

# Protein Folding and Misfolding

## Anfinsen's Principle

- Sequence determines structure
- Spontaneous folding possible
- Minimum free energy state
- Reversible denaturation

## Chaperone Proteins

- Assist protein folding
- Prevent aggregation
- HSP70, HSP90 families
- ATP-dependent mechanisms

## Folding Funnels

- Energy landscape model
- Multiple pathways to native state
- Local minima can trap
- Kinetic vs thermodynamic control

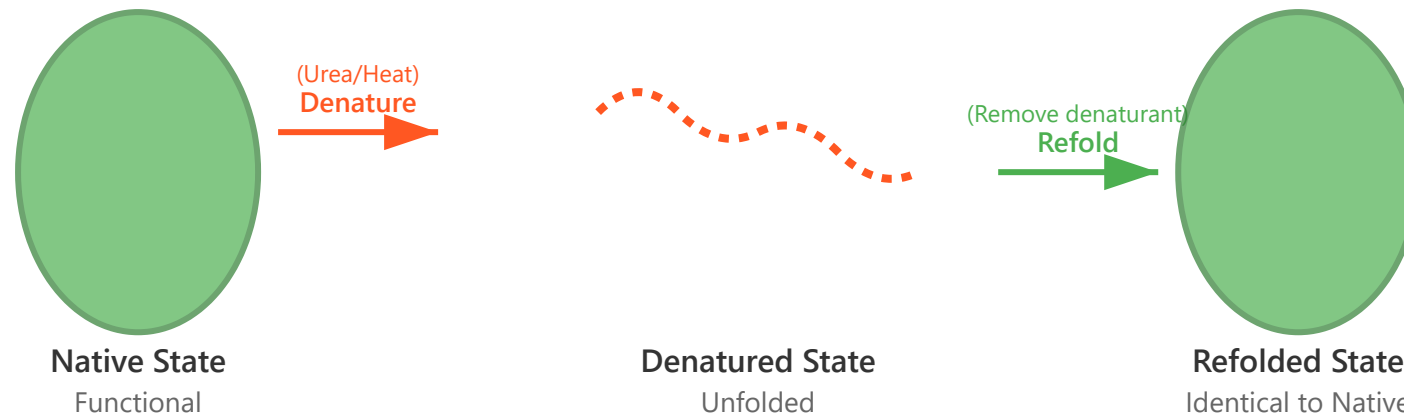
## Misfolding Diseases

- Alzheimer's: A $\beta$  plaques
- Parkinson's:  $\alpha$ -synuclein
- Prion diseases: PrP
- Therapeutic targets

## 1. Anfinsen's Principle: The Foundation of Protein Folding

---

Christian Anfinsen's groundbreaking work in the 1960s demonstrated that the three-dimensional structure of a protein is determined solely by its amino acid sequence. His experiments with ribonuclease A proved that proteins could spontaneously refold after denaturation, earning him the Nobel Prize in Chemistry in 1972.



### Key Experimental Evidence

Anfinsen denatured ribonuclease A using urea and  $\beta$ -mercaptoethanol to break disulfide bonds. Upon removal of these denaturants, the enzyme spontaneously refolded to its native, catalytically active structure with correct disulfide bond formation. This demonstrated that all information needed for proper folding is encoded in the primary sequence.

**Thermodynamic Principle:** The native structure represents the global minimum of free energy under physiological conditions. The protein spontaneously adopts this conformation because it is the most thermodynamically stable state, with optimal balance between enthalpic (hydrogen bonds, van der Waals forces) and entropic contributions.

### Implications

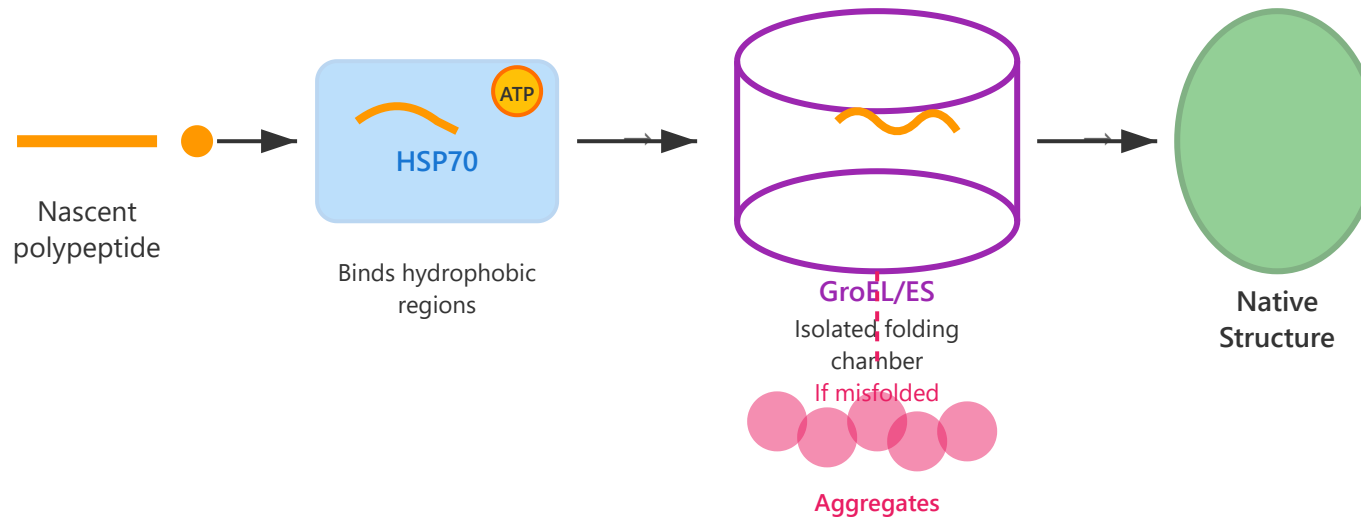
- **Predictability:** In principle, protein structure can be predicted from sequence alone
- **Evolution:** Natural selection acts on sequences to optimize folding and function

- **Mutations:** Changes in sequence can disrupt folding, leading to disease
- **Biotechnology:** Recombinant proteins can fold correctly in heterologous systems

## 2. Chaperone Proteins: Cellular Folding Assistants

---

While Anfinsen's principle shows that spontaneous folding is possible in vitro, the crowded cellular environment presents significant challenges. Molecular chaperones are specialized proteins that facilitate proper folding, prevent aggregation, and help maintain protein homeostasis (proteostasis) in cells.



## Major Chaperone Families

- 1. HSP70 Family:** Binds to hydrophobic patches on nascent or misfolded proteins, preventing aggregation. Uses ATP hydrolysis to regulate binding affinity. Works co-translationally (during synthesis) and post-translationally.
- 2. HSP90 Family:** Involved in later stages of folding, particularly for signaling proteins and kinases. Essential for maturation of many regulatory proteins.
- 3. Chaperonins (GroEL/GroES in bacteria, CCT/TRiC in eukaryotes):** Large barrel-shaped complexes that encapsulate unfolded proteins in an isolated chamber, allowing folding without aggregation. Can accommodate proteins up to ~60 kDa.

**4. Small HSPs:** Hold misfolded proteins in folding-competent states, preventing irreversible aggregation until other chaperones can assist refolding.

**ATP-Dependent Mechanism:** Most chaperones use ATP hydrolysis to power conformational changes that regulate substrate binding and release. This energy input allows chaperones to actively rescue proteins from kinetically trapped misfolded states, going beyond what thermodynamics alone would permit.

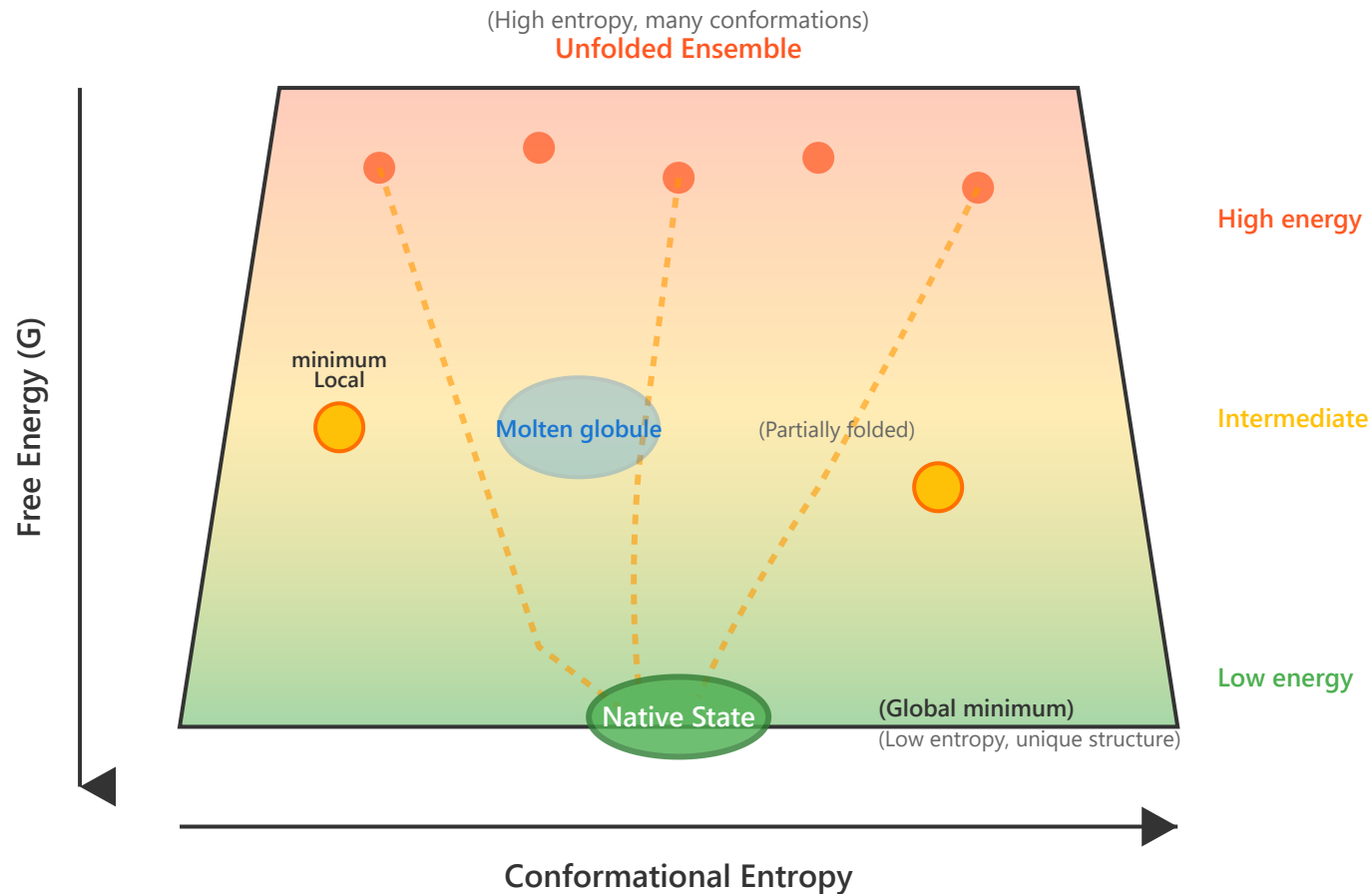
### Cellular Stress Response

Heat shock proteins (HSPs) are dramatically upregulated during cellular stress conditions (heat, oxidative stress, heavy metals). This **heat shock response** helps maintain proteostasis by increasing the cellular capacity to prevent aggregation and refold damaged proteins. Transcription factor HSF1 (Heat Shock Factor 1) mediates this protective response.

## 3. Folding Funnels: The Energy Landscape Perspective

---

The folding funnel model, developed in the 1990s, revolutionized our understanding of protein folding by viewing it as a stochastic search through an energy landscape rather than a predetermined pathway. This framework explains how proteins can fold rapidly despite the astronomical number of possible conformations (Levinthal's paradox).



### The Levinthal Paradox

If a protein randomly sampled all possible conformations to find the lowest energy state, it would take longer than the age of the universe. However, proteins fold in milliseconds to seconds. The solution: **proteins don't search randomly**. The folding funnel shows that local interactions progressively guide the protein toward the native state through multiple parallel pathways, dramatically reducing the search space.

**Key Concept - Funnel Shape:** The funnel narrows as conformational entropy decreases (fewer possible conformations) and free energy decreases (more stable structures). The rough surface represents local energy minima where proteins can temporarily get trapped. The overall downhill slope toward the native state explains why folding can be rapid despite complexity.

## Folding Intermediates

**Molten Globule:** A compact intermediate state with native-like secondary structure but fluctuating tertiary structure. Contains a hydrophobic core but lacks the tight packing of the native state.

**Local Minima:** Kinetic traps where proteins can get stuck in non-native conformations. Chaperones help proteins escape these traps by providing energy input or preventing premature collapse.

**Transition State:** The highest energy barrier along the folding pathway, analogous to the transition state in chemical reactions. Protein engineering studies of the transition state reveal which contacts form early versus late in folding.

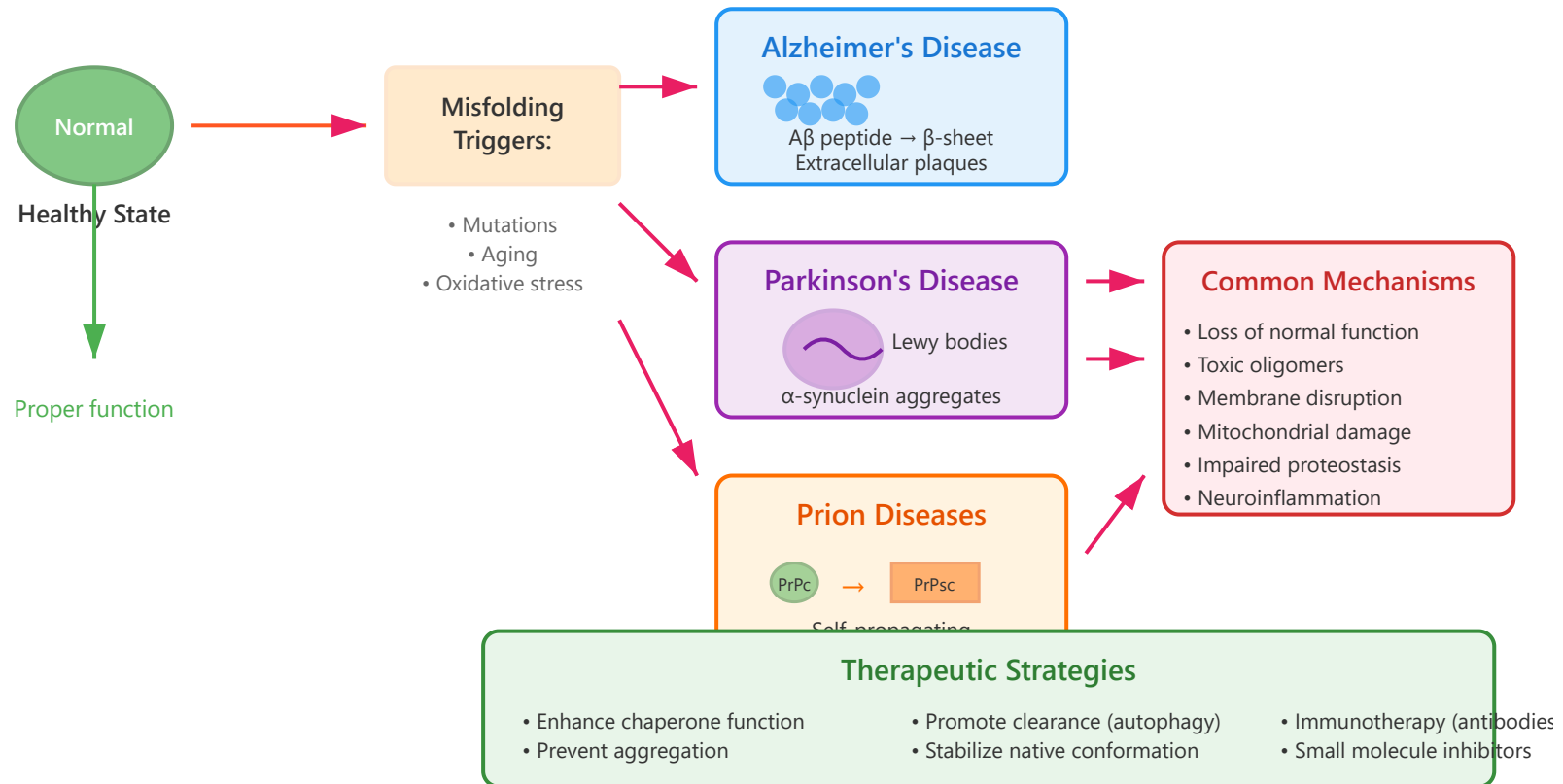
## Kinetic vs. Thermodynamic Control

**Thermodynamic control:** Under ideal conditions, proteins reach the global energy minimum (native state).

**Kinetic control:** In crowded cellular environments or during stress, proteins may get trapped in local minima, leading to kinetically stable but non-native states. This is where chaperones become essential, using ATP energy to overcome kinetic barriers and allow proteins to reach the thermodynamic minimum.

## 4. Misfolding Diseases: When Proteins Go Wrong

Protein misfolding diseases, or proteinopathies, occur when proteins fail to achieve or maintain their native structure, leading to loss of function, toxic gain of function, or formation of pathological aggregates. These diseases particularly affect long-lived cells like neurons, where protein quality control becomes compromised with age.





**Key Protein:** Amyloid- $\beta$  (A $\beta$ ) peptide, derived from cleavage of amyloid precursor protein (APP)

**Mechanism:** A $\beta$  monomers undergo conformational change from  $\alpha$ -helix/random coil to  $\beta$ -sheet structure, forming toxic oligomers and eventually insoluble extracellular plaques. Tau protein also misfolds, forming intracellular neurofibrillary tangles.

**Pathology:** Synaptic dysfunction, neuronal loss, cognitive decline, and dementia. Affects over 55 million people worldwide.

**Current Treatments:** Anti-amyloid antibodies (aducanumab, lecanemab) show modest benefits by clearing plaques, but significant side effects remain. Prevention strategies focus on modifiable risk factors.

## Parkinson's Disease

**Key Protein:**  $\alpha$ -synuclein

**Mechanism:**  $\alpha$ -synuclein misfolds and aggregates into Lewy bodies within dopaminergic neurons of the substantia nigra. The protein normally exists as an unstructured monomer but adopts  $\beta$ -sheet-rich structures in disease.

**Pathology:** Progressive loss of dopaminergic neurons leads to motor symptoms (tremor, rigidity, bradykinesia) and non-motor symptoms (cognitive decline, depression). Second most common neurodegenerative disease.

**Spreading:** Evidence suggests  $\alpha$ -synuclein pathology can spread from cell to cell in a prion-like manner, explaining disease progression patterns.

## Prion Diseases

**Key Protein:** Prion protein (PrP)

**Mechanism:** The normal cellular form (PrP<sup>c</sup>) can be converted into a misfolded,  $\beta$ -sheet-rich scrapie form (PrP<sup>sc</sup>). PrP<sup>sc</sup> acts as a template, catalyzing the conversion of more PrP<sup>c</sup> into PrP<sup>sc</sup> in an autocatalytic, self-propagating manner.

**Unique Features:** Transmissible between individuals and even across species barriers (though with less efficiency). No nucleic acid required for transmission - protein alone carries infectivity.

**Diseases:** Creutzfeldt-Jakob disease (CJD), variant CJD (mad cow disease), kuru, fatal familial insomnia, Gerstmann-Sträussler-Scheinker syndrome. All are invariably fatal with no effective treatments.

**Toxic Oligomer Hypothesis:** Recent research suggests that small, soluble oligomers of misfolded proteins (rather than large insoluble aggregates) may be the primary toxic species. These oligomers can disrupt membranes, impair synaptic function, and seed further aggregation. This has shifted therapeutic focus toward preventing oligomer formation and enhancing clearance.

### Common Themes in Protein Misfolding Diseases

- **Age-related:** Most manifest in older adults as protein quality control systems decline
- **Gain of toxic function:** Aggregates interfere with cellular processes
- **Loss of native function:** Sequestration of functional protein into aggregates
- **Seeding and spreading:** Misfolded proteins can template misfolding of normal proteins
- **Selective vulnerability:** Specific cell types affected despite widespread protein expression
- **Protein degradation failure:** Overwhelmed ubiquitin-proteasome and autophagy systems
- **No curative treatments:** Current therapies only manage symptoms or modestly slow progression

## Future Therapeutic Directions

- 1. Chaperone Enhancement:** Pharmacological upregulation of heat shock proteins to improve protein quality control
- 2. Aggregation Inhibitors:** Small molecules that stabilize native conformations or prevent oligomerization
- 3. Immunotherapy:** Antibodies targeting toxic species for clearance by immune system
- 4. Autophagy Enhancement:** Promoting cellular clearance mechanisms to remove aggregates
- 5. Gene Therapy:** Reducing expression of disease proteins or enhancing protective factors
- 6. Early Intervention:** Biomarker development for pre-symptomatic diagnosis and treatment