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# Biophysical Methods in Target Validation and Hits-to-Leads

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# Acknowledgements

## Astex Biophysics

- Joe Coyle
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- Glyn Williams

- Past Members
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# Outline

- Biophysical Methods and Detection of False Positives
- Hits, Leads and Ligand Efficiencies
- Thermodynamic Properties of Astex  Hits and Leads
- Enthalpy, Entropy and Potency


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# Astex Biophysics


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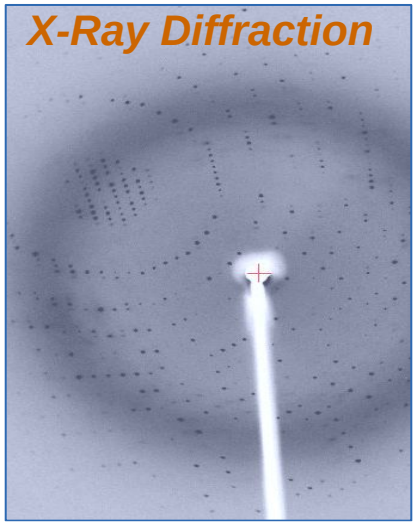
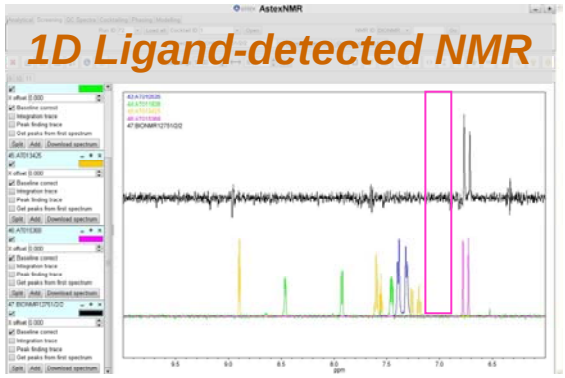
# Biophysical Methods at Astex

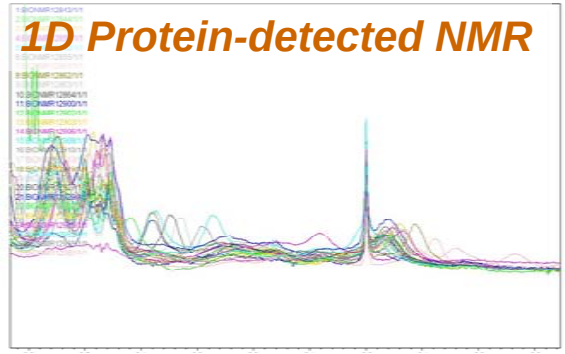
## X-Ray Diffraction



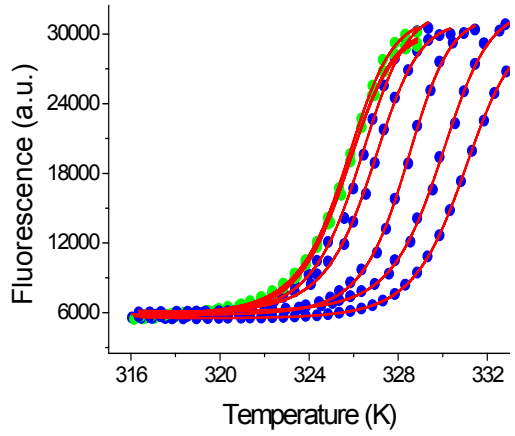
## 1D Ligand-detected NMR



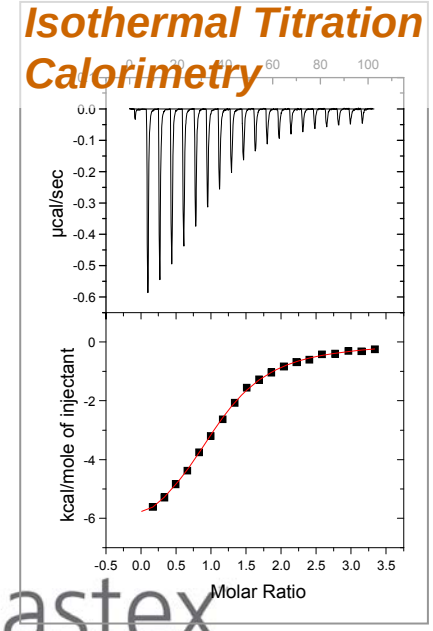
## 1D Protein-detected NMR



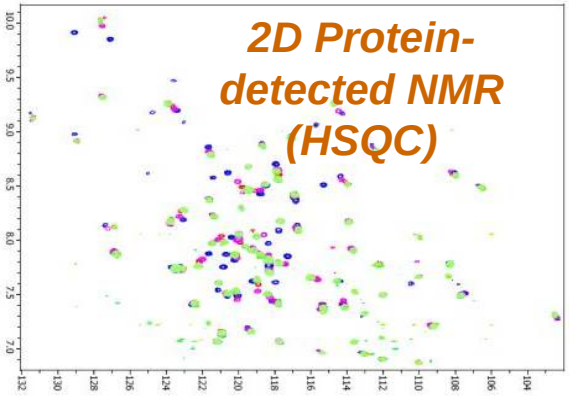
## Thermal Unfolding



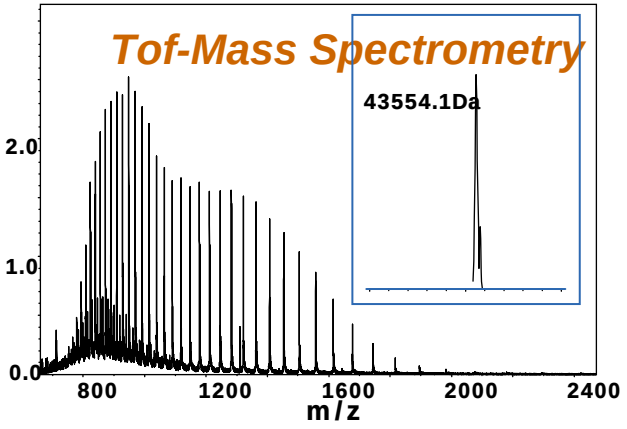

## Isothermal Titration Calorimetry




## 2D Protein-detected NMR (HSQC)



## Tof-Mass Spectrometry





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# False Positives


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# Common Mechanisms & Biophysical Detection

- Redox-activity (Time dependent)
  - Protein Modification [+O, -2H]
    - LC-MS – Δ(Retention Time, Mass)
    - NMR – loss of reducing agents and/or modification of ligand
- Aggregation (Time dependent)
  - Protein
    - DLS, MS, NMR etc
  - Ligand
    - NMR – buffer LOGSY > 0
    - ITC – anomalous ΔH
  - Protein-Ligand complex
    - ITC – anomalous ΔH and slow heat output
    - NMR – protein LOGSY >> 0
- Superstoichiometry
  - Non-specific binding
    - NMR – increased ligand linewidths in presence of protein + no effect of active-site competitor
    - ITC – high stoichiometry/ failure to saturate
  - Local aggregation
    - NMR - increased ligand linewidths in presence of protein, reversed by active-site competitor


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# Fragments and Hits


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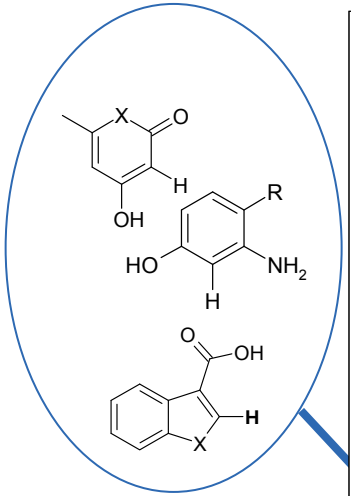
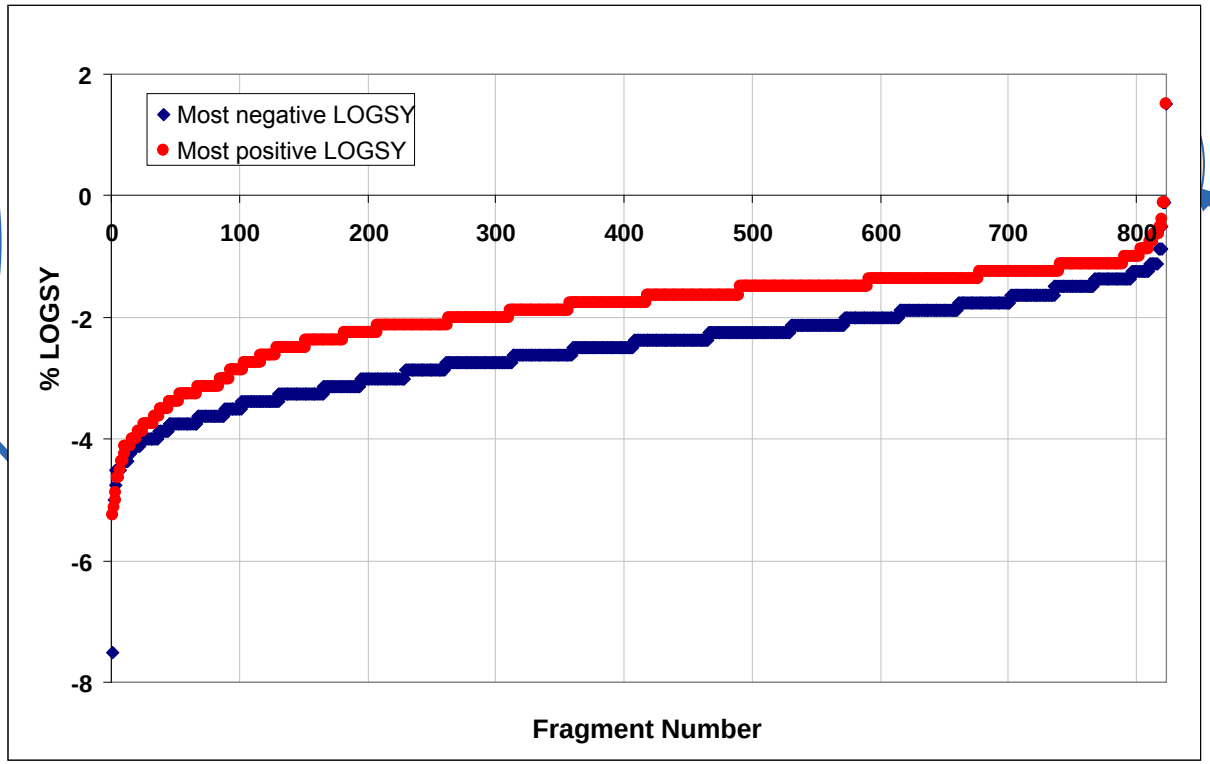
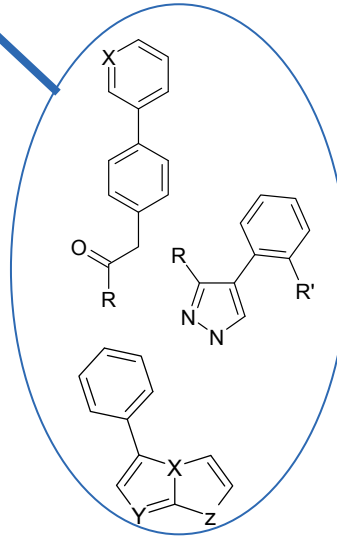
# Phys. Chem. Properties of Astex Fragments in Aq. Buffers

- LOGSY effect is negative for freely-rotating, highly hydrated fragments (depends on $r^{-6}$, $\tau_r$)
- LOGSY effect is small, and may be positive, for poorly hydrated, transiently aggregated fragments





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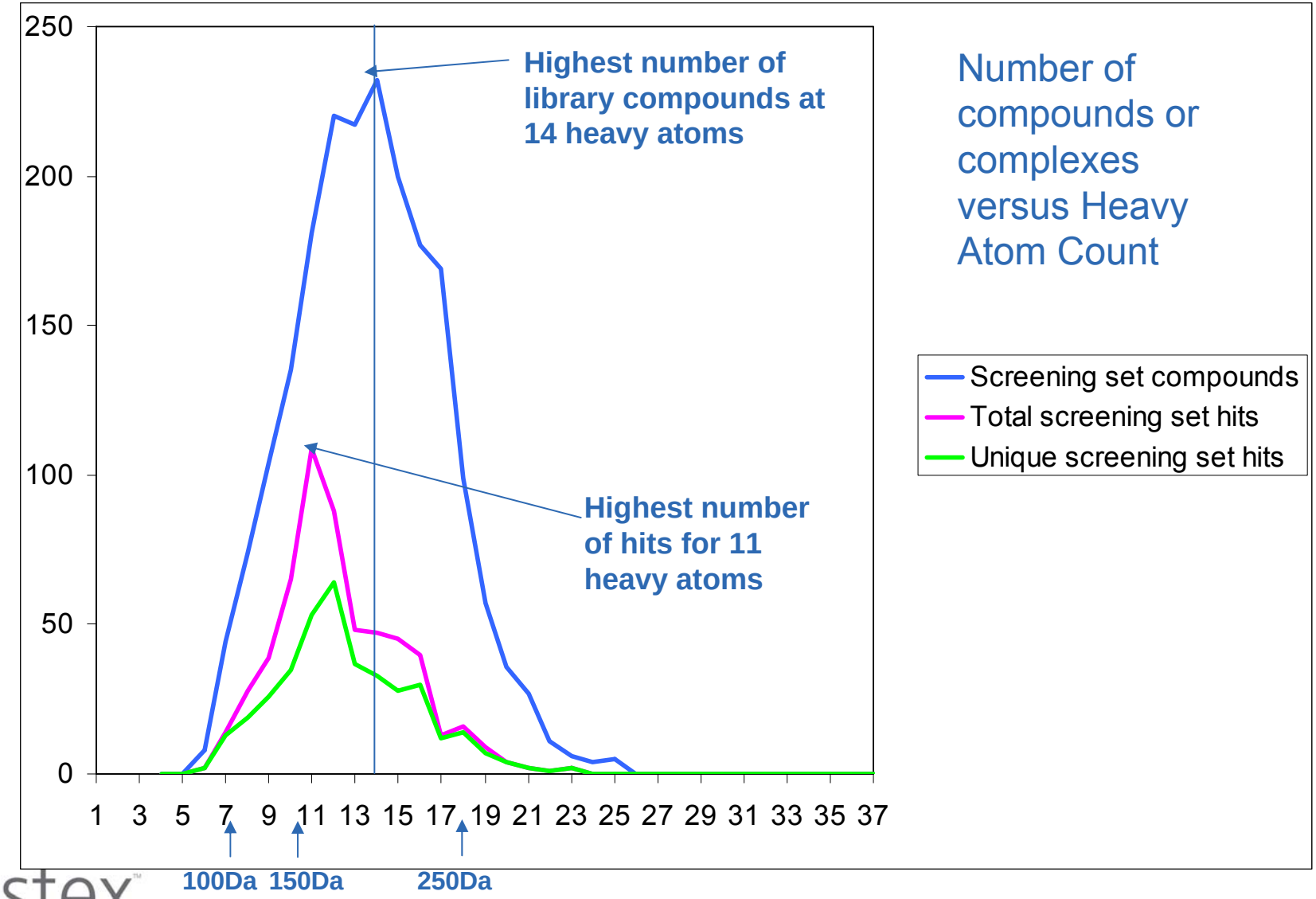
# Molecular Weight Analysis of Pyramid™ (X-Ray) Hits





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# Hits, Leads and Ligand Efficiency


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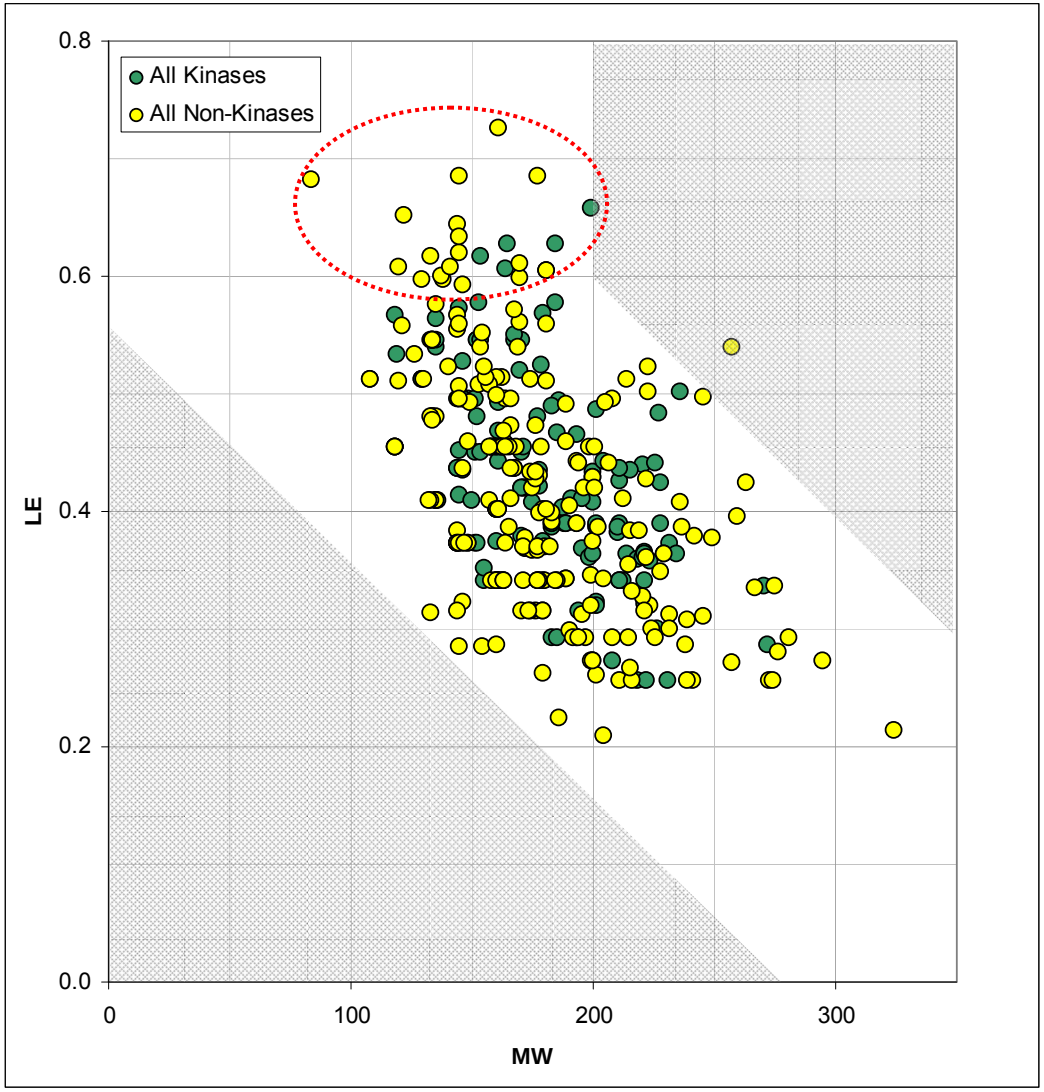
# LE versus MW for Pyramid screening hits




- Historical analysis of X-ray hits from fragments in library (375 complexes)
  - LE's derived from IC50 and Kd data (NMR, ITC)
  - Analysis **excludes** hits with no measurable affinity ("missing" points in bottom left: Kd> 5mM)
  - Higher molecular weight fragments yield lower LE starting points (empty area in top right)
  - 'Best' hits have LE>0.6 and MW<200Da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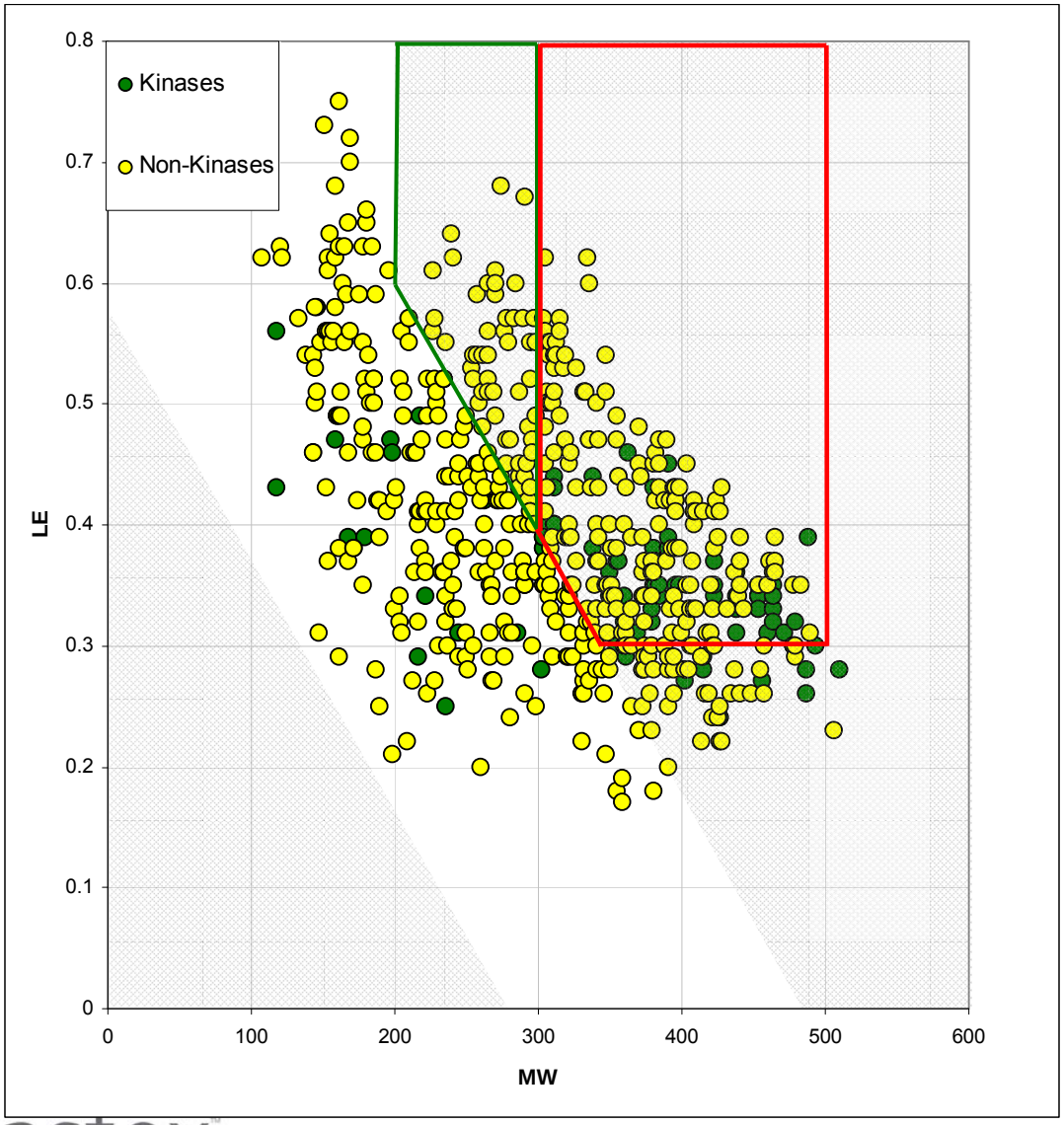
# LE versus MW for All Compounds with ITC data




- Analysis of current ITC data from Astex hits and leads
  - >600 complexes, 19 targets (~100 kinase + ~500 non-kinase)

- Targeted synthesis has produced many 'optimised hits' with LE>0.4 and MW <300

- Structure-based design has made many larger compounds (300-500Da) with good LE (>0.3)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# Energetics of Fragment Binding (*Credo*)

- Fragment binding necessitates the loss of +4.2 ±0.6 kcal/mol of rotational and translational entropy at 25ºC

- The 5% most ligand efficient, validated Astex hits against 17 diverse targets have LE's of 0.65±0.05 and contain 11.5 ± 2.5 non-H atoms = 'optimised hits'
  - The average binding energy of an optimised hit is 0.65*11.5 = -7.5kcal/mol
  - The average *intrinsic* binding energy of an optimised hit is -7.5-4.2 = -11.7kcal/mol
  - The average *intrinsic* ligand efficiency of an optimised hit is 11.7/11.5 ~1 kcal/mol/atm

- Each optimised hit makes 3 interactions with the protein
  - On average each optimised interaction is worth -11.7/3 ≈ -4kcal/mol
  - Compare this with gas phase H-bond strengths (e.g. OH---O=C = -7.4kcal/mol)
  - On average ~4 (11.5/3) non-H atoms are required to form each interaction
    - average size of functional group + linker atoms


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# Potencies of smaller fragments *(reductio ad absurdam)*

- While the best Astex hits have LE=0.65 ±0.05 and 11.5 non-H atoms, a more typical screening hit has LE~0.4 and 13.5 non-H atoms
  - 'typical' Astex screening hits have intrinsic binding energies of ~9kcal/mol or about 3kcal/mol/interaction

- If current Astex hits are ~12 atoms and make 3 optimised interactions. What might be expected from smaller fragments?
  - ***2 optimised* interactions** (~ 8 non-H atoms)
  - Intrinsic binding energy ≈ -2 * 4 = -8kcal/mol
  - <ΔGbind> = -8 + 4.2 = -3.8kcal/mol (LE= 0.45)
  - ***Kd ≈ 2mM***

  - ***2 non-optimised* interactions** (~ 8 non-H atoms)
  - Intrinsic binding energy ≈ -2 * 3kcal/mol = -6kcal/mol
  - <ΔGbind> = -6 + 4.2 = -1.8kcal/mol (LE= 0.23)
  - ***Kd ≈ 50mM***


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# Hits-to-Leads & Group Efficiency (GE)

- Hits (fragments) are grown into leads by adding functional groups which make new interactions with the protein

- If optimal, each additional interaction *could* add up to -4kcal/mol to $\Delta G_{bind}$
  - Since each functional group is (on average) 4 atoms, group efficiencies of 1.0 are feasible

  - More realistically, improvements of -3kcal/mol (GE=0.75) would be expected if the new group is as close to optimal as a typical fragment.

  - If the first fragment has already found all the best 'hotspots', then GE<0.75

- In practice, measured GEs span a larger range than fragment LEs
  - GE>1.0 is not uncommon & GE<0 is quite possible!

  - Improvements in affinity caused by addition of small groups to a fragment are more easily detected than the binding of the small group itself


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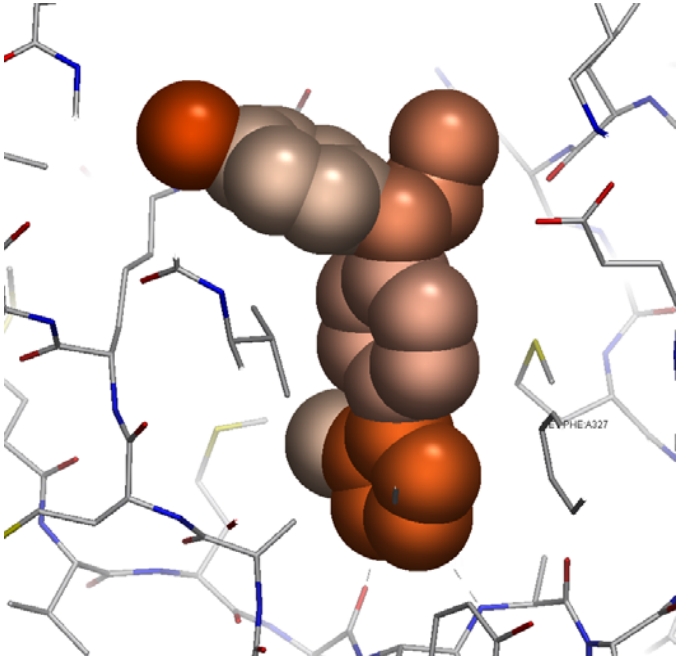
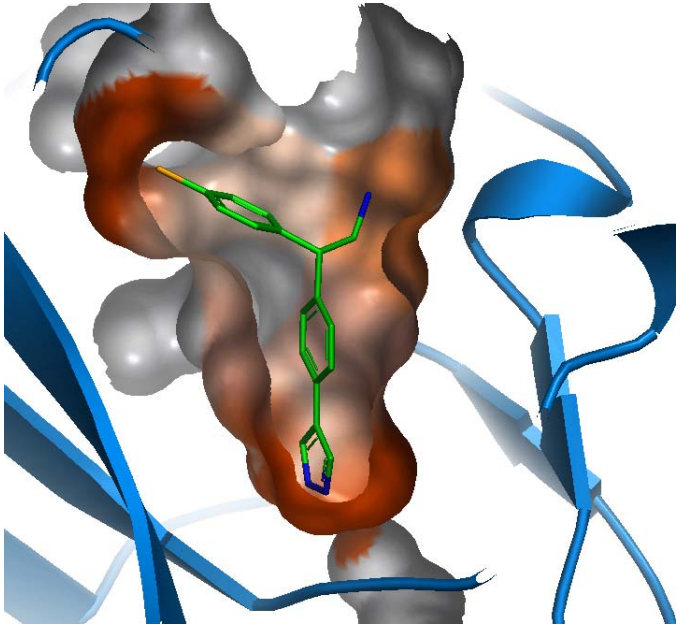
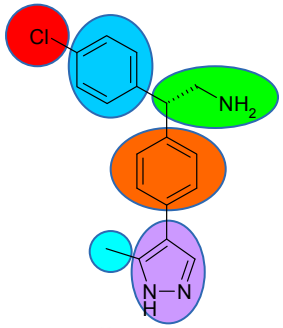
# Group efficiency example - PKB








| Compound | Pyr | Me | Phe1 | EtNH2 | Phe2 | Cl | DG |
|---|---|---|---|---|---|---|---|
| [1] | ✓ | ✓ | ✓ | | | | -6.0 |
| [2] | ✓ | ✓ | ✓ | ✓ | | | -7.6 |
| [3] | ✓ | | ✓ | | | | -5.7 |
| [4] | ✓ | | ✓ | ✓ | ✓ | | -9.0 |
| [5] | ✓ | | ✓ | ✓ | ✓ | ✓ | -10.6 |
| [6] | ✓ | | | | | | -3.1[a)] |
| *dG* | -7.3[b)] | -0.3 | -2.5 | -1.6 | -1.7 | -1.6 | |
| *GE* | 1.5 | 0.32 | 0.42 | 0.54 | 0.28 | 1.6 | |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# Average Thermodynamic Properties


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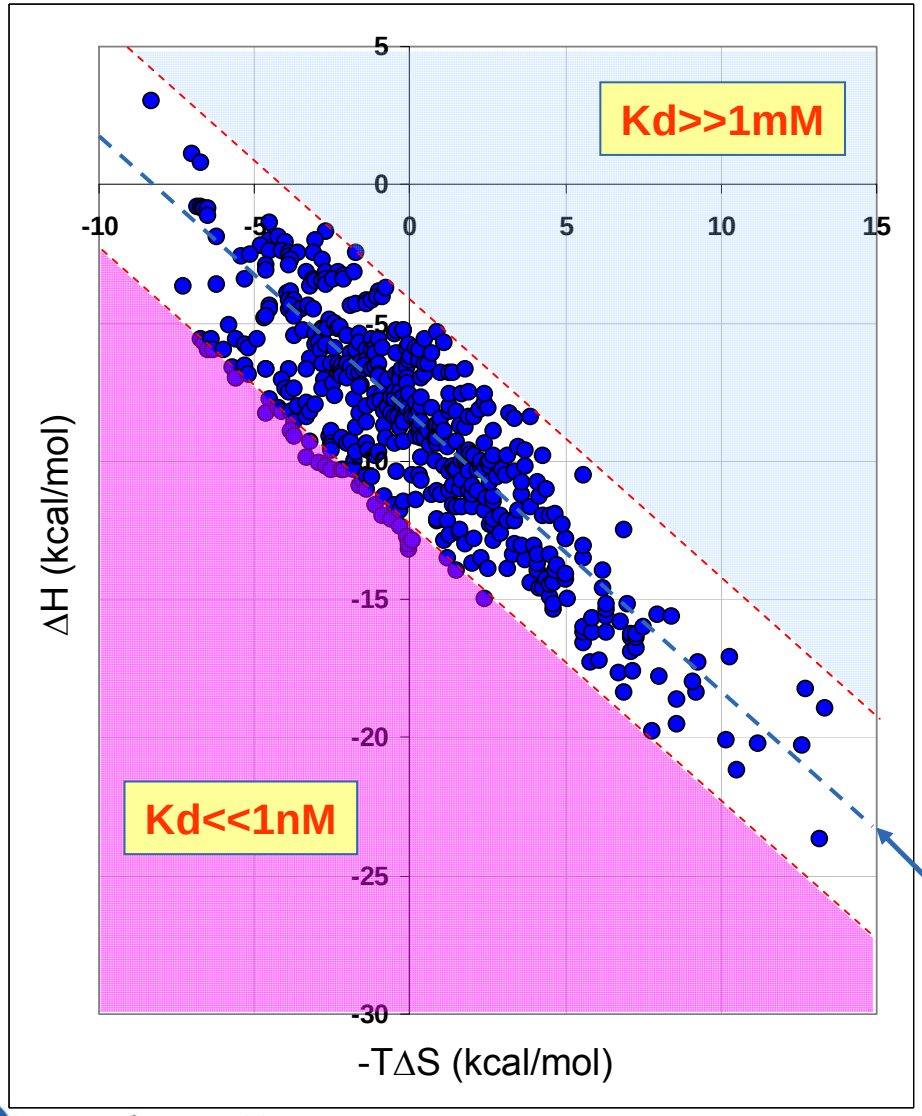
# $\Delta H_{bind}$ vs $-T\Delta S_{bind}$




| | ΔG | ΔH | -TΔS |
|---|---|---|---|
| Ave. value | -8.7 | -8.9 | +0.2 |
| Max-Min | 9.0 | 26.7 | 21.7 |

- All current Astex ITC data with good stoichiometry and reliable ΔH (490 datasets)
- *On average* Astex hits and leads are enthalpy driven with small (~0) entropies of binding
- The range of ΔH and TΔS values is 2-3x the range of ΔG ('Enthalpy-entropy compensation')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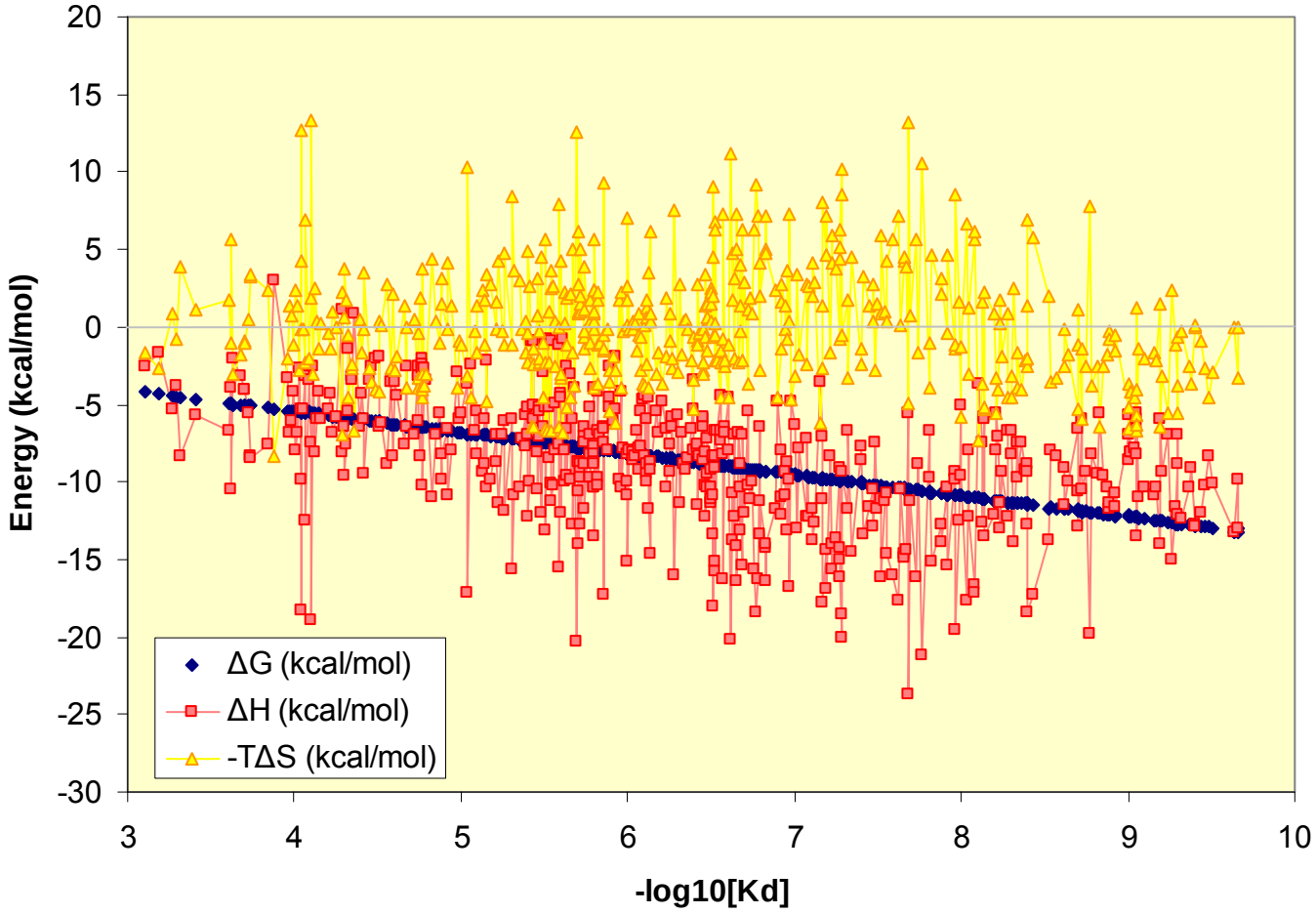
# How do ΔH and -TΔS behave as ΔG improves?

- **Arrange data in order of increasing affinity and plot ΔG, ΔH and -TΔS against $-\log_{10}K_d$**





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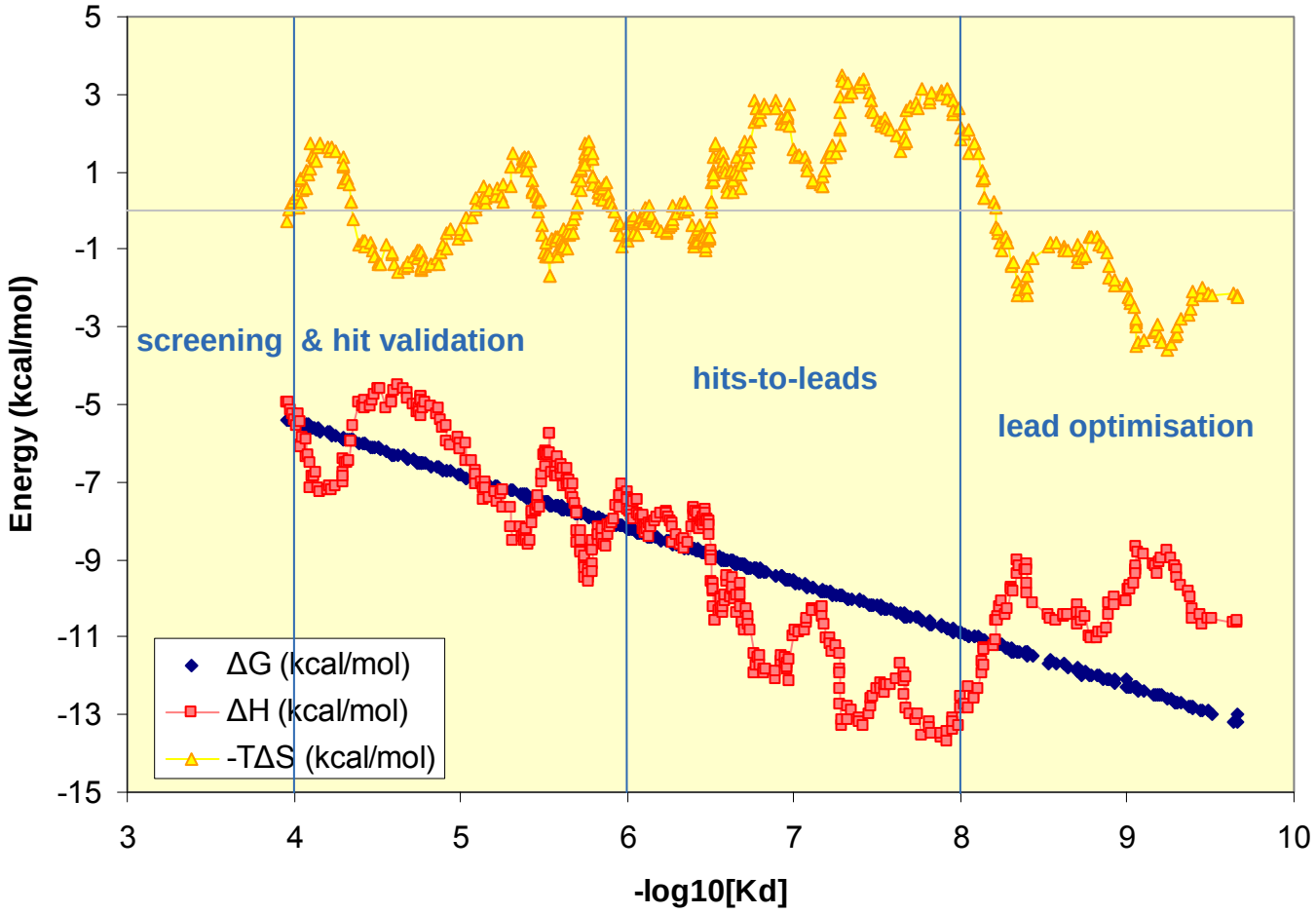
# *On average*, how do ΔH and -TΔS behave as ΔG improves?

- **Arrange data in order of increasing affinity and plot a running (20 point) average of ΔG, ΔH and -TΔS against $-\log_{10}K_d$**





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# *On average*, how do thermodynamic properties change during hits-to-leads?

| All Astex Targets & Ligands | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Affinity Range** | **Description** | **No. of Targets** | **No. of Ligands** | **<ΔG> kcal/mol** | **<ΔH> kcal/mol** | **<-TΔS> kcal/mol** | **<LE> kcal/mol** |
| >100uM | Fragment Hits | 8 | 22 | -4.9 | -5.0 | 0.0 | 0.35 |
| 1uM-100uM | Optimised Hits | 17 | 192 | -7.0 | -7.1 | 0.1 | 0.39 |
| 10nM-1uM | Leads | 15 | 186 | -9.3 | -10.5 | 1.2 | 0.41 |
| <10nM | Optimised Leads | 5 | 90 | -11.9 | -10.1 | -1.8 | 0.45 |


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# Thermodynamic Properties of HSP90 Lead Series


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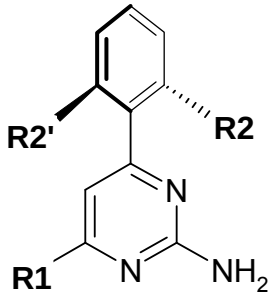
# HSP90: Amino-Pyrimidine Thermodynamics

| HSP90 Amino-pyrimidines | | | | | | |
|---|---|---|---|---|---|---|
| **Affinity Range** | **Classification** | **No. of Ligands** | **<ΔG> kcal/mol** | **<ΔH> kcal/mol** | **<-TΔS> kcal/mol** | **<LE> kcal/mol** |
| >100uM | Fragment Hits | 2 | -4.9 | -5.3 | 0.4 | 0.38 |
| 1uM-100uM | Optimised Hits | 8 | -7.5 | -6.5 | -0.9 | 0.44 |
| 10nM-1uM | Leads | 36 | -9.6 | -8.4 | -1.2 | 0.49 |
| <10nM | Optimised Leads | 6 | -11.4 | -15.4 | 4.0 | 0.55 |

- Binding is enthalpy driven during all phases
- Series is more ligand-efficient than the average (0.35-0.45)
  - HSP90 is highly druggable
- LE improves steadily from “hits” to “optimised leads”
  - cannot be due to attrition of hits and leads with low LE
  - must be due to addition of interactions with good group efficiencies
- This series bucks the average trend that entropy improves in final stages (Kd<10nM)





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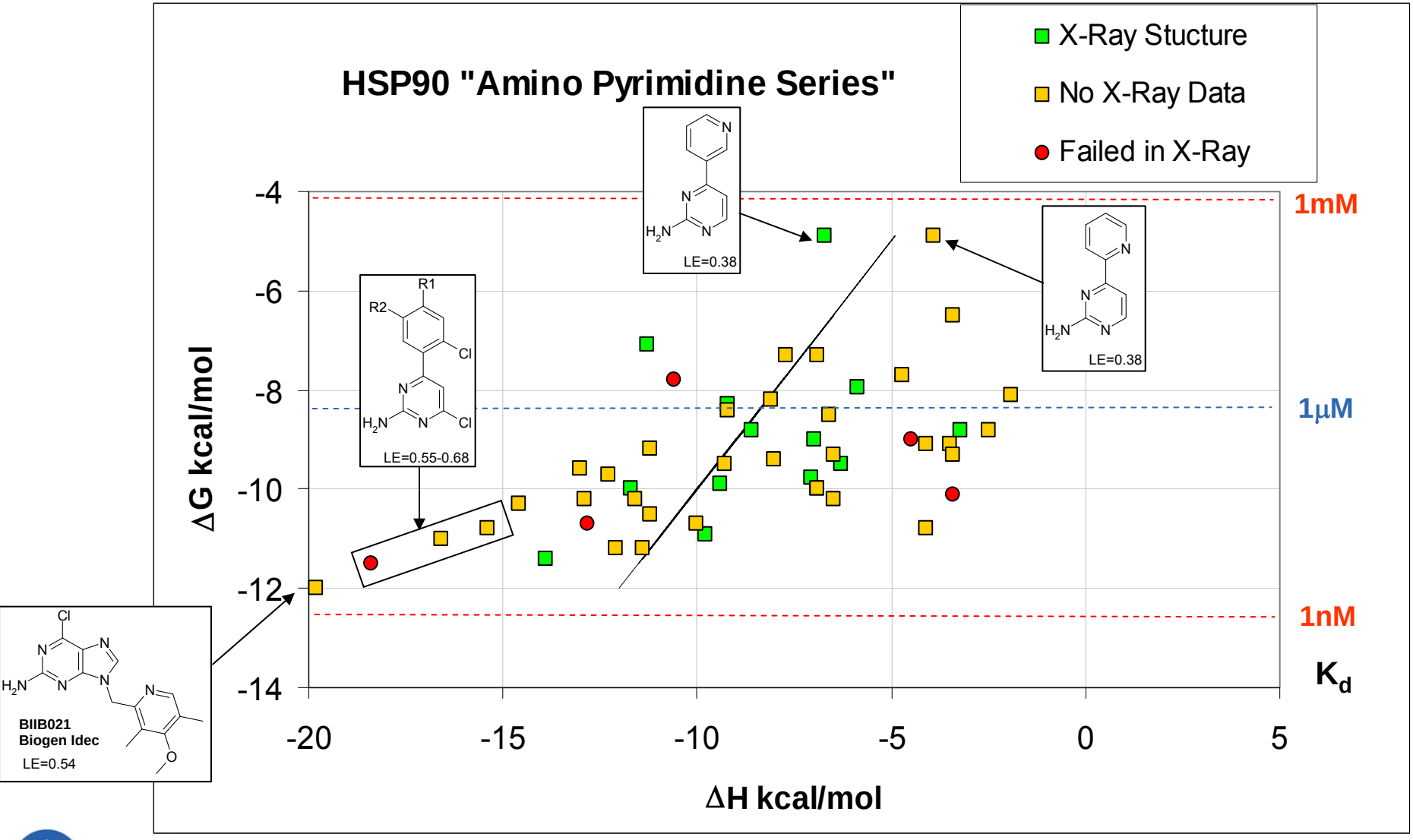
# From Hits to Optimised Leads





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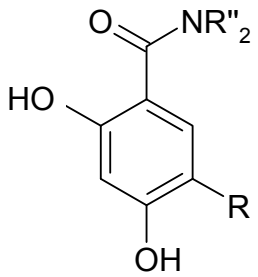
# HSP90: Resorcinol Thermodynamics

| HSP90 Resorcinols | | | | | | |
|---|---|---|---|---|---|---|
| **Affinity Range** | **Description** | **No. of Ligands** | **<ΔG> kcal/mol** | **<ΔH> kcal/mol** | **<-TΔS> kcal/mol** | **<LE> kcal/mol** |
| >100uM | Fragment Hits | 3 | -5.0 | -1.9 | -3.1 | 0.36 |
| 1uM-100uM | Optimised Hits | 1 | -8.0 | -2.6 | -5.4 | 0.35 |
| 10nM-1uM | Leads | 9 | -10.2 | -8.2 | -1.9 | 0.46 |
| <10nM | Optimised Leads | 63 | -12.1 | -9.2 | -2.9 | 0.46 |

- HSP90 Resorcinol series (HSP90 series 1) is distinct from the average
- Binding is entropically driven during the early phases
  - displacement of tightly bound water molecules
- LE *appears* to improve discontinuously
  - very sparse ITC data for “optimised hits” (n=1)
  - in this range, assay (IC50) data give <ΔG>= -6.4kcal/mol & <LE> = 0.41 (n=4)
  - series made rapid progress from hit to 1uM lead (5 compounds)





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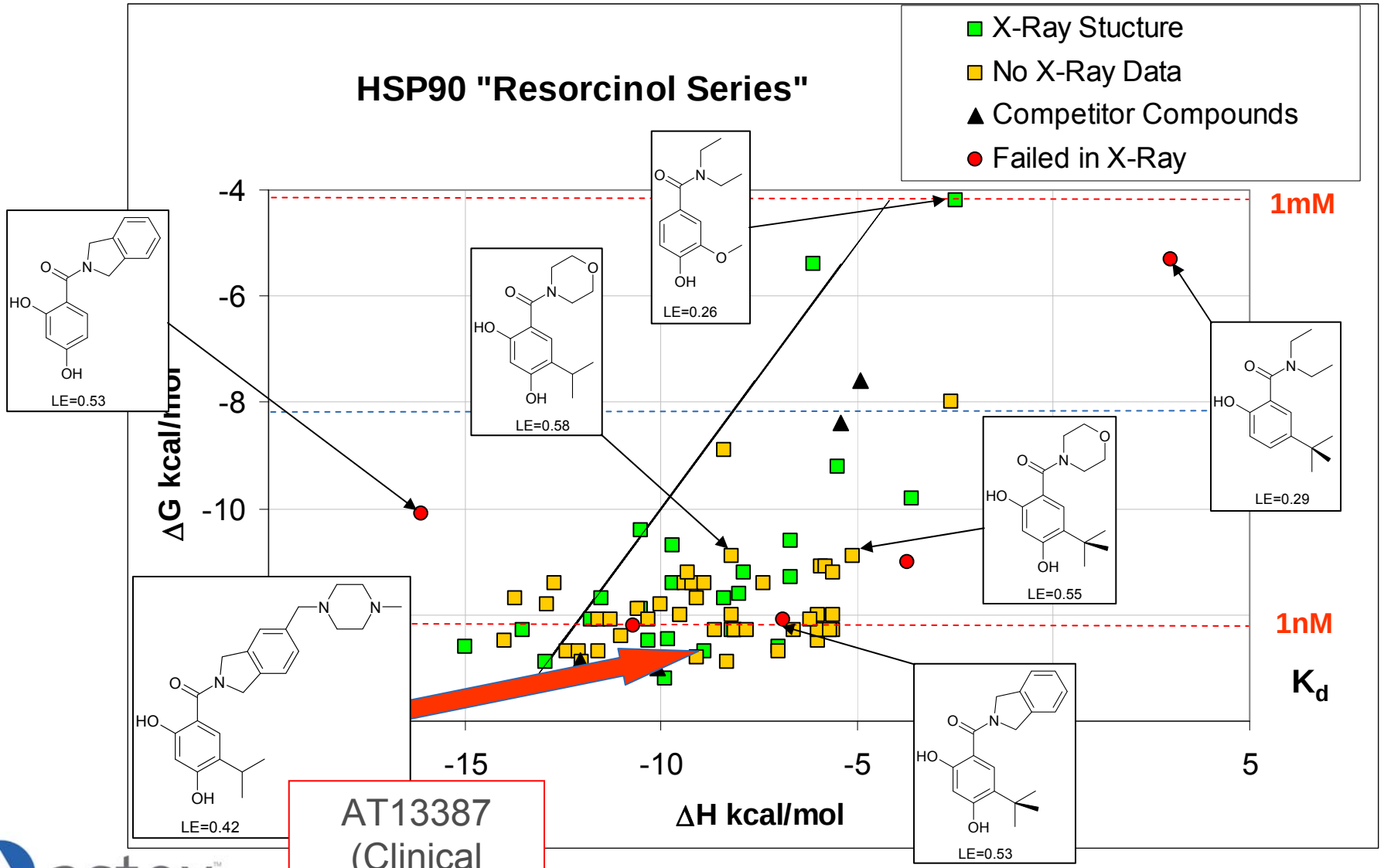
# From Hits to a Clinical Series





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# Summary of HSP90 Thermodynamics

- Amino-Pyrimidine and Resorcinol series are thermodynamically distinct
  - Clinical candidate came from less-enthalpically favoured series (resorcinols)
  - Selection was made on basis of PK/PD properties

- Important growth points on fragment can be more easily identified from changes in $\Delta H$ than $\Delta G$
  - enthalpy-entropy compensation ensures $|\Delta\Delta H| > |\Delta\Delta G|$ when the fragment is modified
  - may be useful to select growth points if X-ray structures were unavailable


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# Enthalpy-Driven, Entropy Driven or just Potent?


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# Free Energy and the Universe

Total Entropy Change of Reactants + Solution

Entropy Change in Solution (buffer, calorimeter etc)

Entropy Change in Reactants (protein + ligand)

$$-\Delta G/T = -\Delta H/T + \Delta S$$

*The Universe* *The Surroundings* *The System*

- The terms “Entropy- or Enthalpy-driven” reflect differences in the proportion of entropy that is created in the *System* or in the *Surroundings* during the reaction
- One way in which this can be quantitatively expressed is to define ‘Index $_{(E\text{-}E)}$’

$$\text{Index}_{(E\text{-}E)} = (\Delta H + T\Delta S)/(\Delta H - T\Delta S)$$

$-T(\Delta S_{Surroundins} - \Delta S_{System})$ $-T\Delta S_{Universe}$


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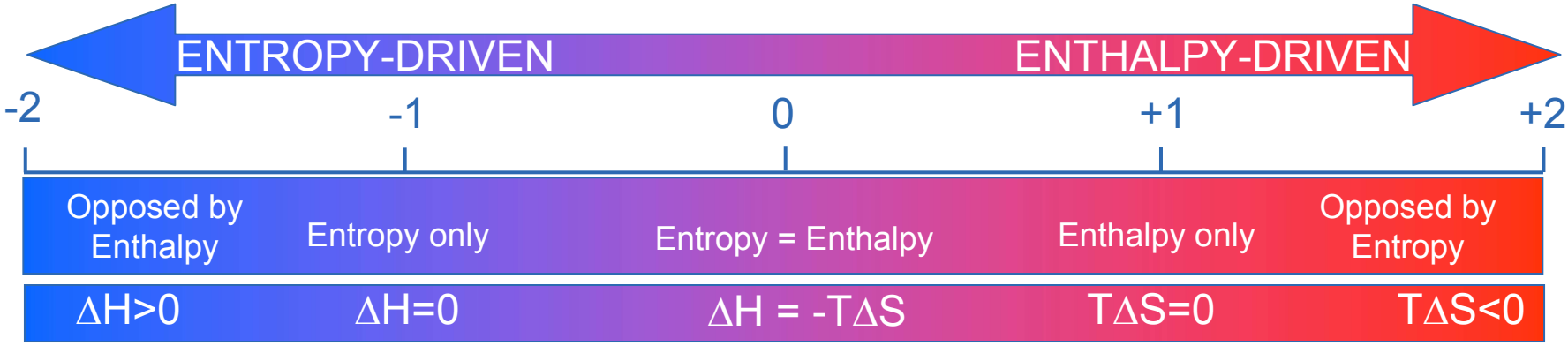
# Index $_{(E\text{-}E)}$

$$\text{Index}_{(E\text{-}E)} = (\Delta H + T\Delta S)/(\Delta H - T\Delta S)$$




Scale is normalised by $\Delta G \Rightarrow$ can be used to compare fragments and leads

- Are enthalpically-driven ligands more ligand efficient?
- Are the most potent Astex compounds more enthalpically-driven?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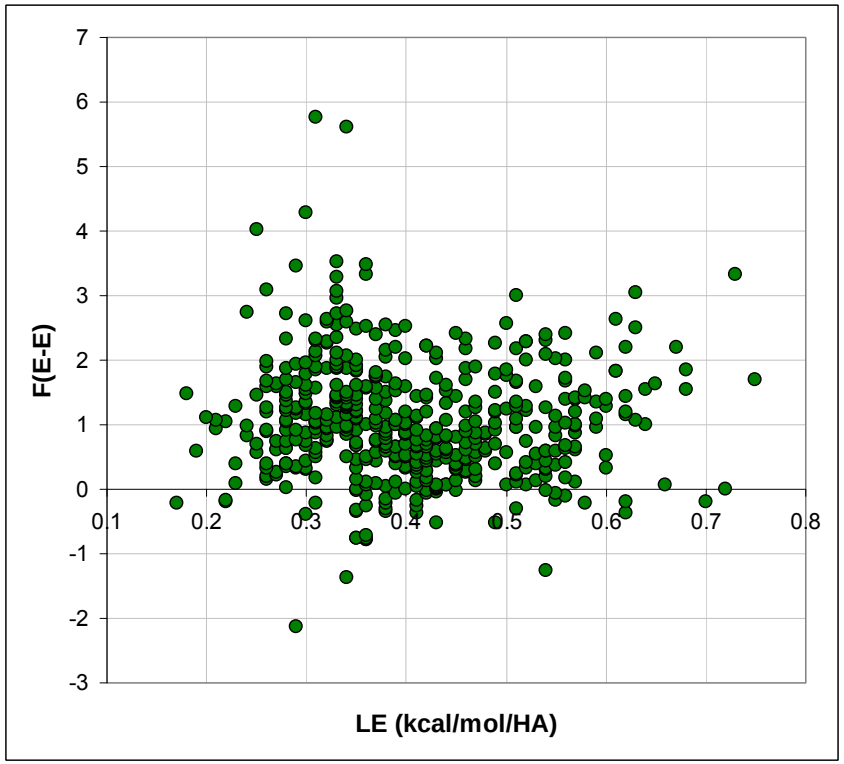
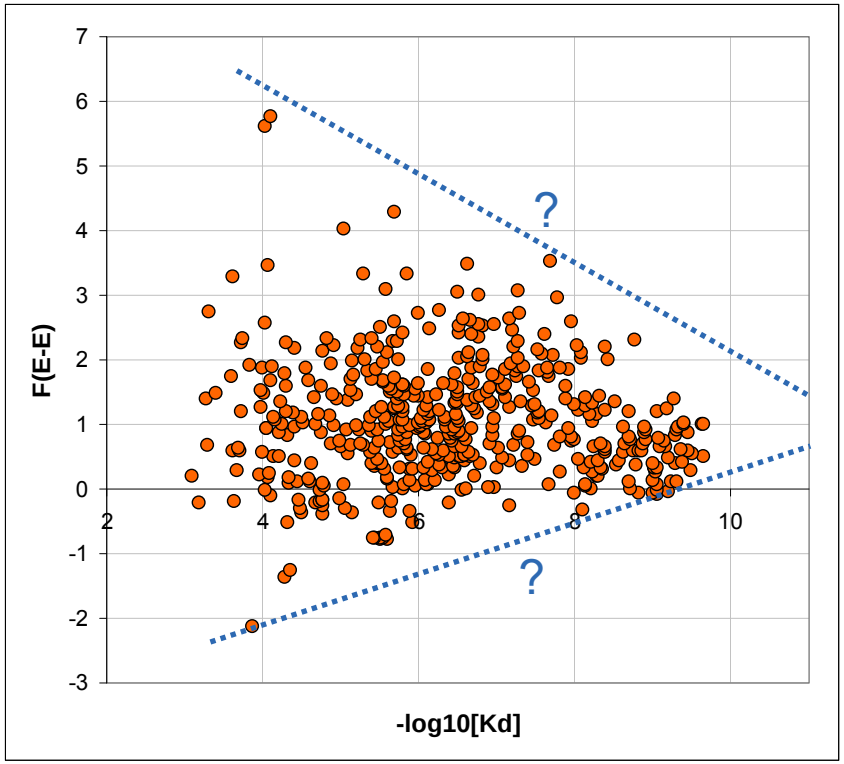
# Index $_{(E-E)}$ vs LE and ΔG (490 Interactions)




No correlation of Index $_{(E-E)}$ with LE

- but largest variation is observed for compounds with low LE

No increase in Index $_{(E-E)}$ with potency

- but most potent compounds have LE between 0 and ~1 (ENTHALPY *and* entropy driven)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# Summary

*'An Investment in Knowledge Always Pays the Best Interest'*

*-Benjamin Franklin*


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# Lessons from Biophysics

- Measurement of weak affinities (mM) adds value to hits and to understanding of targets and libraries (long-term benefits)
  - ITC and NMR provide reliable data at the cost of protein
  - On average, fragment binding is driven principally by enthalpy
  - SBDD maintains favourable enthalpies

- Growing fragments and improving LE has been easier than expected
  - most of the entropic penalty for binding is paid when the fragment is small
  - group efficiencies >1 are feasible, but unlikely unless growing into regions where fragment binding has already been observed ('hot-spots')
  - fragments which make only 2 interactions will be difficult to detect (Kd ~ 2-50mM)
  - changes in $\Delta H$ reveal potential growth points but $\Delta G$ is more readily optimised

- 'Day-to-day' value is in detection of 'false positives' (undesirable MoA)
  - anomalous enthalpies (ITC)
  - line broadening or anomalous LOGSY intensities (NMR)
  - changes in protein mass (Tof-MS)


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