Fragment screening by crystal structure at Diamond

Frank von Delft

Principal Beamline Scientist for I04-1, Diamond

Principal Investigator @ SGC, Oxford University

PSDI 2013, Luzern

5 November 2013

Industrial Liaison Office at Diamond

Pharmaceutical applications

- Macromolecular crystallography (MX)
- Small angle X-ray scattering (SAXS)
- Circular dichroism (CD)
- Infra-red spectroscopy (IR)
- Small molecular crystallography (SMX)
- X-ray powder diffraction (XRPD)

Proprietary access

- Beamtime only
- Remote access
- Mail-in service
- Full analysis service

For more information please visit Jitka and Alex at Diamond stand

diamond

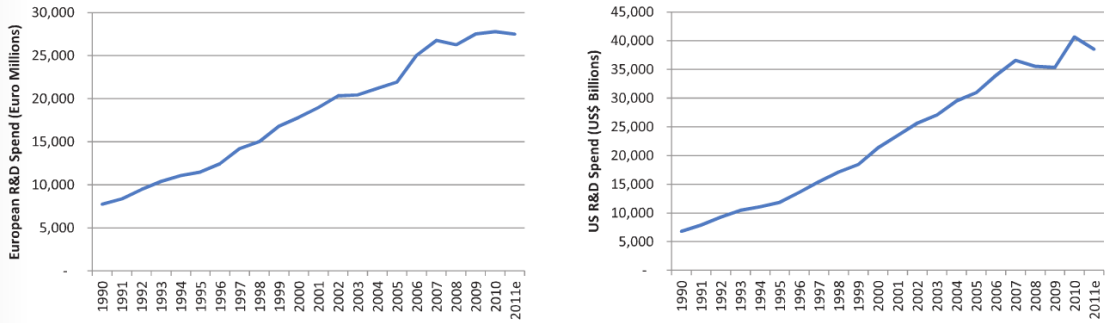
SGC

UNIVERSITY OF OXFORD

Drug discovery: reality check

- Costs have been spiralling – for decades

Figure 1.1. European and US R&D Spending



Source: EFPIA (2012)

Table 1.1. Number of new chemical or biological entities (1990–2009)

Number	1990–1994	1995–1999	2000–2004	2005–2009
Total	215	207	162	146
Average per year	43	41	32	29

Source: EFPIA (2010a)

Table 1.2. Number of new chemical or biological entities (2005-2009)

Year	2005	2006	2007	2008	2009
Number	30	35	25	31	25

Source: EFPIA (2010a)

- Several reasons, not all scientific
- BUT: is the process as smart as it needs to be?

diamond

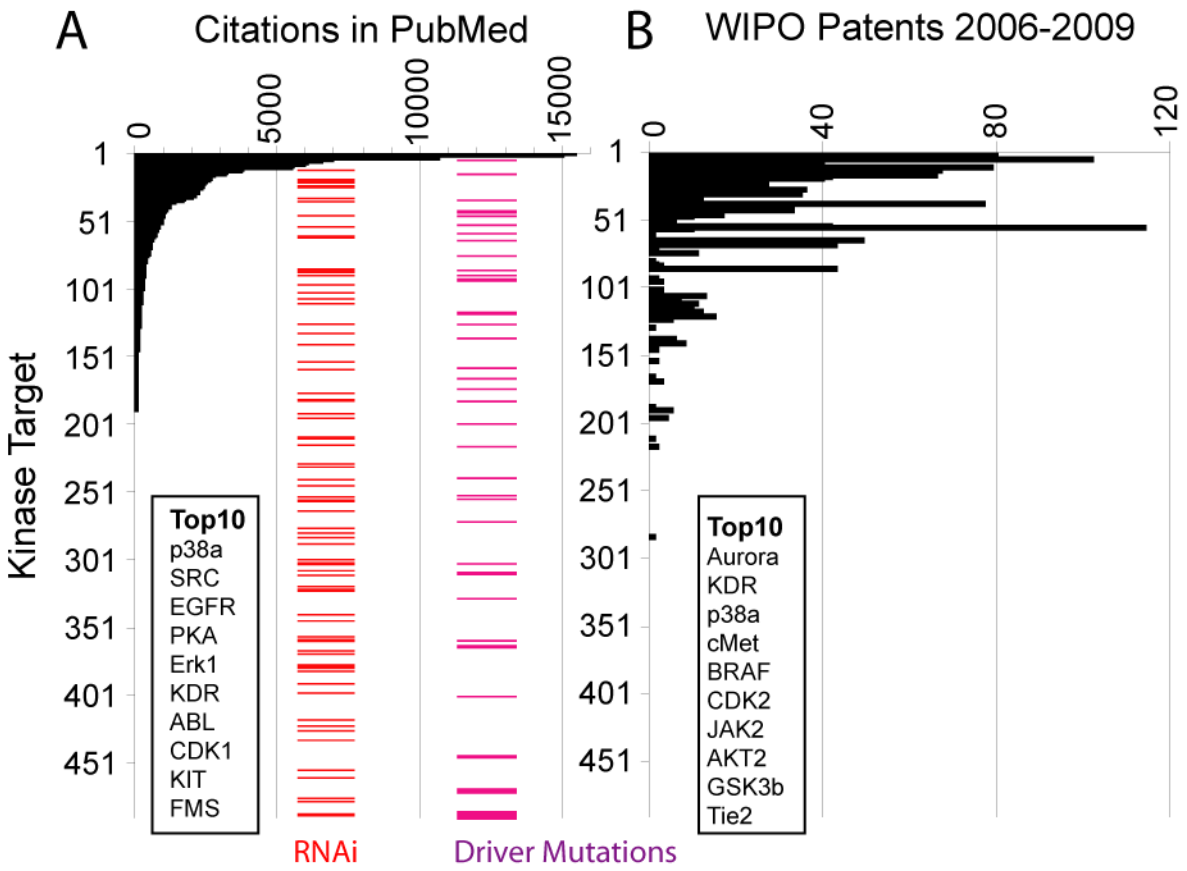
SGC

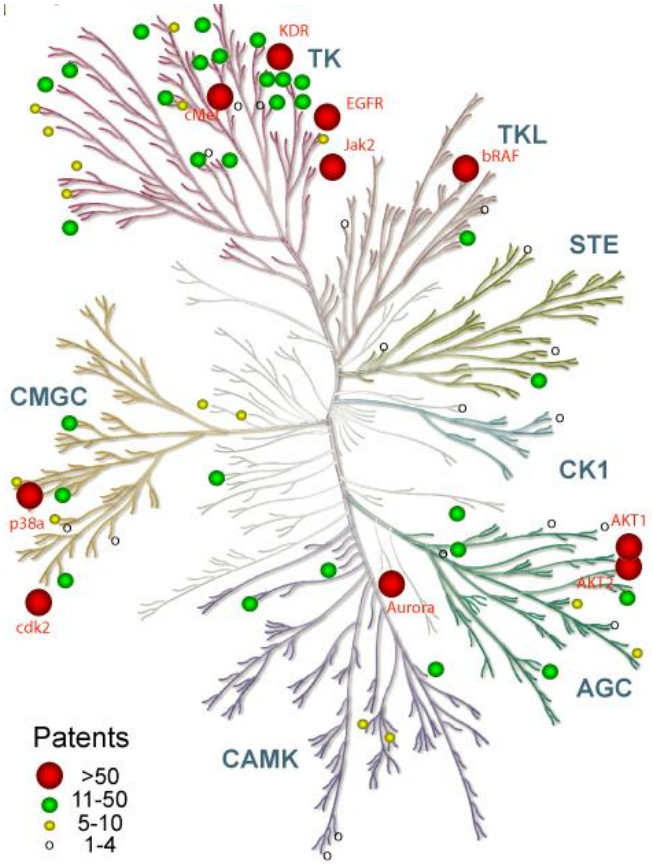
UNIVERSITY OF OXFORD

Targets are selected VERY conservatively

Covering mainly ~10% Kinome
Patents follow public data

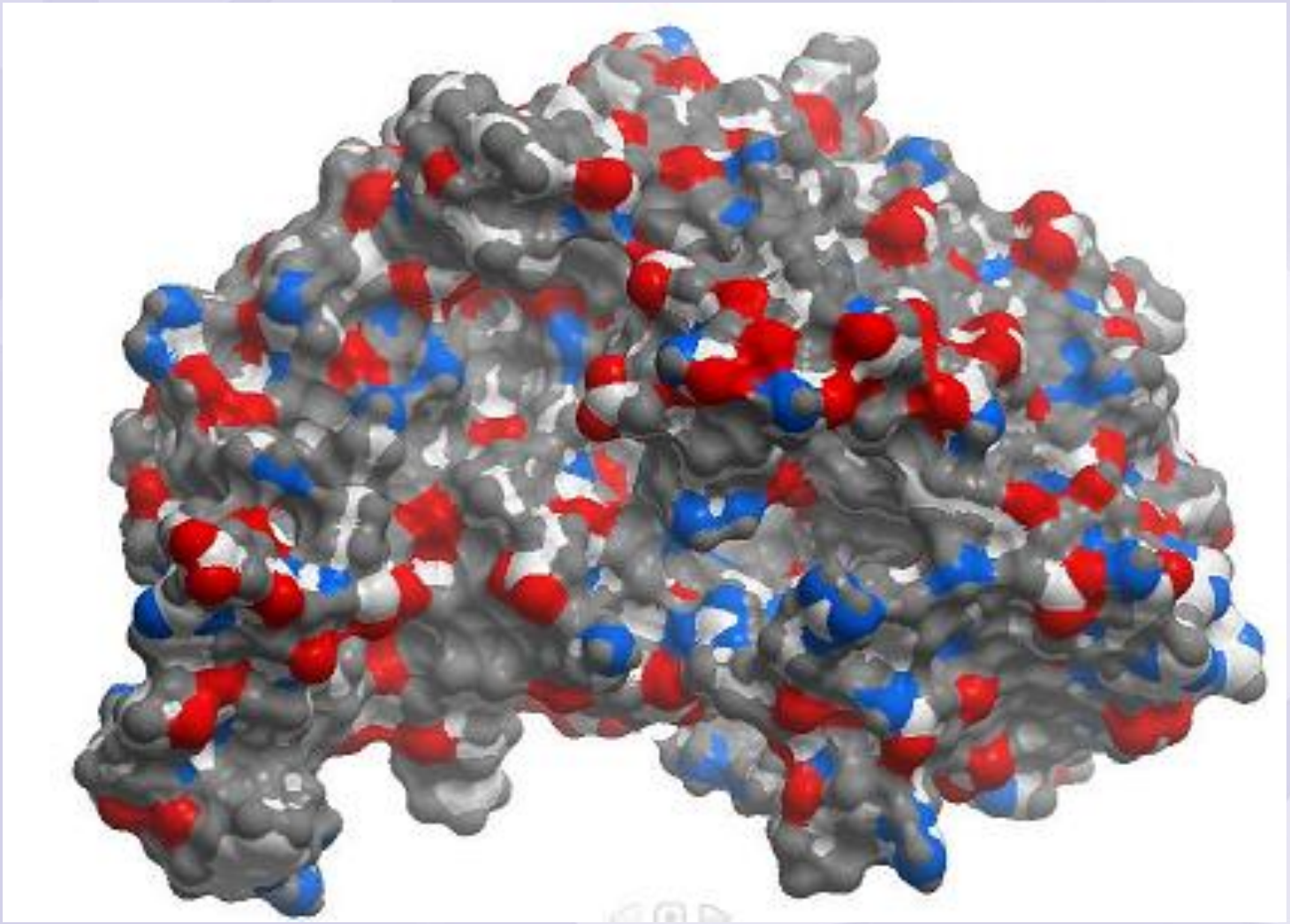
Kinases: > 500 000 papers in PubMed
> 10 000 US patents





Federov, Müller, Knapp (2010), Nat Chem Biol

How structures help Chemistry



diamond

SGC

UNIVERSITY OF OXFORD

DO structures help Chemistry?

- What if protein structure has no compound bound?
- Can binding strength be predicted?

- Algorithms are apparently still rubbish

1983, Blundell *et al*, Nature:

"*We are now using computer graphics programs to investigate the interaction of putative substrates and inhibitors with a view to designing molecules which might be more effective in the treatment of hypertension.*"

2012, Head of Structural Biology at one of most innovative Big Pharmas:

"Well... a structure without a bound ligand does not help chemists very much – though once you have something bound, it's very powerful for ***guiding chemistry****."*

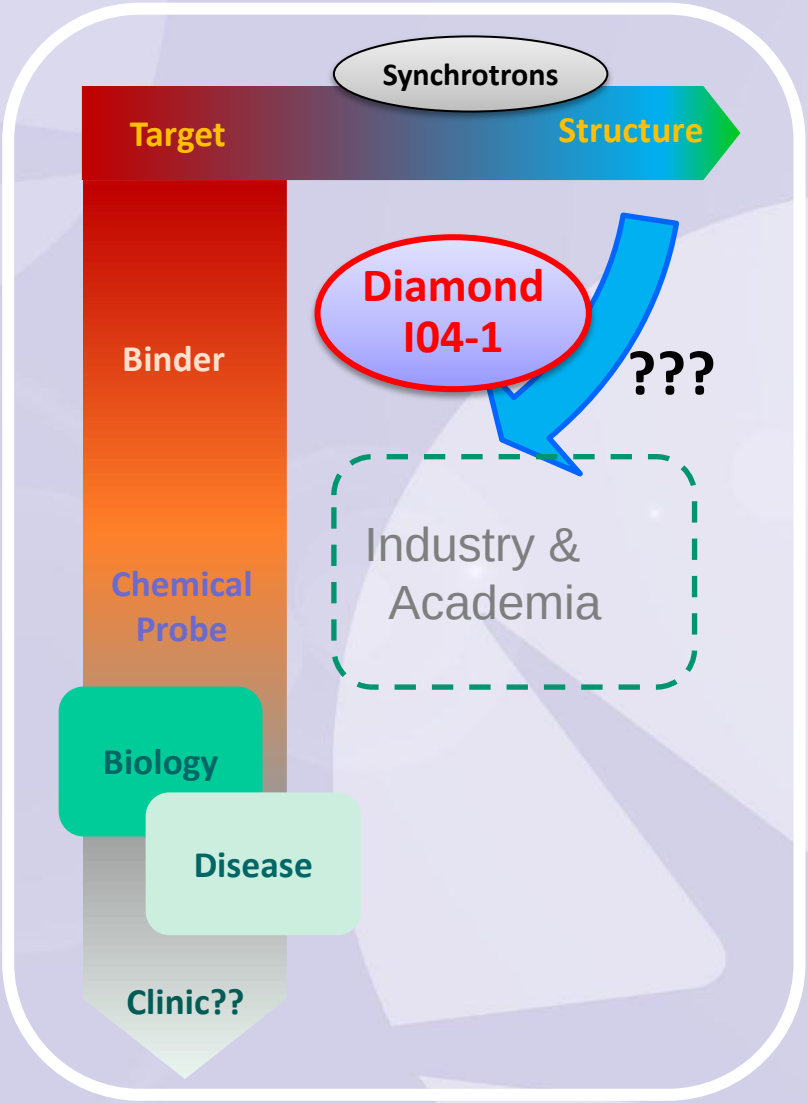
Oi! … what happened to *predicting* chemistry…???

diamond

SGC

UNIVERSITY OF OXFORD

Crystallography's repertoire



diamond

SGC

UNIVERSITY OF OXFORD

Fragments

- How to identify
  - Biophysical techniques: test 100s / 1000s whether they bind
  - Crystal structure: observe 10s / 100s how they bind
  - Compounds: 150-350 Da – bind weakly

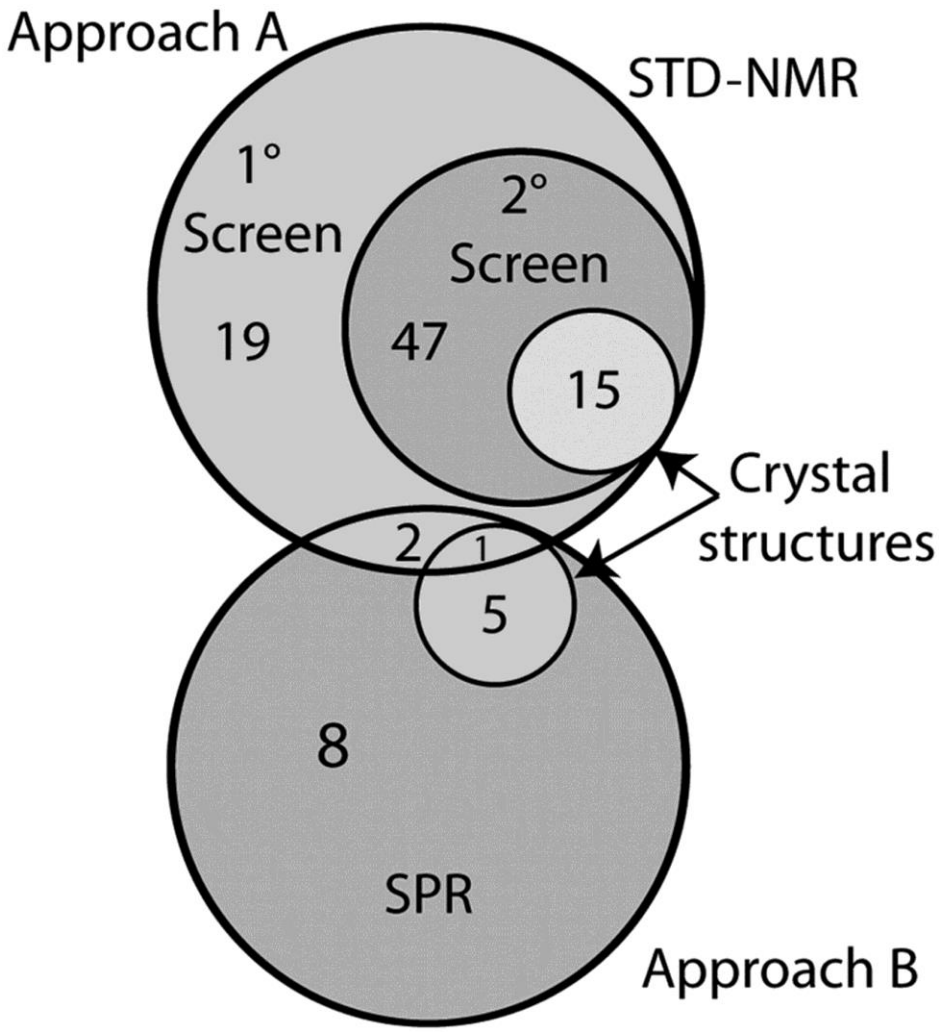
- How to use
  - grow: take one fragment, expand by synthesis
  - link: take 2 fragments, link them up by synthesis
  - descriptive: *ab initio* synthesis (??)

diamond

SGC

UNIVERSITY OF OXFORD

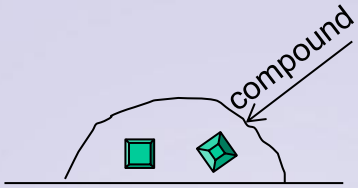
Figure 3. Venn diagram showing the numbers of compounds identified at each screening step and the overlap of hits found by the two approaches.



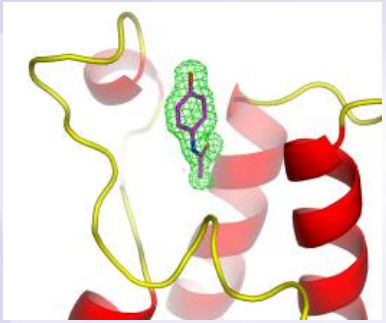
Wielens J et al. J Biomol Screen 2012;18:147-159

Screening by crystal structure

- Getting compound in: "simply" add to crystal (soak)



- Crystal structure – fast: calculate Fourier maps
  - (if crystals are identical)



- Speed of experiment:
  - 10 years ago: **10-100 min** / crystal
  - Meanwhile: Hotter beams & Dectris detectors & robots & algorithms
  - Now: **<2 min** / crystal
  - realistic: 100s datasets / day (!!!)

➔ test binding directly by X-ray structure

- **Read-out is binary: yes/no** *(unlike biophysics)*
- smaller compounds (150-200 Da) *(unlike biophysics)*
- Ensemble of hits: **collectively informative**

diamond

SGC

UNIVERSITY OF OXFORD

Besides, why can't EVERYBODY do fragments?

- Old, established technique… but can YOU do it?

- Massive logistical overhead

➔ Job for a facility!!

diamond

SGC

UNIVERSITY OF OXFORD

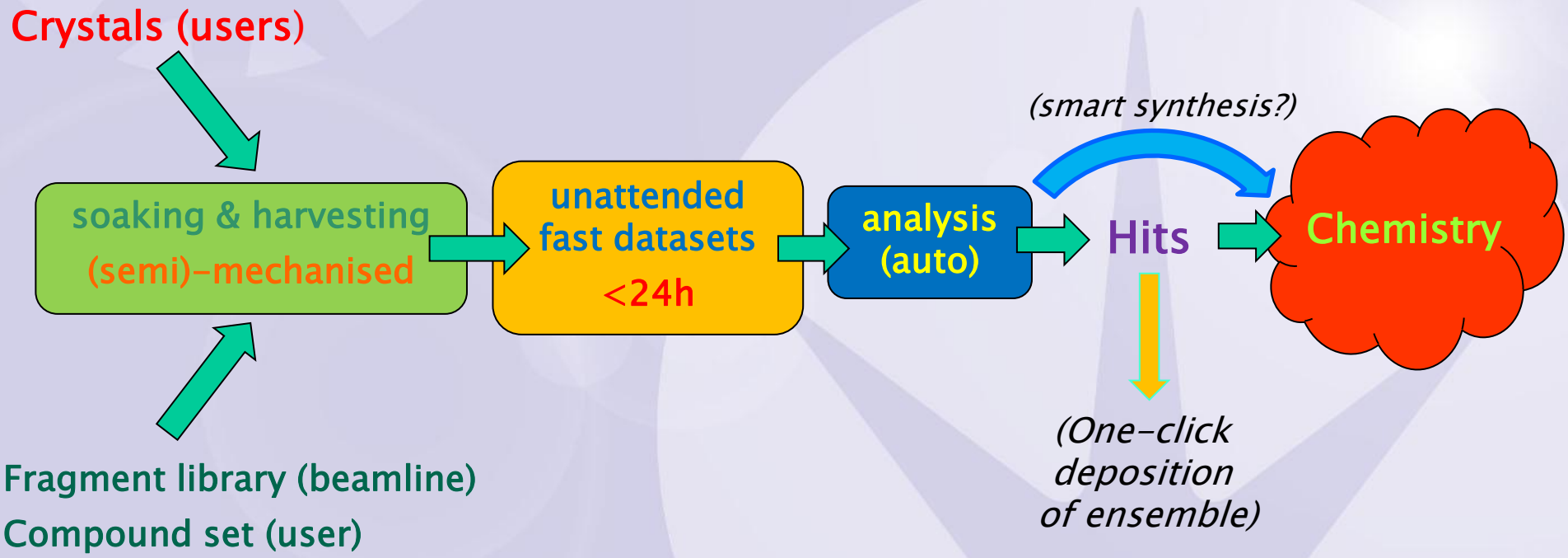
Diamond I04-1

- Fixed wavelength side-station
- Focus: stabililty, high-throughput
- Since 2010
- Joined: Dec 2012

SGC
UNIVERSITY OF OXFORD

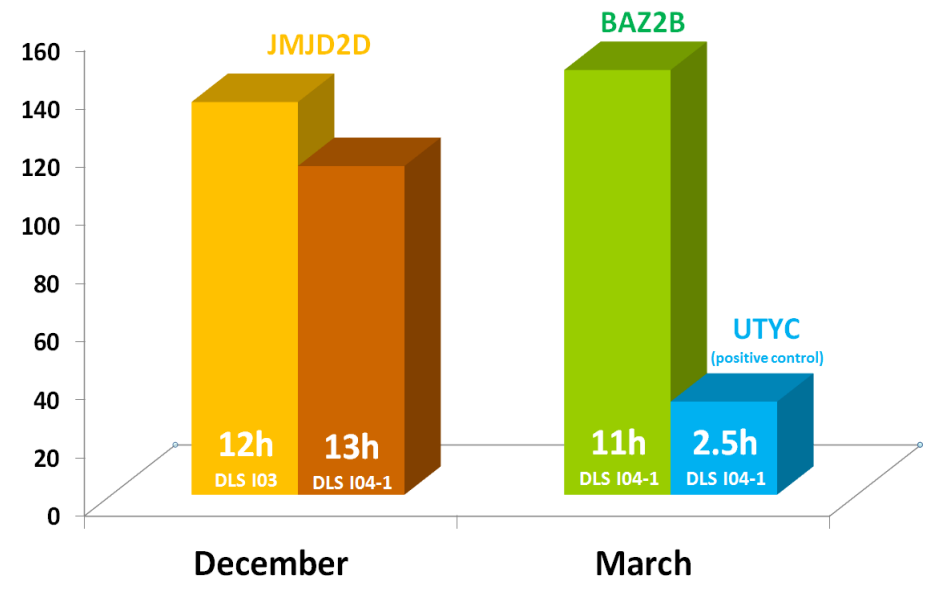
What will the beamline provide

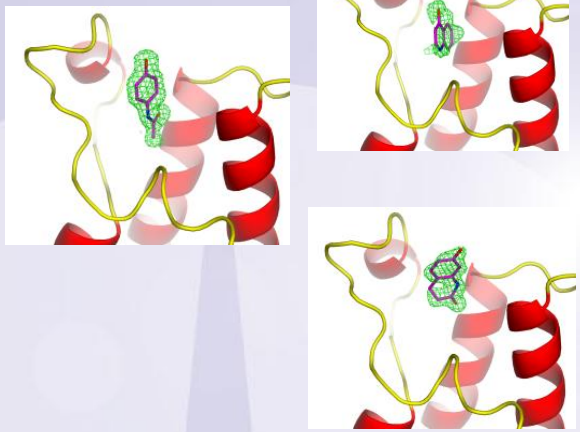
- Setting up as facility for **X-ray screening of fragment soaks**
  - *Old technique – so make available to users*
  - *User: soaking <u>at beamline</u> and collect rapidly*



Datasets: current capability

- Before any optimization:





- Can achieve: **~350 datasets/24h**

- Since May: autocentring with unattended operation
  - **>20 datasets / hr** *(theoretically: >400 / day)*
  - Crystals must match loop
  - (Mark Williams, I03; Richard Fearn, GDA)

diamond

SGC

UNIVERSITY OF OXFORD

Datasets: implementation focus

Immediate

- Duty cycle — *3.5-4min ➔ ~2min* (goal: 1.4min: 1000/day)
- Reliability — *auto-align beamline, eliminate robot fails*
- More photons — *undulator gap, CRLs*

2014

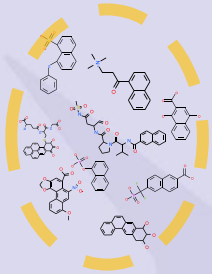
- Robot — *easy loading, fast exchange*
- Centring — *offline review, omplex shapes, diffraction-based*
- Sample logistics — *Tracking pins, soaks, pucks…*
- Dataset evaluation — *Rapid visual assessment of maps & stats*

diamond

SGC

UNIVERSITY OF OXFORD

What else will it take



Many compounds

↓

Many crystals

↓

Many frozen crystals

↓

Beamline robot

↓

Many datasets

↓

Many calculations

↓

Many evaluations

↓

2-10% will have compound bound…

Fragment library(ies)
Low-volume dispensing robot
Robot-assisted harvesting

} *Lab 36*

Loop logistics

Upgrade robot: high capacity, fast exchange

…

SciSoft: fastdp, Xia2, dimple

CCP4 GUI2 (?)

diamond

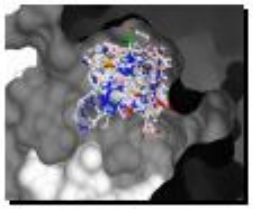
SGC

UNIVERSITY OF OXFORD

Precedent: *e.g.* Janssen strategy

Fragment Progression to Lead Declaration

Primary Library

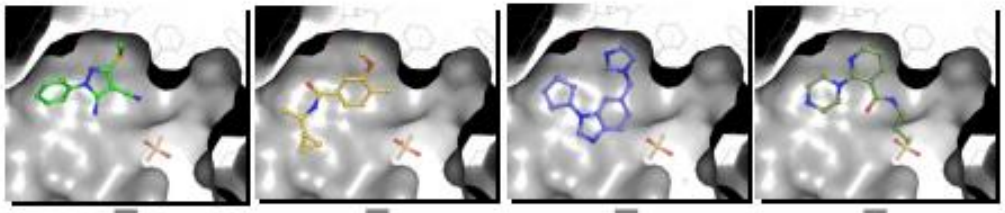


KHK

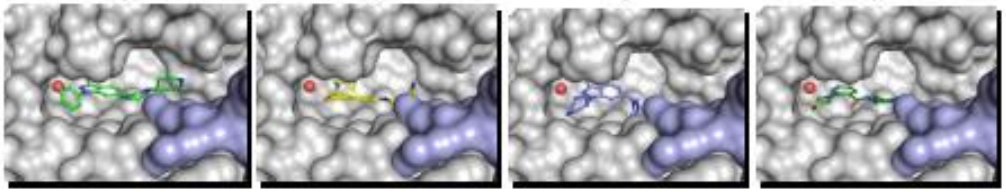
848 fragments	Number of hits	Hit Rate (%)
X-ray structure	44	5.2

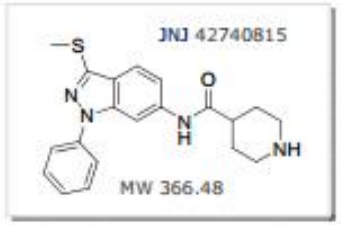
Progressed 6 without affinity guidance

Secondary Library
628 compounds
design + synthesis

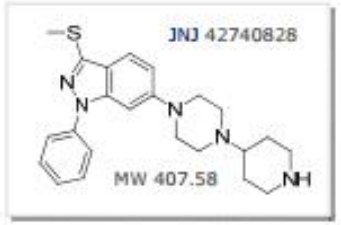


Tertiary library
510 compounds
design + synthesis





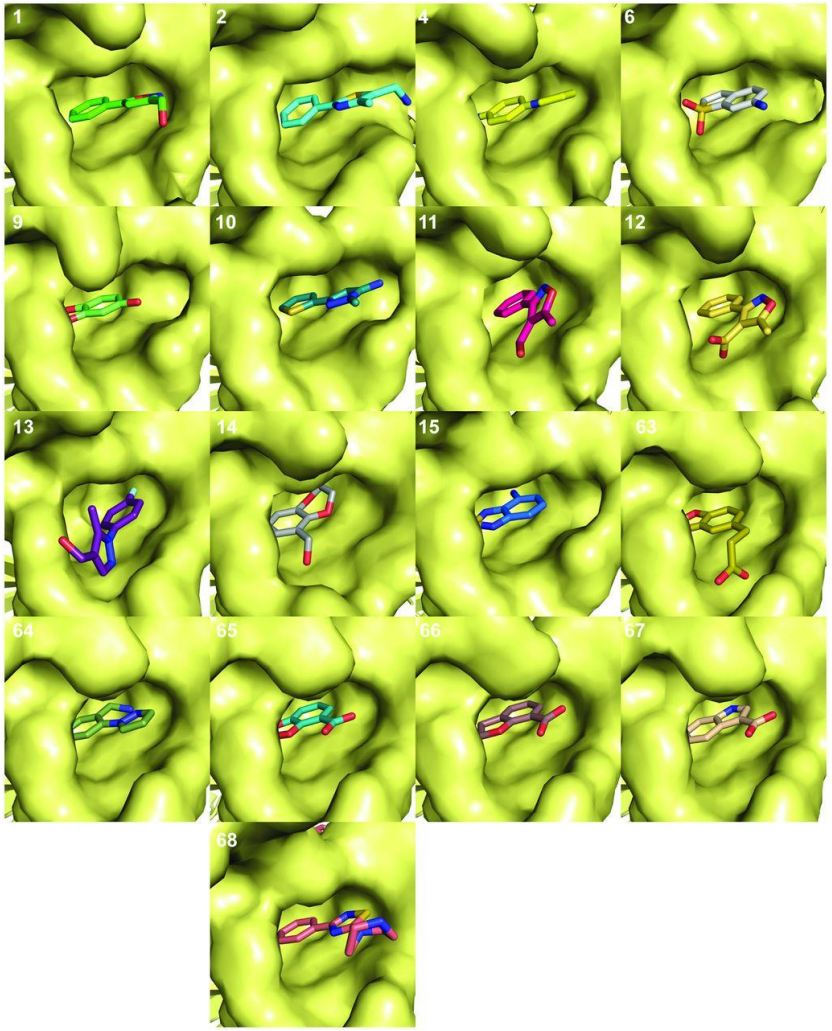
Declared 2 leads



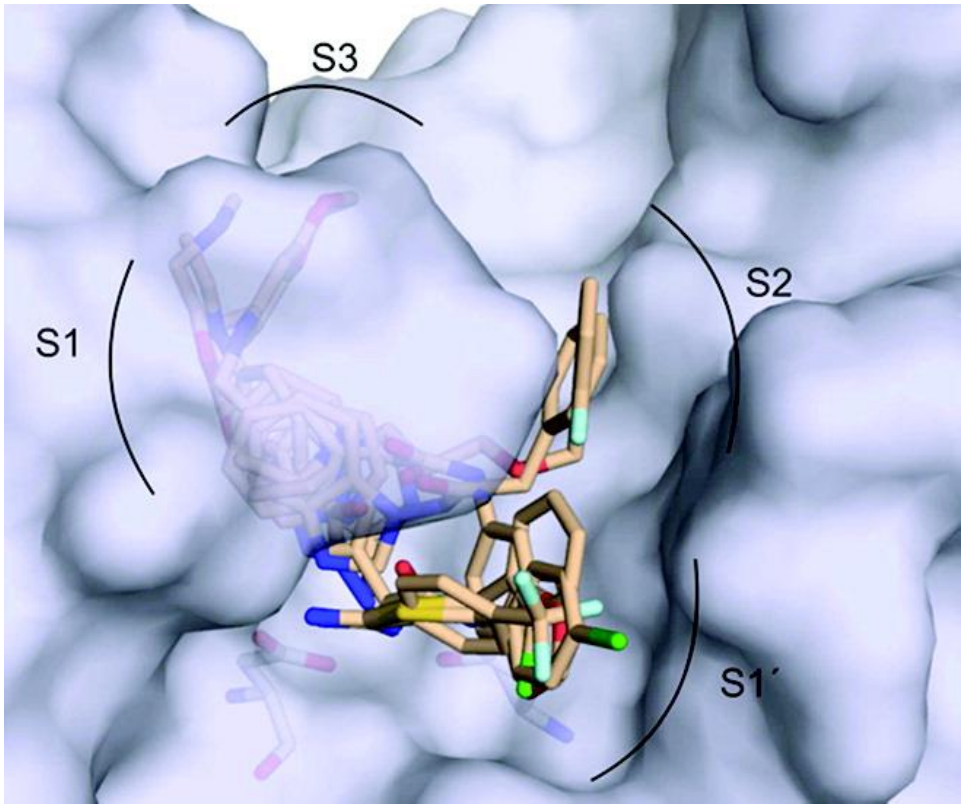
Gibbs et al., (2010) *J. Med. Chem.* **53**, 7979–7991

Premature to rank when binding is weak

Figure 2. Crystal structures of fragments bound to the integrase core domain (IN) fragment binding pocket.



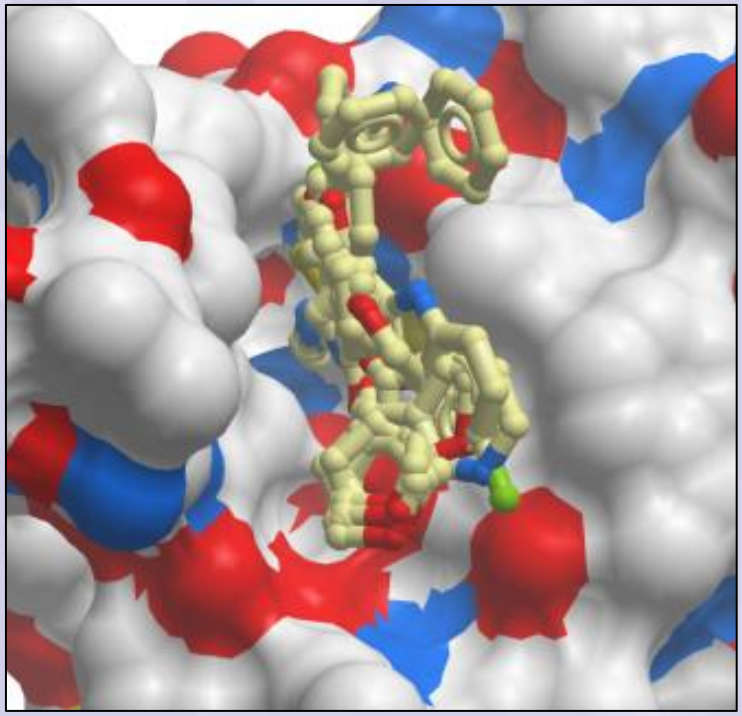
Wielens J et al. J Biomol Screen 2012;18:147-159

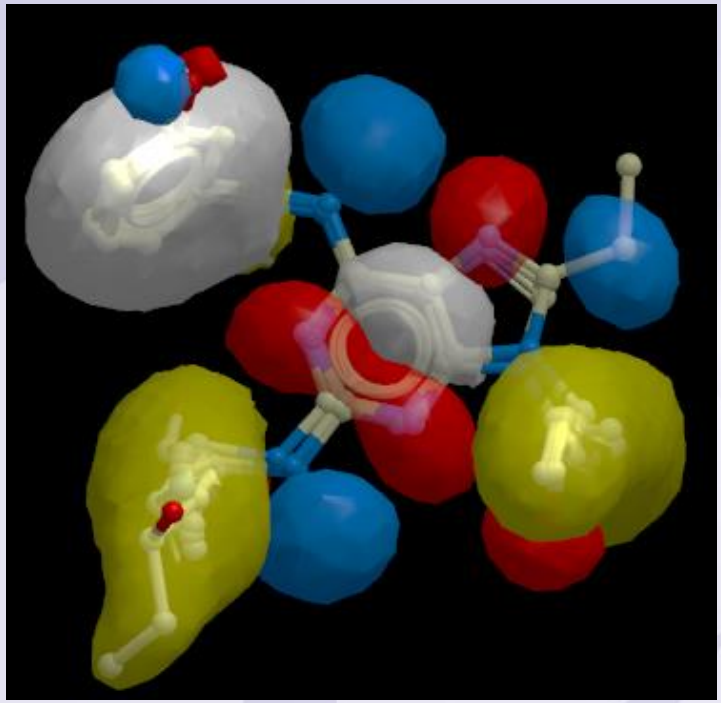


Overlay of all 11 fragment structures. The binding pocket is in surface representation, and specificity pockets are indicated. Carbon atoms are colored in salmon, nitrogen in blue, oxygen in red, chlorine in green, and fluorine in cyan.

Published in: Helene Köster; Tobias Craan; Sascha Brass; Christian Herhaus; Matthias Zentgraf; Lars Neumann; Andreas Heine; Gerhard Klebe; *J. Med. Chem.* **2011,** 54, 7784-7796.
DOI: 10.1021/jm200642w

“Chemical microscope”





- Co-crystals: crystallize the conformation that binds best
- Soaking: characterize the crystallized conformation

diamond

SGC

UNIVERSITY OF OXFORD

Klebe library:
don't look for hits; characterize instead

Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

A Small Nonrule of 3 Compatible Fragment Library Provides High Hit Rate of Endothiapepsin Crystal Structures with Various Fragment Chemotypes†

Helene Köster,[‡,#] Tobias Craan,[‡,#] Sascha Brass,[‡] Christian Herhaus,[§] Matthias Zentgraf,[∥] Lars Neumann,[⊥] Andreas Heine,[‡] and Gerhard Klebe*[,‡]

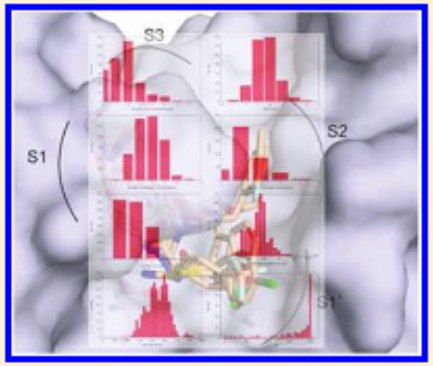
[‡]Department of Pharmaceutical Chemistry, Philipps University Marburg, Marbacher Weg 6, 35032 Marburg, Germany

[§]Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt, Germany

[∥]Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorfer Straße 65, 88397 Biberach/Riß, Germany

[⊥]Proteros Biostructures, Bunsenstr. 7a, D-82152 Martinsried, Germany

ABSTRACT: Druglike molecules are defined by Lipinski's rule of 5, to characterize fragment thresholds, they have been reduced from 5 to 3 (Astex's rule of 3). They are applied to assemble fragment libraries, and providers use them to select fragments for commercial offer. We question whether these rules are too stringent to compose fragment libraries with candidates exhibiting sufficient room for chemical subsequent growing and merging modifications as appropriate functional groups for chemical transformations are required. Usually these groups exhibit properties as hydrogen bond donors/acceptors and provide entry points for optimization chemistry. We therefore designed a fragment library (364 entries) without strictly applying the rule of 3. For initial screening for endothiapepsin binding, we performed a biochemical cleavage assay of a fluorogenic substrate at 1 mM. "Hits" were defined to inhibit the enzyme by at least 40%. Fifty-five hits were suggested and subsequently soaked into endothiapepsin crystals. Eleven crystal structures could be determined covering fragments with diverse binding modes: (i) direct binding to the catalytic dyad aspartates, (ii) water-mediated binding to the aspartates, (iii) no direct interaction with the dyad. They occupy different specificity pockets. Only 4 of the 11 fragments are consistent with the rule of 3. Restriction to this rule would have limited the fragment hits to a strongly reduced variety of chemotypes.



diamond

SGC

UNIVERSITY OF OXFORD

Soaking approach

- Probably Good Thing:
  - Force minor hits by soaking at high concentration (>100mM)
  - Increase hit rate by using small fragments

- Consequences:
  - Identify solvent best tolerated by crystal
  - need fragments in multiple solvents

- Currently at I04-1
  - Maybridge 1000 – “can’t go wrong”
  - Edelris 280 – natural product-like
    - Small (<250), highly soluble
    - Follow-up compounds off-the-shelf
  - (Not yet solubilized… ☹)

WE DO NOT YET KNOW THIS IS GOOD

diamond

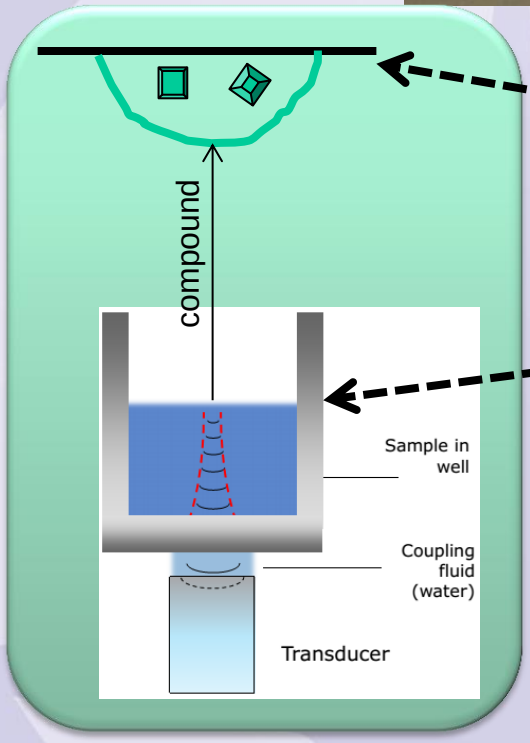
SGC UNIVERSITY OF OXFORD

ECHO for soaking

Labcyte Technology

Move Liquids with Sound

- No physical contact
  - Perfect sample integrity
  - Low energy transfer
  - Consistent drop size
  - 2.5 nL or 25 nL transfers
  - 200-500 droplets/second
- Transfer into inverted plate
  - Surface tension, electrostatics hold liquid in place
- Touchless - eliminate nozzles and tips from transfer process
  - Improve reliability and precision
  - Eliminate washing and pipette tips
  - Eliminate potential for cross contamination



Crystal plate is inverted

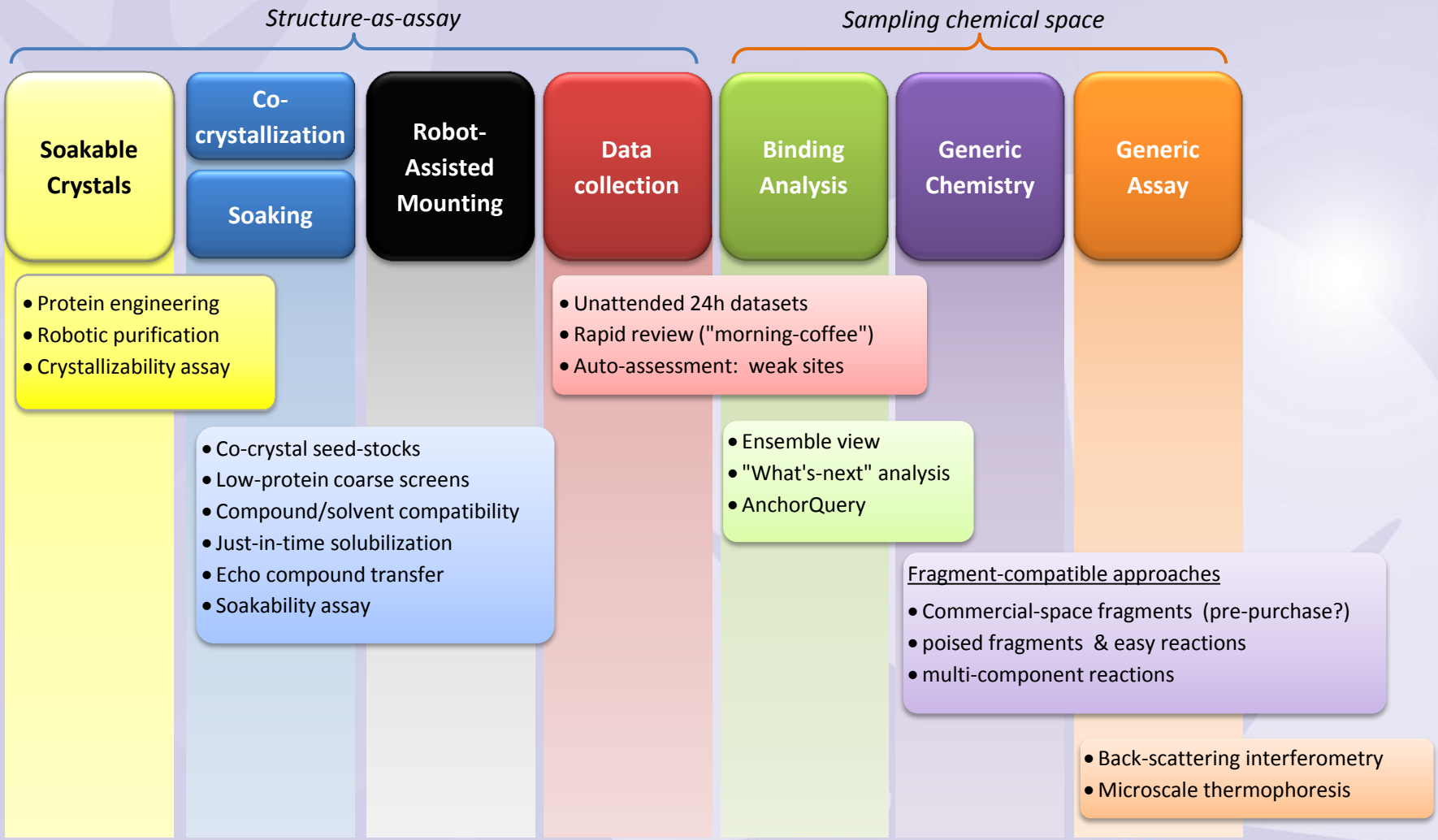
Entire library on 1536 plate

diamond

SGC

UNIVERSITY OF OXFORD

Challenges



diamond

SGC

UNIVERSITY OF OXFORD

Upstream challenge: "simply soaking" – HA!

- Compound: must be soluble enough, easy to transfer
- Crystal: must be amenable to soaking
- Investigating in both groups,
  - How to generate alternative crystal forms (SGC)
  - How test soakability (on-the-fly cocktails?) (SGC, Diamond)
  - Timing and geometry of soaking
- **RECRUITING! 3 postdoc positions:**
  - **@SGC: Running fragment screens on high-value targets**
  - **@SGC: Rapid protein engineering for alt. crystal forms**
  - **Diamond: Soaking best practice**

diamond

SGC UNIVERSITY OF OXFORD

Acknowledgements

Diamond I04-1

Jose Brandao-Neto
Alice Douangamath
Keith Henderson

SGC-PX

Tobias Krojer
Melanie Vollmar
John Raynor
Nathan Wright
Lauren Crawley
Jia Tsing Ng
Anthony Bradley
Nick Pearce

Diamond:

I04-1 construction:
Andy Marshall, Geoff Preece, Tim Whitewood, James O'Hea, Ralf Flaig, Martin Walsh
I03: Katherine McAuley, Mark Williams
SciSoft: Graeme Winter, Alun Ashtun, ...
GDA: Richard Fearn, Jun Aishima, Paul Hathaway, ...
MX village, Controls, ...

UK Government & Wellcome Trust!

SGC (lots!!!):

Brian Marsden, Paul Brennan, Nicola Burgess-Brown, Claire Strain-Damerell, Chas Bountra

Oxford University:

Charlotte Deane, Martin Smith, Martin Booth

SGC FUNDING PARTNERS: AbbVie, Boehringer Ingelheim, the Canada Foundation for Innovation, the Canadian Institutes for Health Research, Genome Canada, GlaxoSmithKline, Janssen, Lilly Canada, the Novartis Research Foundation, the Ontario Ministry of Economic Development and Innovation, Pfizer, Takeda, and the Wellcome Trust.