Vernalis

York Structural Biology Laboratory

THE UNIVERSITY of York

HYMS THE HULL YORK MEDICAL SCHOOL

For slides – r.hubbard@vernalis.com

Progressing fragments for challenging targets

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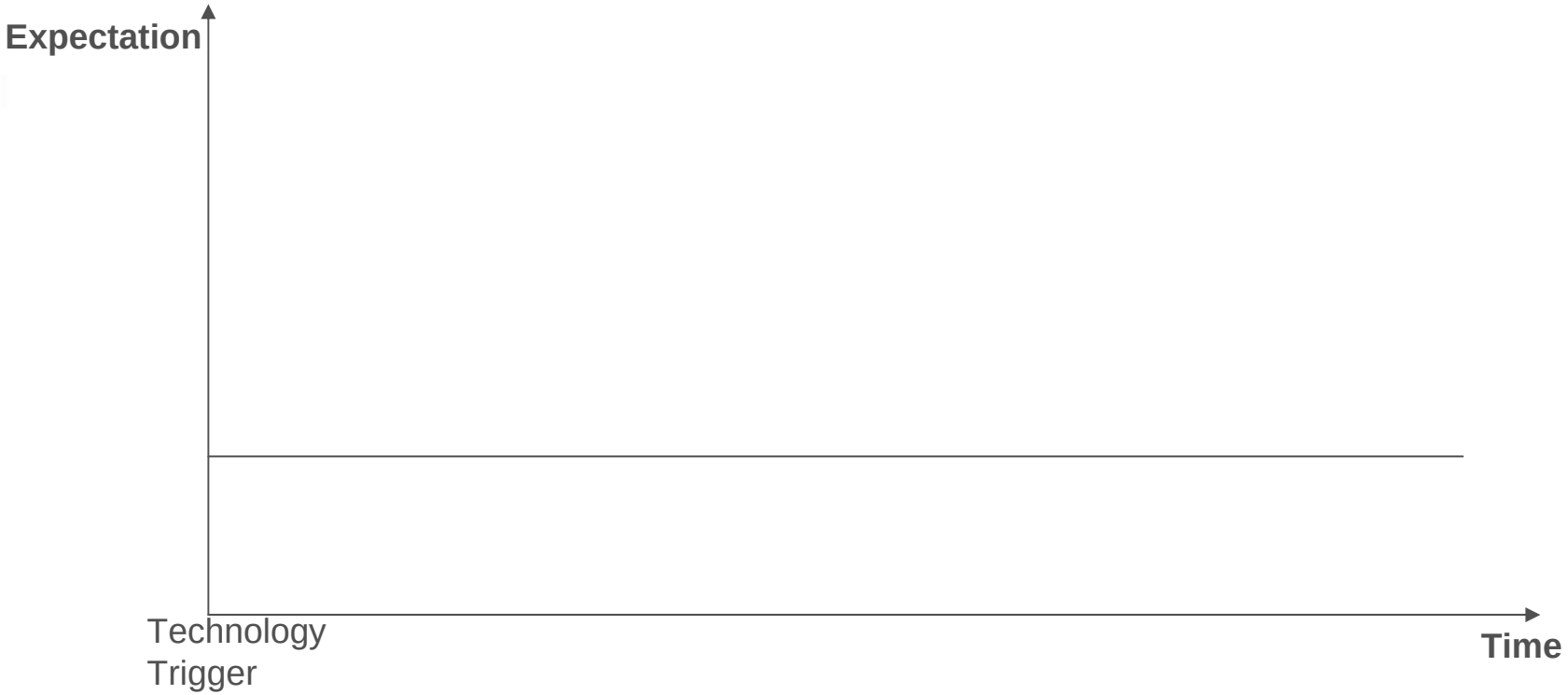
YSBL & HYMS, Univ of York, UK

Fragments 2013

Trends for new technologies

- In the beginning – lots of excitement
  - Which can lead to hype and over-selling



Analysis / terms initially proposed by Gartner group (Murcko)

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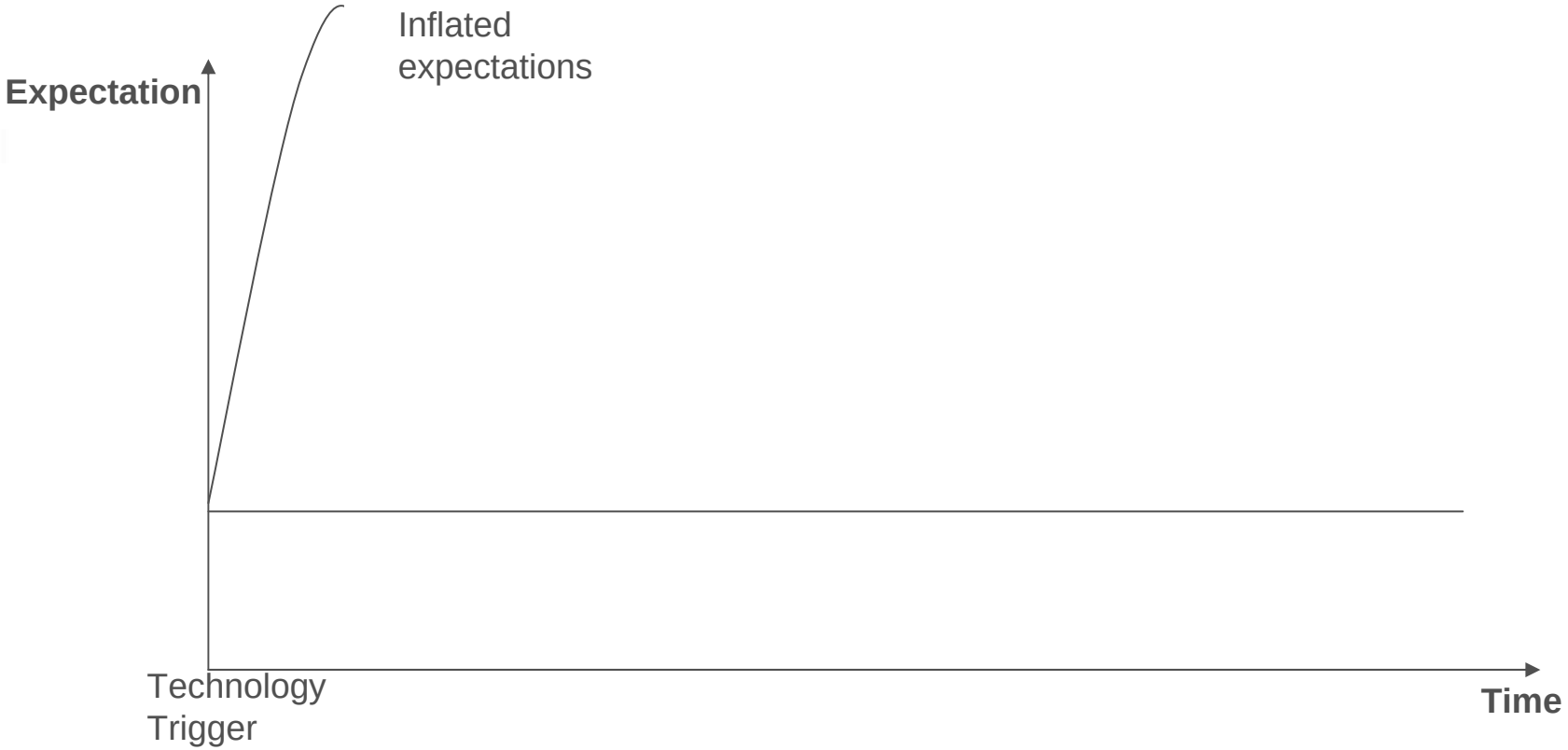

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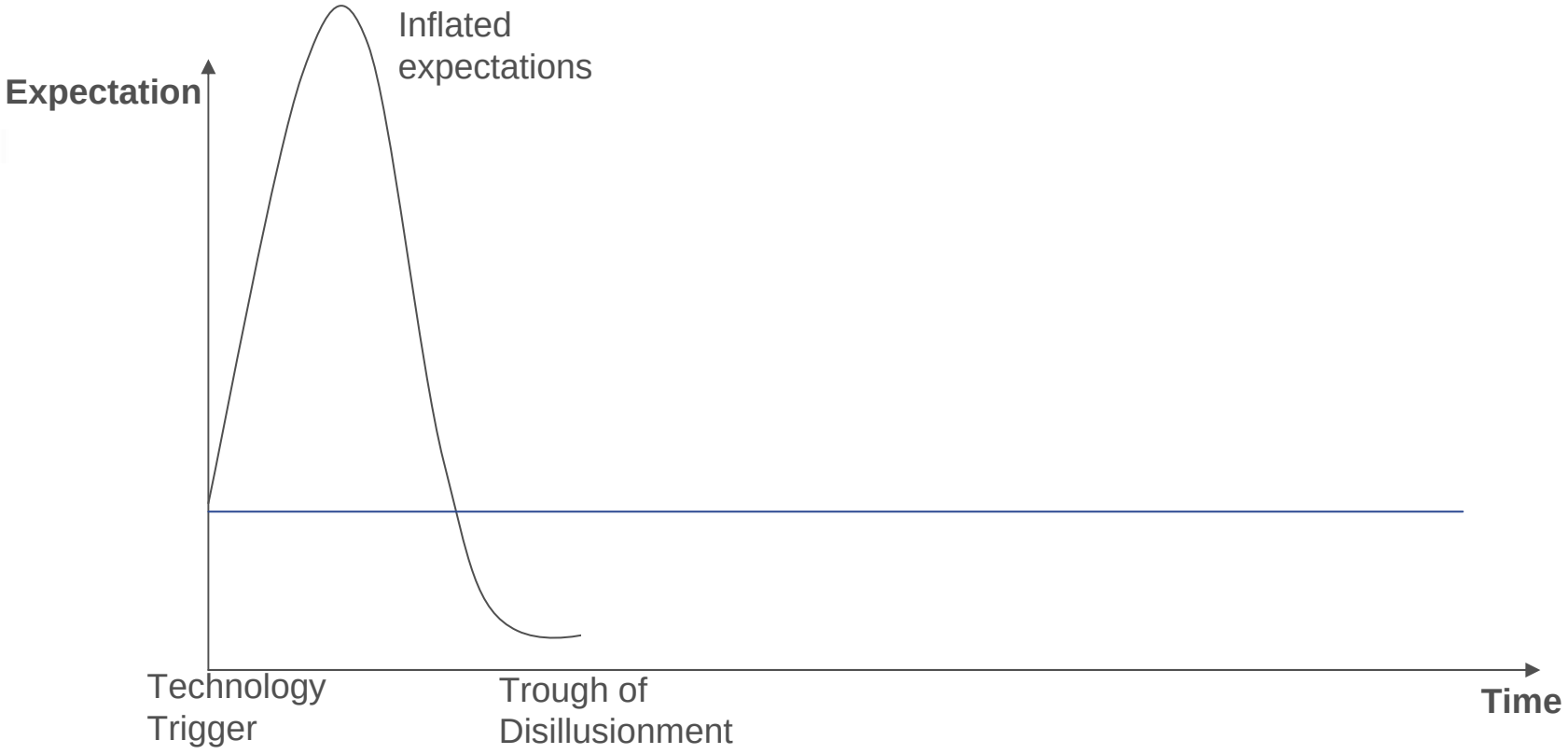
Trends for new technologies

- Too rapid (often inexpert) deployment
  - It doesn't work - disillusionment



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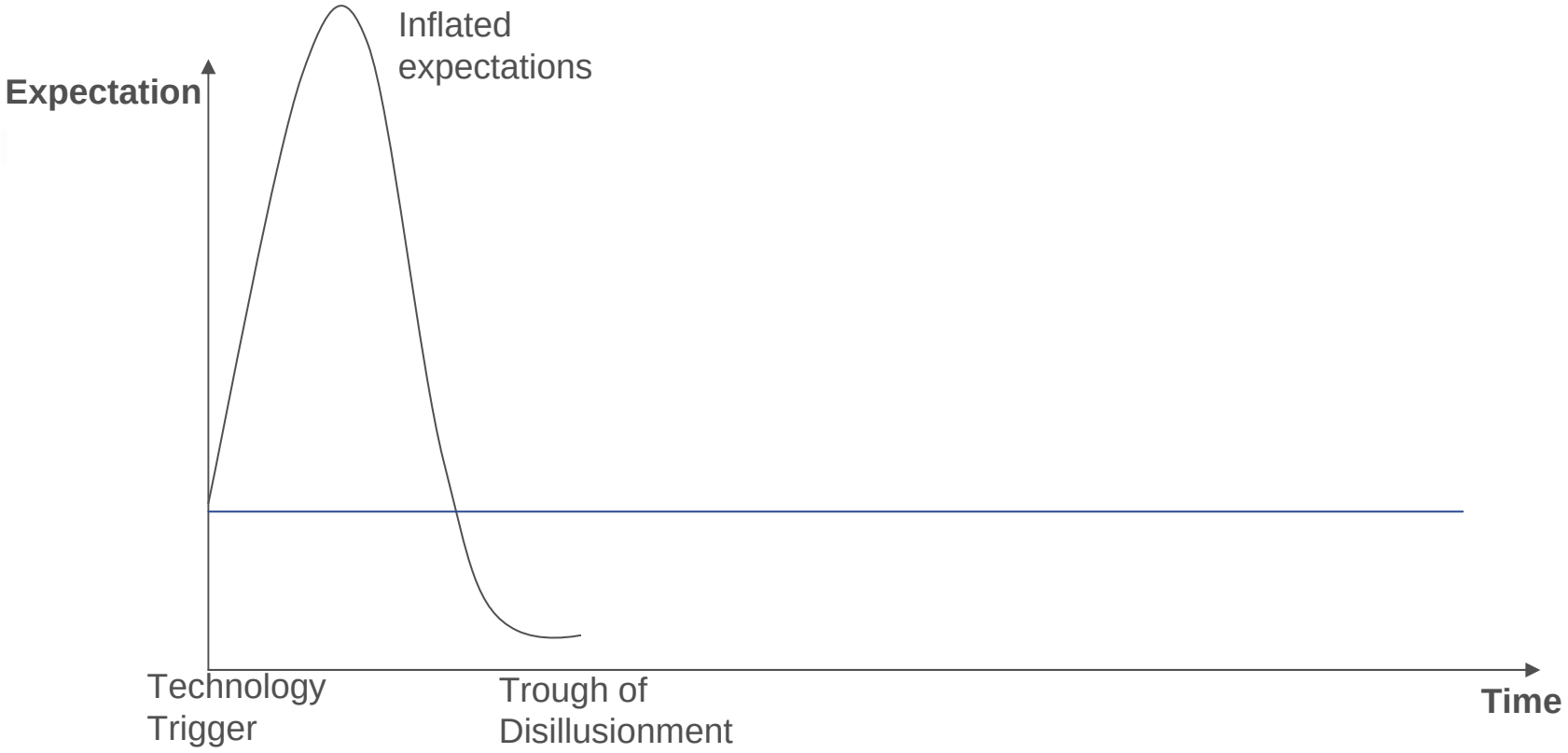
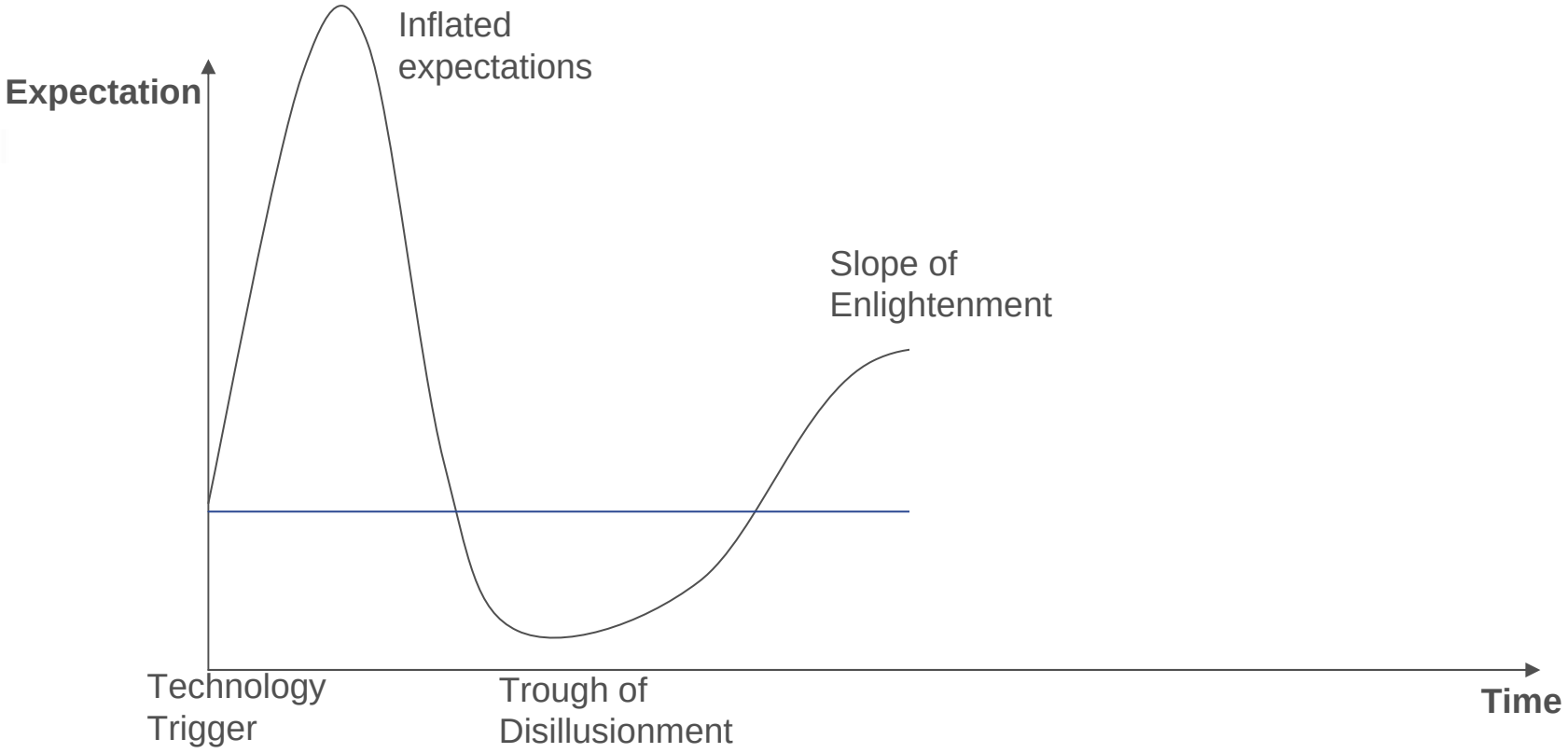


Analysis / terms initially proposed by Gartner group

Trends for new technologies

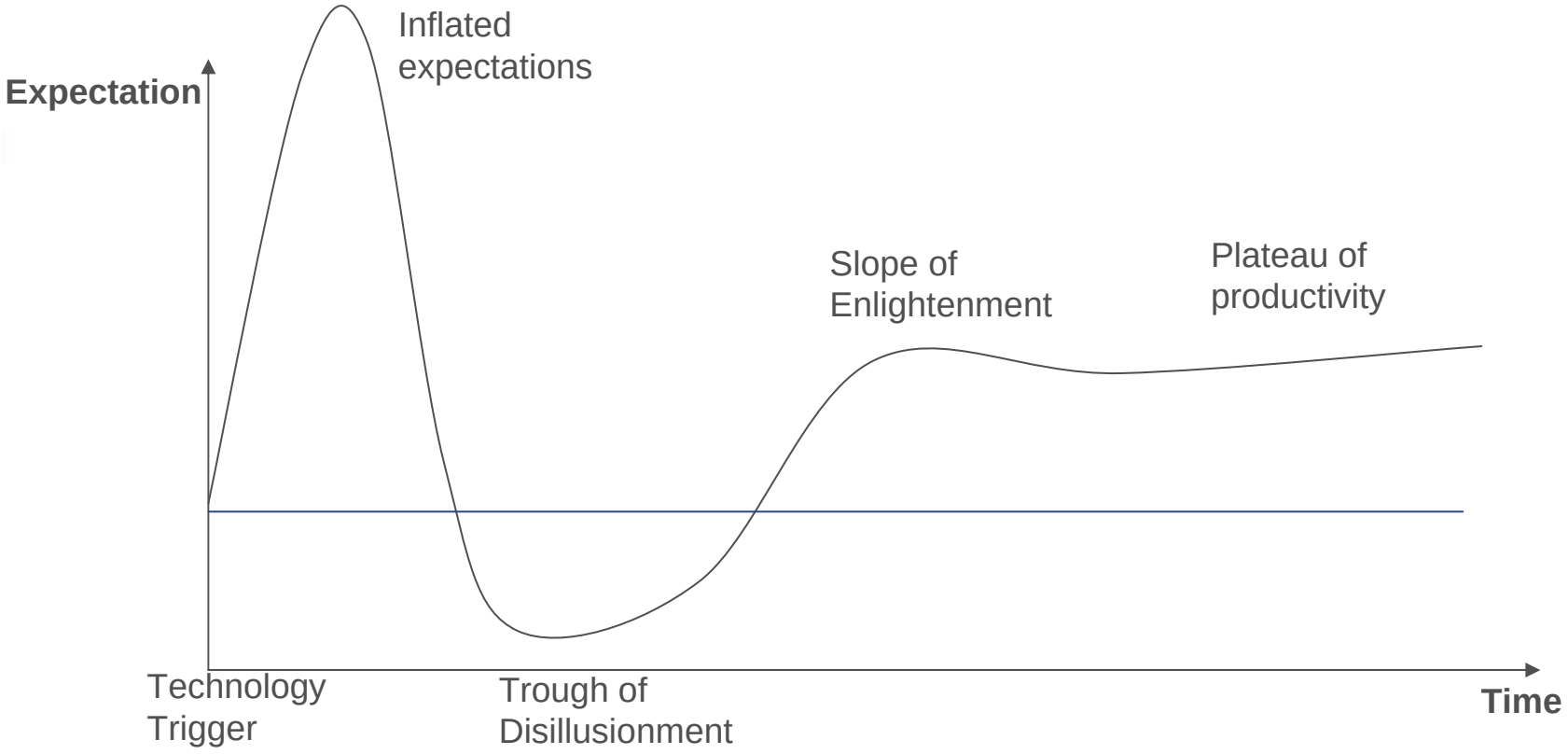
- Eventually, expertise grows
  - Begin to understand how and where to apply methods



Analysis / terms initially proposed by Gartner group

Trends for new technologies

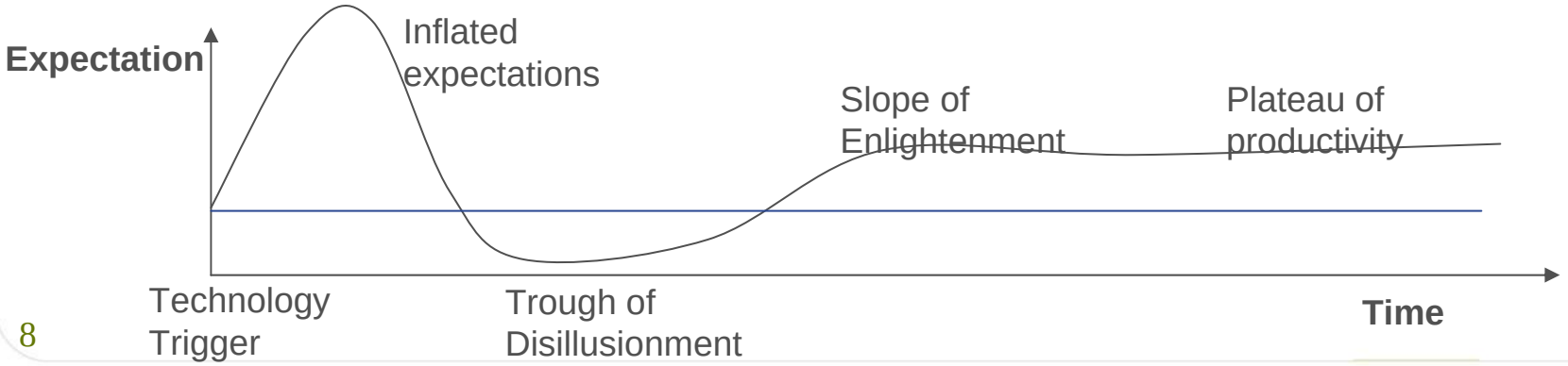
- Learn how to integrate the methods into the process
  - Add to productivity



Analysis / terms initially proposed by Gartner group

Fragments

- The ideas established in the molecular modelling / structural biology community during the 1980s and early 1990s
- First reduced to practice by Abbott in SAR by NMR approach in mid 1990s
  - Other pharmaceutical companies unable to replicate success
- Approaches developed in small pharma companies in late 1990s / early 2000s
  - Astex, Vernalis, Plexxikon, SGX ….. and underground in large pharma …
- Underpinning concepts developed in the 2000s – complexity, ligand efficiency
- Success has led to increased use
  - Different aspects of FBLD are on different parts of this curve
    - And in different organisatiions



Fragments 2013 talk

Vernalis

- Summary from 2009
  - Where we were 4 years ago
- New approaches / ideas for conventional targets
  - Screening methods
  - Off-rate screening for fragment to hit optimisation
- How to approach non-conventional targets
  - What is a non-conventional target?
  - Methods for determining binding mode
  - Issues – assays, plasticity, compound properties, 3D
- Final remarks

Fragments 2013 talk

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- Summary from 2009
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SeeDs process - 2008

Structural Exploitation of Experimental Drug Startpoints*

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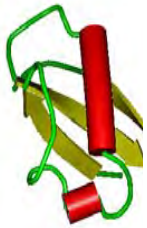
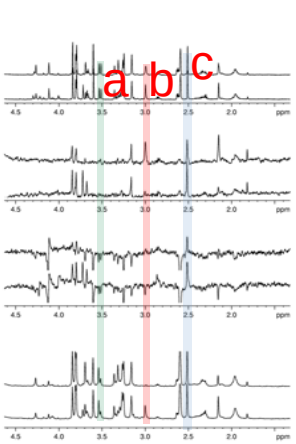
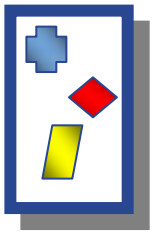
Target



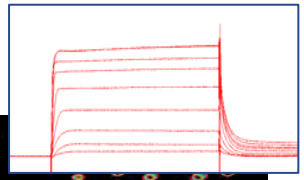


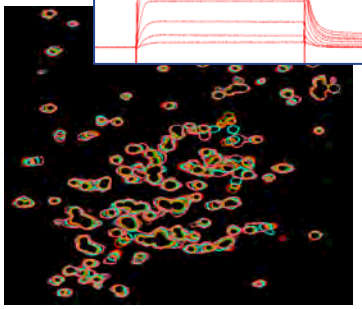
NMR Competitive Binding Experiment



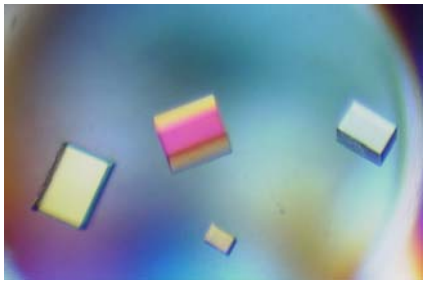
Validation

Biacore

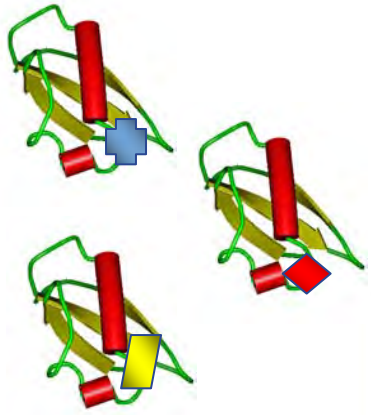




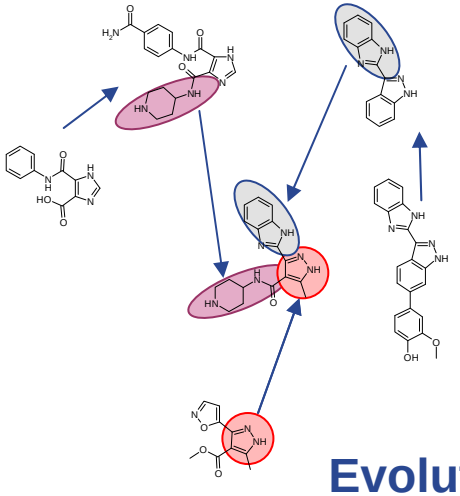
HSQC

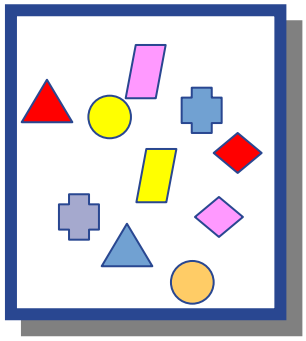


X-ray Structures



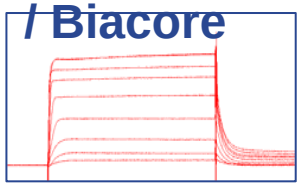
Evolution





Fragment Library

Wet assay / Biacore



*Hubbard et al (2007), Curr Topics Med Chem, **7**, 1568

Chen & Hubbard (2009), JCAMD, **23,** 603

Finding fragments

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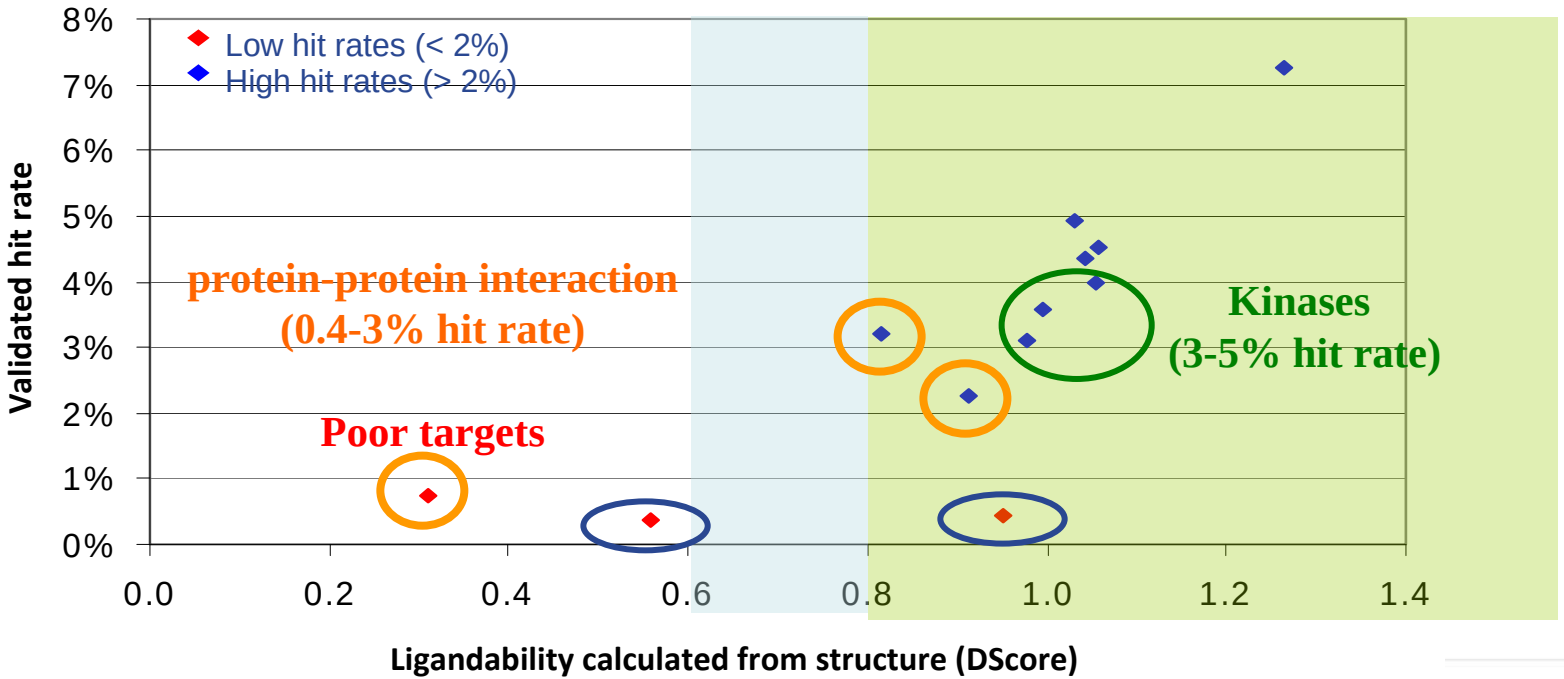
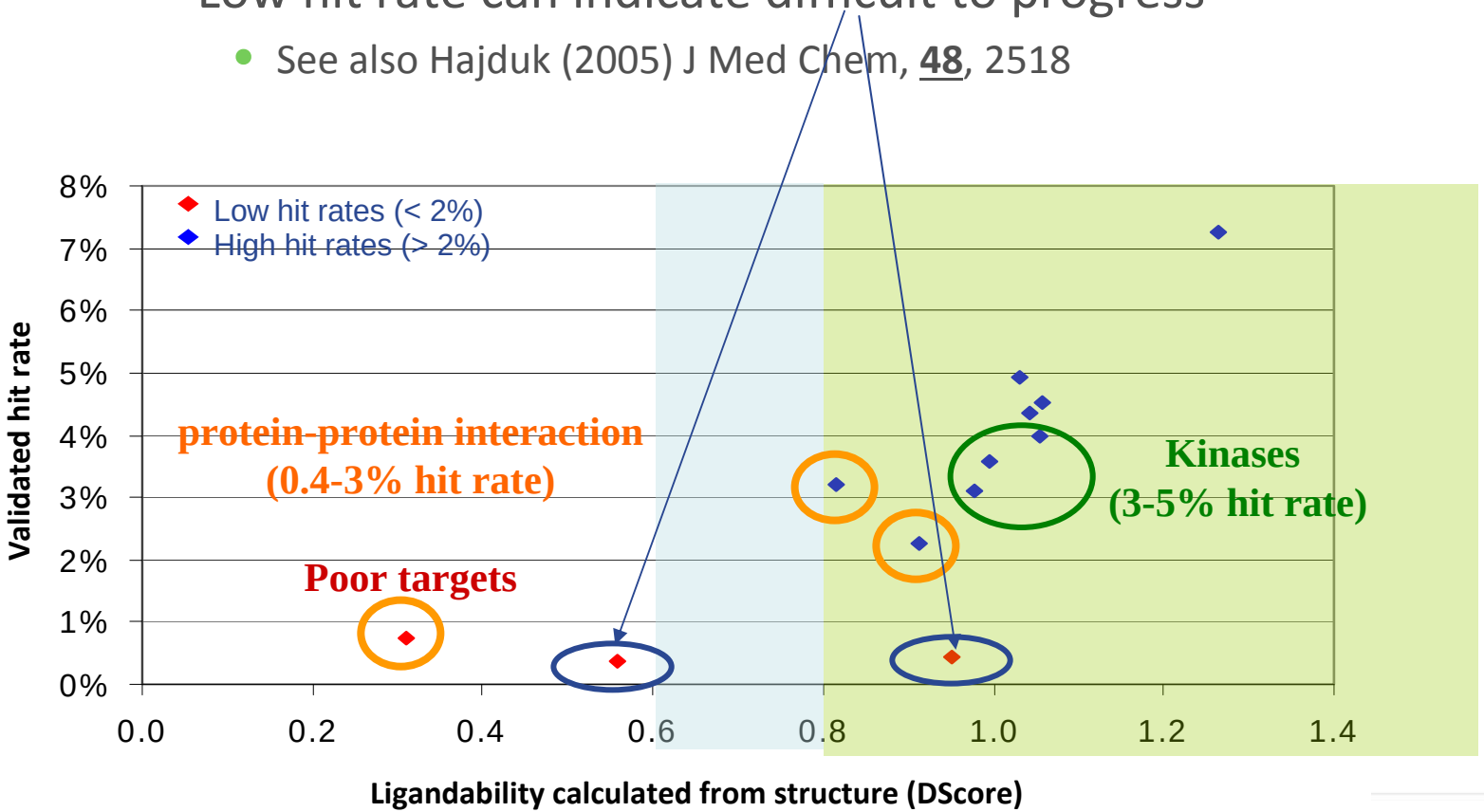
- Finding fragments that bind is not difficult
  - A good way of assessing target "ligandability"



Chen & Hubbard (2009), JCAMD, **23,** 603

Finding fragments

- Finding fragments that bind is not difficult
  - A good way of assessing target "ligandability"
  - Low hit rate can indicate difficult to progress
    - See also Hajduk (2005) J Med Chem, **48**, 2518



Using fragments

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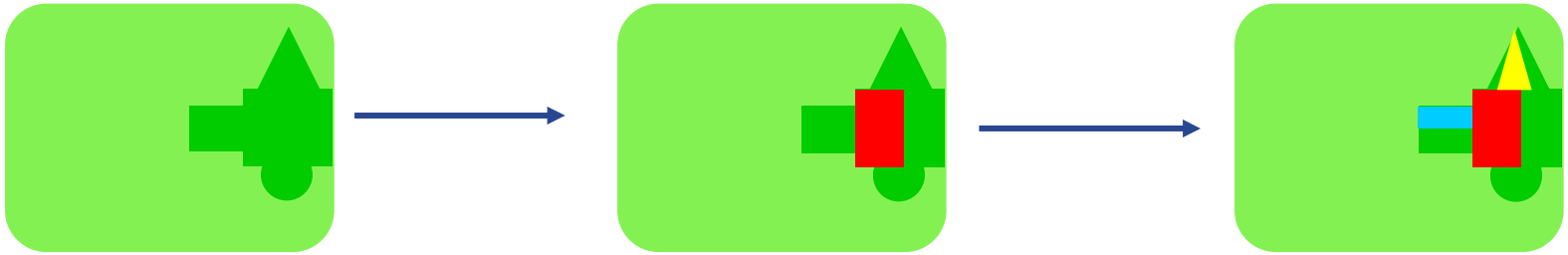
- Growing fragments



Using fragments

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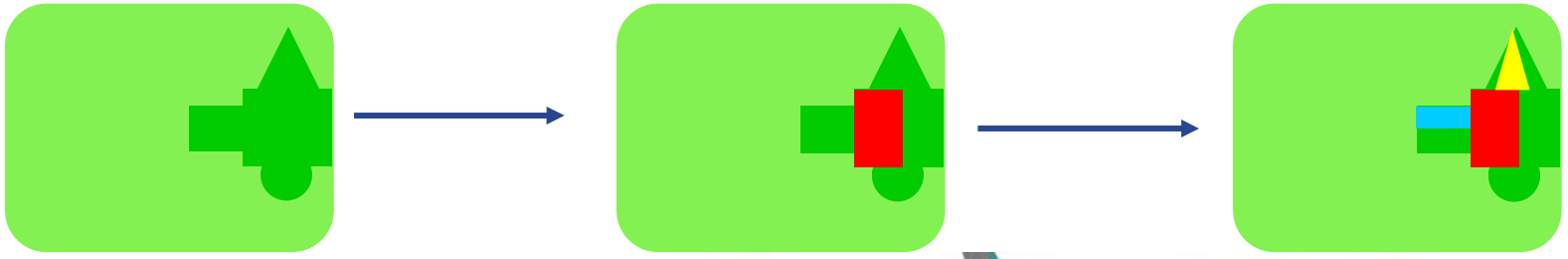
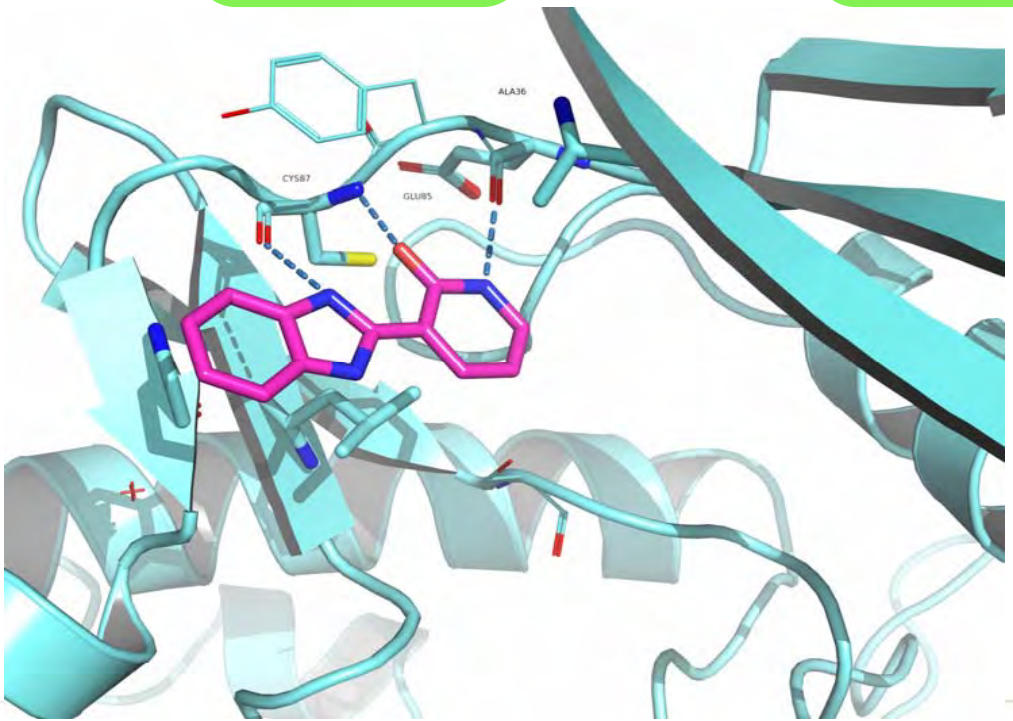
- Growing fragments – CHK1 example



Chk-1 IC_{50} >100μM

LE ~ 0.39



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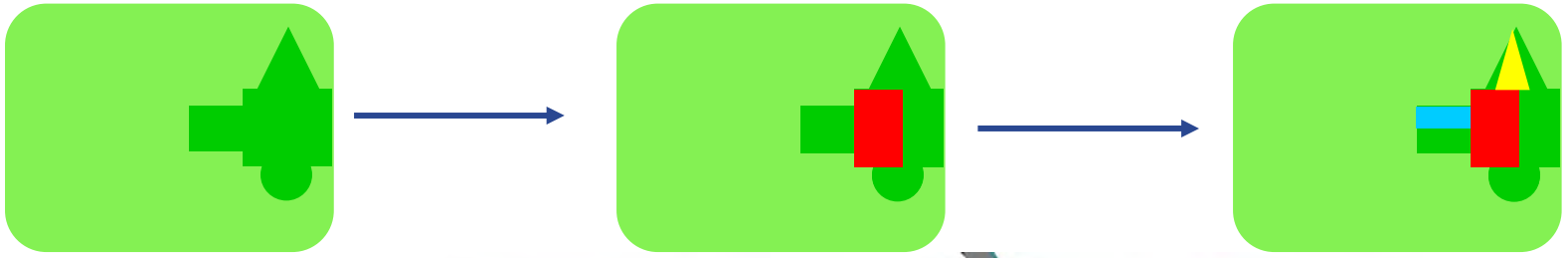
- Growing fragments – CHK1 example

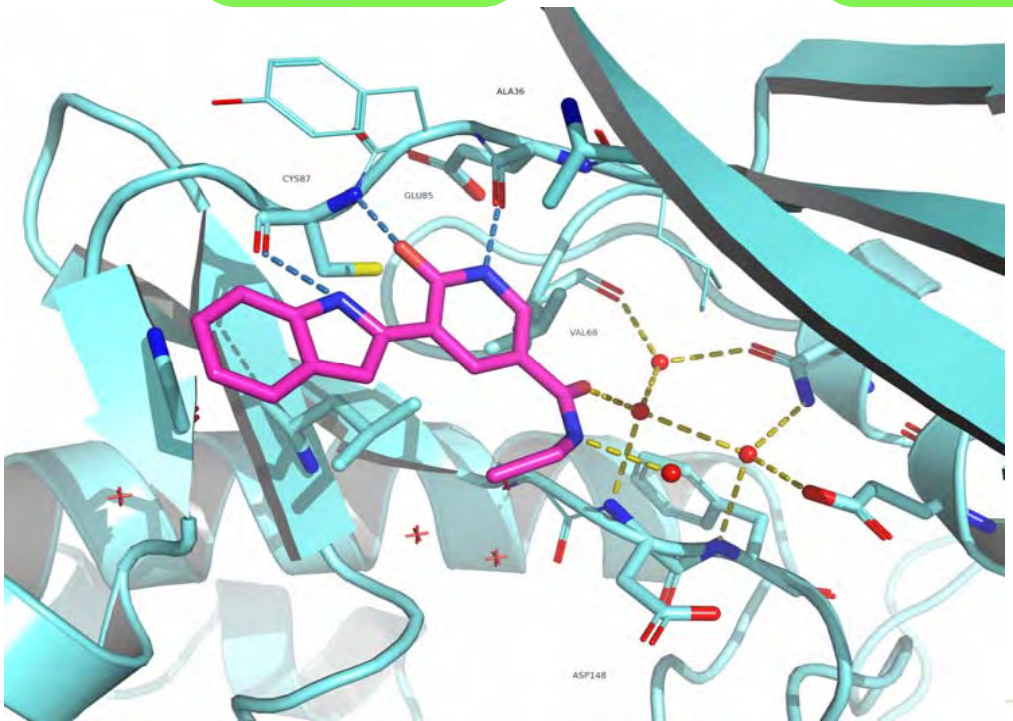


Chk-1 IC_{50} = 5μM

LE = 0.39

GI_{50} HCT116 >80µM

pH2AX (MEC) – inactive



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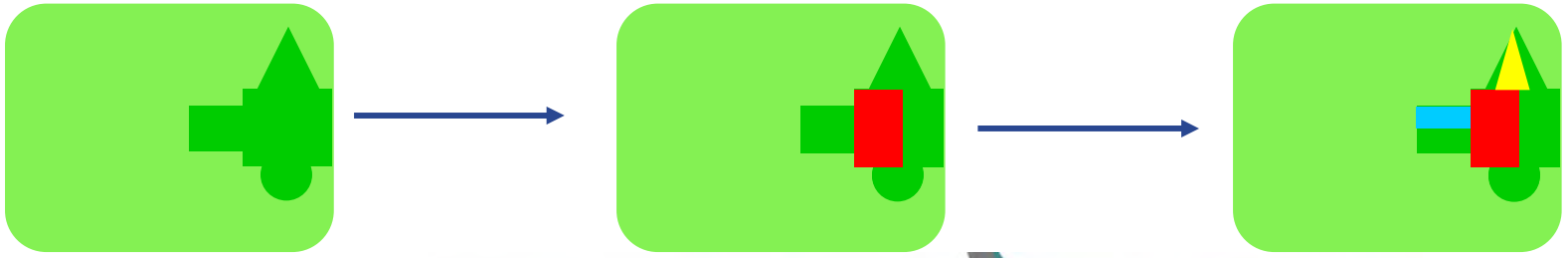
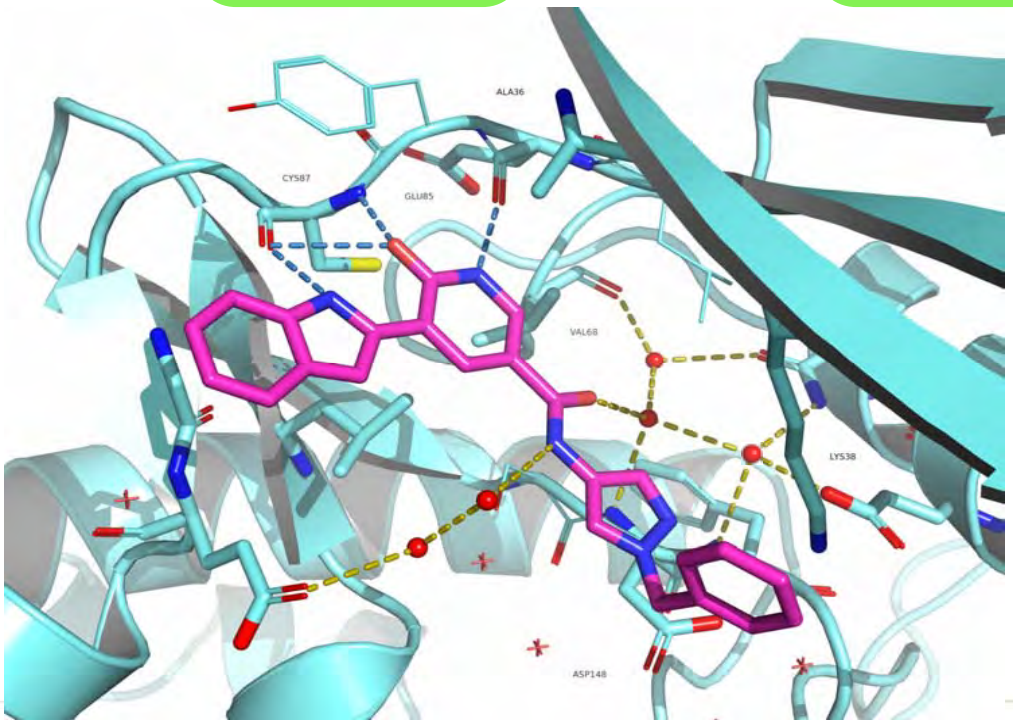


Chk-1 IC_{50} = 0.2μM

LE = 0.33

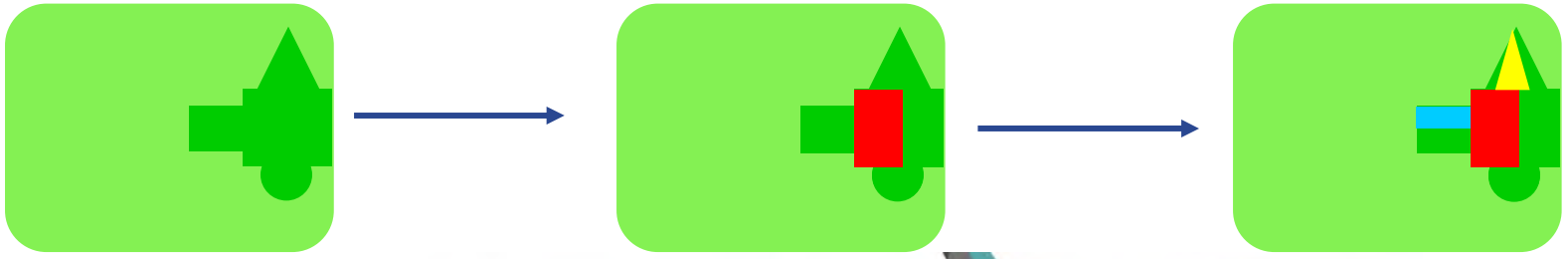
GI_{50} HCT116 = 4µM

pH2AX (MEC) = 7µM



Using fragments

- Growing fragments – CHK1 example

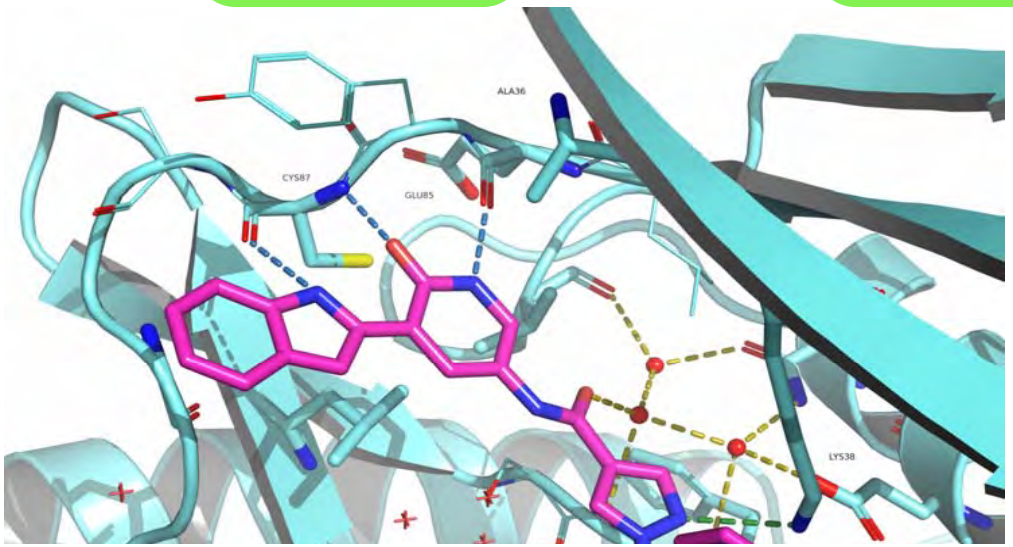


Chk-1 IC_{50} = 0.013μM

LE = 0.39

GI_{50} HCT116 = 1.8µM

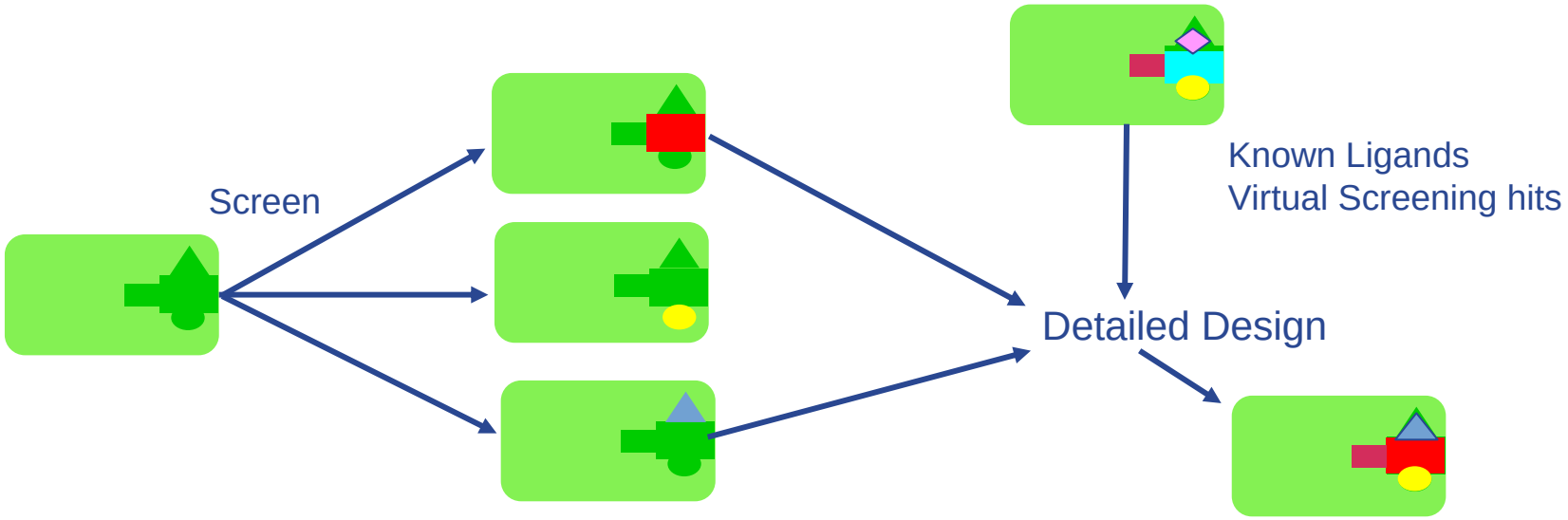
pH2AX (MEC) =0.2µM



Series members further optimised to identify Candidate V158411

Using fragments

- Merging – HSP90 example



HSP90 – BEP800 story

Brough et al (2009) J Med Chem **52**,4794-4809
Roughley et al (2012) Top Curr Chem **317**, 61

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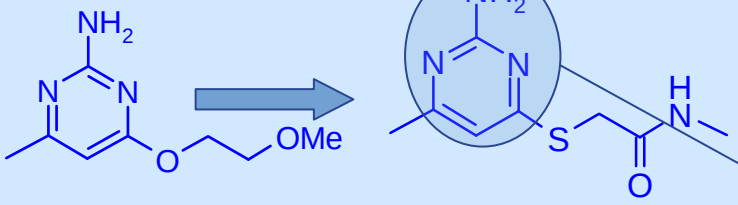
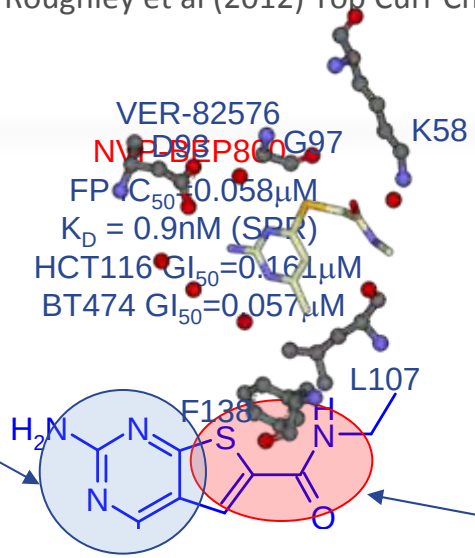
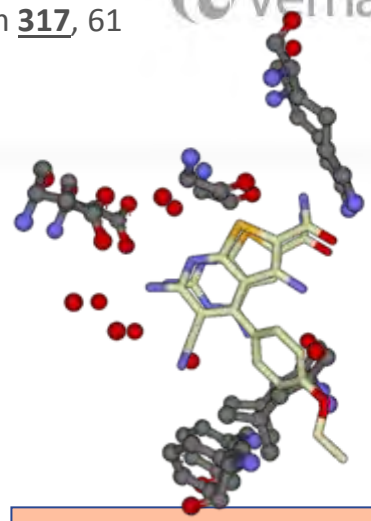
Fragment **Evolved fragment**



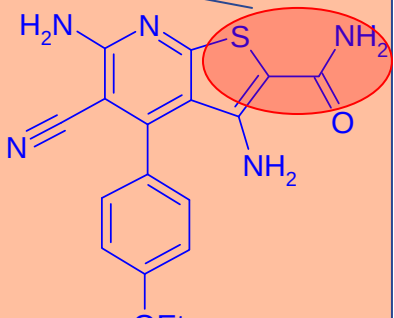
VER-26734
FP IC_{50}>5mM

VER-52959
FP IC_{50}=535µM

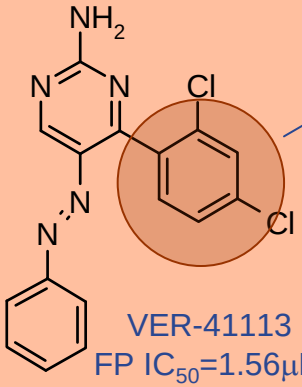




Virtual Screening Hit

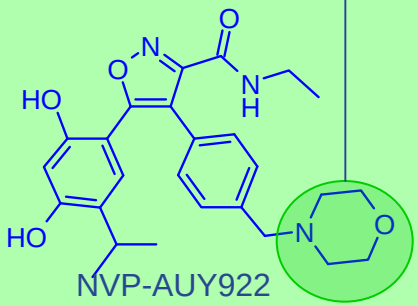


VER-45616
FP IC_{50}=0.9µM



VER-41113
FP IC_{50}=1.56µM

Virtual Screening Hit

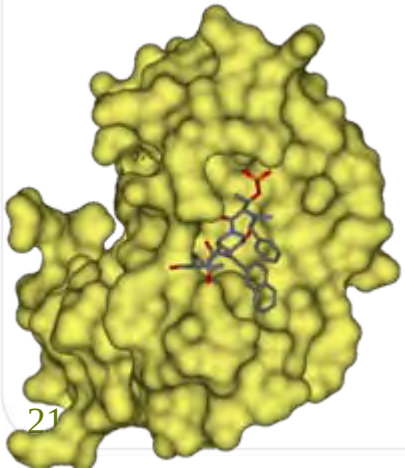


NVP-AUY922
Vernalis Phase II candidate (FBLD / SBDD derived)

Pin1 Story

Vernalis

- Proline isomerase – persuasive biological rationale that key oncology target
  - Structure available + D-peptide tool compound



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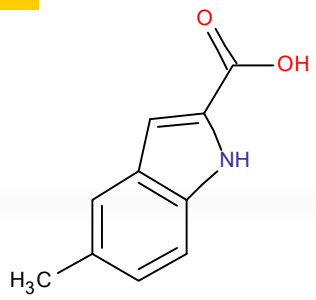
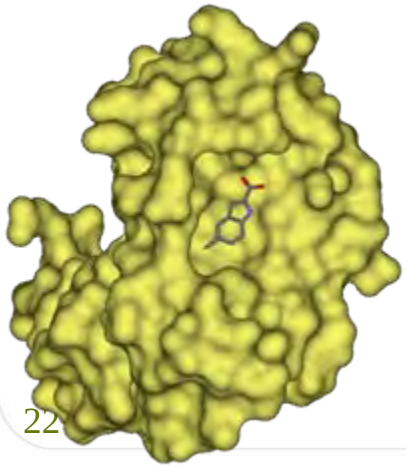


- Proline isomerase – persuasive biological rationale that key oncology target
- Fragments identified: fragment to hit evolution
  - No correlation between biophysical and enzyme assays



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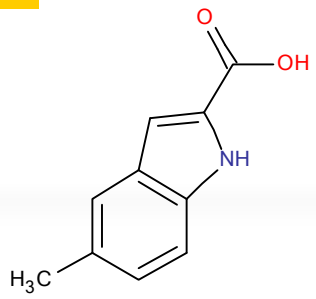
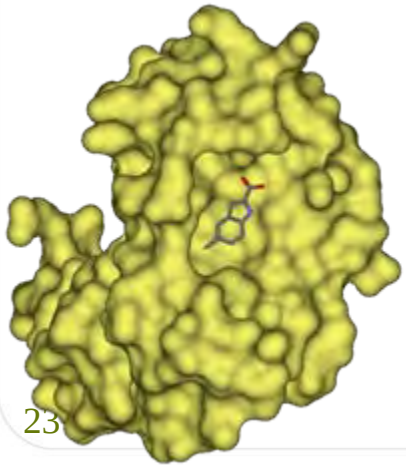
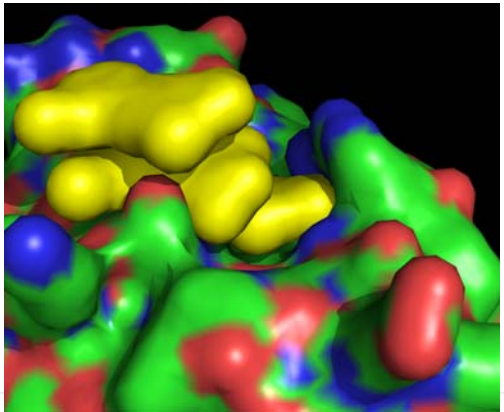
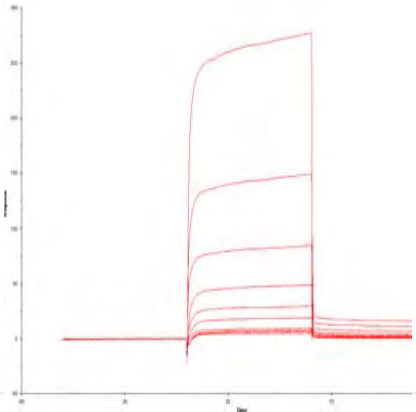


- Proline isomerase – persuasive biological rationale that key oncology target
- Fragments identified: fragment to hit evolution
- Issue with over-binding – multiple copies of fragment binding to the protein – SPR and Xray







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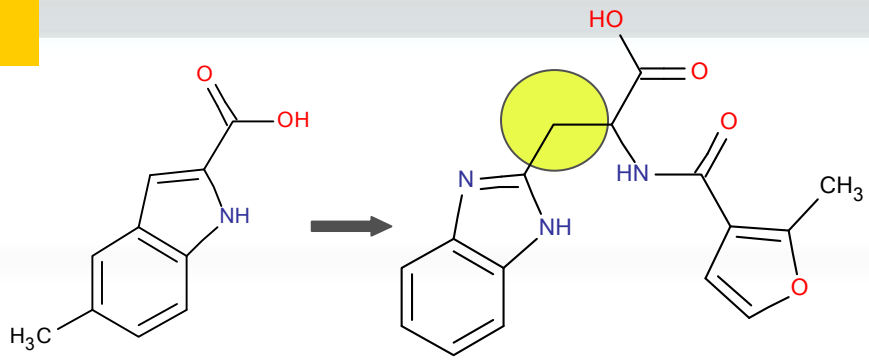
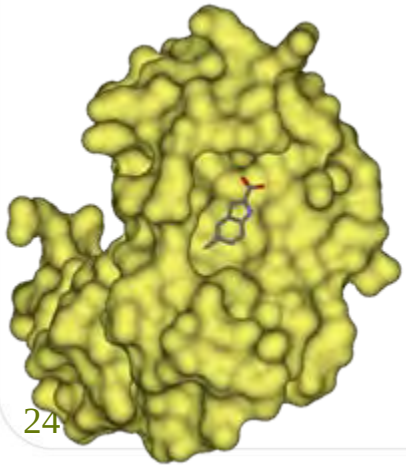
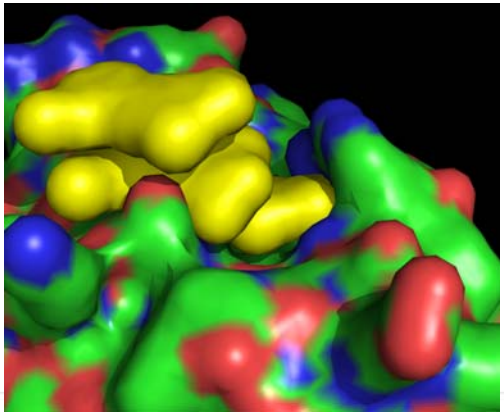
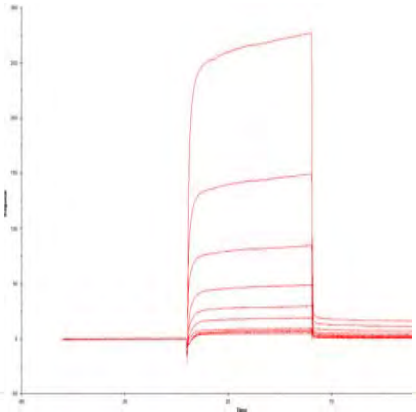


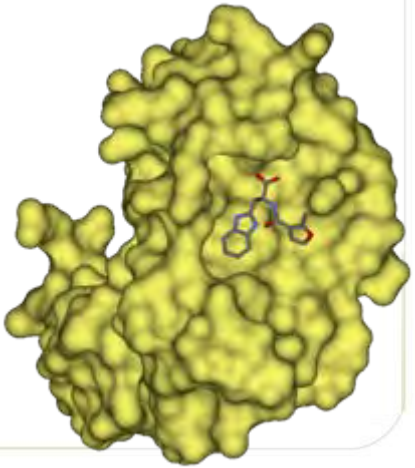
Potter AJ et al, Bioorg Med Chem Lett. 2010; 20:586-590

- Proline isomerase – persuasive biological rationale that key oncology target
- Fragments identified: fragment to hit evolution
- Issue with over-binding – multiple copies of fragment binding to the protein – SPR and Xray
- Designed 3D fragment – progressed multiple series < 100nM on target showing cellular activity









Fragments 2013 talk

Vernalis

- Summary from 2009
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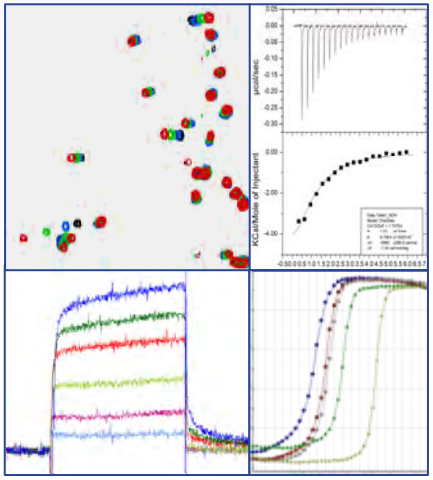
The Vernalis process

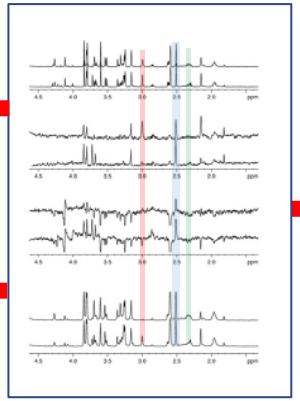
Vernalis

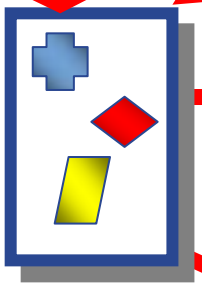
Target

Screen by SPR, DSF, WAC, biochem or binding assay, Xray

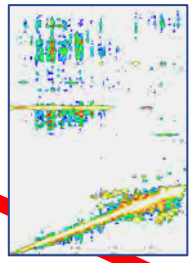
Virtual screen; literature; library screen







Hits



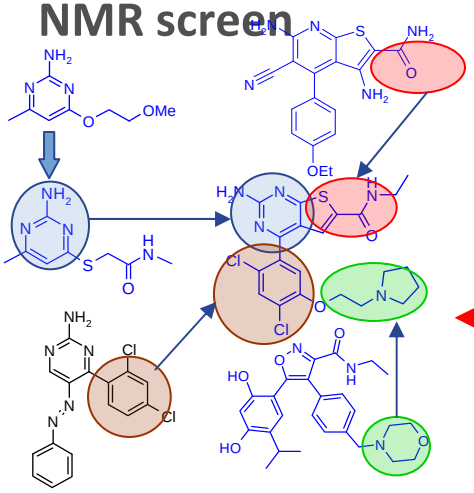
Characterisation

Fragment Library

~ 1200 compounds

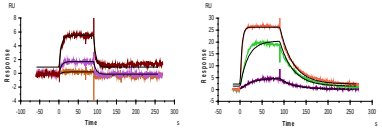
Ave MW 190

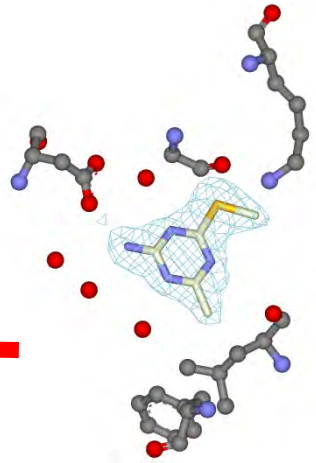
Competitive NMR screen



Optimise fragment

Fragment to hit : SAR by catalog off-rate screening





X-ray or NMR guided model

Drug?

Design, Build & Test

Some comments on fragment screening

Hubbard & Murray (2011), Meth Enzymology, **493**, 509

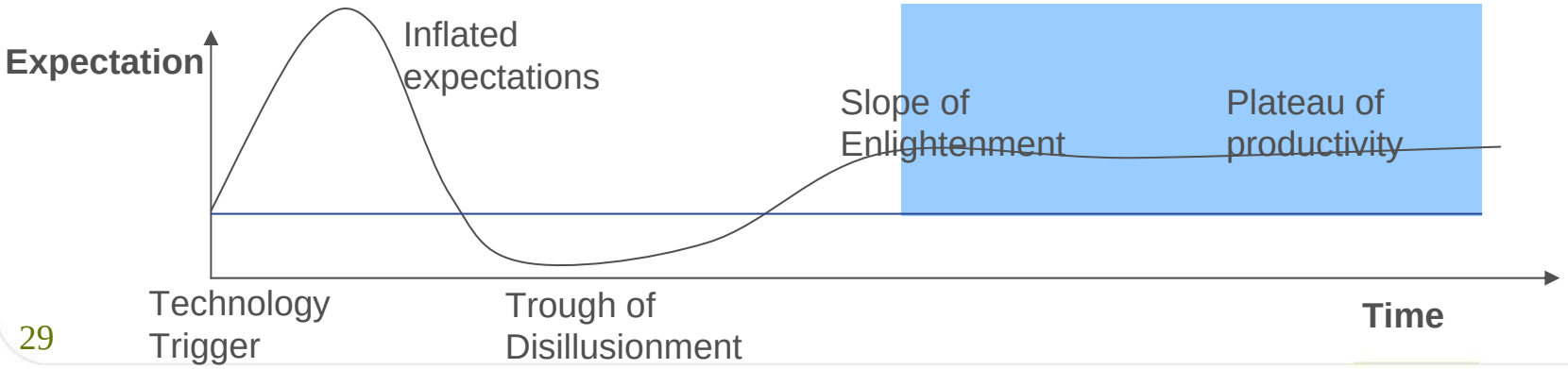
- For "good" target sites (many enzymes):
  - If assays configured correctly
    - Same hits identified by ligand observed NMR and SPR
      - validated hits tend to give crystal structures
    - Careful QC of fragment library – attention to assay conditions
  - There can be lots of false negatives from screening by X-ray
    - Requires suitable crystal system
  - "Wet" assays can work sometimes
    - But high concentrations can confound the assay
  - Thermal melt methods unreliable
- For non-conventional targets (such as protein-protein):
  - Many issues
    - Overbinding, problems due to properties of compounds and target
  - Cross-validate binding by different techniques

Leads generated for many Targets

- Disclosed targets include:
  - Kinases: CDK2, Chk1, PDPK1, PDHK1, Pim1, STK33, Pak4
  - ATPases: DNA gyrase, Grp78, HSP70 and HSP90
  - protein-protein interaction targets: Pin1 and Bcl-2
  - FAAH and tankyrase
- Undisclosed targets include:
  - kinases with unusual binding sites
  - protein-protein interaction targets
  - novel classes of enzymes in large multi-domain complexes

Summary – fragments and conventional targets

- Fragment screen to assess targets
- Fragments alongside HTS and knowledge-based
  - You always find something new
- Fragments to hits for conventional targets is relatively straightforward
  - (when crystal structures / good models available)
  - The main issues are organisational and cultural
    - Discuss !!



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Exploiting the dissociation rate constant

Vernalis

$$K_D = \frac{k_{off}}{k_{on}} \quad \frac{(s^{-1})}{(M^{-1}s^{-1})}$$

$$PL \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} P + L$$

Exploiting the dissociation rate constant

Vernalis

$$K_D = \frac{k_{off}}{k_{on}} \quad \frac{(s^{-1})}{(M^{-1}s^{-1})}$$

- Cannot improve association rate constant above ~10^{-8} $M^{-1}s^{-1}$
  - No point in drug discovery, too many membranes in the way!
- Dissociation rate constant can be infinite (ie covalent!)
- We have seen that it is the key driver of potency
  - As have others (review Copeland, Future Med. Chem. 3(12), 2011)

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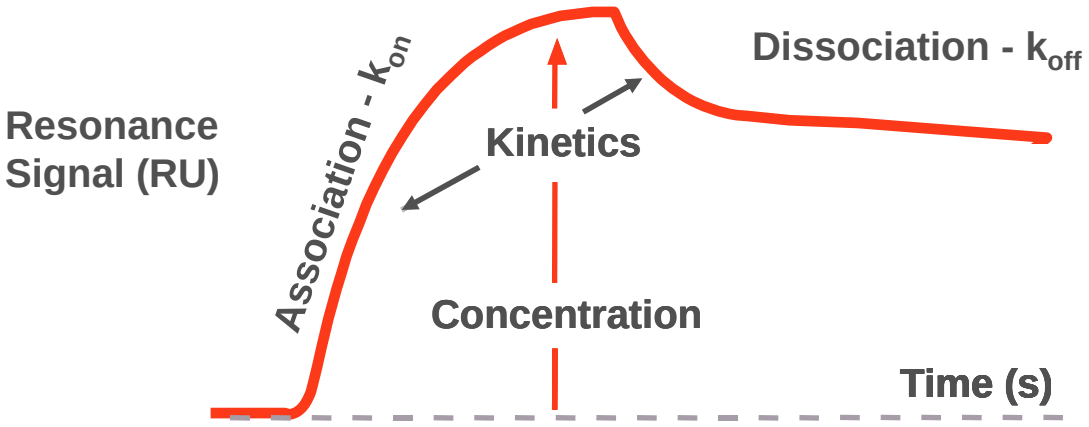

- Surface plasmon resonance – a way to measure kinetics
  - FOCUS ON THE Off-RATE - k_{off}

Exploiting the dissociation rate constant

Vernalis

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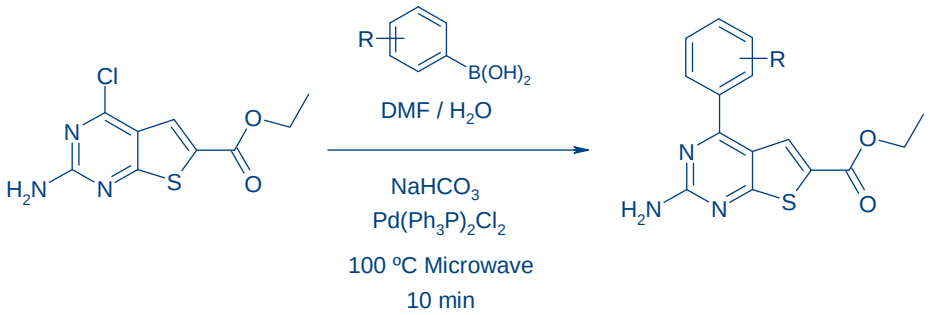
- Dissociation rate constant can be infinite (ie covalent)
- We have seen that it is the key driver of potency
  - As have others (review Copeland, Future Med. Chem. 3(12), 2011)
- Independent of concentration
  - Exploit this to assess unpurified reactions, off-rate screening (ORS)

Off rate screening (ORS): Example

- Historical Hsp90: thienopyrimidines
  - 200 nM – 5 μM IC_{50} by FP assay
- Re-prepared compounds by Suzuki reaction



- Minimal work-up
  - Evaporate, partition
  - Purity 50 – 80 % (LCMS)
- Screened by ORS

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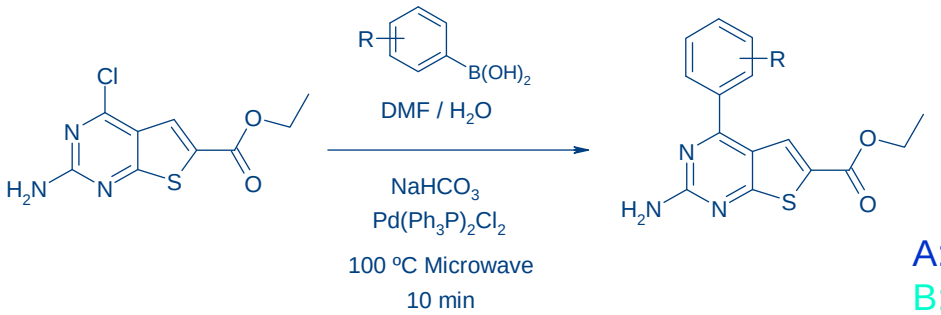
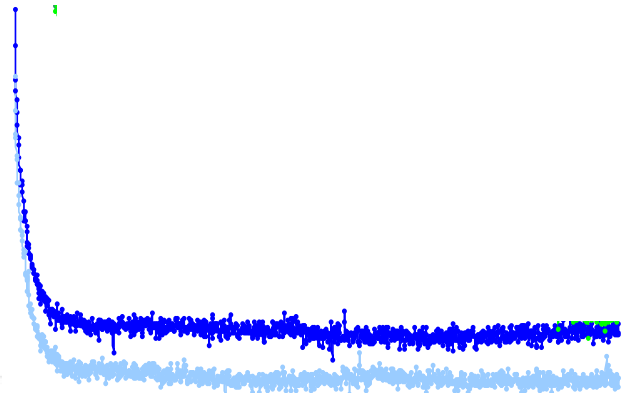


A: Pure starting material
B: Faux reaction

- Minimal work-up
  - Evaporate, partition
  - Purity 50 – 80 % (LCMS)
- Screened by ORS



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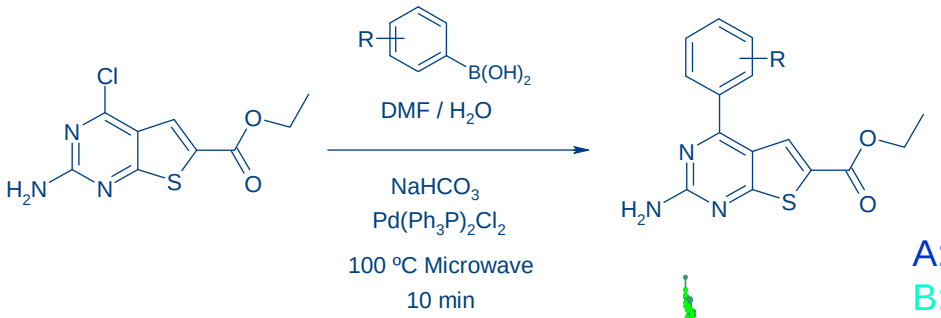

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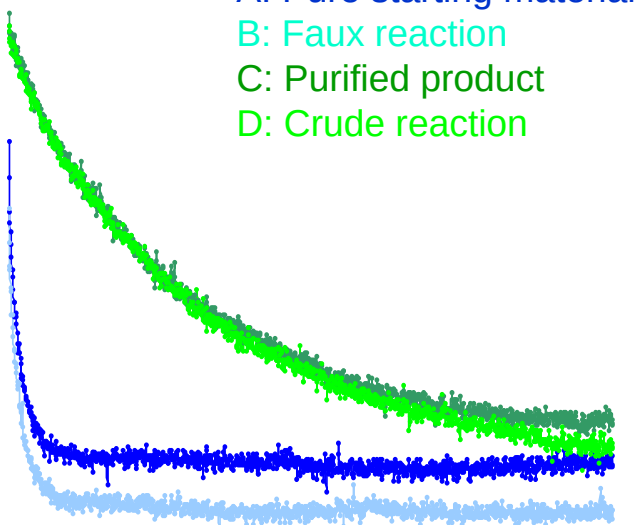
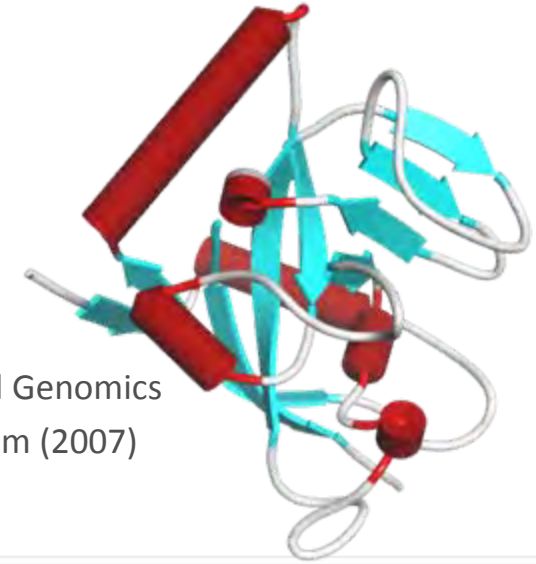


Tankyrase - Introduction

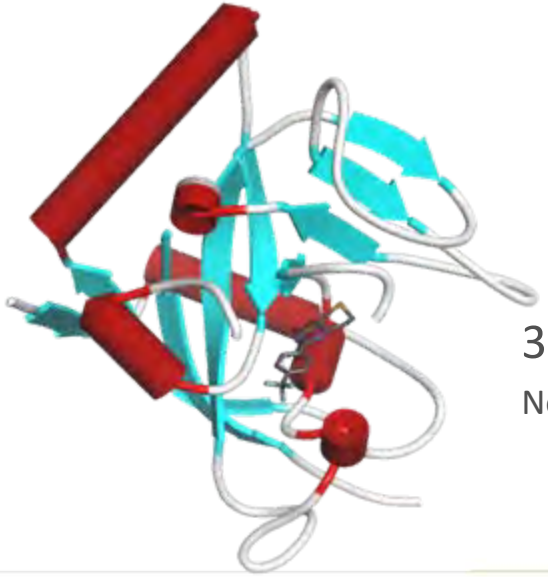
Vernalis

- Axin is targeted for turnover through poly-D-ribose labelling by Tankyrase. This removes axin, which has a role in stabilising β-catenin
- Inhibition of Tankyrase has been proposed to enhance the degradation of β-catenin, an indirect way of affecting the WNT-pathway
- Crystal structure of tankyrase available in 2007



2RF5

Structural Genomics Consortium (2007)



3UH4

Novartis (2012)

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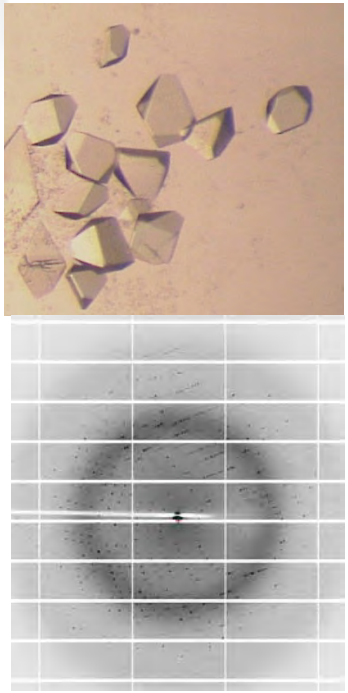
- Crystal structure of tankyrase available in 2007

- At Vernalis:
  - Initially – low levels of protein production – insufficient material for fragment screen by NMR
  - Able to produce large numbers of apo-crystals that preliminary trials showed were suitable for ligand soaking

Tankyrase - Hit identification



Crystals

↓

Soaked fragments in pools of 8

↓

Data collection (up to 1.6Å resolution)

↓

Streamlined structure solution

↓

62 hits from 1563 fragments

↓

Characterised by SPR and TSA

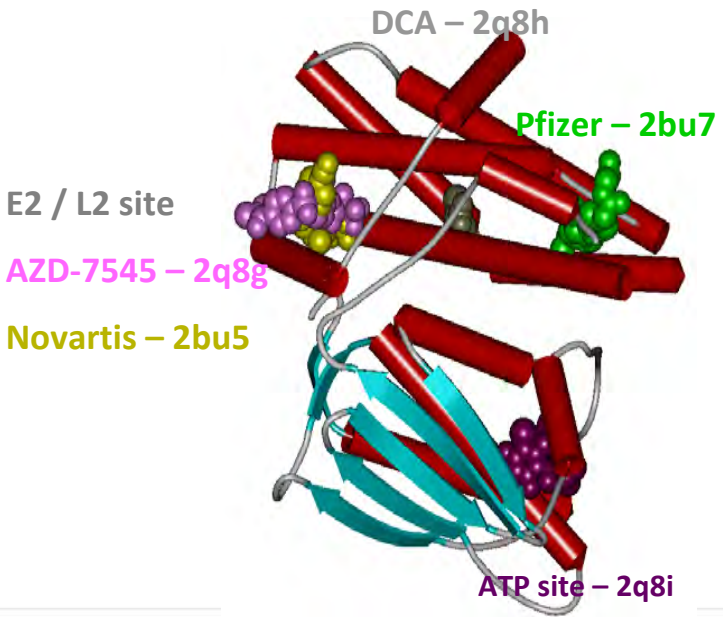
Tankyrase - Fragment to hit evolution

Vernalis

- The most attractive fragments were not suitable for rapid chemistry
- Designed a modified fragment
- Off-rate screening libraries identified vectors and substituents
  - Crystals soaked directly with reaction mixtures
- Lead Series driven using combinations of tools
  - Computational chemistry
  - X-ray crystallography
  - SPR (ORS), DSF
  - Medicinal Chemistry
- Properties
  - 5 nM vs TNKS2, high ligand efficiency (0.60)
  - Affects PD markers in cells (stabilises Axin2, inhibits WNT pathway)
- Tools to probe the biology

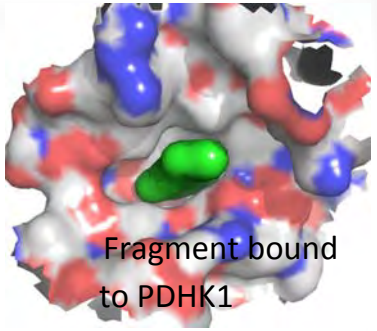
PDHK – an unusal kinase

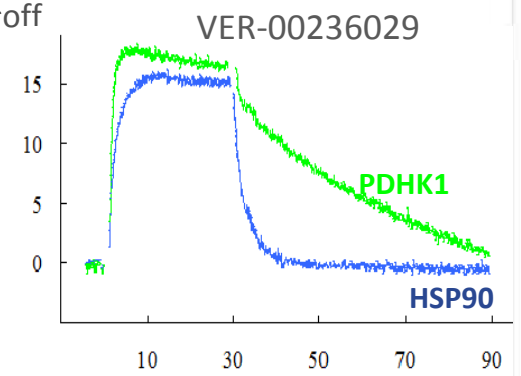
- GHKL family protein (like HSP90)
- Other companies have targetted for diabetes
  - AZ and Novartis in the 1990s – various allosteric sites
- Vernalis investigated as a cancer metabolism target
  - Off-rate screening to selectively evolve a fragment

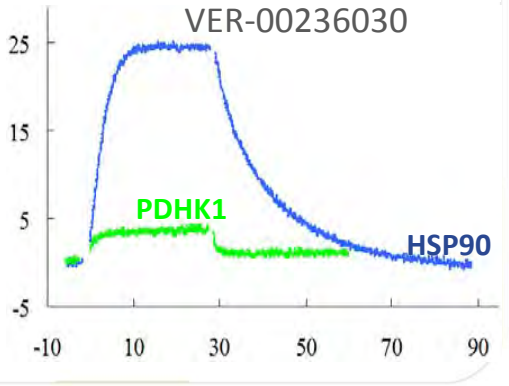


PDHK project

- Initial fragment hit for ATP site – also binds to HSP90
- Structure shows which vector(s) to explore
- Parallel libraries synthesised and screened by SPR
  - Using the off-rate screening method – changes in k_{off}
  - One SPR channel with HSP90, one with PDHK
- Can identify PDHK selective compounds
  - And HSP90 selective compounds







Fragments 2013 talk

Vernalis

- Summary from 2009
  - Where we were 4 years ago
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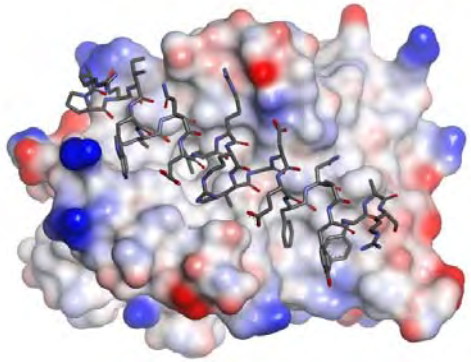
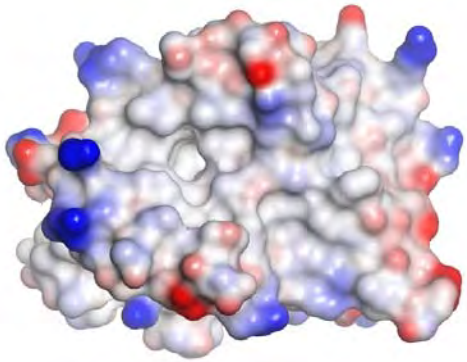
Non-conventional targets



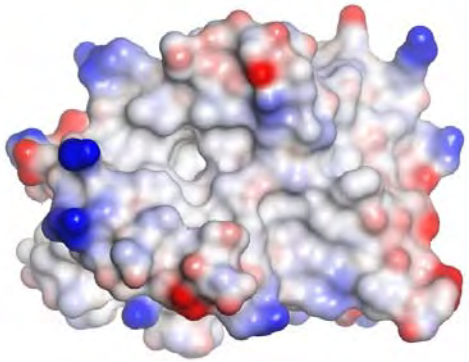
Vernalis

Non-conventional targets



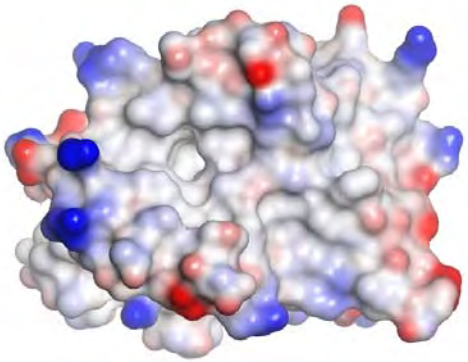
- Can find fragments that bind
  - Orthogonal biophysical methods can validate and characterise fragment binding

Non-conventional targets



- Can find fragments that bind
- Evolution requires robust model of fragment binding
- Best model is from X-ray structure
  - But sometimes high affinity ligand required for structure

Non-conventional targets



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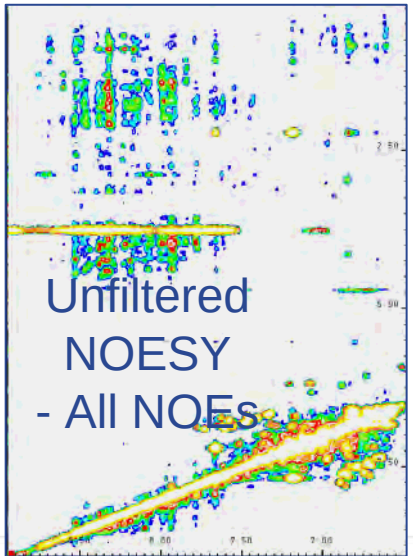
- NMR methods can provide sufficient quality of model
  - Experiments can be filtered to reveal just the interactions between protein and ligand



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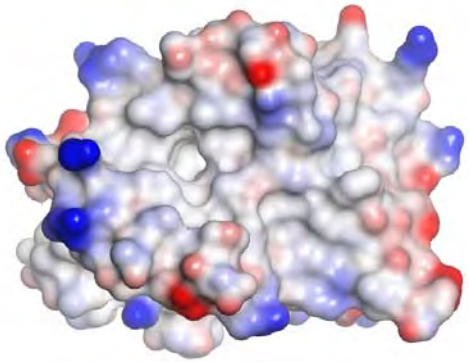

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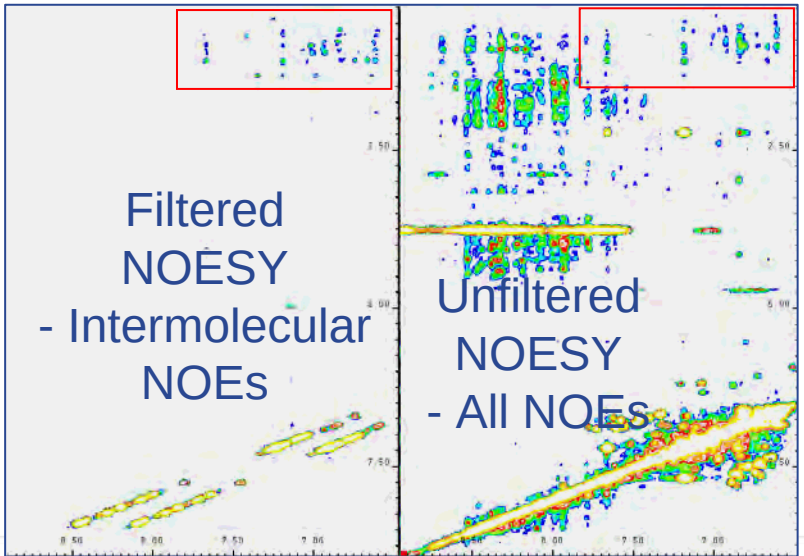
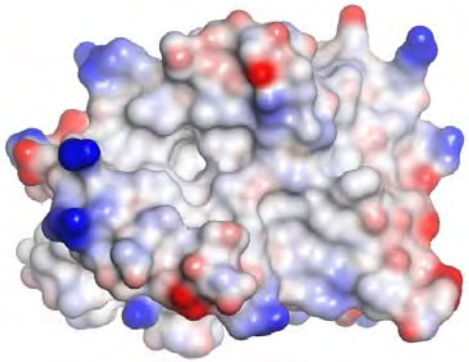


Protein/ligand

Non-conventional targets

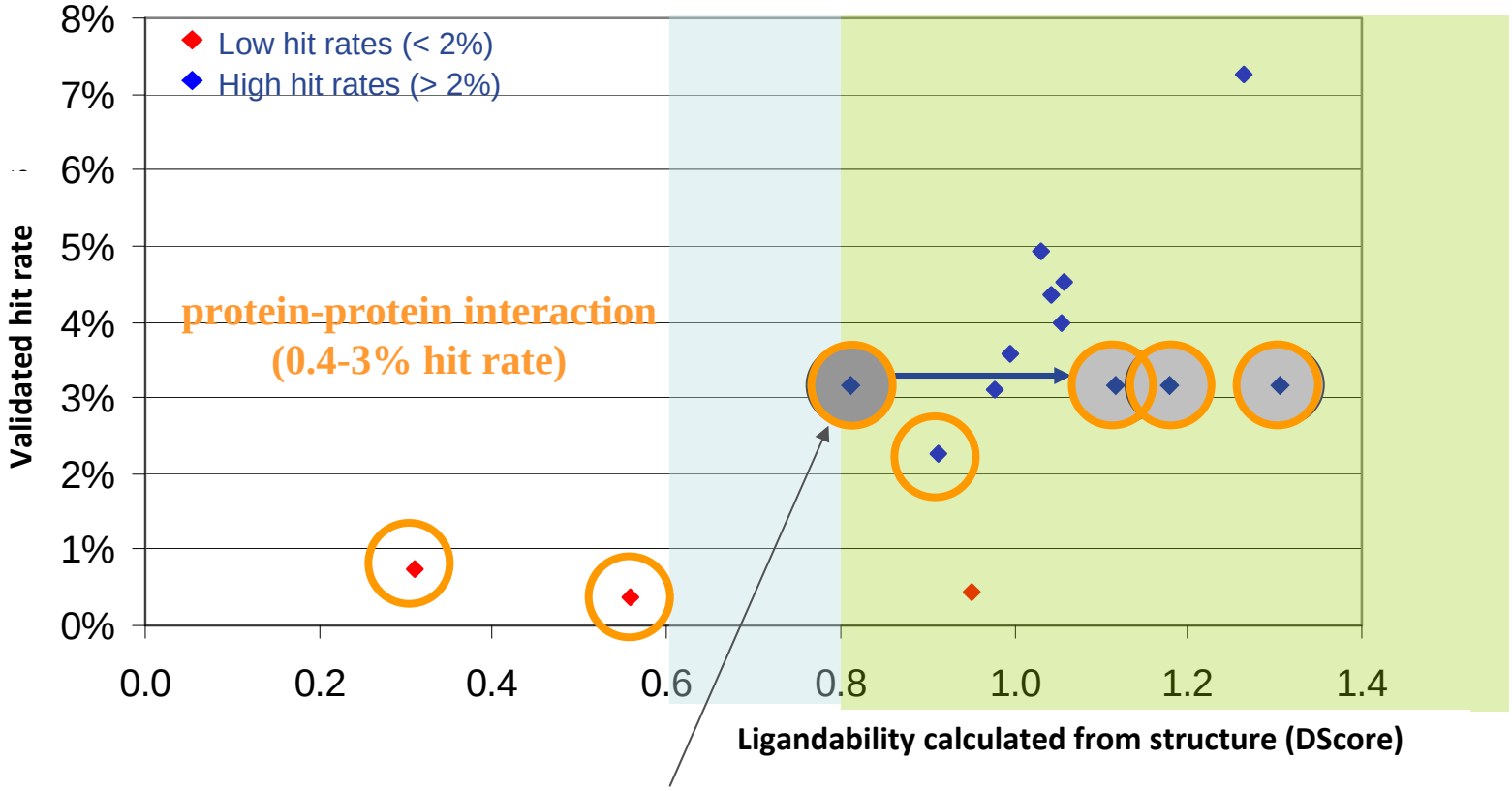


Vernalis

- Can find fragments that bind
- Evolution requires robust model of fragment binding
- Best model is from X-ray structure
- NMR methods can provide sufficient quality of model
  - Experiments can be filtered to reveal just the interactions between protein and ligand
  - Have developed leads from fragments using NMR models
  - High affinity ligands give X-ray structures that confirm model

Video of Bcl-2 plasticity

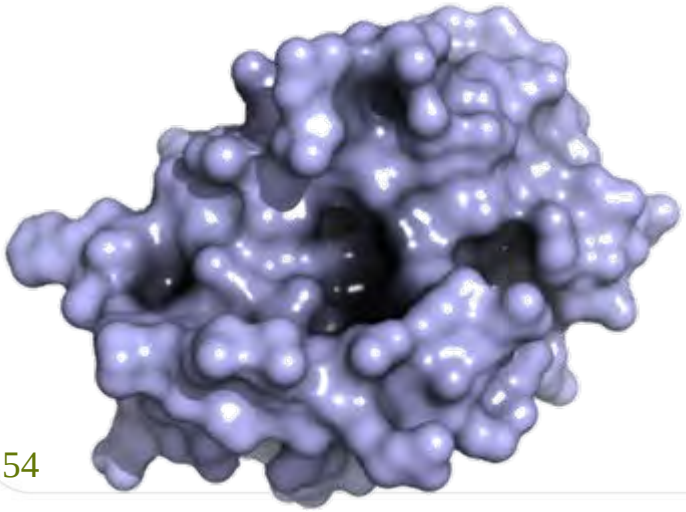
Bcl-2 - ligandability

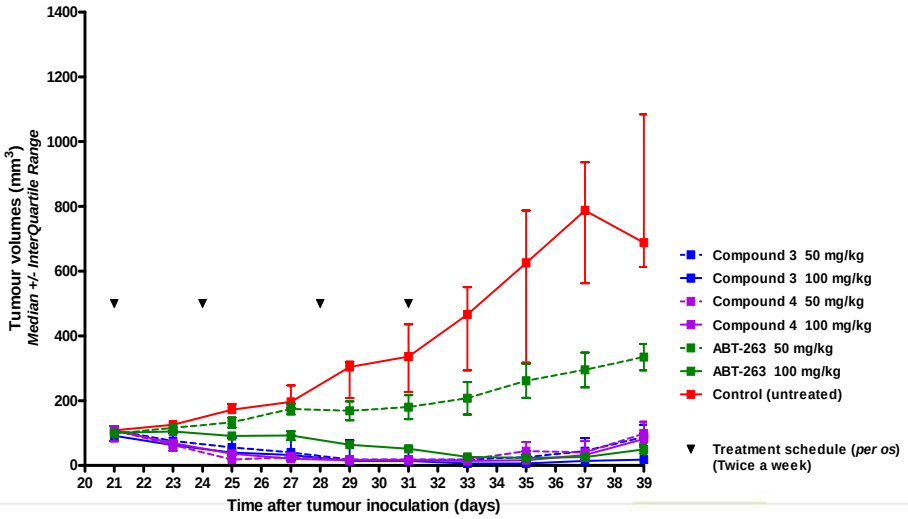


- changes dramatically as ligands explore available pockets / flexibility

Selective Bcl-2 inhibitor program

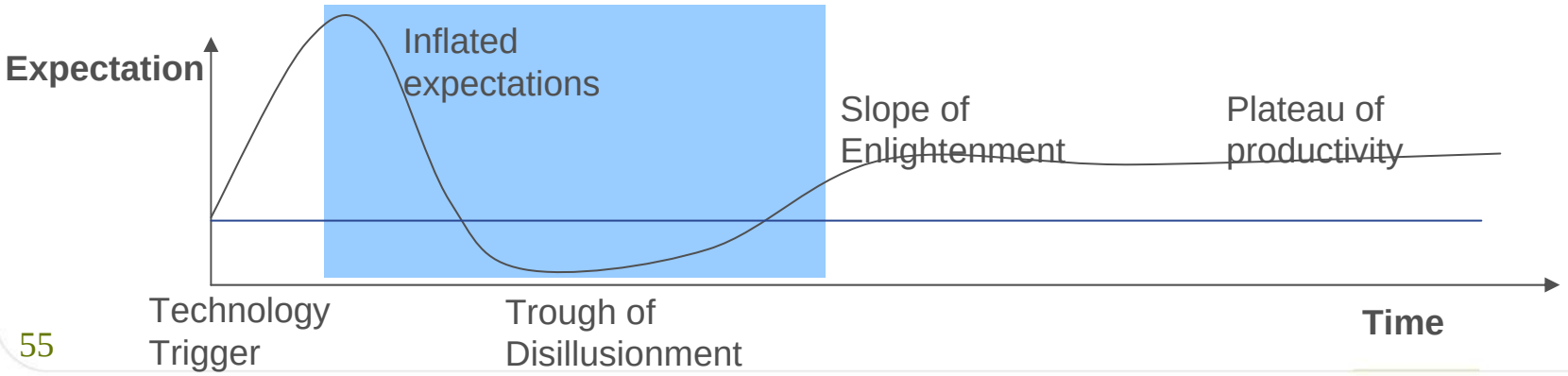
- Structure-guided optimisation has generated selective Bcl-2 inhibitors
  - MW < 780; > 100-fold selective over other BH_3 domains
  - Sub-10 nM efficacy in cell models of AML
  - *In vivo*, rapid and strong apoptotic response in RS4;11 xenograft models, both iv and oral
  - Platelet sparing (cf ABT-263)
  - Key was biophysical assays to assess cell penetration and compound aggregation





Summary - non-conventional targets

- For non-conventional targets
  - And where structures are hard to obtain
  - It takes time – and not yet clear how often fragments can be successful
  - Integration with biophysical methods can be key
  - Also – a commitment to the long haul



Fragments 2013 talk

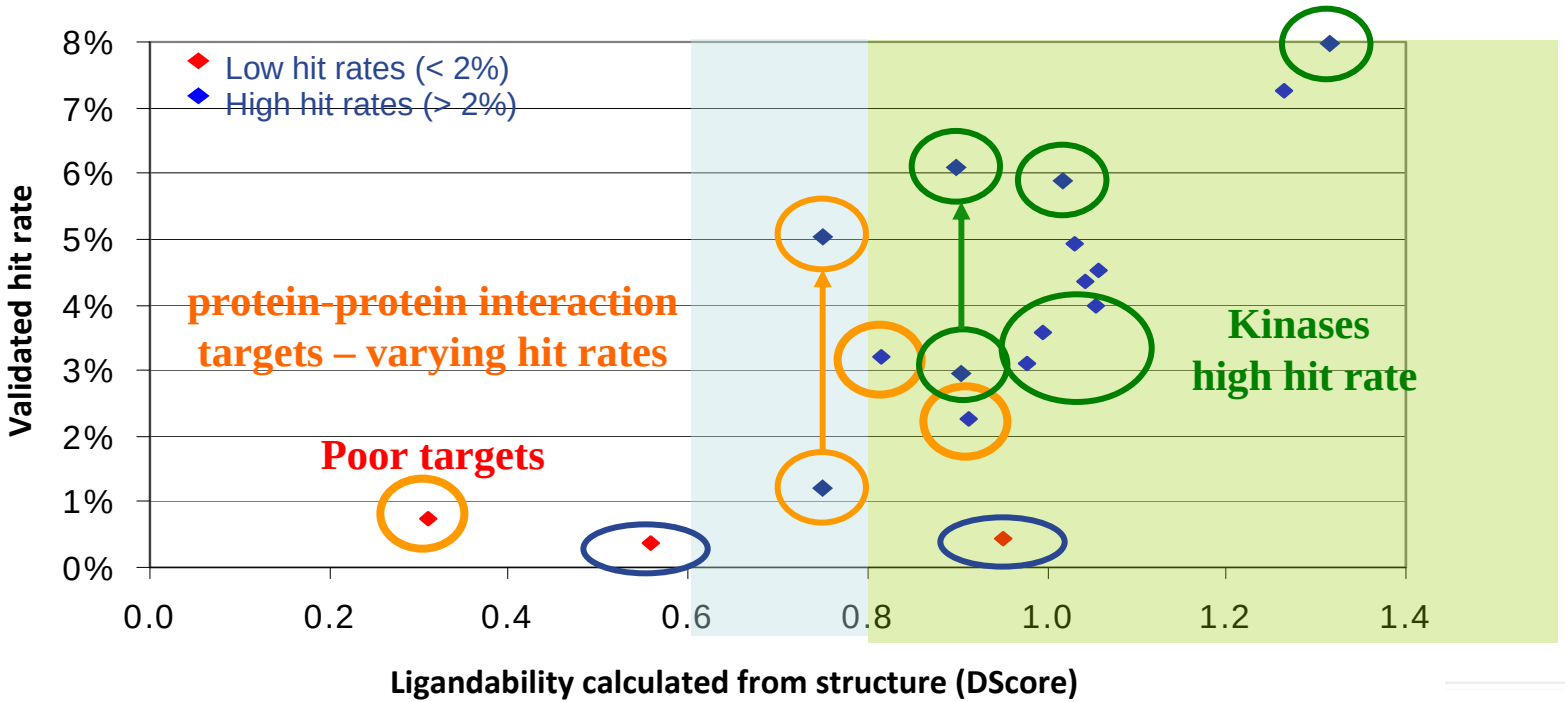
Vernalis

- Summary from 2009
  - Where we were 4 years ago
- New approaches / ideas for conventional targets
  - Screening methods
  - Off-rate screening for fragment to hit optimisation
- How to approach non-conventional targets
  - What is a non-conventional target?
  - Methods for determining binding mode
  - Issues – assays, plasticity, compound properties, 3D
- Final remarks

Updated from Chen & Hubbard (2009), JCAMD, **23,** 603

Finding fragments - issues

- For some targets, it can take time to configure a robust assay
  - For fragment screening but also hit progression
  - Protein construct, assay conditions, etc, etc



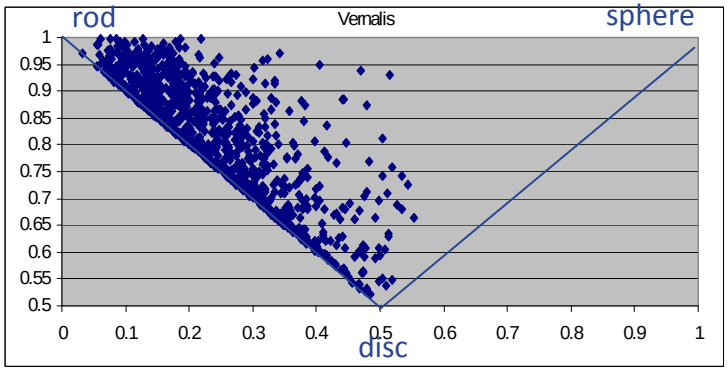
3D fragments

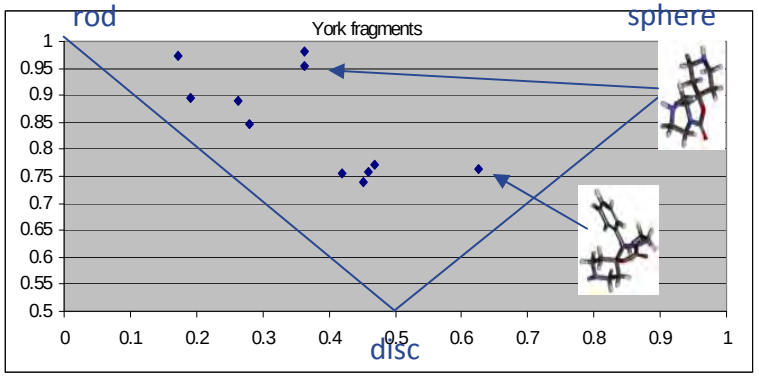
Vernalis

- Most of the compounds in fragment libraries are commercially available small molecules
  - Medicinal chemist emphasis on chemical tractability
  - Some use privileged fragments from existing drugs
- Most of the fragments are flat heterocycles
  - This is fine for some targets (kinases, ATPases)
  - Perhaps limiting for other (new) target classes
- Some initiatives underway to introduce more 3D fragments
  - The challenge will be synthetic tractability
  - A York initiative

York 3D fragments

- Peter O'Brien has developed chemistry for adding lithiated N-Boc heterocycles **2** (formed from **1**) to heterocyclic, symmetrical ketones
- The resulting fragments have distinctive 3D shapes, presenting useful looking pharmacophores
  - Shape measured by principle moments





York 3D fragments

- Peter O'Brien has developed chemistry for adding lithiated N-Boc heterocycles 2 (formed from 1) to heterocyclic, symmetrical ketones
- The resulting fragments and lead-like compounds have distinctive 3D shapes, presenting useful looking pharmacophores
- Project underway to explore the chemistry
  - Generate 500 member library
  - See how it performs against various targets

Concluding remarks

Vernalis

- Straight forward to find fragments for most sites on most proteins
  - Opportunities for new “3D” fragments?
- The challenge is knowing what to do with the fragments
  - Off-rate screening allows exploration of vectors
  - Evolving fragments in absence of structure?
- For conventional targets
  - Lots of starting points; opportunity for “good” medicinal chemistry
  - Issue in some organisations is integration with medicinal chemistry
- For non-conventional targets
  - Provides starting points when other techniques fail
  - Close integration with biophysics is crucial; takes time and commitment
- Not necessarily faster – patience required
  - But hopefully better

End

Vernalis

York Structural Biology Laboratory

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THE UNIVERSITY of York

- References in the slides acknowledge who did the work

- FBLD conference
  - 2008 – San Diego
  - 2009 – York
  - 2010 – Philadelphia
  - 2012 – San Francisco
  - 2014 – Basle
  - http://www.fbldconference.org

Vernalis Research Overview

- Approximately 60 staff in research
  - Based in Cambridge, UK (Granta Park)
  - Recognised for innovation and delivery in structure and fragment-based drug discovery
  - Structure-based drug discovery since 1997
  - Distinctive expertise combining X-ray, NMR, ITC and SPR to enable drug discovery against established and novel, challenging targets
- Portfolio of discovery projects
  - Six development candidates generated in the past six years
  - Protein structure, fragments and modelling integrated with medicinal chemistry
  - Internal Vernalis projects in oncology
  - Collaborations across all therapeutic areas
    - e.g. oncology, neurodegeneration, anti-infectives
- Aim to establish additional collaborations during 2013 / 14