Protein Engineering

Protein Stability in Cells

Individual proteins turn over at different rates. Their half-lives can vary from a few minutes to several weeks.

Rapid protein turnover ensures rapid response to constantly changing conditions.

The rate of hydrolysis of a protein can be inversely related to the stability of its tertiary structure: misfolded and unfolded proteins are quickly degraded.

Half-live - Time for half of the protein pool to become denatured or degraded

Turnover – Proteins are continually synthesized and degraded in all cells

Life-span – Time from protein synthesis to its degradation

Proteins have variable life-spans

Half-lives	Time
Ornithine decarboxylase	11 min
RNA polymerase I	90 min
Tyrosine aminotransferase	120 min
hexokinase	4 h
PEPcarboxylase	5.0 h
Aldolase	118 h
GAPDH	130 h
cytochrome *c*	150 h
collagen	1 year

The half life of a cystosolic protein is determined by a large extent by the N-terminal residue
(The N-end rule related to to ubiquitination, Varshavsky, 1986)

Protein Quality control in the cells

- Protein damage takes place continuously in every cell of an organism
- Truncated translational polypeptide products, missfolded intermediates, and unassembled subunits of oligomeric protein complexes frequently expose hydrophobic regions susceptible to aggregation.
- Nonnative posttranslational modifications and disulfide bridges also affect protein folding and stability.
- Native proteins are continuously endangered by high-energy radiation and a number of reactive chemicals and metabolic by-products that cause oxidation, carbonylation, nitrosylation, and other modifications.
- Cells have to cope with protein damage in order to survive.
- Degradation and synthesis of proteins is tightly regulated in cells.

What we can change?

- Changes in physico-chemical properties
- e.g. improvement in thermostability
- e.g. improvement in stability in organic solvents
- Changes in conformation (e.g. α-helix)
    - » Changes in catalytic properties
    - » Increase in Vmax
    - » Decrease in Km
    - » Changes in specificity, sensitivity
- Changes in pH & temperature optimum
- An increase in **pH or thermal stability** may allow the protein to be used under conditions where it would normally be **denatured**.

Cofactor Requirement and Increase Specificity

- The abolishment of the need for a **cofactor** may be beneficial where under certain industrial conditions a cofactor has to be constantly provided.

- Increase **specificity** of the enzyme decreases undesirable side reactions.

Improving Stability

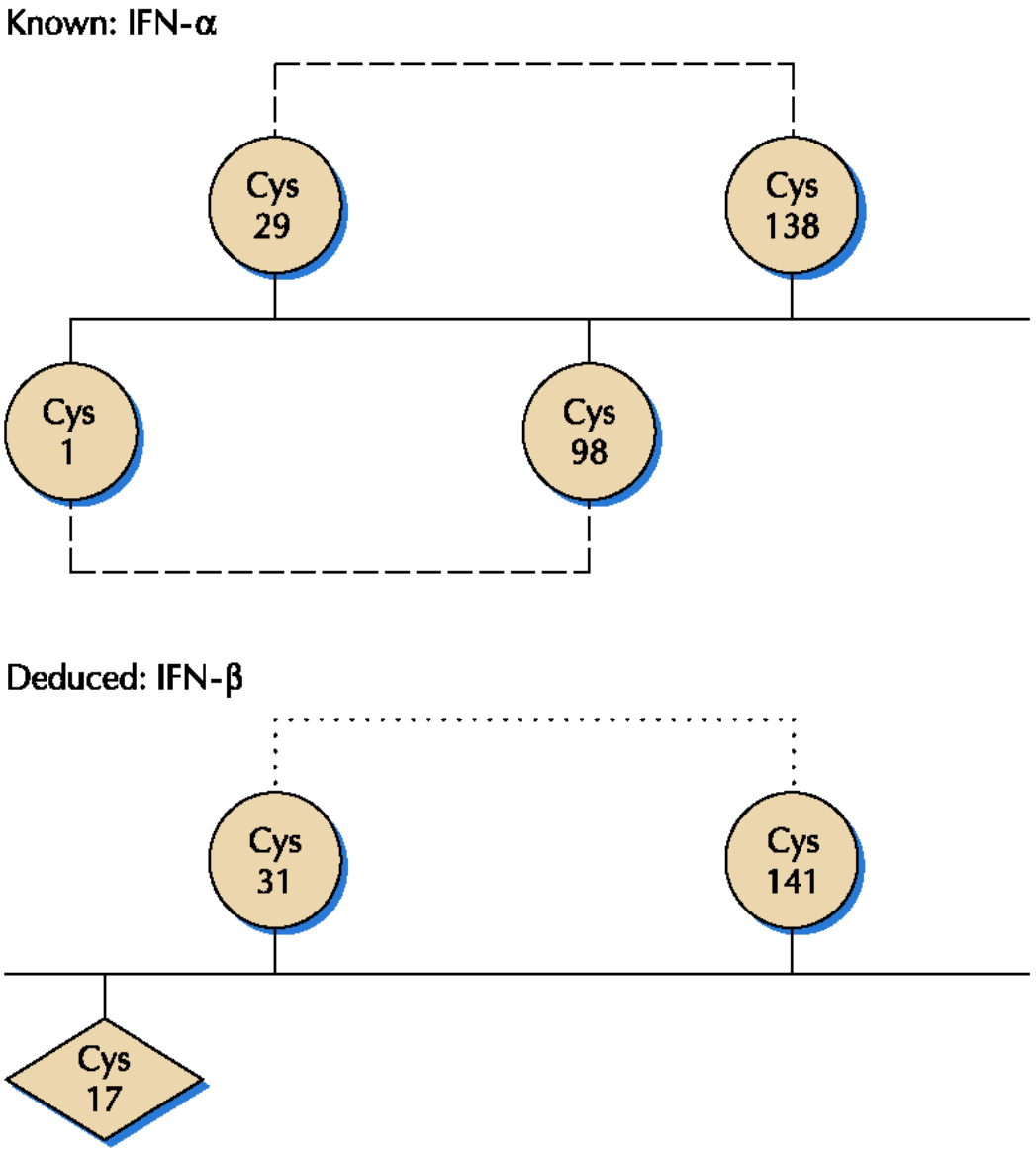
- Protein stability can be increased by creating a molecule, which will **not readily unfold** under unfavorable conditions.
- Protein stability can be improved by:
  - Adding **disulphide bonds**
  - Replacing **labile amino acids**
  - Reducing the number of **free S-H** groups.

Adding Disulphide Bonds

- **Disulphide bonds** can significantly **stabilize** the native structure of proteins.

- This effect is presumed to be due to the **decrease in configuration chain entropy** of the unfolded polypeptide.

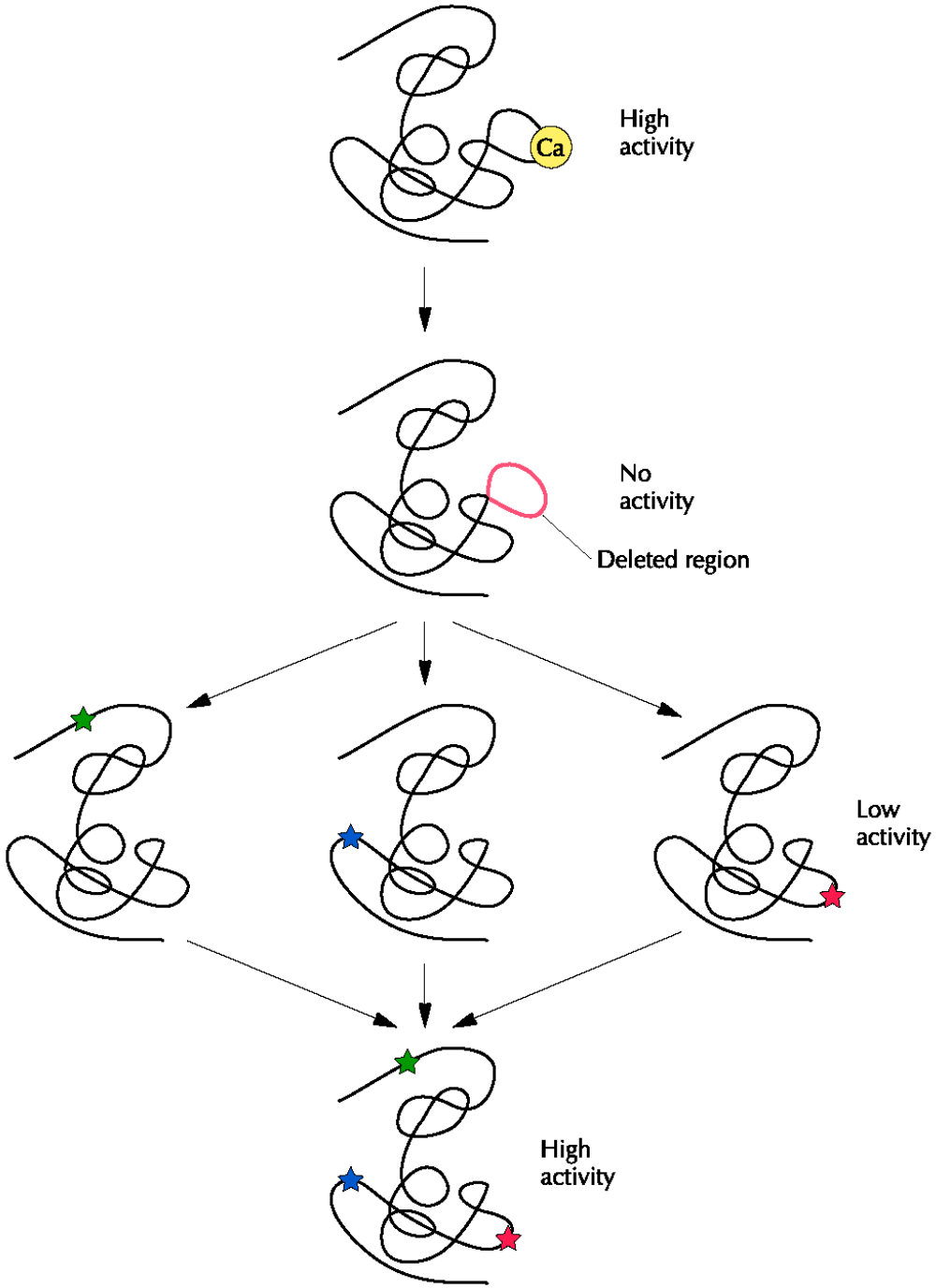
Elimination of Free Sulfhydryl Residues



- Free sulfhydryl groups allow dimer/oligomer formation, especially at high protein concentrations and upon storage
- Change cys to acceptable alternative amino acid (e.g. ser)

Changing Labile Amino Acids

- When proteins are exposed to high temperatures deamidation occurs.
- **Deamidation** $\rightarrow$ release of NH_3
- Asparagine $\rightarrow$ Asparatic acid
- Glutamine $\rightarrow$ Glutamic acid
- The loss of the **amide groups** may result in the lost of activity of the affected enzymes.



Modifying Metal Requirements

- Serine protease for laundry detergent
- Requires calcium
- Calcium induces conformation change necessary for activity
- Modification of amino acid sequence to achieve conformation and stability without calcium

Industrial Use of Enzymes

Although thousands of enzymes are used for industrial purposes, only about 20 enzymes represent 90%

Modifying these enzymes can yield improvements in the heavily utilized processes.

Table 8.1 Some industrial enzymes and their commercial uses

Enzyme	Industrial use(s)
α-Amylase	Beer making, alcohol production
Aminoacylase	Preparation of L-amino acids
Bromelain	Meat tenderizer, juice clarification
Catalase	Antioxidant in prepared foods
Cellulase	Alcohol and glucose production
Ficin	Meat tenderizer, juice clarification
Glucoamylase	Beer making, alcohol production
Glucose isomerase	Manufacture of high-fructose syrups
Glucose oxidase	Antioxidant in prepared foods
Invertase	Sucrose inversion
Lactase	Whey utilization, lactose hydrolysis
Lipase	Cheese making, preparation of flavorings
Papain	Meat tenderizer, juice clarification
Pectinase	Clarifying fruit juices, alcohol production
Protease	Detergent, alcohol production
Rennet	Cheese making

Engineered of Xylanase

- Xylanase:
  - Degrades hemicellulose, reducing the need for bleaching wood pulp fiber
  - Need for temperature stable variant
  - Computer modeling suggested sites for SS bonds
  - Improved variant produced
  - **Introduction of S-S bonds** and increased thermostability up to 60°C.

- The use of xylanase reduces the amount of chemical bleaching agent and the amount of polluting by-products.

Engineering of lysozyme

- **Wild type lysozyme** has **2 cysteine** residues and **no disulphide bonds**.

- Site-directed mutagenesis was used to **introduce new cysteine residues** and new **internal S-S bonds** between amino acids:

Human Pancreatic Ribonuclease

- Ribonuclease from bull semen **(bsRNase)** can act as an **antitumorigenic agent**.
- The protein is taken up by tumor cells where it **degrades rRNA** blocking protein synthesis.
- The dimeric form of the protein is joined by **two S-S bridges**.
- Formation of dimer is further supported by changing:
  - Glu 28→ Leu
  - Arg 31, 33 →Cys
  - Asp 34 → Lys
- When this mutant was expressed in *E. coli* and solubilized it was a good candidate for an anti-cancer agent.

Triose Phosphate Isomerase

- **Triose phosphate isomerase** catalyses the interconversion of dihydroxyacetone and phosphate to glyceraldehyde –3 phosphate during glycolysis.
- The enzyme (*S. cerevisiae*) consists of 2 identical subunits and each subunit has **2 asparagine residues** which contributes to its thermal sensitivity.
- Using oligonucleotide directed mutagenesis:
  - Asn 14 $\rightarrow$ Ile
  - Asn 78 $\rightarrow$ Thr
- Resulted in **enhanced thermostability**.
- When both Asn $\rightarrow$ Asp the resulting protein was unstable even at **room temperature**.