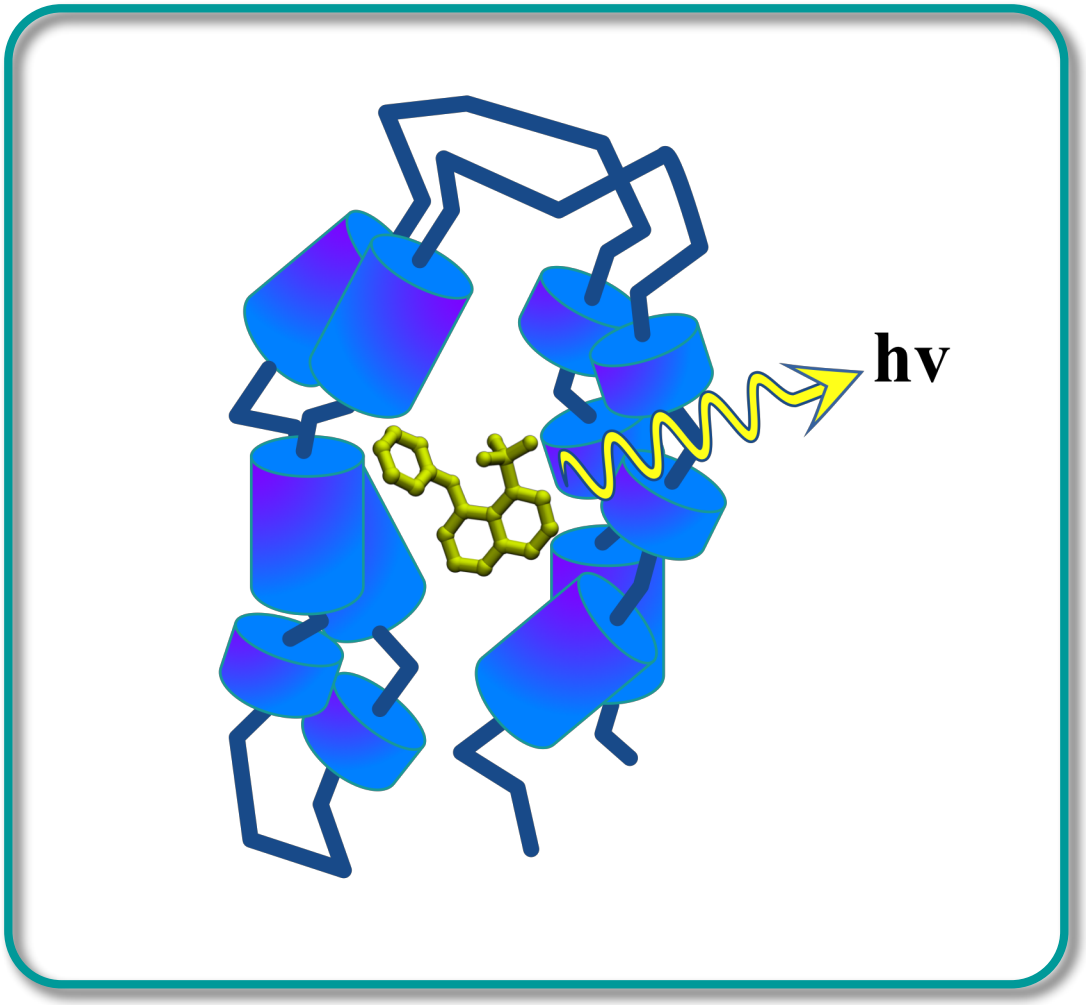
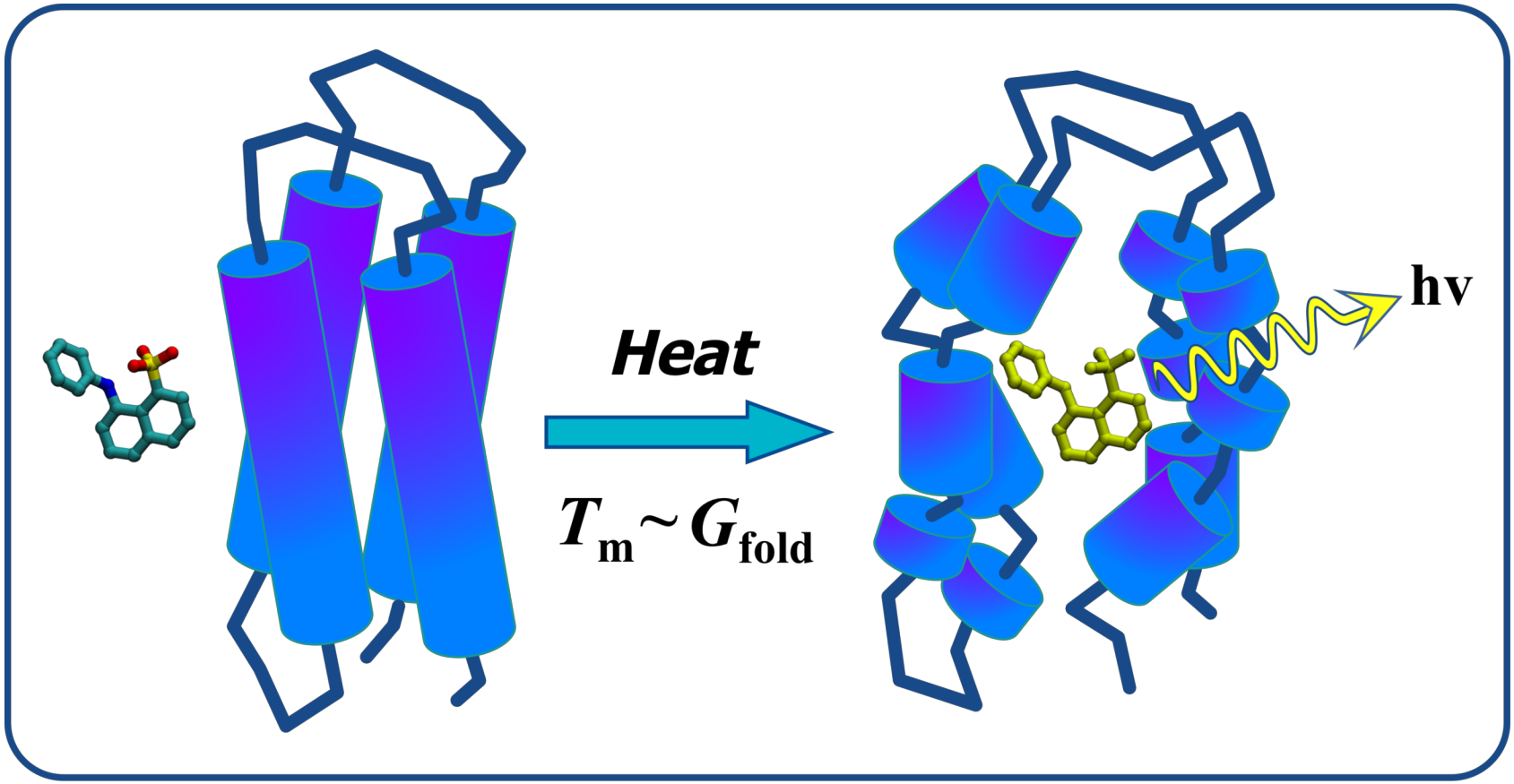
Thermofluor (aka Differential Scanning Fluorimetry)

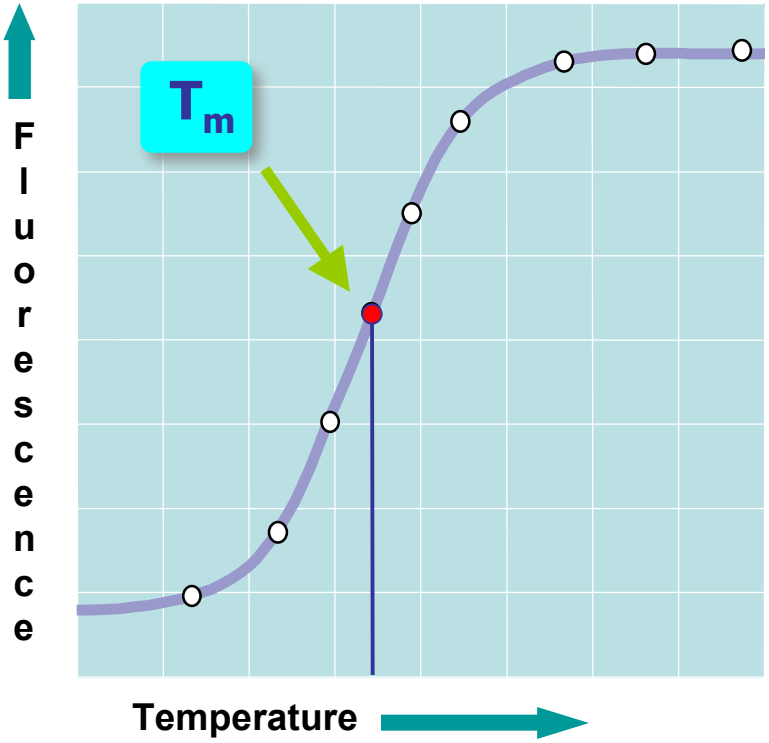


Thermofluor

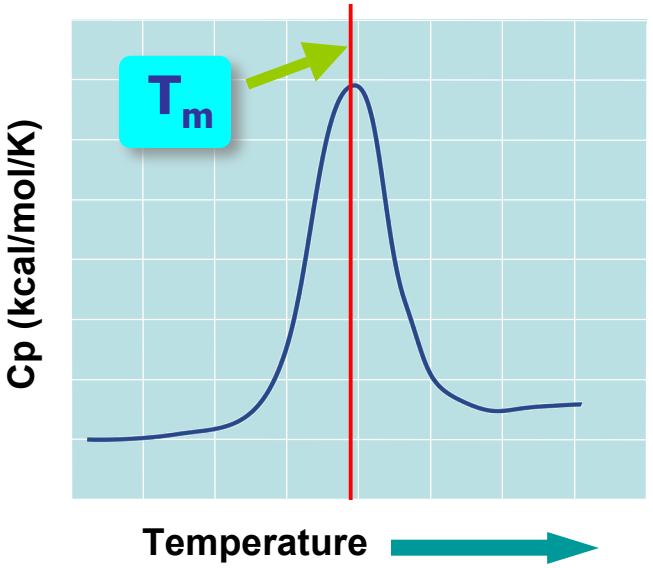


- Thermofluor gives an optical readout of protein melting as e.g. observed with DSC.
- Optical readout is much more sensitive than direct thermal measurement.
- TF consequently allows parallel measurements in 384 well-plates using fluorescent imaging plate readers.

ThermoFluor & DSC



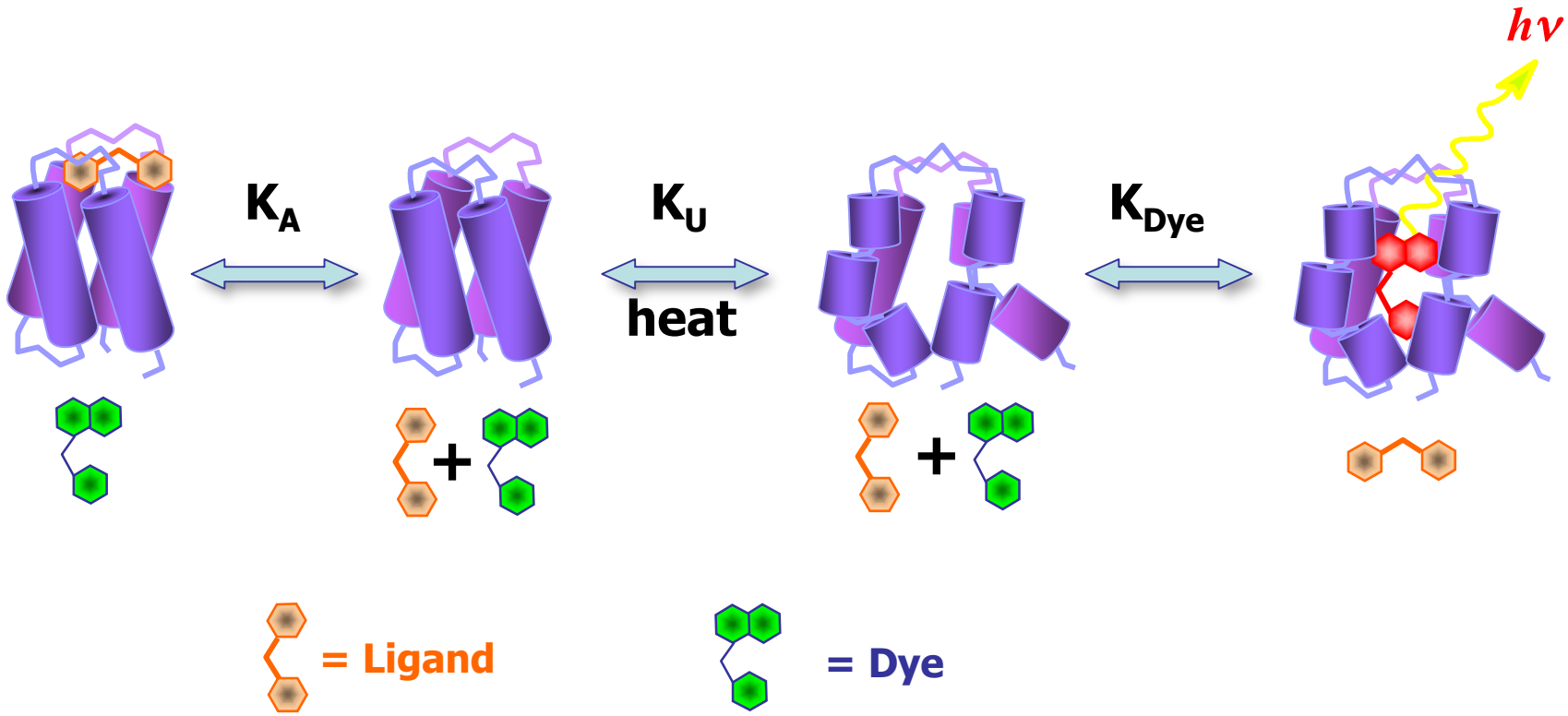
DSC Measurement of T_m
(Proteins melt cooperatively at a T_m reflecting their stability)



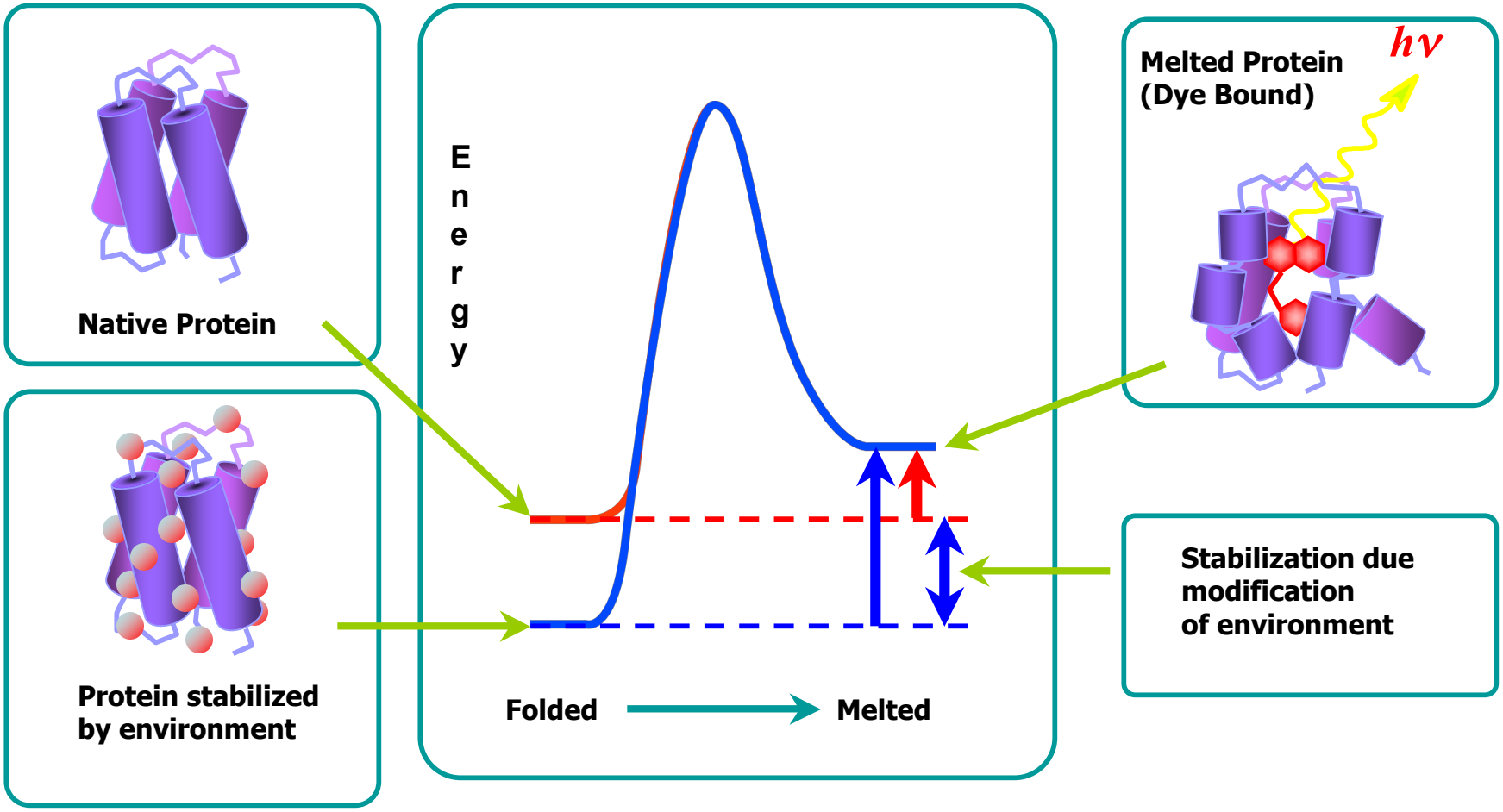
Thermofluor Applications

- **Optimization of solution conditions (pH, Ionic strength, cofactors, metals, etc.) for protein stabilization.**
  - **Protein preparation and biochemistry**
  - **Optimization of crystallization conditions**
- **Measurement of ligand binding affinity**
  - **Drug screening**
  - **Analysis of binding cooperativity between multiple ligands**
    - **Inhibitors plus substrates or cofactors.**
    - **Agonists vs. antagonists (e.g. NHR ligands vs. activator & repressor peptides).**
  - **Use of "functional probe libraries" incorporating substrates, metabolic intermediates, & cofactors to "decrypt" proteins of unknown function.**
  - **Use of structural analog libraries to determine detailed energetics of cofactor binding (e.g. ATP bound to different kinases).**

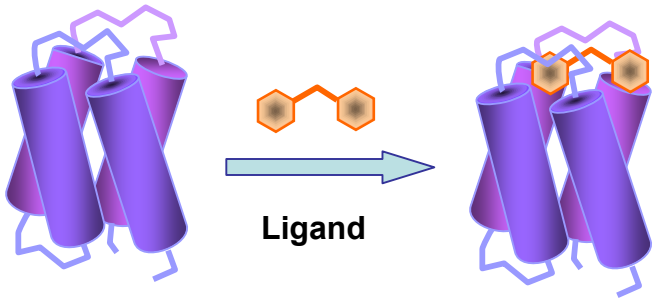
Ligand-Induced Thermal Stabilization measured using Fluorescence Readout

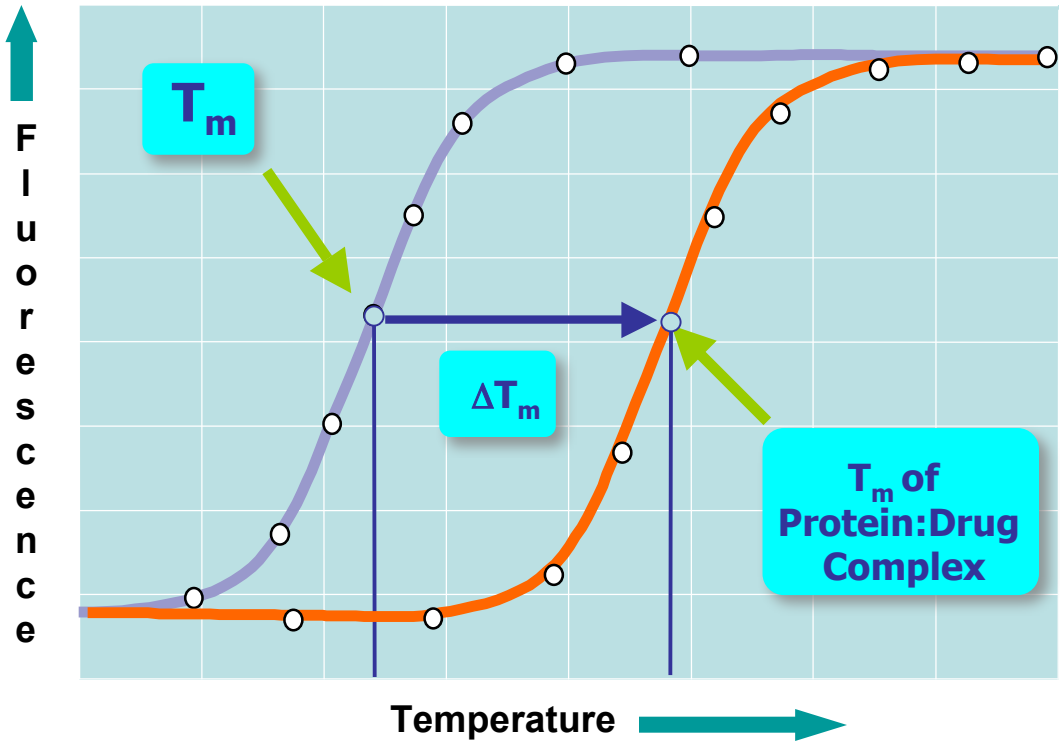


Thermofluor Can Measure Protein Stabilization

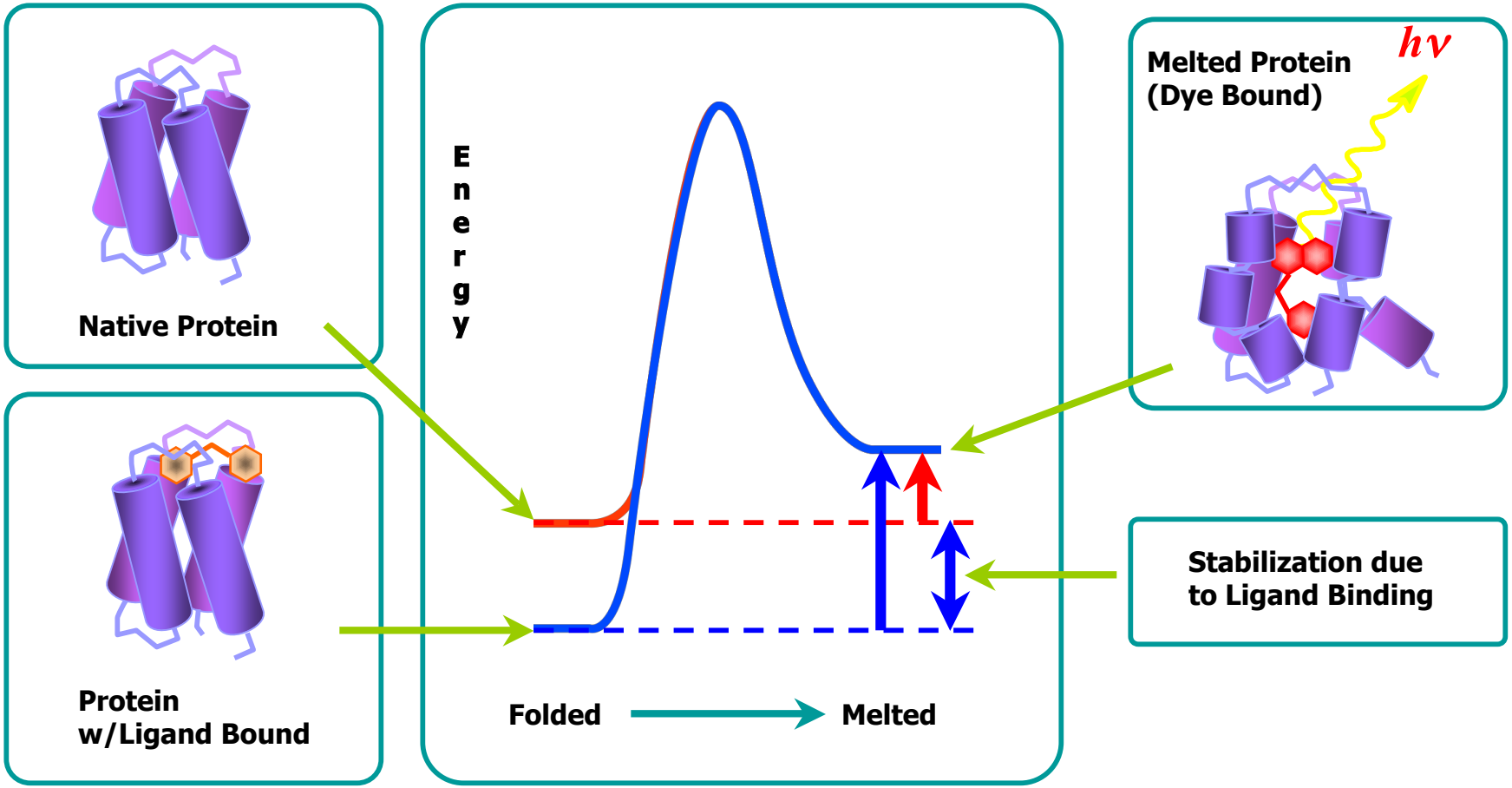


Ligand Binding Can Stabilize Protein Structure

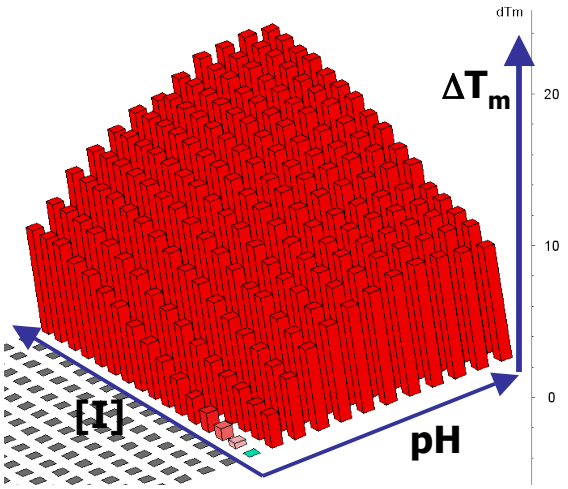




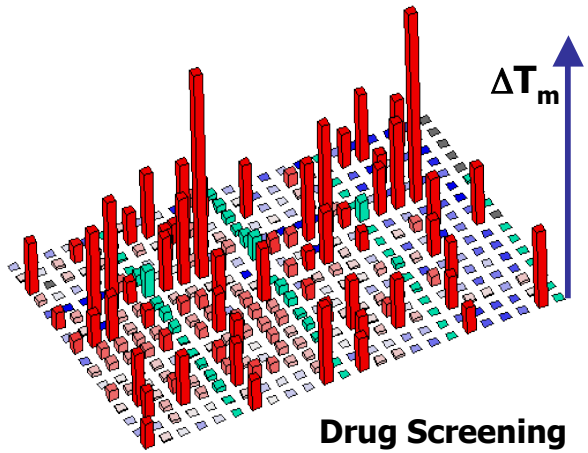
Thermofluor Can Measure Energetics of Ligand Binding



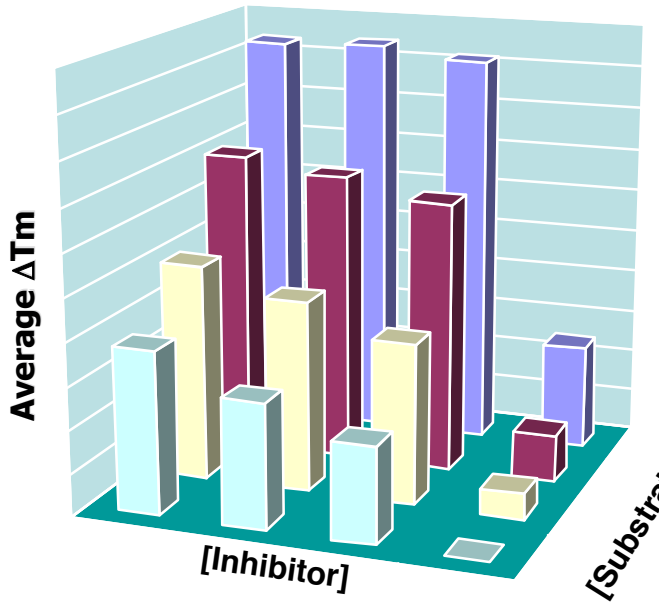
Thermofluor Applications



Protein Stability Optimization

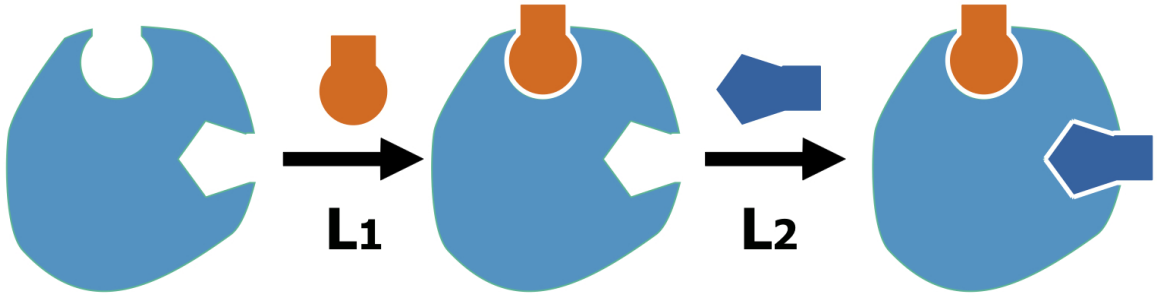


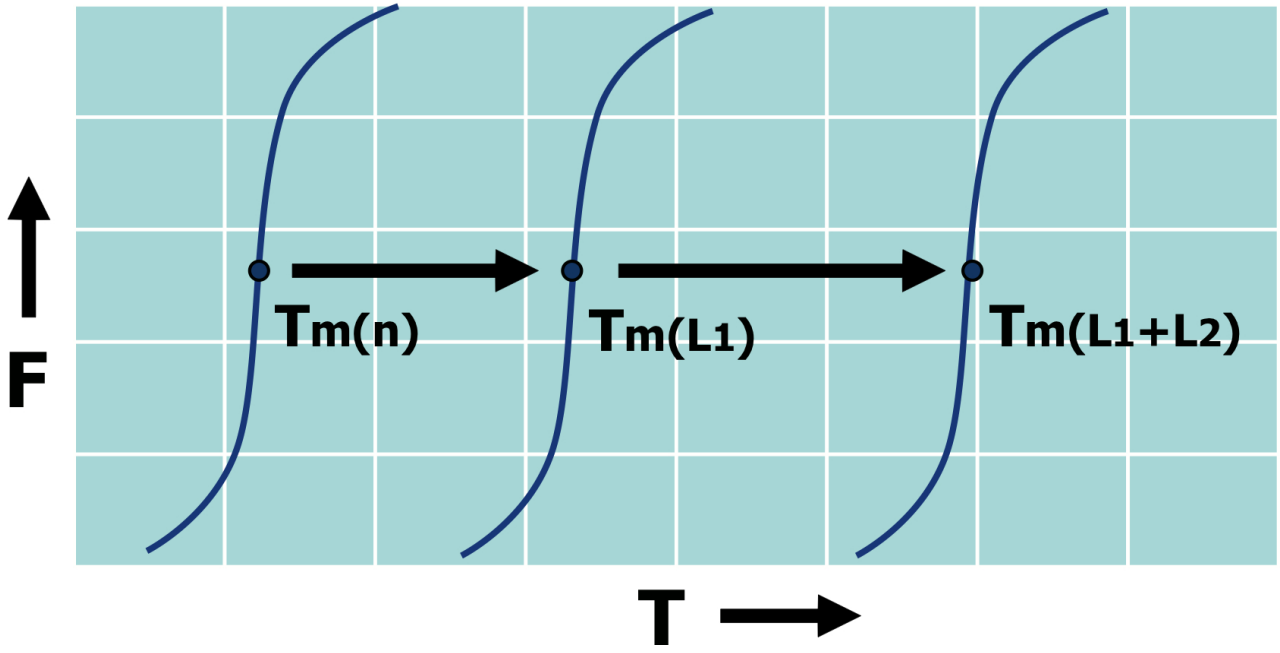
Drug Screening



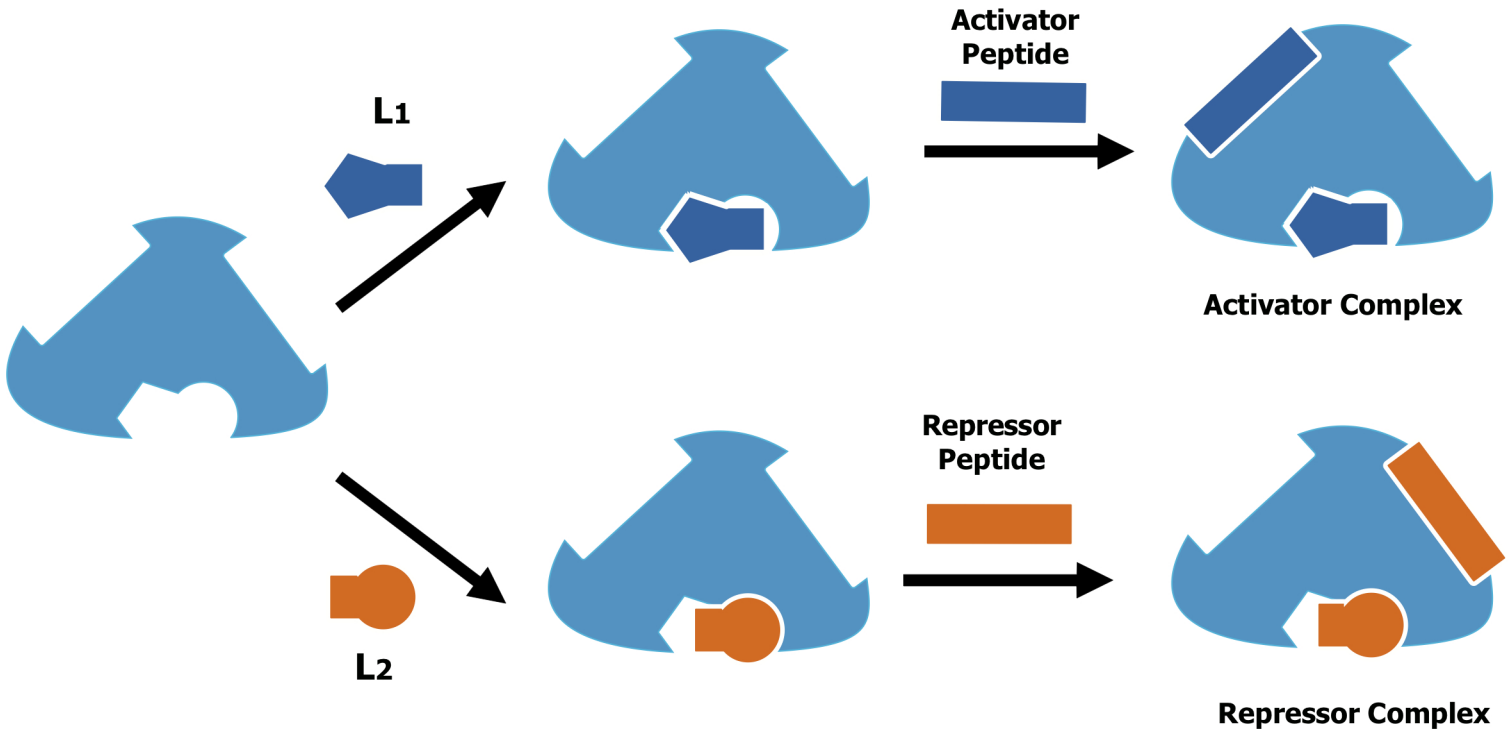
Analysis of cooperativity between substrate and inhibitor binding

Multiple Ligand Binding

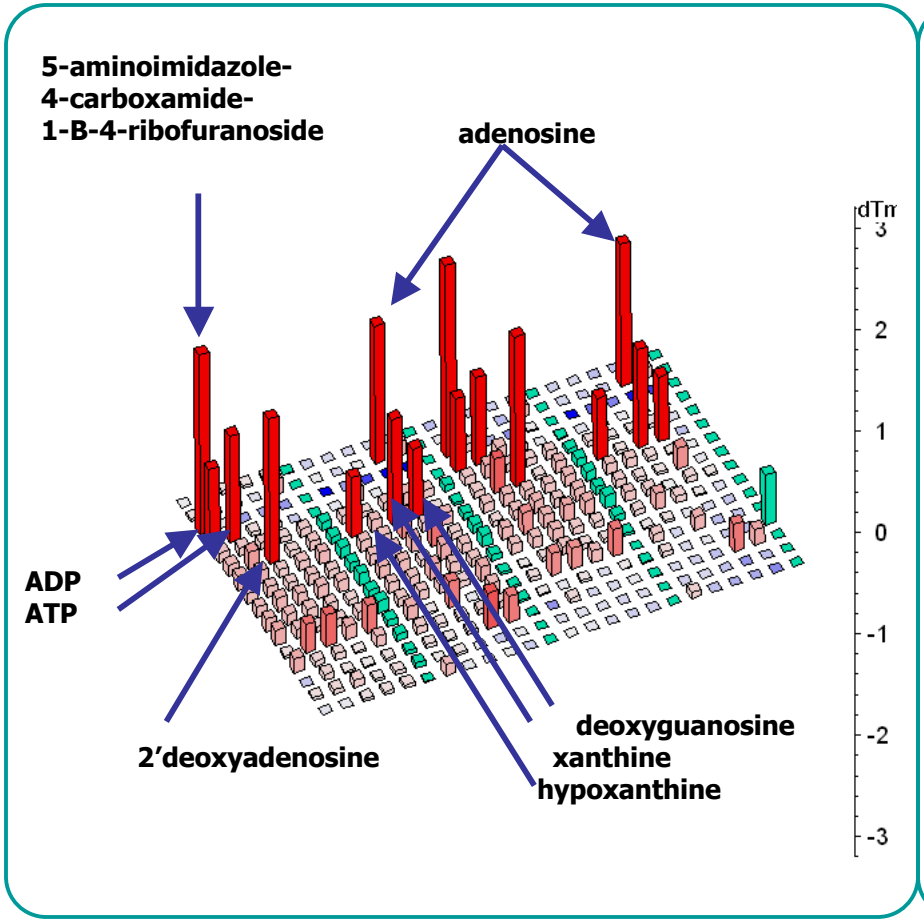




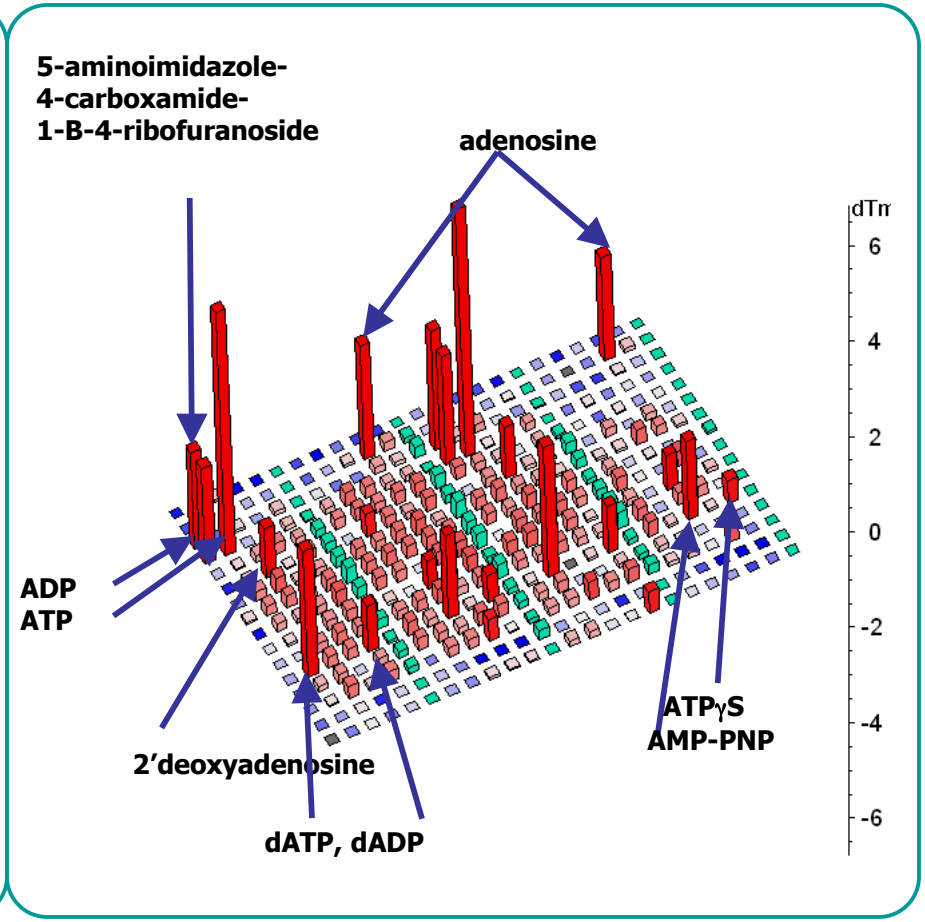
Differentiating "Activators" & "Repressors"
(e.g. for Nuclear Hormone Receptors)



Mapping Kinase ATP Binding Energetics by Thermofluor using an ATP Analog Library



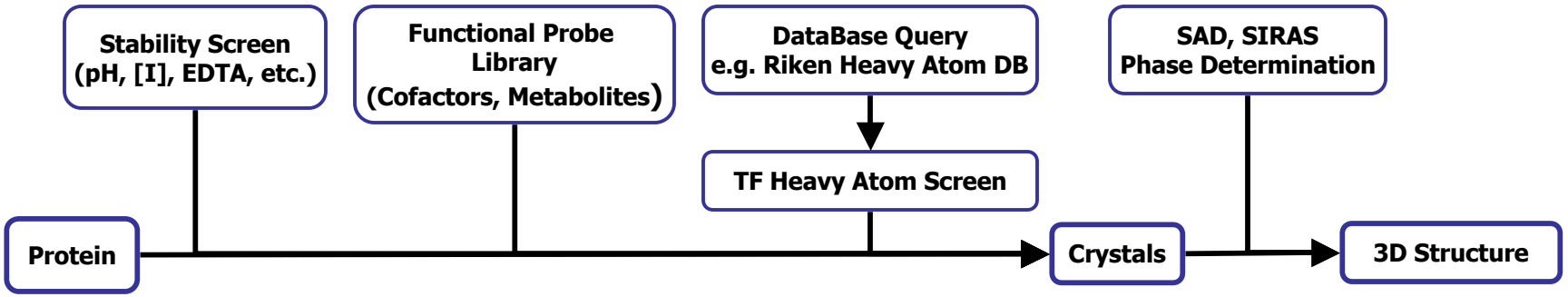
Kinase "A"



Kinase "B"

Conclusion: Different kinases have evolved different specific interactions to maintain ATP-binding capacity.
Implication: ATP-analog drugs can be specific for individual kinases. (Thought for many years to be untrue.)

Thermofluor-Assisted High-Throughput Crystallography



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