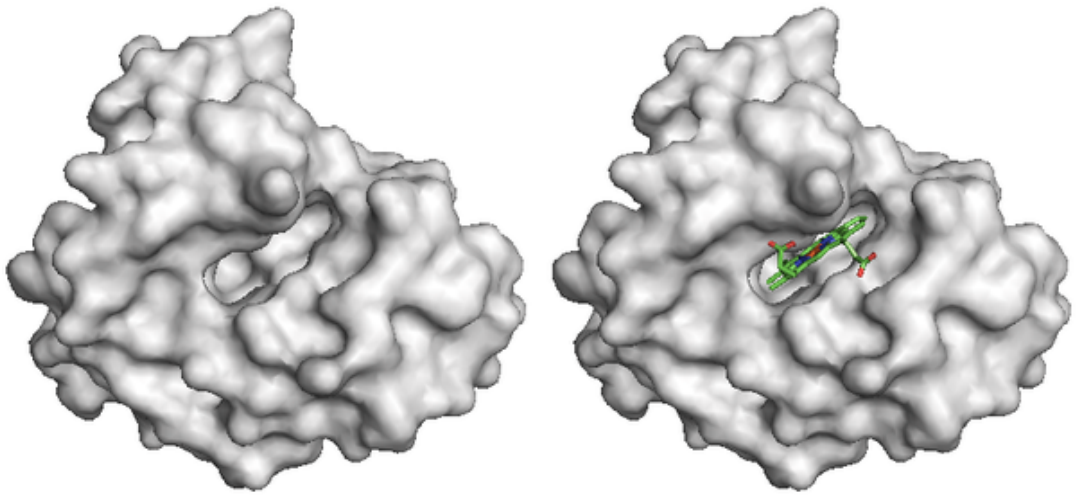


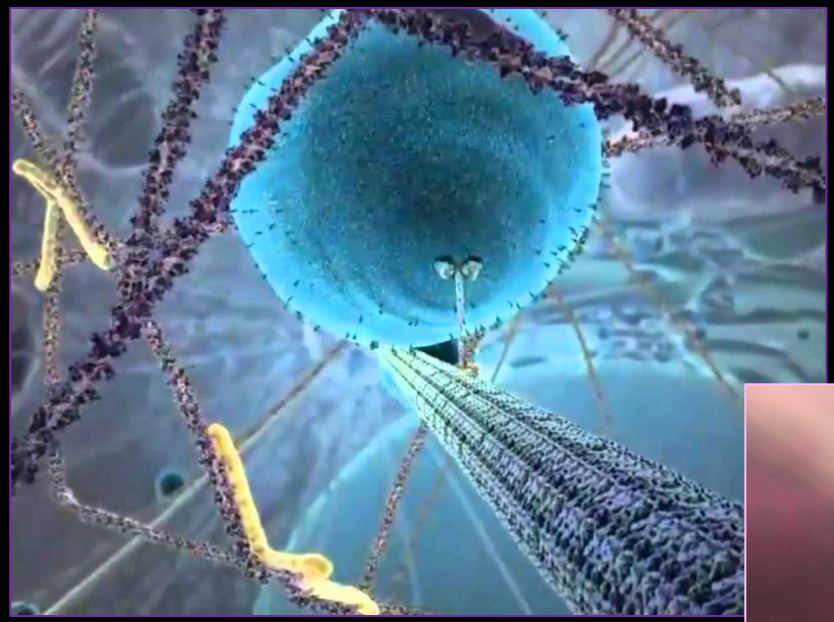
L4 – Quantitative Evaluation of Binding Interactions

November 21, 2019

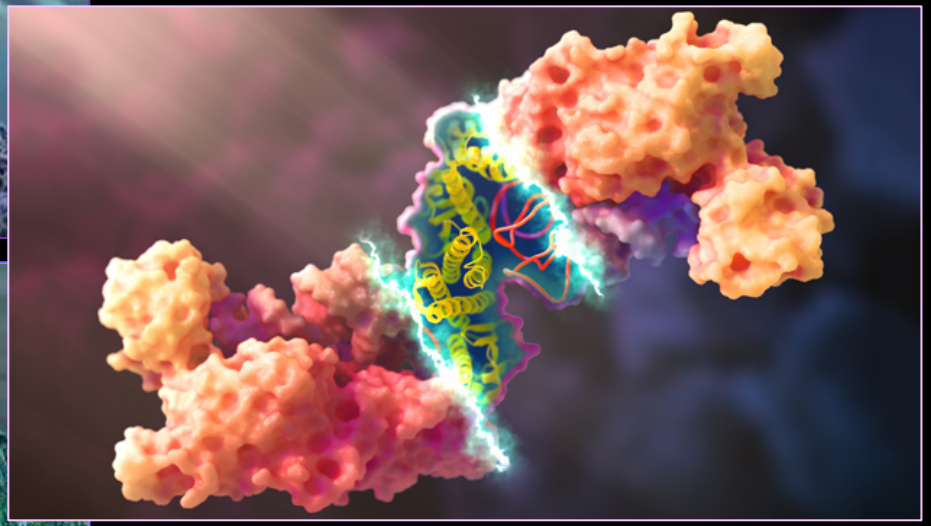
Molecular recognition is ubiquitous in biology

proteins, lipids, sugars, nucleic acids, metabolites, antibodies

The Inner Life of the Cell – Alain Viel, Harvard



https://www.youtube.com/watch?v=FzcTgrxMzZk



8 minute video – watch it while you are running an experiment

Basic language of binding interactions

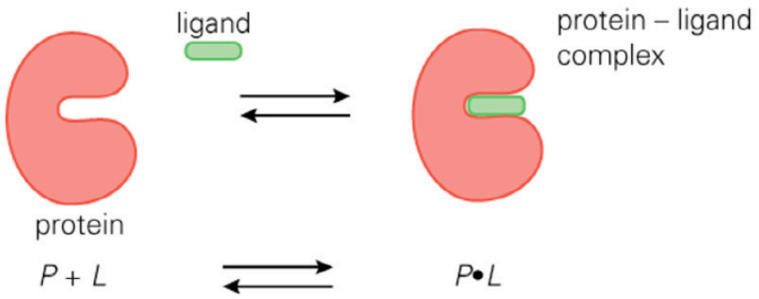
Affinity: strength of the interaction, measured by the corresponding decrease in free energy upon binding

Specificity: relative strength of interaction for a 'cognate' and 'non-cognate' receptor-ligand complex

There are two basic types of non-covalent interactions: simple binding and allosteric

Some binding interactions are 'simple' equilibria – each encounter is independent

simple interaction



Adapted from Kuriyan, The Molecules of Life, Chapter 12, Molecular Recognition

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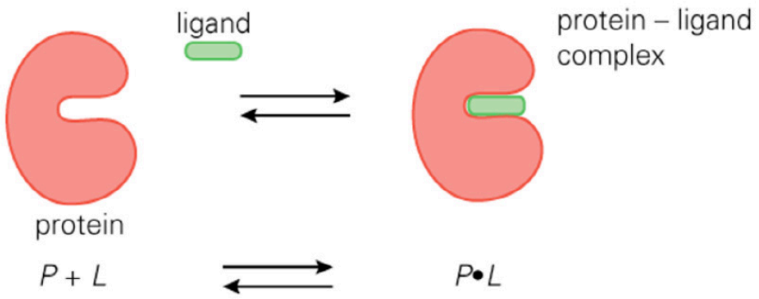
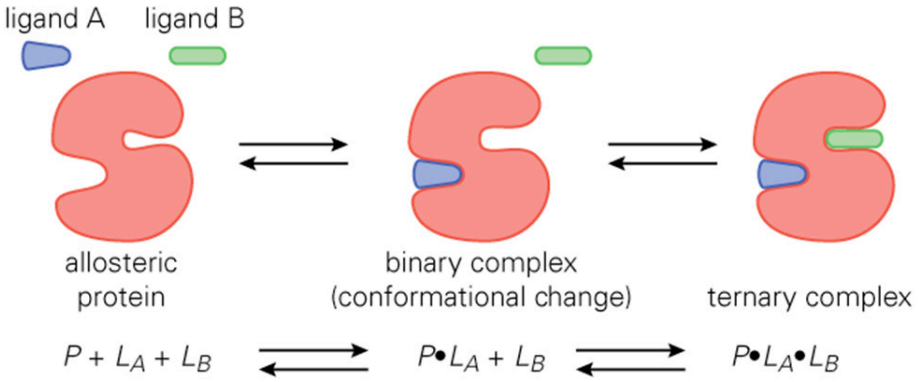


Others are more complex, involving allostery, where one ligand binding event alters the affinity for another ligand

allosteric interaction



Adapted from Kuriyan, The Molecules of Life, Chapter 12, Molecular Recognition

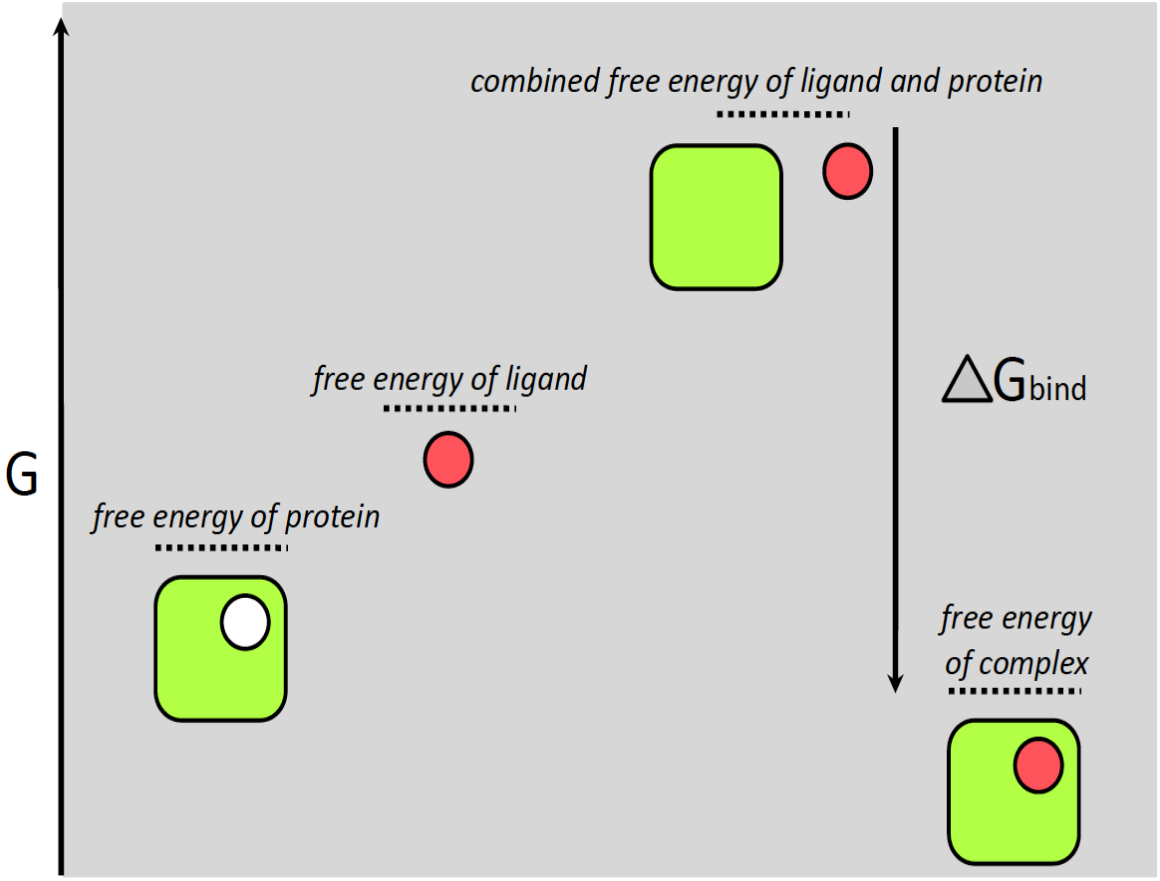
Thermodynamics provide insight into molecular interactions

As you learned in 20.110, we can think about the following binding-related terms thermodynamically:

- affinity and specificity
- contribution of entropy and enthalpy
- dependence on temperature
- contributions of chemical groups on the ligand and/or the receptor

This information can in turn be used to understand a system and to alter the system (e.g. drug design)

Relationship of ligand binding free energy to association constants



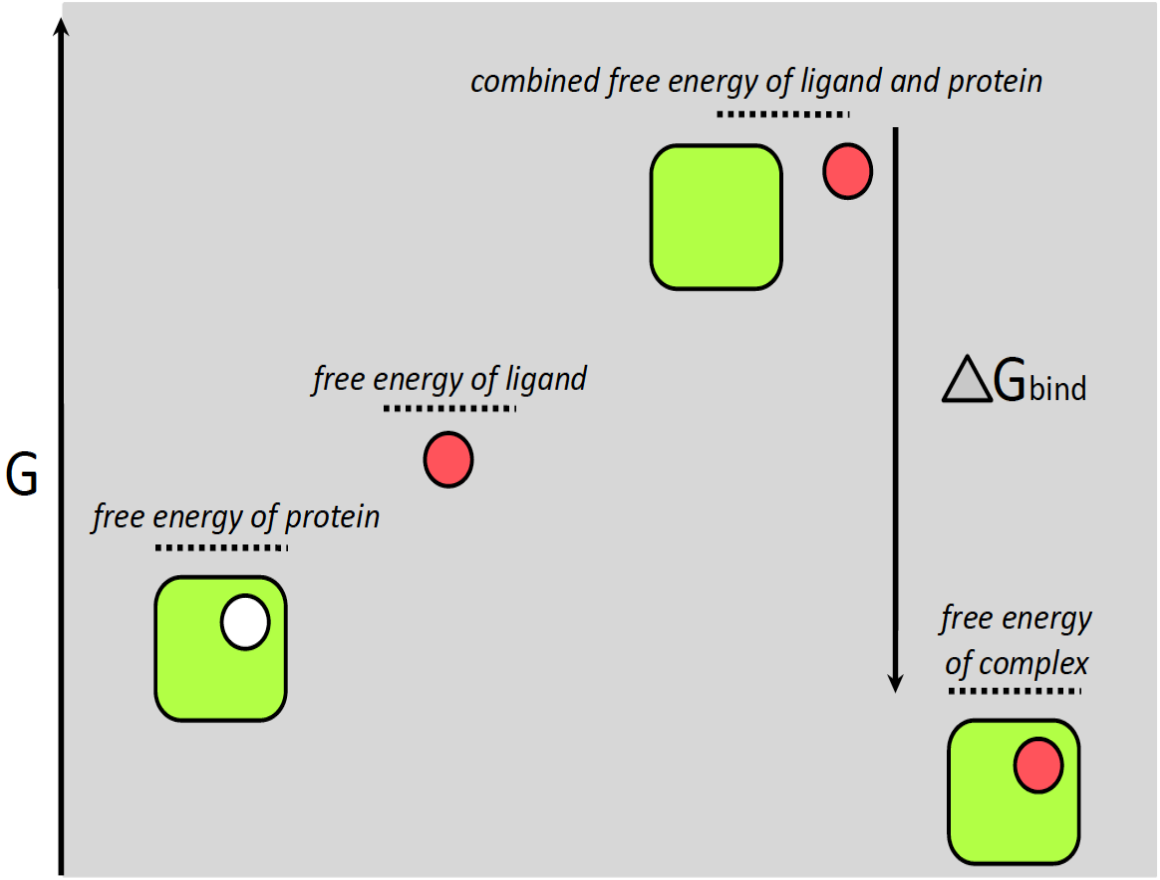
From 20.110:

$$\Delta G^{\circ}_{bind} = -RT \ln K_A$$

$$K_D \underset{\text{(dissociation constant)}}{} = \frac{[P][L]}{[P \cdot L]} = \frac{1}{K_A}$$

$$\Delta G^{\circ}_{bind} = +RT \ln K_D$$

Relationship of ligand binding free energy to association constants



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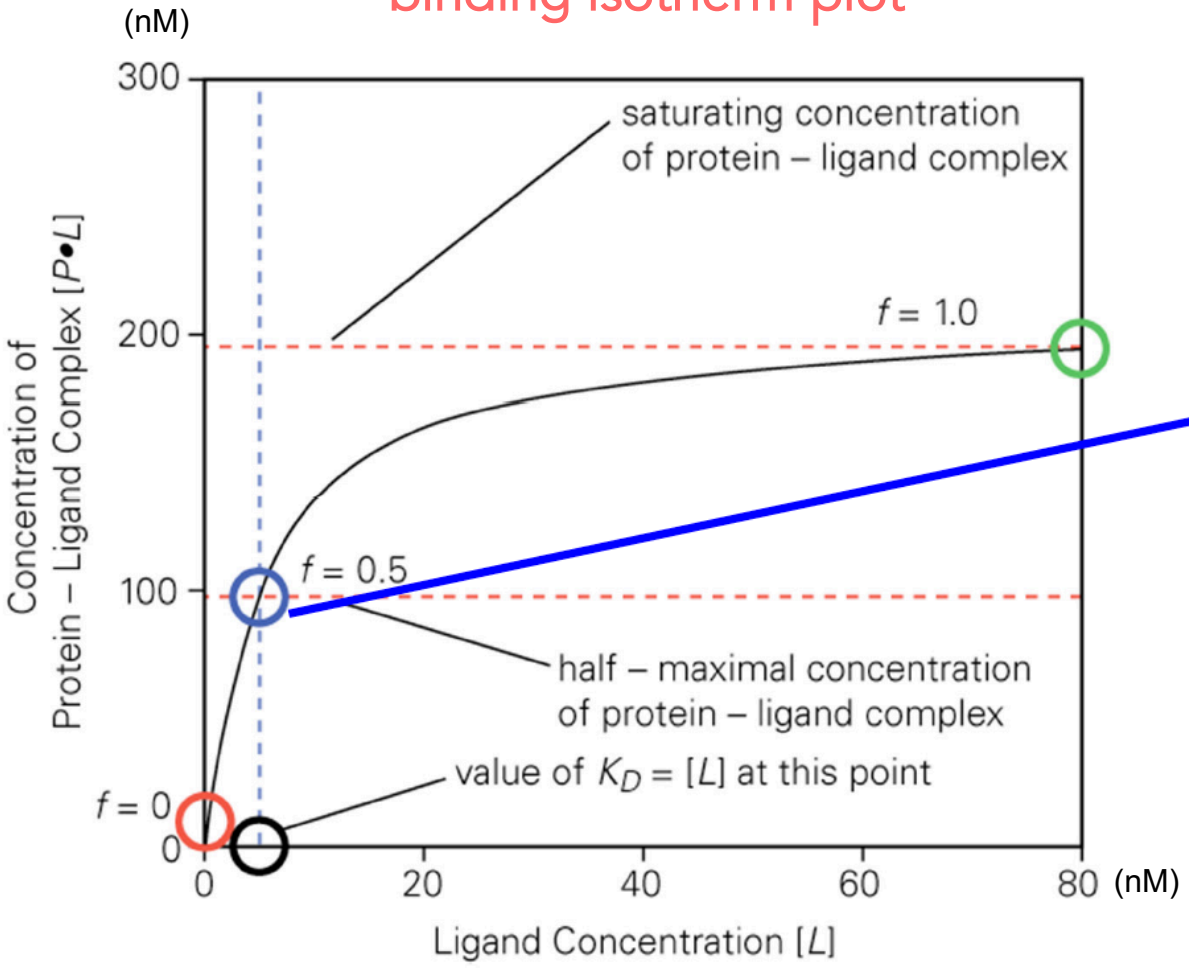
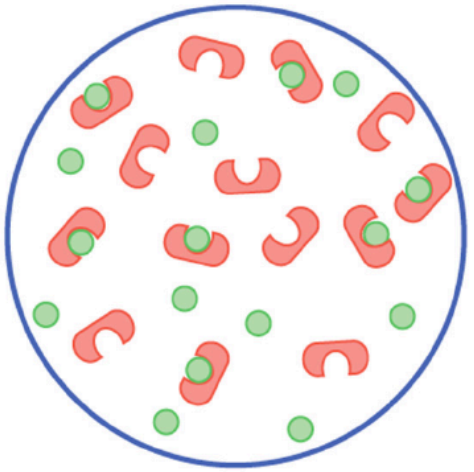
$$\Delta G^{\circ}_{bind} = +RT \ln K_D$$

Binding isotherms are half maximal at $[L] = K_D$

'binding isotherm plot'



$R \bullet L \leftrightarrows R + L$

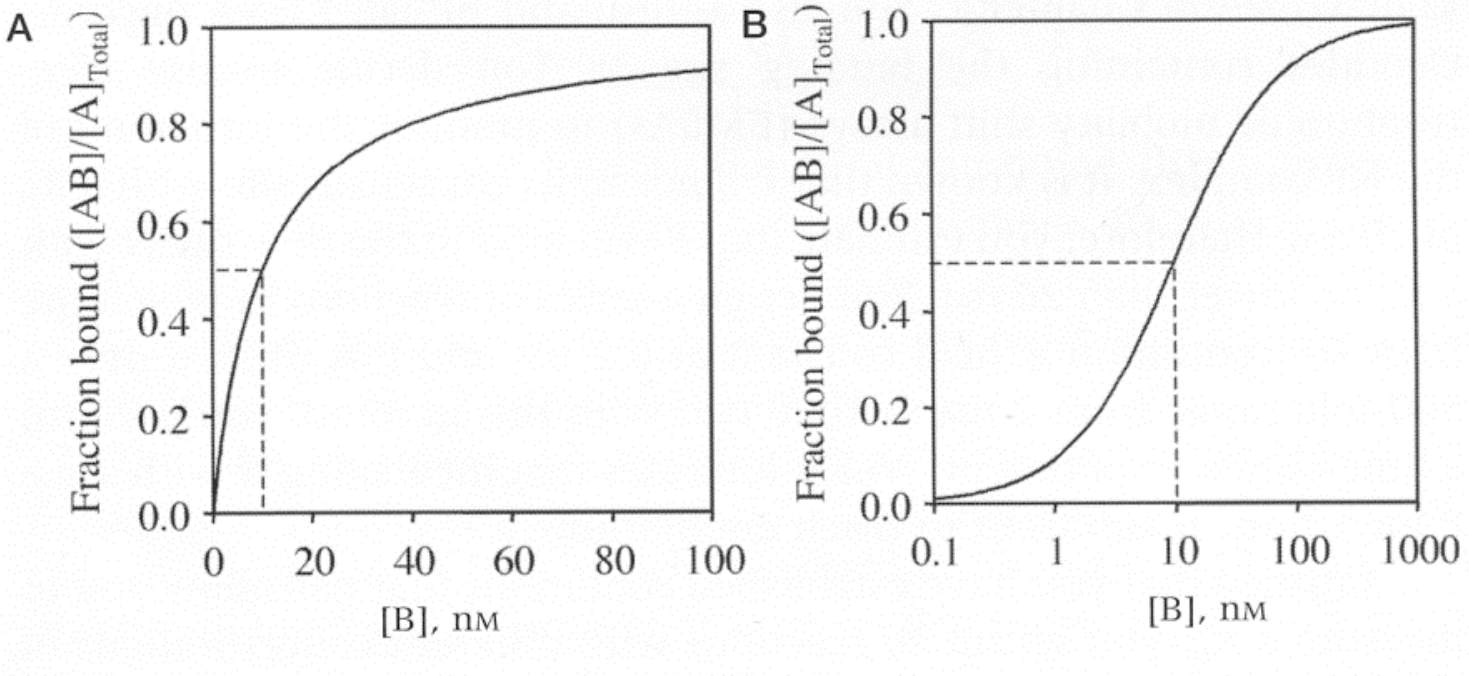


intermediate ligand concentration: $f = 0.5$
$K_D = [L]$ when $f = 0.5$

steady-state
equilibrium analysis

Logarithmic vs. Linear display of data

historic convention *current convention*



as a corollary, choose your concentrations wisely:

1, 3, 10, 30, 100, 300 nM

vs.

50, 100, 150, 200, 250, 300 nM

Range of biologically important interactions

Type of Interaction	K_D (molar)	ΔG^0_{bind} (at 300K) kcal/mol
Enzyme:ATP	~1×10^{-3} to ~1×10^{-6} (millimolar to micromolar)	**-4 to -8 kcal/mol**
signaling protein binding to a target	~1×10^{-6} (micromolar)	**-8 kcal/mol**
Sequence-specific recognition of DNA by a transcription factor	~1×10^{-9} (nanomolar)	**-12 kcal/mol**
small molecule inhibitors of proteins (drugs)	~1×10^{-9} to ~1×10^{-12} (nanomolar to picomolar)	**-12 to -17 kcal/mol**
biotin binding to avidin protein (strongest known non-covalent interaction)	~1×10^{-15} (femtomolar)	**-21 kcal/mol**

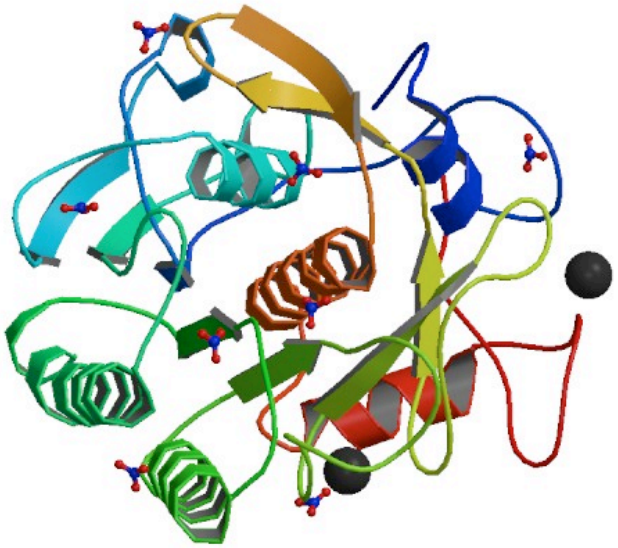
higher K_D value
weaker interaction

lower K_D value
stronger interaction

Adapted from Kuriyan, The Molecules of Life, Chapter 12, Molecular Recognition

Specificity in molecular recognition

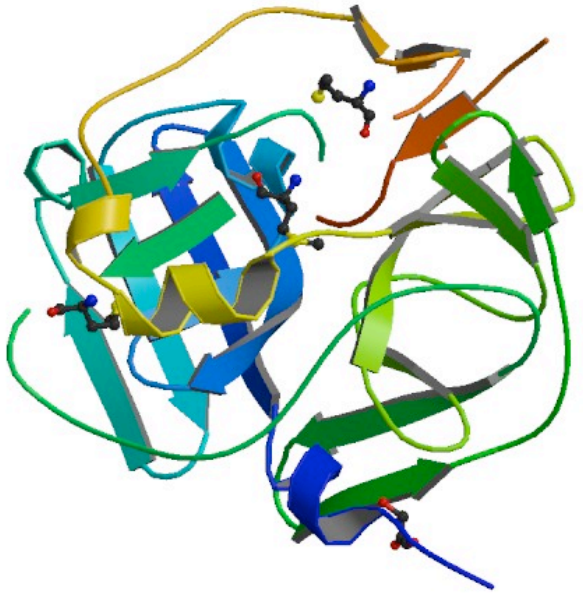
discrimination among targets



Proteinase K

low specificity

Aliphatic/X
Aromatic/X

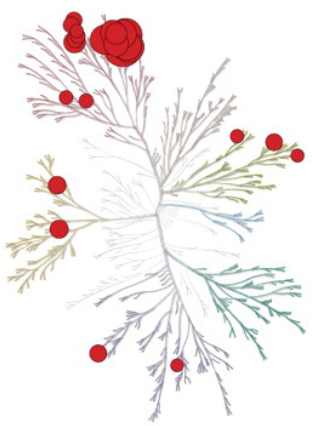


Tobacco Etch Virus (TEV) protease

high specificity

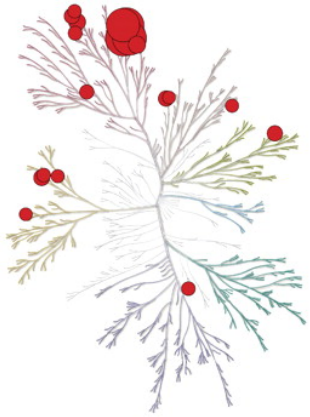
Glu-X-X-Tyr-X-Gln/Ser

Specificity in molecular recognition – kinase drugs



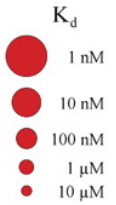
AC220

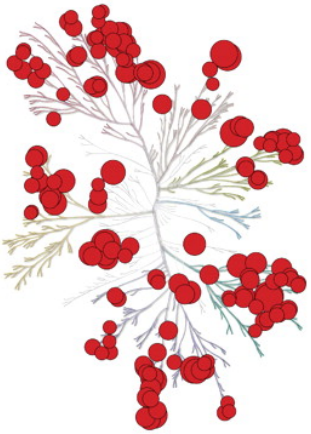
CEP-701



MLN-518

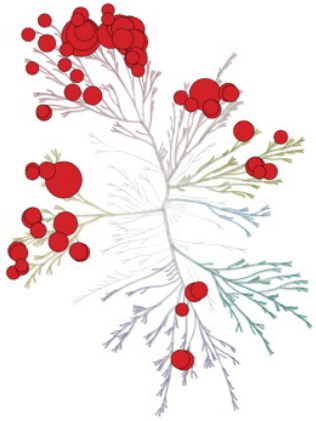
binding constants





PKC-412

CGP-52421



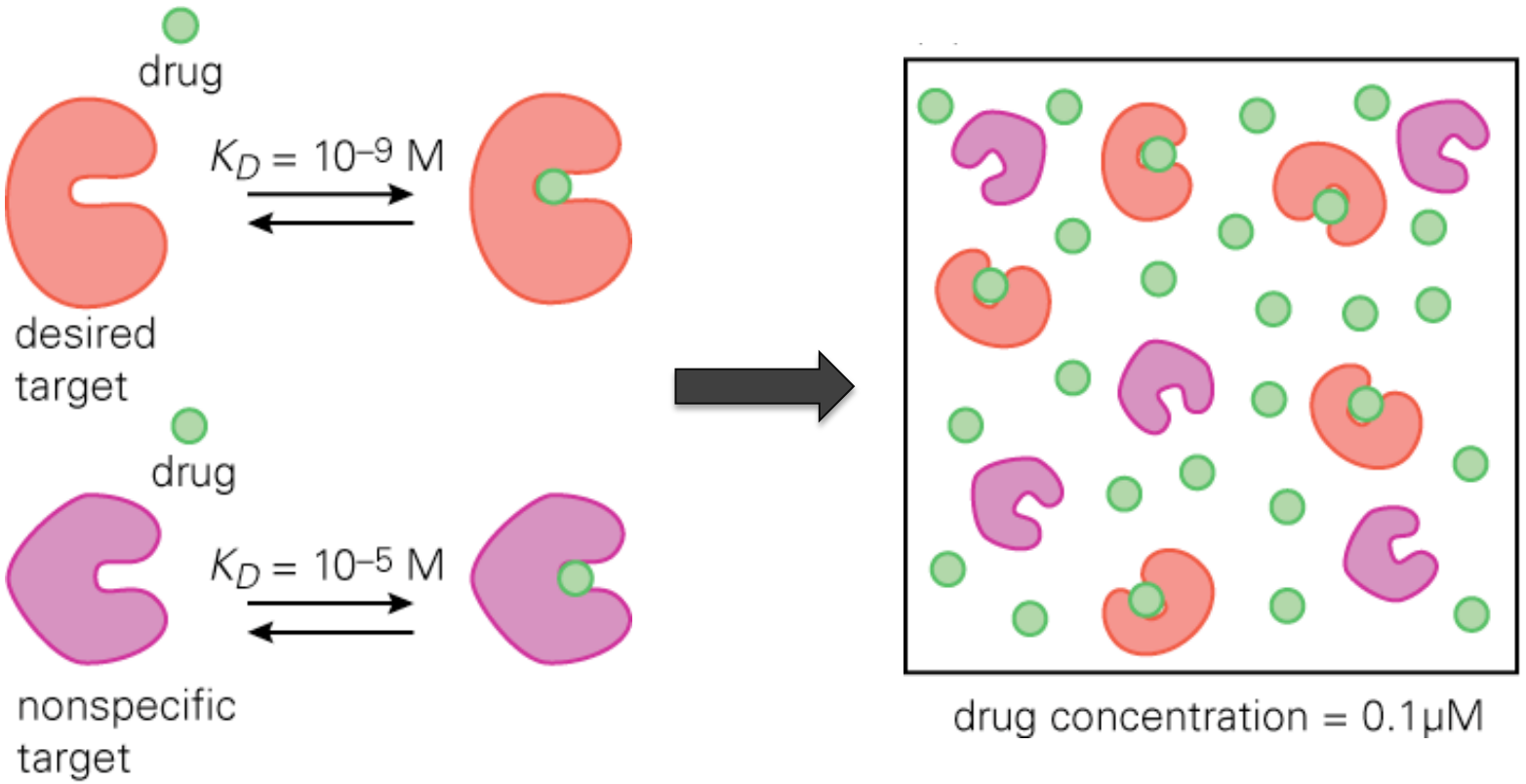
Sorafenib

Sunitinib

Adapted from Zarrinkar et al, Blood (2009), 114: 2984-2992

Specificity in drug binding – fractional saturation

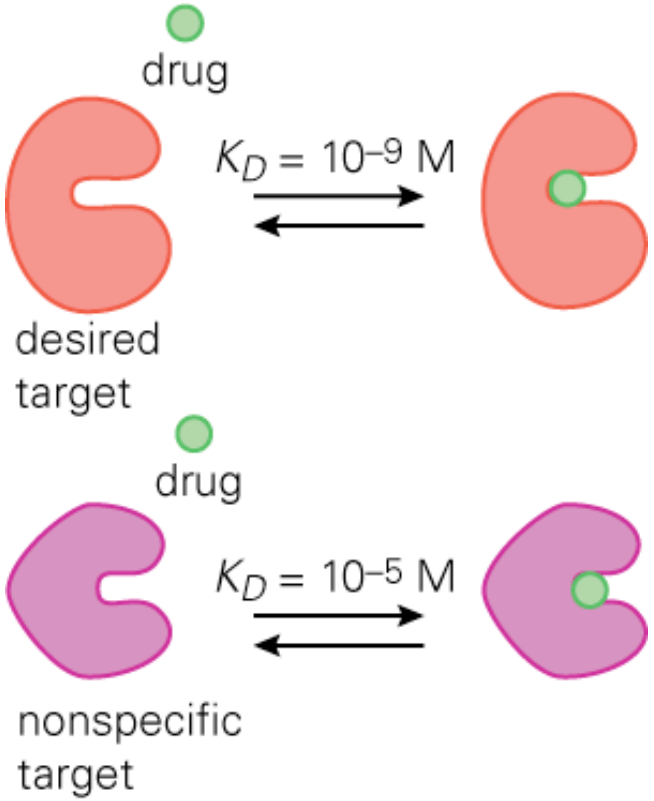
deliver the drug at a concentration below the K_D for non-cognate target



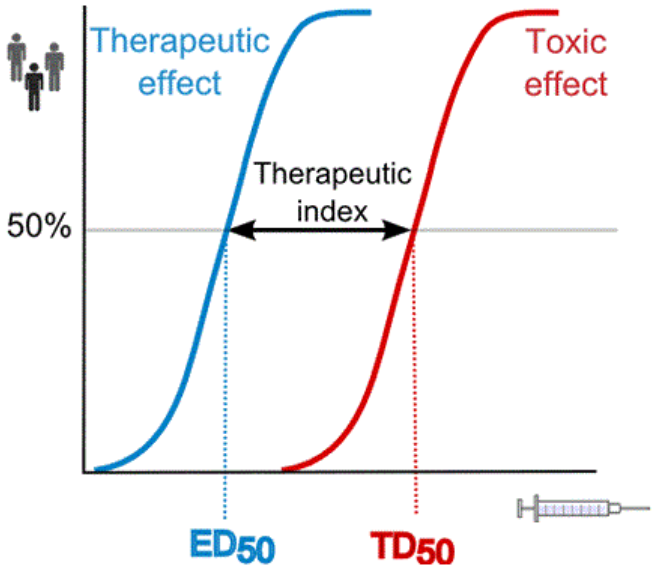
Adapted from Kuriyan, The Molecules of Life, Chapter 12, Molecular Recognition

Specificity in drug binding – fractional saturation

deliver the drug at a concentration below the TD_{50} in patients



impact therapeutic effects?



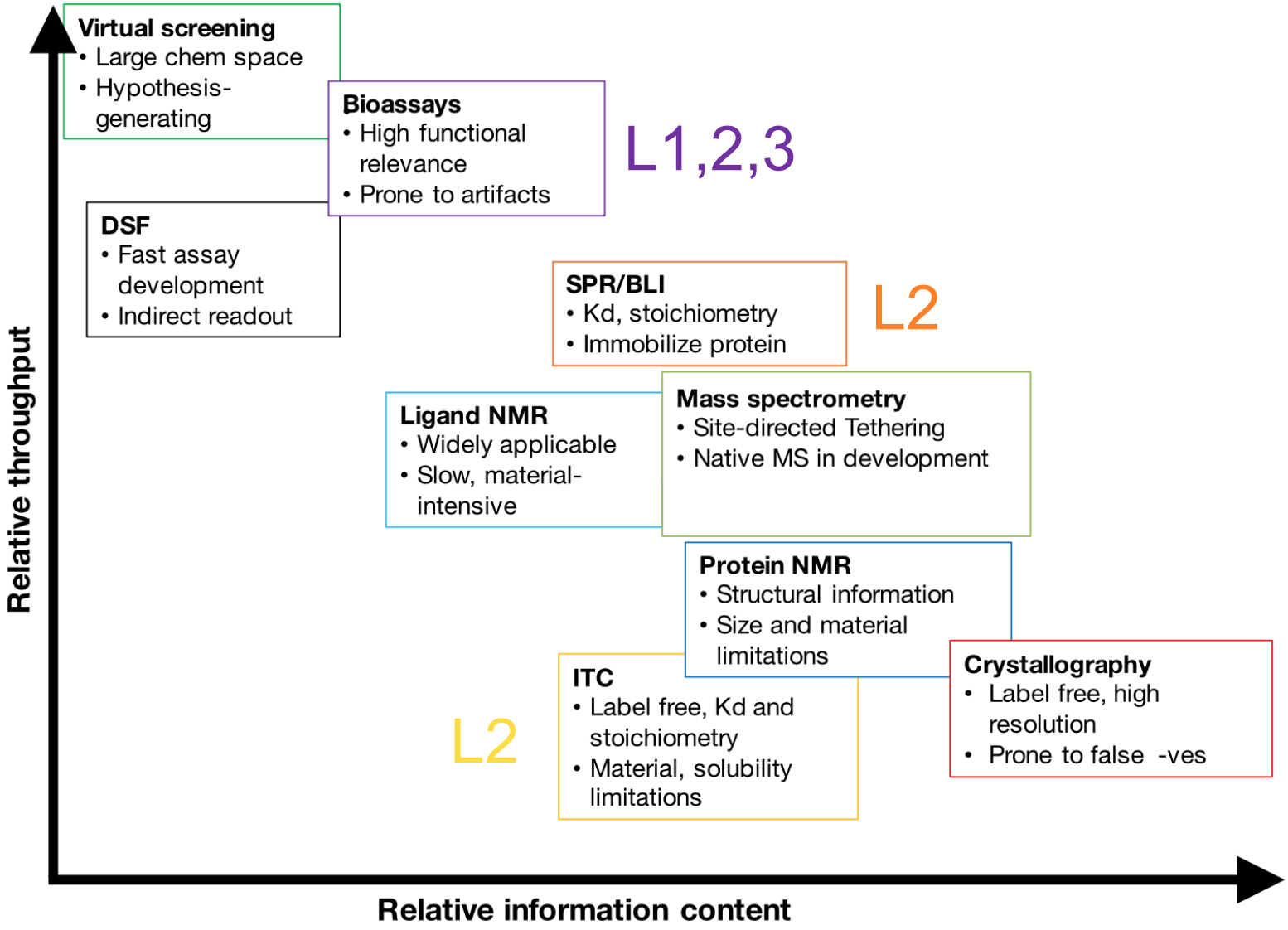
ED_{50} = effective in 50% patients

TD_{50} = toxic in 50% patients

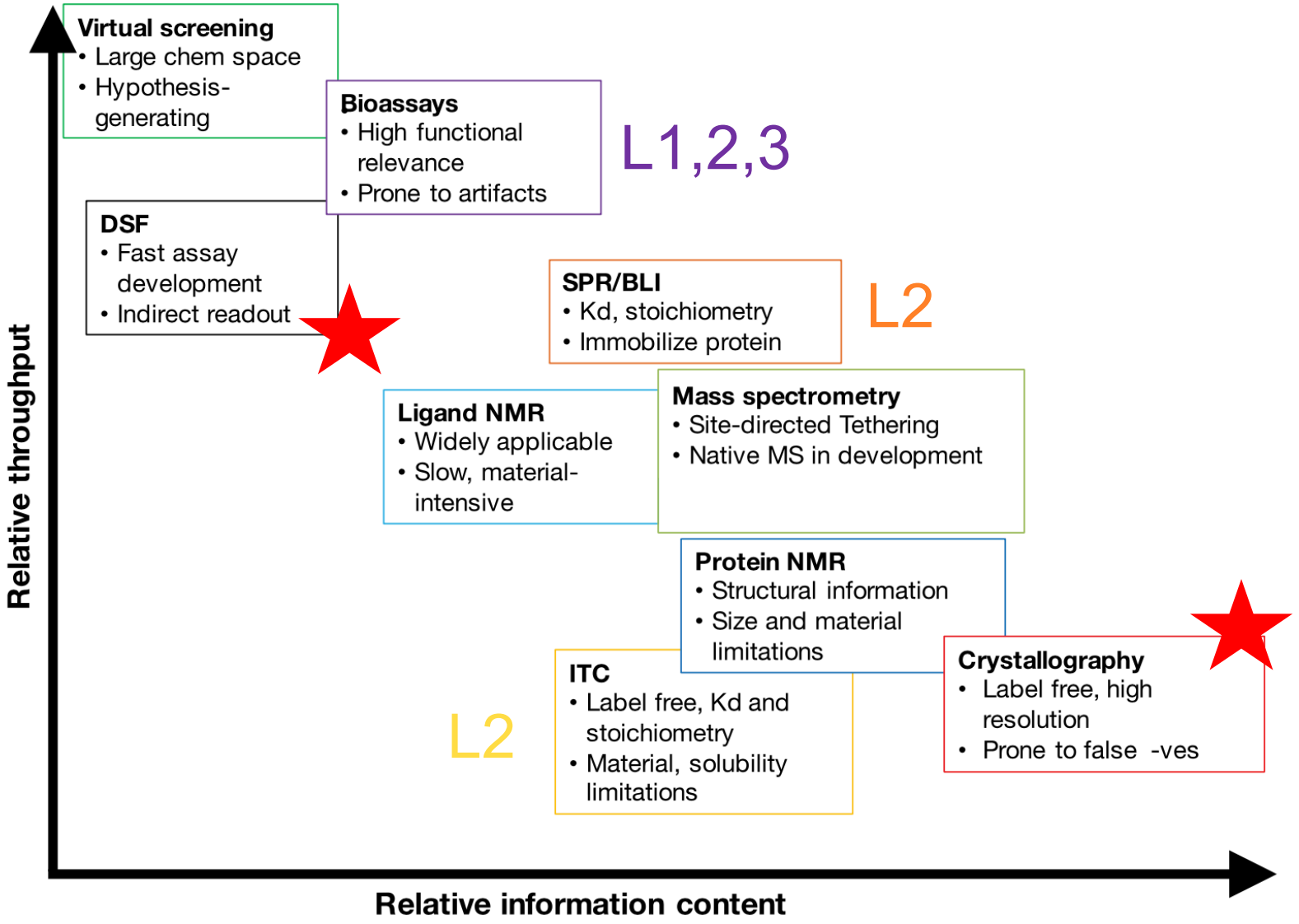
Adapted from Kuriyan, The Molecules of Life, Chapter 12, Molecular Recognition

But how do we go about measuring these K_D values in a laboratory setting?

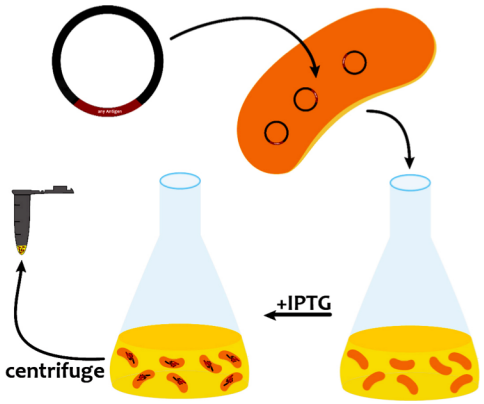
Methods to evaluate binding interactions



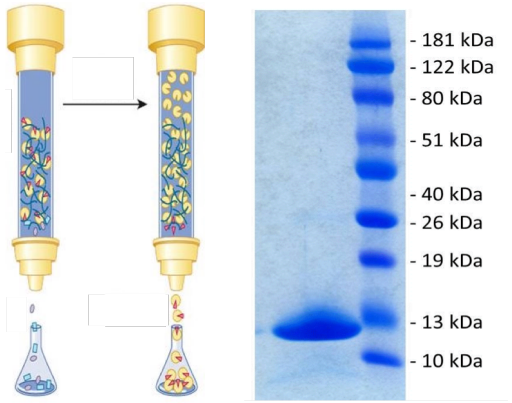
Methods to evaluate binding interactions



20.109 path to evaluate FKBP12 ligands

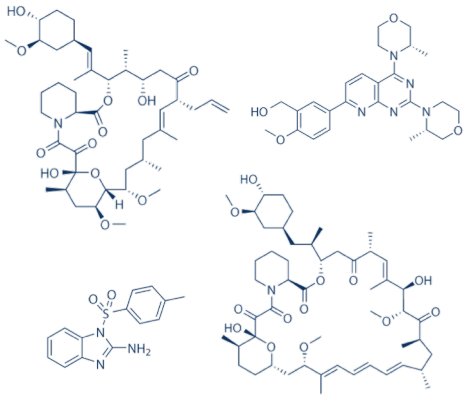


in silico cloning; overexpress FKBP12

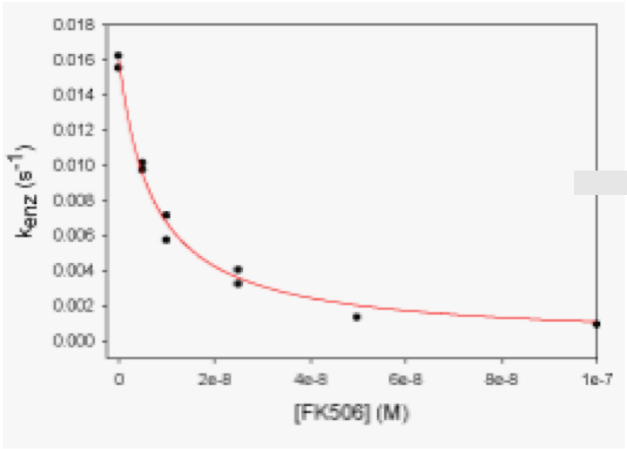


purify and analyze FKBP12

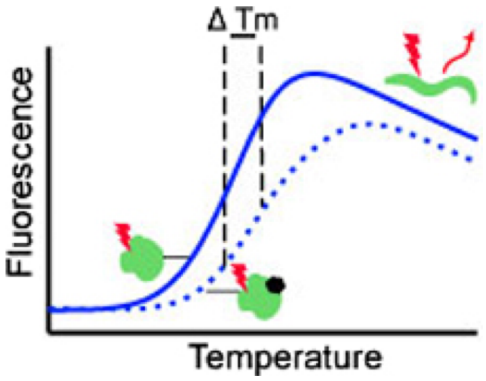
+



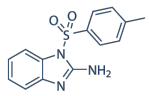
re-analyze screen data



test FKBP12 and ligands
in enzyme activity assay



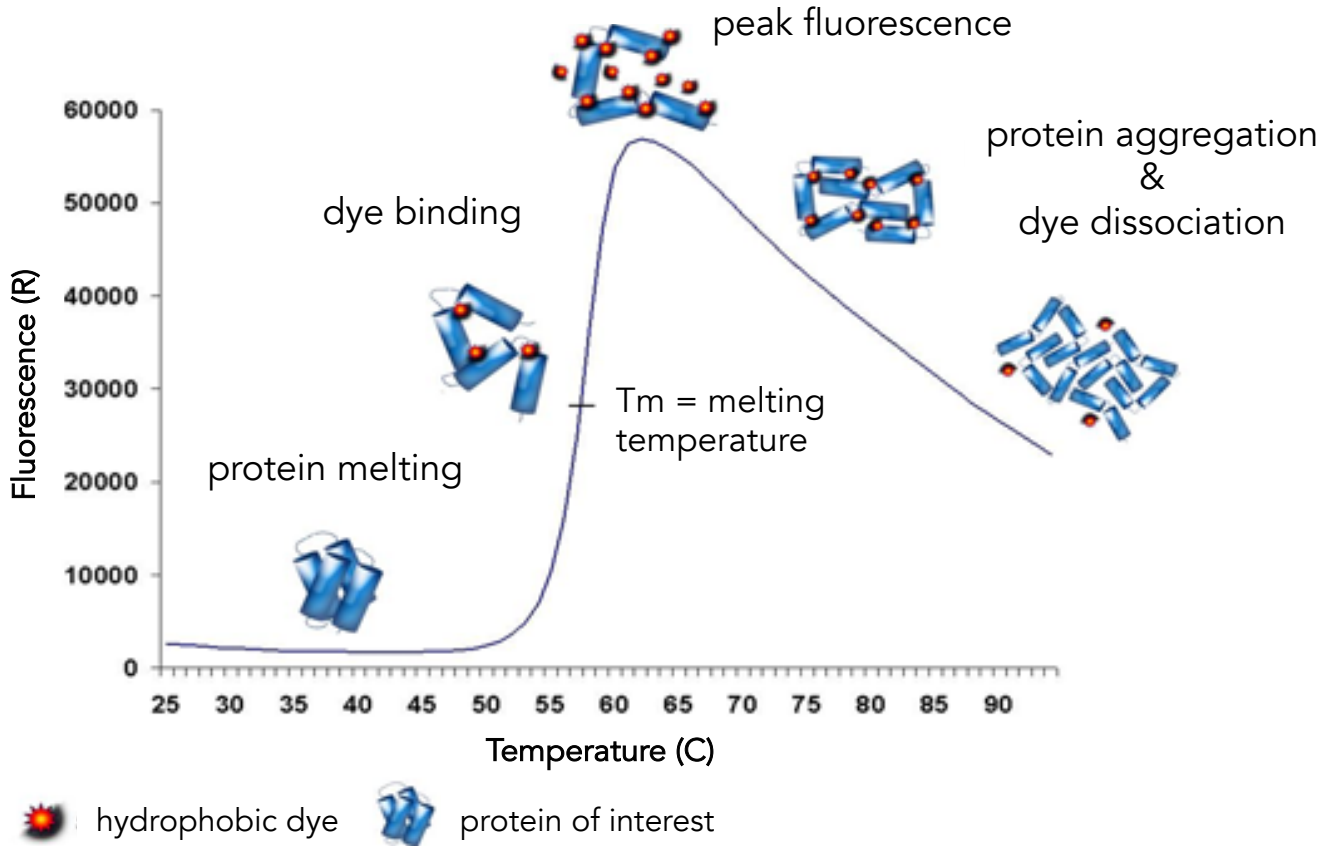
test ligands in FKBP12
binding assay



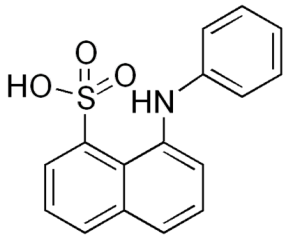
IC_{50} = X μM
ΔT = Y °C

complete data analysis
prioritize best ligands

Measuring a thermal melt profile for a protein



Dyes used to detect protein unfolding

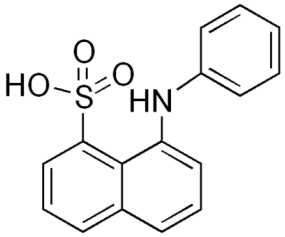


ANS

8-anilinonapthalene-1-sulfonic acid

(1965)

Dyes used to detect protein unfolding



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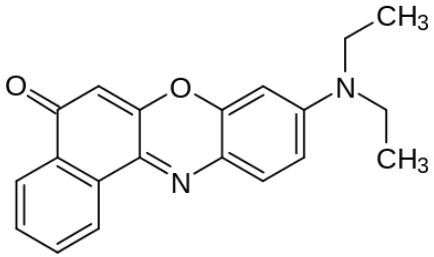


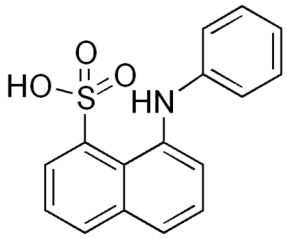
Nile Red

9-diethylamino-5-benzo[a]phenoxazinone
(1985)

solvatochromic

Nile Red under visible and
UV light in different solvents

Dyes used to detect protein unfolding



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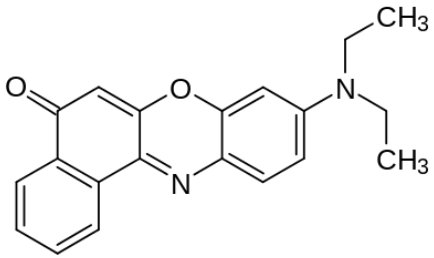

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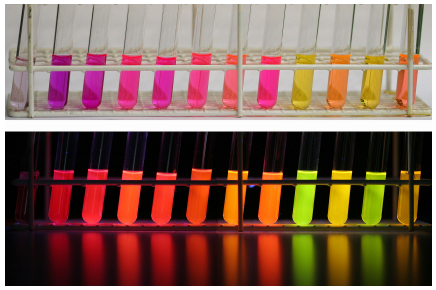
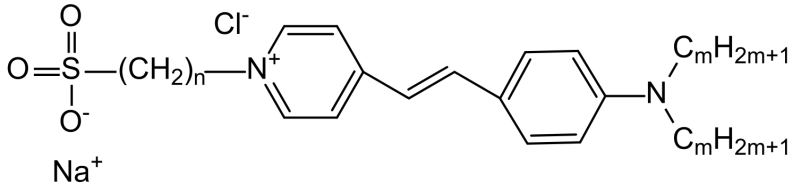


solvatochromic

Nile Red under visible and
UV light in different solvents

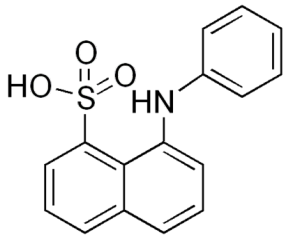


SYPRO® Orange

Most common dye for DSF/TS
(2004)

binds nonspecifically to hydrophobic surfaces;
water quenches fluorescence

Dyes used to detect protein unfolding



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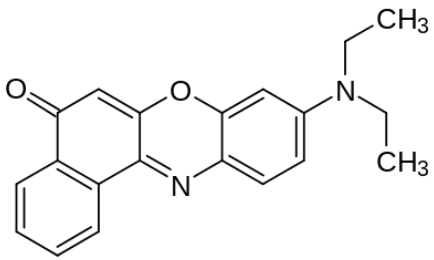

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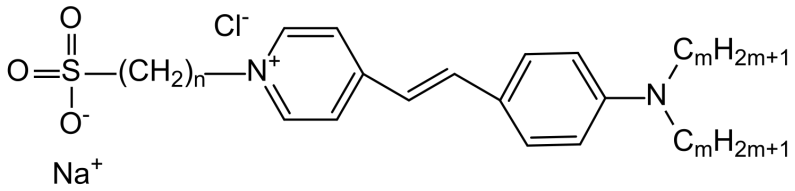
Nile Red under visible and UV light in different solvents



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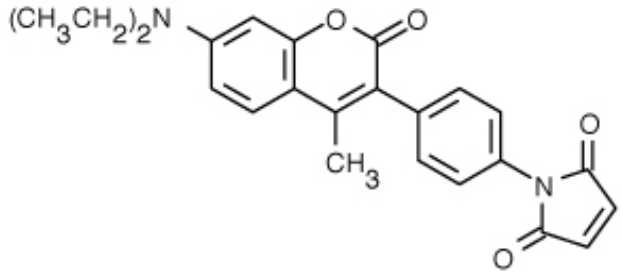
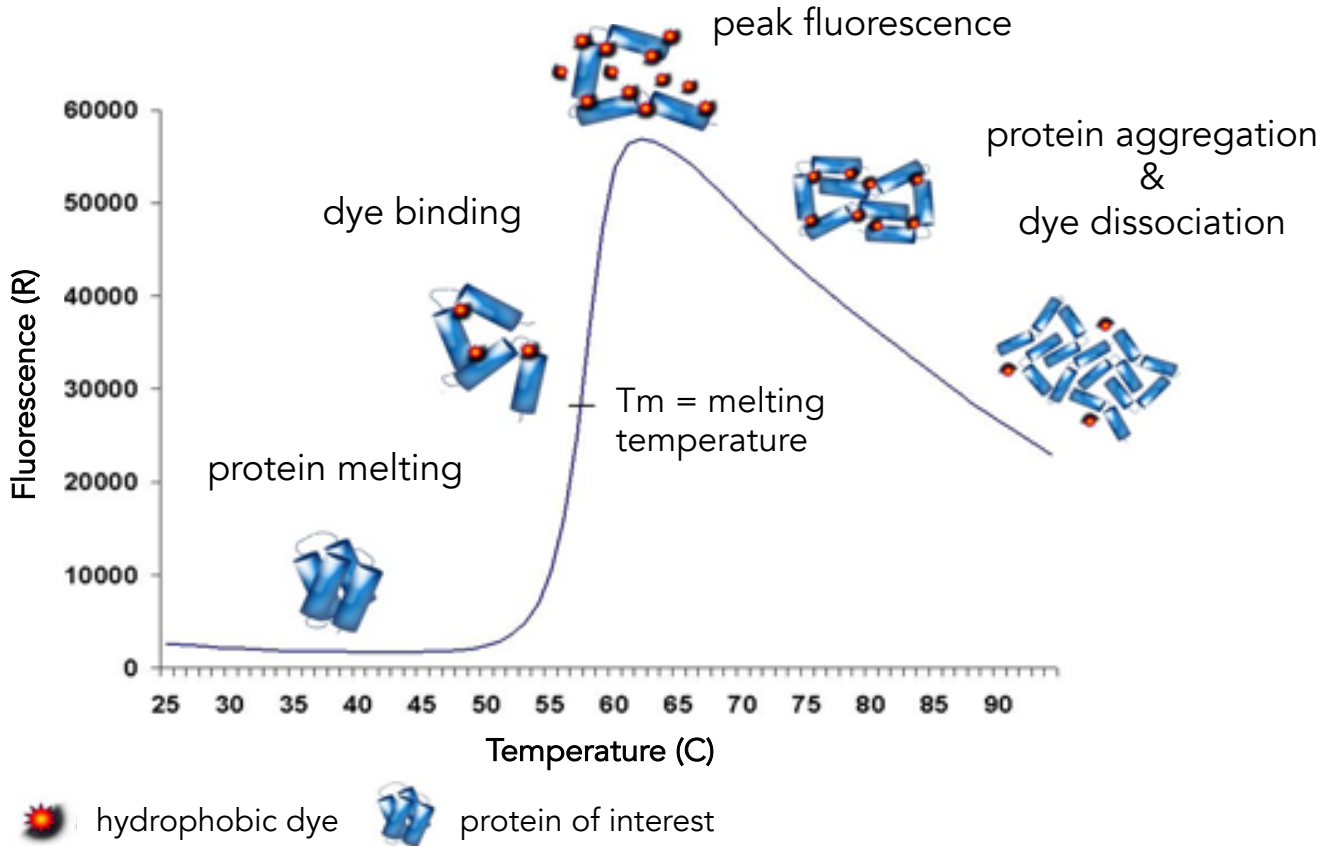


CPM

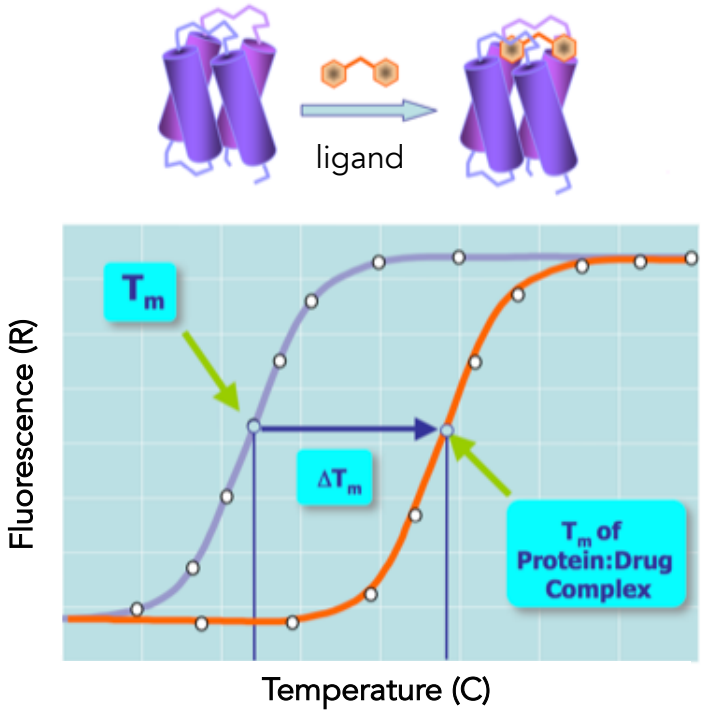
N-[4-(7-diethylamino-4-methyl-3-coumarinyl)phenyl]maleimide
(2008)

only fluoresces after reacting with Cys residues

What happens when you add a small molecule?

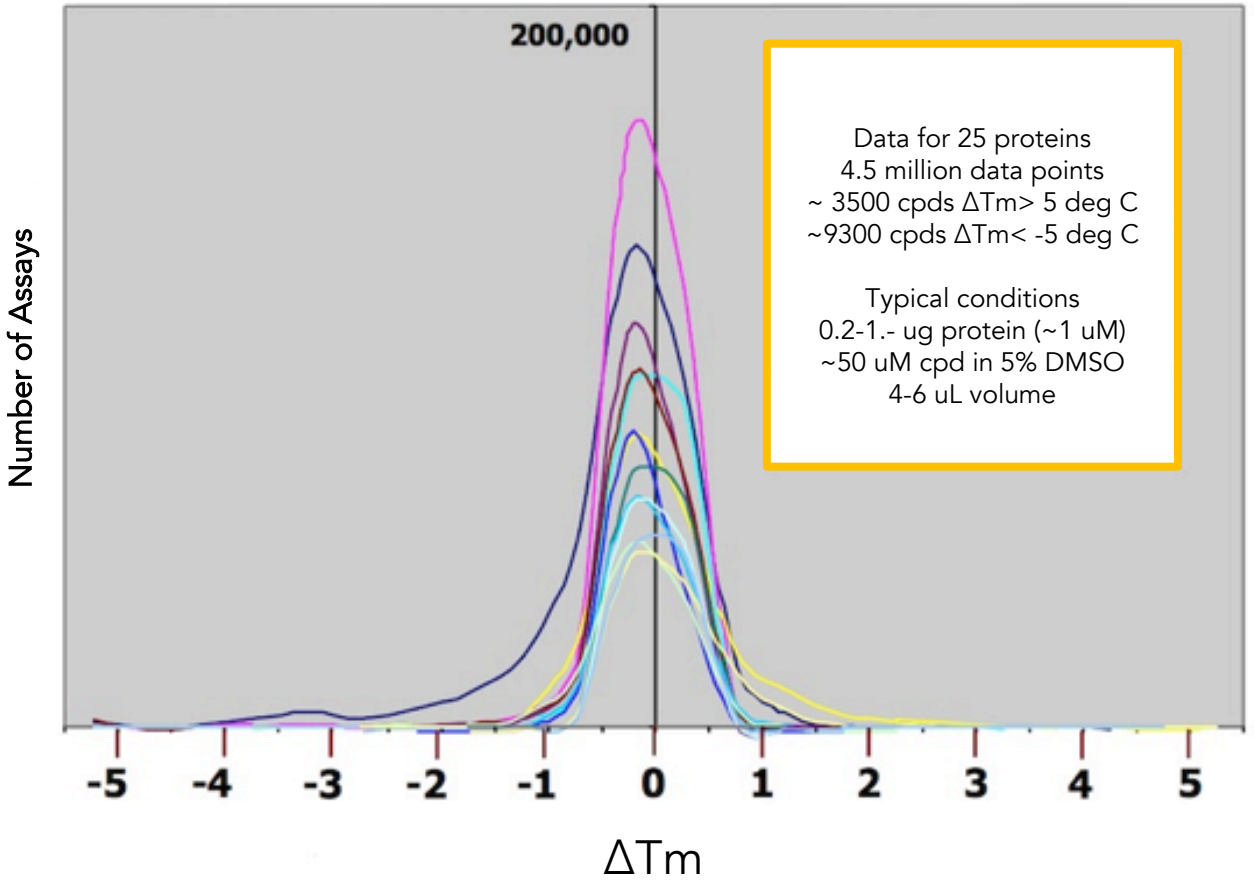


Thermal shift assays with small molecules



Adapted from Dr. Salemme at thermofluor.org

Real thermal shift screens with small molecules

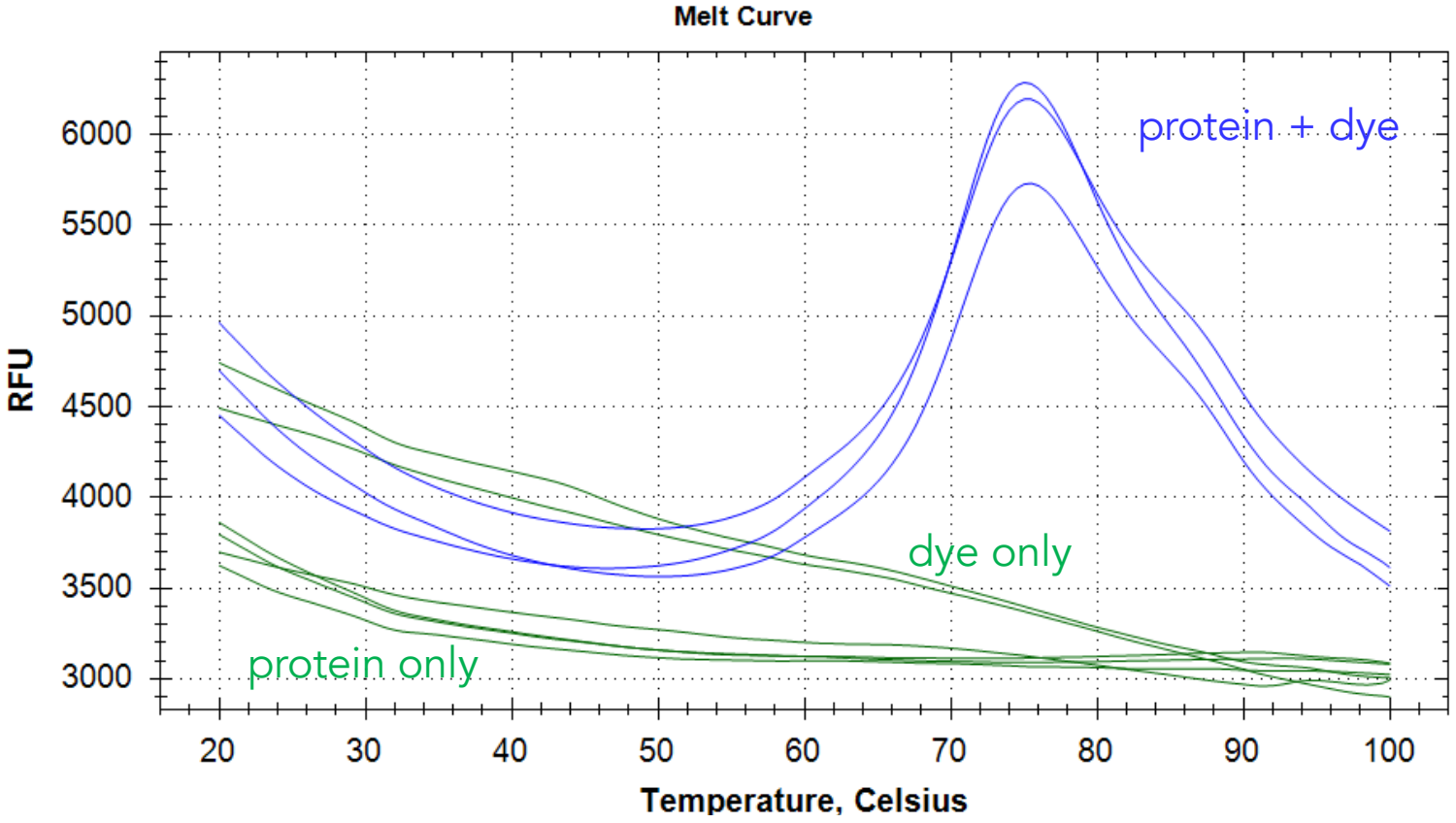


preferential ligand binding to unfolded states?

Adapted from Dr. Salemme at thermofluor.org

Real results from thermal shift studies

assay development

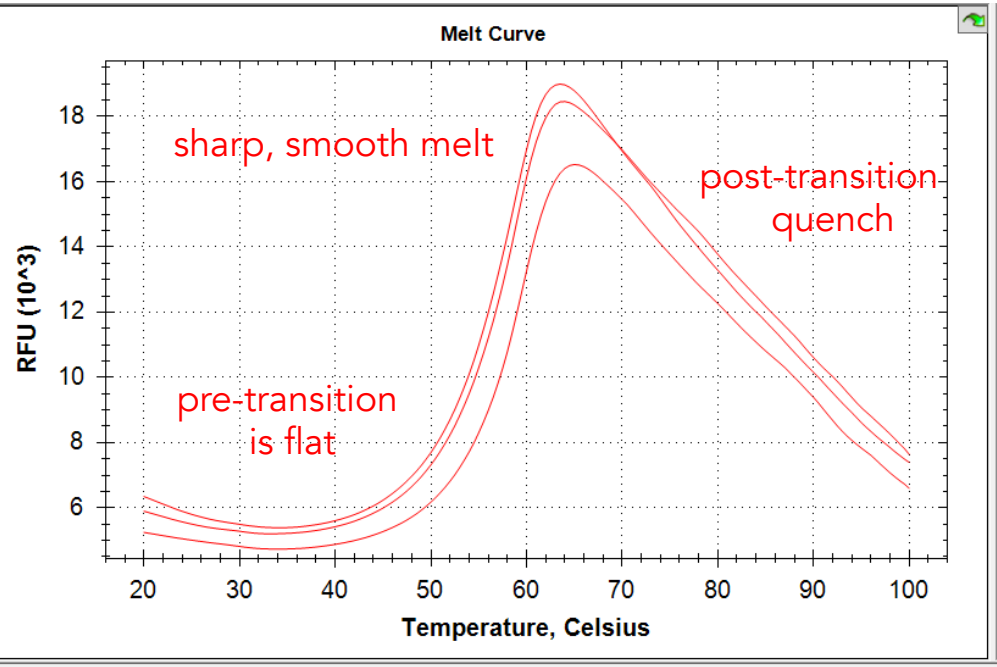


consider optimizing buffer conditions – pH, cofactors

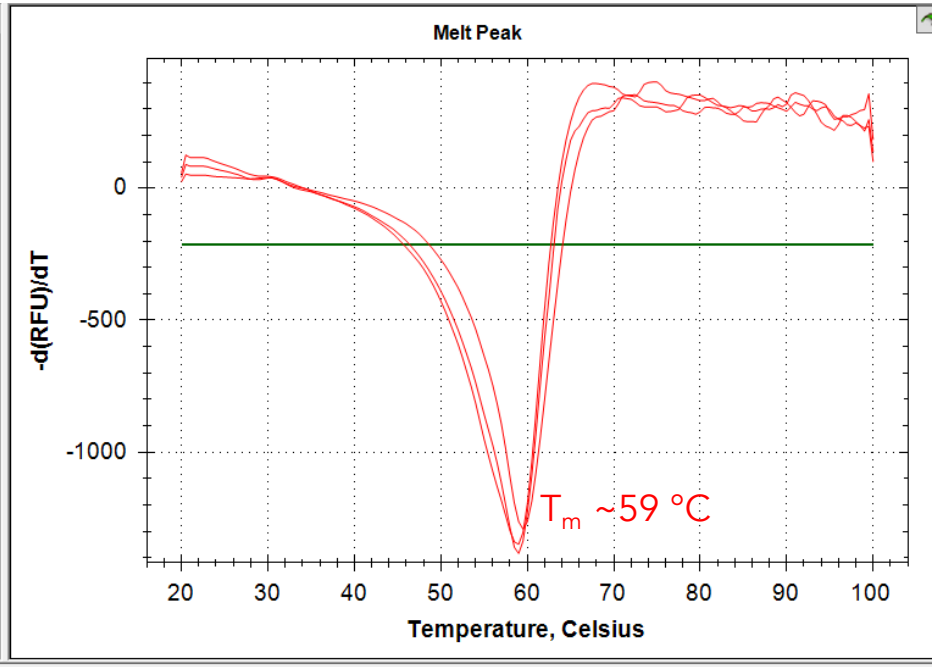
Adapted from Collaborative Crystallisation Centre

Real results with thermal shift assays

three replicates for a single experiment



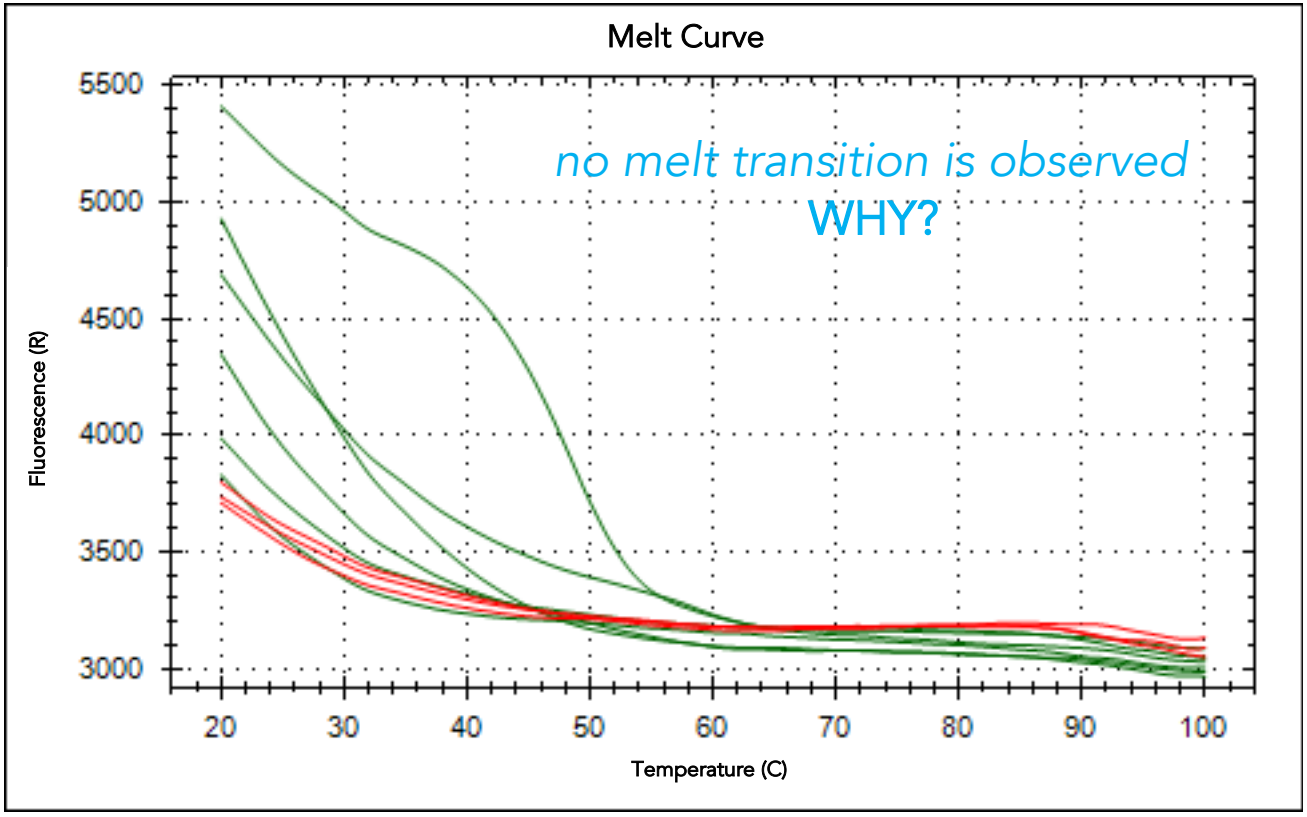
raw fluorescence thermal curves



first derivative representation

Adapted from Collaborative Crystallisation Centre

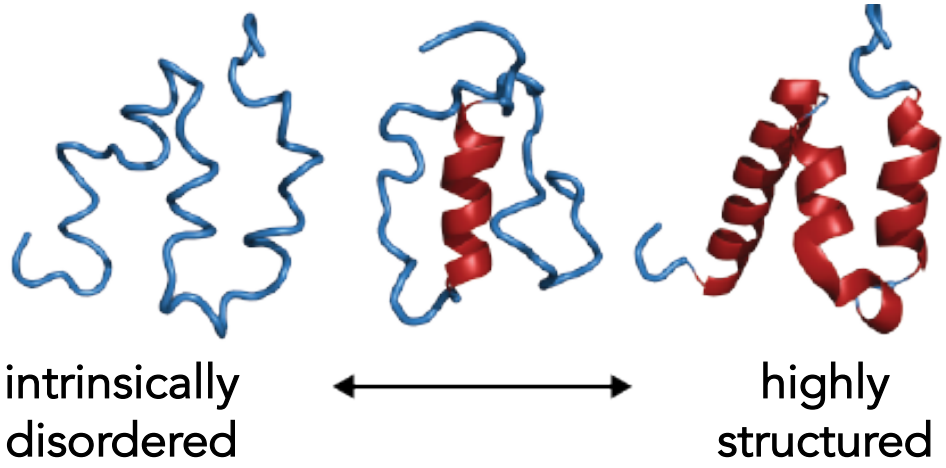
Real results with thermal shift assays



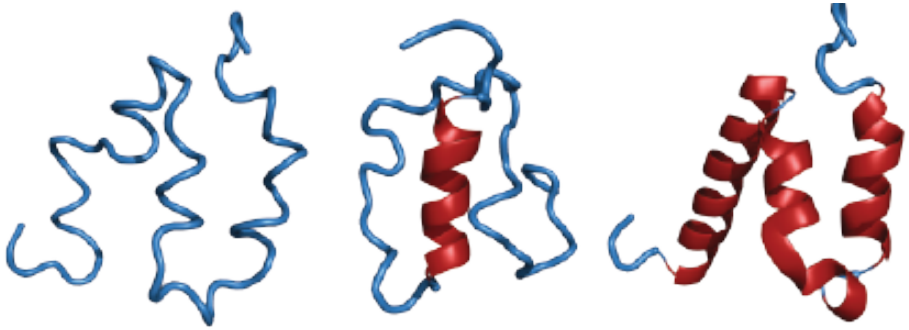
raw fluorescence thermal curves

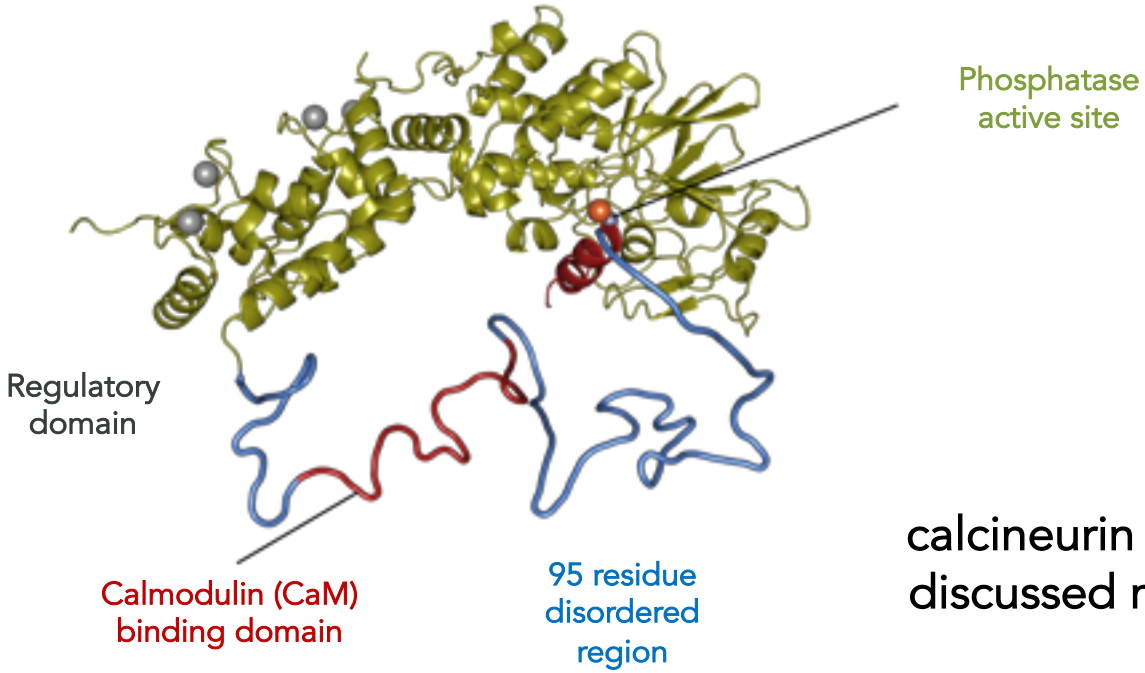
Adapted from Collaborative Crystallisation Centre

Protein disorder continuum



Protein disorder continuum

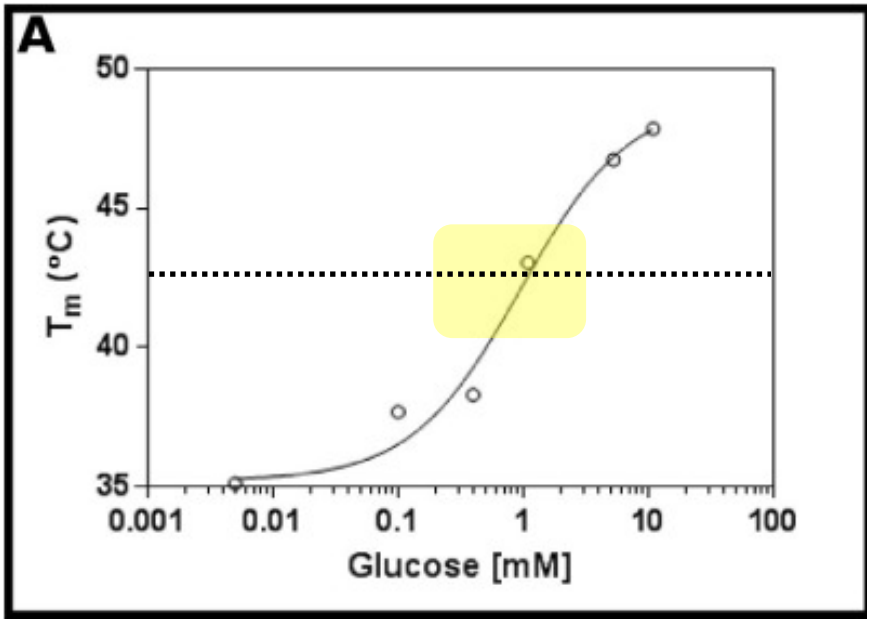




calcineurin will be discussed more in L5

Determining apparent dissociation constants

hexokinase (receptor) and glucose (ligand)



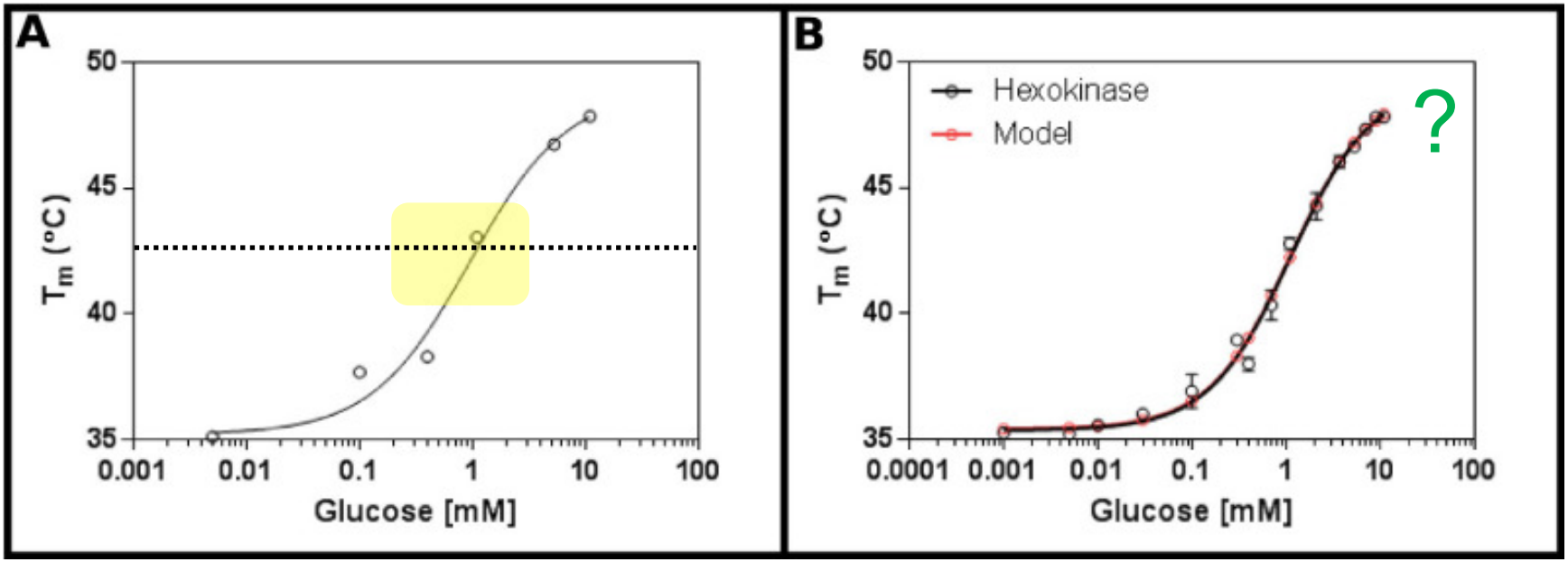
Experiment 1:

test a wide range of glucose concentrations

K_D is likely between 0.2 and 1.7 mM

Determining apparent dissociation constants

hexokinase (receptor) and glucose (ligand)



Experiment 1:

test a wide range of glucose concentrations

K_D is likely between 0.2 and 1.7 mM

Experiment 2:

test 16 concentration of glucose
fit to single binding event model (red)

apparent K_D ~ 1.12 +/- 0.05 mM

Determining apparent dissociation constants

Step-by-step protocols with more examples

jove Journal of Visualized Experiments www.jove.com

Video Artic http://www.jove.com

Determination of Protein-ligand Interactions Using Differential Scanning Fluorimetry

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URL: http://www.jove.com/video/51809
DOI: doi:10.3791/51809

Keywords: Biophysics, Issue 91, differential scanning fluorimetry, dissociation constant, protein-ligand interactions, StepOne, cooperativity, WcbI.

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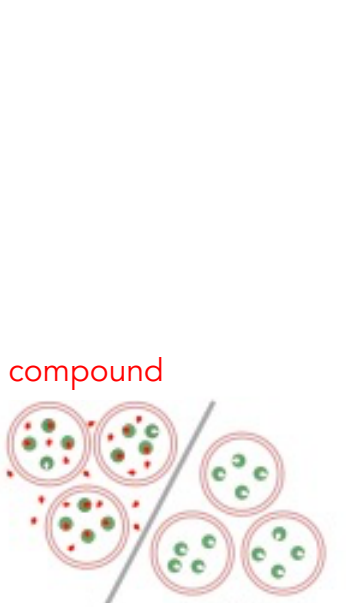
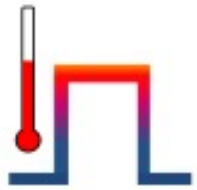
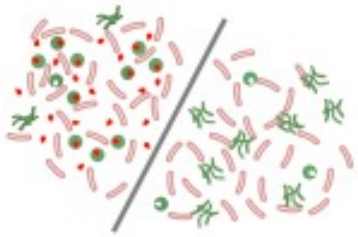
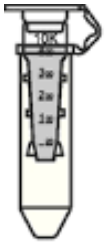
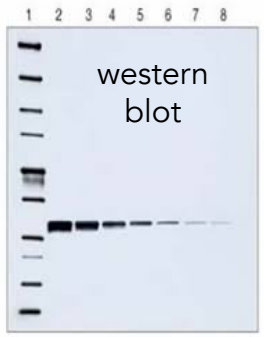
Target engagement in cells:
cellular thermal shift assays (CETSA)

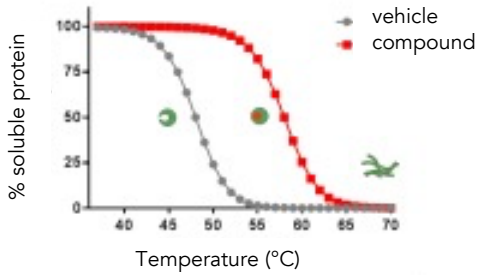








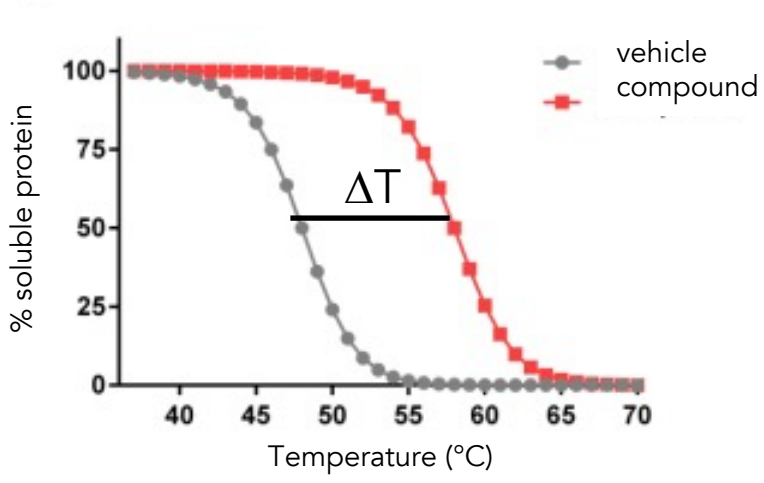




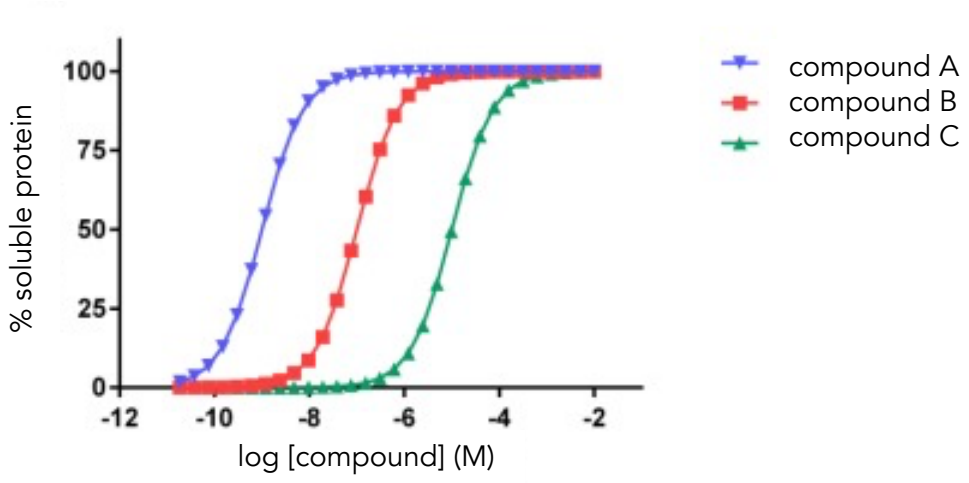
compound treatment in cells → heating and cooling → lyse cells → separation (optional) → detection

Anticipated results from CETSA assays

T_{agg} curve



ITDRF curves

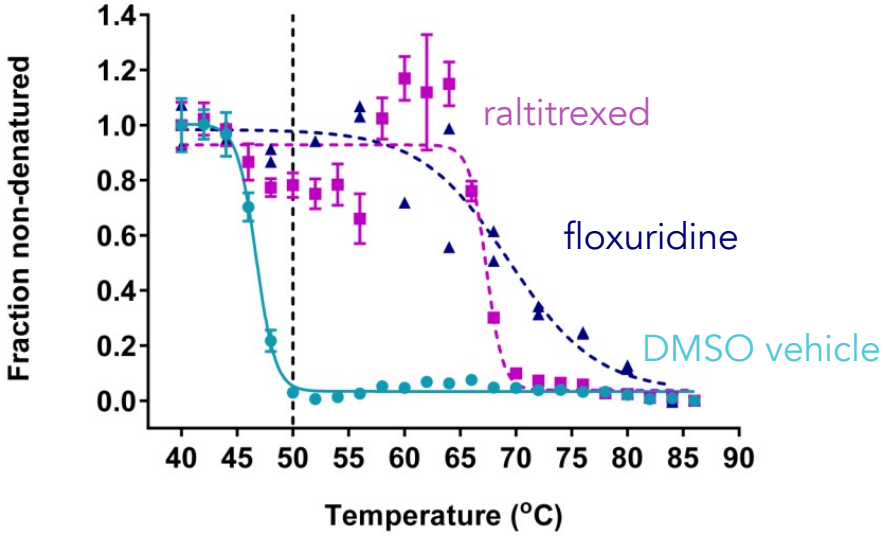


IsoThermal Dose Response Fingerprint
'apparent potencies' at single temp

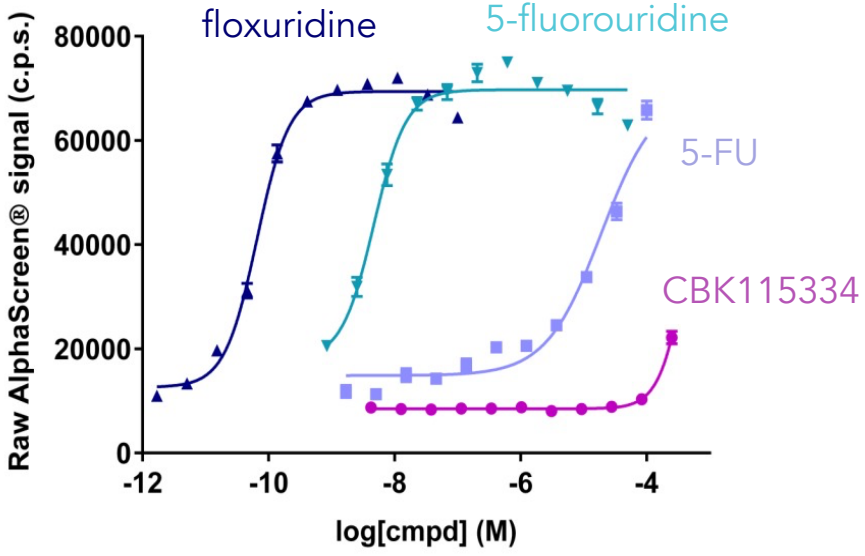
Real results from CETSA assays

thymidylate synthase drugs in K562 cells

T_{agg} curve

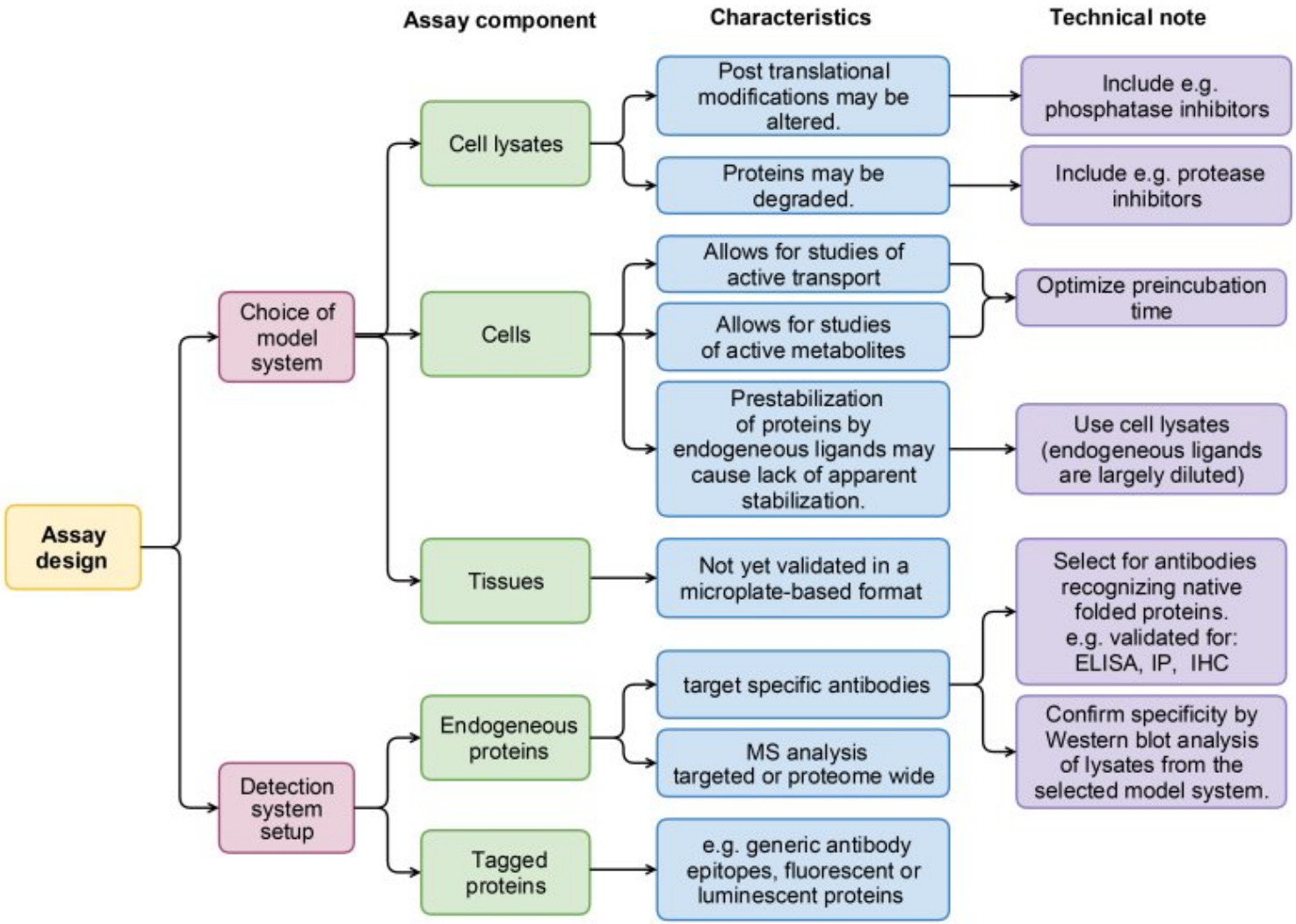


ITDRF curves at 50 °C



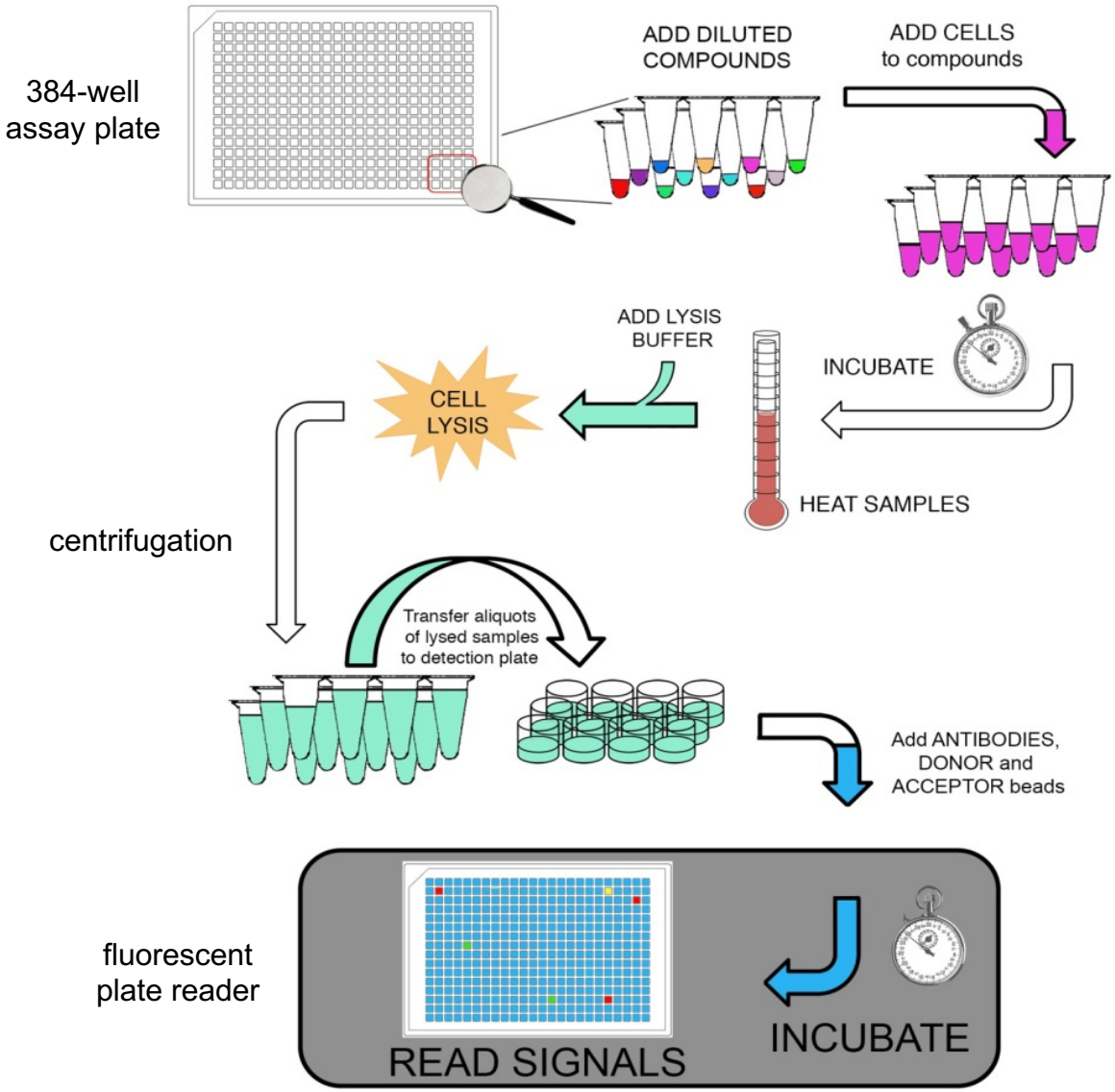
quadruplicate data from one independent experiment

General considerations for CETSA design



Adapted from the NIH Assay Guidance Manual

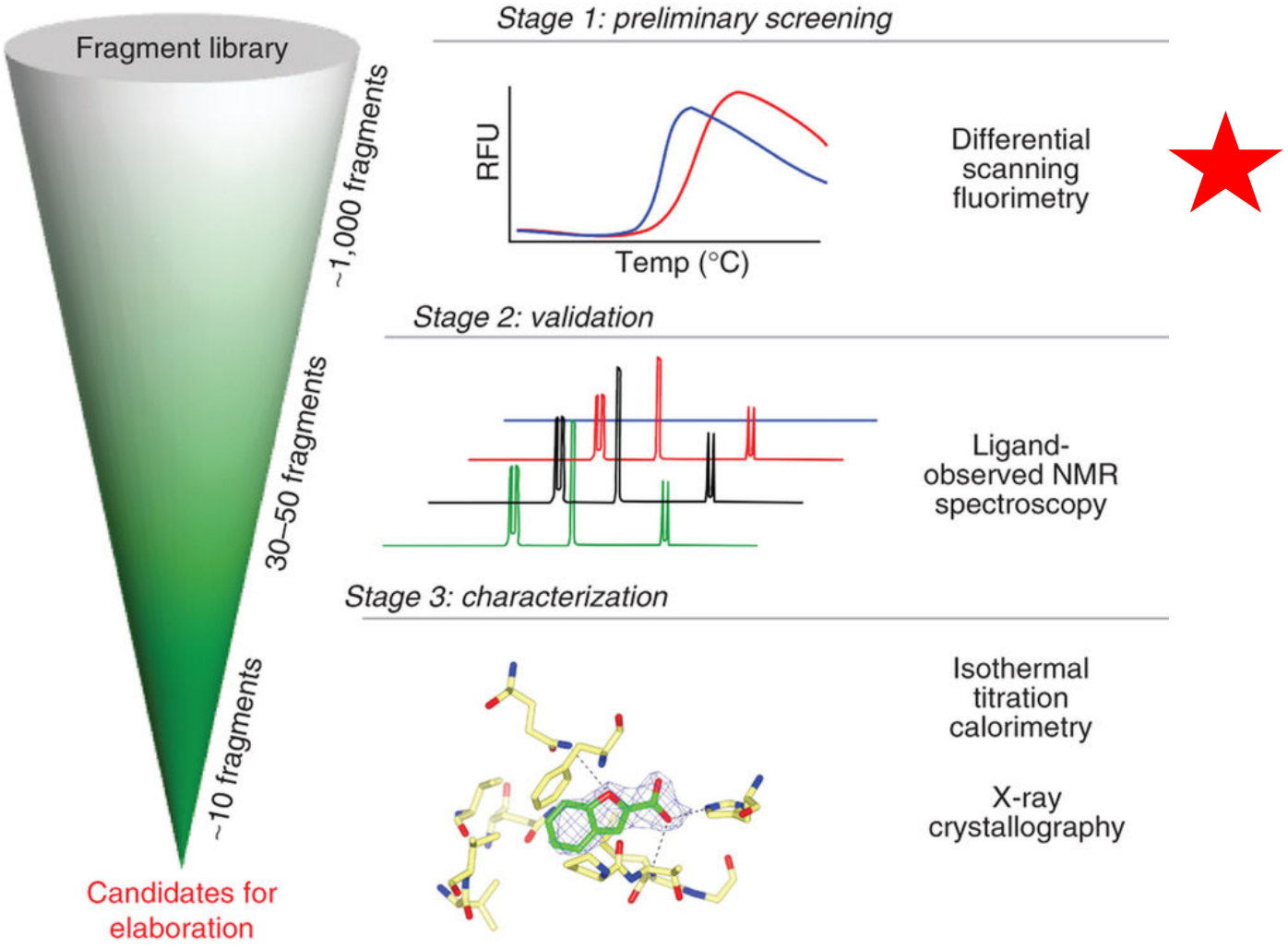
CETSA for high-throughput screening



Adapted from the NIH Assay Guidance Manual

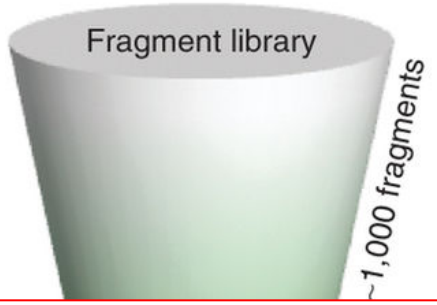
Small molecule stabilizers to aid crystallization

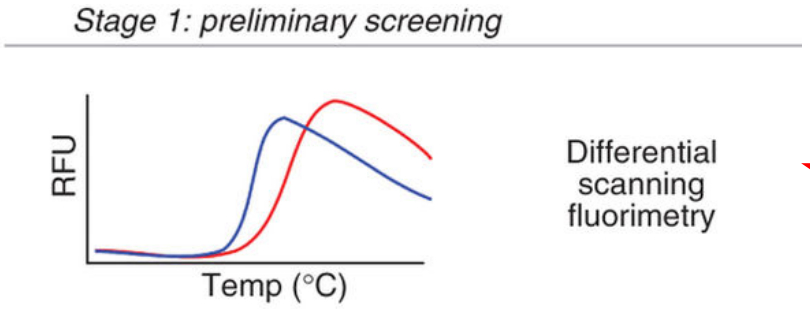
improving structural biology efforts



Small molecule stabilizers to aid crystallization

improving structural biology efforts





PNAS

Chemical screening methods to identify ligands that promote protein stability, protein crystallization, and structure determination

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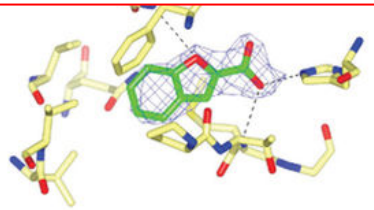
Candidates for elaboration



X-ray crystallography