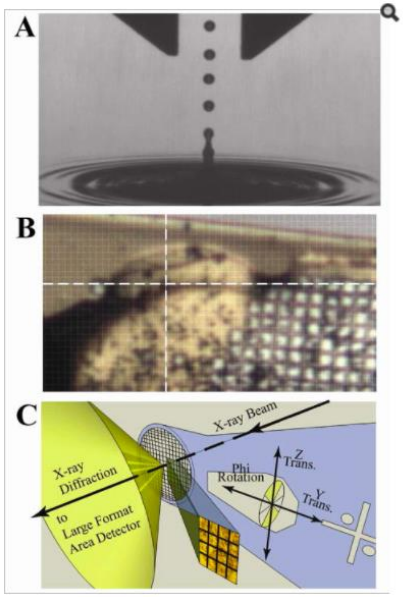
Drug Discovery at the Speed of Sound Workshop

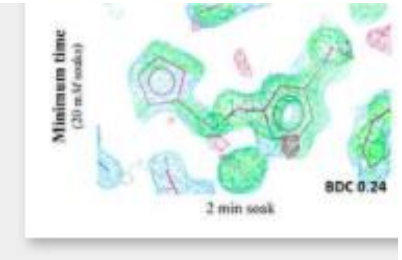
30th January 2018

Matthew Groves
(m.r.groves@rug.nl)

Acoustic dispensing in high-throughput crystallography

1. Current approaches (2011-
2. Future trends
3. X-tream





First* use of Acoustic dispensing in crystallography

Slurries of microcrystals mounted onto grid raster

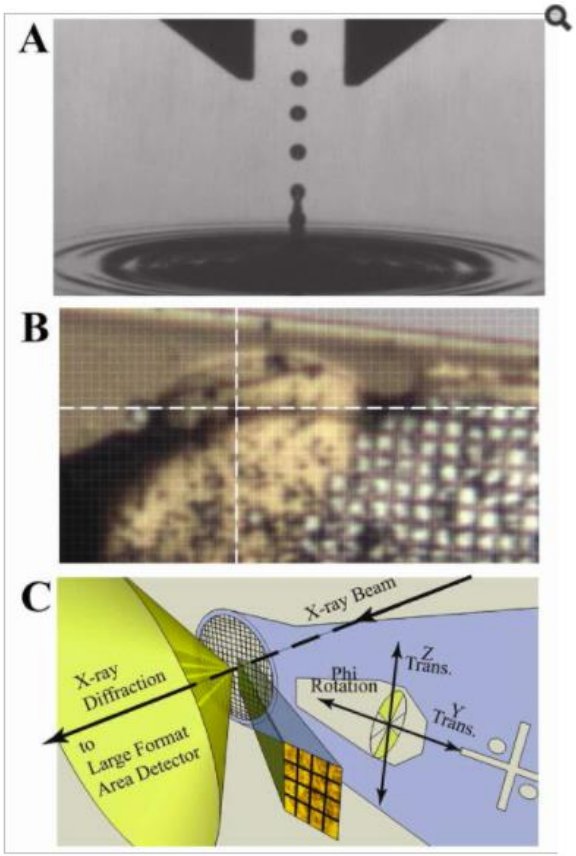
Leveraged developments around FEL approaches in both data processing (serial crystallography) and sample handling (liquid jets on FELs).

Misconceptions:

“Microcrystals are easier to grow” (Chapman, 2010)

Beam flux means moving to serial crystallography is inevitable

Figure 1



Soares *et al* Biochemistry 2011

- NSLS II User meeting 2010
- Wood EW, Loomis AL. Philosophical Magazine Series. 1927;7(4):417–436.

"I used to say the evening that I developed the first x-ray photograph I took of insulin in 1935 was the most exciting moment of my life. But the Saturday afternoon in late July 1969, when we realized that the insulin electron density map was interpretable, runs that moment very close."

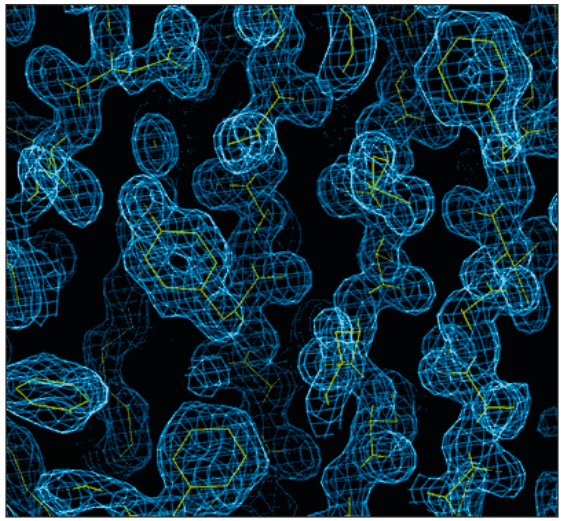
— Dorothy Hodgkin

Insulin and lysozyme

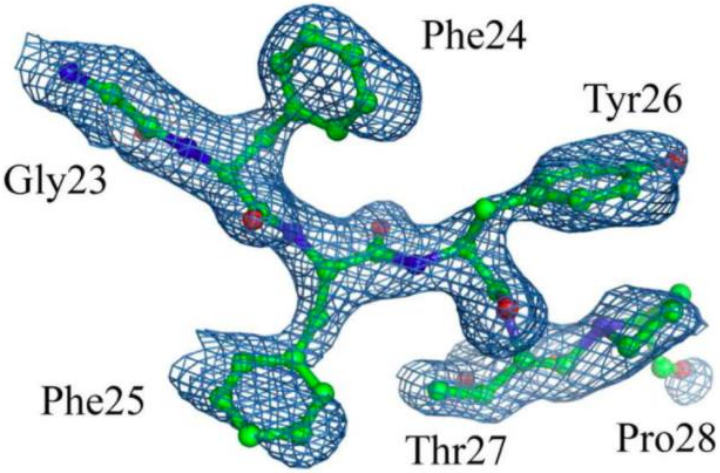
Standard test cases

Approach suffers from serial approaches to data collection

Other non-AD dispensing techniques

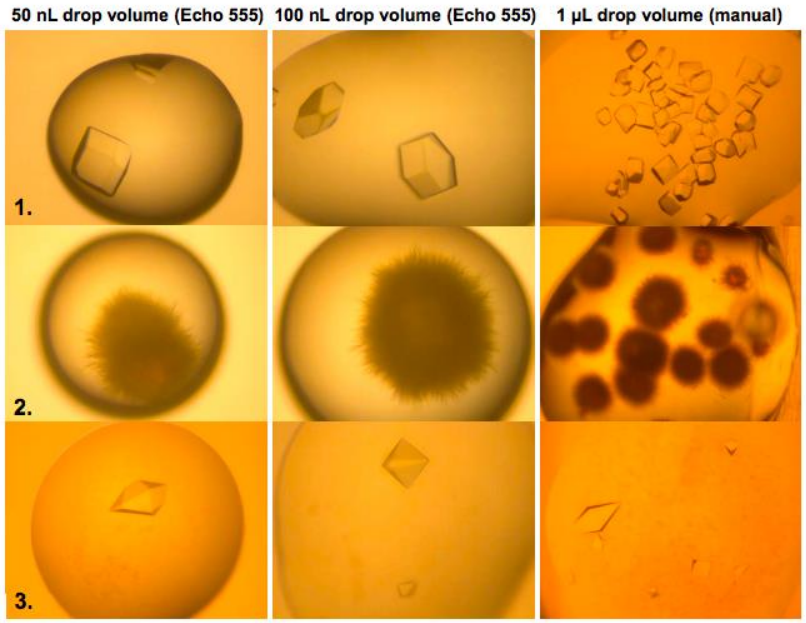


Standard approaches



Soares *et al* Biochemistry 2011

AD in crystal growth as an addition to miniaturization



Summary

- The ability of the Echo liquid handler to precisely dispense volumes as low as 2.5 nL allows for miniaturization of protein crystallography trials – saving on reagents and protein.
- Touchless fluid transfer eliminates carryover and contamination.
- Dynamic Fluid Analysis™ allows for transfer of protein and most sparse matrix screens with a single fluid class setting, eliminating the need for multiple settings or changing calibrations.
- Eliminates liquid handling consumables, ie: pipette tips, and the associated costs and waste.
- High-concentration stock components can be transferred on the fly to quickly generate grid screening conditions.

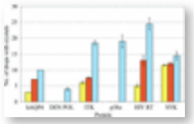
Source: Labcyte poster
https://www.labcyte.com/media/pdf/POS-Protein-Crystallography-SLAS-2012.pdf
&
Villaseñor AG, Wong A, Shao A, Garg A, Kuglstatter A, Harris SF. Acta Crystallogr D Biol Crystallogr. 2010;66:568–576.

Low volume pipetting reaches similar sample efficiency

“Ink jet” approaches

No true (quantum) leap in throughput or range of experiments possible

AD in crystal growth as an addition to current technologies

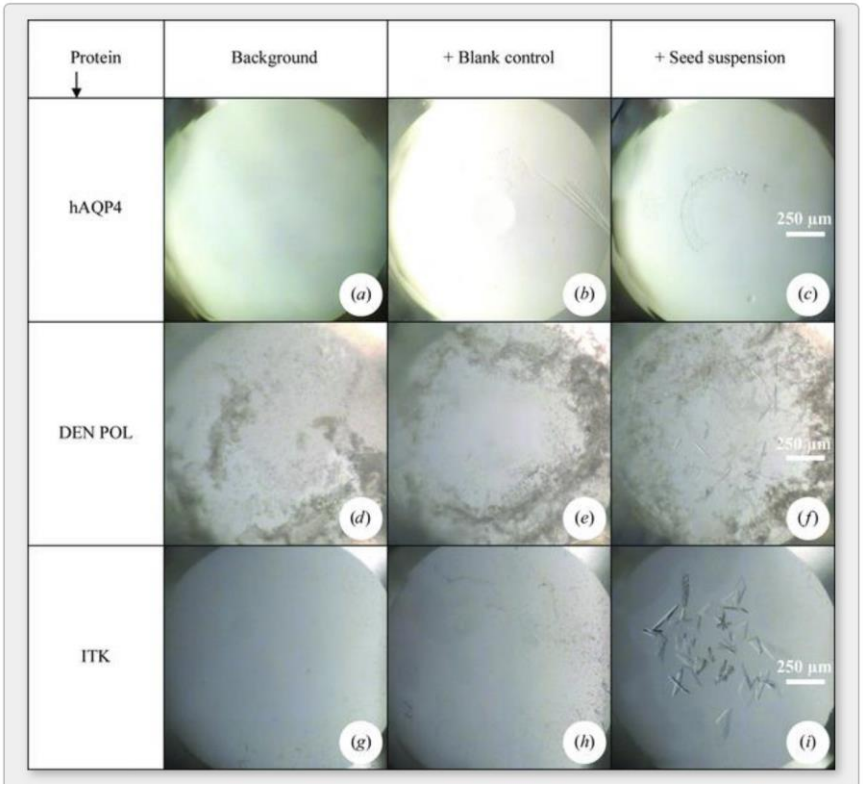


Acoustic matrix microseeding: improving protein crystal growth with minimal chemical bias

A. G. Villaseñor, A. Wong, A. Shao, A. Garg, A. Kuglstatter and S. F. Harris

A crystal seeding technique is introduced that uses acoustic waves to deliver nanolitre volumes of seed suspension into protein bias from chemical components in the seed-carrying buffer suspension. Using this technique, it was found that while buffer cor boosting the number of sparse-matrix conditions that yield protein crystals.

Keywords: crystal seeds; **acoustic liquid dispensing**; **protein crystallization**.



AD in crystal growth as an addition to current technologies

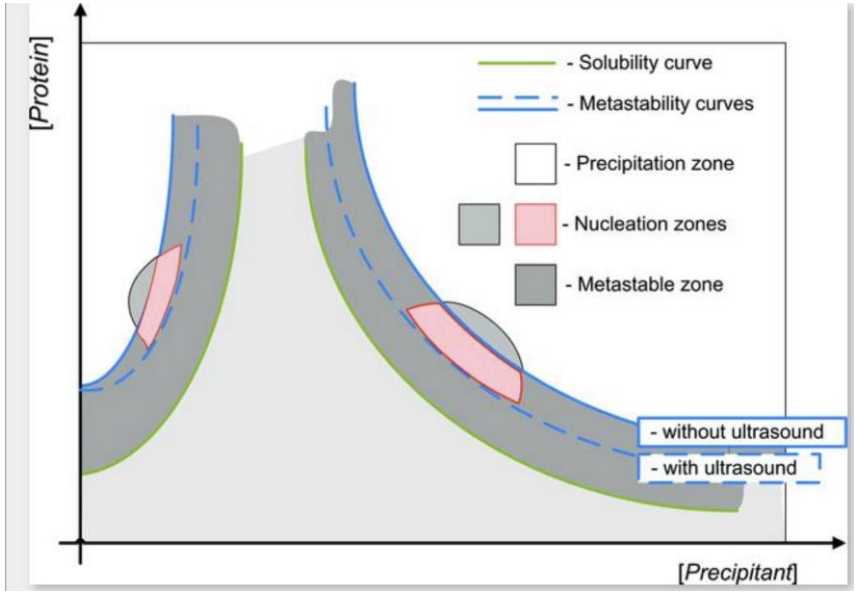
Potential use of ultrasound to promote protein crystallization

Rosa Crespo,[a] Pedro M. Martins,[a,b]* Luís Gales,[a,b] Fernando Rocha[c] and Ana M. Damas[a,b]

[a]Grupo de Estrutura Molecular, IBMC – Instituto de Biologia Molecular e Celular, Porto, Portugal, [b]ICBAS – Ir
*Correspondence e-mail: pmartins@ibmc.up.pt

(Received 12 May 2010; accepted 12 October 2010; online 9 November 2010)

This work shows promising applications of ultrasound in promoting protein crystallization, which is important egg-white lysozyme were carried out with and without ultrasonic irradiation using commercial crystallization technique. The same effect was confirmed by the increased number of conditions leading to the formation of have better diffraction properties. In fact, X-ray diffraction data sets collected using 13 lysozyme crystals (se the preliminary diffraction results also suggest a promising application in crystal quality enhancement.



AD in fragment-based screening approaches

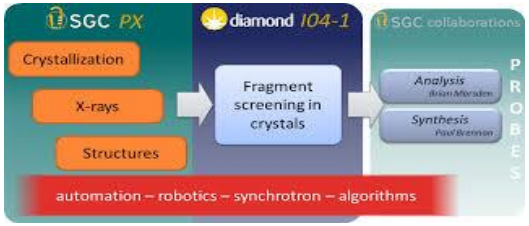
AD/X-ray synergy realised?

Pioneered by T L Blundell FRS FMedChem in Astex

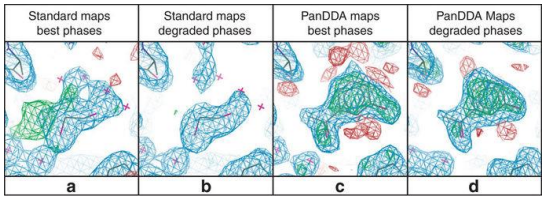
Multiple low affinity starting points can be found

Cocktail soaks

Reproducibility



astex pharmaceuticals



Article | OPEN

A multi-crystal method for extracting obscured crystallographic states from conventionally uninterpretable electron density

Nicholas M. Pearce, Tobias Krojer, Anthony R. Bradley, Patrick Collins, Radosław P. Nowak, Romain Talon, Brian D. Marsden, Sebastian Kelm, Jiye Shi, Charlotte M. Deane & Frank von Delft

Nature Communications **8**, Article number: 15123 (2017)
doi:10.1038/ncomms15123

Received: 01 November 2016
Accepted: 03 March 2017
Published online: 24 April 2017

AD in fragment-based screening approaches

ECHO as a solution to fragment soaks

Precise volume w/o pipetting

(Partial) high-throughput

Implemented within Xchem at Diamond Lightsource Ltd, UK

RESEARCH PAPERS

Acta Cryst. (2017). D**73**, 246-255
https://doi.org/10.1107/S205979831700331X
Cited by 6

OPEN ACCESS

Gentle, fast and effective crystal soaking by acoustic dispensing

P. M. Collins, J. T. Ng, R. Talon, K. Nekrosiute, T. Krojer, A. Douangamath, J. Brandao-Neto, N. Wright, N. M. Pearce and F. von Delft

The steady expansion in the capacity of modern beamlines for high-throughput data collection, enabled by increasing X-ray brightness, capacity of robotics and detector speeds, has pushed the bottleneck upstream towards sample preparation. Even in ligand-binding studies using crystal soaking, the experiment best able to exploit beamline capacity, a primary limitation is the need for gentle and nontrivial soaking regimens such as stepwise concentration increases, even for robust and well characterized crystals. Here, the use of acoustic droplet ejection for the soaking of protein crystals with small molecules is described, and it is shown that it is both gentle on crystals and allows very high throughput, with 1000 unique soaks easily performed in under 10 min. In addition to having very low compound consumption (tens of nanolitres per sample), the positional precision of acoustic droplet ejection enables the targeted placement of the compound/solvent away from crystals and towards drop edges, allowing gradual diffusion of solvent across the drop. This ensures both an improvement in the reproducibility of X-ray diffraction and increased solvent tolerance of the crystals, thus enabling higher effective compound-soaking concentrations. The technique is detailed here with examples from the protein target JMJD2D, a histone lysine demethylase with roles in cancer and the focus of active structure-based drug-design efforts.

Keywords: **fragment screening**; **crystal soaking**; **acoustic droplet ejection**; **Diamond Light Source I04-1**; **Structural Genomics Consortium**; **XChem**.

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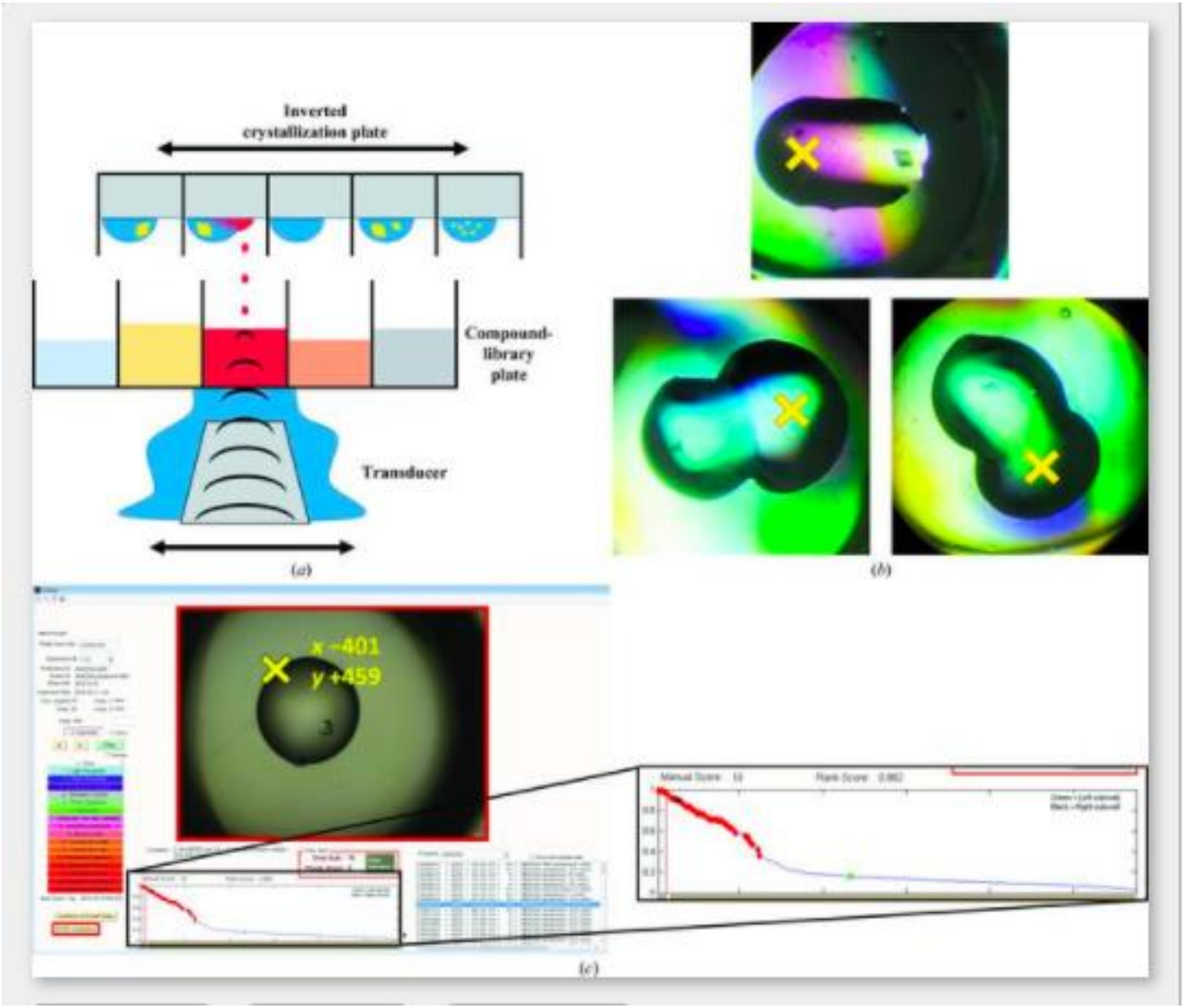
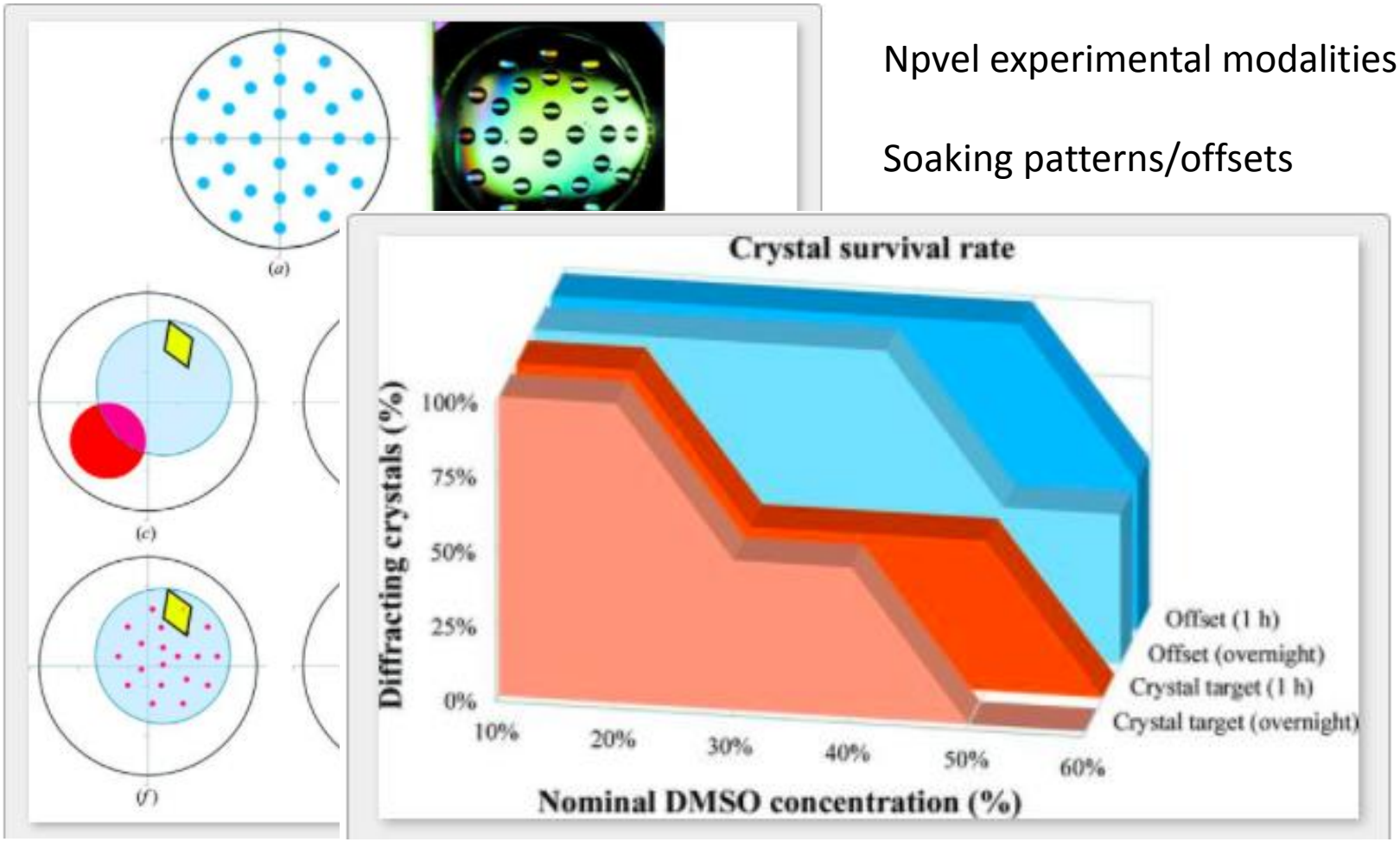
AD in fragment-based screening approaches



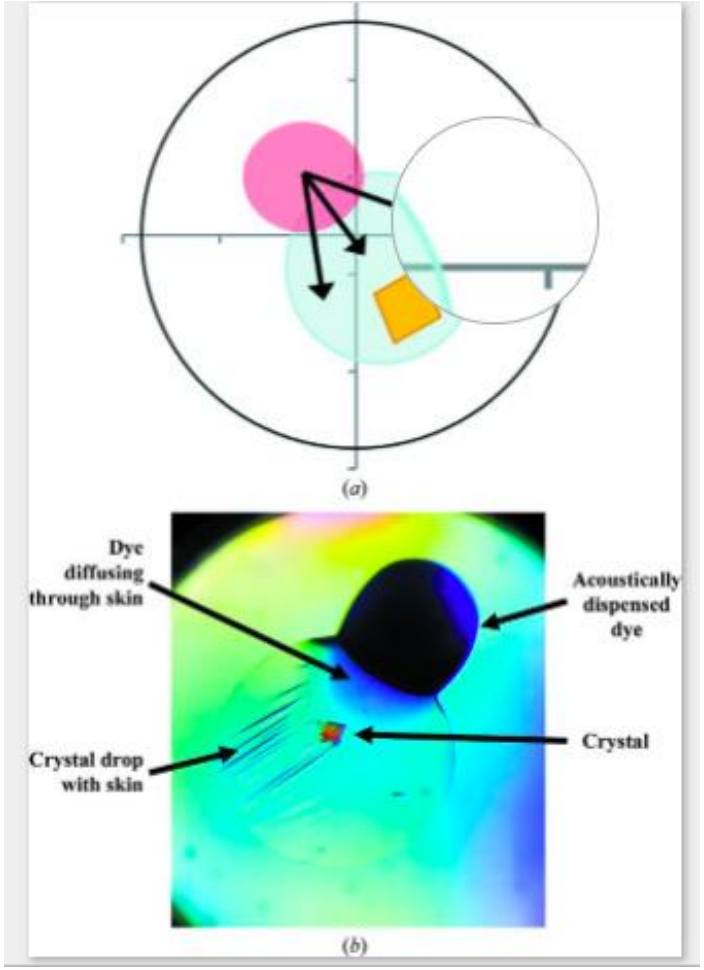
AD in fragment-based screening approaches



Npvel experimental modalities

Soaking patterns/offsets

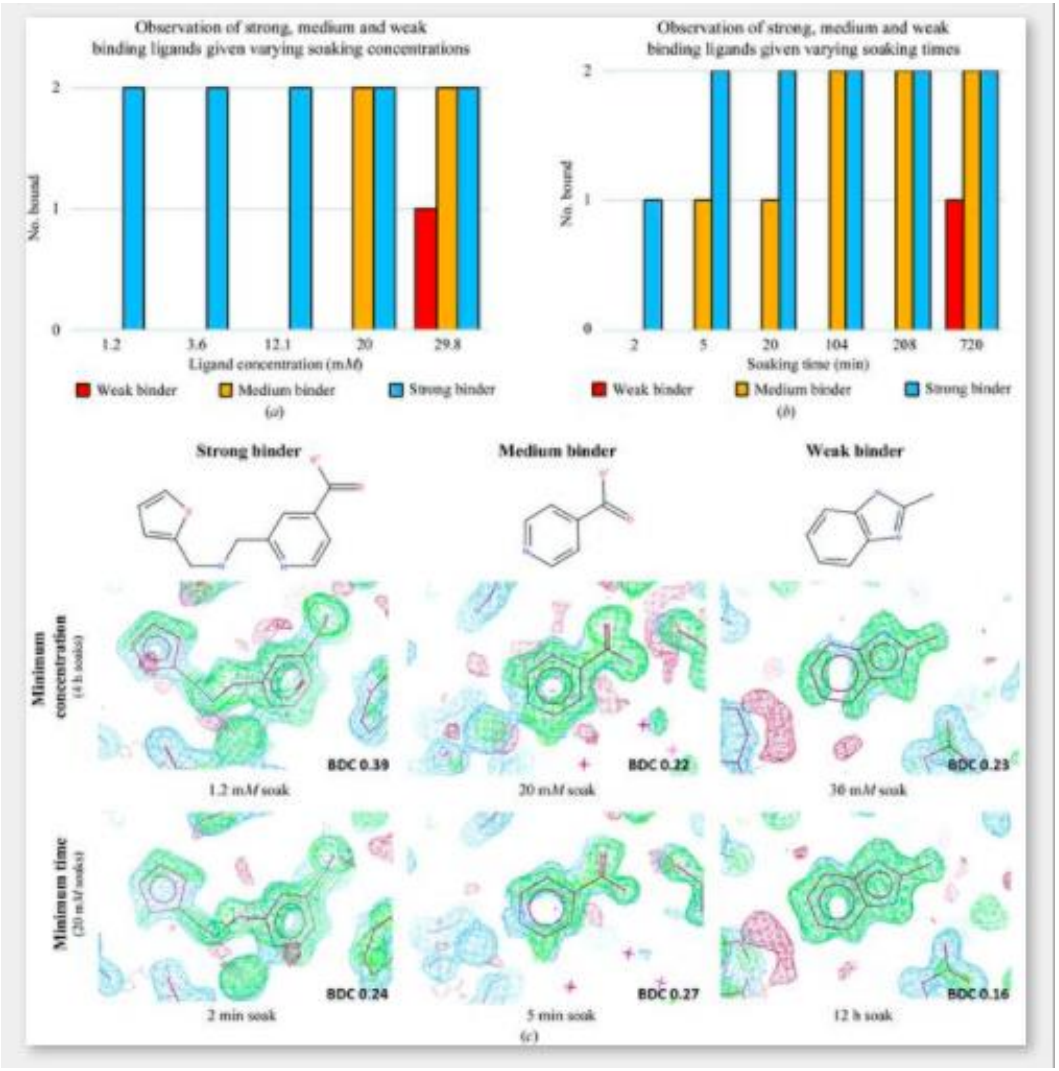
AD in fragment-based screening approaches



Novel experimental modalities

Soaking patterns/offsets

Concentration (gradient) control



Npvel experimental modalities

Soaking patterns/offsets

Concentration (gradient) control

Improve detection of weak/mediun binders?

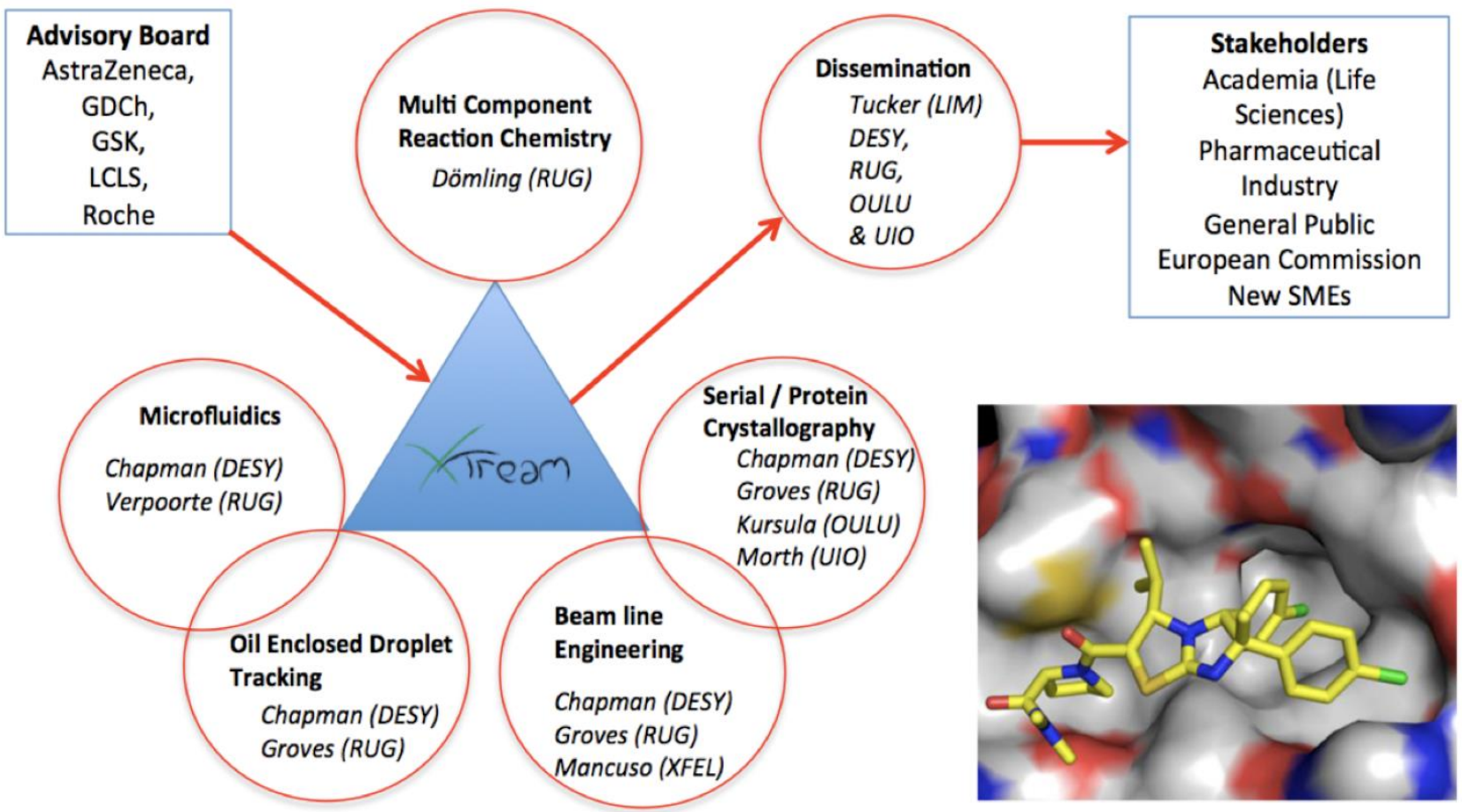


Figure 1: The role of the X-tream Consortium in Supporting Target-Led NSM Generation

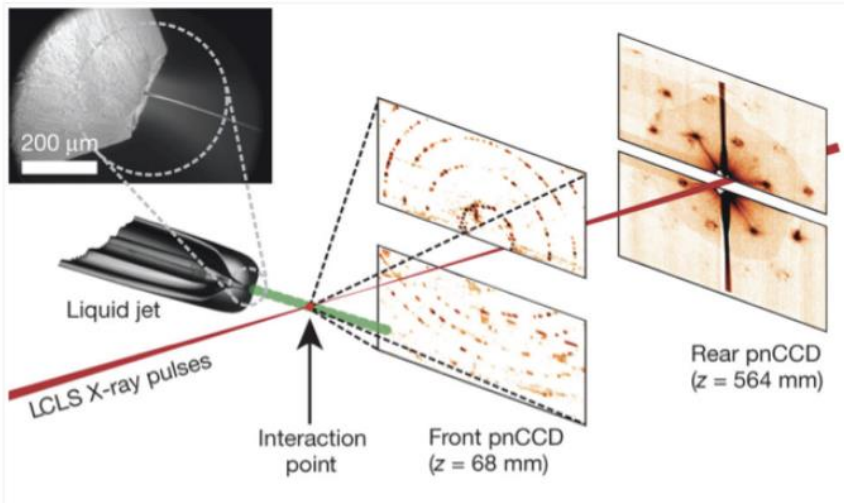


Figure 2a: Schematic of a serial crystallography experiment (source: Chapman *et al.,* 2011)

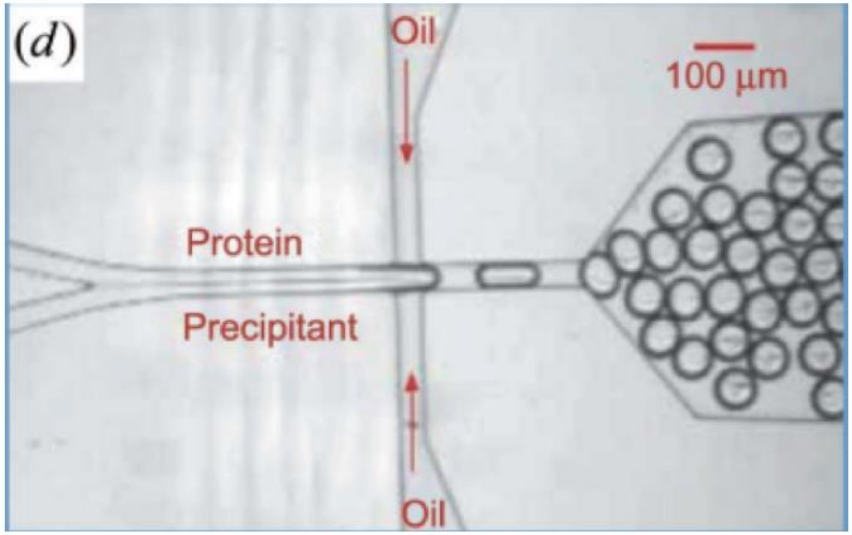


Figure 2: Creation of oil-enclosed droplets, each containing a single protein crystal (source: Heymann *et* al., 2014).

X-tream aims to leverage MCR chemistry at point of data collection

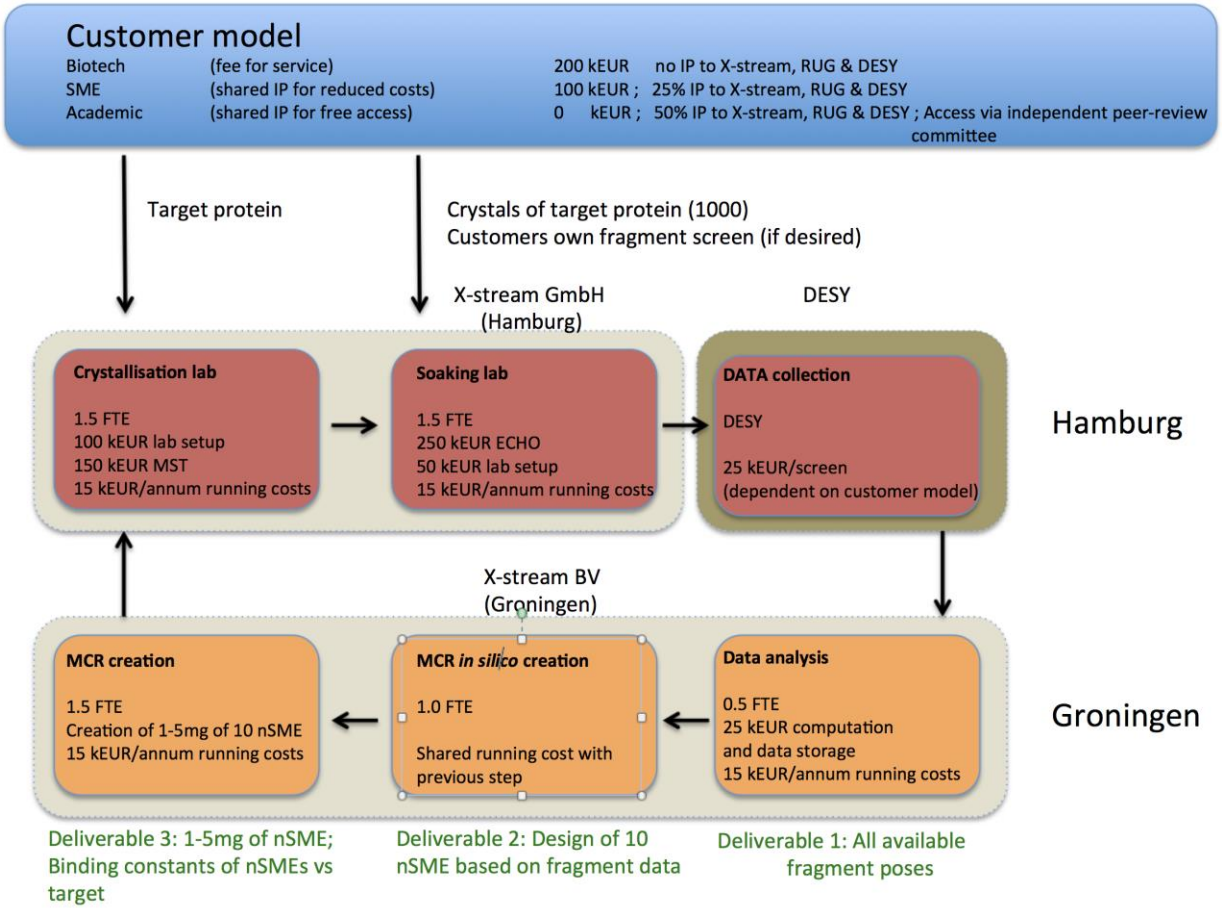
Structural & biophysical (MST/DSF) evolution of compounds

Initial concept using microfluidics and OEDS,

Milestones

Demonstrate AD can provide same support of compounds transfer

Demonstrate reactions be “soaked” w/o work-up & loss of diffraction?



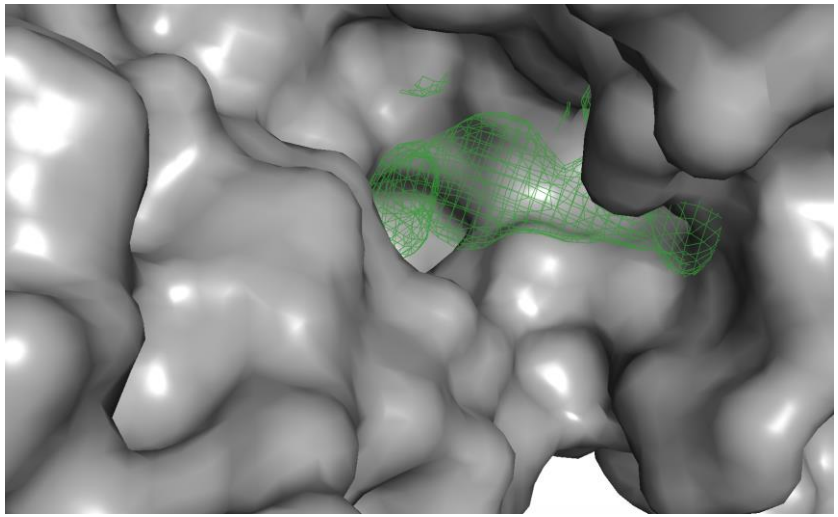
575 kEUR		start-up costs (equipment)
6 FTE @ 75 kEUR	= 450	personnel costs/annum
60 kEUR/annum		running costs/annum
75 kEUR		operation cost *per* project under fee for service (ie. no shared IP)

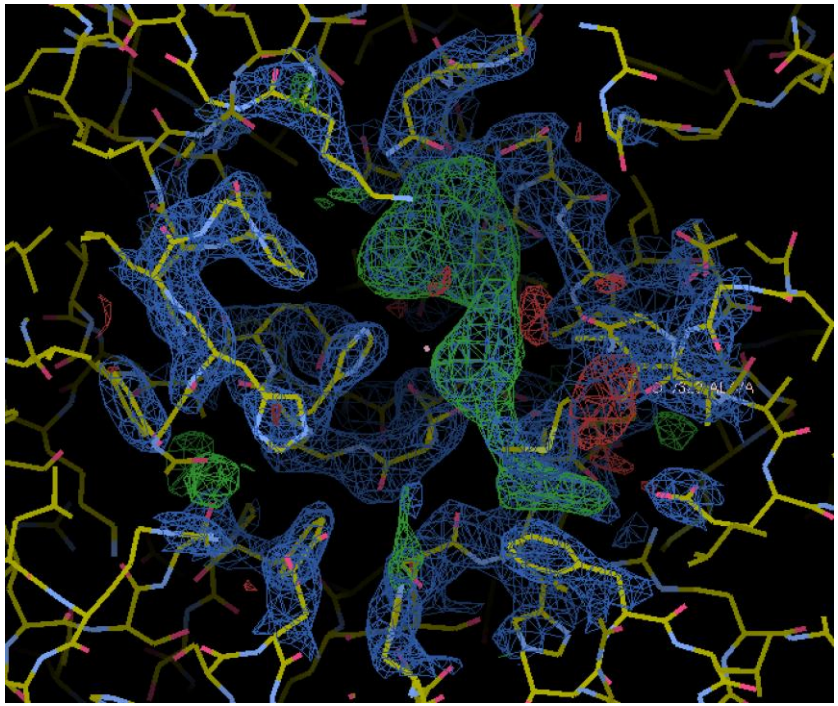
Start-up & 1st year operation: **1.085 MEur**
Thereafter annual running costs: **510 kEur/annum**

Proof-of-principle expts. Dec 2017

150 cocktail soaks on 3 biomedically relevant systems

| | | |





DXS – ESKAPE and pesticide target (MtDXS)

H1N1 – viral target

PfATC – malarial target

Implementation of AD within DDSoS:

1. As part of an initial fragment screening campaign to provide starting points for development

2. As an integrated checkpoint of control of binding modes

3. As a fully integrated step post-binding detection (would require synchrotron placement)

4. As part of a binding detection step (FELS)

Implementation of AD within DDSoS:

1. **As part of an initial fragment screening campaign to provide starting points for development**

No further method development required (Plug-n-play?)

Standard approaches as implemented at various Xray sources

Likely to find initial weak binders and binding modes

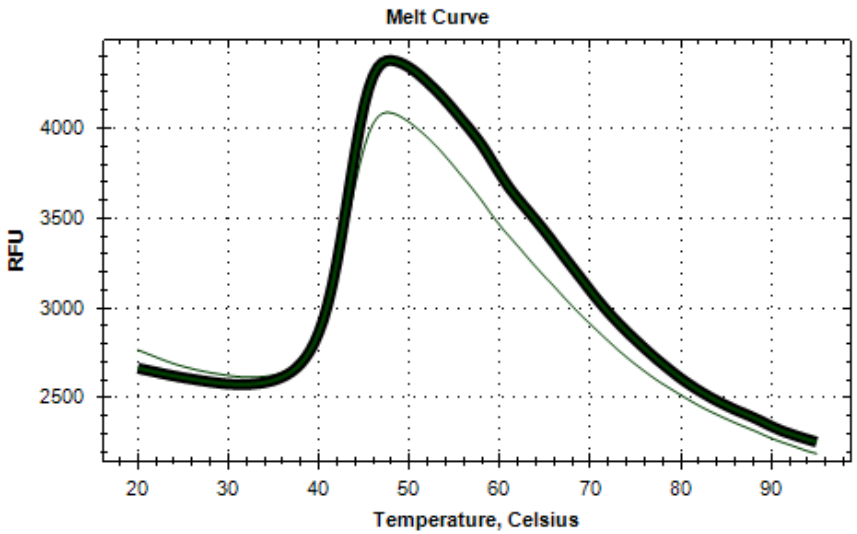
Implementation of AD within DDSoS:

1. As part of an initial fragment screening campaign to provide starting points for development

2. **As an integrated checkpoint of control of binding modes**

   Minor method development required (transport of reaction mixes to crystals)

   DSF experiments successful in MCR-style reaction conditions


Melt Curve
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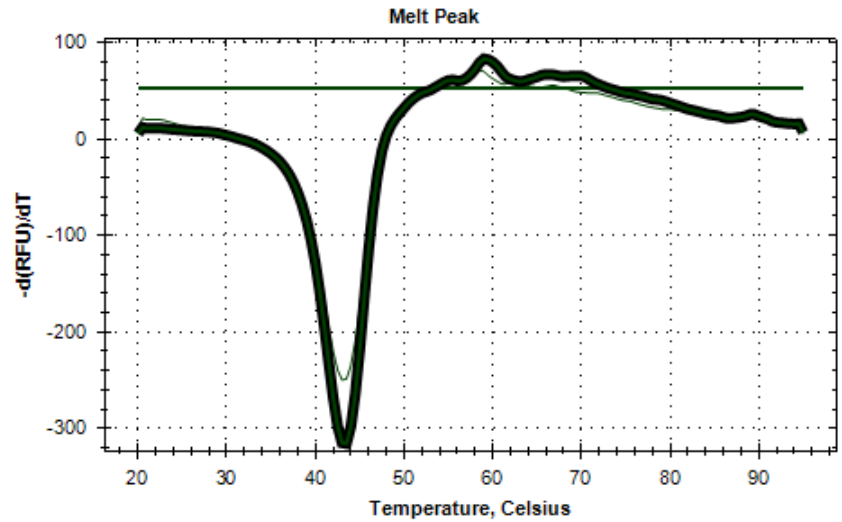
Temperature, Celsius

1:1


Melt Peak
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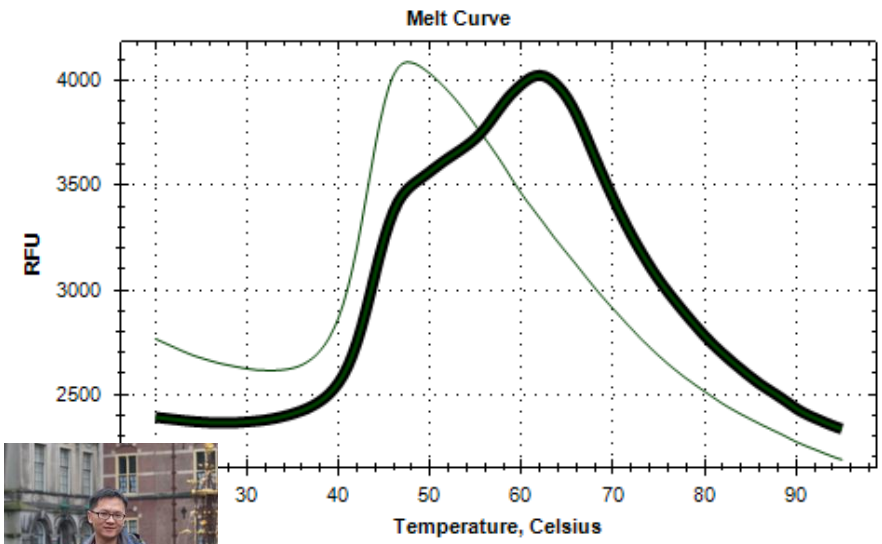
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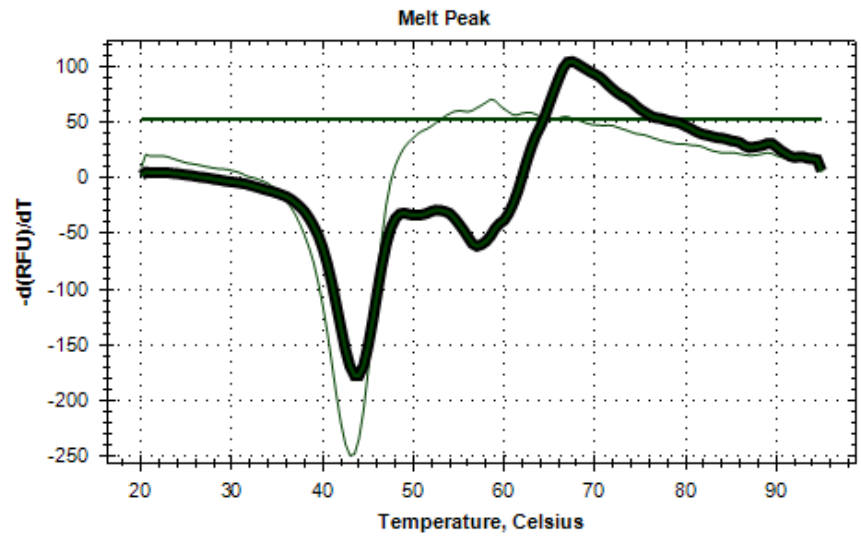
Melt Peak
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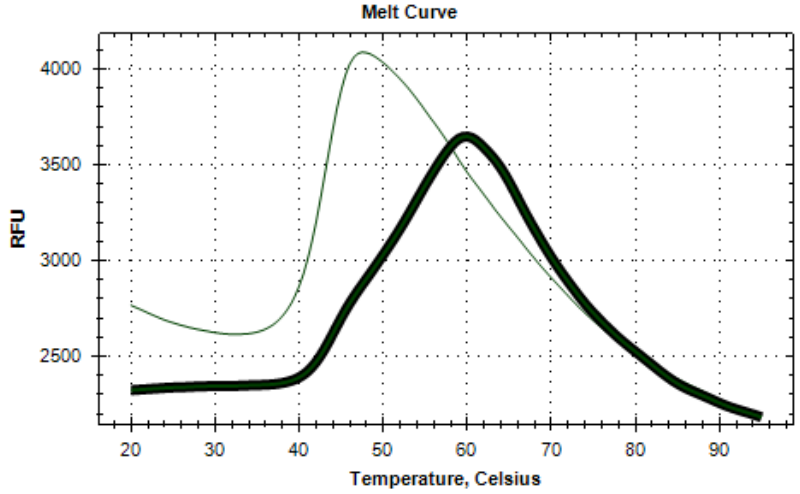

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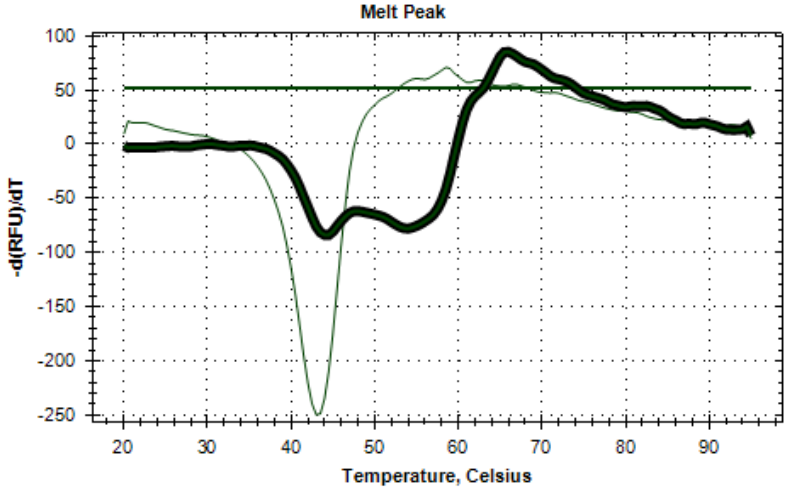

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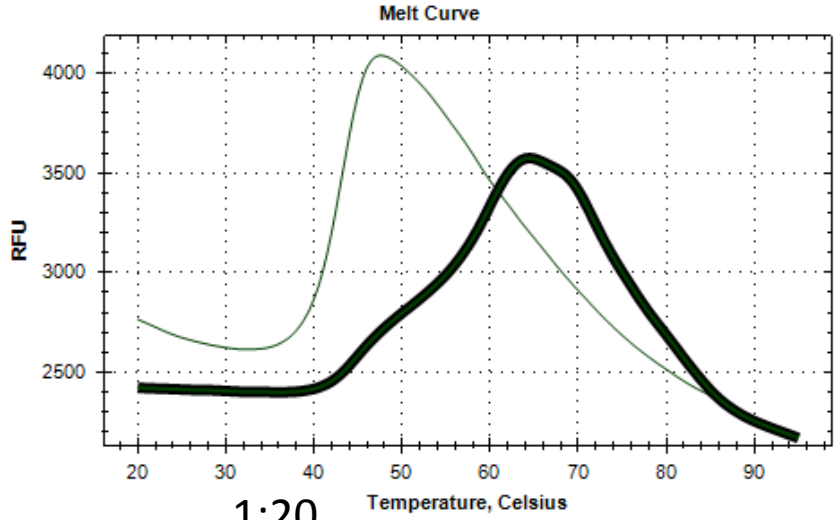

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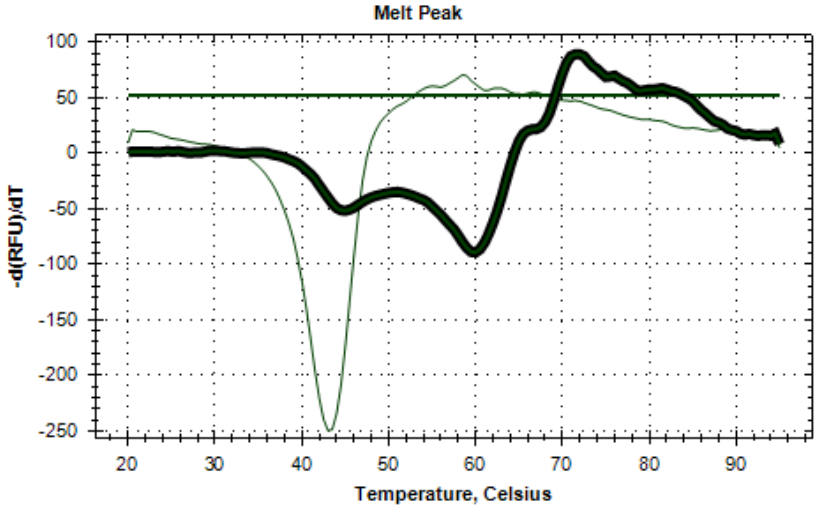

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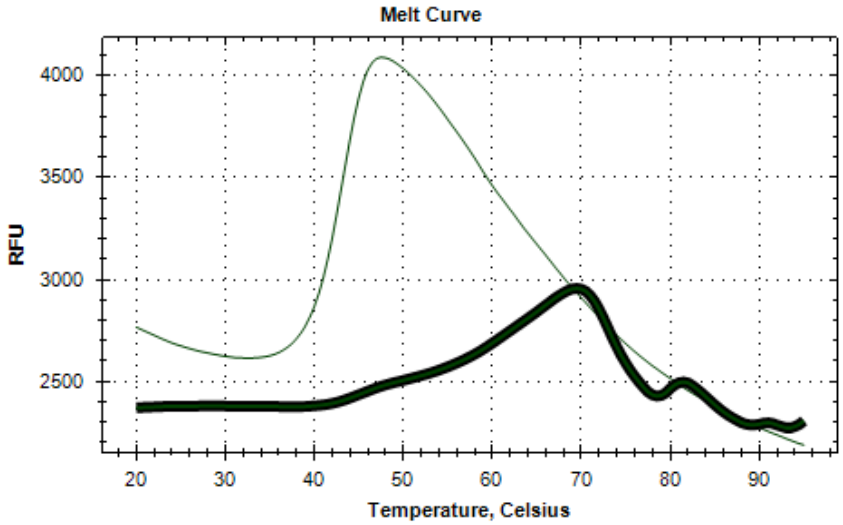
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Temperature, Celsius

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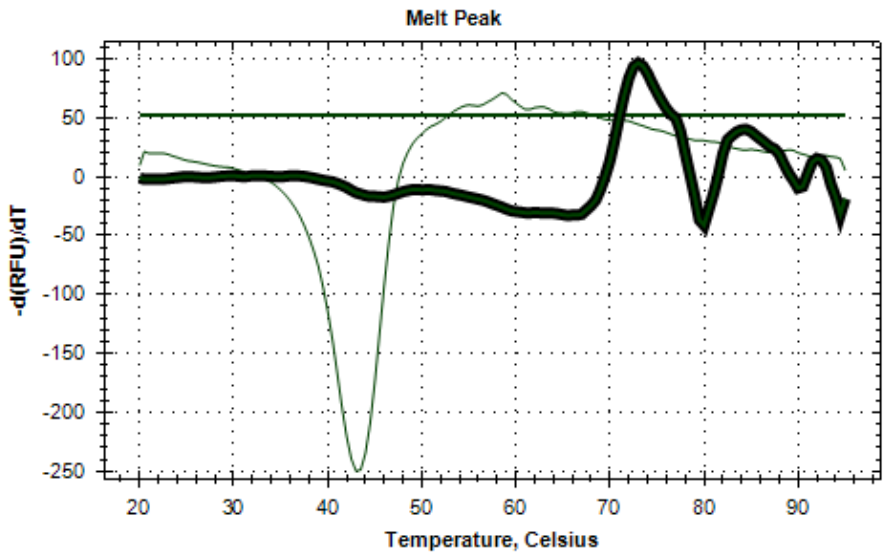
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Temperature, Celsius

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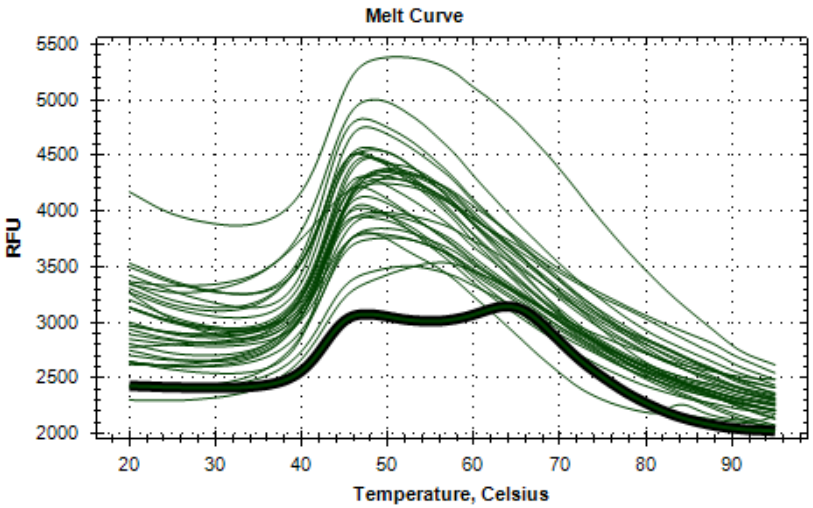

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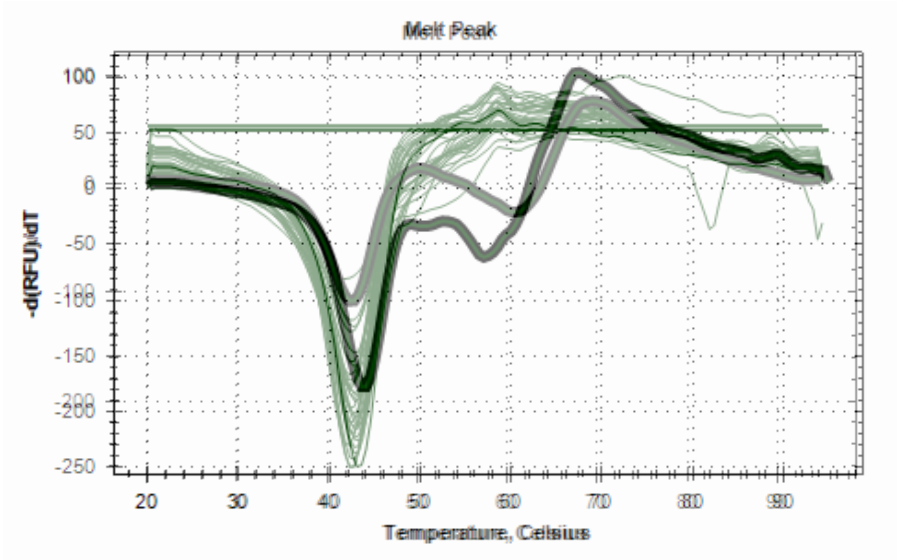

1:10

Overview of DSF result from plate EG as solvent, contain 31 compounds, 1 is positive

Ethylene Glycol does not disturb measurement

Possible to identify without work-up

Implementation of AD within DDSoS:

1. As part of an initial fragment screening campaign to provide starting points for development

2. As an integrated checkpoint of control of binding modes

3. **As a fully integrated step post-binding detection (would require synchrotron placement)**

   Method development required (transport of many reaction mixes to crystals)

   DSF experiments successful in MCR-style reaction conditions

   Only successful experiments analysed structurally

   Optimally placed near beamline

Implementation of AD within DDSoS:

1. As part of an initial fragment screening campaign to provide starting points for development

2. As an integrated checkpoint of control of binding modes

3. As a fully integrated step post-binding detection (would require synchrotron placement)

4. **As part of a binding detection step (FELS)**

   Major method development required (transport of all reaction mixes to many crystals)

   Syncrotron sources unlikely to cope with demands – FEL refresh rates coupled to AD

Outlook

AD is here to stay in fragment-based screening

Major current strength is in fragment detection, but forseaeble that similar approaches can be implemented as part of a Med Chem evolution pipeline (DDSoS/X-tream)

Reactions mixtures are tolerated by proteins (DSF) – very interesting!

Reaction mixtures tolerated by crystals (initial data)

True integration will require automation and immediate access to beamtime (parasitic/dedicated mode)