Protein Stability

Biotechnology & Biomedicine

Adrian Velazquez-Campoy

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

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Stability (‘*stabilitas*’)

Capability of maintaining structural/functional properties in space and along time, or recovering them after perturbations

- **Conformational (structure)**
  thermodynamic/kinetic
- **Chemical (chemical integrity)**
- **Colloidal (solubility/aggregation)**
- **Biologic (degradation)**

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Interest for studying protein stability?

- Tackling a complex problem (non-branched polymer, 20 different subunits, auto-assembly)
- Understand regulation of function (expression, trafficking, interaction, stress, degradation)
- Evaluate impact of mutations associated with pathologies
- Stabilize proteins for industrial and biotechnological applications
- Formulate and develop biomedical biologic products
- Develop new therapeutic drugs
- Perform quality control in biologic products manufacture

Working in the lab or developing a protein-based application requires putting the protein into a harsh environment, resulting in loss of stability

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Singh et al. 3 Biotech 2016 6(2):174

+ **Forensic**
Environmental
Scientific
Clinical
…

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Protein stability depends on:

- Sequence (net charge, charge distribution, hydropathy profile, mutations, natural variability…)
- Posttranslational modifications (deletion, phosphorylation, hydroxylation, acetylation, methylation…)
- External conditions (*T*, *P*, pH, μ…) and excipients/solutes
- Chemical modifications (deamidation, oxidation, proteolysis and hydrolysis, β-elimination, racemization…)
- Interacting molecules (ligands, osmolytes…)
- Presence of surfaces and interfaces/interphases

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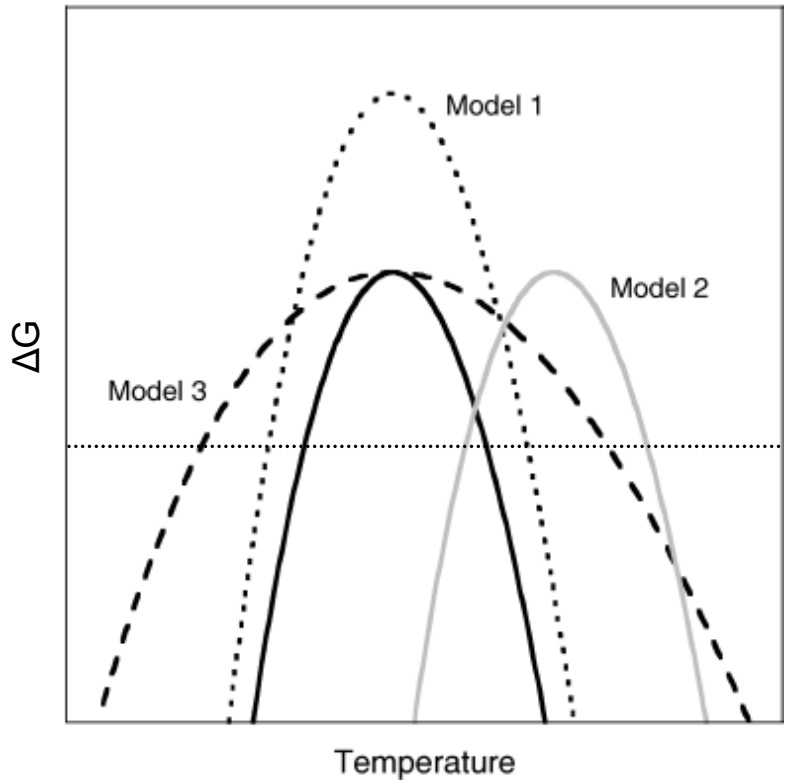
Protein stabilization



$\Delta G = \Delta H - T\Delta S$ Stabilization → increase ΔG

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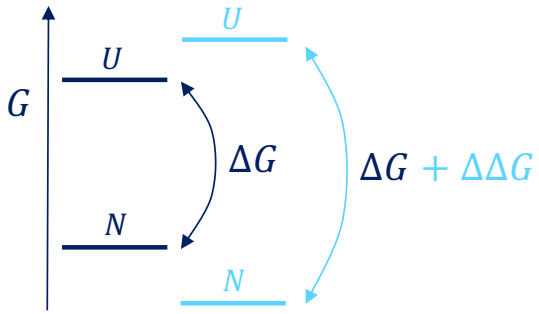
Protein stabilization

- Development of biotechnological and industrial applications based on proteins
- Development of biomedical biologic products based on proteins

Stabilization:

- Rational (directed mutagenesis; *Smith & Mullis, 1993*)
- Random or "irrational" (directed evolution; *Arnold, 2018*)



$\Delta G = \Delta H - T\Delta S$ Stabilization → increase ΔG

In order to increase ΔG, we can:

- Increase energy of state *U* (by decreasing ΔS)
- Decrease energy of state *N* (by increasing ΔH)

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Protein stabilization

Decrease ΔS:
- Introducing Pro
- Substituting Gly
- Introducing S-S
- Changing molecular topology
- Immobilizing on surface

Increase ΔH:
- Introducing and/or redistributing electrostatic interactions
- Optimizing α-helix sequences (charges, hydrophobicity, helix propensity)
- Improved internal packing

Optimizing sequences: phylogenetic analysis and analysis of homologous proteins (e.g., thermophilic organisms, ancestral proteins)

Extrinsic optimization: solutes and additives that specifically and/or unspecifically modulate the protein stability

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EXTRINSIC FACTORS:
pH, ionic strength, type/concentration of buffering agents, type/concentration of additives

STABILIZING AGENTS:
aminoacids, other proteins (e.g., albumin), osmolytes (e.g., TMAO), polymers (e.g., Ficoll, PEG, dextran), polyanions and polycations (e.g., heparin), polyamines, sugars (e.g., sucrose, trehalose), glycopolymers, polar organic solvents (e.g., DMSO), salts (e.g., NaCl, GuHCl at low concentration)

MECHANISMS TO STABILIZE PROTEINS BY FORMULATION:
Preferential interaction, non-specific interaction with surface hydrophobic pockets or charged amino acids, specific ligand binding, molecular crowding…

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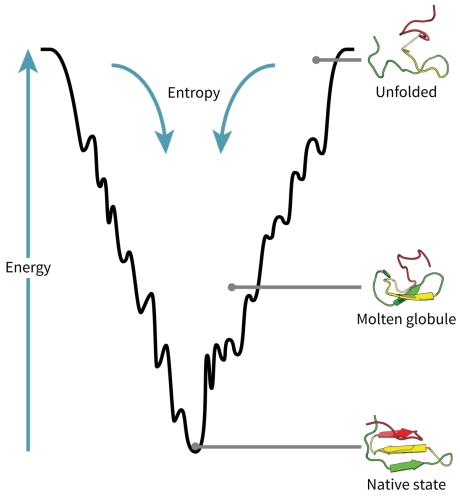

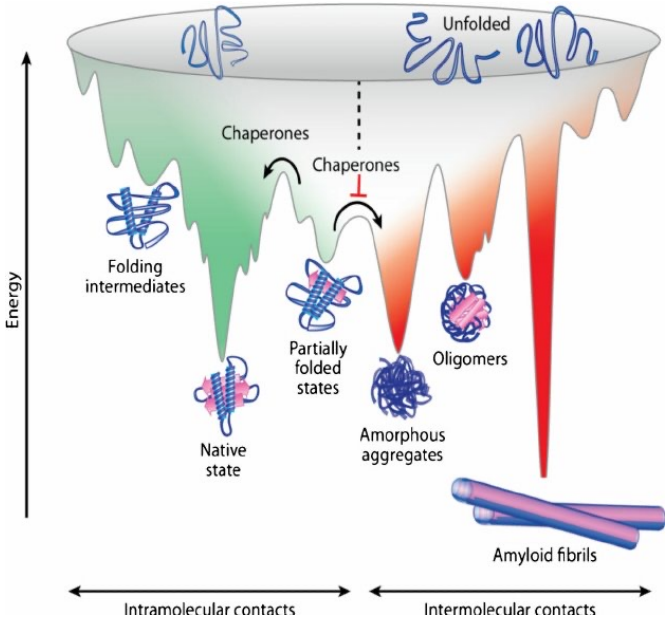

J. Inherit. Metab. Dis. 2014, 37:505-523

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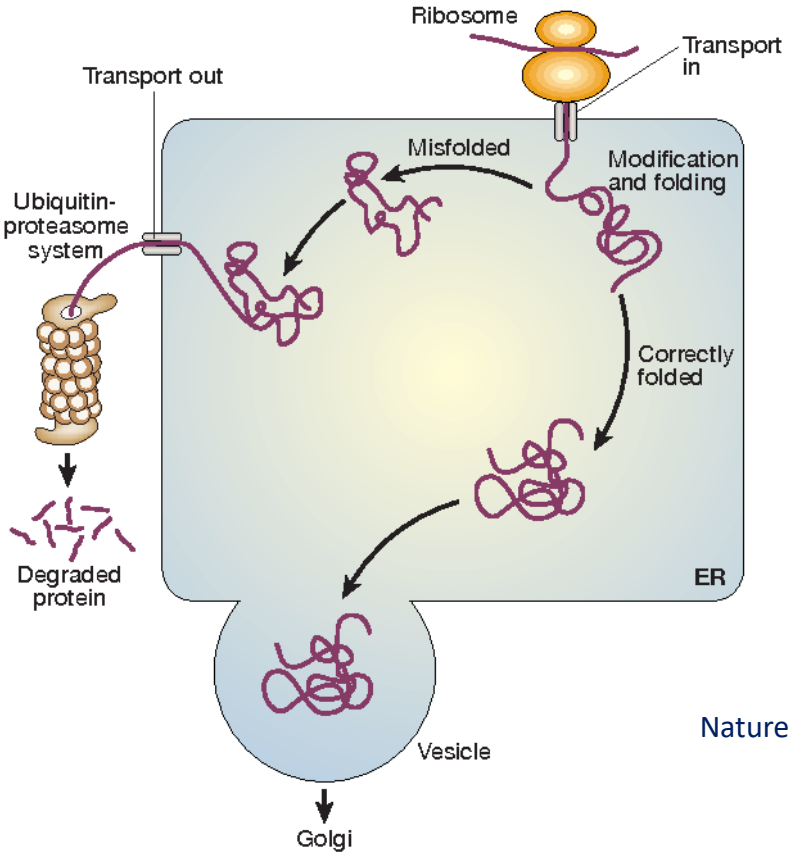
Protein life-cycle



Nature 426, 884–890 (2003)

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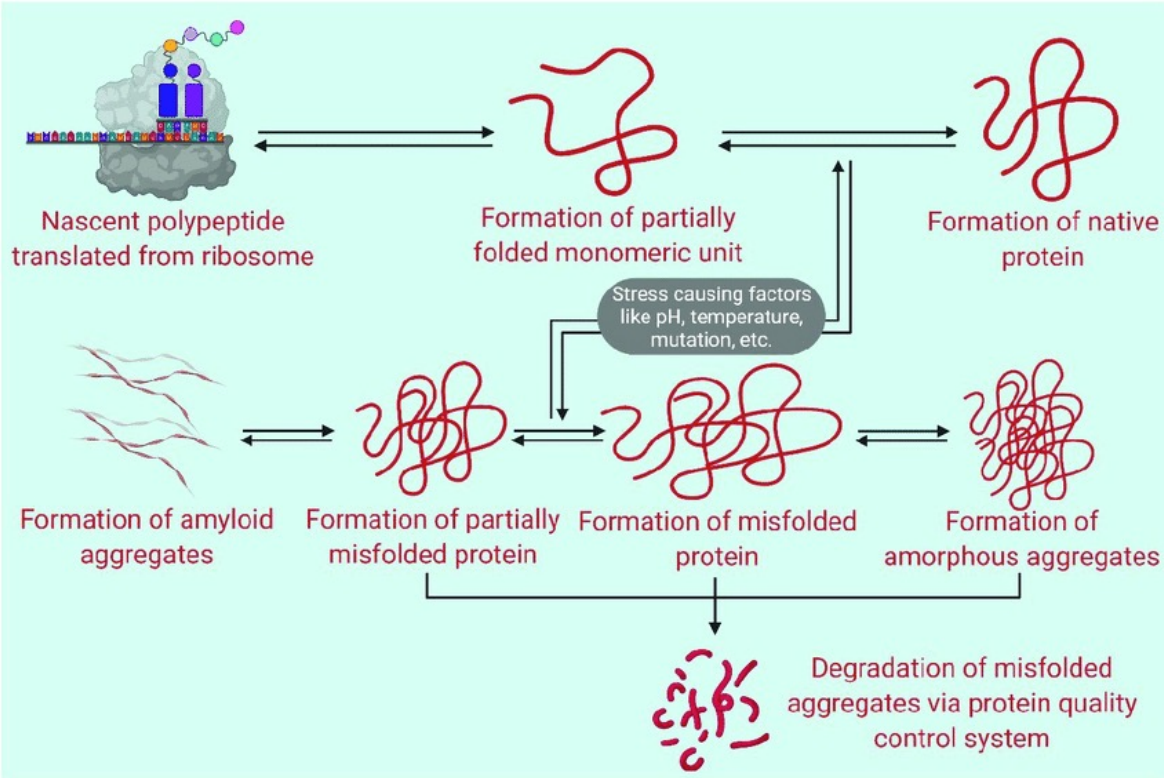
Aggregation & conformational (folding) diseases



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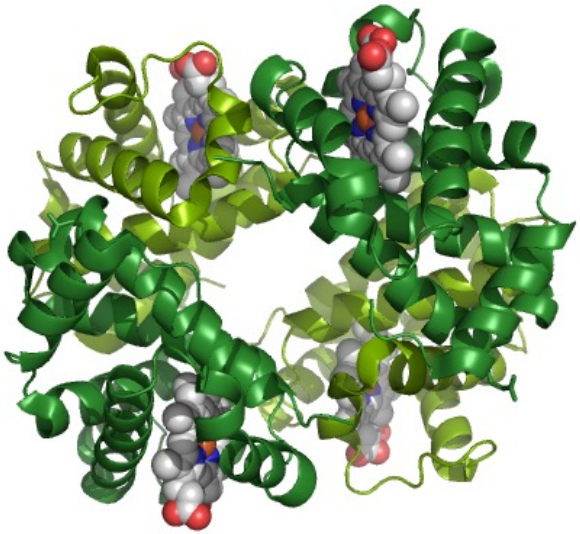
What happens if a protein malfunctions?



Hemoglobin
4 peptide chains ($\alpha_2\beta_2$)
574 aminoacids
O_2 and CO_2 transport
Blood pH maintenance

Glu6(β) → Val

Polymerization
Erythrocytes deformation
Fast elimination of erythrocytes
Sickle cell disease
Protection against malaria (?)

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Conformational diseases or proteinopathies

The energy landscape in protein folding is rough; proteins may be trapped into intermediate partially folded states, resulting in misfolded proteins

Protein conformational disease refers to the pathology involving a protein that adopts abnormal conformational states, altering its function within the cell

Multiple phenomena: loss of function, toxic gain of function, improper degradation and localization, aggregation, fibril formation…

Problem: many associated mutations leading to highly variable phenotypes

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Possible treatments

Palliative treatment for primary or secondary processes

Reduce expression of protein (e.g., siRNAs, antisense oligos)

Reduce misfolding and/or aggregation (pharmacochaperones)

Remove aggregates (e.g., antibodies, vaccines)

Molecular replacement

Gene therapy

Transplant

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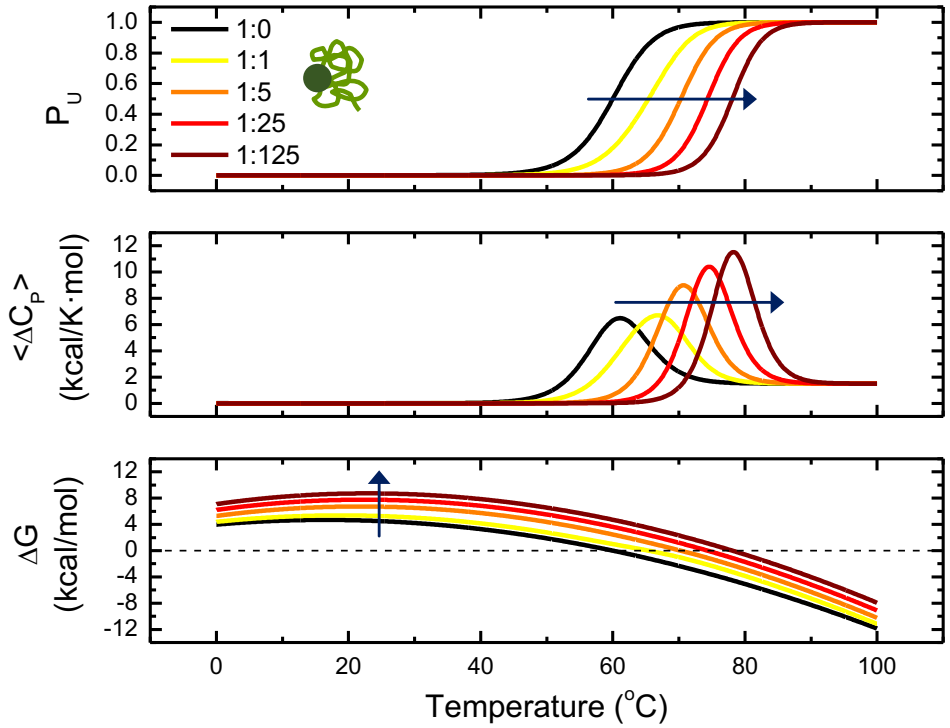
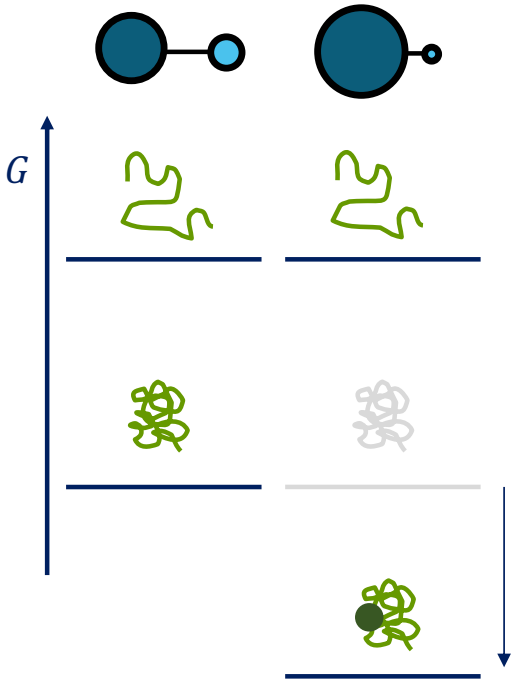
Ligand interaction induces stabilization





$$\Delta G([L]) = \Delta G + RTln(1 + K_N[L])$$

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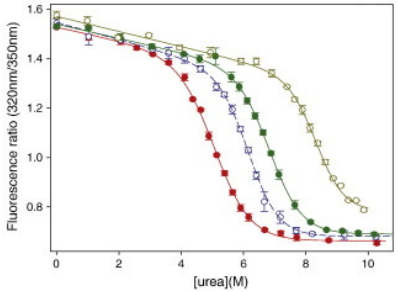
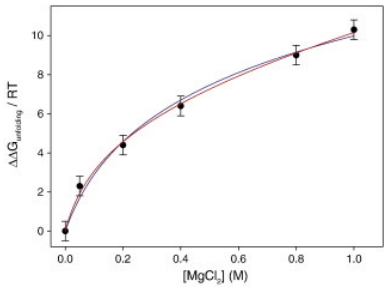
Ligand interaction induces stabilization

ribonuclease T1





Preferential interaction

$$\Delta\Delta G([L]) = nRTln(1 + K[L])$$

n 1.9 ± 0.2

K_{Mg} 10 ± 2 M-1

- **Direct evidence of interaction**
- **(gross) Estimation of interaction affinity**

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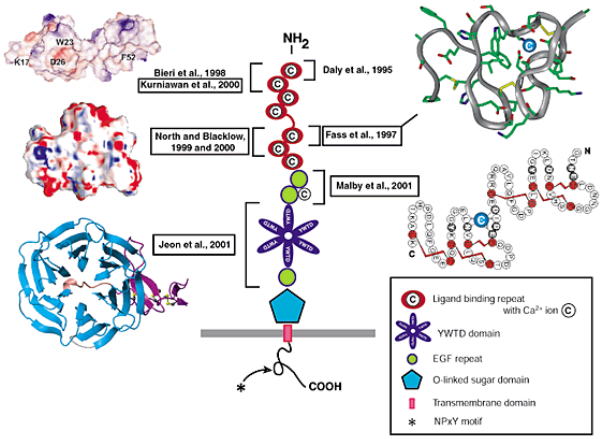
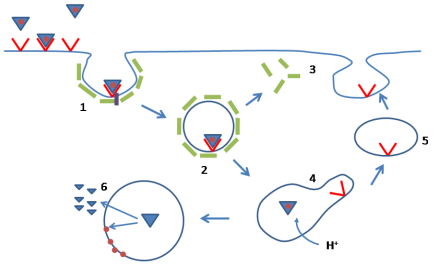
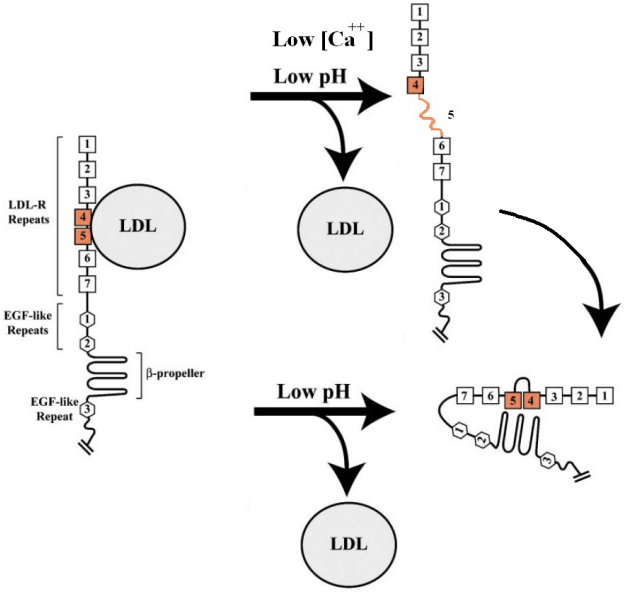
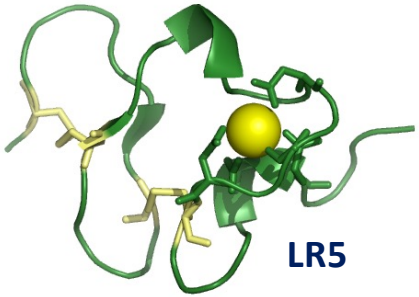
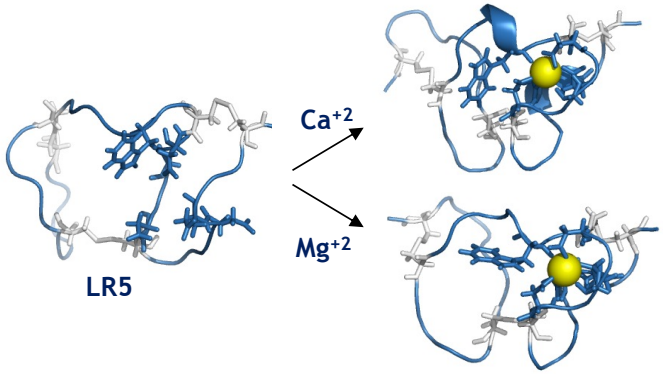
Hyperlipidemia (LDLR)











J. Biol. Chem. 2008, 283:22670-22679
Proteins 2010, 78, 950-961

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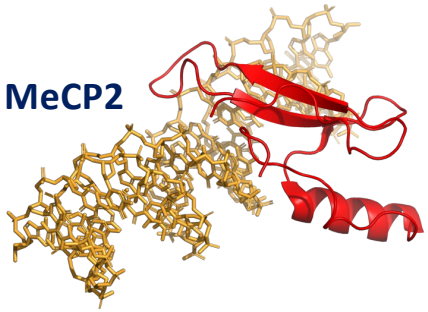
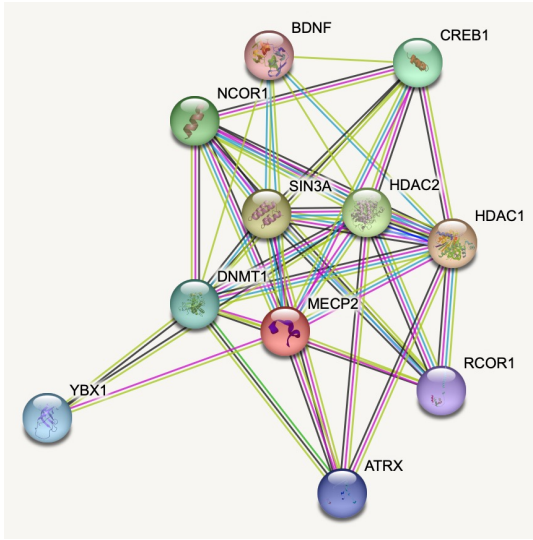
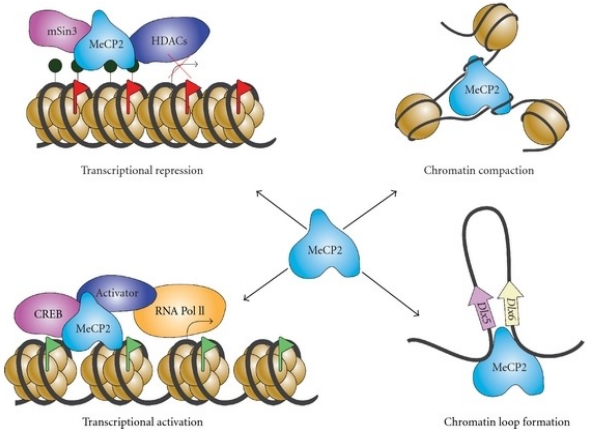
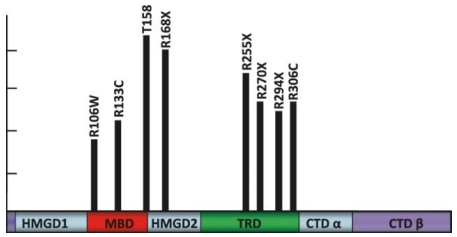
Rett syndrome & MeCP2 duplication syndrome (MeCP2)







Neural Plast. 2012;2012:415825



Biochem. Cell Biol. 89(1):1-11

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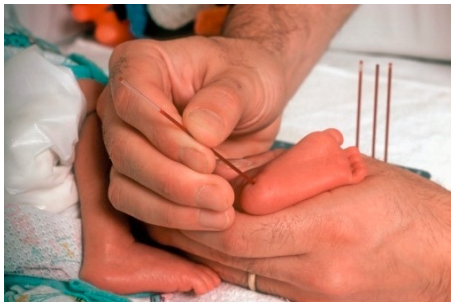
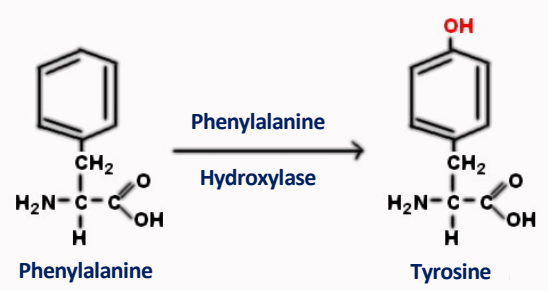
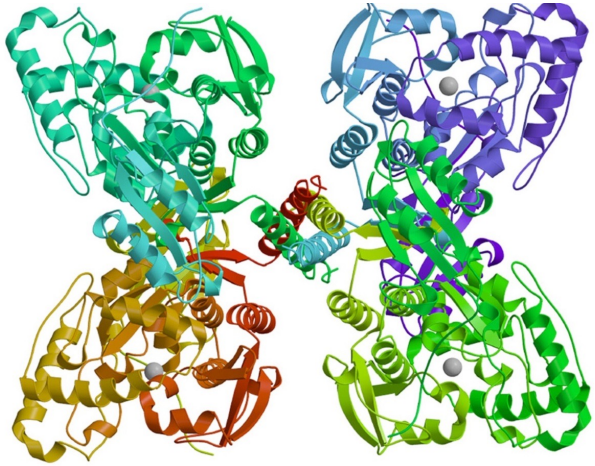
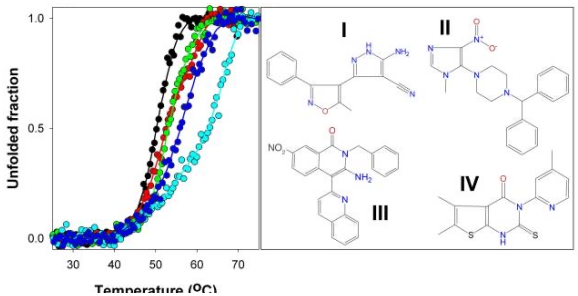
Phenylketonuria or hyperphenylalaninemia (PAH)







PAH



J. Clin. Invest. 2008 118:2858-67

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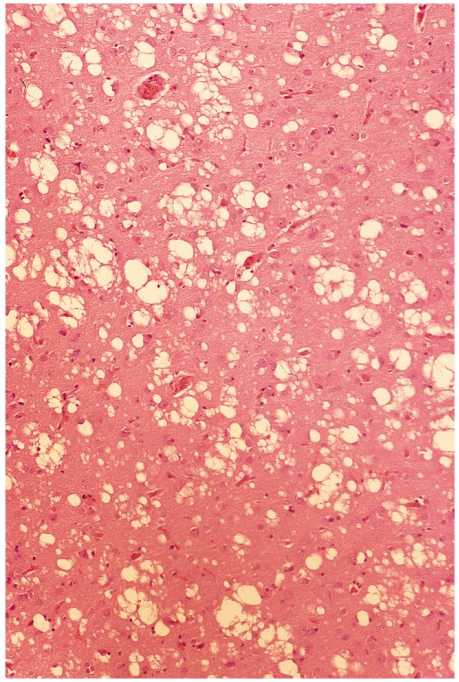
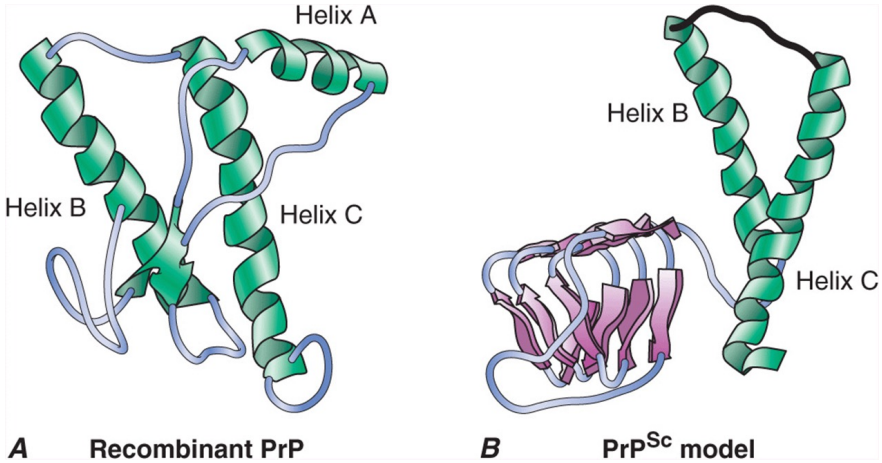
Prion’s diseases (prion proteins)





PrP	PrP^{Sc}
α-helical structure	β-sheet structure
Susceptible to proteases	Resistant to proteases
Monomeric form	Aggregated form
Stable monomers	Unstable monomers leading to amyloid aggregates
Normal resistance levels	Extreme resistance to radiation and strong solvents
Soluble in detergents	Insoluble in detergents

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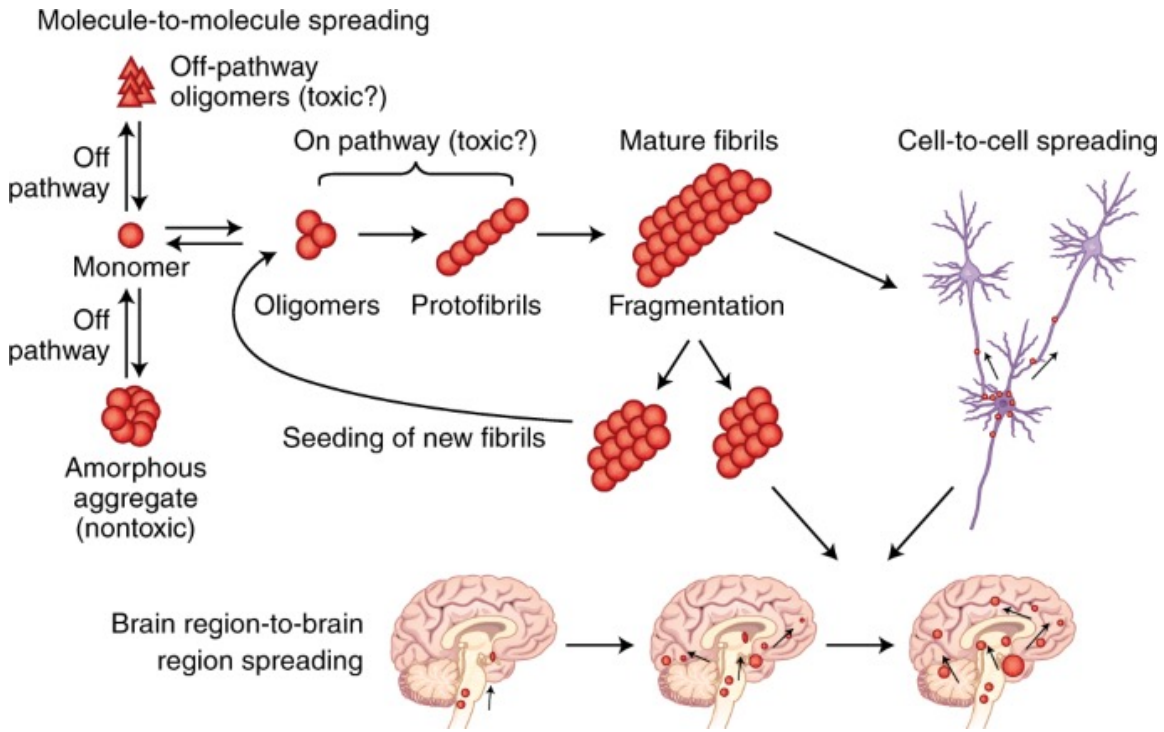
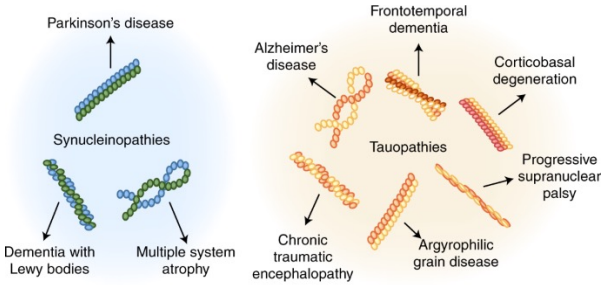
Parkinson, tauopathy, Alzheimer, Huntington…
(α-synuclein, tau, Aβ-peptide, huntingtin…)





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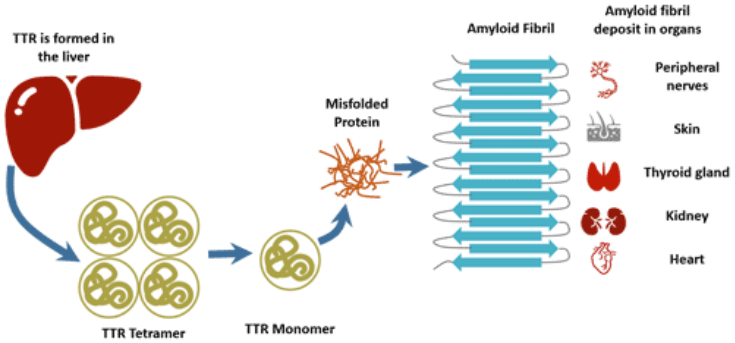
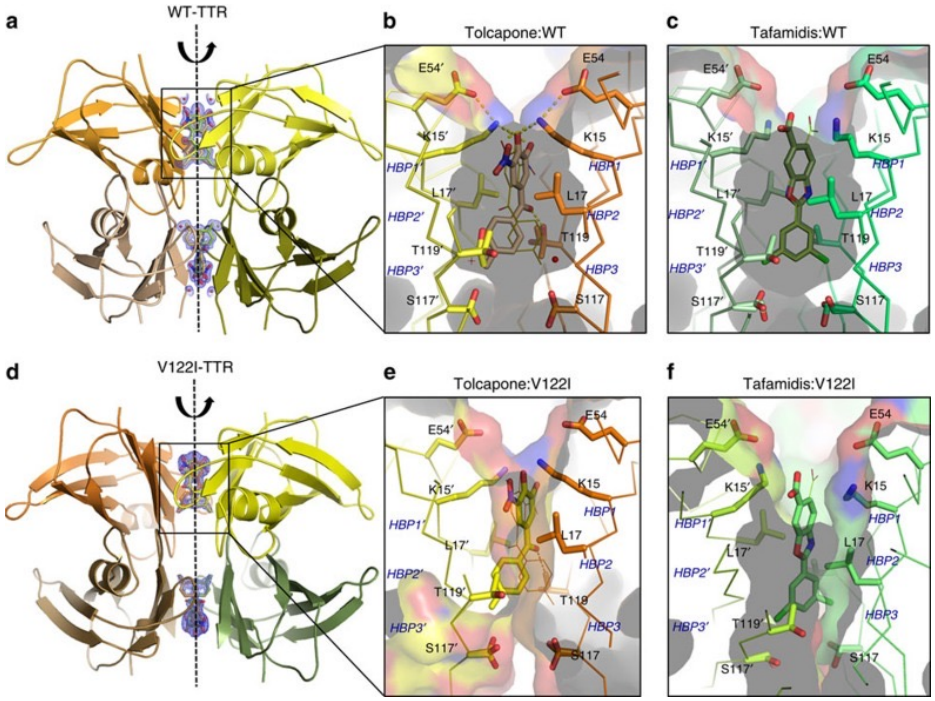
Familial Amyloid Polyneuropathy (TTR)





TTR

Nat. Commun. 2016, 7:10787

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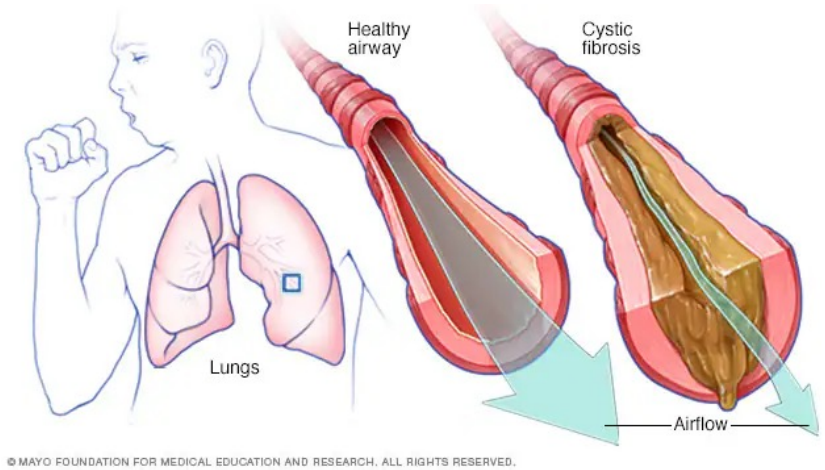
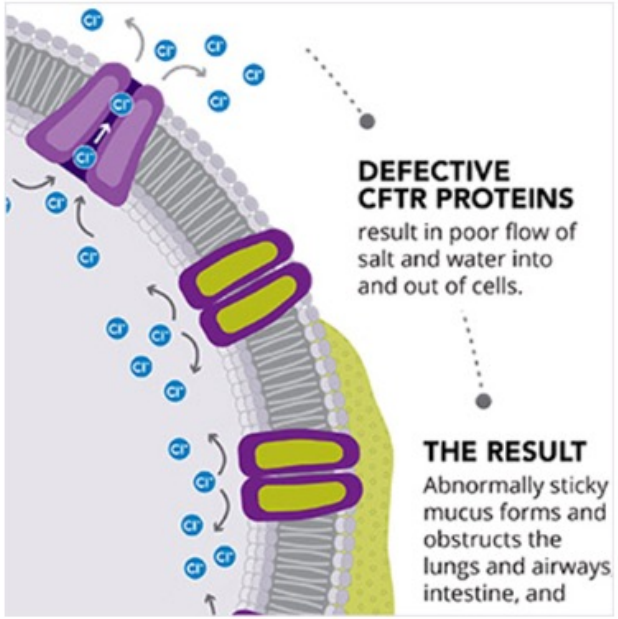
Cystic fibrosis (CFTR)



CFTR



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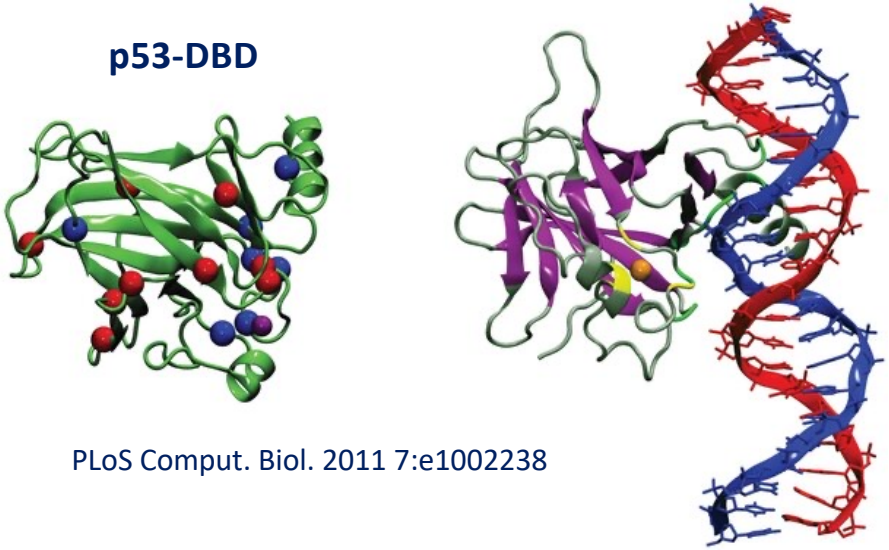
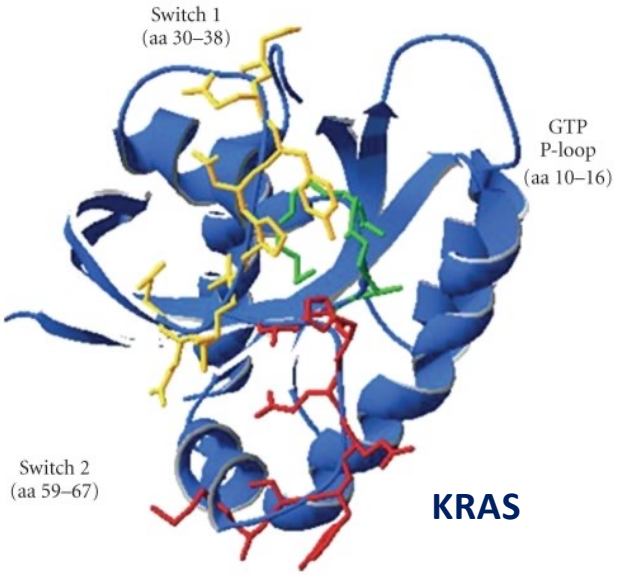
Cancer

p53-DBD



PLoS Comput. Biol. 2011 7:e1002238



KRAS

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