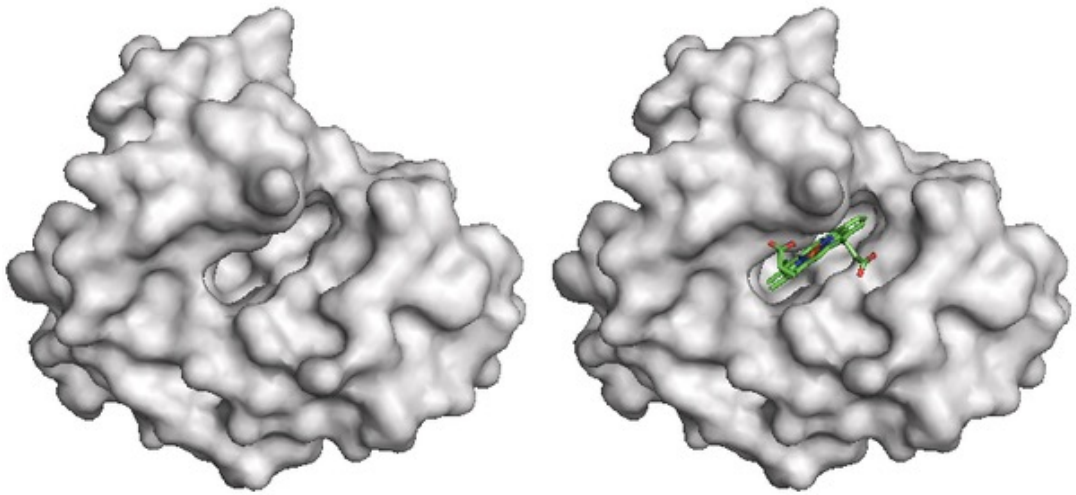
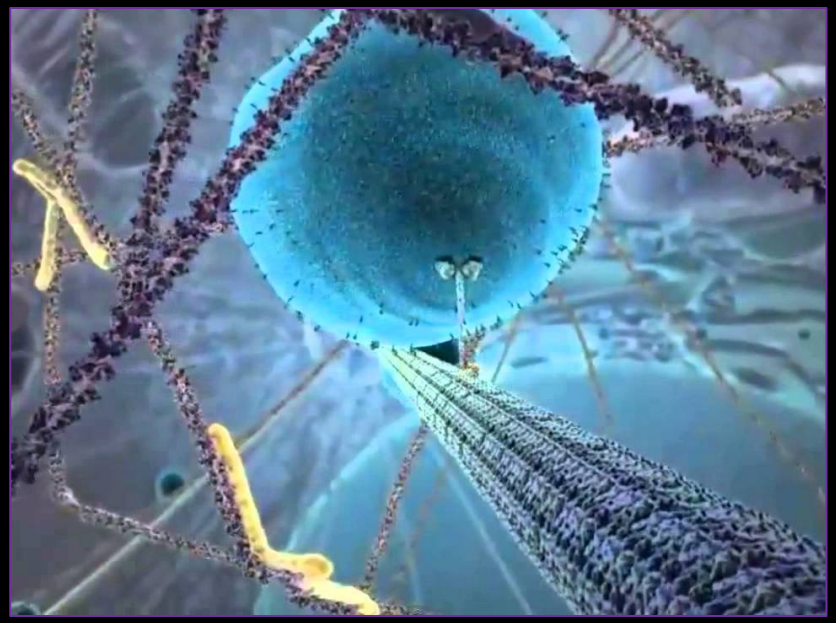
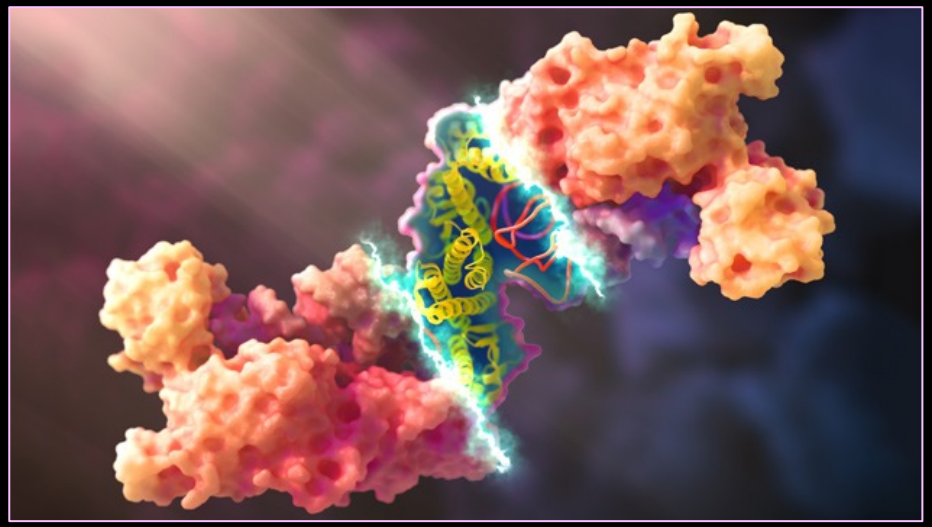


L4 – Quantitative Evaluation of Binding Interactions

February 15, 2022

The Inner Life of the Cell – Dr. 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

from 20.110

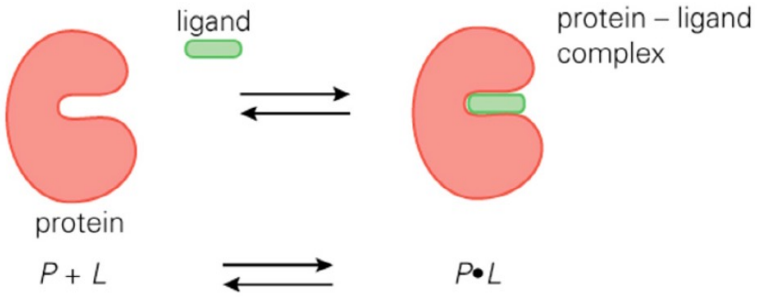
Affinity: strength of the interaction, measured by the corresponding decrease in free energy upon binding

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

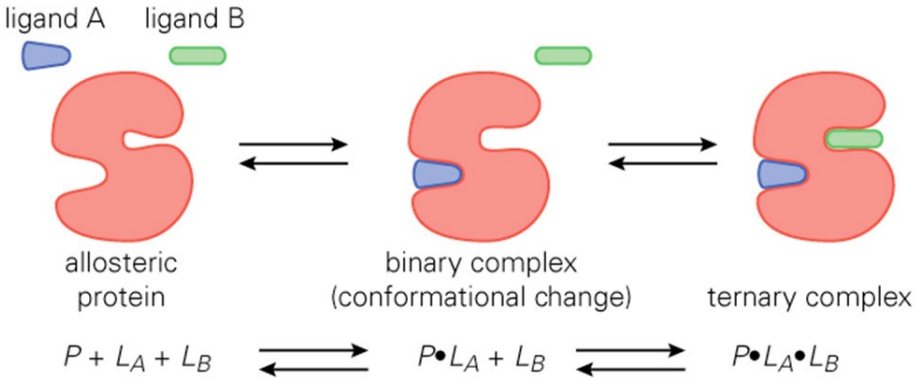
Some binding interactions are 'simple' equilibria – each encounter is independent

simple interaction



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

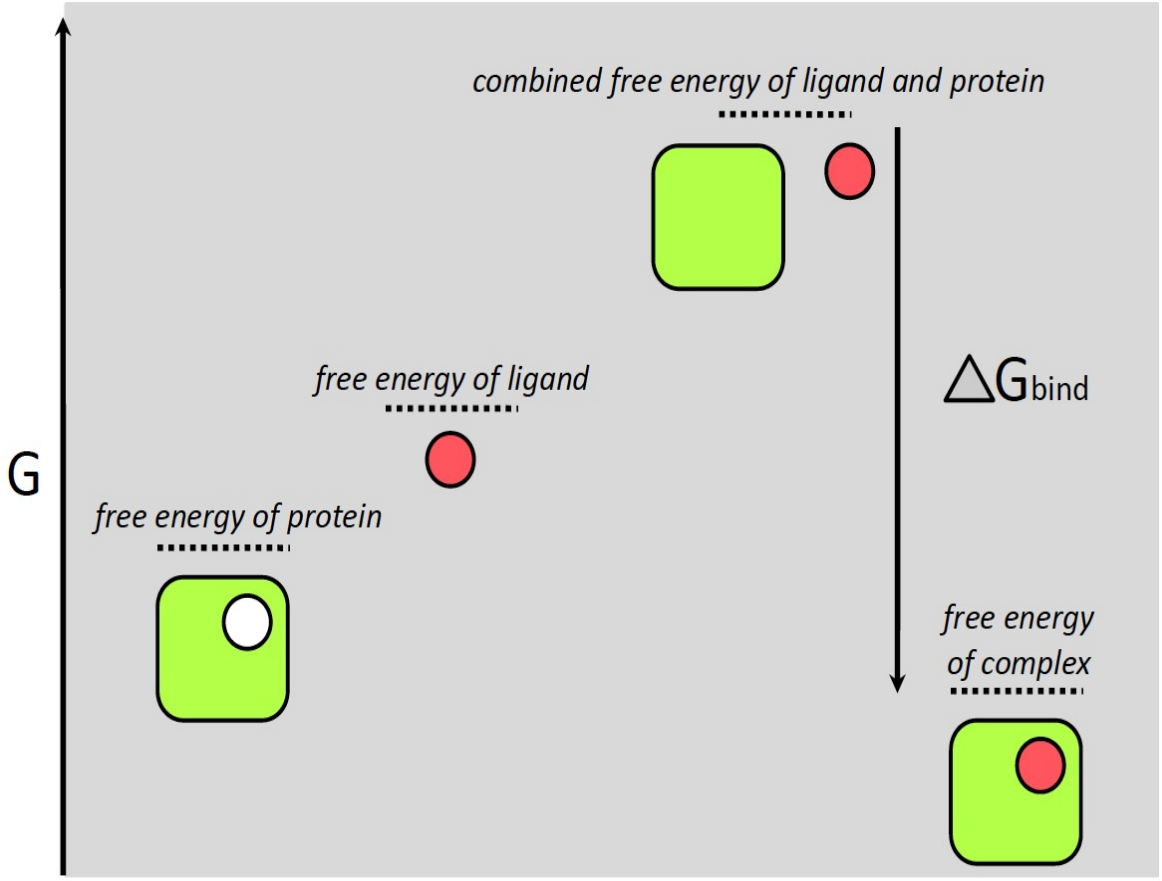
Thermodynamic analyses provide insight into molecular interactions

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:

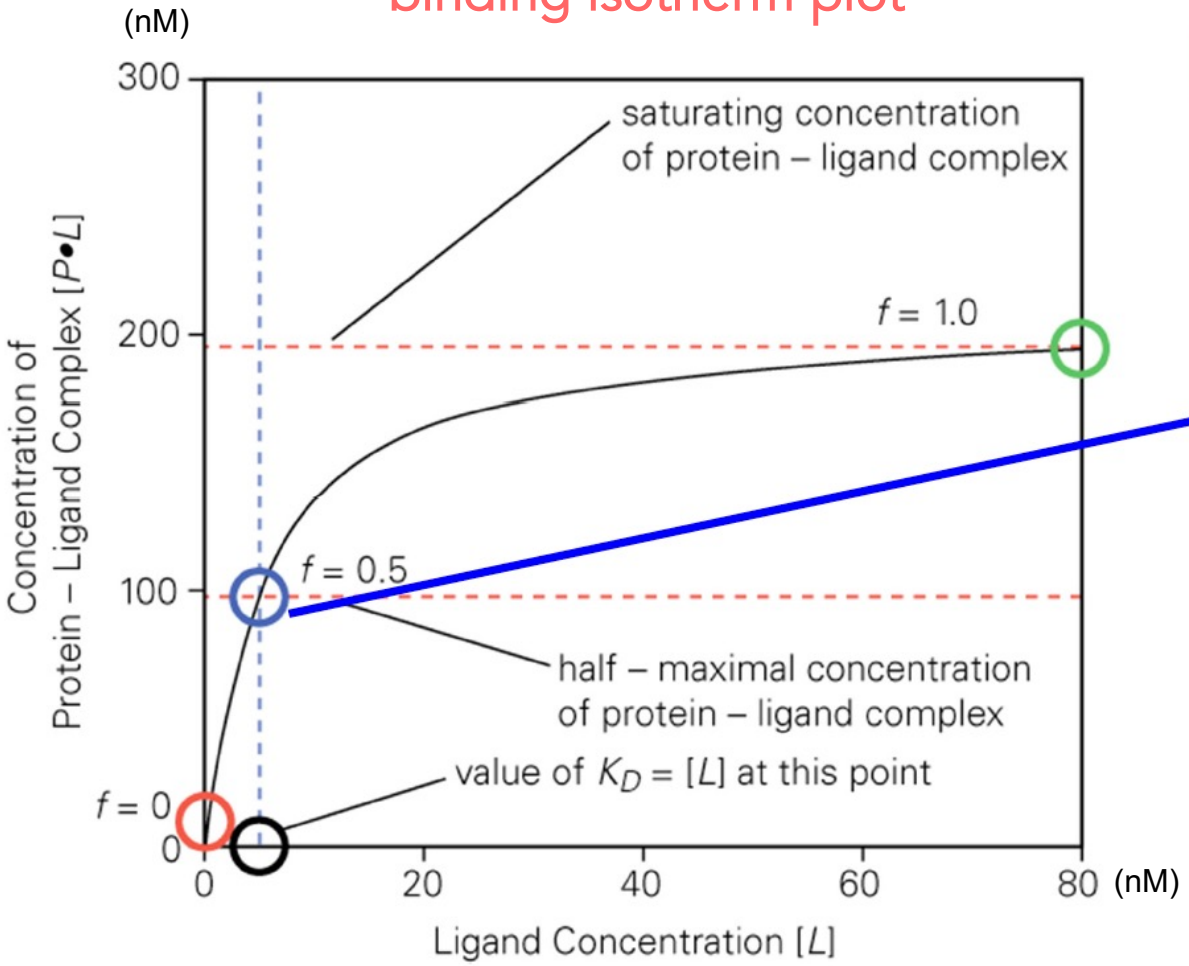
$$\Delta G^{\circ}_{bind} = -RT \ln K_A$$

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

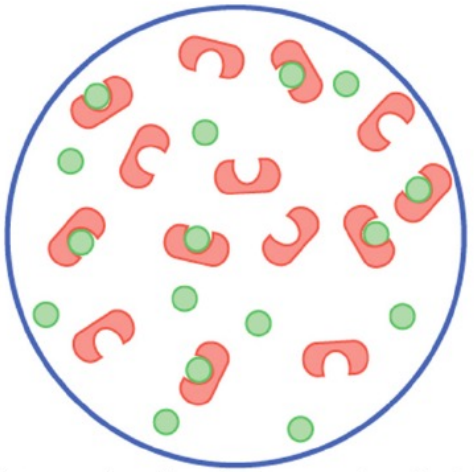
$$\Delta G^{\circ}_{bind} = +RT \ln K_D$$

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

'binding isotherm plot'



$R \bullet L \leftrightarrows R + L$

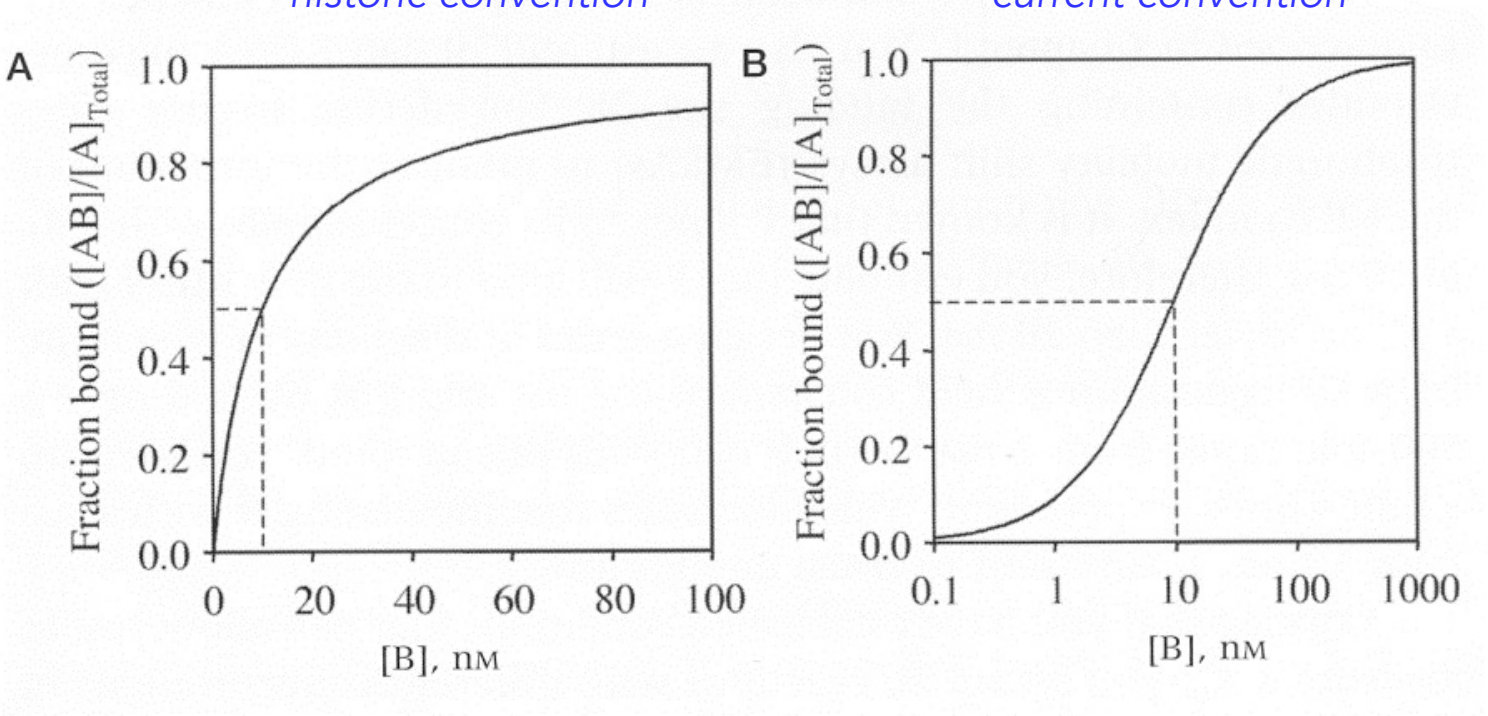


intermediate ligand concentration: $f = 0.5$
$K_D = [L]$ when $f = 0.5$

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**

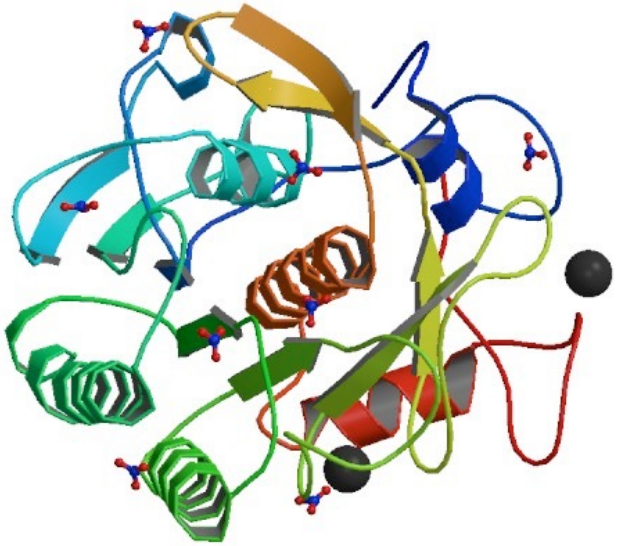
higher K_D value
weaker interaction

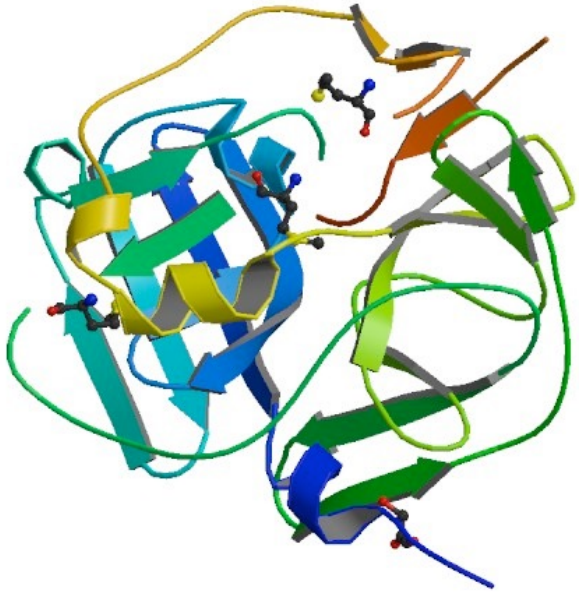
lower K_D value
stronger interaction

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

Lab Use - DNA/RNA preps

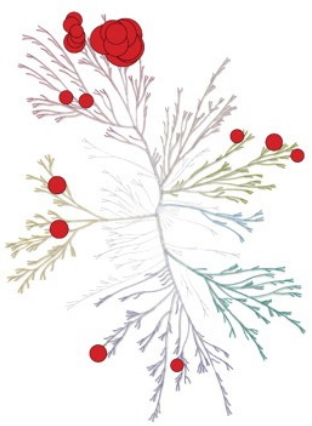
HRV 3C Protease

high specificity

Leu-Glu-Val-Leu-Phe-Gln/Gly-Pro

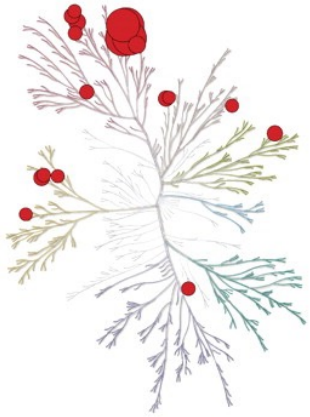
Lab Use – cleaving fusion proteins

Specificity in molecular recognition – kinase drugs



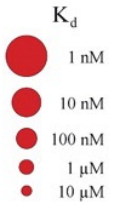
AC220

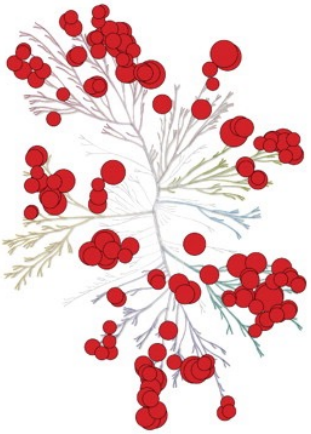
CEP-701



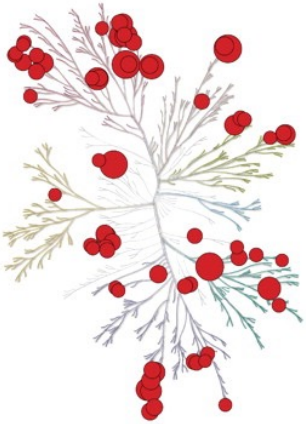
MLN-518

binding constants

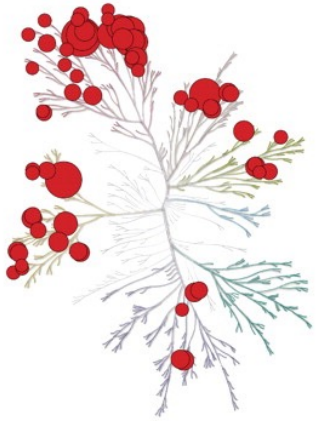




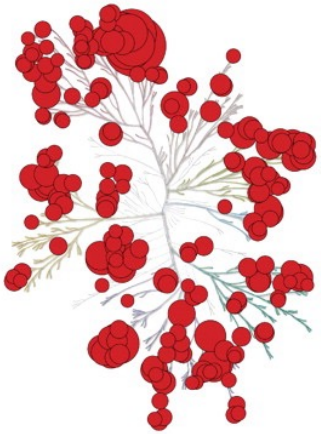
PKC-412



CGP-52421



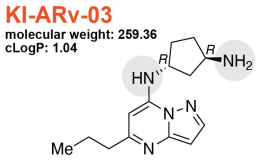
Sorafenib

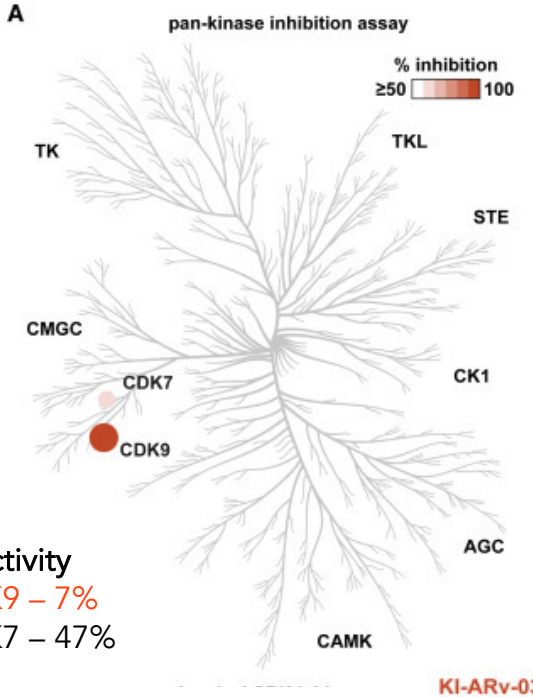


Sunitinib

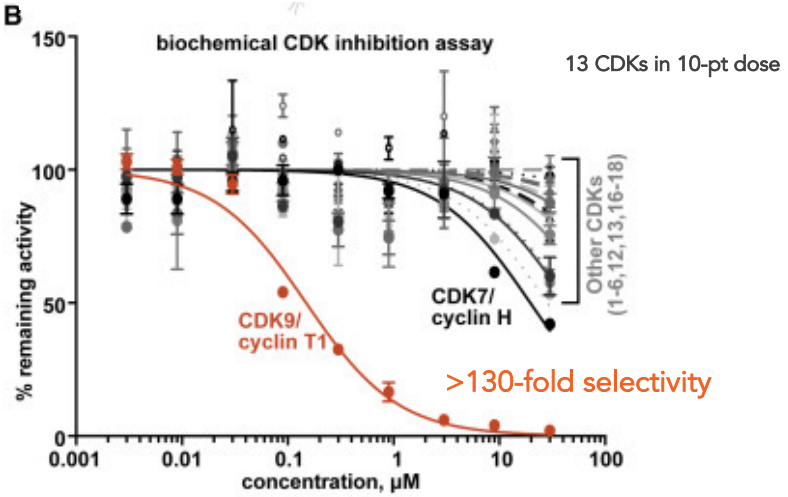
Adapted from Zarrinkar et al, Blood (2009), 114: 2984-2992

Recent example from my lab

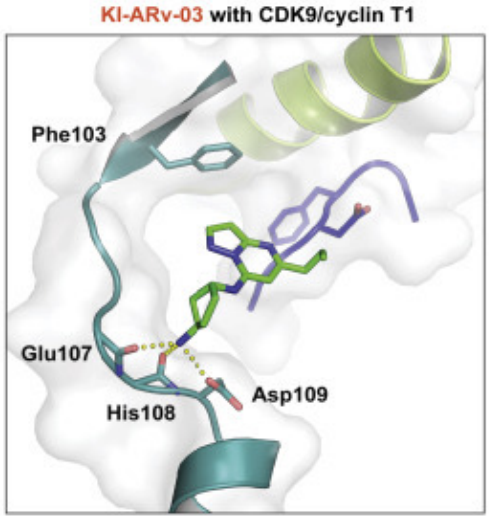




% activity
CDK9 – 7%
CDK7 – 47%

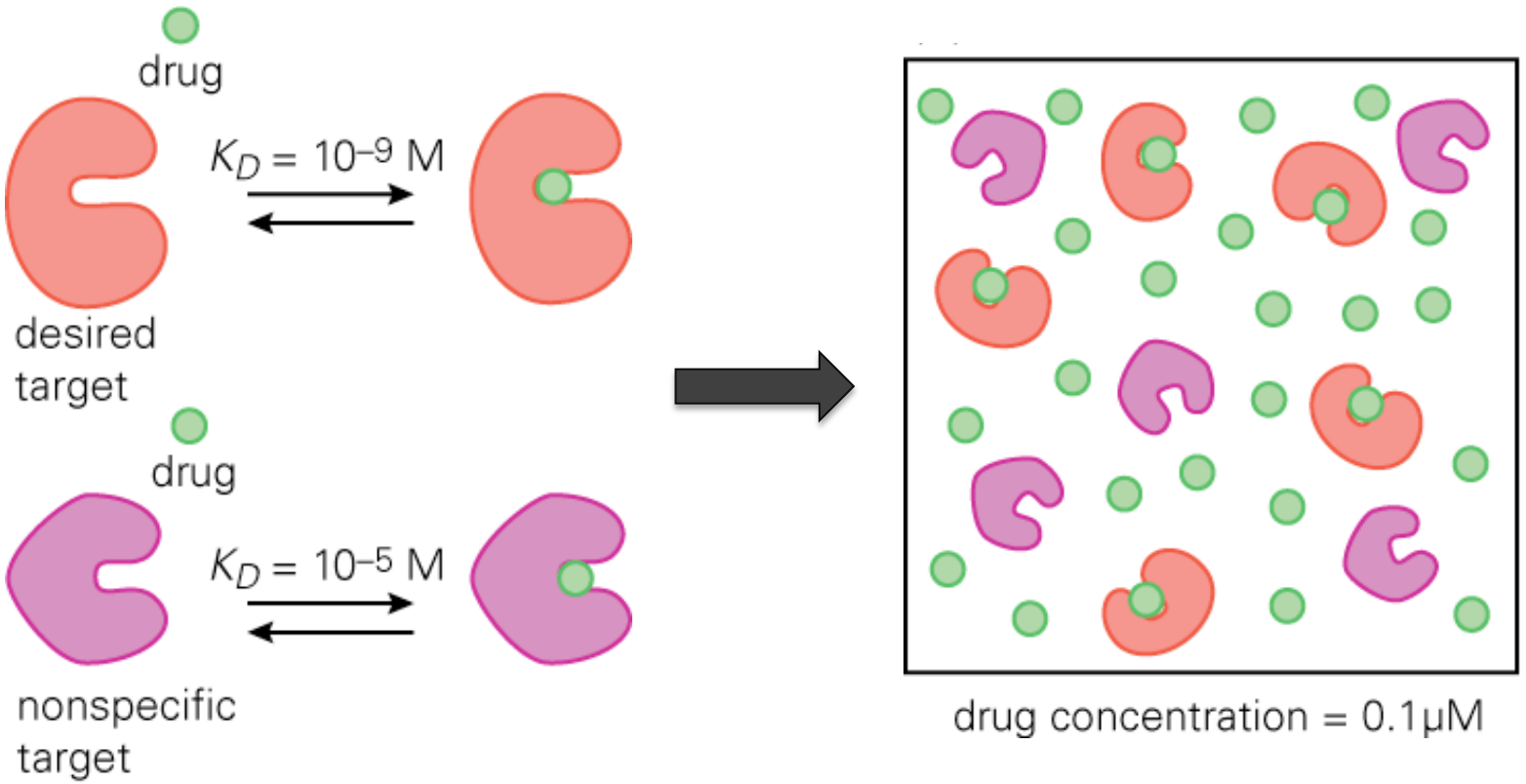


CDK9 IC_{50} = 0.15 μM (45 μM ATP)
CDK7 IC_{50} = 20.1 μM



Specificity in drug binding – fractional saturation

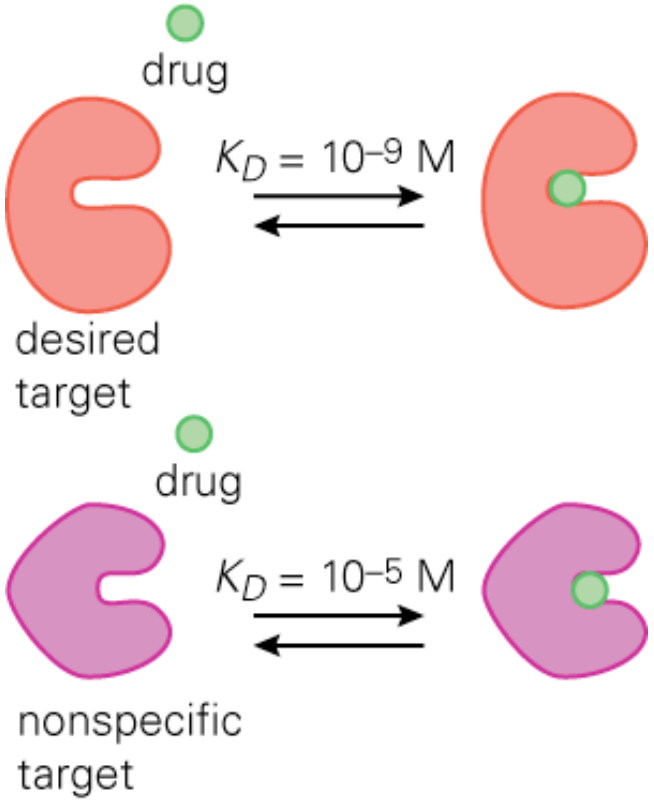
deliver the drug at a concentration below the K_D for non-cognate target



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

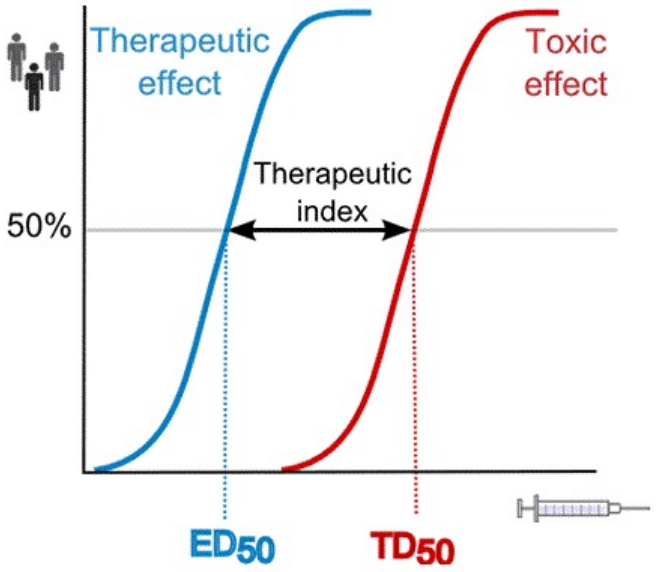
Specificity in drug binding – fractional saturation

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



impact therapeutic effects?

'Therapeutic Window'

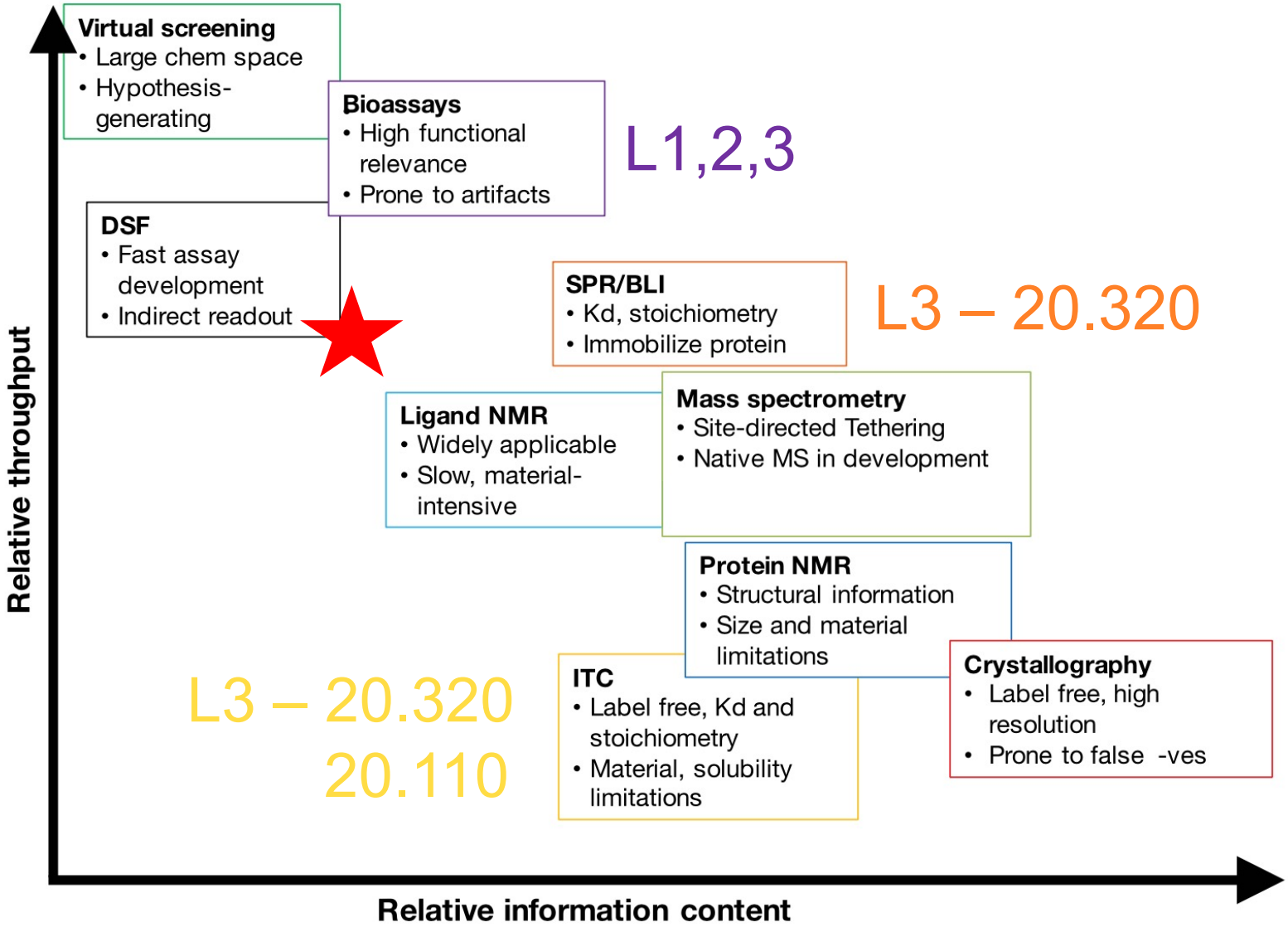


ED_{50} = effective in 50% patients

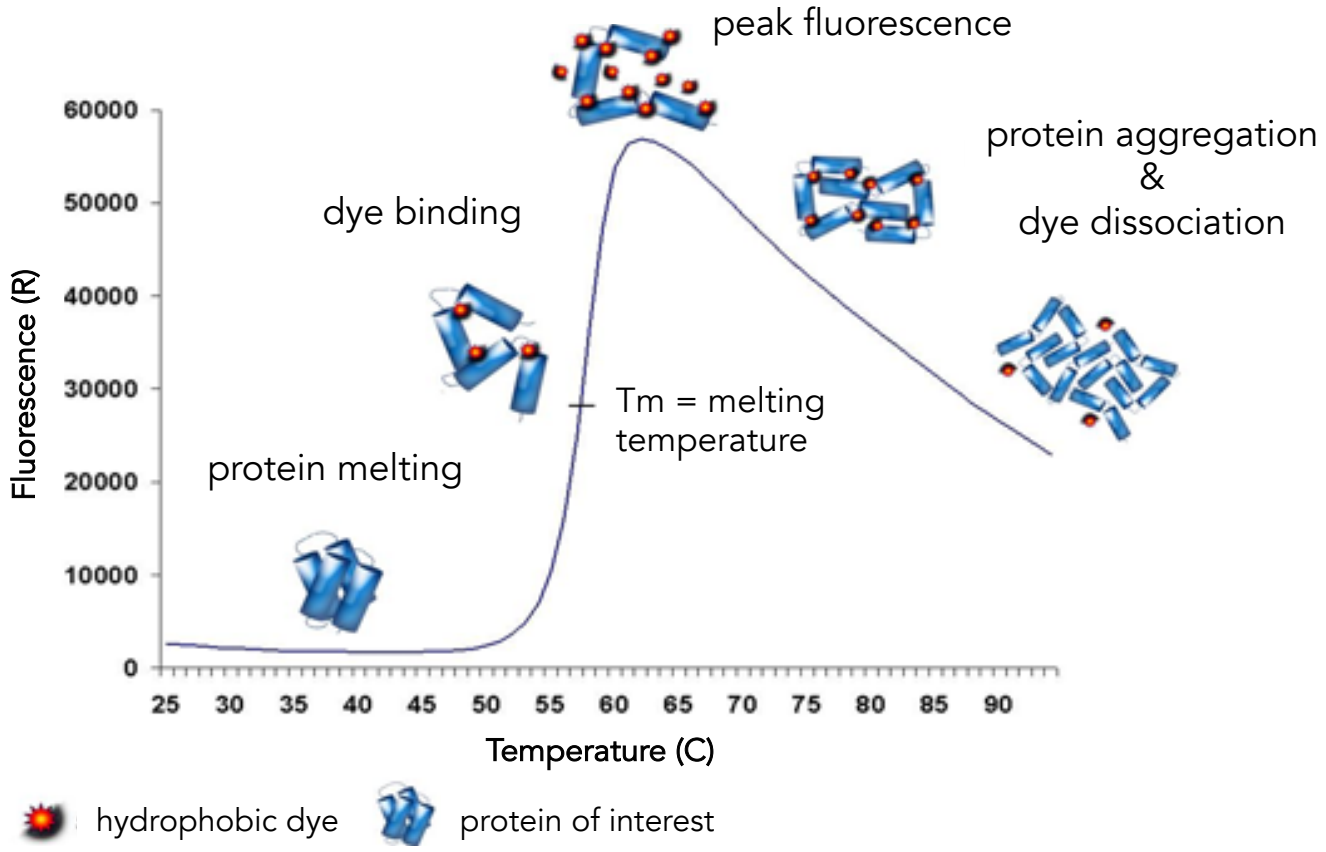
TD_{50} = toxic in 50% patients

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

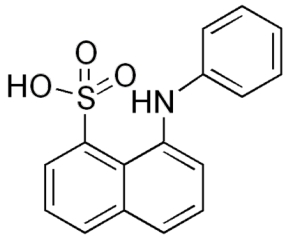
Methods to find or evaluate binding interactions



Measuring a thermal melt profile for a protein

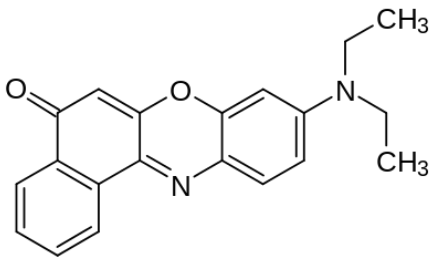


Dyes used to detect protein unfolding



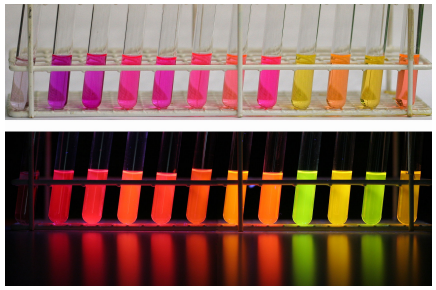
ANS

8-anilinonapthalene-1-sulfonic acid
(1965)



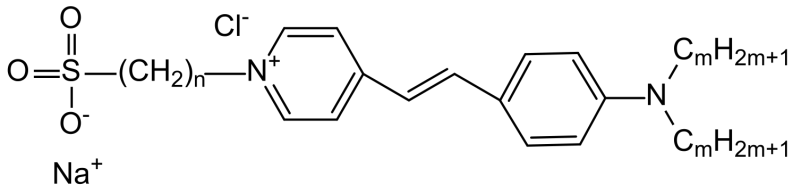
Nile Red

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



solvatochromic

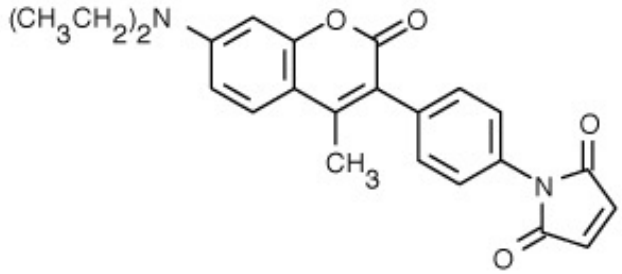
Nile Red under visible and UV light in different solvents



SYPRO® Orange

Most common dye for DSF/TS
(2004)

binds nonspecifically to hydrophobic surfaces; water quenches fluorescence

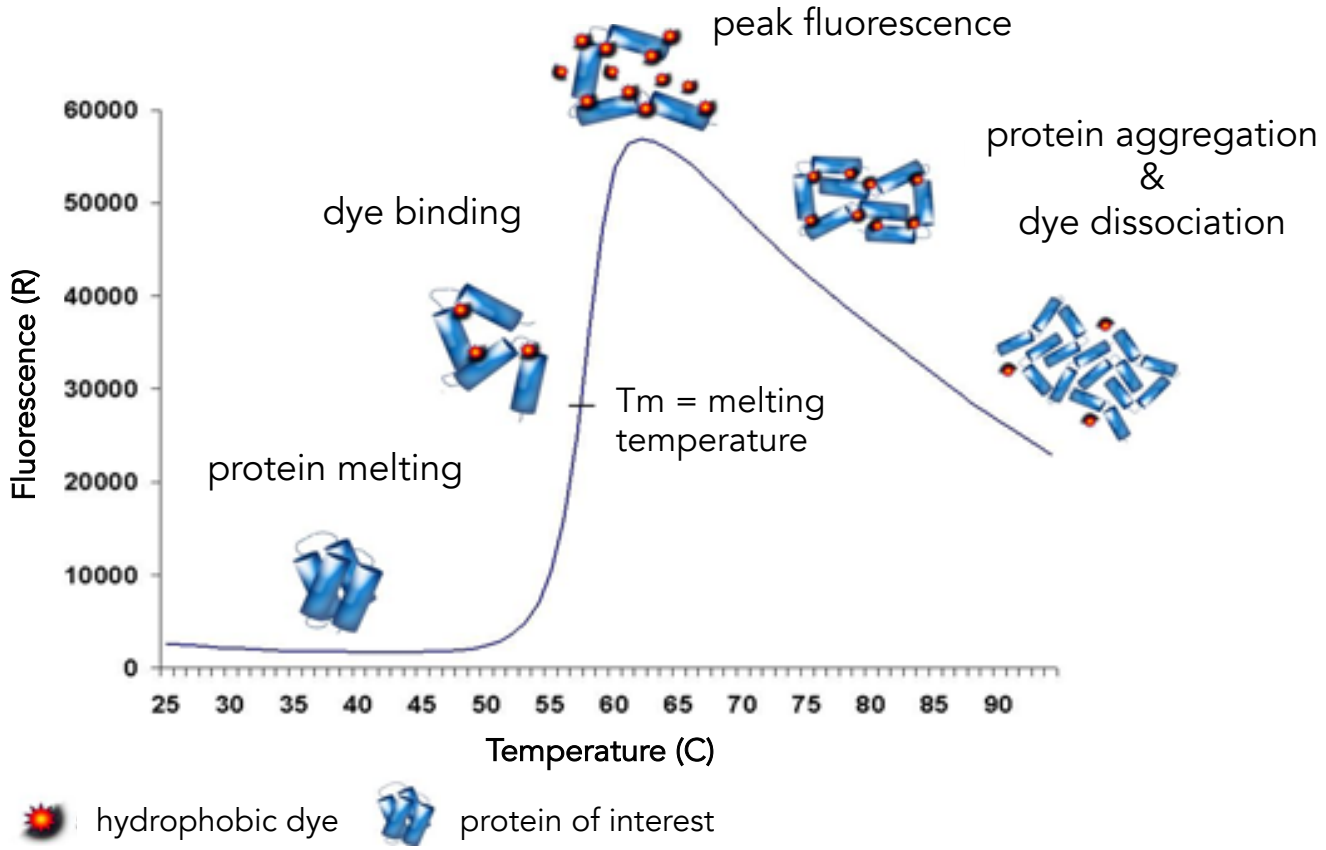


CPM

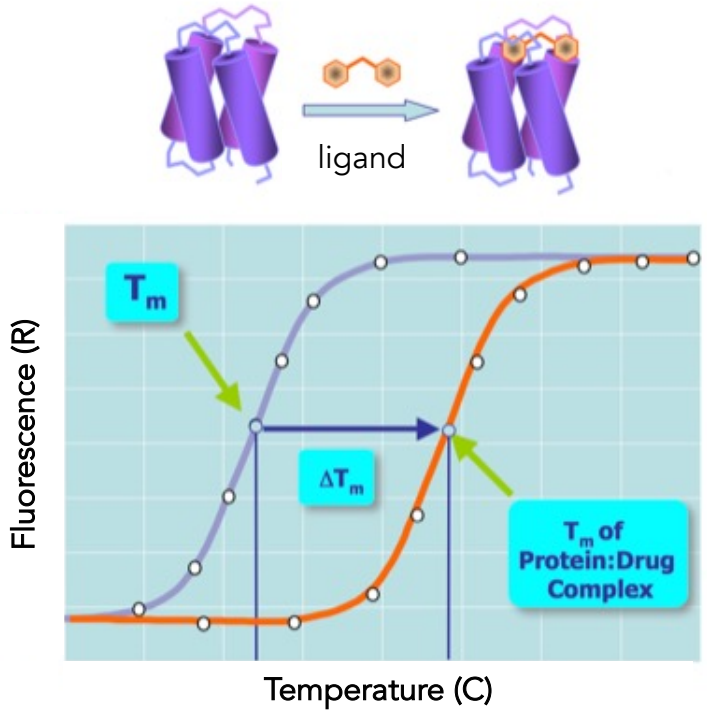
N-[4-(7-diethylamino-4-methyl-3-coumarinyl)phenyl]maleimide
(2008)

only fluoresces after reacting with Cys residues

What happens when you add a small molecule?

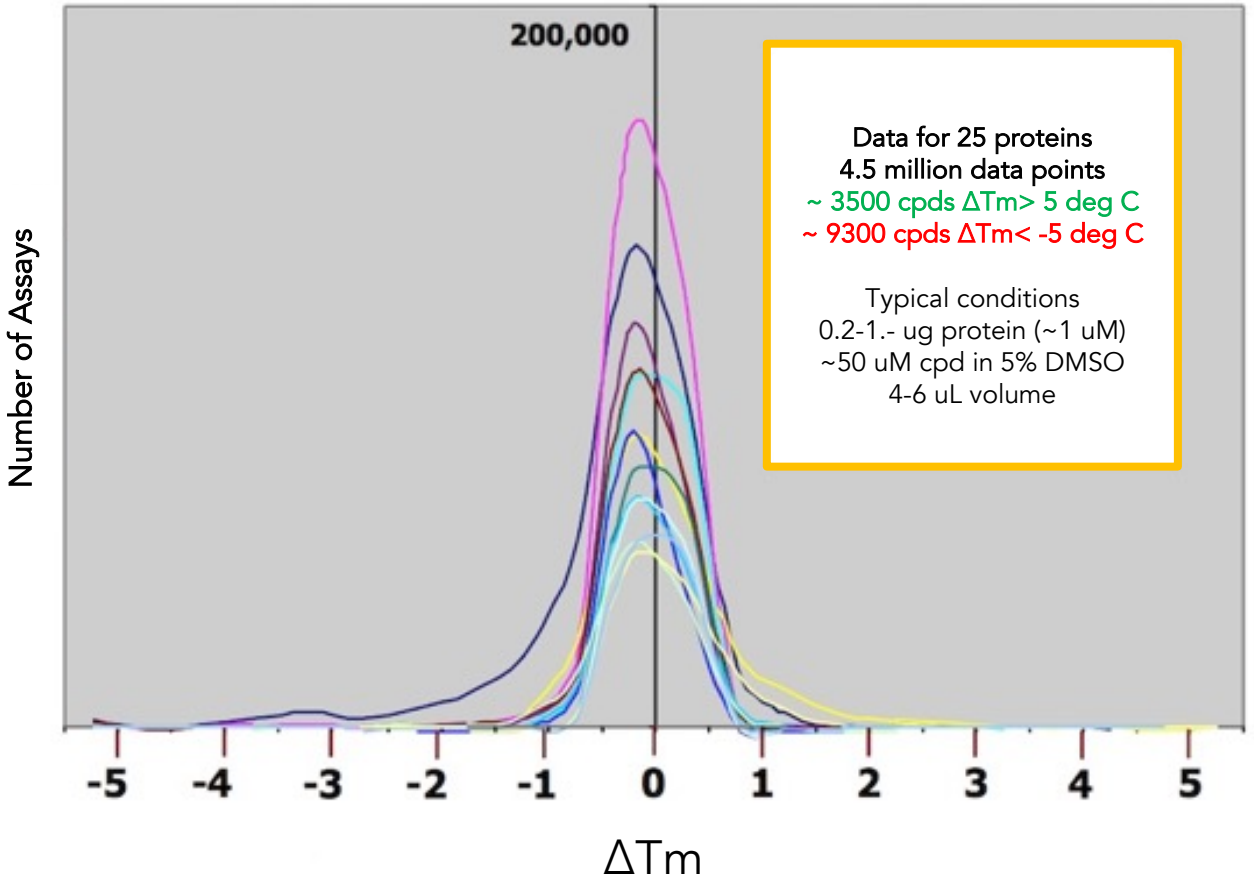


Thermal shift assays with small molecules



Adapted from Dr. Salemme at thermofluor.org

Real thermal shift screens with small molecules

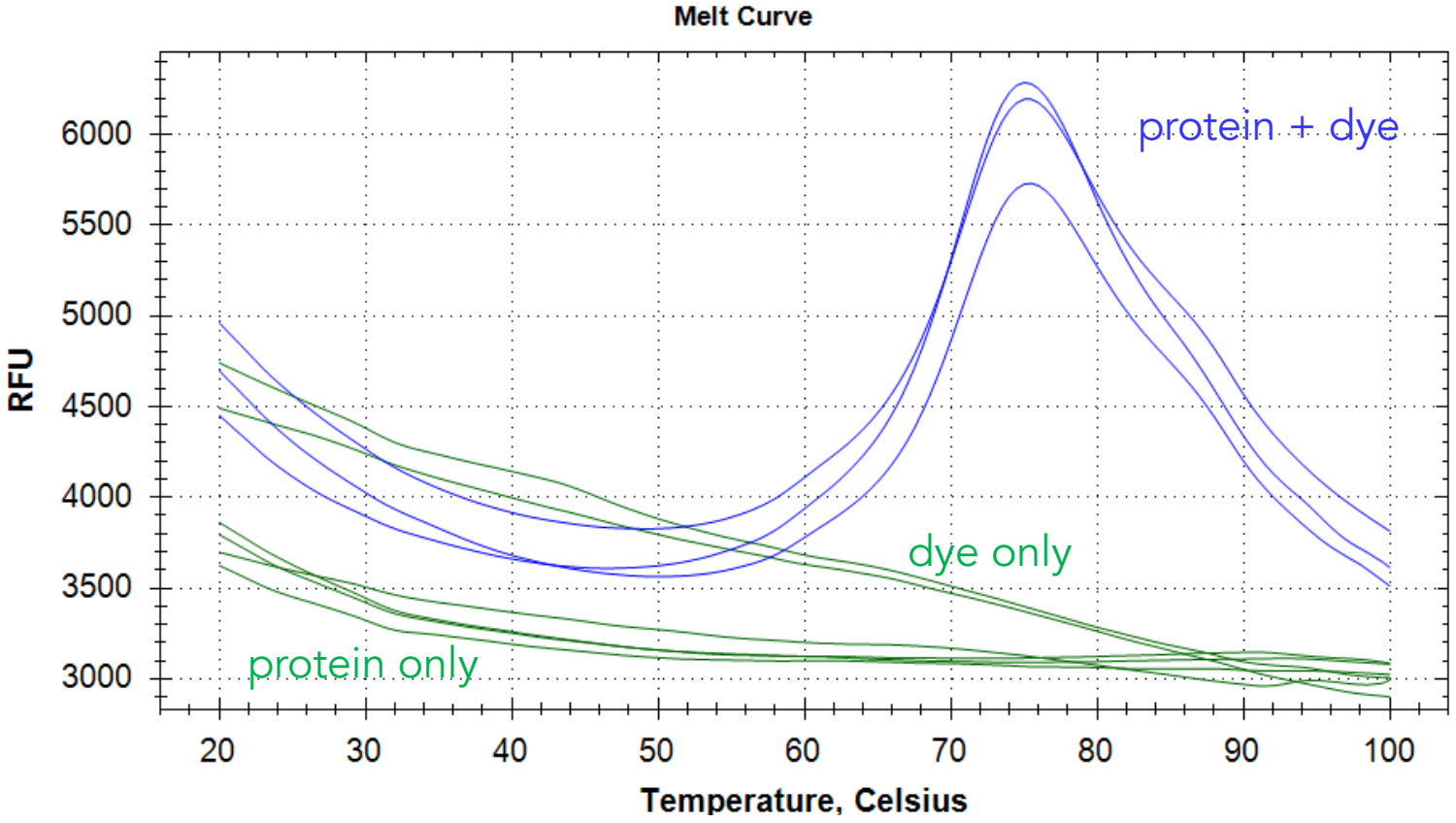


preferential ligand binding to unfolded states?

Adapted from Dr. Salemme at thermofluor.org

Real results from thermal shift studies

assay development

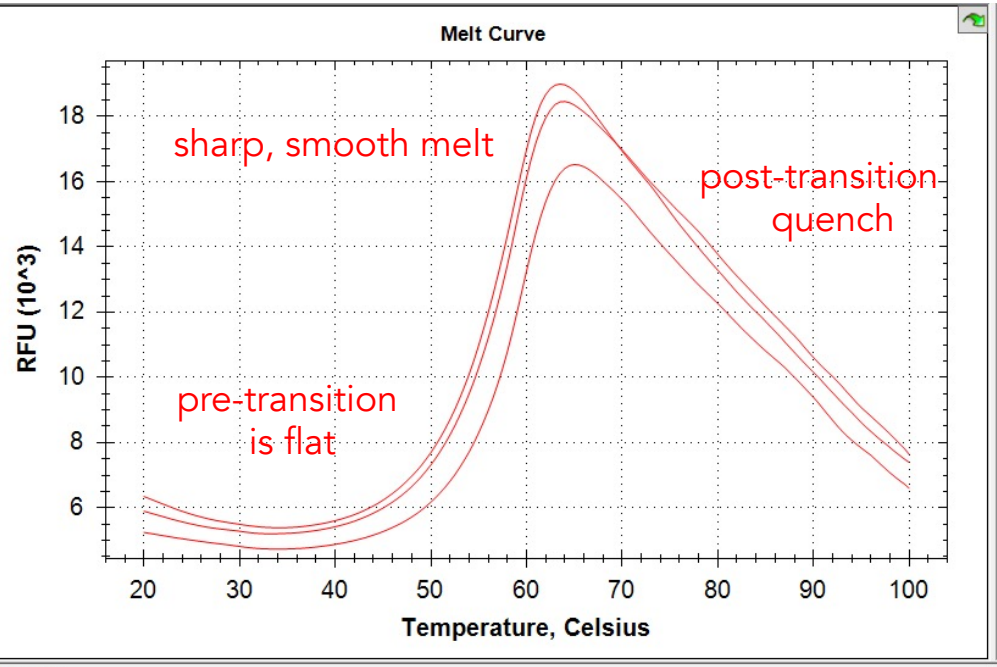


consider optimizing buffer conditions – pH, cofactors

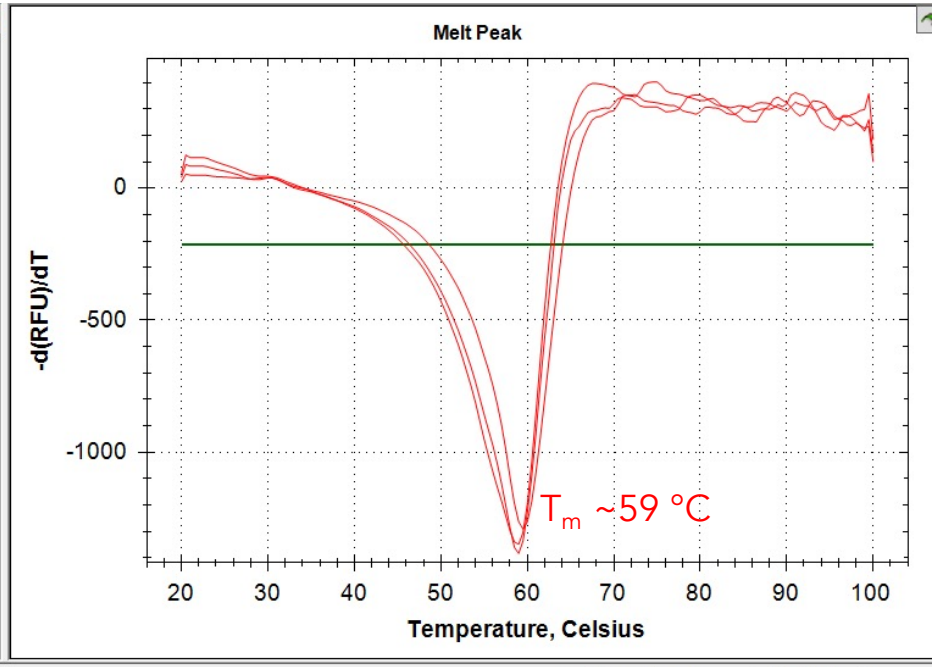
Adapted from Collaborative Crystallisation Centre

Real results with thermal shift assays

three replicates for a single experiment



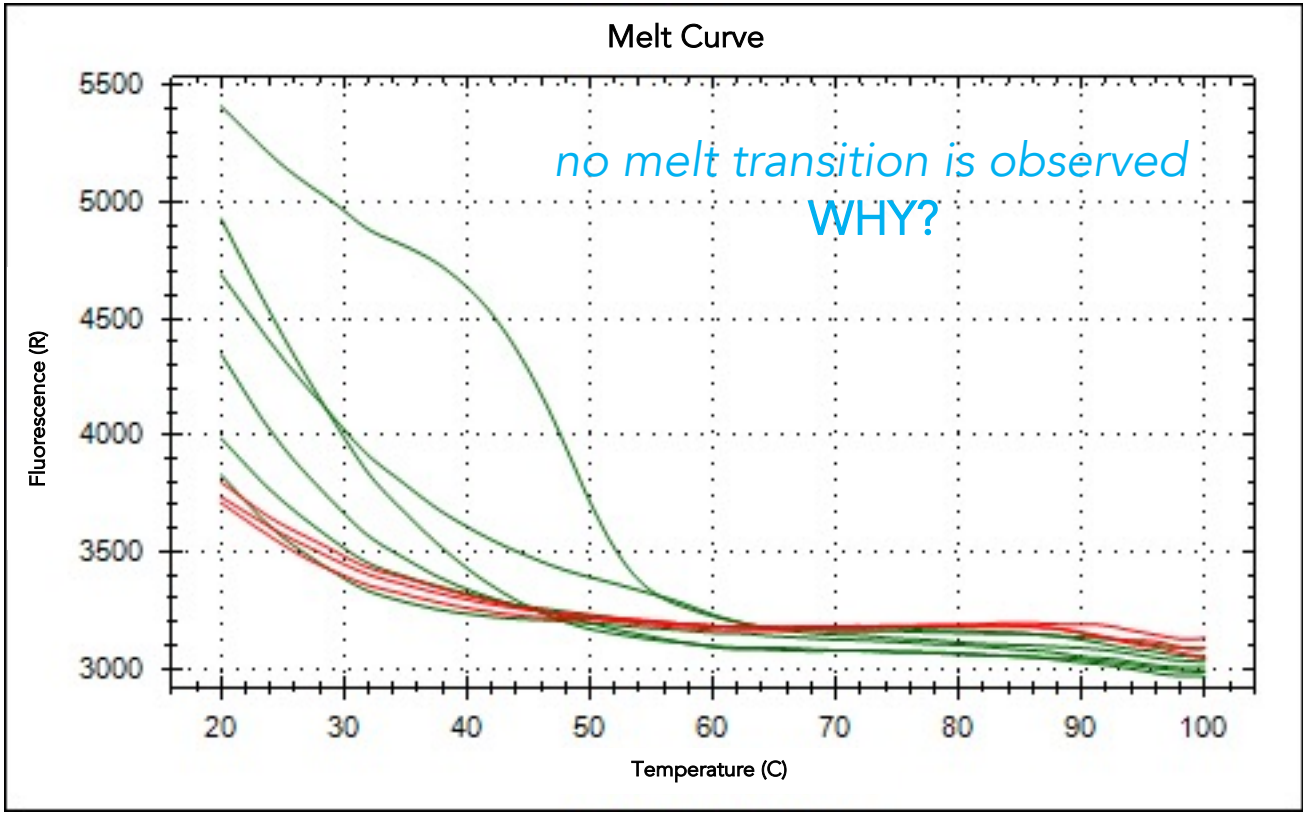
raw fluorescence thermal curves



first derivative representation

Adapted from Collaborative Crystallisation Centre

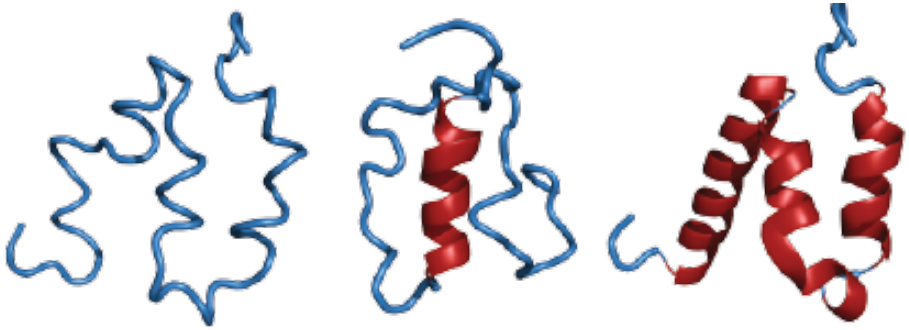
Real results with thermal shift assays

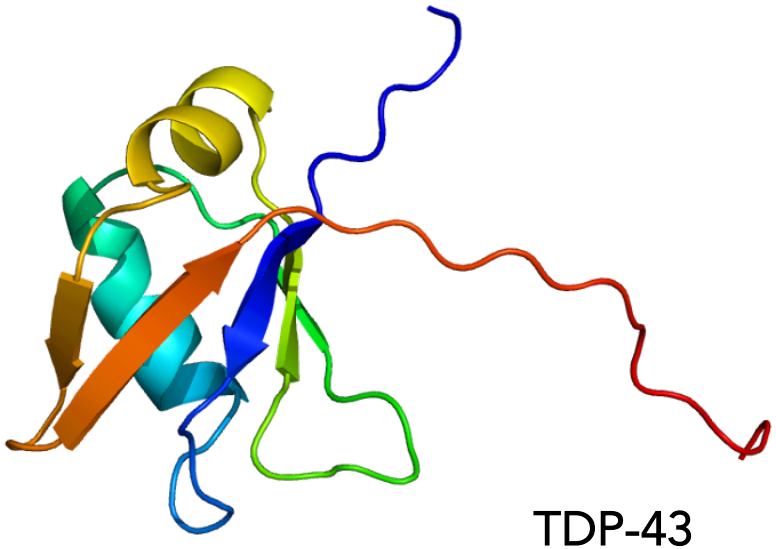


raw fluorescence thermal curves

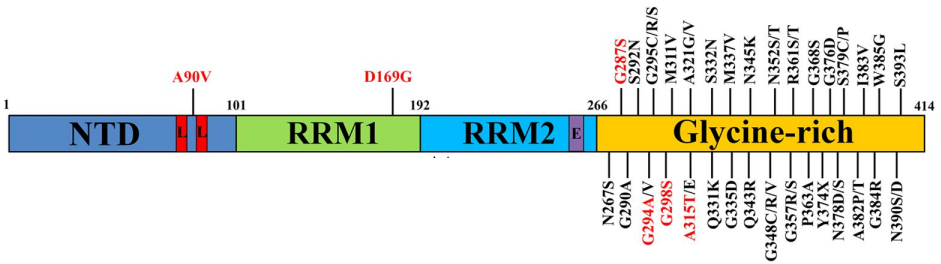
Adapted from Collaborative Crystallisation Centre

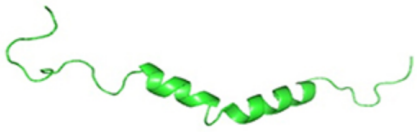
Protein disorder continuum





TDP-43



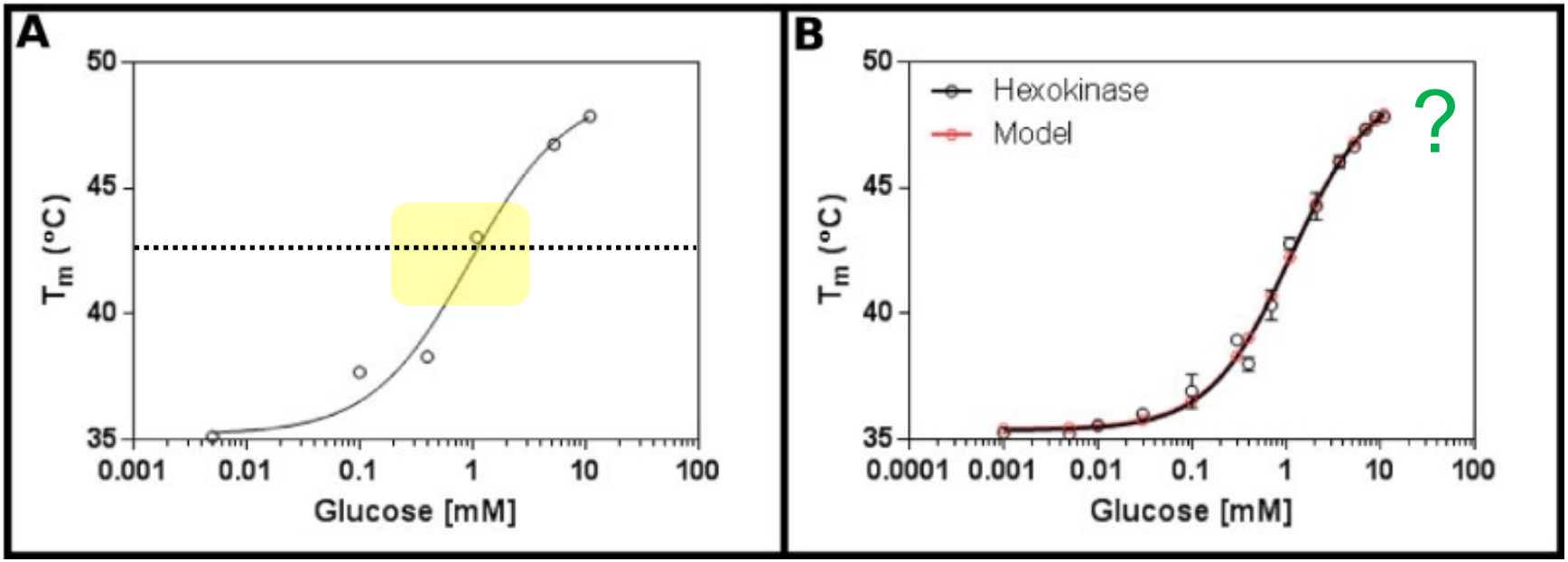


C-term
aa 311-360
PDB ID = 2N3X

Low complexity domain (LCD)
tendency to aggregate

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

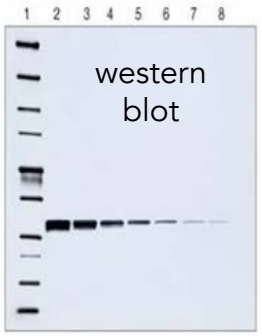
Experiment 2:

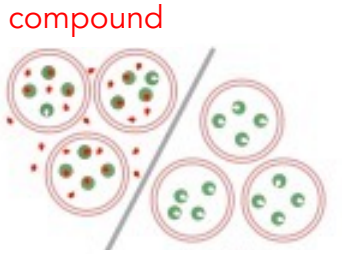
test 16 concentration of glucose
fit to single binding event model (red)

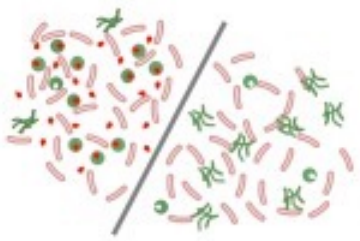
apparent K_D ~ 1.12 +/- 0.05 mM

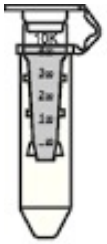
Target engagement in cells: cellular thermal shift assays (CETSA)

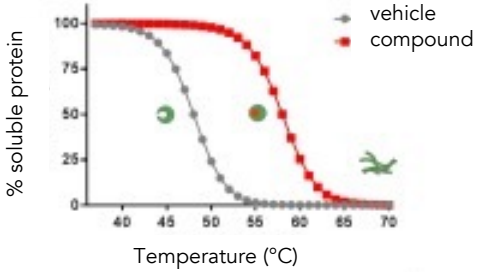
Monitor levels of soluble proteins







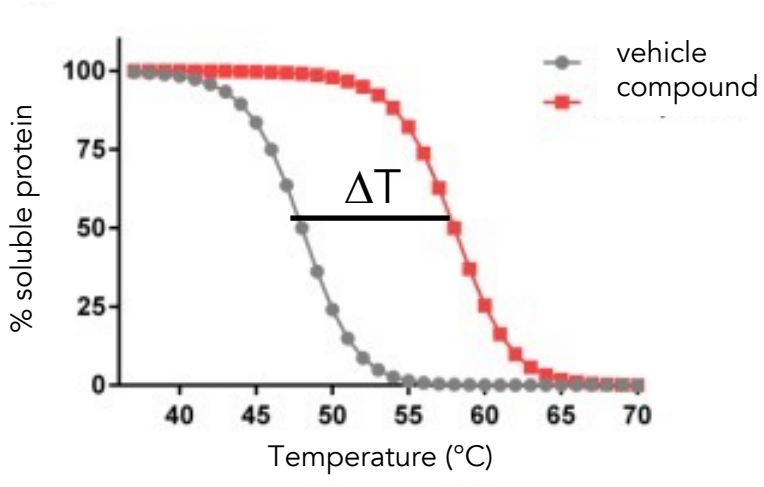




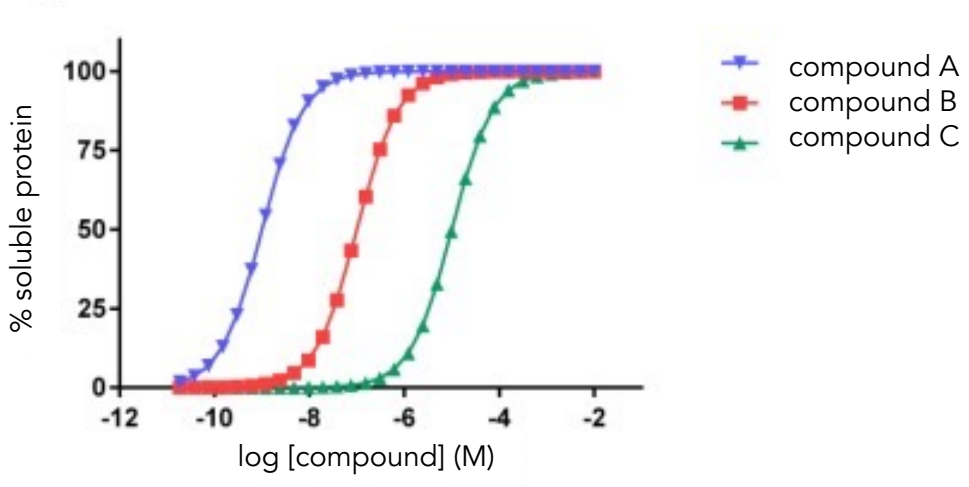
compound treatment in live cells → heating and cooling → lyse cells → separation (optional) → detection

Anticipated results from CETSA assays

T_{agg} curve



ITDRF curves

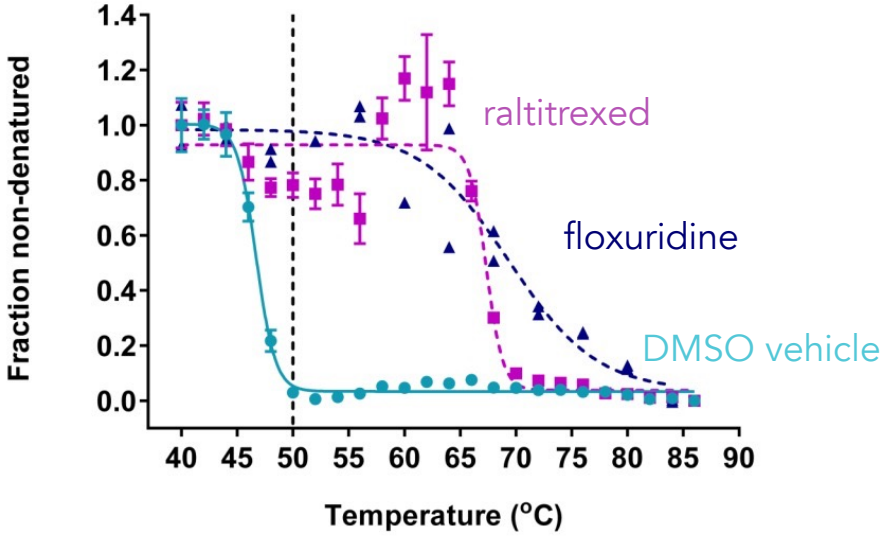


IsoThermal Dose Response Fingerprint
'apparent potencies' at single temp

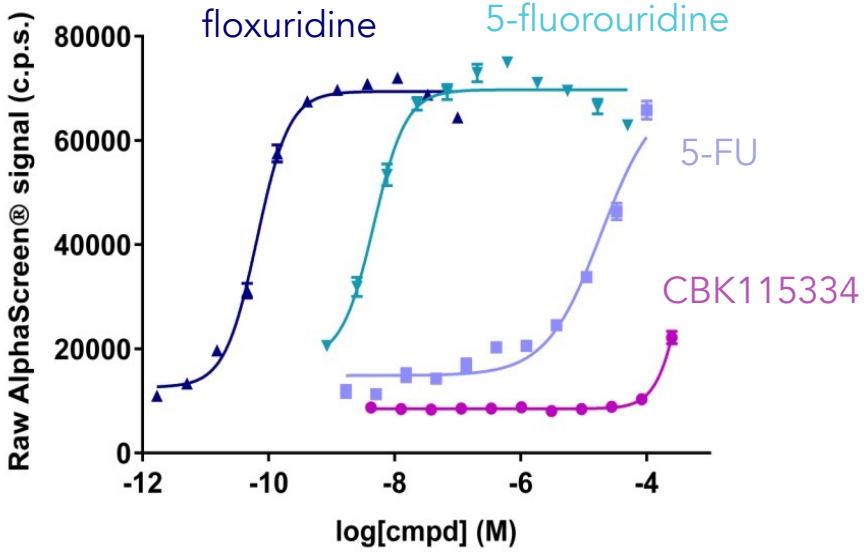
Real results from CETSA assays

thymidylate synthase drugs in K562 cells

T_{agg} curve

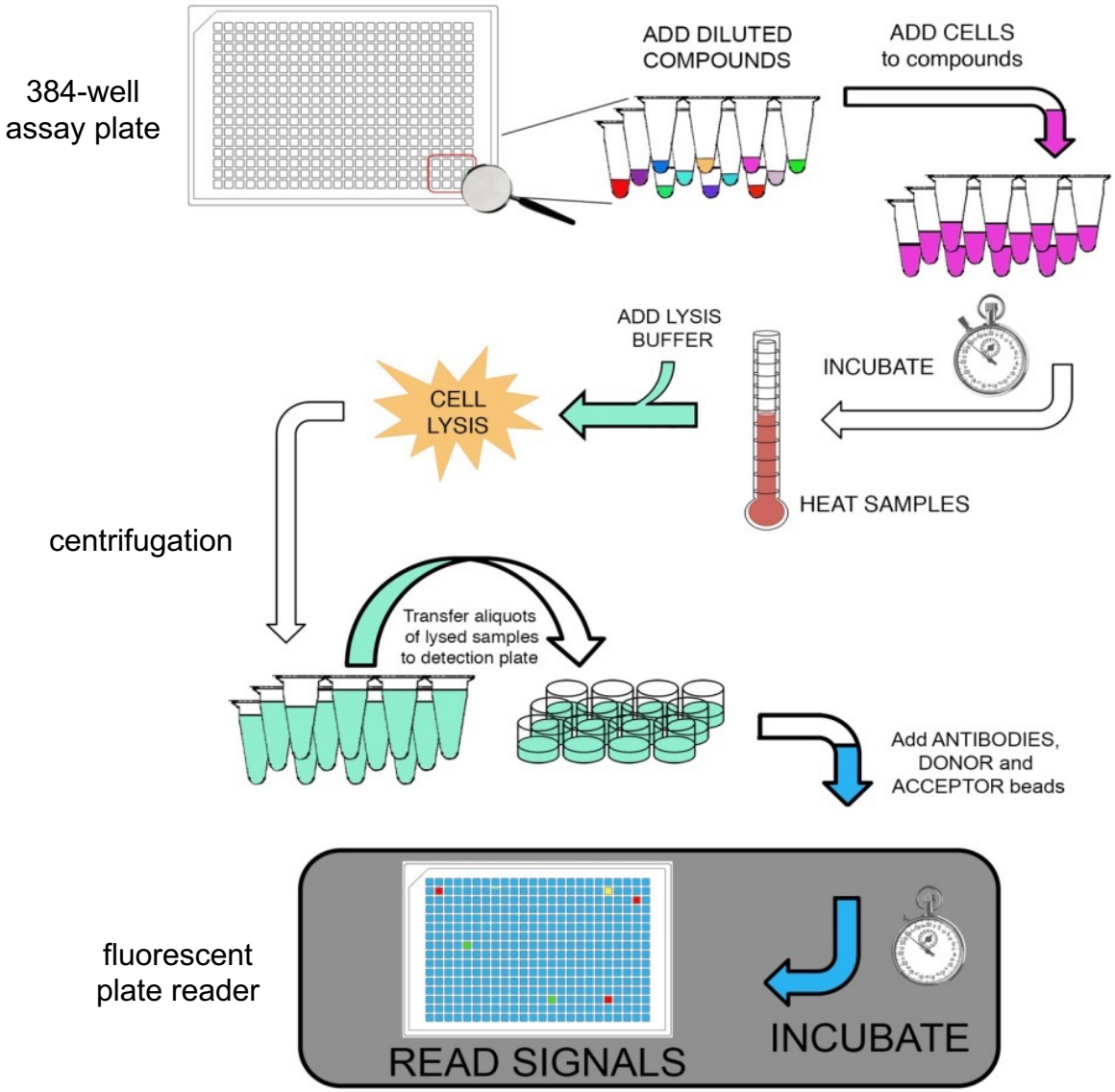


ITDRF curves at 50 °C



quadruplicate data from one independent experiment

CETSA for high-throughput screening



Adapted from the NIH Assay Guidance Manual

CETSA for target identification of drugs

Cell Chemical Biology
Minireview

Small-Molecule Target Engagement in Cells

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Monitoring how, when, and where small molecules engage their targets inside living cells is a critical step in chemical biology and pharmacological research, because it enables compound efficacy and confirmation of mode of action to be assessed. In this mini-review we summarize the currently available methodologies to detect and prove direct target engagement in cells and offer a critical view of their key advantages and disadvantages. As the interest of the field shifts toward discovery and validation of high-quality agents, we expect that efforts to develop and refine these types of methodologies will also intensify in the near future.

Workflow for novel drug target identification