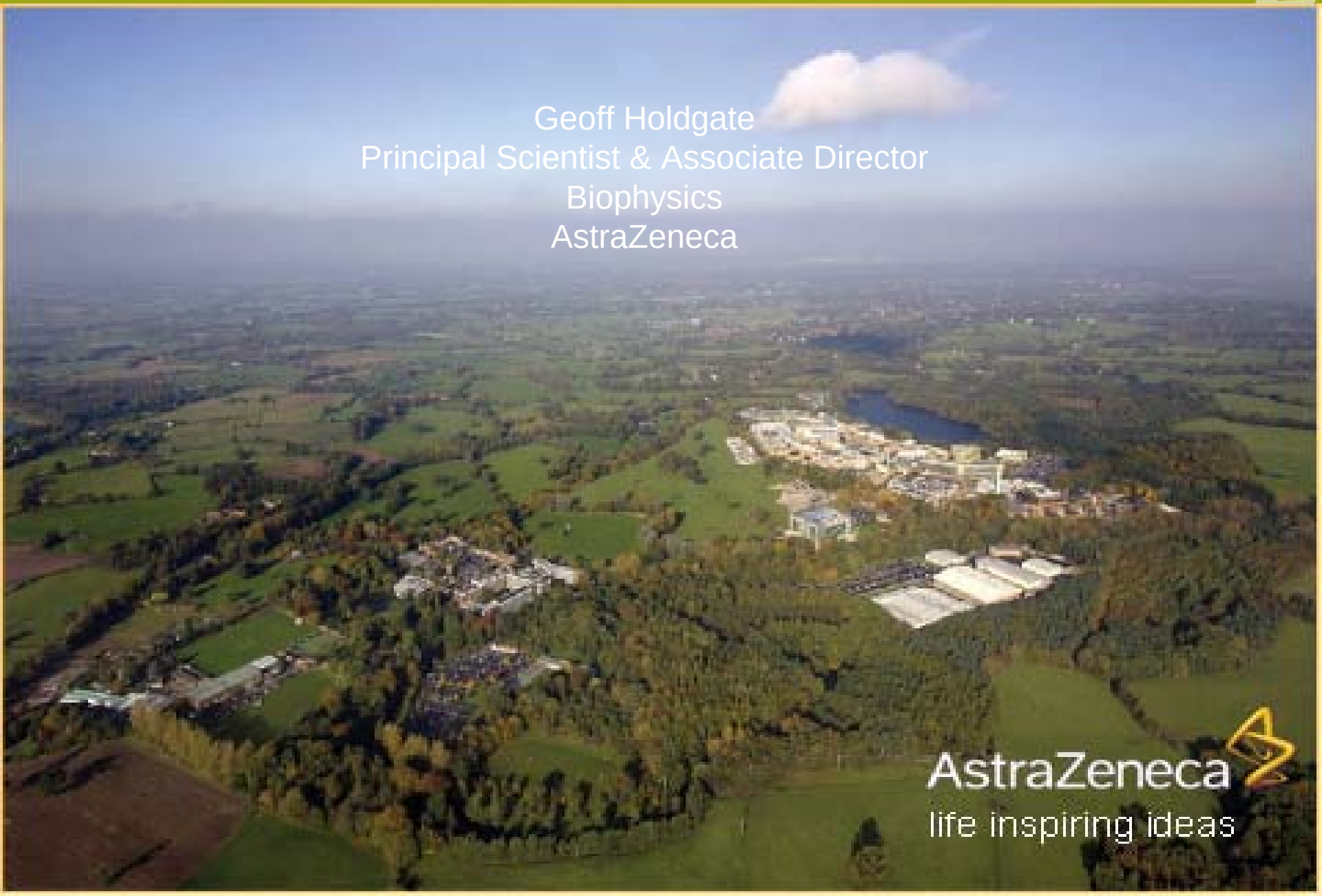
Exploiting Kinetics & Thermodynamics in Drug Discovery



Summary

Why are kinetics and thermodynamics important

Recent interest

Methods for measuring kinetic and thermodynamic parameters

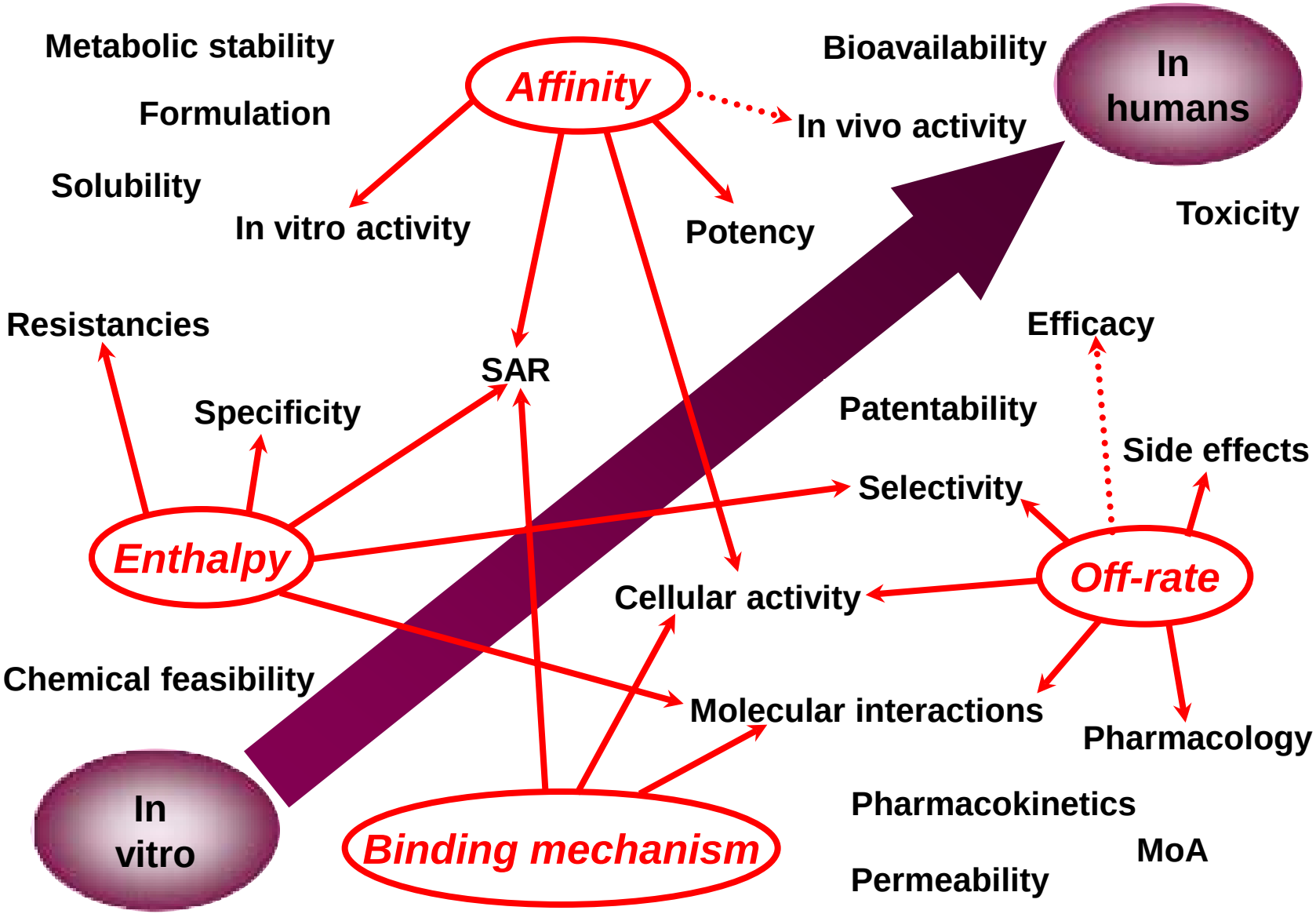
Utility and Issues of measurement

Potential use in design

Influencing Medicinal Chemists

Key take home messages

Why Kinetics and Thermodynamics are important



Kinetics & Thermodynamics

Recent Interest - Kinetics

There has been a lot of interest recently in impact of interaction kinetics, especially residence time, in drug discovery. This is based on the fact that a drug in a body is in an ”open” system; concentration fluctuates over time.
In such a system, interaction kinetics is perhaps a more relevant thing to look at / optimise than equilibrium constants (K_d or K_i)

- Copeland RA. et al. Drug-target residence time and its implications for lead optimization, Nature Drug Discovery, 2006

- Tummino PJ et al. Residence time of Receptor-Ligand complexes and its effect on biological function, Biochemistry, 2008

Kinetics & Thermodynamics

Recent Interest - Thermodynamics

Interest in thermodynamics was raised again recently with the presentation of data on the statins and HIV Protease inhibitors. This was based around later compounds having a more favourable negative enthalpy. However, many believe that this is a simplistic view, and certainly the definition ‘best in class’ is a complex, and not scientific parameter

- Freire; Do Enthalpy and Entropy Distinguish First in Class From Best in Class? Drug Discovery Today 2008
- Freire; A thermodynamic approach to the optimization of drug candidates Chem Biol Drug Design, 2009
- Ferenczy & Keseru; Thermodynamics guided lead discovery and optimization, Drug Discovery Today, 2010

Measuring binding kinetics

Methods

SPR – direct methods

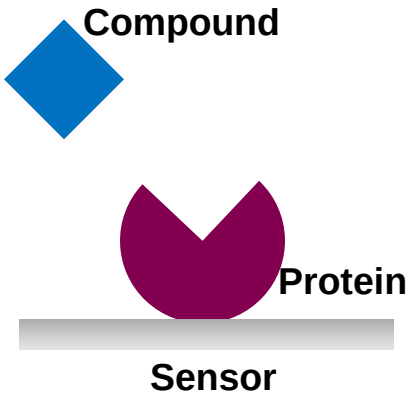
SPR / OWG – competition methods

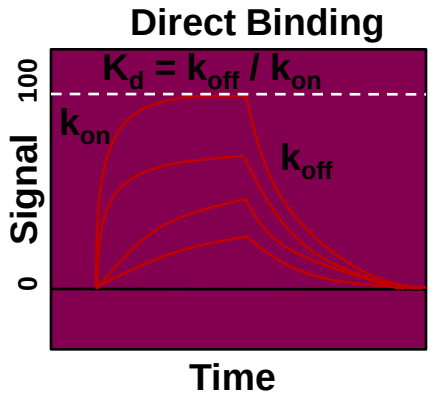
Other reporter displacement assays

Enzyme kinetics

SPR & OWG

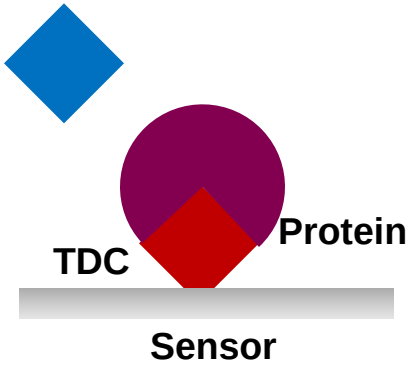
Direct and Competition methods

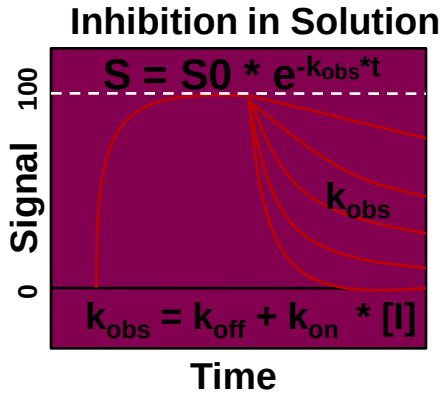




SPR:

- Label-free, real-time flow cell system
- Target immobilisation (-NH, -SH, -OH, SA)
- Extensive assay development
- Limited throughput





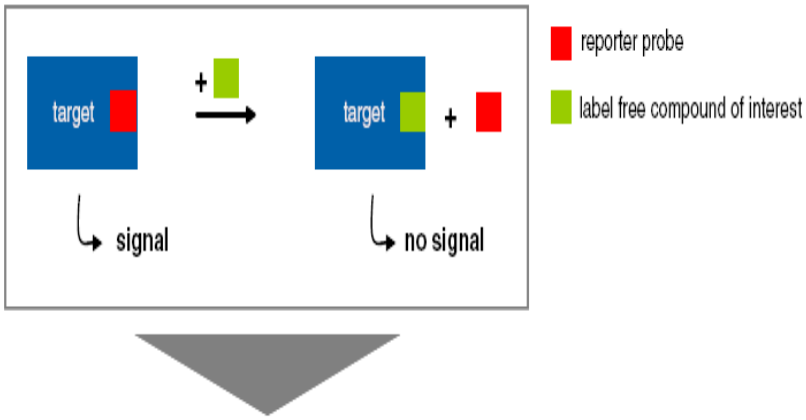
OWG:

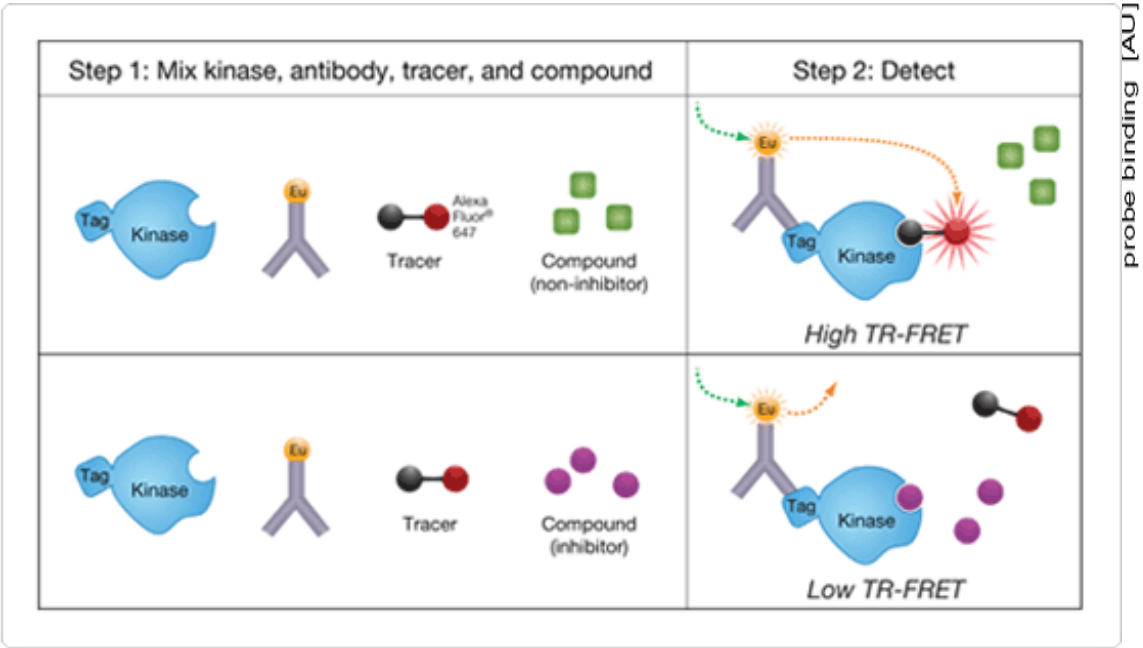
- Label-free, real-time plate systems
- Immobilisation of TDC
- Rapid assay development
- Increased throughput

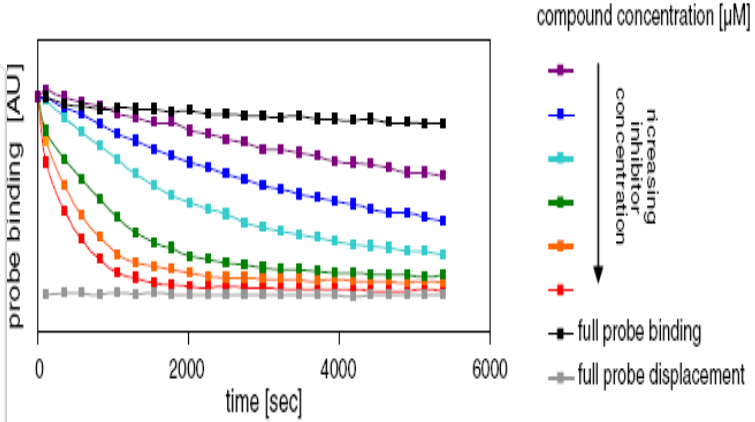
Reporter Displacement Assays

Often fluorescence-based

Fig. 2 Assay principle of reporter displacement assay. Binding of the reporter probe generates a specific signal. Displacement of the reporter probe by a competing compound of interest results in signal loss. By analyzing the kinetics of signal loss at various compound concentrations values as K_d, k_{on}, k_{off} and residence time can be calculated

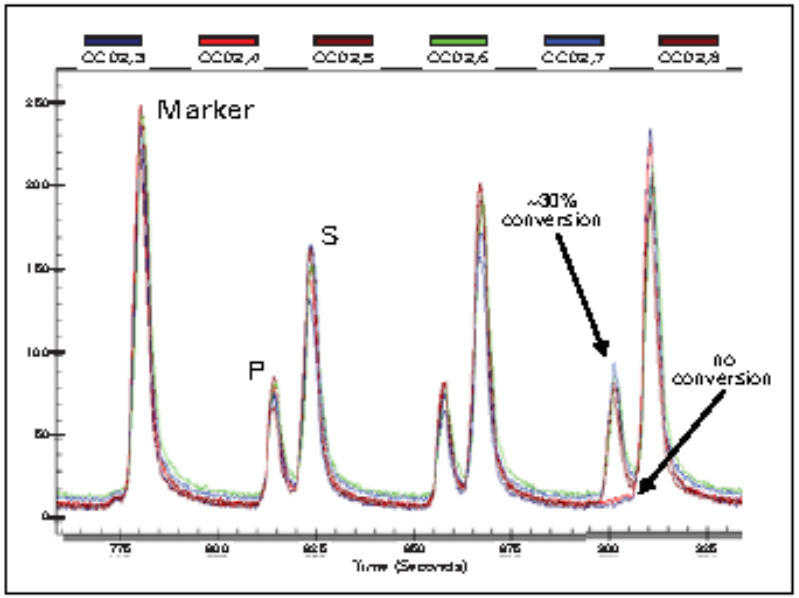


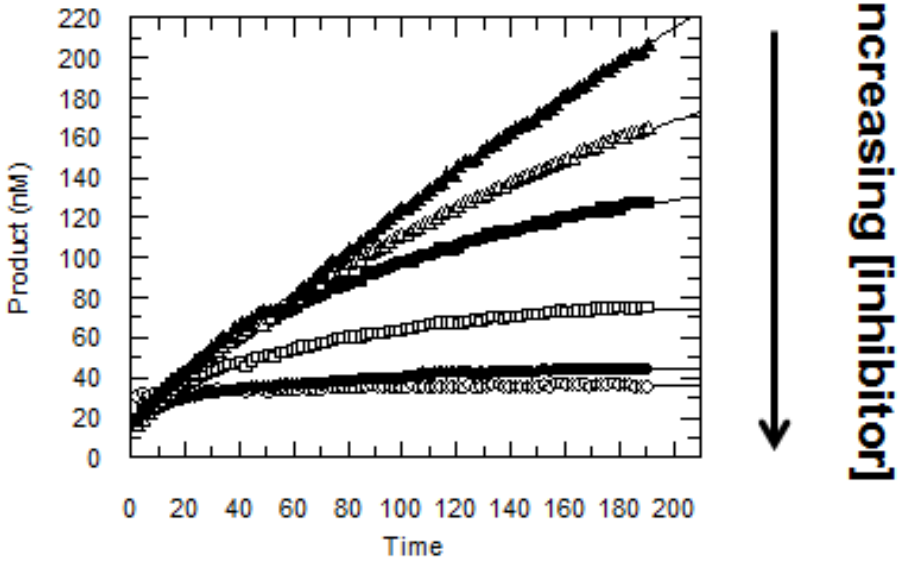


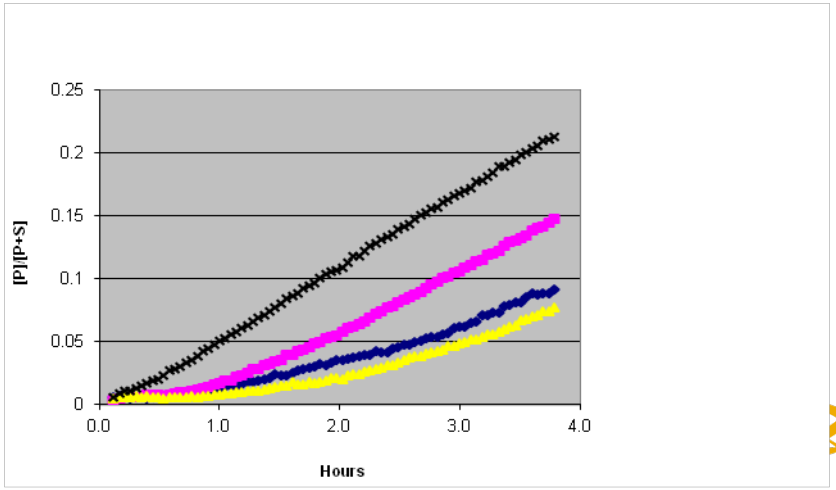


Enzyme Kinetics

Caliper and other continuous assays

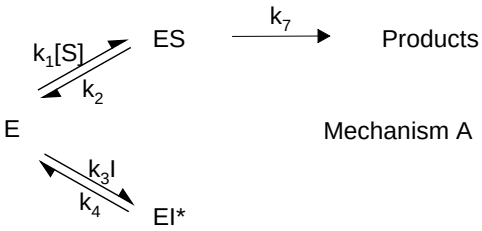


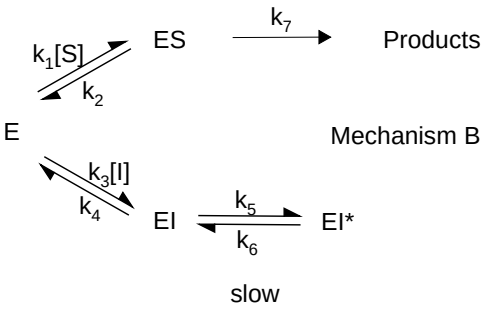


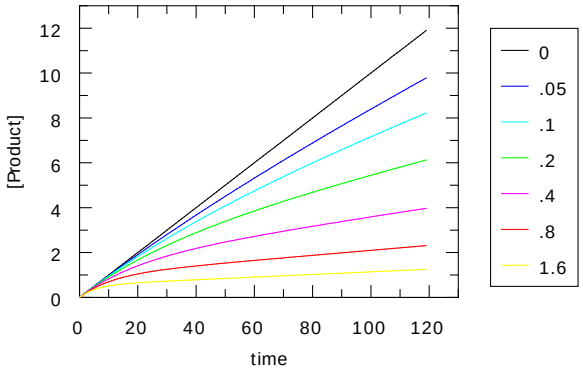


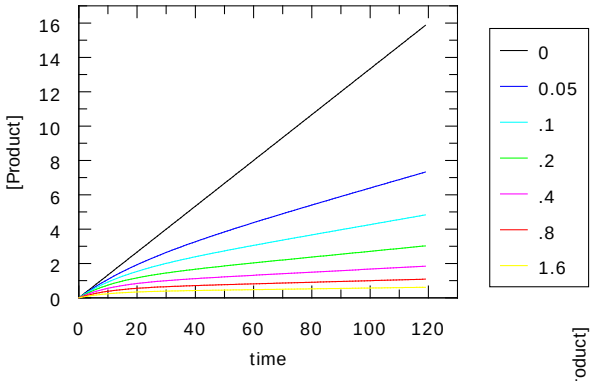
Kinetic Binding Mechanisms

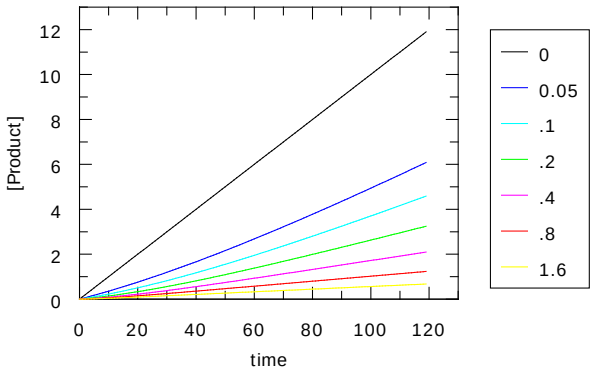
1 or 2 step?

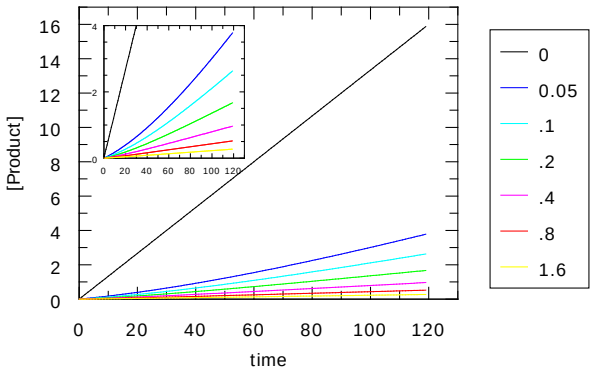


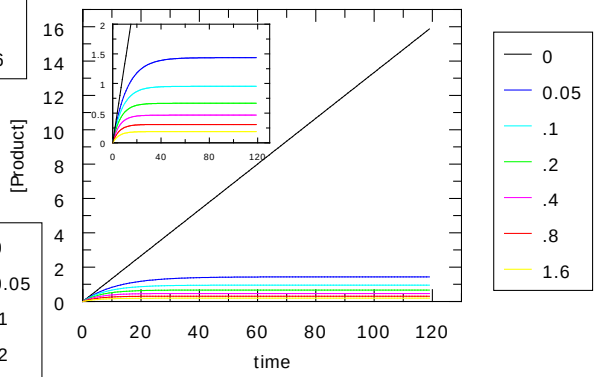












Measuring binding kinetics

Utility

One binding affinity many different kinetic contributions – allows differentiation between compounds

Duration of Action, clinical benefit

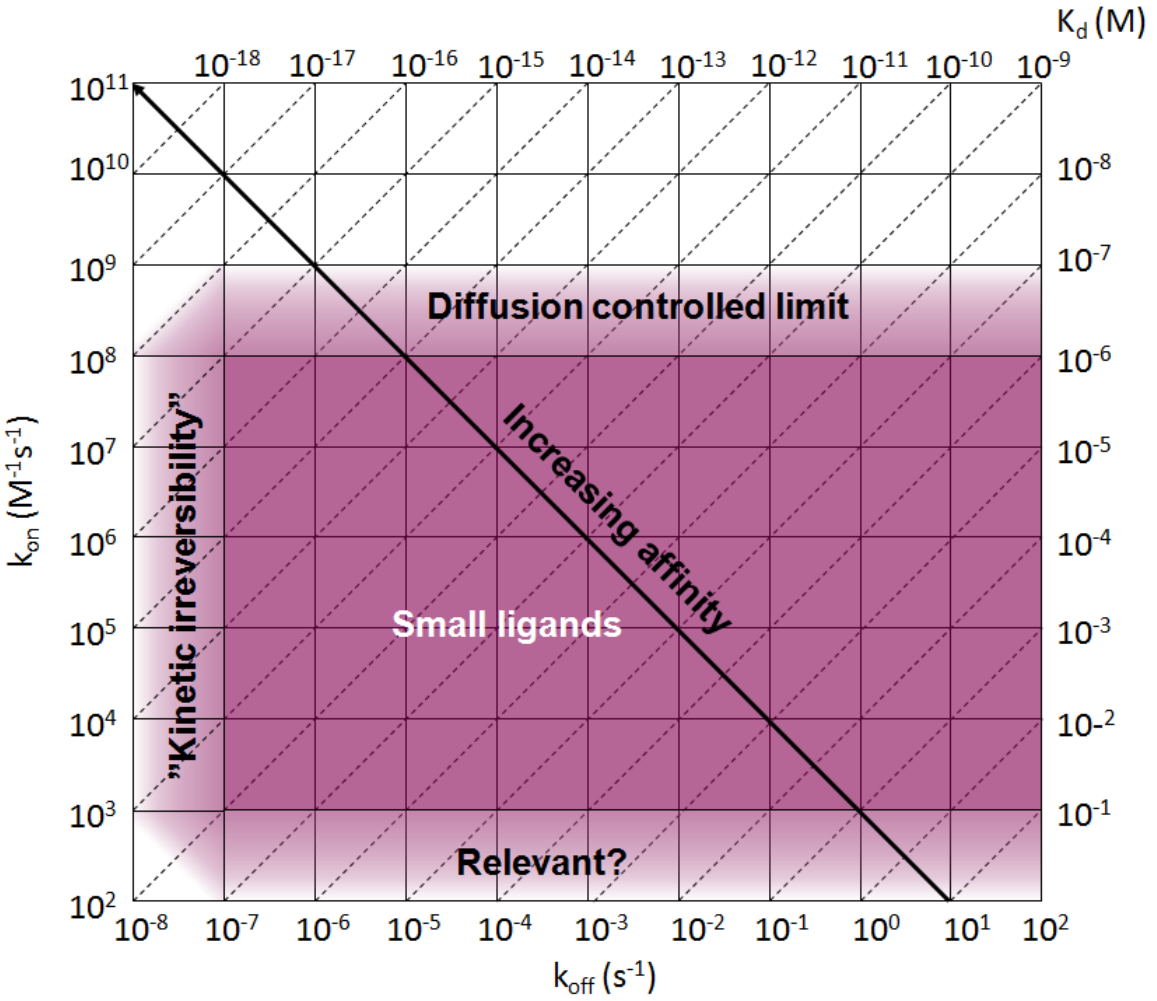
- Long residence time provides long lasting PD effect that can outlast PK: meaning less frequent dosing, thus better patient compliance
- Safety benefit, less off target tox (especially if drug released quickly from the body after dissociation)

Therapeutic differentiation, choice

- 2 drugs binding to the same target can have 2 different physiological responses
- In certain case fast kinetics needed: when compromise needed between mechanism-based toxicity and efficacy

Safety

- Slow kinetics, lower concentration of drugs, better efficacy, minimize off target tox



Measuring binding kinetics

Issues

Standard assays may not reach steady state for slow-binding compounds, as they do not allow time for the compound to exert their full equilibrium

Many slow-binding interactions can be described by a 2 step mechanism, involving initial encounter between the protein and ligand, followed by a slow conformational change leading to tighter binding – sometimes these different mechanisms can be hard to distinguish

Measuring binding thermodynamics

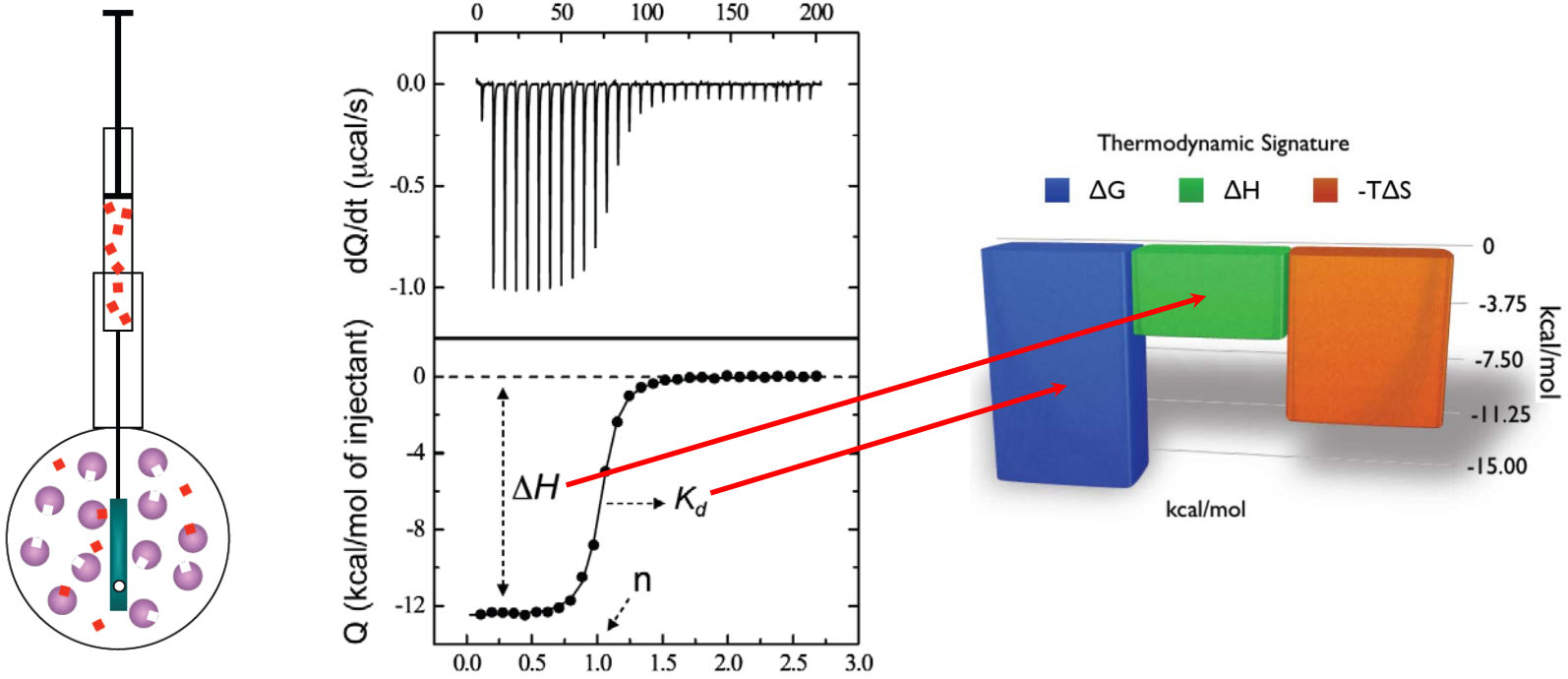
Methods

Calorimetric methods

Van’t Hoff approaches

Measuring binding thermodynamics

Isothermal Titration Calorimetry



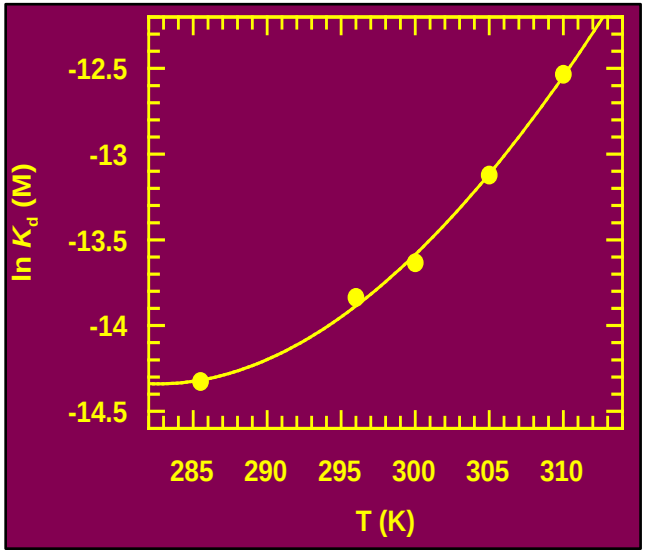
Measuring binding thermodynamics

Van't Hoff Analysis

$\Delta H°$ <u>indirectly</u> from T dependence of K_d

Need to allow for $\Delta H°$ changing with T

Magnitude of ΔCp & $\Delta H°$ dependent on each other → large SE



$$\ln K_d = \ln K_d^{\,ref} - \left[\frac{\Delta H^{\circ ref} - T^{ref}\Delta Cp}{R}\left(\frac{1}{T^{ref}} - \frac{1}{T}\right) + \frac{\Delta Cp}{R}\ln\frac{T}{T^{ref}} \right]$$

Measuring binding thermodynamics

Utility

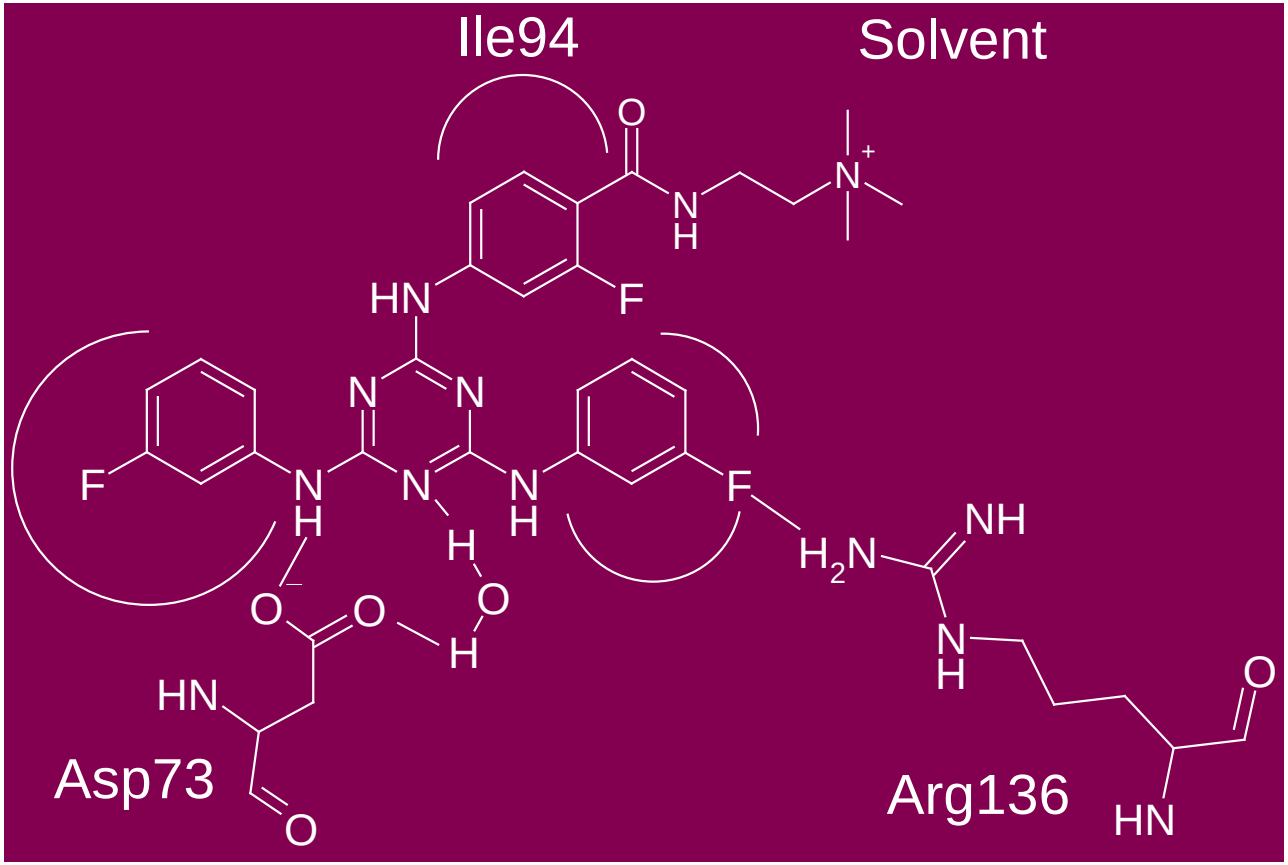
One binding affinity many different thermodynamic contributions – allows differentiation between compounds

Triazine / Gyrase G24 complex

3 pockets, ↑ burial: Ile94, Arg136, Asp73

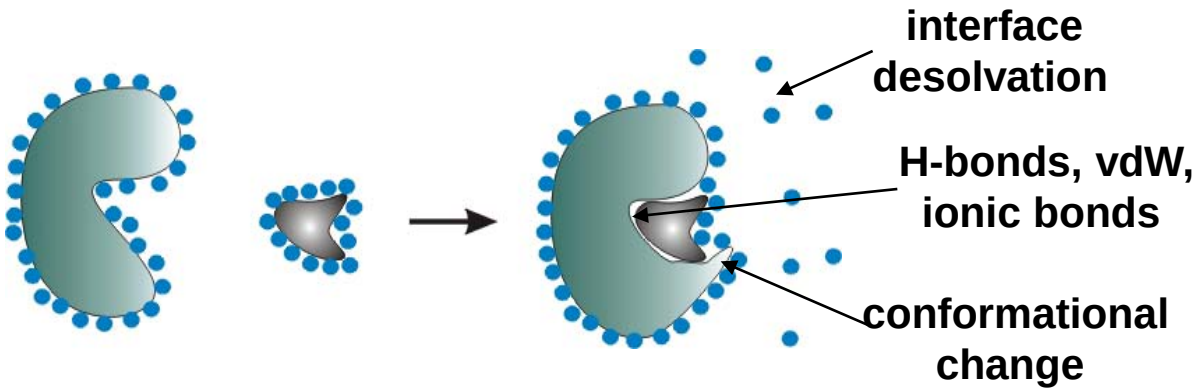
Symmetry in cpds, but $\Delta\Delta H°$ > 2 kcal/mol suggests changed binding mode confirmed by NMR

Monitoring thermodynamic profile allows optimisation in different ways



Measuring binding thermodynamics

Issues - Data can be difficult to rationalise



$$\Delta G^\circ = RT \ln K_d = \Delta H^\circ - T\Delta S^\circ$$

$$\Delta G = \Delta H_{int} + \Delta H_{dsolvLig} + \Delta H_{dsolvProt} - T\Delta S_{confLig} - T\Delta S_{confProt} - T\Delta S_{dsolvLig} - T\Delta S_{dsolvProt} - T\Delta S_{T+RLig} - T\Delta S_{T+RProt}$$

Enthalpy

Contributions from forces within the complex (H-bonding, v d Waals, electrostatic)

Penalty from desolvation processes (polar surfaces >>unpolar)

Entropy

Contribution from surface desolvation = increase of disorder

Penalty from formation of rigid structures = loss of degree of freedom

Penalty from loss of translational and rotational freedom

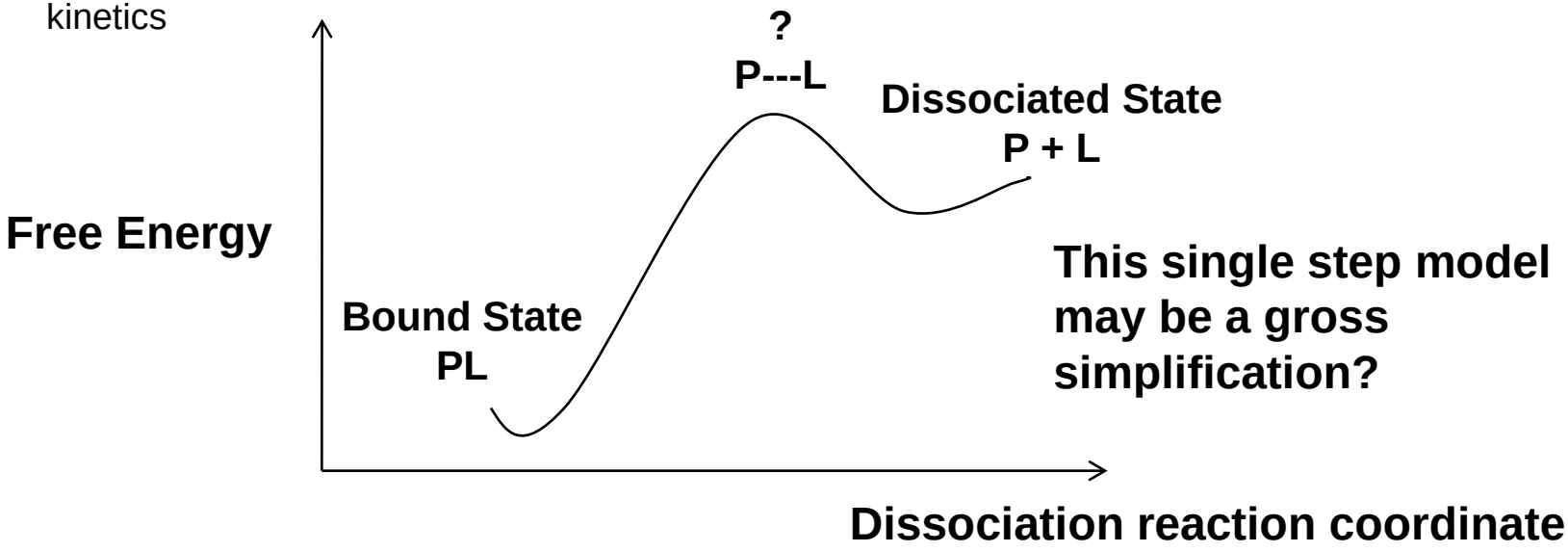
Kinetics in Compound Design

Present & Future Challenge

Challenge to medicinal chemistry is to find compounds that bind with sufficient potency - measured in assays that assume an equilibrium binding event
We have a language to discuss intermolecular interactions that are based on equilibrium considerations

If the challenge was designing a compound that has a slow off-rate for binding, where would designers begin?

Hydrogen bonds, pi-pi interactions, salt bridges, hydrophobic interactions – all these things are related to the two ends of an equilibrium and we have no idea what goes on in between – even if we did we have no idea what effect changing a molecule's structure and therefore interactions with a protein would do to the kinetics



Kinetics in Compound Design

So where are we upto?

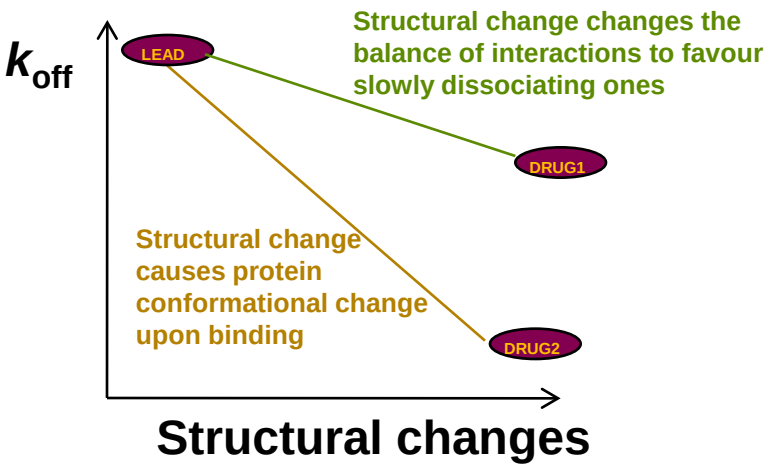
Disparate examples in the literature and in-house of small numbers of compounds against a small set of targets

Most rationalizations of changes in k_{off} depend upon protein conformational changes

Predicting protein conformational changes remains at the forefront of computational and experimental capability

Compound design is still dominated by equilibrium considerations even if only in the thought processes going on

Is it enough to make a compound bind more tightly which ought to have a parallel effect on off-rate (not necessarily a simple relationship here though)?



We don't know how to do either of these at the moment but can see simple things that could be done to do the green one. There ought to be general rules here.

The orange one feels like it will always be target specific and will therefore involve large collaborative efforts and will be challenging to do on a timescale that impacts on medicinal chemistry projects – so far most examples have been serendipitous events that have been post-rationalized.

Kinetics in Compound Design

Computational Capabilities

Recent developments in DFT have created functionals that are able to better model dispersion based interactions (aromatic and other hydrophobic)
These same functional types also claim to be better at modelling for interactions away from the minima

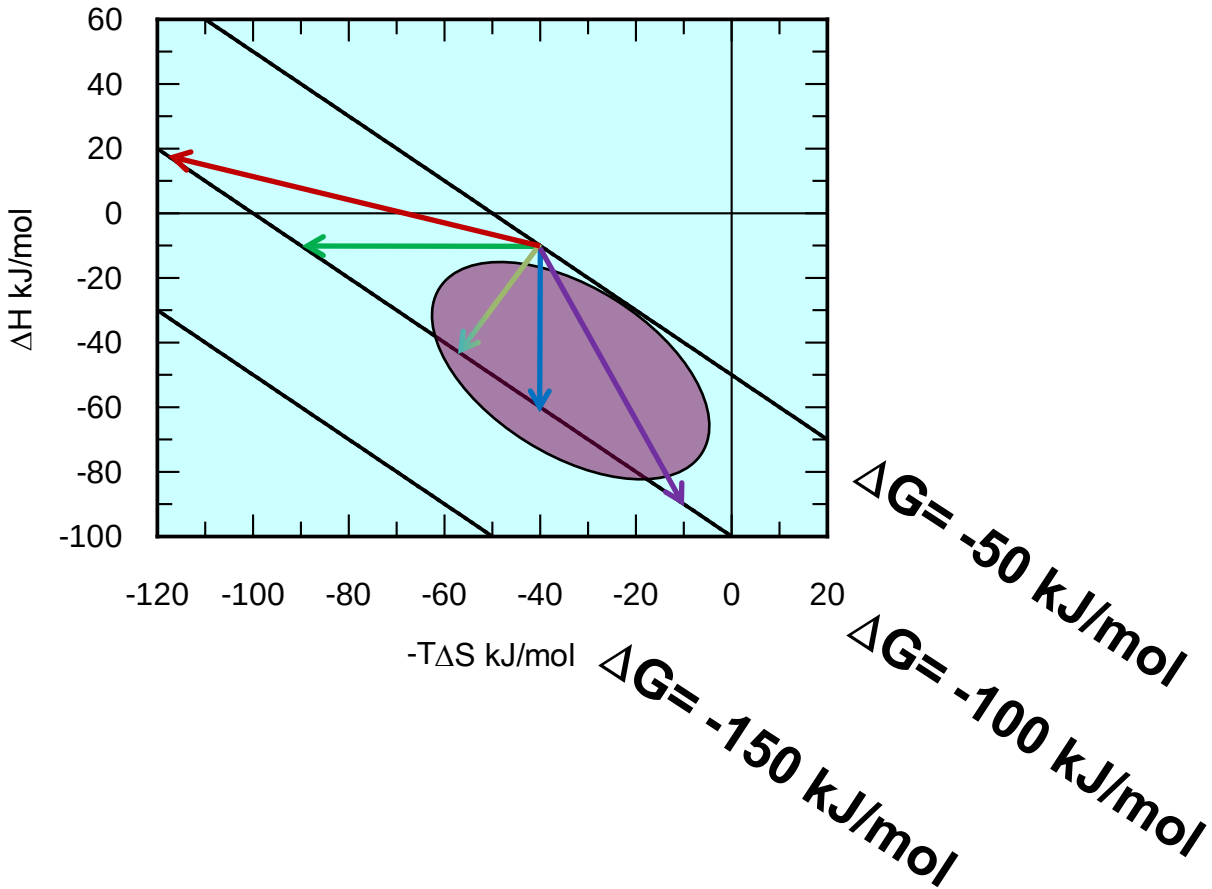
Recent dynamics data have suggested that the detection of transient pockets in proteins might be able identify where conformational change effects might be possible

These two are both VERY early and so far with little validation as tools for compound design BUT are encouraging signs

Thermodynamics in Compound Design

Opportunities

LG start points are small molecules or fragments with typically μM to mM affinity
Affinity of drugs needs to be around 3 – 6 orders of magnitude higher
Achieving this change means lowering the Gibbs binding free energy by 17.1 – 34.3 kJ/mol



Increasing affinity means making ΔG more negative
This can be achieved by:
1.Making ΔH alone more negative
2.Making -TΔS alone more negative
3.A combination of changes in ΔH and -TΔS together being negative

Most drugs are entropically driven

Evidence may suggest that enthalpic driven binding can be useful

So should Med chem. efforts be concentrated in this region, where enthalpy is the driving force in affinity optimisation?

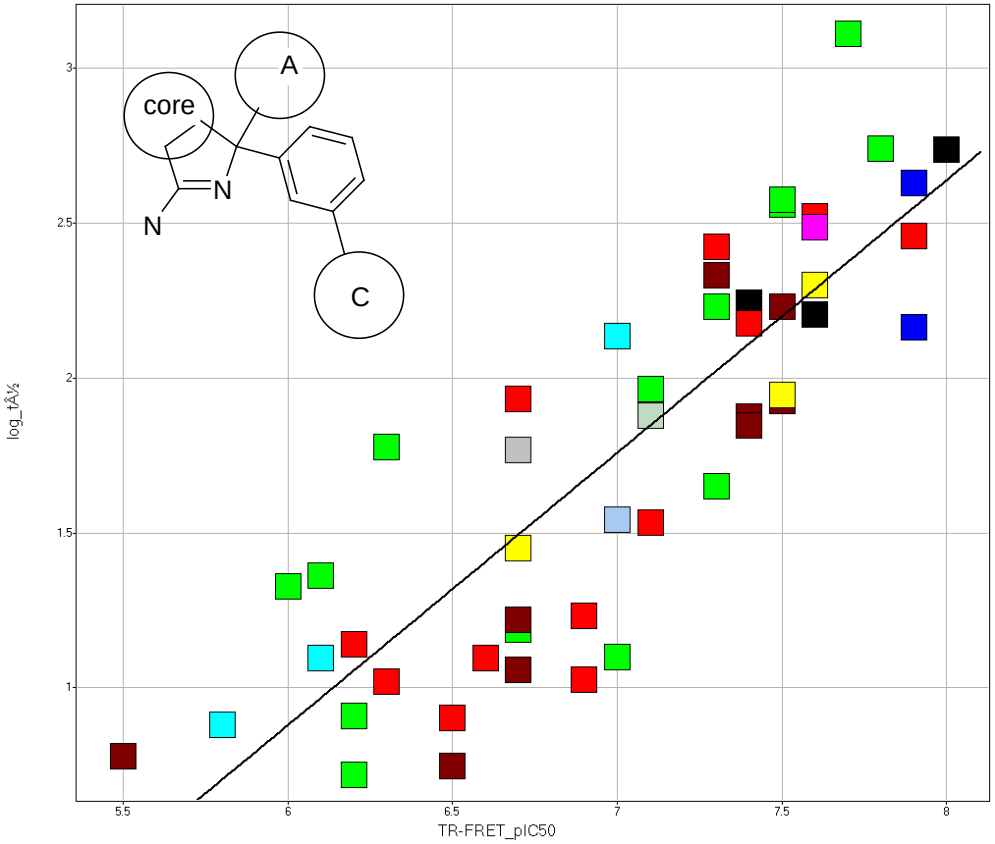
Retrospective and real time data collection

Kinetics & Thermodynamics

Have they helped?

Kinetics

Protease 1 data

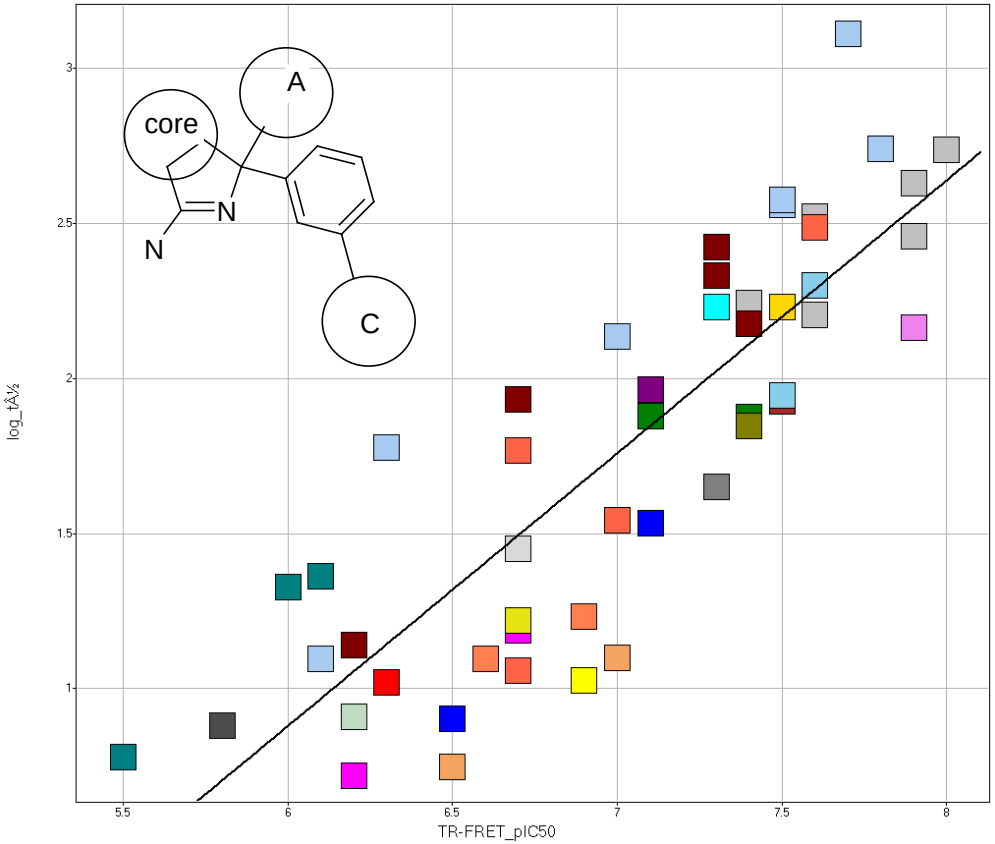


Colored by chemical series (core)

- **Nice correlation between pIC50 and off-rate**
- **Independent of chemical series**

Kinetics

Protease 1 data

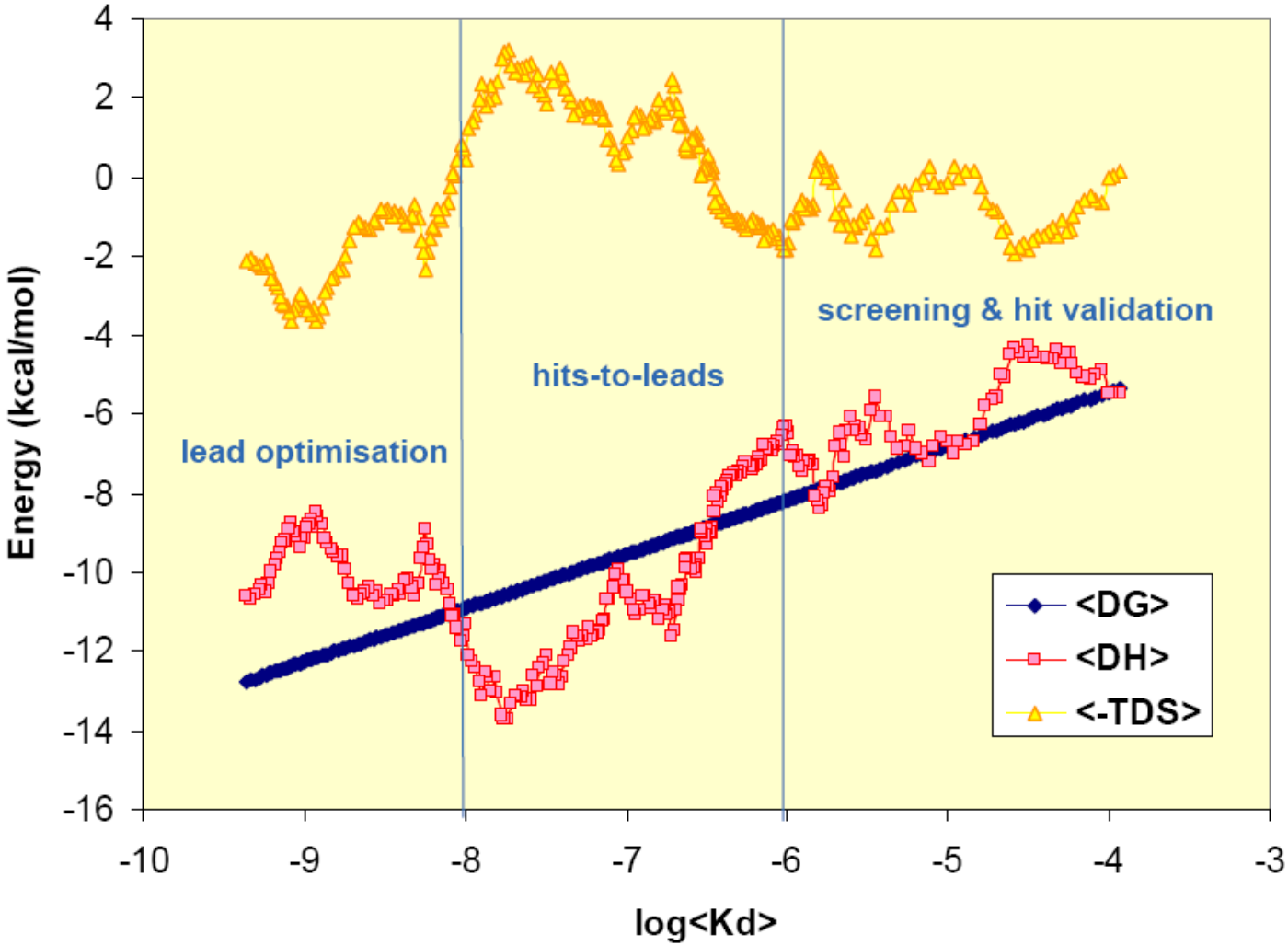


Color by A-ring substructure

- **We have seen that the A-ring has a great impact on potency.**
- **Independent of core**
- **From this plot we observe that different A-rings behave differently in the kinetics assay.**

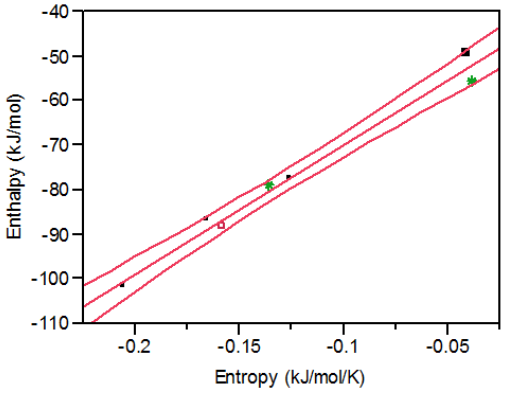
Thermodynamics

Astex data

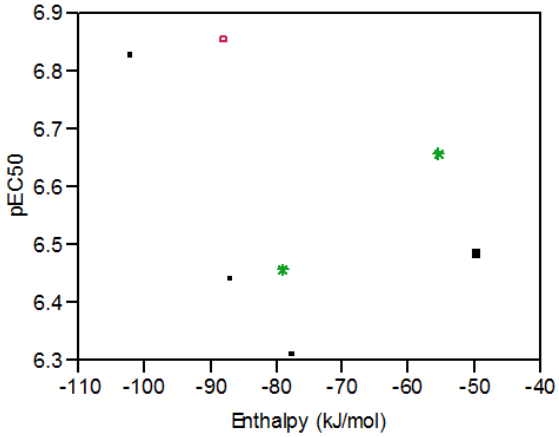


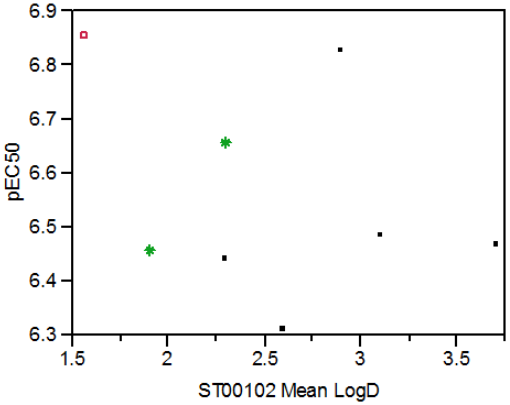
Thermodynamics

Kinase data



Across the series, and including CD1 from previous series, observe enthalpy-entropy compensation
ΔG very similar for all compounds, but larger variations in the individual contributions from enthalpy and entropy observed



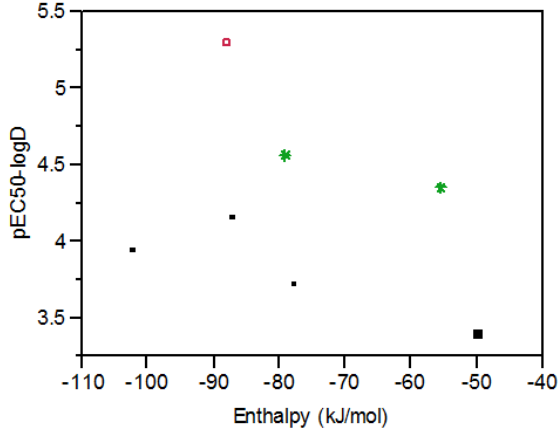


Green stars :optimised compounds from 2nd series
Red square : CD1, original series

Project reduced Log D, and retained potency

Other compounds represent changes made in doing this, where crystal structures available

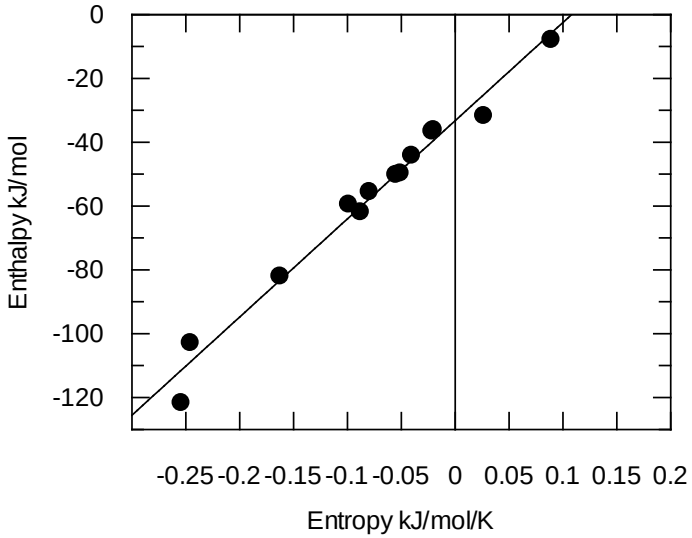
Most significant changes in SAR believed to be through H-bonding interactions with ordered waters

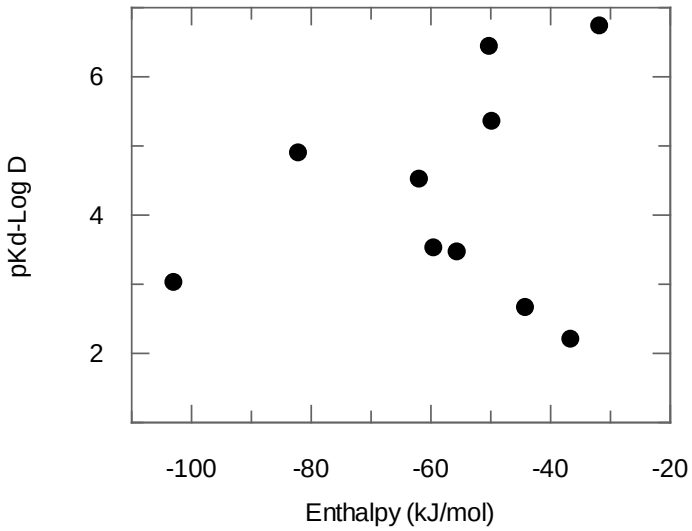


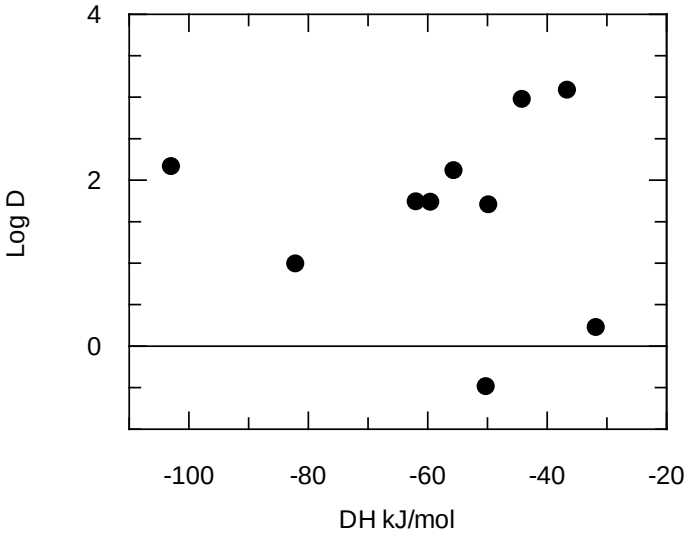
Looking for a correlation between pEC_{50} –Log D and enthalpy
This was not observed

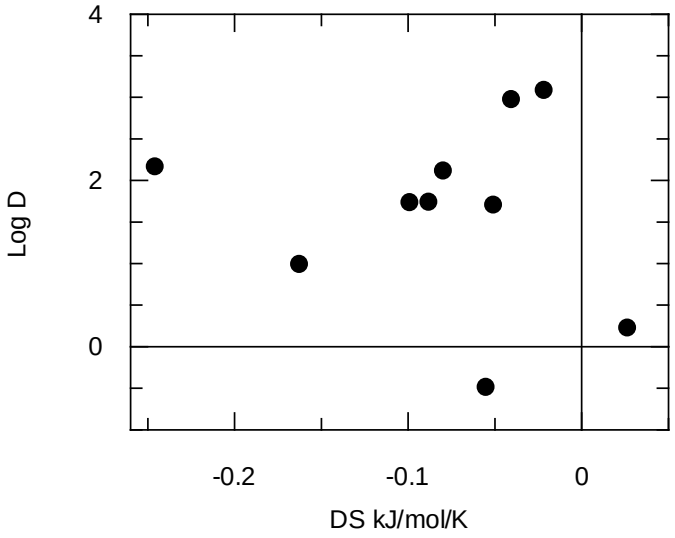
Thermodynamics

Synthase Data



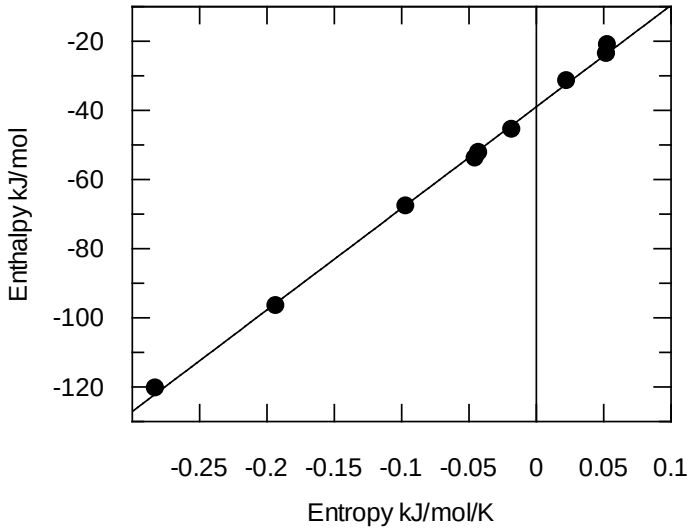


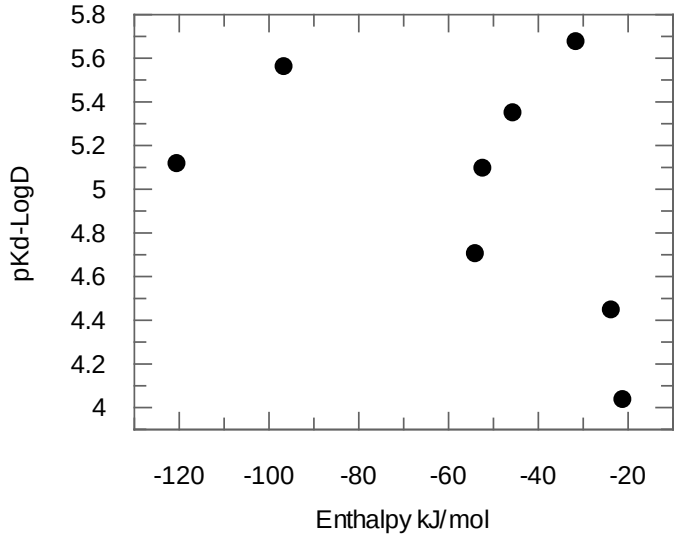


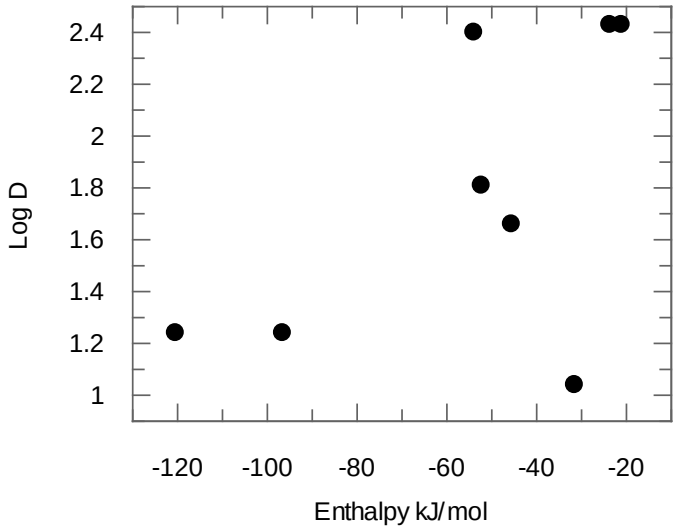


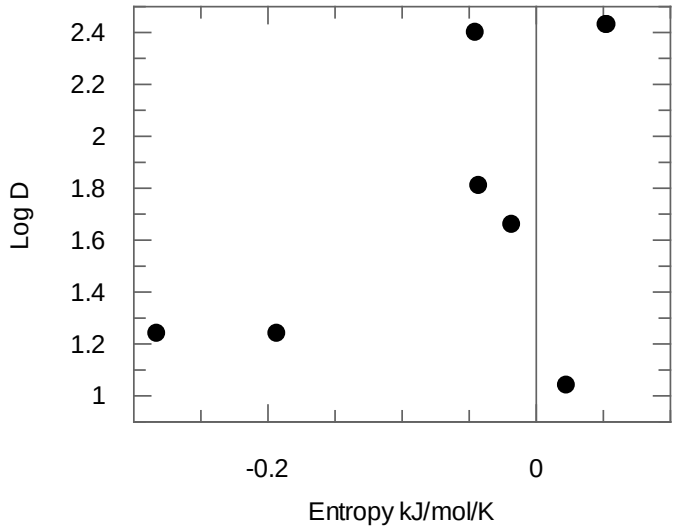
Thermodynamics

Protease 2 data









Using Kinetics and Thermodynamics in Design

Med. Chemists current views

Enthalpy and entropy not associated with local binding effects

General explanations derived from few anecdotal observations

Analysis in literature is simplistic

Should collect enthalpy and entropy data where we can

Spreading theory is important

Danger is to measure things because we can

Problem is neither thermodynamics nor kinetics but wrong biochemical mechanism

How can it add value?

Data are not critical

Not convinced

Kinetics has more utility

Understanding is not sufficiently mature

Influencing Medicinal Chemists

Future work - Building our understanding

Our ability to design *at will* small-molecule ligands that inhibit or modulate protein recognition events is currently a distant dream, because there are still significant gaps in our understanding of molecular recognition events

IMI

Kinetics for Drug Discovery

CASE collaboration with Sarah Harris (Leeds)

The overall aim of the present project is to measure the vibrational entropy of ligands in the free and bound states in a suitable model system of pharmaceutical interest, and to assess the feasibility of re-designing ligands so that losses in vibrational entropy on binding are minimised

Exploiting Kinetics & Thermodynamics

Key Take Home Messages

Shape, Dynamics and Interactions

Guidelines for Optimisation

Utility of Biophysical Methods

Shape, Dynamics & Interactions

Some Key Messages

Both the protein and ligand are flexible

Static picture does not robustly represent reality

Small differences in the shape of the protein and ligand can invalidate assumptions based on a static picture

Important to consider water as an extension to the binding site

Binding occurs as a result of the complex in solution having less free energy than the partners in solution

Changes away from the molecular interface may influence affinity

Use structure and small changes to give deeper understanding and opportunities for binding to other parts of the protein

Guidelines for Optimisation

Some Key Messages

Number and nature of protein ligand interactions (H-bonds, hydrophobic contacts) may influence binding kinetics

Optimising enthalpy – difficult to do, have to over come E-E compensation.
Select enthalpic hits in the first place; identify loactions that contribute favourably to enthalpy; introduce H-honds (worth 4 to 8 kJmol for neutral / ionic bonds of ideal geometry) which do not introduce significant structuring, which do not reduce desolvation, and which have optimal geometry; eliminate groups which contribute unfavourable enthalpy; optimise van der Waals binding – a good fit ensures atom efficient binding

Optimising entropy – exploit opportunities to release weakly bound water molecules in the binding site and around the ligand; minimise conformational flexibility in the ligand; exploit the hydrophobic effect (worth 6 kJ/mol for each methylene group added to the ligand) – but be cautious of just increasing lipophilicity.

Utility of Biophysical Methods

Use Should Help Inform the Design Process

Drug design paradigm of emphasising affinity improvement will need to change

Kinetic & thermodynamic considerations around the most favourable attributes and start points can be useful:

- Kinetics: rapid (fast on fast off) vs transient (slow on, fast off) vs slow (slow on, slow off)
- Thermodynamics: enthalpic hits may facilitate optimisation – relative ease of entropic gains versus enthalpic gains

We should take advantage of the strengths that biophysical methods provide and combine them with structural methods to provide fully annotated start points

Reducing model system artefacts by the use of orthogonal methods is valuable

Approaching protein-ligand interactions from multiple view points – both in terms of methods but also industry and academia will eventually lead to impact in Lead Generation

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