# Classroom Challenge

Protein Fold & Function Exploration – Student Guide

# Instructions:

1. You will be assigned a protein to research.
2. Read the teaser question carefully - it should guide your investigation. You do not need to answer it, but it frames what might be interesting about the protein.
3. Using textbooks, UniProt, and scientific literature, find and describe in your own words:

* Biological role and cellular localization
* Folding dynamics, fold stability, free energy barriers, sidechain interactions
* Assembly, oligomerization, or polymerization
* Cofactors or ligands required
* Interactions with binding partners or related proteins
* Polymorphisms (mutations), isoforms, or variants

Prepare a paragraph summarizing your findings using concepts from your protein physics course. You will be asked to present your findings to another student (group).

## Example for Students – Calmodulin

How does a calcium-binding protein act as a dynamic regulator of multiple enzymes?

Calmodulin is a calcium-binding protein that functions as a sensor and regulator for numerous enzymes and signalling proteins in the cytoplasm. Its amino acid sequence folds into two globular domains connected by a flexible linker, each containing EF-hand motifs that coordinate calcium ions. Binding of calcium stabilizes specific sidechain interactions and lowers the free energy barrier for interaction with target proteins, such as kinases and ion channels. In the absence of calcium, the domains sample multiple conformations; calcium binding shifts the population toward the active, high-affinity state. Cofactors like calcium are essential, and the local ionic environment and pH modulate folding kinetics and binding interactions. Variants in EF-hand residues or post-translational modifications can alter calcium affinity or partner specificity, highlighting the relationship between sequence, fold, and function.

## Proteins

1. **☐ Spider Silk (Spidroins)**   
   How does a soluble protein suddenly become an incredibly strong fiber?
2. **☐ Hemoglobin**  
   How does one mutation turn a lifesaving oxygen carrier into a fiber that blocks blood vessels?
3. **☐ Prions (PrP)**  
   How can a protein’s shape be contagious?
4. **☐ Actin**  
   How does a single protein create a dynamic scaffold inside cells?
5. **☐ Tubulin**  
   How do dimers assemble into dynamic highways inside the cell?
6. **☐ GABAA\_AA​ Receptor**  
   How can one receptor mediate both inhibition and diverse pharmacology in the brain?
7. **☐ Green Fluorescent Protein (GFP)**  
   How does a barrel of protein glow on its own?
8. **☐ Lysozyme**  
   How does a small enzyme efficiently break bacterial cell walls?
9. **☐ Ferritin**  
   How can a protein cage safely store thousands of iron atoms?
10. **☐ Immunoglobulins (Antibodies)**  
    How can one protein fold encode endless antigen specificity?
11. **☐ Bacteriorhodopsin (Membrane Protein)**  
    How does a tiny retinal molecule trigger proton pumping across a membrane?
12. **☐ Aquaporin (Membrane Protein)**  
    How does aquaporin let water pass but block protons?
13. **☐ Alpha-synuclein (Intrinsically Disordered Protein)**  
    Why does a protein with no stable fold become toxic when aggregated?
14. **☐ CFTR (Membrane Protein)**  
    How can deletion of one amino acid ruin a vital chloride channel?
15. **☐ Beta-barrel Outer Membrane Proteins (OmpA)**  
    How do β-barrels form stable pores in membranes?
16. **☐ Tardigrade Disordered Proteins (TDPs)**  
    How can a completely disordered protein save an organism from drying out?
17. **☐ Ubiquitin**  
    How can eight amino acids encode a universal cellular “post-it note”?
18. **☐ Green Opsin / Rhodopsin (Membrane Protein)**  
    How does a single chromophore tune vision to different colors?
19. **☐ Concanavalin A (Circular Permutation)**  
    How can reshuffling a protein’s sequence leave its fold intact?
20. **☐ Hydrophobins (Fungal Amphipathic Proteins)**  
    How can a tiny protein coat both water and oil surfaces?
21. **☐ Interferon**  
    How does a secreted signaling protein trigger antiviral responses across cells?
22. **☐ Hedgehog**  
    How does a morphogen signal through both lipid modifications and receptor interactions to control development?

# Teachers reference

## 0. Calmodulin

**Teaser:** How does a calcium-binding protein act as a dynamic regulator of multiple enzymes?

**Prompt Questions:**

* What processes does it regulate? Where in the cell is it found?
* How does calcium binding affect folding? Which sidechains are critical? Are there folding intermediates or energy barriers?
* Does it function as a monomer or oligomer? What binding partners does it interact with?
* Are there isoforms or post-translational modifications affecting function?

**Expanded Example Answer:**  
Calmodulin is a cytoplasmic calcium-binding protein that regulates enzymes and signaling pathways, including muscle contraction, neurotransmission, and metabolism. Its amino acid sequence folds into two globular EF-hand domains connected by a flexible linker. In the absence of calcium, the domains sample multiple conformations, corresponding to shallow minima in the free energy landscape. Calcium binding stabilizes hydrogen bonds and hydrophobic contacts, lowering the free energy barrier for the active conformation. Sidechains coordinating calcium and hydrophobic patches for protein-protein interactions are correctly positioned only in this state. Calmodulin functions as a monomer but interacts with numerous targets such as kinases and ion channels. Post-translational modifications, like phosphorylation, can modulate calcium affinity and binding specificity. This illustrates the interplay between fold, ligand-induced conformational dynamics, and cellular function.

## 1. Spider Silk (Spidroins)

**Teaser:** How does a soluble protein suddenly become an incredibly strong fiber?

**Prompt Questions:**

* What is the biological function of spider silk? Where is it stored in the spider?
* How is solubility maintained in the gland? What triggers polymerization?
* How does the protein assemble into fibers? Are there oligomers or intermediate states?
* Are there multiple spidroin isoforms with different mechanical properties?

**Expanded Example Answer:**  
Spidroins are stored in spider silk glands as soluble, partially folded proteins. Fiber formation is triggered during spinning by a combination of pH change, ion gradients, and shear stress. These environmental changes lower the free energy barrier for β-sheet-rich fiber formation and promote alignment of repetitive motifs. Sidechain interactions, especially between hydrophobic and charged residues, stabilize the emergent fibers. The proteins assemble from monomeric or small oligomeric precursors into long polymeric fibers. Different isoforms, such as MaSp1 and MaSp2, have distinct amino acid compositions, leading to variations in elasticity and tensile strength that are critical for different types of silk fibers.

## 2. Hemoglobin

**Teaser:** How does one mutation turn a lifesaving oxygen carrier into a fiber that blocks blood vessels?

**Prompt Questions:**

* What is hemoglobin’s role and where is it found?
* How does its fold facilitate oxygen binding? Are there cooperative interactions?
* How do the α and β subunits assemble? What triggers polymerization in HbS?
* What are the functional consequences of different isoforms or mutations like HbF or HbS?

**Expanded Example Answer:**  
Hemoglobin is a tetrameric protein in red blood cells responsible for oxygen transport. Each subunit binds a heme group, and the α2β2 assembly allows cooperative oxygen binding. The native fold of each subunit is stabilized by hydrophobic core packing, hydrogen bonds, and salt bridges. In sickle cell hemoglobin (HbS), a single amino acid mutation creates a hydrophobic patch on the β-chain surface, promoting polymerization into fibers under deoxygenated conditions. These fibers distort red blood cells into a sickle shape, impairing blood flow. Hemoglobin isoforms like HbF and HbA differ in subunit composition and oxygen affinity, illustrating how small sequence variations impact fold, assembly, and function.

## 3. Prions (PrP)

**Teaser:** How can a protein’s shape be contagious?

**Prompt Questions:**

* What is the biological function or pathogenic role?
* How does the fold change between normal and misfolded forms? Are there intermediate states?
* How does aggregation or polymerization occur?
* Are there polymorphisms or strains that influence disease?

**Expanded Example Answer:**  
Prion protein (PrP) normally folds into an α-helical conformation, primarily in neurons. Misfolding induces a β-sheet-rich state that can template conversion of other PrP molecules, propagating the misfolded conformation. The free energy barrier between normal and pathogenic forms is influenced by environmental factors and local sidechain interactions. Misfolded PrP assembles into amyloid fibrils, forming insoluble aggregates. Different prion strains correspond to structurally distinct aggregates, demonstrating how slight sequence or conformational differences can influence propagation and disease phenotype.

## 4. Actin

**Teaser:** How does a single protein create a dynamic scaffold inside cells?

**Prompt Questions:**

* What is actin’s role and where is it localized?
* How do folding and nucleotide binding influence stability?
* How do monomers assemble into filaments? Are there intermediates?
* Are there isoforms with functional differences?

**Expanded Example Answer:**  
Actin is a cytoplasmic protein forming the dynamic cytoskeleton. Monomeric G-actin folds into a globular structure stabilized by hydrophobic core packing and hydrogen bonds, with ATP or ADP influencing stability. Filamentous F-actin assembles via nucleation, elongation, and treadmilling, with transient oligomeric intermediates. Sidechains critical for inter-subunit interactions stabilize the filament. Isoforms (α, β, γ) show tissue-specific expression and affect filament dynamics and interactions with actin-binding proteins, linking folding and assembly to cellular structure and motility.

## 5. Tubulin

**Teaser:** How do dimers assemble into dynamic highways inside the cell?

**Prompt Questions:**

* What is tubulin’s role and localization?
* How do α/β subunits fold and what cofactors are required?
* How do dimers polymerize into microtubules? What are the dynamics?
* Are there isoforms or modifications that influence function?

**Expanded Example Answer:**  
Tubulin α/β heterodimers fold with the assistance of chaperones and bind GTP. Folding stabilizes the subunit interface and prepares the GTP-binding site. Dimers polymerize into microtubules, which exhibit dynamic instability driven by GTP hydrolysis. Sidechain interactions and lattice contacts stabilize the polymer. Isoforms of α- and β-tubulin and post-translational modifications regulate microtubule stability, interactions with motor proteins, and cellular organization, illustrating how folding, nucleotide binding, and polymerization coordinate cellular function.

## 6. GABA\_A Receptor

**Teaser:** How can one receptor mediate both inhibition and diverse pharmacology in the brain?

**Prompt Questions:**

* What is the receptor’s role and localization?
* How do subunits fold and assemble in the membrane?
* What is the oligomerization state? How does ligand binding affect conformation?
* Are there isoforms or subunit variants affecting pharmacology?

**Expanded Example Answer:**  
GABA\_A receptors are pentameric chloride channels located in neuronal membranes that mediate inhibitory neurotransmission. Each subunit folds co-translationally in the ER, forming a stable α/β/γ subunit architecture. Assembly into a pentameric channel ensures proper gating and chloride conductance. Ligand binding induces conformational changes that open or close the pore. Multiple α, β, and γ subunit isoforms generate receptors with distinct kinetics and pharmacological profiles, illustrating how fold, assembly, and subunit composition integrate to produce functional diversity.

## 7. Green Fluorescent Protein (GFP)

**Teaser:** How does a barrel of protein glow on its own?

**Prompt Questions:**

* What is GFP’s role and localization?
* How does folding enable chromophore formation?
* Does GFP oligomerize? Are there intermediate states?
* Are there variants with altered fluorescence properties?

**Expanded Example Answer:**  
GFP is a cytoplasmic protein that fluoresces autonomously, widely used as a reporter. It folds into a β-barrel structure enclosing the chromophore. Correct folding aligns key sidechains to catalyze chromophore cyclization and maturation. GFP is primarily monomeric, though some engineered variants dimerize. Spectral variants (YFP, CFP) alter sidechain interactions in the chromophore pocket, shifting emission wavelengths. Folding dynamics influence fluorescence efficiency, connecting structure to function.

## 8. Lysozyme

**Teaser:** How does a small enzyme efficiently break bacterial cell walls?

**Prompt Questions:**

* What is its role and localization?
* How does folding stabilize the active site? Any folding intermediates?
* Is it monomeric or does it form higher-order structures?
* Are there variants or mutations affecting stability or activity?

**Expanded Example Answer:**  
Lysozyme is a small, secreted enzyme that hydrolyzes bacterial cell walls. It folds into a compact α/β structure stabilized by hydrogen bonds, hydrophobic packing, and disulfide bridges. Correct folding positions residues in the active site for catalysis. Lysozyme is generally monomeric, though misfolded variants can aggregate. Species differences and point mutations alter fold stability and enzymatic efficiency, illustrating how sequence affects free energy landscapes, sidechain interactions, and functional performance.

## 9. Ferritin

**Teaser:** How can a protein cage safely store thousands of iron atoms?

**Prompt Questions:**

* What is its biological role and where is it located?
* How does folding stabilize monomers?
* How do monomers assemble into the 24-mer cage? Any intermediates?
* Are there isoforms affecting storage properties?

**Expanded Example Answer:**  
Ferritin monomers fold into four-helix bundles stabilized by hydrophobic cores and hydrogen bonds. Twenty-four monomers assemble into a hollow spherical cage capable of storing thousands of iron atoms. Sidechain interactions at subunit interfaces stabilize the assembly. Isoform composition (H/L subunits) varies across tissues, influencing iron nucleation rate and storage efficiency. Folding and assembly link directly to functional iron storage and release dynamics in cells.

## 10. Immunoglobulins (Antibodies)

**Teaser:** How does a Y-shaped protein recognize virtually any antigen?

**Prompt Questions:**

* What is its role and cellular localization?
* How does the fold of variable and constant domains stabilize the structure?
* How do monomers assemble into functional antibodies?
* Are there isoforms, splice variants, or hypervariable loops affecting specificity?

**Expanded Example Answer:**  
Immunoglobulins are secreted or membrane-bound proteins critical for adaptive immunity. Each antibody consists of two heavy and two light chains, with variable and constant domains folding into immunoglobulin folds stabilized by β-sheets and disulfide bonds. Disulfide linkages and noncovalent interactions assemble chains into a functional Y-shaped molecule. Hypervariable loops in variable domains create antigen-binding diversity. Isoforms, class switching, and alternative splicing further modify effector functions and localization, linking fold, sequence, and assembly to immune recognition.

## 11. Bacteriorhodopsin

**Teaser:** How does a small membrane protein convert light into a proton gradient?

**Prompt Questions:**

* What is its cellular localization and role?
* How does its fold stabilize the retinal cofactor?
* How does oligomerization affect function?
* Are there variants that change absorption or efficiency?

**Expanded Example Answer:**  
Bacteriorhodopsin is an integral membrane protein in halophilic archaea that pumps protons using light energy. It folds into seven transmembrane α-helices forming a pocket for the retinal cofactor. Photoisomerization of retinal triggers conformational changes that transport protons. Bacteriorhodopsin forms trimers in the membrane, and inter-subunit interactions stabilize structure and optimize function. Sequence variants can shift absorption spectra or alter proton pumping efficiency, showing how membrane fold, cofactor interaction, and oligomerization drive energy conversion.

## 12. Aquaporin

**Teaser:** How do cells move water rapidly without losing ions?

**Prompt Questions:**

* What is its role and membrane localization?
* How do monomers fold into functional water channels?
* How do monomers assemble into tetramers?
* Are there isoforms with tissue-specific water permeability?

**Expanded Example Answer:**  
Aquaporins are integral membrane proteins forming selective water channels in plasma and organelle membranes. Each monomer folds into six transmembrane helices creating a narrow pore. Tetramerization stabilizes the complex and may affect gating. Isoforms differ in expression across tissues and organelles, modifying water permeability and response to physiological cues. Folding, oligomerization, and sidechain interactions within the pore ensure selective water transport without ion leakage.

## 13. Alpha-synuclein

**Teaser:** How does a protein that is normally disordered form pathological aggregates?

**Prompt Questions:**

* Where is it localized and what is its normal function?
* How does its intrinsic disorder affect folding and interactions?
* How do oligomers and fibrils form?
* Are there mutations that change aggregation propensity?

**Expanded Example Answer:**  
Alpha-synuclein is a neuronal protein localized mainly in presynaptic terminals, involved in vesicle trafficking. It is intrinsically disordered in solution, lacking a stable tertiary structure under normal conditions. Upon environmental triggers or mutations, it can form β-sheet-rich oligomers and amyloid fibrils. Sidechain interactions in the NAC region drive aggregation. Familial Parkinson’s disease mutations (A30P, A53T) increase aggregation propensity, showing how disorder, local sequence, and environmental factors affect folding, oligomerization, and pathology.

## 14. CFTR

**Teaser:** How does a misfolding-prone channel lead to cystic fibrosis?

**Prompt Questions:**

* What is CFTR’s role and membrane localization?
* How do nucleotide-binding domains and transmembrane domains fold?
* How does oligomerization or trafficking affect function?
* Are there isoforms or mutations affecting folding and disease?

**Expanded Example Answer:**  
CFTR is a chloride channel in epithelial membranes. It folds co-translationally into two transmembrane domains, two nucleotide-binding domains, and a regulatory domain. Proper folding and assembly are assisted by chaperones. Mutations like ΔF508 destabilize the fold, prevent ER export, and reduce channel activity. Folding defects demonstrate how subunit architecture, domain interactions, and sidechain packing influence trafficking, assembly, and functional consequences in disease.

## 15. Beta-barrel Outer Membrane Proteins

**Teaser:** How do proteins fold in a membrane environment to form a barrel?

**Prompt Questions:**

* What is their biological role and localization?
* How do β-strands fold to form a barrel?
* How do oligomerization and insertion into membranes occur?
* Are there variants or loops affecting function?

**Expanded Example Answer:**  
Beta-barrel proteins reside in bacterial outer membranes, forming pores for transport. Individual β-strands fold and hydrogen bond to create a cylindrical barrel. Sidechain interactions stabilize the barrel interior and loops define specificity and gating. Assembly involves chaperones and the BAM complex for membrane insertion. Sequence variations and loop differences modulate pore properties and specificity, demonstrating how folding, membrane insertion, and oligomerization enable selective transport.

## 16. Tardigrade Disordered Proteins

**Teaser:** How can a highly disordered protein protect cells from desiccation?

**Prompt Questions:**

* Where are they localized and what is their function?
* How does intrinsic disorder contribute to protection?
* Do they oligomerize or form condensates?
* Are there isoforms adapted to different stresses?

**Expanded Example Answer:**  
Tardigrade disordered proteins (TDPs) are cytoplasmic proteins that protect macromolecules during desiccation. They are intrinsically disordered, lacking stable secondary or tertiary structure. Upon drying, they can form amorphous solids or liquid-like condensates, stabilizing cellular components. Sidechains mediate weak interactions for protection without forming rigid aggregates. Isoforms differ across species and tissues, illustrating how intrinsic disorder and transient assembly confer stress resilience.

## 17. Ubiquitin

**Teaser:** How does a tiny protein tag everything for destruction?

**Prompt Questions:**

* What is its role and localization?
* How does folding stabilize the protein?
* How does it interact with ligases and target proteins?
* Are there variants or modifications that alter signaling?

**Expanded Example Answer:**  
Ubiquitin is a small, highly conserved protein that tags other proteins for proteasomal degradation. It folds into a compact β-grasp fold stabilized by hydrophobic interactions and hydrogen bonds. Its surface residues interact with E1, E2, and E3 ligases to covalently attach to lysines on target proteins. Post-translational modifications and ubiquitin-like variants regulate signaling pathways beyond degradation, showing how fold, sidechains, and interactions determine diverse cellular functions.

## 18. Green Opsin / Rhodopsin

**Teaser:** How does a G-protein coupled receptor convert light into a signal?

**Prompt Questions:**

* What is the localization and physiological role?
* How do seven transmembrane helices fold to bind retinal?
* Does oligomerization affect function?
* Are there isoforms or spectral variants?

**Expanded Example Answer:**  
Rhodopsins are GPCRs in photoreceptor membranes. Seven transmembrane helices fold to create a retinal-binding pocket. Light-induced retinal isomerization triggers conformational changes that activate transducin. Oligomerization may stabilize the receptor and affect signaling. Spectral variants and opsin isoforms tune light absorption to different wavelengths, illustrating how membrane fold, cofactor interactions, and subunit assembly determine sensory function.

## 19. Concanavalin A

**Teaser:** How does a plant lectin selectively recognize sugars?

**Prompt Questions:**

* What is its role and localization?
* How does the fold create carbohydrate-binding sites?
* Does it oligomerize for function?
* Are there isoforms with different binding specificity?

**Expanded Example Answer:**  
Concanavalin A is a plant lectin that binds specific carbohydrates, mainly in vacuoles. It folds into a β-sandwich structure creating precise sugar-binding pockets. Monomers can assemble into dimers or tetramers to enhance multivalent binding. Isoforms show slight sequence variations altering binding specificity and stability, demonstrating how fold and assembly optimize recognition and biological activity.

## 20. Hydrophobins

**Teaser:** How can tiny fungal proteins self-assemble at interfaces to change surface properties?

**Prompt Questions:**

* What is their biological role and localization?
* How does the fold allow amphipathic surface formation?
* Do they oligomerize or form amyloid-like layers?
* Are there isoforms with different assembly behavior?

**Expanded Example Answer:**  
Hydrophobins are small fungal proteins that coat aerial structures and spores. They fold into a compact β-barrel stabilized by disulfide bonds, exposing hydrophobic patches on the surface. These patches mediate self-assembly at hydrophobic-hydrophilic interfaces into amyloid-like layers. Different classes of hydrophobins vary in sequence and assembly propensity, affecting surface wettability and protective functions. The interplay of fold, surface chemistry, and oligomerization underlies their unique interfacial properties.

## 21. Interferon

**Teaser:** How does a secreted signaling protein activate antiviral defenses?

**Prompt Questions:**

* What is its cellular source and secretion pathway?
* How does folding stabilize the cytokine and its receptor-binding interface?
* Does it oligomerize or form complexes with receptors?
* Are there isoforms with distinct receptor specificity or potency?

**Expanded Example Answer:**  
Interferons are secreted cytokines that induce antiviral states in target cells. They fold into α-helical bundles stabilized by hydrophobic core interactions and hydrogen bonds, presenting receptor-binding surfaces. Interferon binds its heterodimeric receptor, forming a signaling complex that activates JAK/STAT pathways. Isoforms like IFN-α, IFN-β, and IFN-γ differ in sequence, receptor affinity, and cellular response, illustrating how fold, surface interactions, and complex formation determine signaling specificity and potency.

## 22. Hedgehog

**Teaser:** How does a secreted morphogen convey positional information during development?

**Prompt Questions:**

* Where is it produced and secreted?
* How does the protein fold to maintain activity and resist degradation?
* How does it interact with receptors or co-factors?
* Are there isoforms or post-translational modifications affecting signaling range?

**Expanded Example Answer:**  
Hedgehog proteins are secreted morphogens that regulate tissue patterning. They fold into a structured signaling domain stabilized by disulfide bonds, which maintains a precise conformation for receptor binding. Hedgehog interacts with Patched and other co-receptors to initiate downstream signaling. Post-translational modifications, such as lipidation, regulate secretion, diffusion, and signaling range. Isoforms in different organisms or developmental contexts fine-tune activity, demonstrating how fold, cofactor interaction, and modification orchestrate spatial signaling during development.