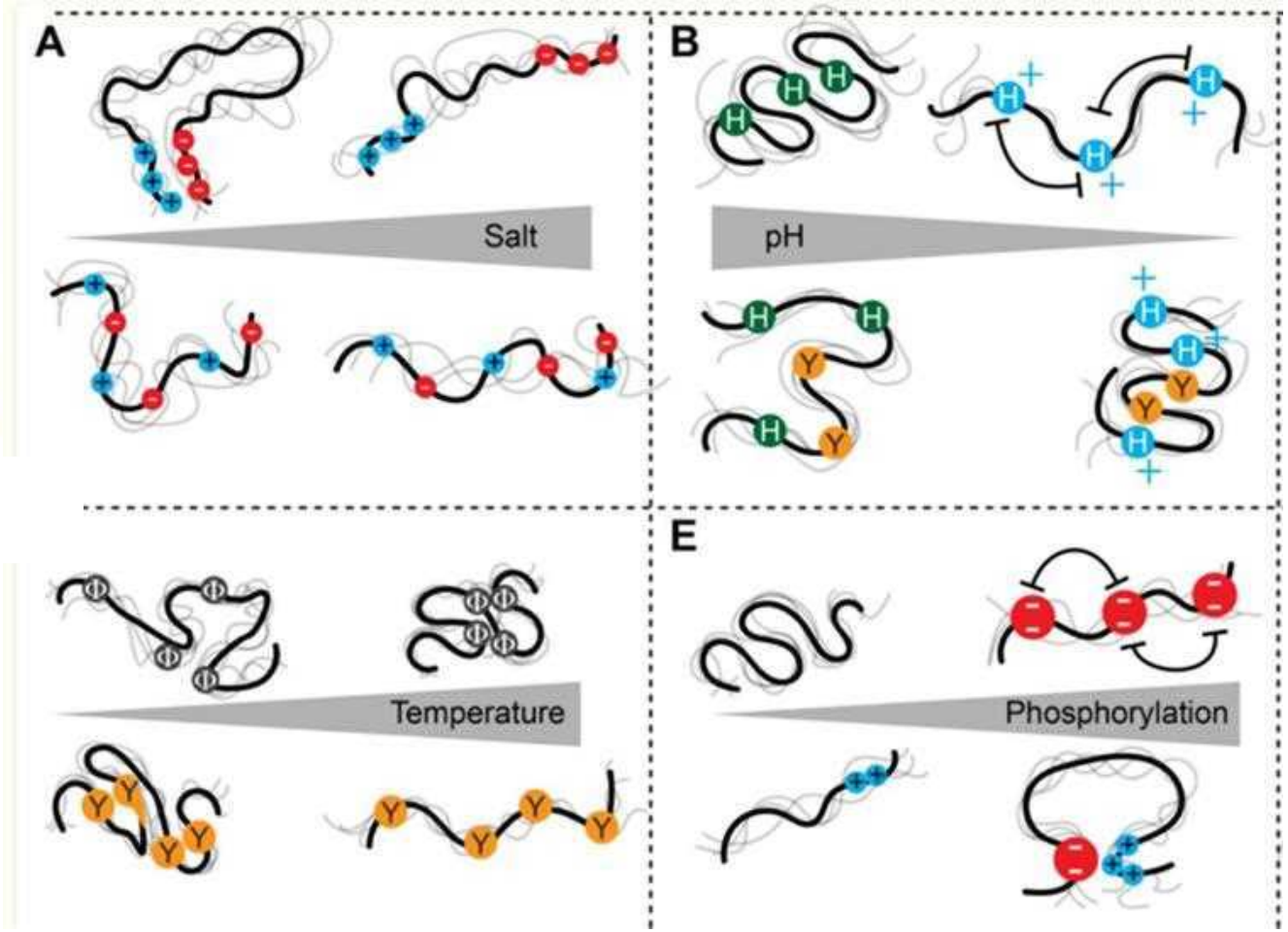
Intrinsically disordered proteins



Intrinsically disordered proteir | Protein with intrinsically disordered regions | Structured protein

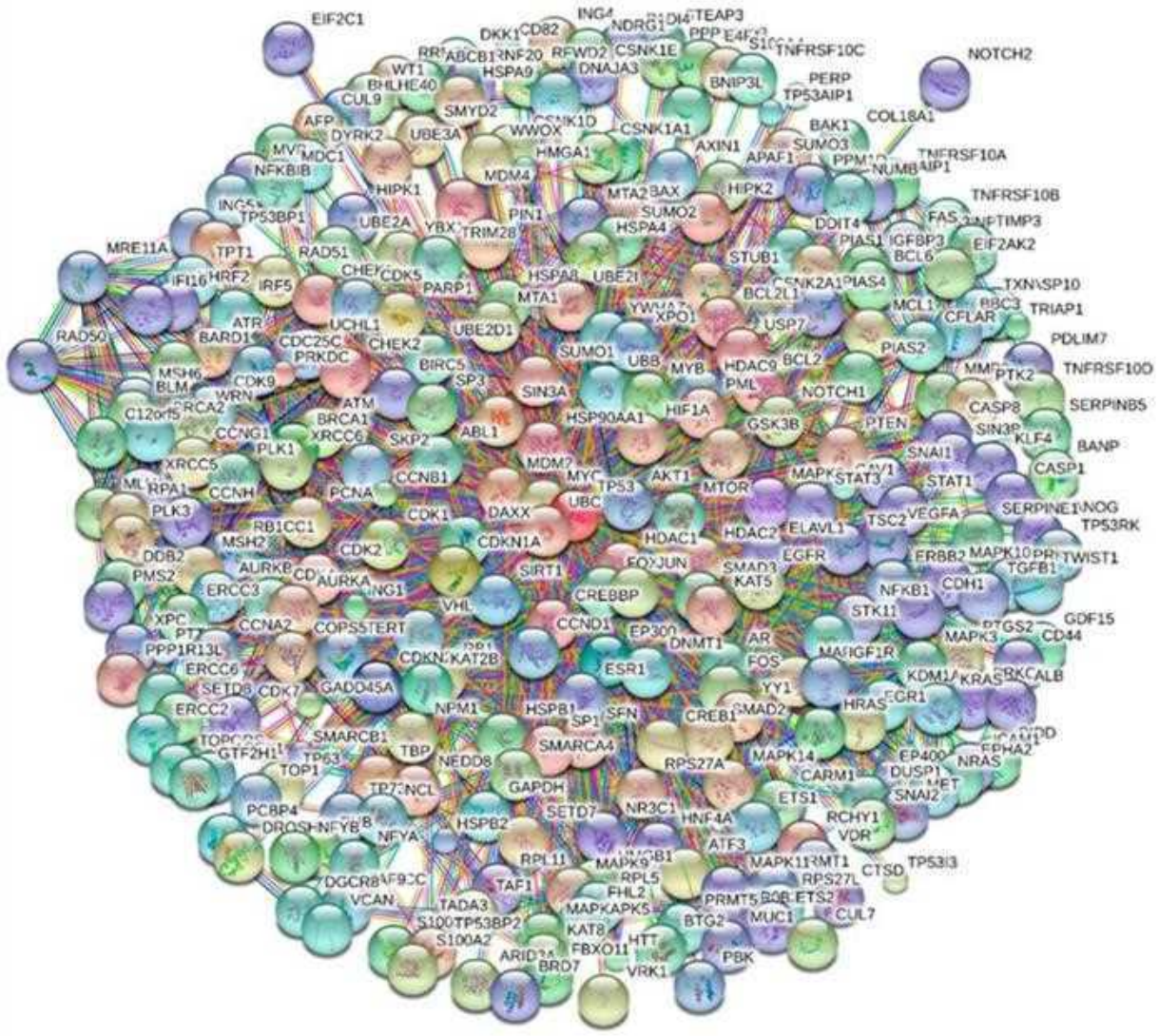
Proteins that lack stable secondary and tertiary structure in significant regions are termed intrinsically disordered proteins (IDPs).

- 40% of all mammalian proteins are disordered.
- 70% of all signaling proteins contain extended disordered regions.
- IDPs reside at the hub of multiple intracellular signaling cascades.



Changes in pH, temperature, acid-base balance, salt balance, cellular redox status or PTM can modify the intrinsically disordered regions and induce conformational rearrangements of the protein

Disorder has its advantages



Analysis of the human **p53** interactome (UniProt ID: P04637) using the STRING computational platform, which generates a network of predicted associations centered on the query protein

- Advantages of Intrinsically Disordered Proteins (IDPs) in Cellular Signaling:
- Multivalent Binding Capacity: Disordered sequences can bind to multiple partners, often employing different structures for different interactions.
- Precise Signal Processing: They respond accurately and quantitatively to cellular signals and possess the capacity for complex information processing.
- Dynamic and Transient Interactions: Molecular interactions are transient; IDPs exchange binding partners and compete for binding to central hub proteins.
- Regulation of Scarce Hubs: This dynamic competition is crucial for interacting with central hub proteins, which are often present in limited quantities and have short lifespans.
- Fine-Tuning by PTMs: These interactions are finely tuned by post-translational modifications (PTMs).
- Functional Versatility as Switches: PTMs enable IDPs to function as molecular switches and rheostats, dynamically controlling signaling pathways.

Protein Structure Modeling

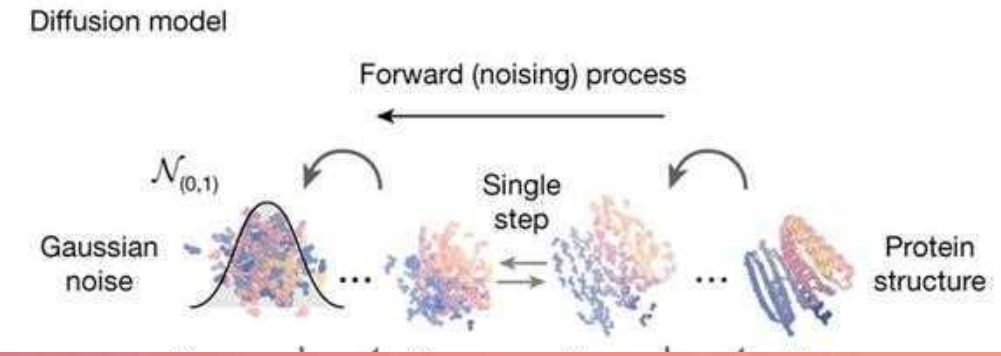
AlphaFold2 (from DeepMind/EMBL-EBI): Uses deep learning to predict protein structure with accuracy comparable to experimental methods. It predicts not only the structure but also its confidence (pLDDT) for each residue.

Availability: The AlphaFold Database contains predicted structures for nearly all known proteins. The source code is also available.

RoseTTAFold (from the Baker Lab): A similar spirit algorithm that also utilizes deep learning. It competes with AlphaFold2 in terms of accuracy.

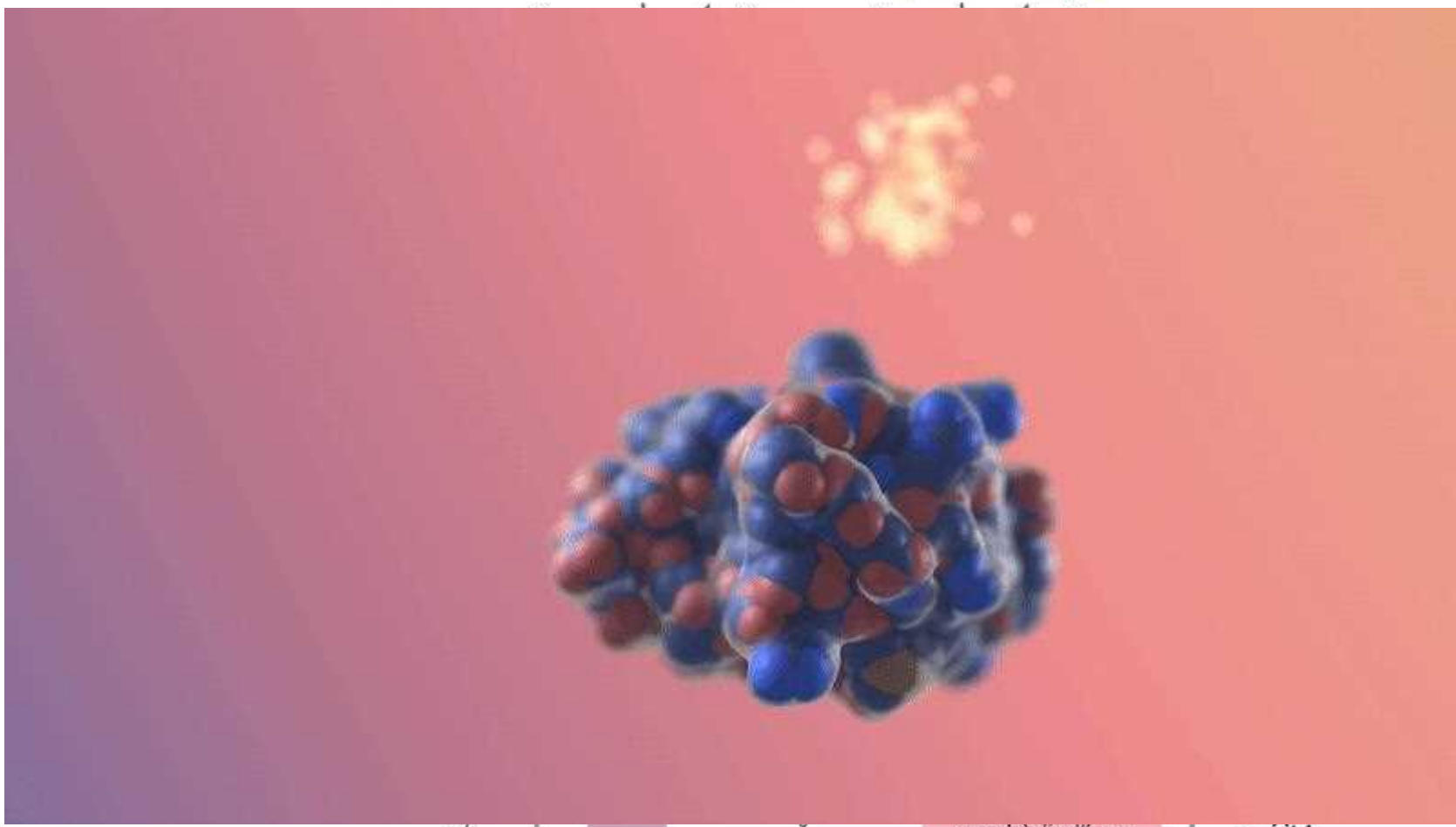
Protein Structure Modeling

RoseTTAFold

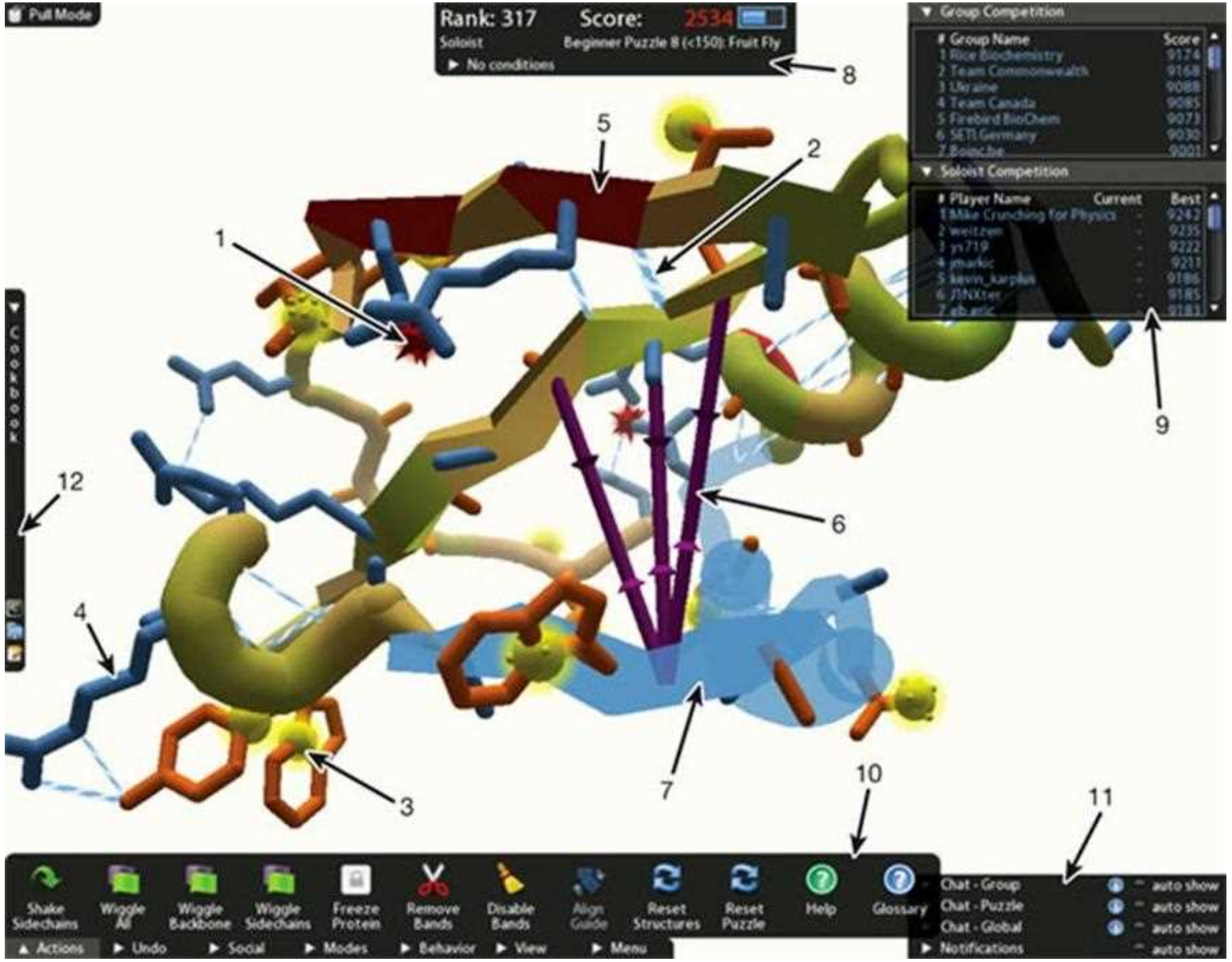


RFdiffusion was developed based on the RoseTTAFold (RF) architecture, utilizing a system for representing protein structures as sets of local residue coordinate frames. Each residue is described by:

- The coordinates of its Cα atom.
- The rigid orientation of its three-atom N-Cα-C backbone fragment.



Protein Structure Modeling

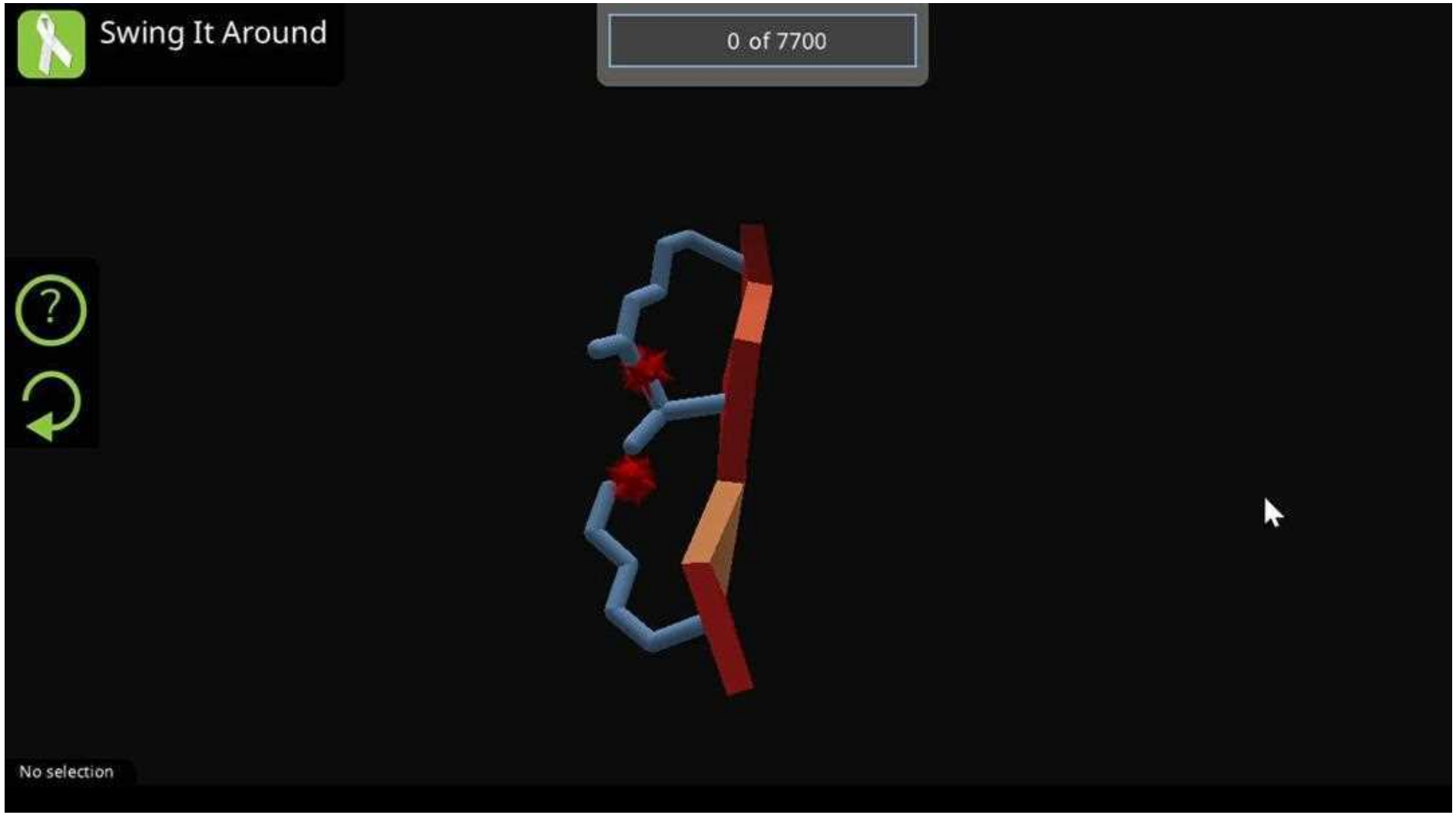


A group of American researchers has recently created a useful online game called **"Foldit" ("Fold It").** By playing it, you can contribute to scientific progress, and participation is open not only to certified specialists but also to ordinary people.

The game presents you with one of the most important, extensive, and tedious tasks in biology—determining the correct conformation of a protein from its amino acid sequence. However, all the tedium is gone in the game. On the screen, you see a colorful chain that needs to be folded according to specific rules. Points are awarded for correct solutions, and each player has their own rating. You can form groups and solve problems collectively—all while advancing the field of biology.

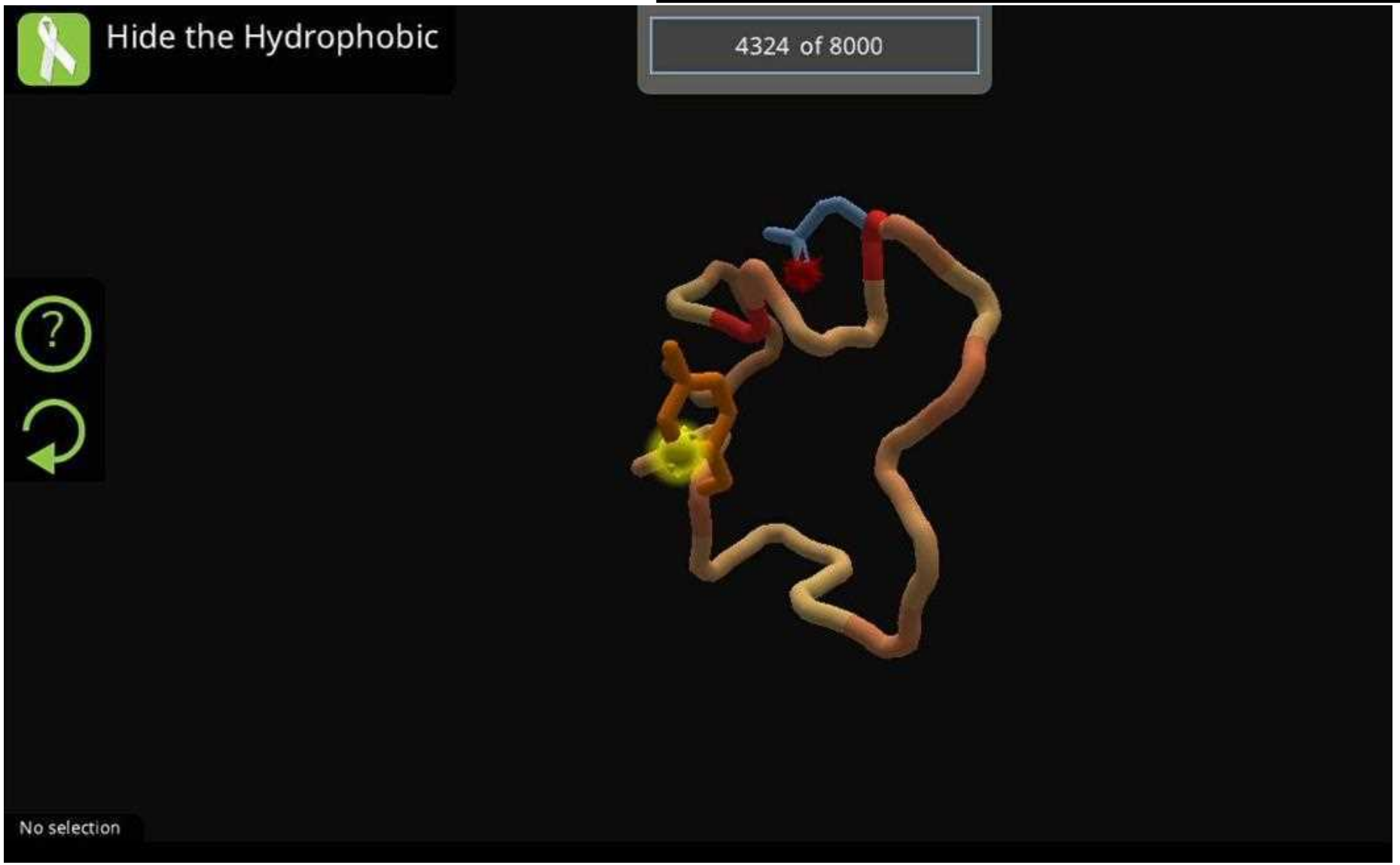
Swing it around

Level Objective: To teach the player how to effectively rotate and move parts of the protein to avoid clashes and find the optimal conformation



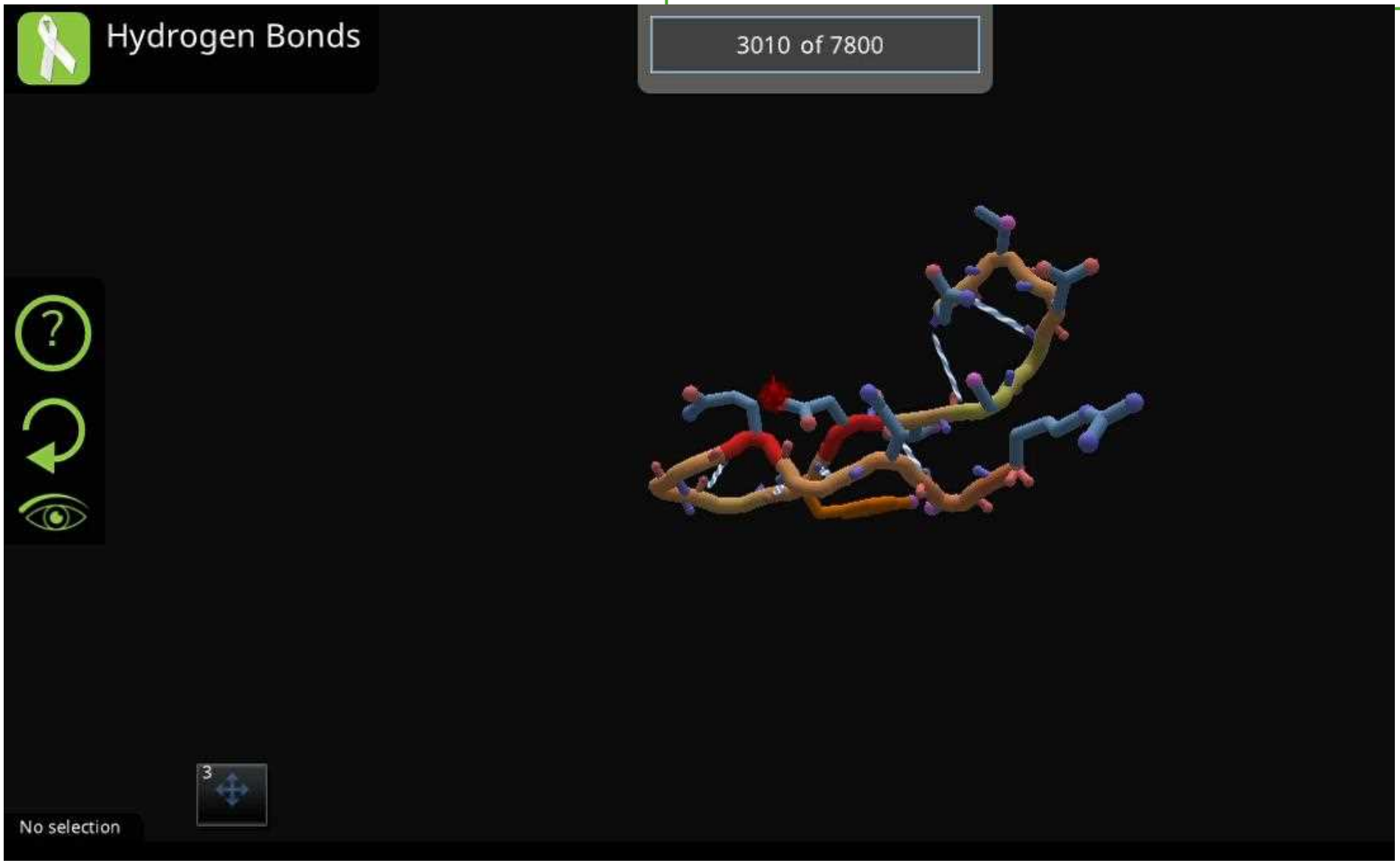
Hide of the hydrobionic

Level Objective: To teach the player how to properly hide **hydrophobic (water-repelling)** regions of the protein inside its structure to achieve stability.



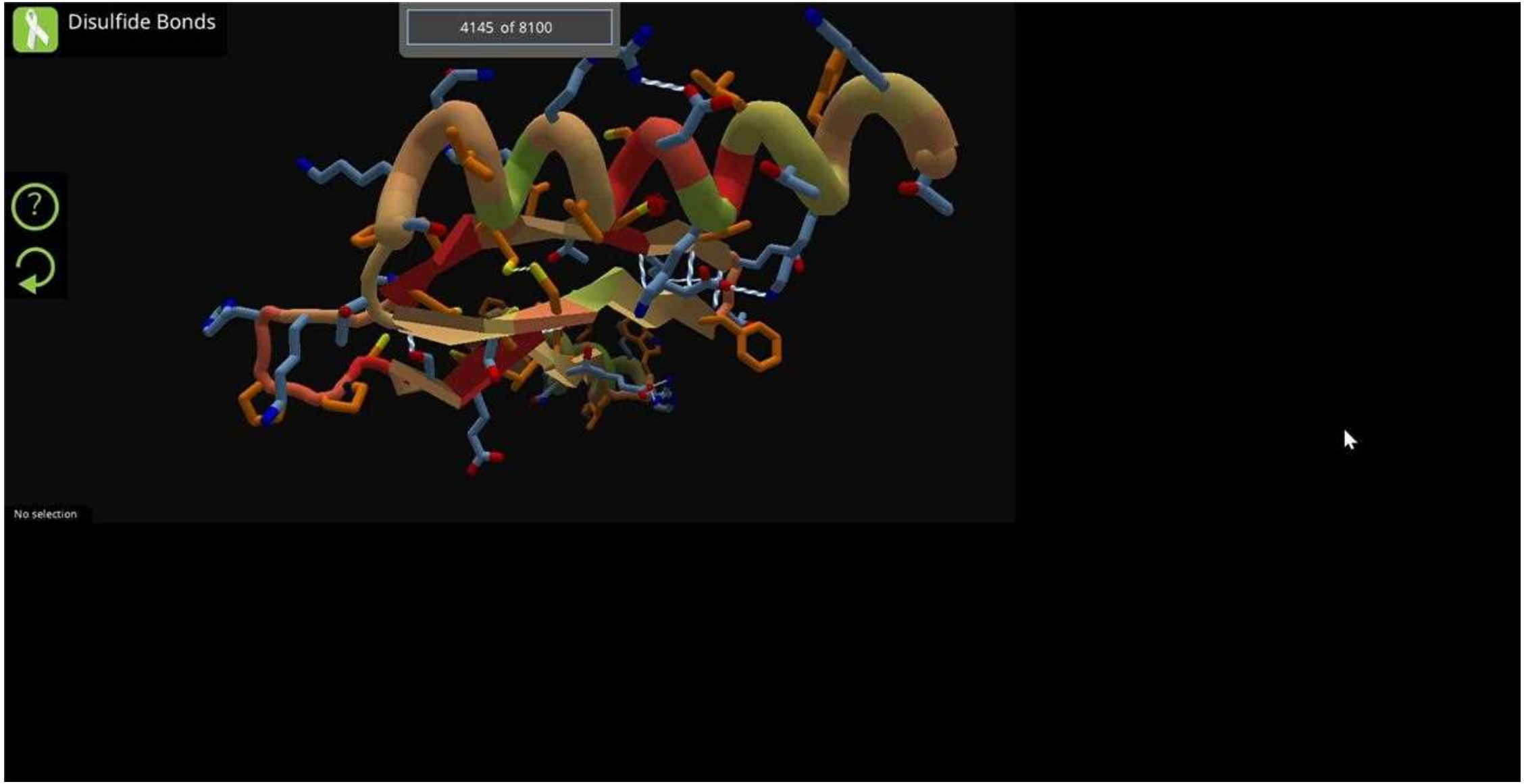
Hydrogen Bonds

Objective: To teach the player to identify and optimize **hydrogen bonds** within the protein structure to enhance its stability.



Disulfide Bonds

Objective: To teach the player to locate and form **disulfide bridges (S-S bonds)** between cysteine and methionine residues to stabilize the protein's 3D structure.

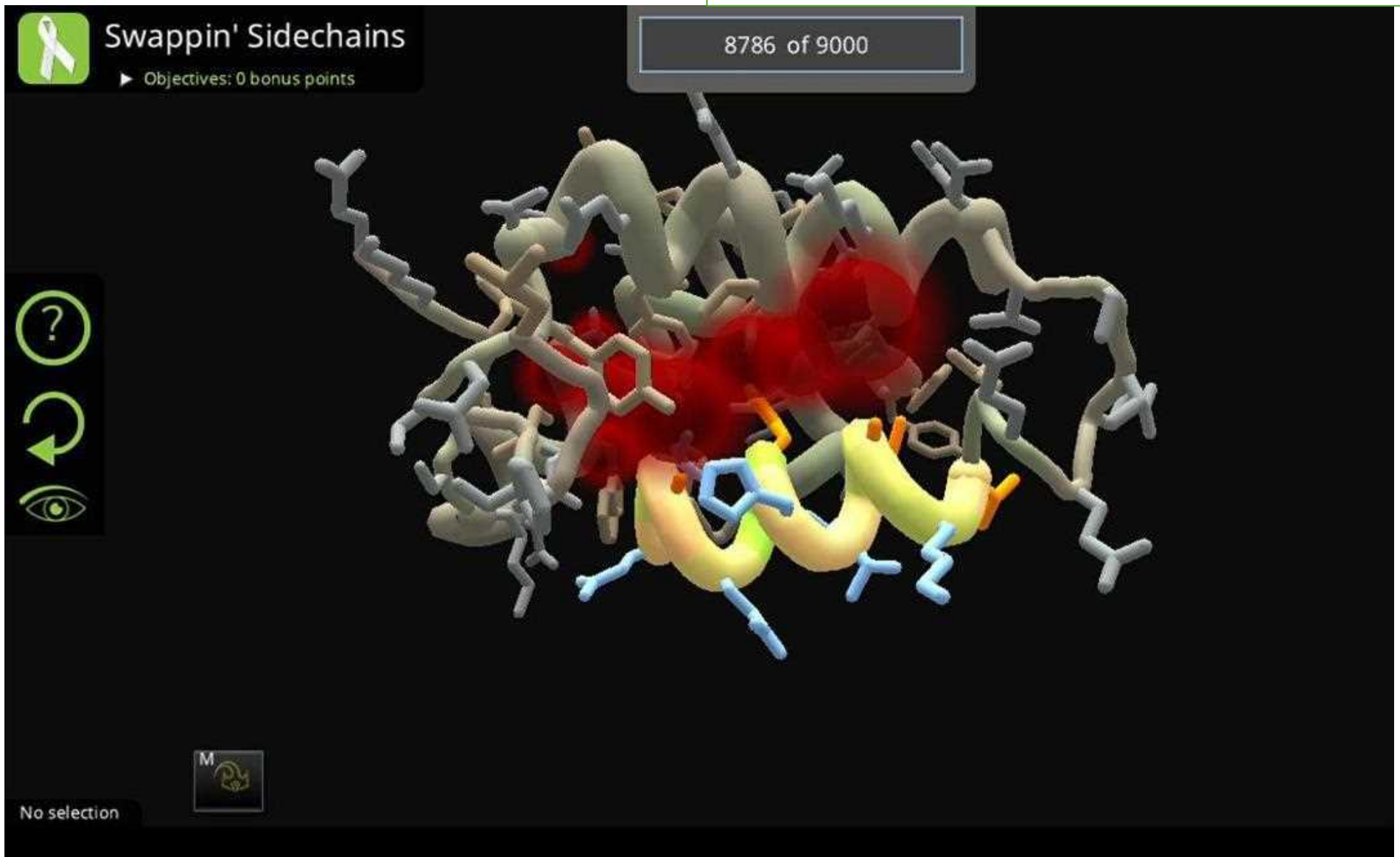


Intro to Design

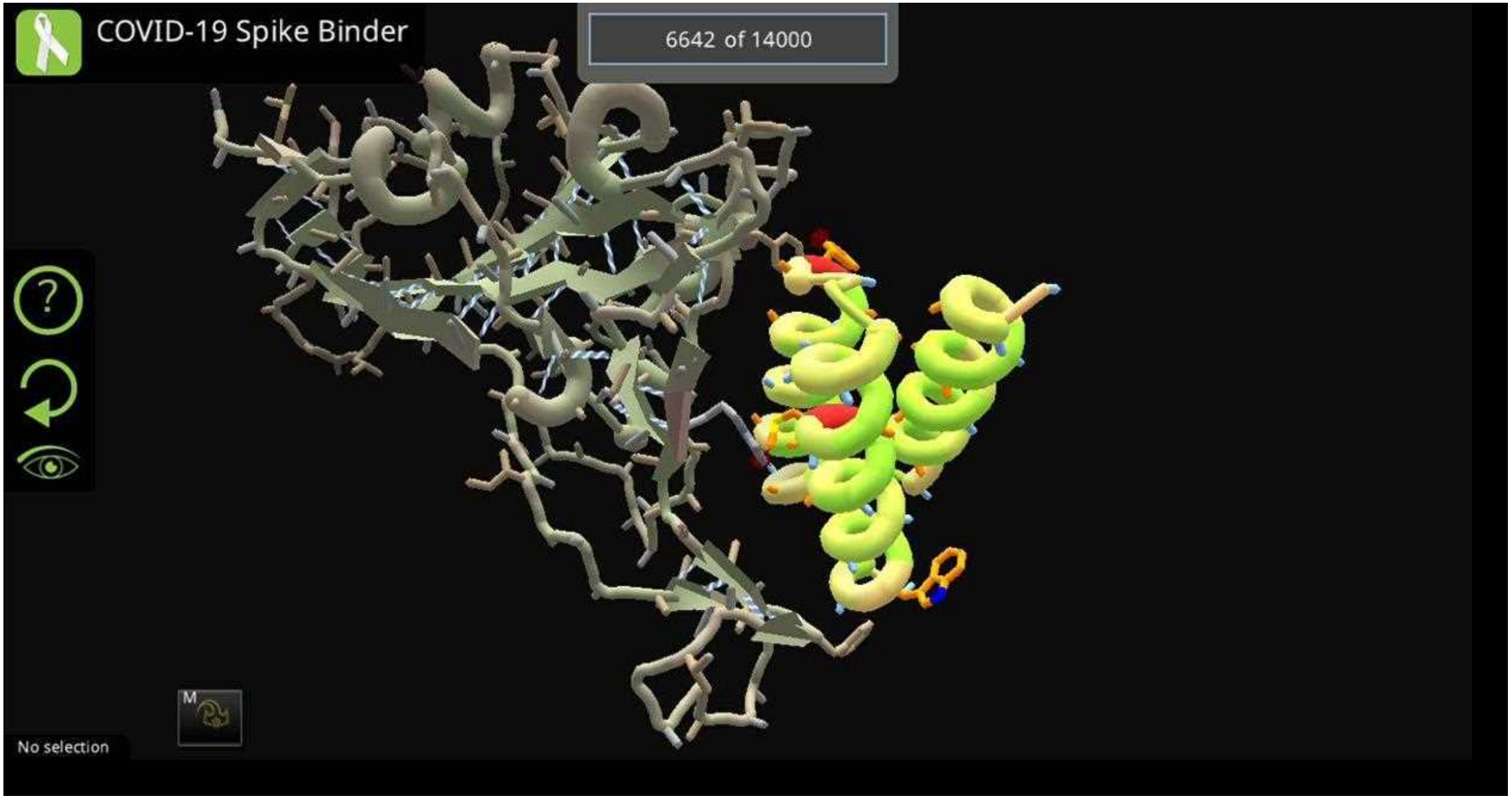
Objective: To introduce the player to the basics of protein design by replacing excessively large amino acids with those possessing smaller side chains, hiding **hydrophobic amino acids inside** the protein core, and positioning hydrophilic ones on the surface.

Swappin’ Sidechains

Objective: To teach the player to optimize the protein structure by selecting **optimal amino acid side chains**. The goal is to minimize voids/empty spaces inside the protein (highlighted in red).



COVID-19 Spike Binder



Thank you for your attention!