
Drug Discovery Strategies for CNS Protein Folding Diseases

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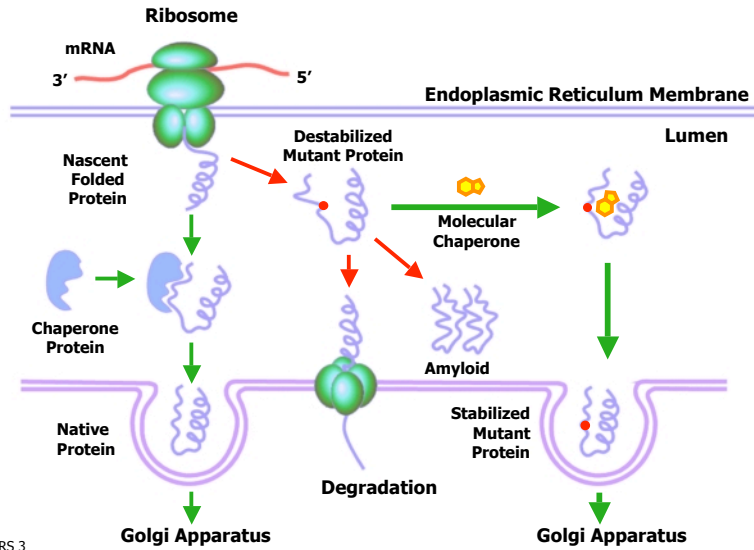
FRS 1

Drug Discovery Strategies for CNS Protein Folding Diseases

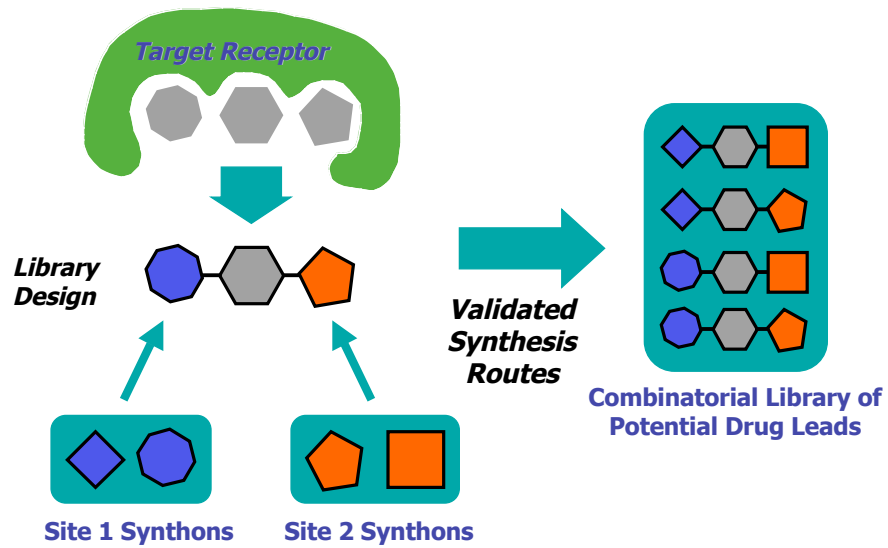
- **Introduction**
 - **Library Design: Strategies & Issues**
 - **High Throughput Assays of Protein Stability**
 - **Structure-Specific Assays of Protein Stability**
 - **Summary**
-

FRS 2

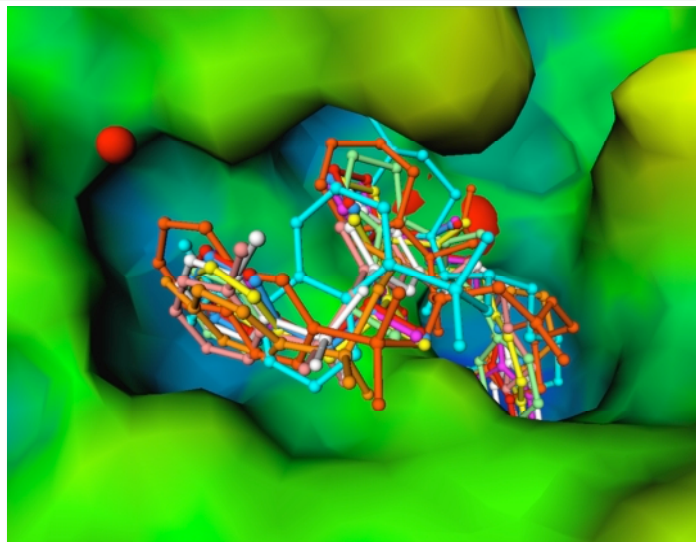
Protein Folding Stabilizers as Synthetic Molecular Chaperones



Structure-Based Library Generation & Synthesis (Mostly Effective for Enzymes)



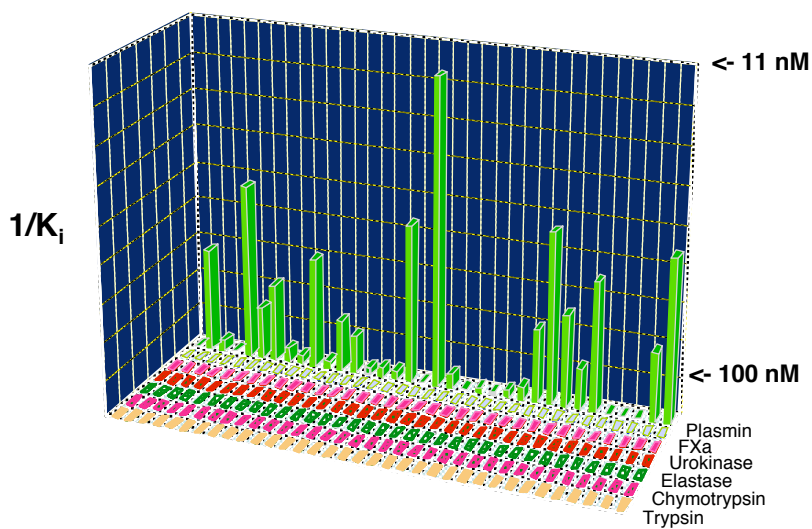
Scaffold Diversity for Protease Inhibitors



FRS 5

Superimposed Crystal Structures of Thrombin Inhibitors

High Throughput Chemistry: SAR Development



FRS 6

Enzyme Inhibitors as Molecular Chaperones

Pro

- Enzyme inhibitors are generally easy to design

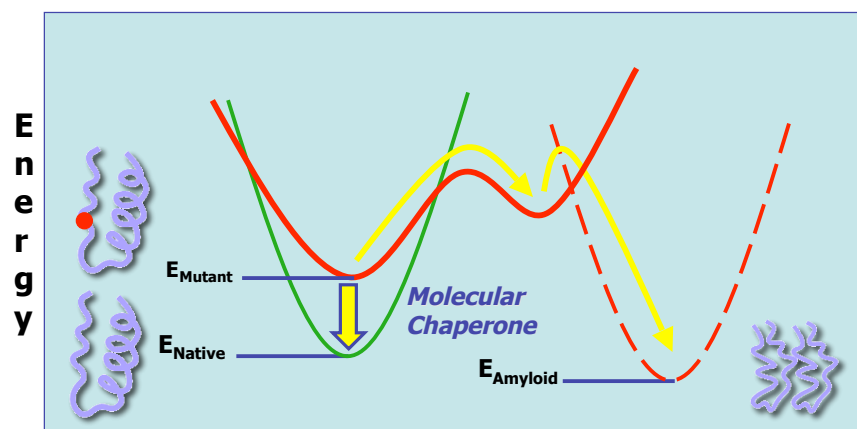
Con

- Many protein misfolding targets are not enzymes
- Enzyme inhibition may frustrate the key objective: Recovery of activity

=> *Need for screening measurements of protein stability*

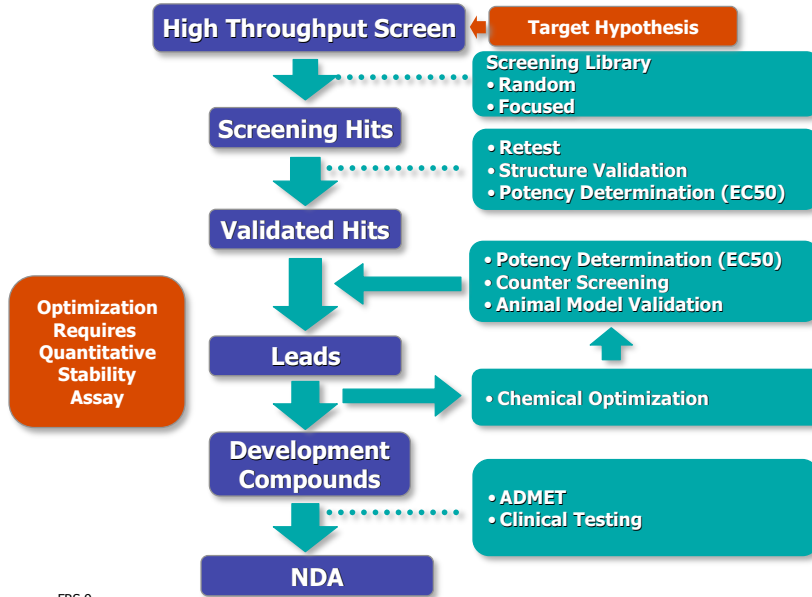
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Protein Energy Landscapes

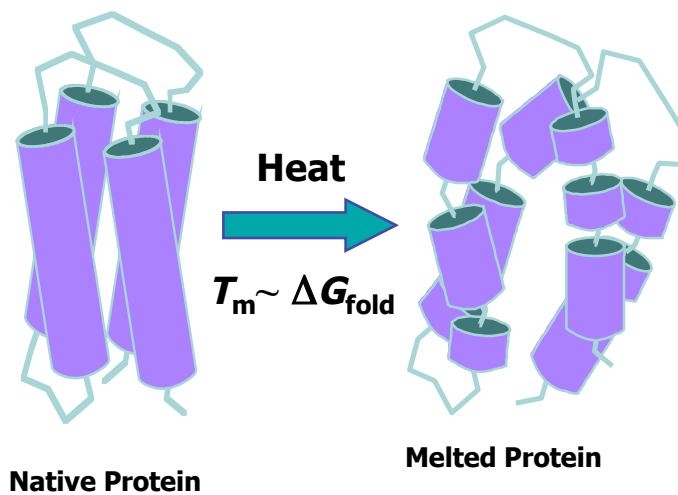


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Drug Discovery & Development

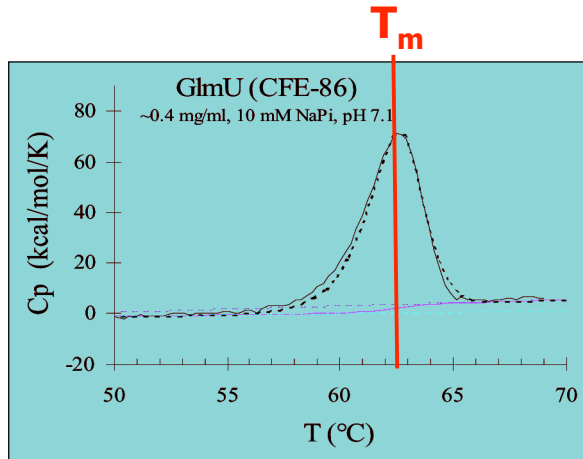


Thermal Phase Transitions: Melting of Proteins



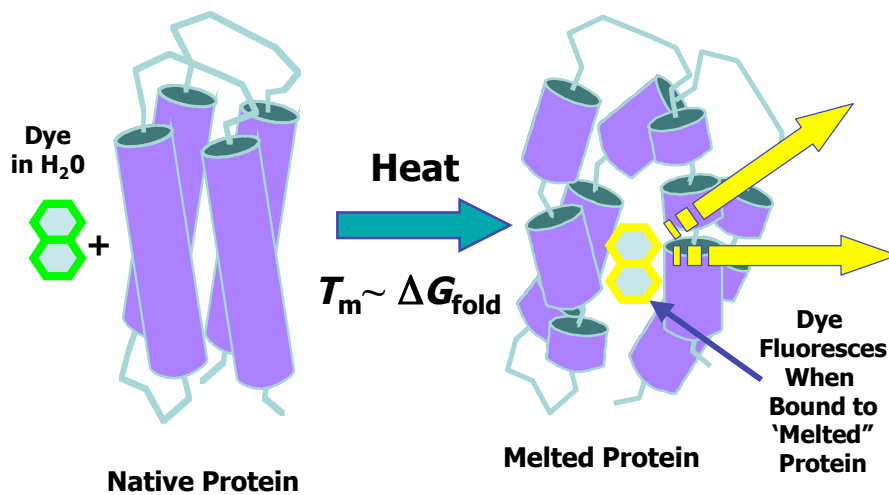
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Differential Scanning Calorimetry (DSC)



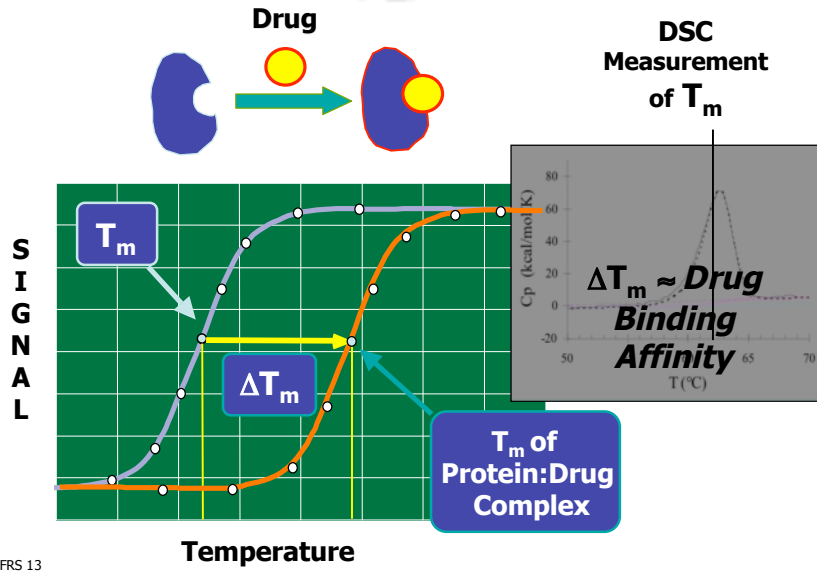
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Fluorescence Detection of Protein Melting

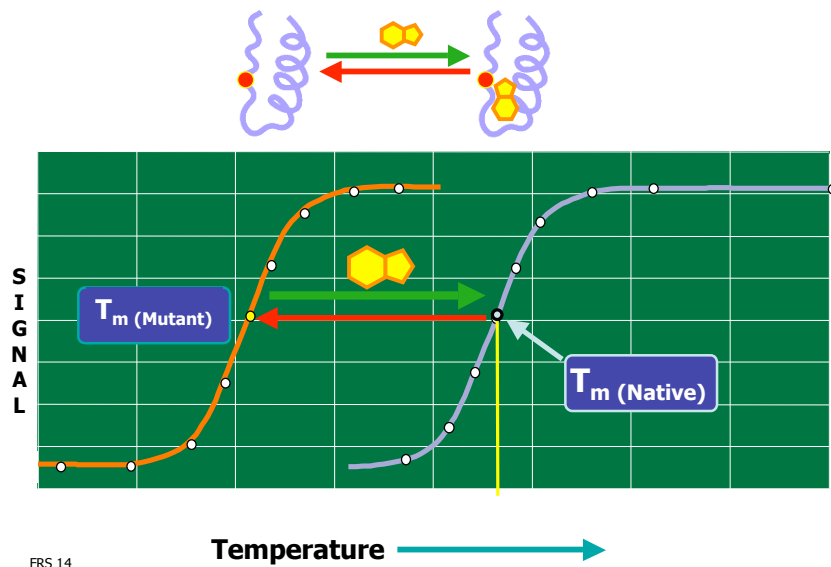


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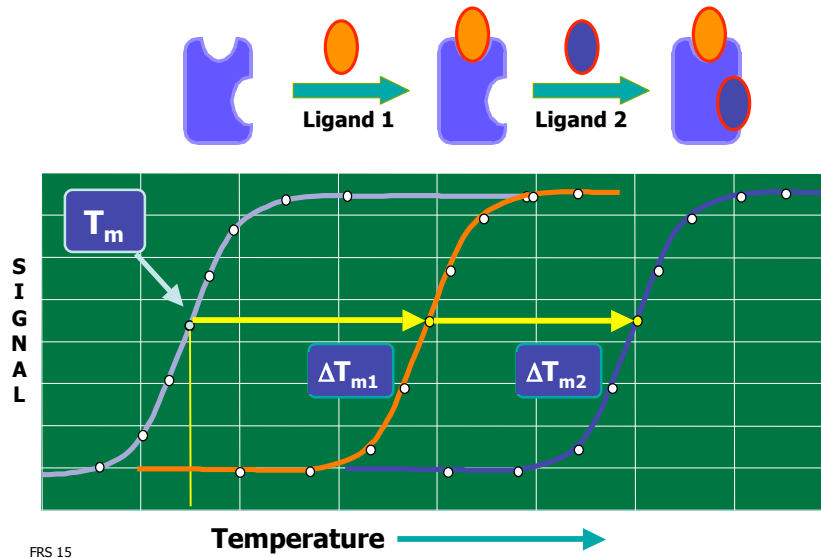
Effect of Drug Binding on Protein Melting Temperature (T_m)



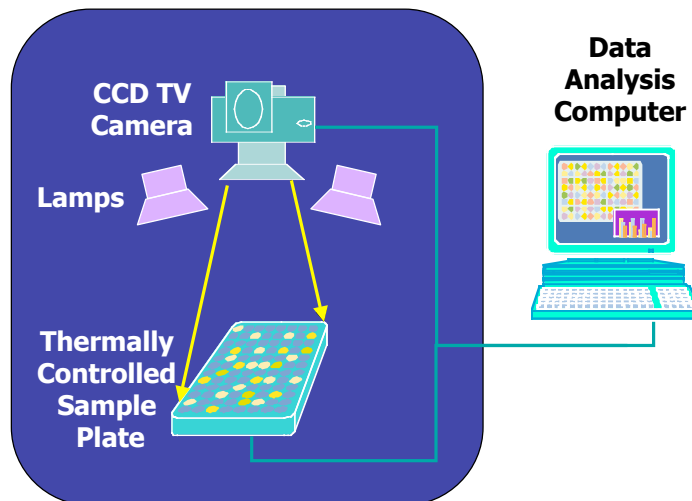
Native and Mutant Protein Stabilities & Role of Molecular Chaperones



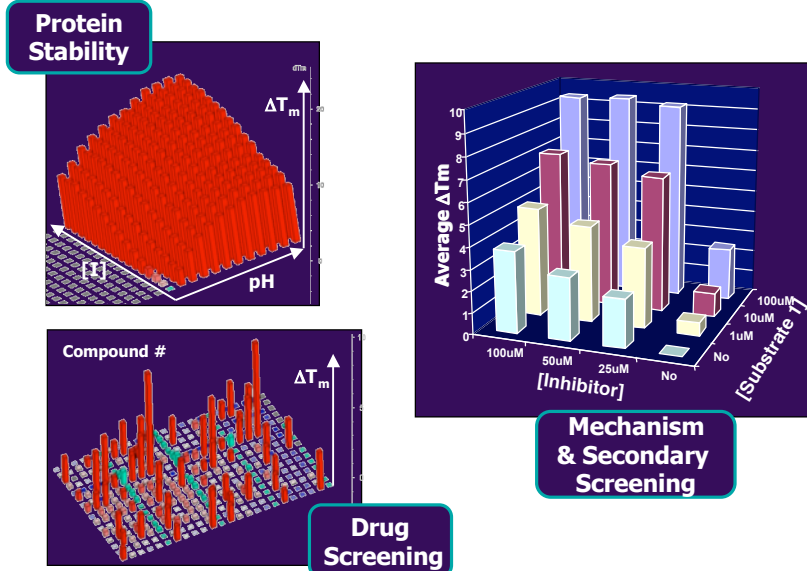
Effect of Multiple Ligand Binding Events on Protein Melting Temperature (T_m)



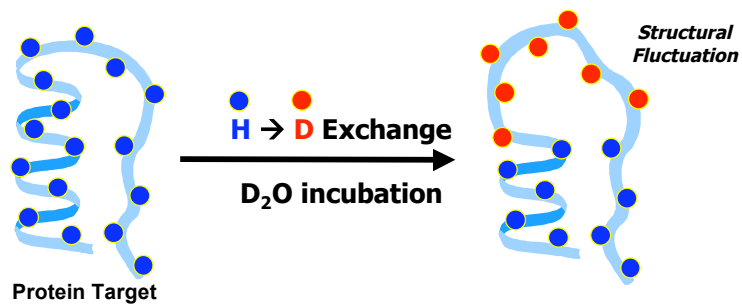
ThermoFluor[®] Instrument



ThermoFluor in Use



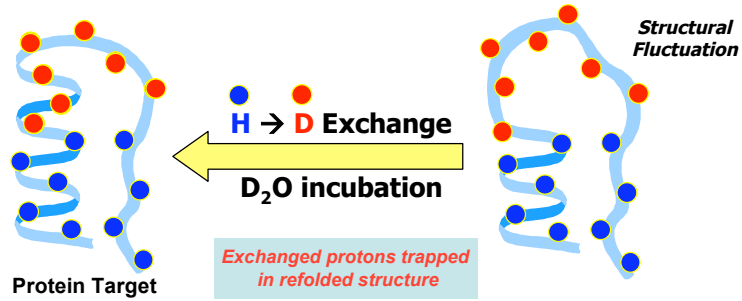
Structural Analysis of Proteins using H/D-Exchange



- Protein dynamics cause amide H (H^1) atoms to exchange for D (H^2) atoms.
- Patterns and rates of exchange can map effects of drug binding and protein:protein interactions.
- Automated analysis using mass spectroscopy

FRS 18

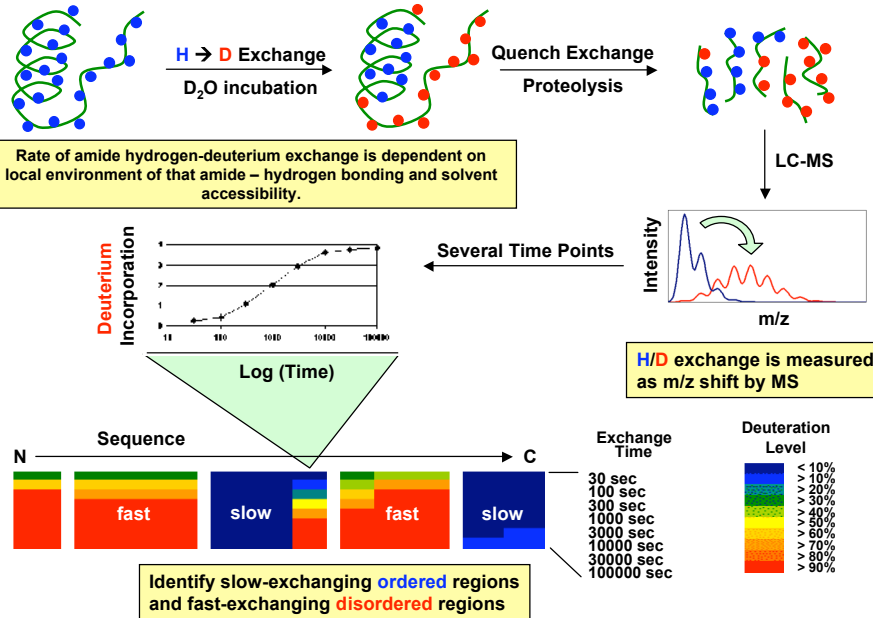
Structural Analysis of Proteins using H/D-Exchange



- Protein dynamics cause amide H (H¹) atoms to exchange for D (H²) atoms.
- Patterns and rates of exchange can map effects of drug binding and protein:protein interactions.
- Automated analysis using mass spectroscopy

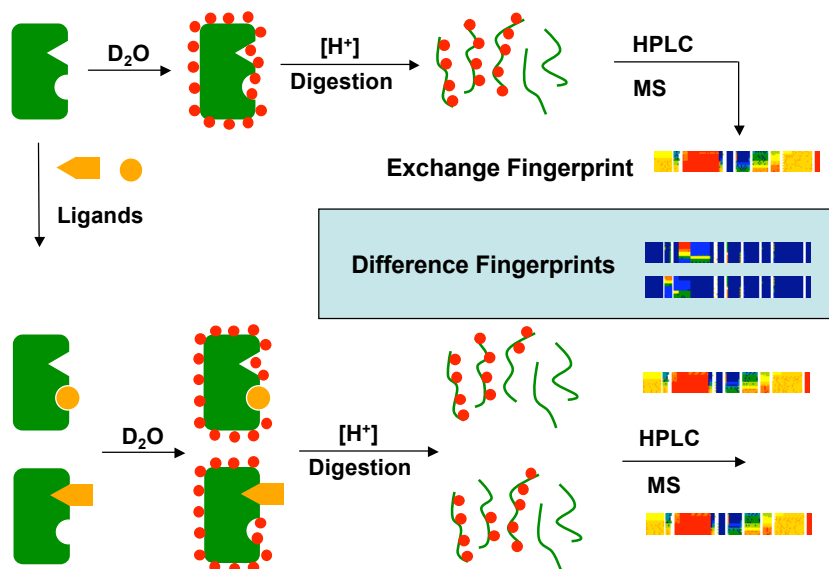
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H/D-Ex Analysis of Proteins



FRS 20

H/D-Ex : Effects of Ligand Binding



FRS 21

Summary

- **Inhibitors can be a useful approach for creating artificial chaperones for misfolding proteins that are enzymes**
 - Conventional drug design strategies can be used
 - Accompanying inhibition of activity contrary to desired result
- **Biophysical methods offer solutions for finding artificial chaperones and provide quantitative optimization capability**
 - HT DSC methods measure protein stabilization due to ligand binding and can distinguish multiple potential chaperone binding sites
 - HXDX can provide detailed information characterizing dynamic structural effects in proteins and how they are effected by ligand binding

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References

Chemical Genomics

Chemical Genomics as an Emerging Paradigm for Postgenomic Drug Discovery. F.R. Salemme, *Pharmacogenomics*. 2003; 4(3):257-267

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Combinatorial Informatics in the Post-Genomics Era, D. Agrafiotis, V. Lobanov, F.R. Salemme *Nature Drug Discovery*. 2002; 1:338-346

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