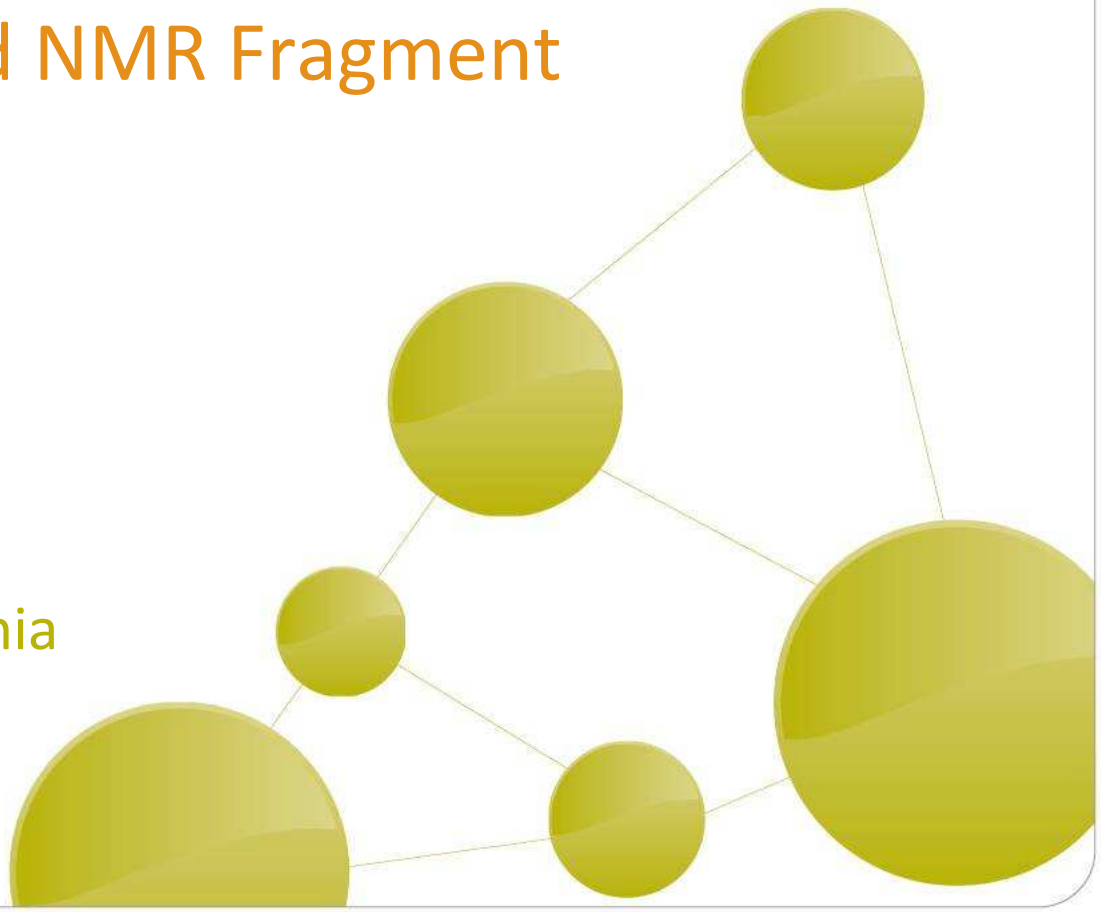
Vernalis

Ligand Observed NMR Fragment Screening

Ben Davis

Vernalis R&D

FBLD 2010, Philadelphia



Acknowledgements

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- **NMR**
  - Krisztina Fehér
  - Richard Harris
  - Heather Simmonite
- **X-ray crystallography**
  - Allan Surgenor
  - Pawel Dokurno
  - Lisa Baker
  - David Robinson
- **SPR**
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- **ITC**
  - Sian Moss
- **Protein Purification**
  - Julia Mathews
  - Neil Whitehead
  - Terry Shaw
  - Peter Kierstan
- **Fragments**
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  - Rod Hubbard
  - Iien Chen
  - James Davidson
- **Biochemical assays**
  - Jenifer Borgognoni
  - Melanie Wong
  - Jalanie D'Alessandro
  - Lindsey Terry
- **Vernalis**
  - Many other people
- **Bcl-2**
  - Olivier Geneste
  - Patrick Casara
  - John Hickman

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Ribosome projects

Which technique to use for fragment screening ?

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- NMR, SPR, CE, DSF, X-ray, Biochemical assays

…

- If it's well configured, and the library is good, all will give results
- Understand limitations of the technique and cross validate with other methods

- In our hands, NMR has proven to be robust and reliable
  - But there are limitations

Screening for Binding by NMR

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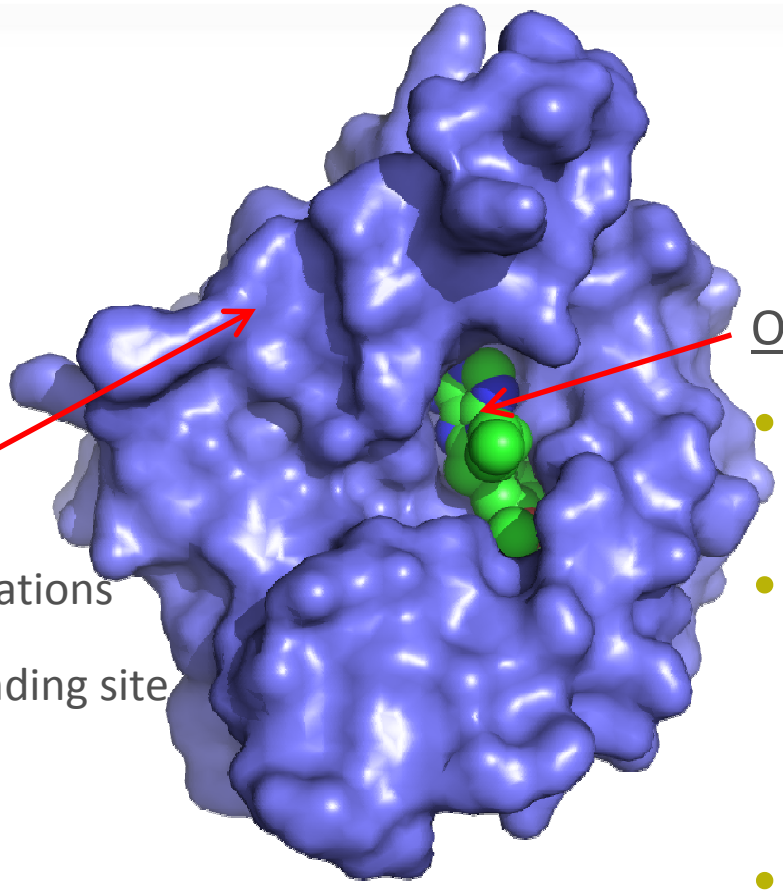

Observe Receptor

- Chemical shift perturbations
- Direct indication of binding site
- Size restricted
  - < 30-40 kDa or so
- Quantity of material
  - Large amounts of isotopically labelled protein

Observe Ligand

- Usually the free state of the ligand
- Modulation of ligand spectrum by interaction with receptor in bound state
- Less demanding on receptor supply and properties
- Infer binding site

Evolution of Fragment Screening at Vernalis

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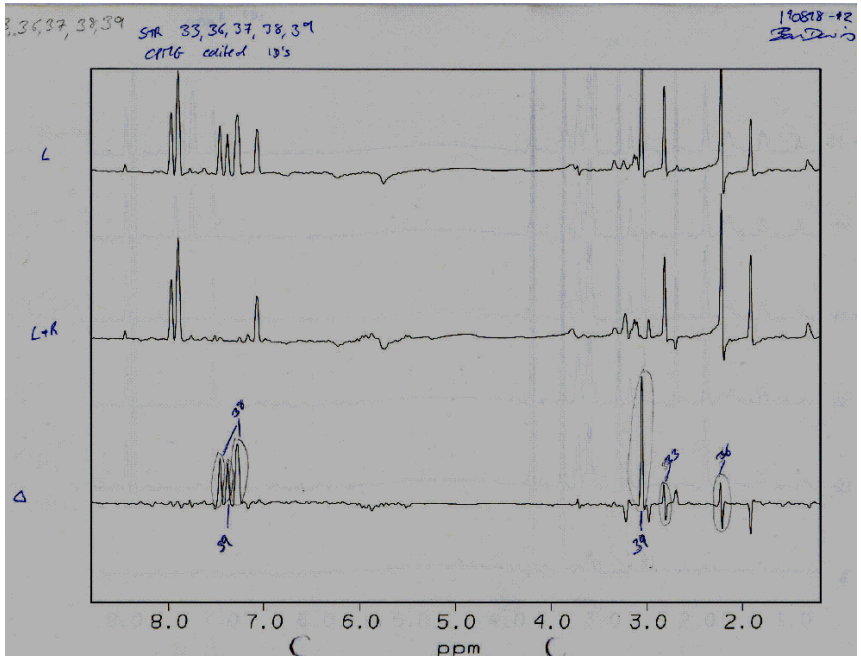
- Early fragment work on RNA targets
  - RiboTargets (’98-’01)
  - RNA supply major issue
  - Size of receptors
    - Ribosomal subunits
- Ligand observed screening
- Fast, reliable, but …
  - Specificity ?
- <u>Competition step</u>
  - Binding & displacement
  - Just as useful for protein targets



Initial Library Development

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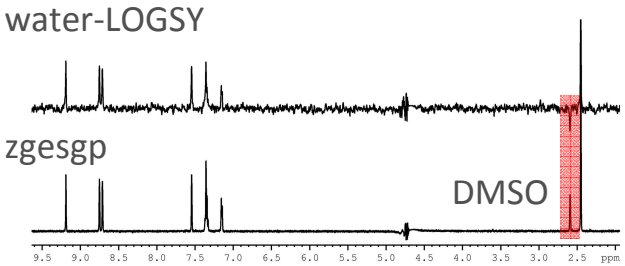
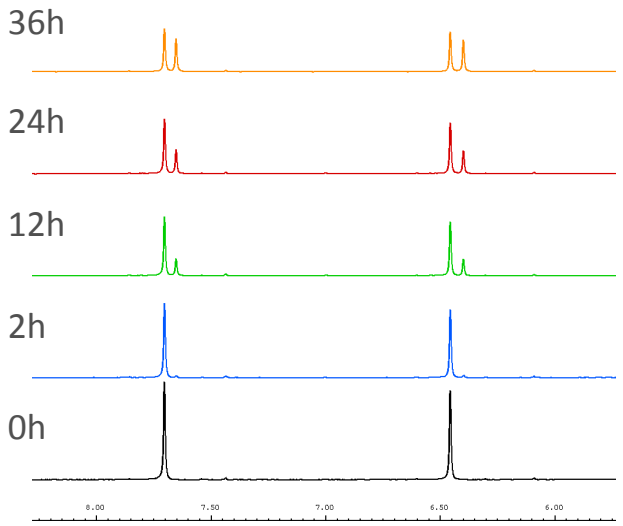
- Design criteria
- QC of library
  - Structure verification
  - Purity
  - Self association
    - Water-LOGSY of isolated compound
  - Aqueous stability
    - 24h in relevant buffer

Self association



Aqueous stability



Baurin et al (2004) JCICS 2004 **44** 2157-66

Dalvit et al (2006) Curr Drug Discov Tech **3** 115-24

Fragment library QC

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- Initial characterisation
  - Sample : 500 µM compound in aqueous solution
  - 1D ^{1}H NMR
    - Repeat after 24 hours for stability test
    - Spectra stored in AMIX SBASE
  - 1D waterLOGSY
  - 1D ^{1}H, ^{13}C NMR in DMSO if required
    - LCMS if required
- QC library ~ 12 monthly
  - 1200-1500 compounds
  - Long term stability
  - Reorder or remove – library maintenance

QC failures

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- Self association
  - positive water-LOGSY spectrum of free compound
  - 1-2% for in-house library
  - Up to 5% for vendor fragment libraries
- 24 hour aqueous stability
  - Up to 5% for both in-house and vendor libraries
  - Often not predictable which compounds will degrade
- Long term stability in DMSO
  - Up to 10% per year show signs of degradation
  - 200mM d6-DMSO, room temperature storage in dark

Combining experimental results

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- Many NMR ligand observed binding experiments
  - Each suffers from experimental artefacts

STD : Direct irradiation of upfield resonances

LOGSY : Positive LOGSY spectra from self association

T_2 filtered : Unexpected relaxation rates (structure)

- Acquire data using several experiments
  - Assess whole dataset rather than single experiment
  - Prioritise ligands showing consistent behaviour

Assessing Improvements

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- Competition step
  - No competition step : no crystal structures from any putative fragment hits
  - Competition step : 16/17 fragment hits crystallised
- Combination of experiments
  - Hit in all 3 experiments: 70-80% of hits crystallise
    - (2/3 experiments 40%, 1/3 experiments rarely)
  - Many fragments where multiple crystallisation conditions / constructs have been tried before crystal structure obtained
- Consistent binding data is used to define a hit, rather than observation of a crystal structure

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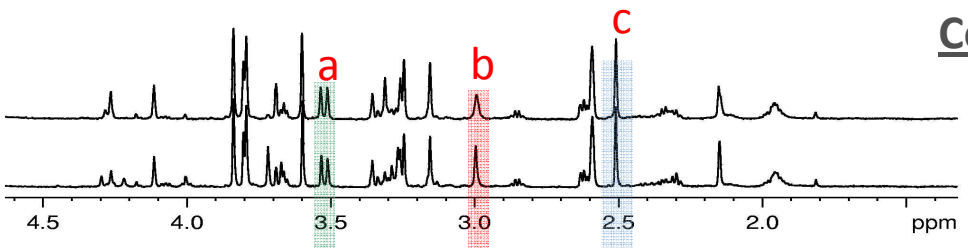
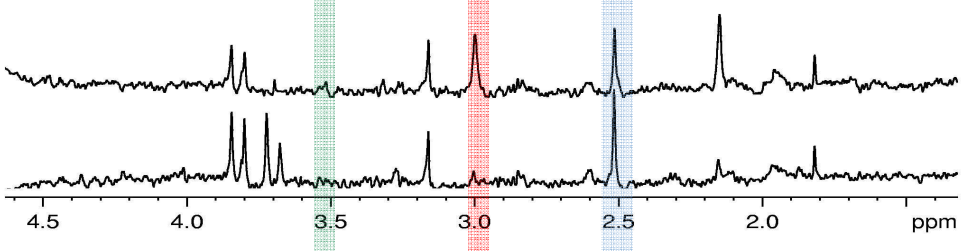
Experiment **Competitor**

1D

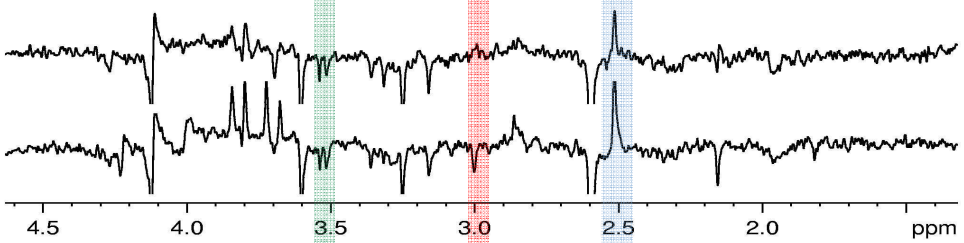


STD



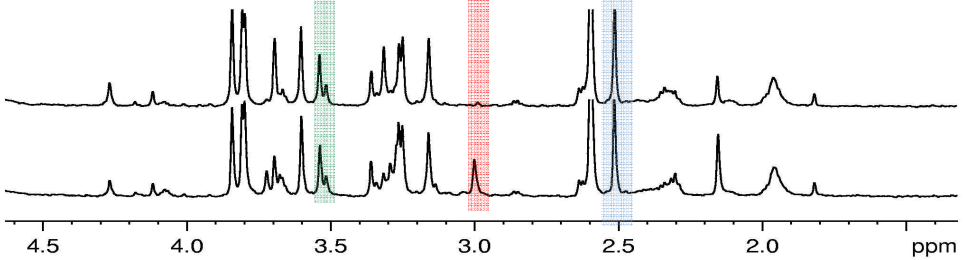
$$I_{obs} = f\left(P_{bound}\right)$$

LOGSY



$$I_{obs} = f\left(P_{bound} - P_{free}\right)$$

Relaxation filtered



$$I_{obs} = f\left(P_{free}\right)$$

Compound b binds and is displaced by competitor in all experiments

Screening the Library - NMR Competitive binding experiments

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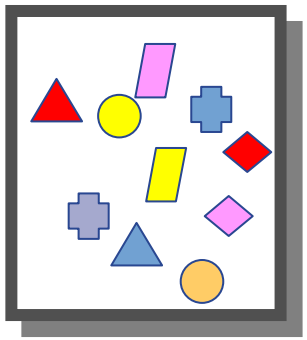
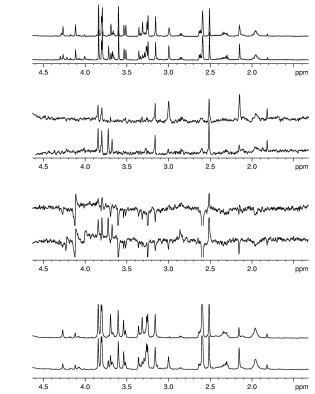
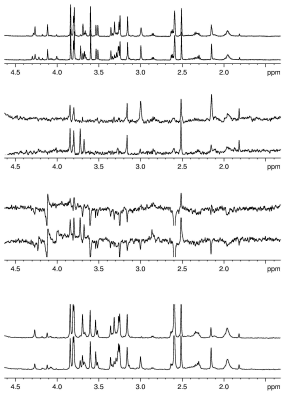


Mixture of fragments + target

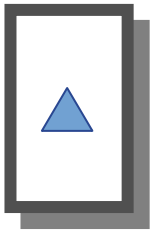


NMR experiments identify ligands

Add competitor

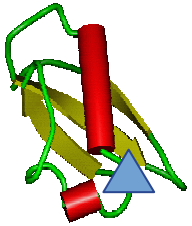


Has competitor displaced the ligand ?

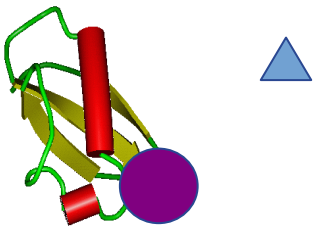


1-10% hit rate

Target + ligand



Target + competitor, ligand displaced



The FBLD Process at Vernalis

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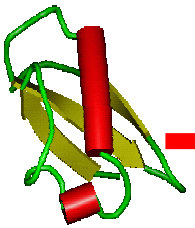
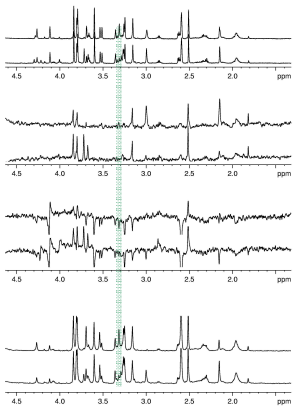
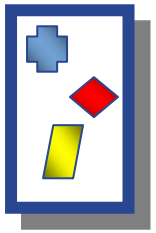


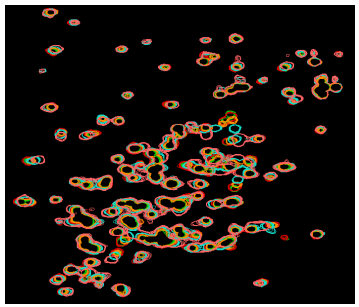
Target

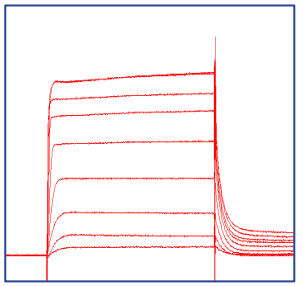


Screen library

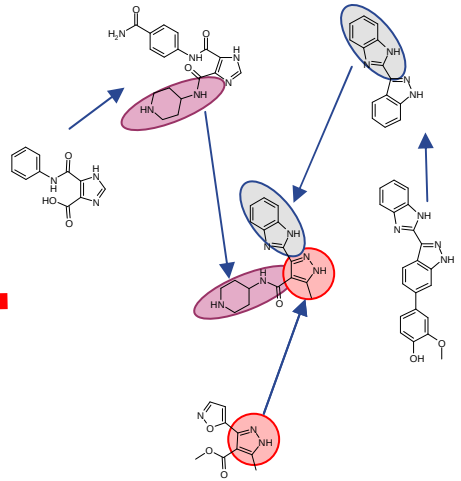


Hits

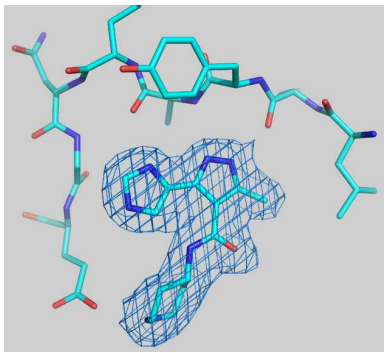




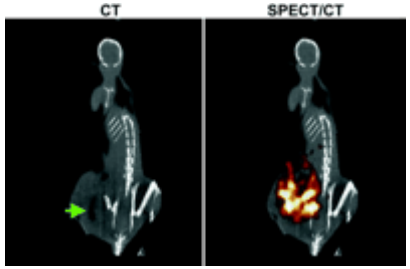
Validation



Design, Build & Test



Structure Determination

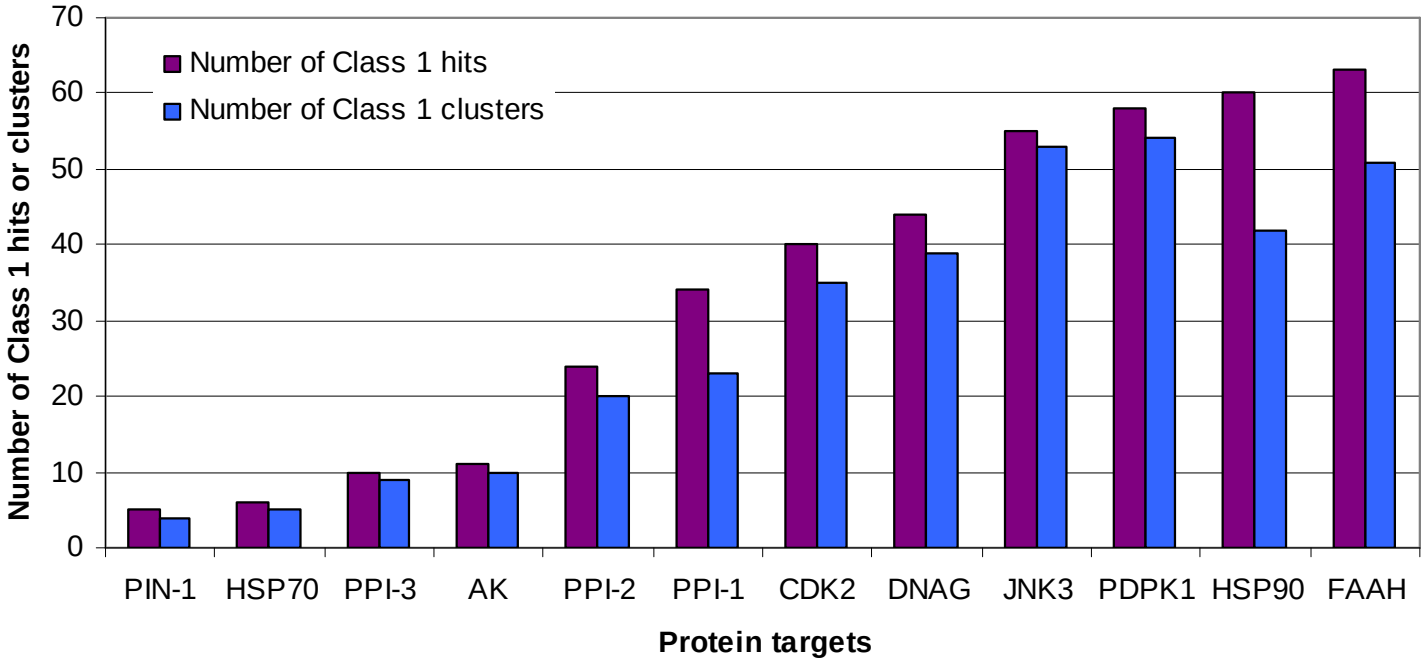


Drug?

Brough *et al* (2008), J. Med. Chem. **51** 196

Brough et al (2009), J. Med. Chem. **52** 4794

Fragment Screening - hit diversity



- Average: 34 Class 1 hits (~ 2.5% hit rate)
  - 29 chemical series (clustering @70%)

Chen & Hubbard (2009) J Comput Aided Mol Des **23** 603-20

Fragment Hits to Leads ?

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- Average 34 validated hits per target
  - All “preferred” chemical structures
- Prioritisation for evolution and progression ?
- Characterisation
  - X-ray structures
  - Biophysical methods (NMR, SPR, ITC, thermal melt)
- Prioritisation where no crystal structure ?
  - Confidence to allocate chemistry resource

NMR as a structural tool

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- NMR structures
  - Time consuming
  - Structure generation much slower than med-chem cycle requires
  - Data is incremental
- NMR guided models
  - Chemical shift perturbations (CSP)
  - STD Group Epitope Mapping (STD-GEM)
  - Interligand NOEs (ILOE)
  - 3D ^{13}C-edited, $^{13}C^{15}N$-filtered NOESY (X-filtered NOESY)
    - Detect NOEs between ^{13}C labelled protein and ligand
    - Observed via ligand (bound state)

X-filtered NOESY

(3D ^{13}C-edited, ^{13}C^{15}N-filtered NOESY)

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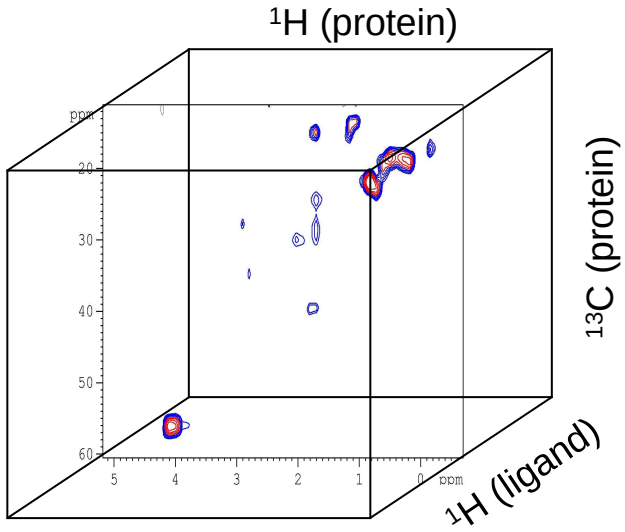

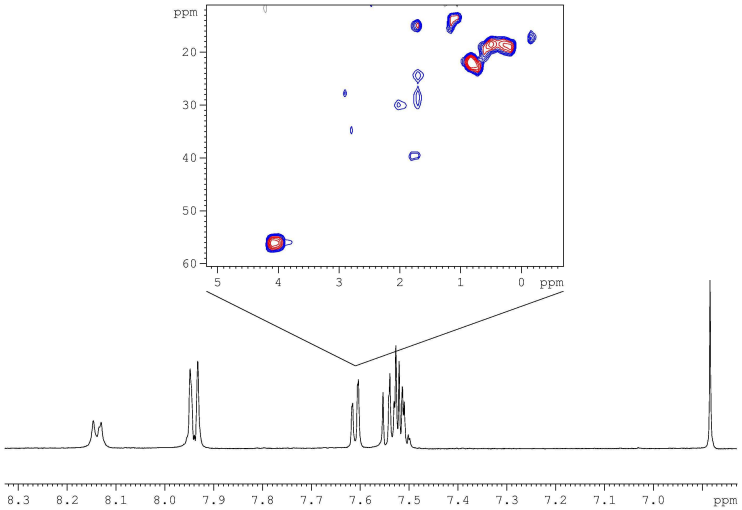

- ^{15}N^{13}C labelled protein, unlabelled ligand
- NOE only from (^{1}H, ^{13}C)(protein) to (^{1}H, ^{12}C)(ligand)
- Unambiguous detection of receptor-ligand NOEs
- Use NOEs to guide modelling

NOE mapping : Bcl-2

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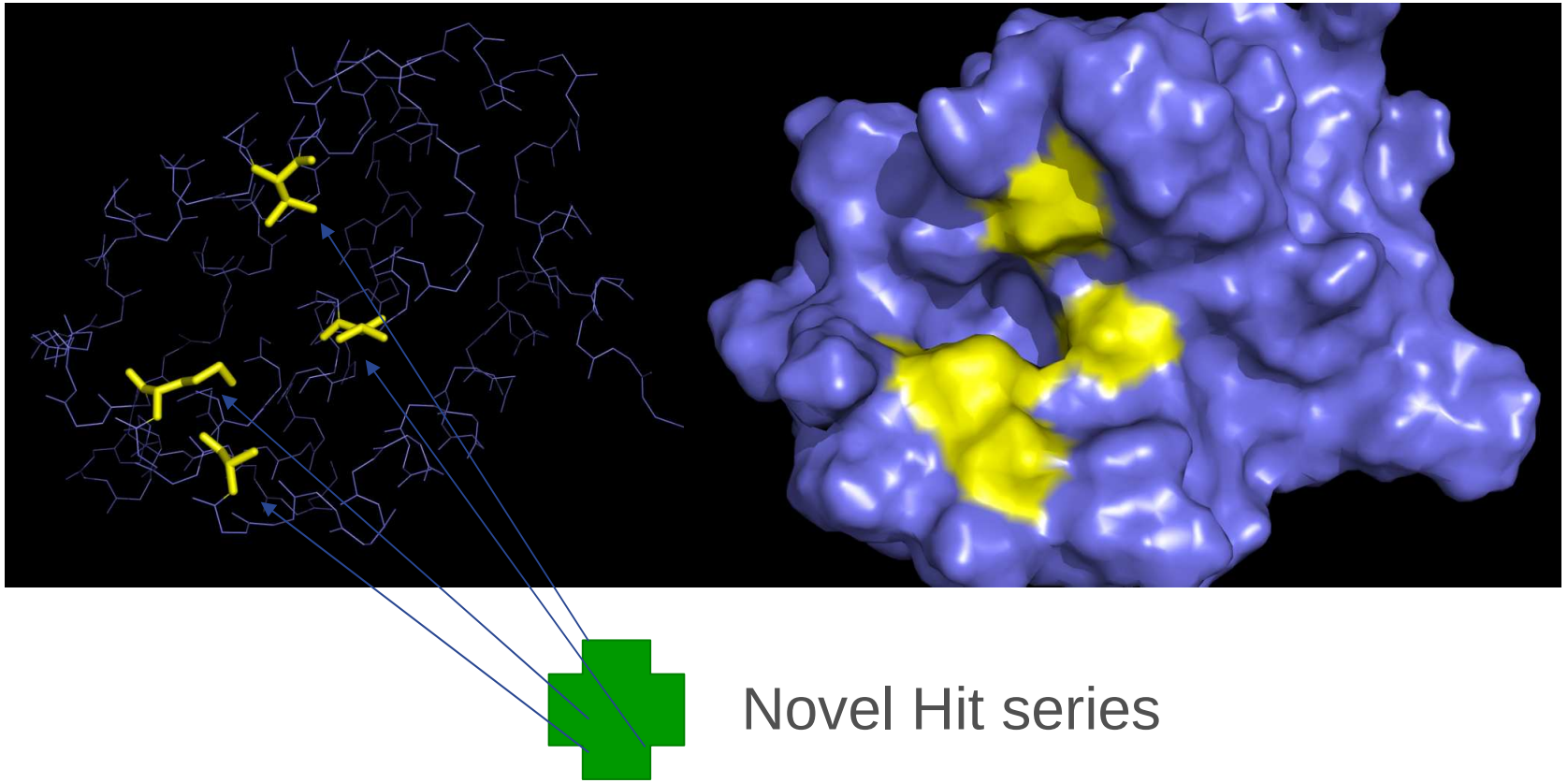

Novel Hit series

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NGM (NMR Guided Model) : Bcl-2

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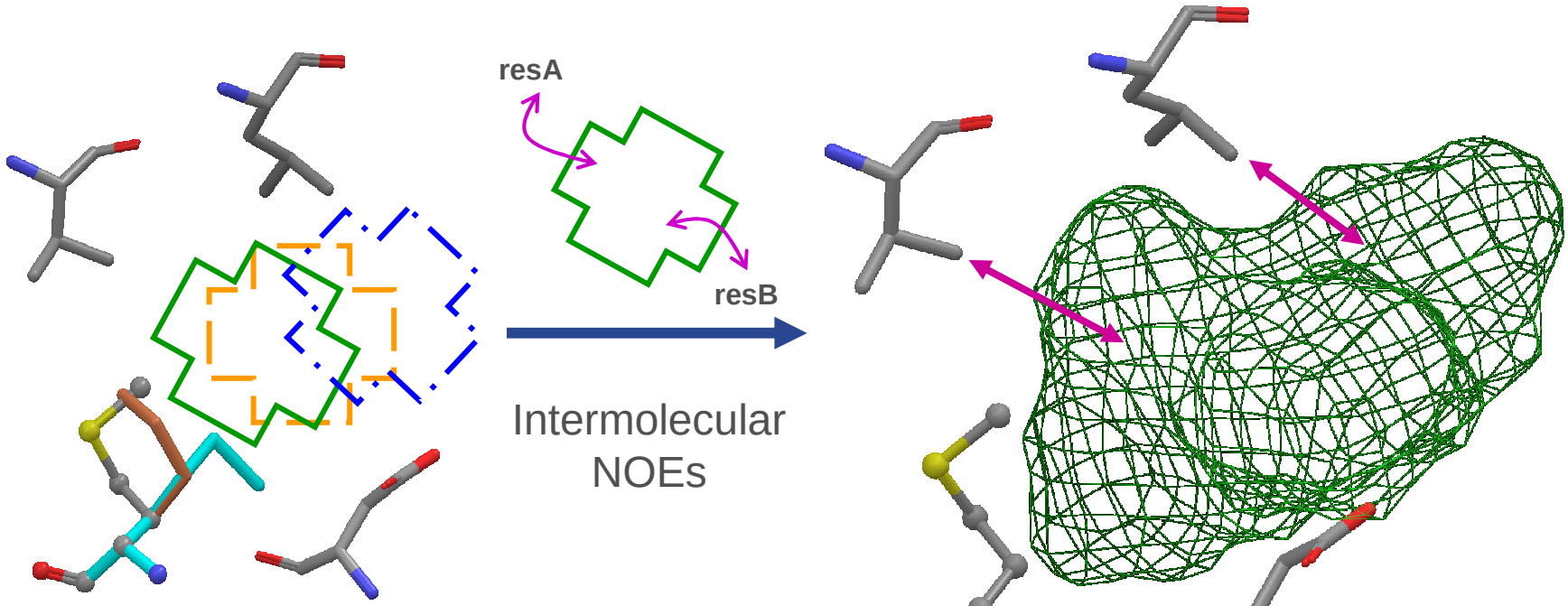
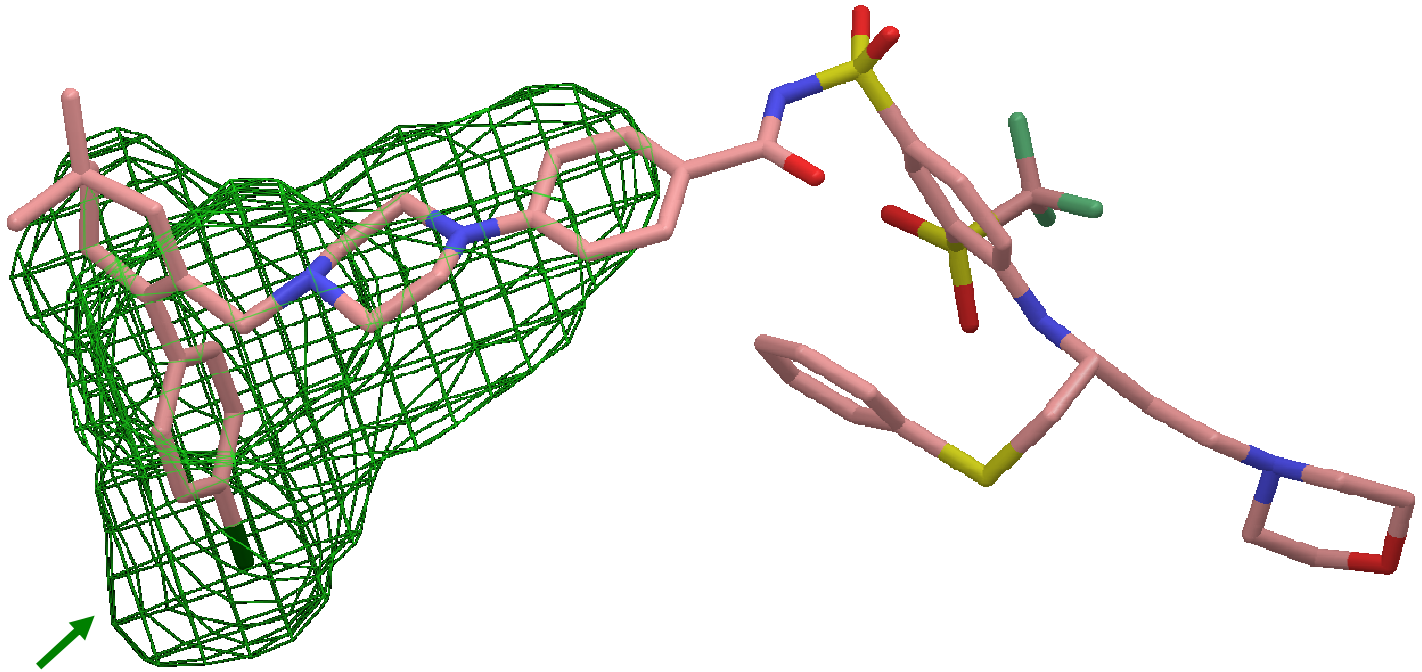


Multiple docking solutions
considering protein flexibility

NGM (wire)

NGM: Superposition with Crystal structure



NGM (wire)

ABT-263 Crystal structure

Non obvious bioisostere

NGM: Generate tool compound

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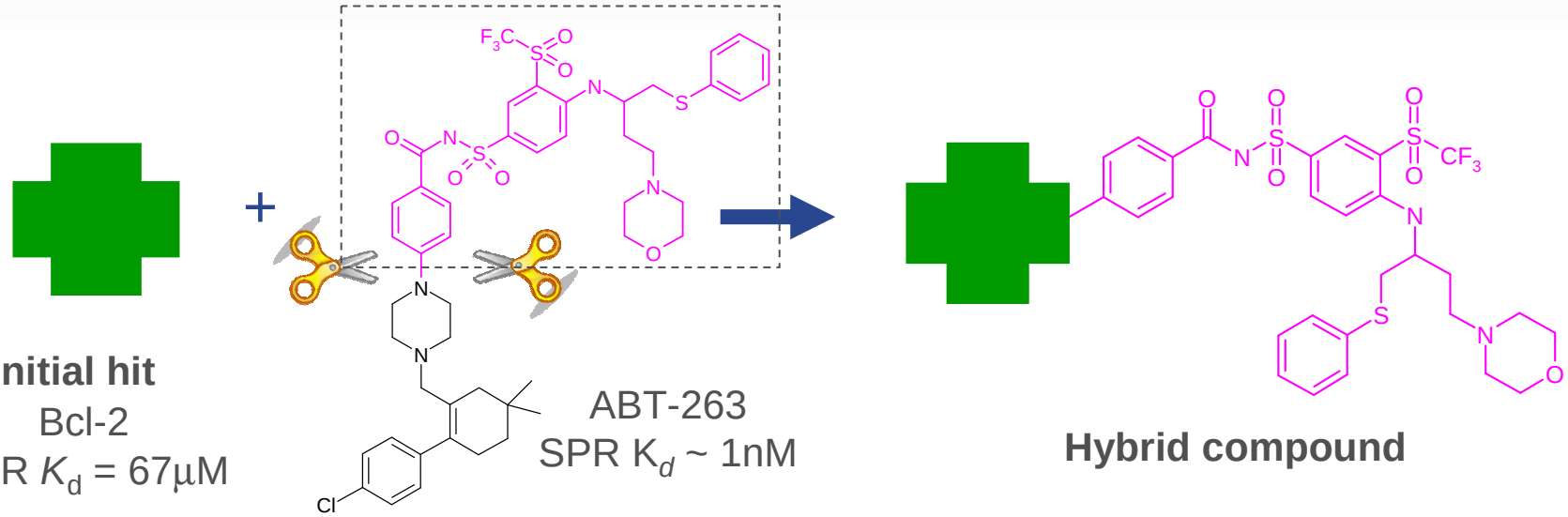
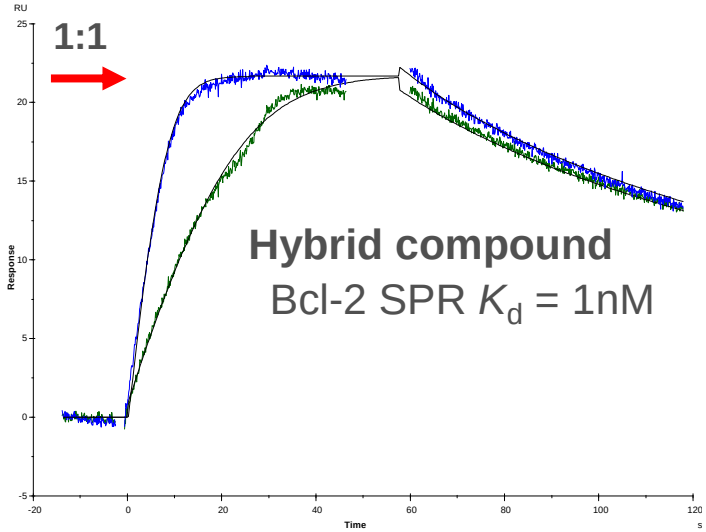
Fragment FP IC_{50} > 10uM



Initial hit
Bcl-2
NMR K_d = 67µM

ABT-263
SPR K_d ~ 1nM

Hybrid compound



Hybrid compound
Bcl-2 SPR K_d = 1nM

NGM: Crystal structure of tool compound in Bcl-2

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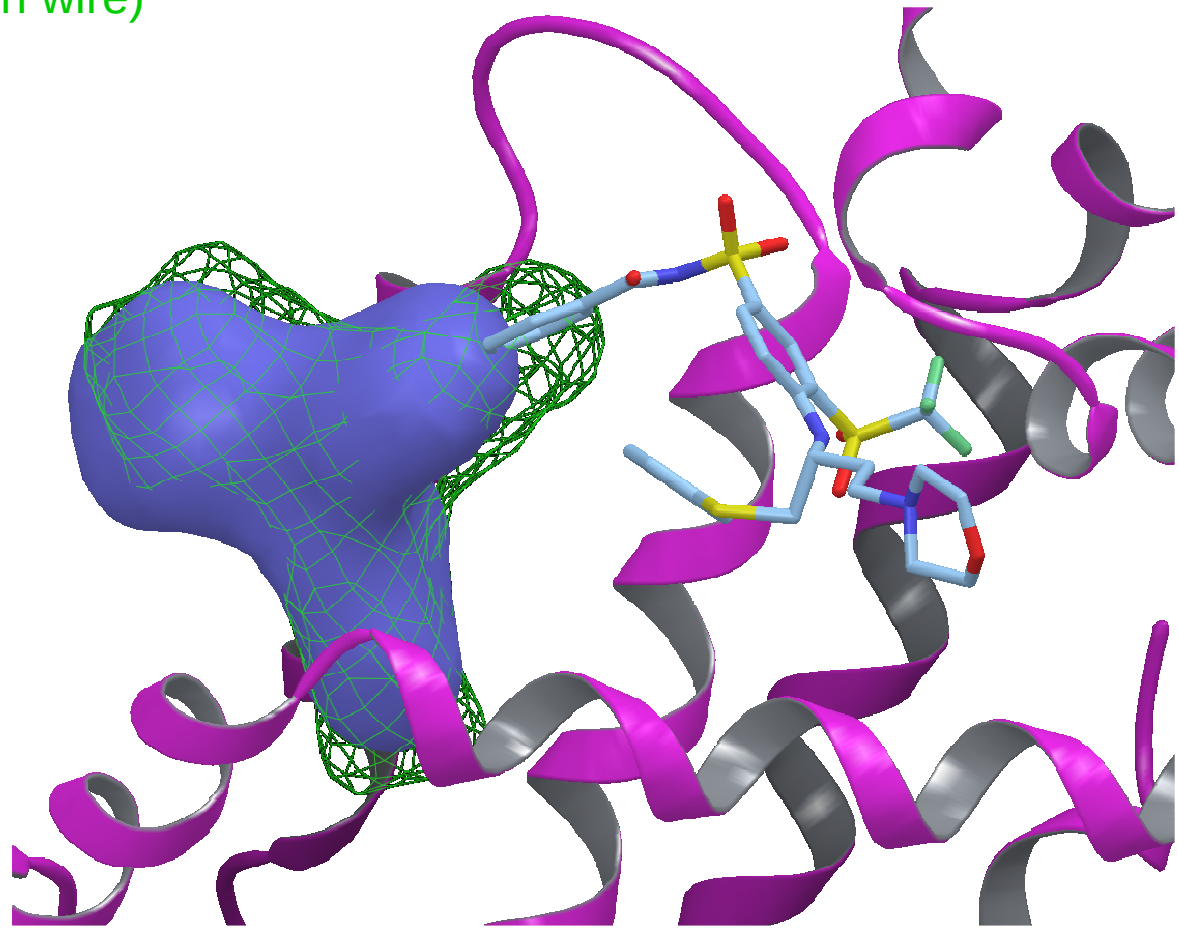
- Tool compound crystal structure (blue surface, pink ribbons)
- NGM (green wire)



NMR Guided Models : Project application

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- ~22 kDa protein-protein interaction target
  - Compounds currently in lead optimisation
- Does not crystallise in a useable form
  - Active site occluded by crystal packing
- Can we drive the medicinal chemistry based on structural data from NMR ?
  - Turn around time fast enough to meet medchem demands
  - 2 people in bio NMR group
  - 600 MHz NMR spectrometer with cryoprobe

NMR driven fragment evolution (1)

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FBLD 2010

- Fragment screen completed & characterised
  - Ligand observed, competition with protein binder
  - 40 hits validated and characterised
  - No fragments crystallised
- Backbone and selected sidechains assigned
  - Active site contains well resolved methyl groups
- Experience with other projects
  - 3D ^{13}C-edited, $^{13}C^{15}N$-filtered NOESY
  - Chemical shift perturbation (CSP)
  - STD-GEM

NMR driven fragment evolution (2)

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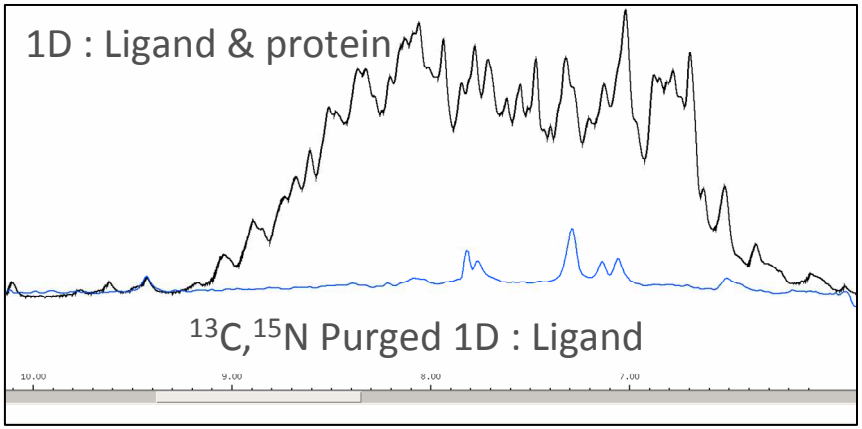
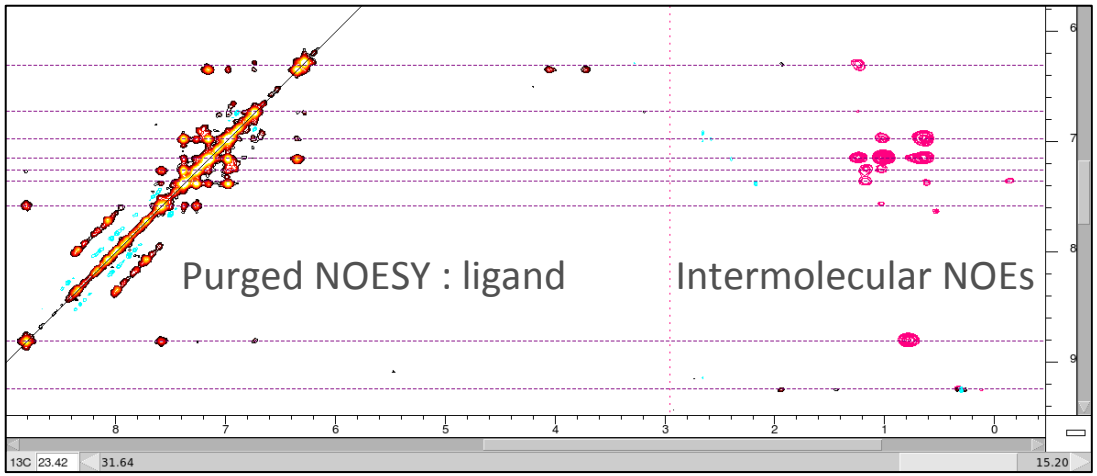
FBLD 2010

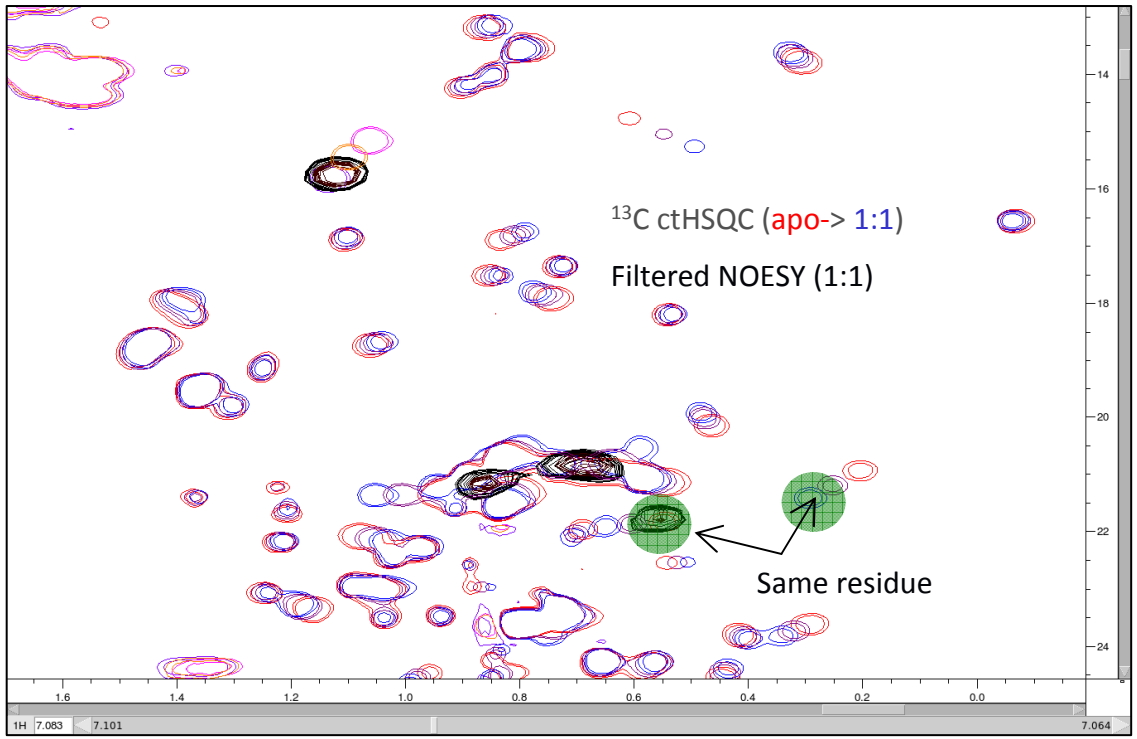
- $^{13}C^{15}N$ labelled protein
- Assign ligand in bound state using purged experiments
- Intermolecular NOEs readily assigned to ligand resonances

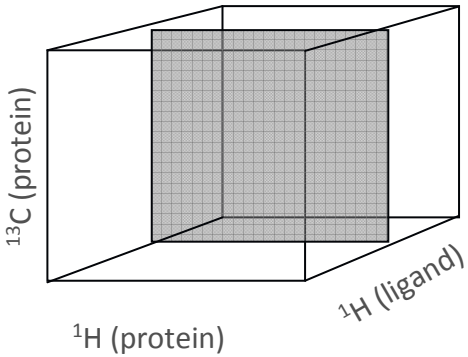




NMR driven fragment evolution (3)

- 3D ^{13}C-edited, $^{13}C^{15}N$-filtered NOESY spectra
  - Focus on methyl region : improved resolution
  - Slices at ligand resonance frequencies





NMR driven fragment evolution (4)

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- 90 2D and 3D ^{13}C-edited, $^{13}C^{15}N$-filtered NOESY spectra acquired over 18 months
- On average :
  - 1 week between data acquisition and assignment of intermolecular NOEs
  - 1 week between NOE assignment and generation of NMR guided model (NGM)
- NGMs generated using pose filtering based on observed NOEs
  - Consider series SAR
- Interaction & iteration between modelling and NMR groups

NMR driven fragment evolution (5)

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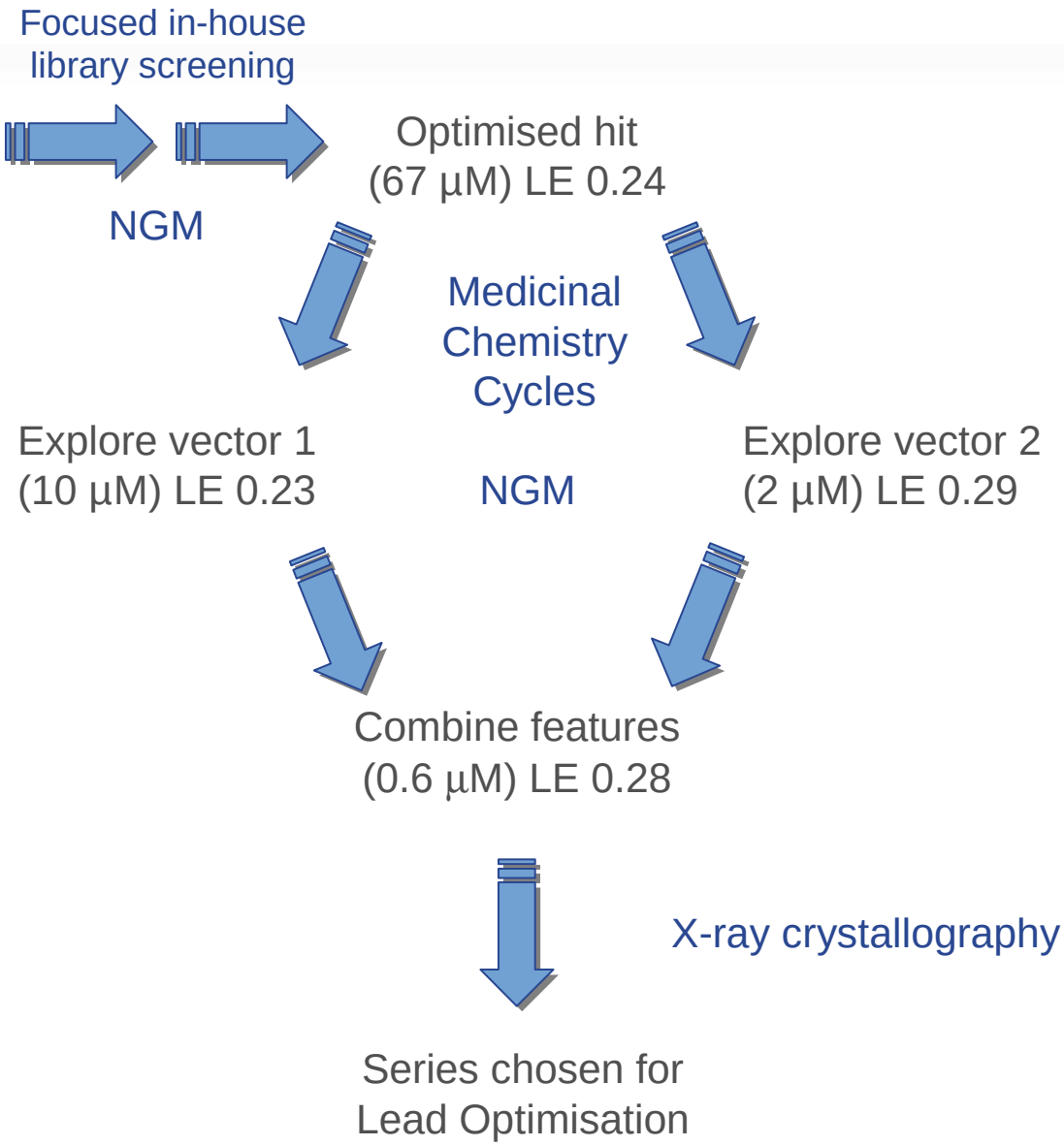
- Average 2 weeks from data acquisition to NGM
- Sufficiently fast to guide medicinal chemistry program
- Over 18 months, ligand K_d values went from mM to sub µM
  - Series now in lead optimisation
- Potent (sub µM) ligands crystallise in bound form
  - Generate crystal structures of potent ligands
  - Consistent with NGMs
    - NGM obtained 4 months before crystal structure

Conclusions

Initial fragment hits
Multiple series
Kd ~0.5-2mM
LE 0.23-0.19



About Vernalis

Vernalis
FBLD 2010

- A small pharmaceutical company
  - Frovatriptan on the market and programmes in clinical development
  - Formed from merger of RiboTargets, Vernalis, British Biotech and others between 2004-2006
- Research Programmes
  - Fragment and structure-based discovery against oncology targets
  - Collaborations with large and small pharma
- Based in Granta Park, south of Cambridge, UK
  - ~60 people in research