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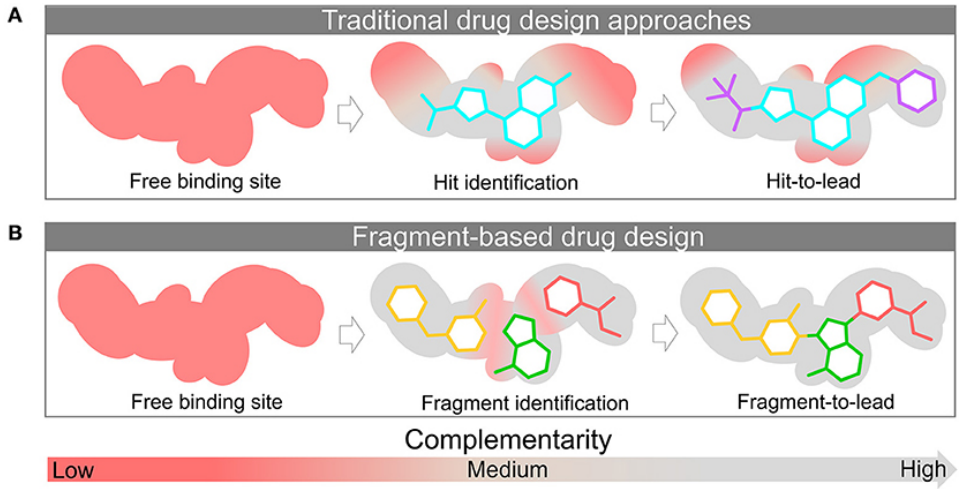
May Sharpe :: Group Leader MX Samples :: Paul Scherrer Institut

HTP Crystallographic Screening for Fragment Based Drug Discovery at the SLS

SLS MX User Meeting 2022, 1st March 2022, PSI

Fragment Based Drug Discovery (FBDD)

- FBDD is a productive alternative to traditional screening (HTS)
- Screen much smaller collections of much simpler compounds
- Allows efficient exploration of chemical space
- Identify fragments hits, then grow or link to increase potency
- Pharma partners already use FBDD
- Increasing use in academia
- Usually screen with biophysical techniques to identify hits

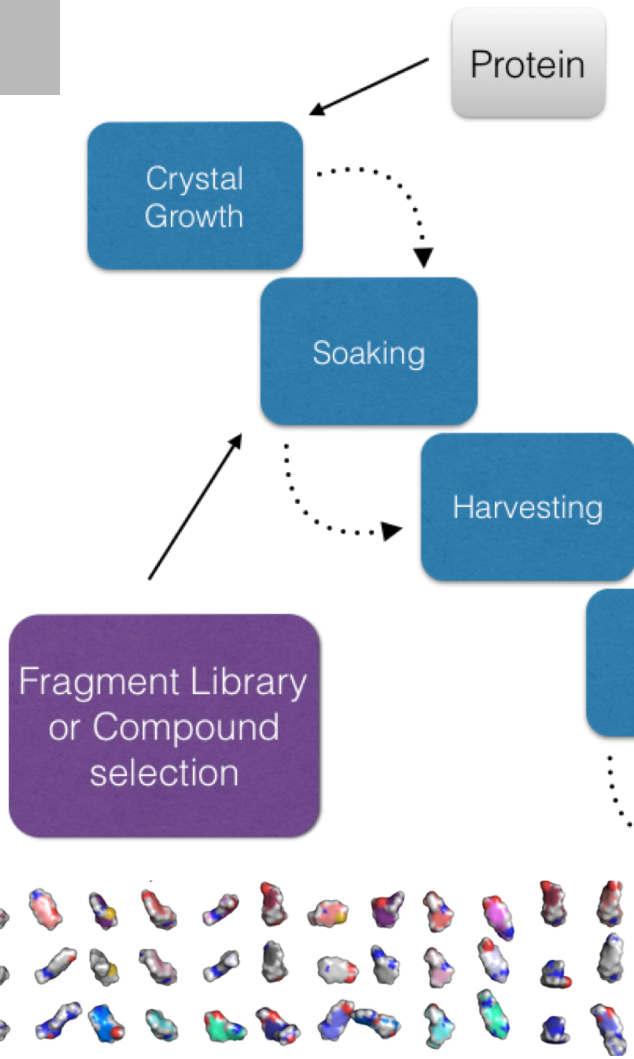


De Souza Neto *et al.*, (2020). Frontiers in Chemistry 8

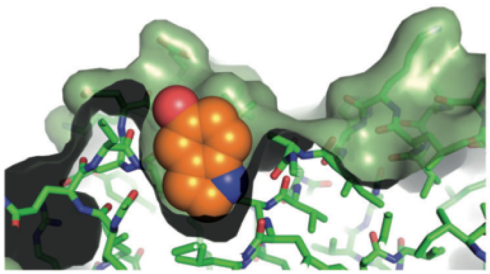
Crystallography for FBDD

- Crystallography works extremely well as a primary screen
- Crystallography essential for follow-up of hits from other techniques
- Conditions favour occupancy for weak binders
- High information content (hit, poise, feasibility of elaboration)
- Other synchrotrons have also set up facilities for this i.e Diamond (Xchem), ESRF (CrystalDirect), Max IV (FragMax) and HZB Fragment screening facility
- Technology enabled by increasing automation and speed of data collection
- **Crystallographic FBDD Pipeline implemented at the SLS**

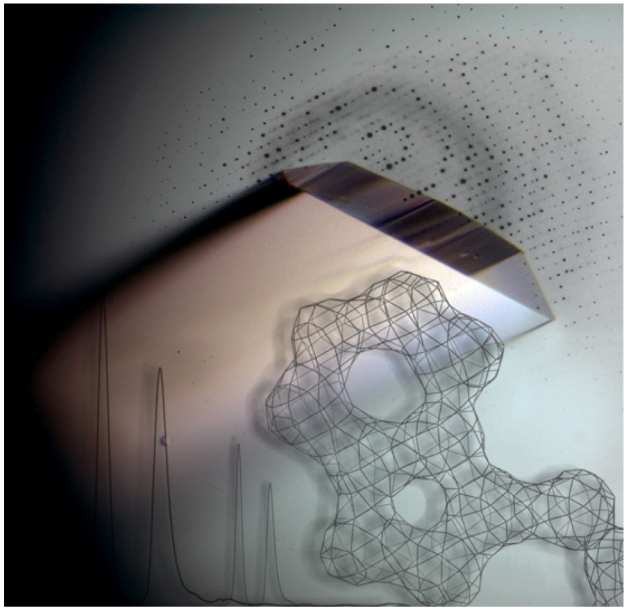
Fast Fragment and Compound Screening (FFCS) Pipeline at the SLS



- Project started in October 2017
- Led by Justyna Wojdyla
- Pipeline implemented at request of SLS beamline partners
- In operation since 2019
- Service and Collaborative academic projects



Fragment Logistics



- Key component of pipeline
- Link compounds to crystals to datasets
- Automated, detailed and robust
- In-house design: FFCS Database + GUI
- Relational database as fragment campaign hub
- Generates input files and collects output files for all devices
- Organises crystal plates, cryo protection, soaking and fishing

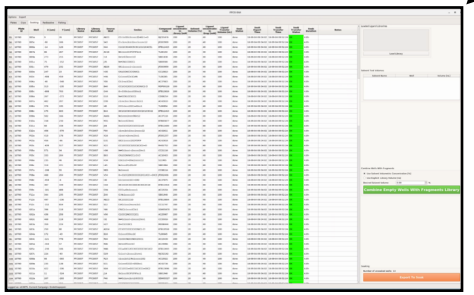
mongoDB®

Crystal Growth | Soaking | Harvesting | Sample Delivery | Data Collection | Data Processing +Refinement | Structures!

Fragment Logistics

Rockimager

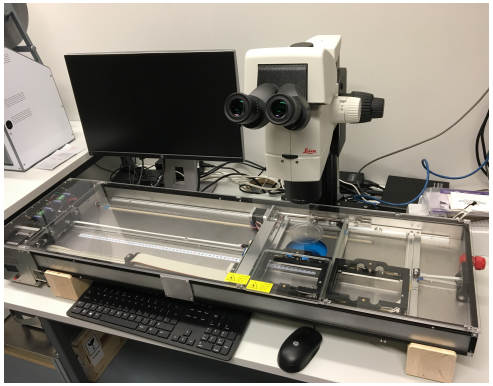
1



FFCS DB

2

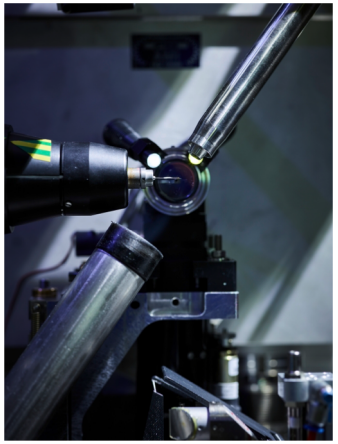
3



Shifter

Echo

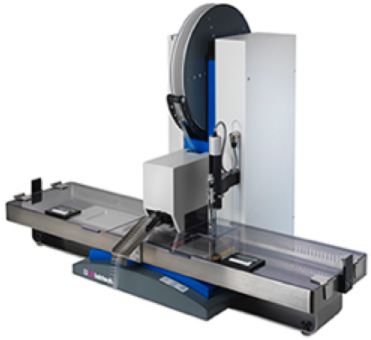
4

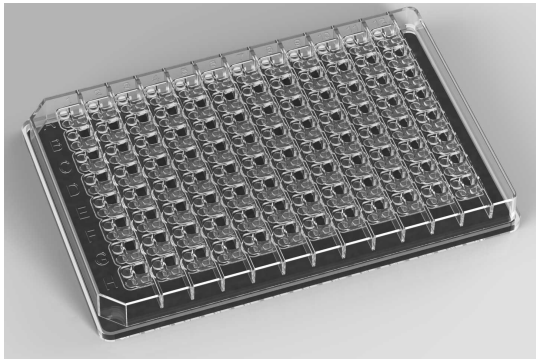


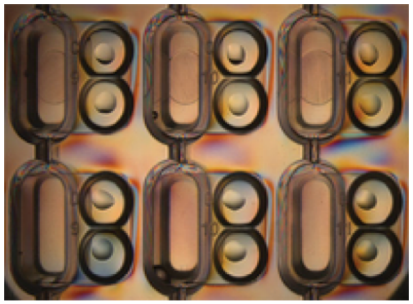
Beamline

Growing and Locating Optimal Crystals

- Need large numbers of high-quality protein crystals
- Need to reproduced in 96 well plates
- Typical drop volumes 200- 400nl
- Need to identify which drops contain suitable crystals and location in drop







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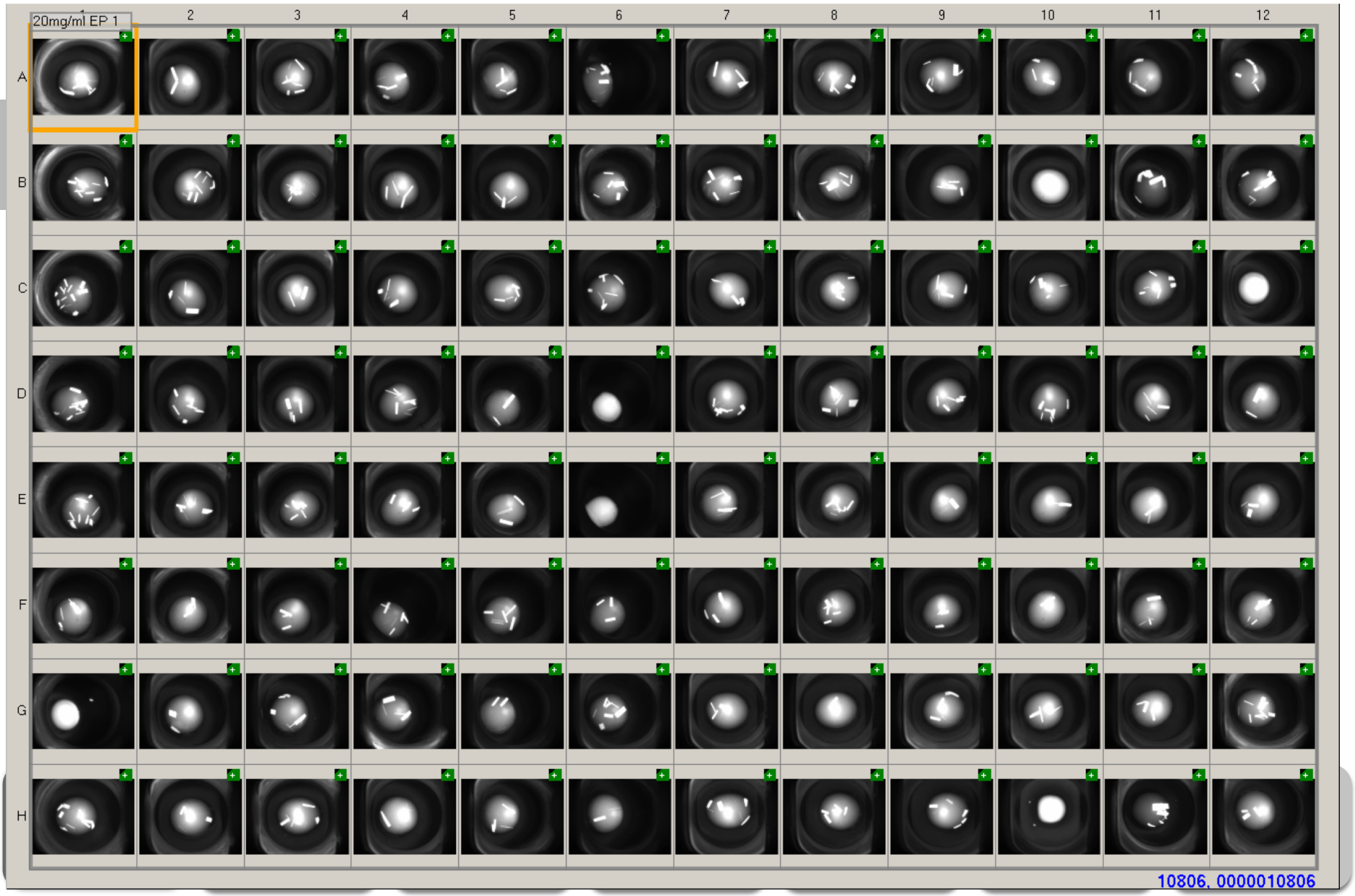
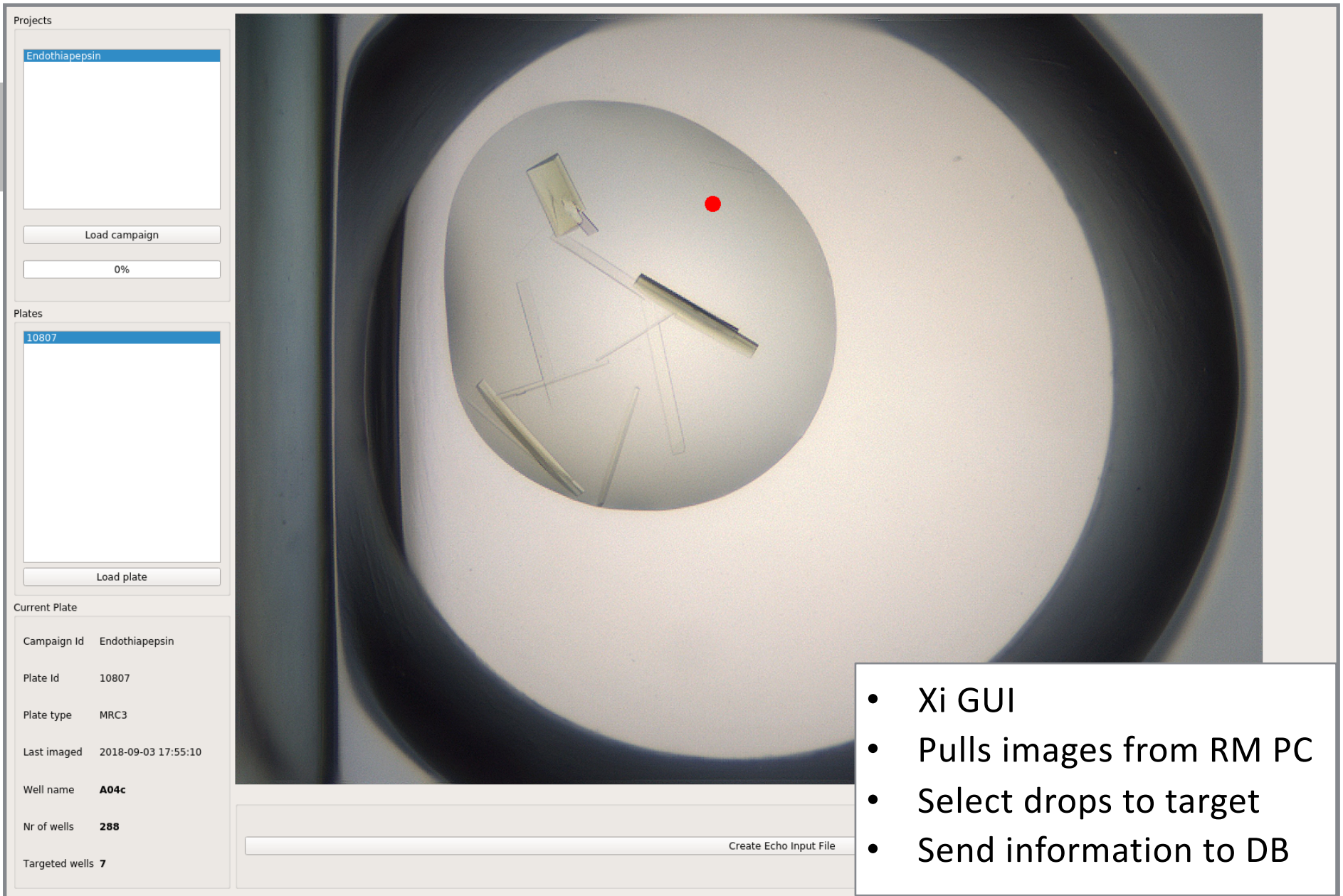
Crystal Identification



Crystal Identification



- Xi GUI
- Pulls images from RM PC
- Select drops to target
- Send information to DB

Crystal Soaking

Options Expert

Plates | Cryo | Soaking | Redissolve | Fishing

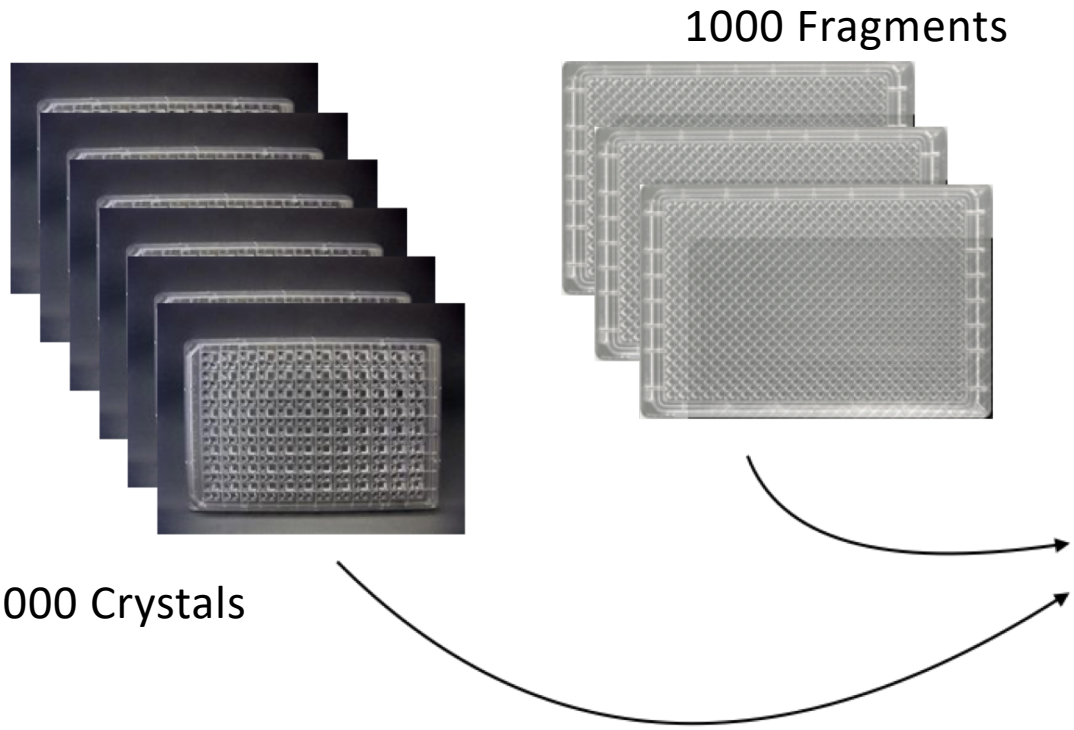
	Plate Id	Well	X [um]	Y [um]	Library Name	Library Barcode	Source Well	Smiles	Compound Code	Ligand oncentratio In ibrary [mM]	Solvent Volume [%]	Ligand oncentratio In Drop [mM]	Ligand Transfer Volume [nL]
55	10780	D05a	9	26	FFCS057	FFCS057	AB33	CCc1c(O)c2ccccc2[nH]c1=O	RJC01476	200	20	40	100
56	10780	D05c	-60	166	FFCS057	FFCS057	S43	Cl.c1cnc2c(c1)ccc1ccccnc12	JFD03909	200	20	40	100
57	10780	D06a	-14	126	FFCS057	FFCS057	K44	C1C2C3C4OC5C3C1C1C2C4C51	BTB12415	200	20	40	100
58	10780	D07a	86	267	FFCS057	FFCS057	AC18	NCc1ccc(C(F)(F)F)cc1	TL00150	200	20	40	100
59	10780	D09a	273	215	FFCS057	FFCS057	I14	Oc1c(Cl)cccc1Cl	SB01838	200	20	40	100
60	10780	E01a	75	-152	FFCS057	FFCS057	J35	N#CN1CCOCC1	SB00580	200	20	40	100
61	10780	E01c	479	232	FFCS057	FFCS057	AB26	OCc1ccccc1-c1ccccc1	JFD03959	200	20	40	100
62	10780	E03a	247	23	FFCS057	FFCS057	H35	CN1CCN(C)C(CN)C1	CC13813	200	20	40	100
63	10780	E03c	-408	459	FFCS057	FFCS057	H46	Cc1cccc(C)c1C#N	TL00285	200	20	40	100
64	10780	E04a	-40	-548	FFCS057	FFCS057	Y08	Clc1nsc(Cl)n1	AC37603	200	20	40	100

- Soaking coordinates transferred to DB
- Fragment Library loaded and assigned to drops
- Input file for Echo created and transferred to Echo PC
- Echo run results transferred to DB

Collins *et al.*, (2017). Acta Cryst. D73, 246-255

Crystal Soaking

- Fragments transferred to the crystal containing drops using a Labcyte Echo 550 acoustic liquid handler
- Coordinates transferred from FFCS Database



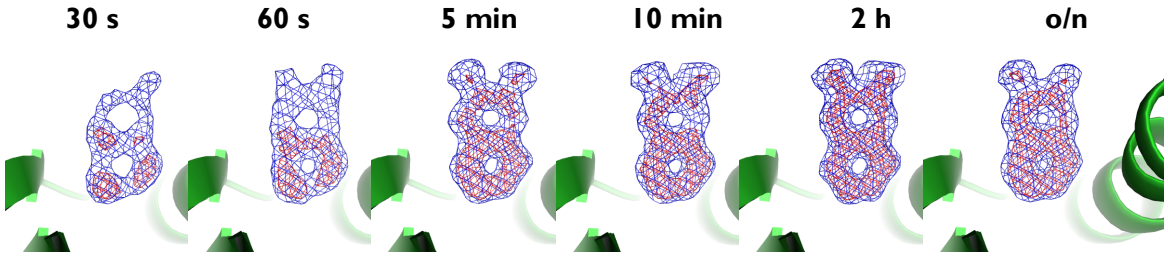
Crystal Growth | **Soaking** | Harvesting | Sample Delivery | Data Collection | Data Processing +Refinement | Structures!

Crystal Soaking

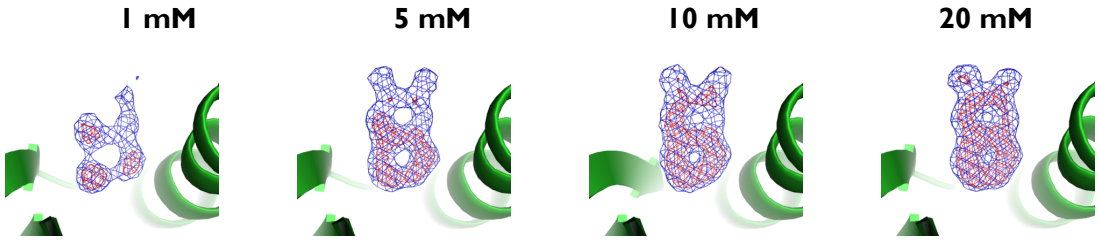
- Soaking parameters first need to be optimized
- Maximum soaking time (1– 24 hours) and maximum DMSO soaking concentration (5 -30%) determined

Blue Density: 1.0 σ
Red Density: 2.0 σ

Soaking Time:



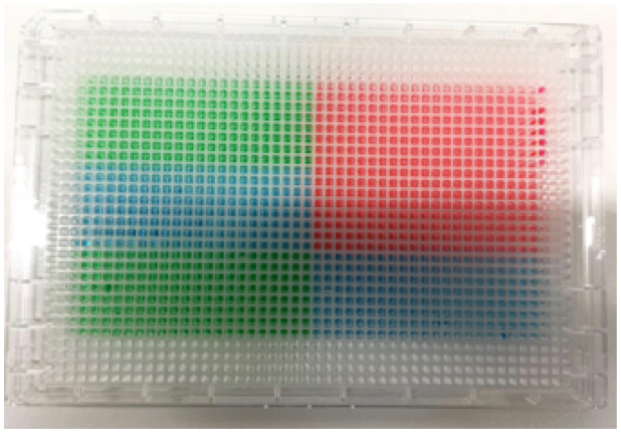
Soaking Concentration:



Crystal Growth | **Soaking** | Harvesting | Sample Delivery | Data Collection | Data Processing +Refinement | Structures!

Fragment and Compound Library Storage

- Stored in hermetically sealed dry environment (N2 purged)
- Heat sealed plates
- Limit number of times opened
- Extensive advice from Idorsia Compound Library and Screening
- Idorsia 1056 library and Maybridge 2500 library
- Utilize any library stored in Echo compatible plates

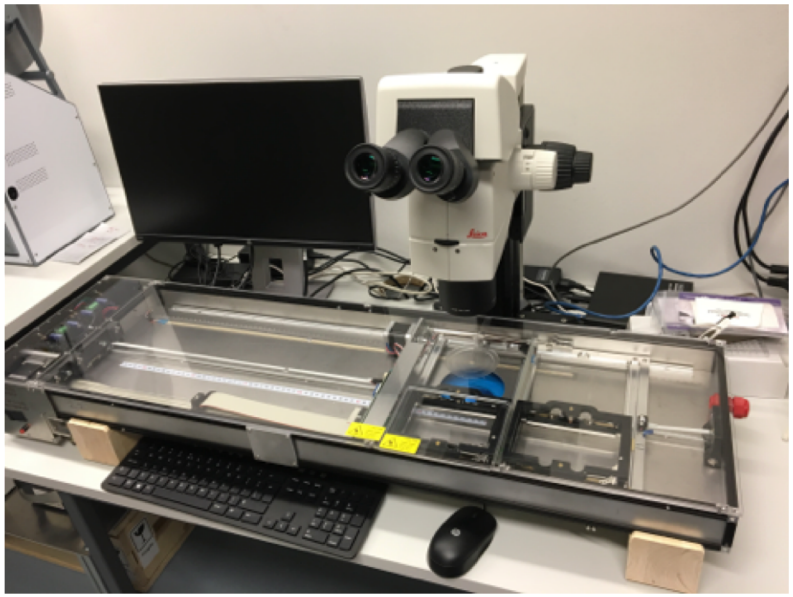


Crystal Growth | **Soaking** | Harvesting | Sample Delivery | Data Collection | Data Processing +Refinement | Structures!

Crystal Harvesting

- Utilize Shifter robot (Oxford Lab Technology)
- Manually assisted harvesting
- Shifter input file generated by FFCS DB
- Automates all repetitive steps
- Can freeze 100-150 crystals per hour
- Barcoded pucks

Developed at Diamond Light Source



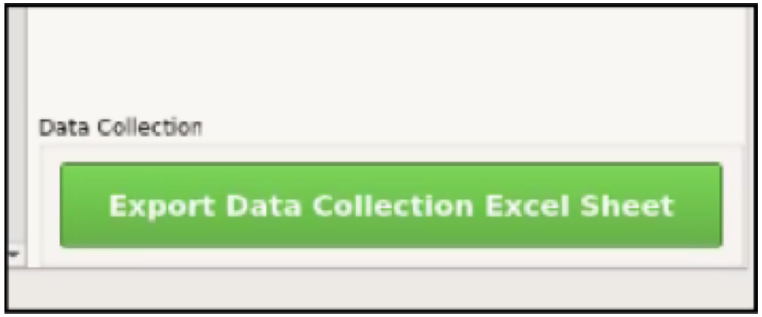
Wright *et al*., (2021). Acta Cryst. D77, 62-74

Sample Delivery

Options Expert

Plates | Cryo | Soaking | Redissolve | Fishing

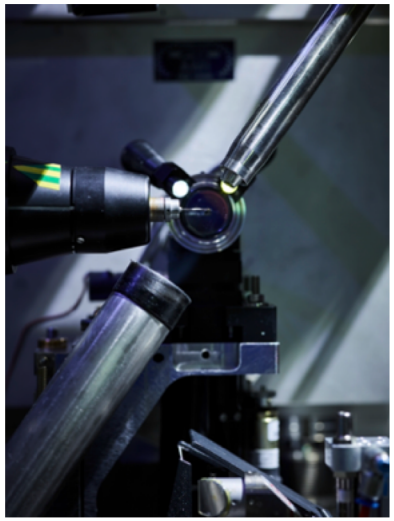
	Plate Id	Well	Fishing Result	Fishing Time	Fishing Duration	Puck Barcode	Puck Position	
49	10780	C12a	OK: Mounted Clear	18-08-30 15:45:59	00:01:20	CFSLS010	3	-C
50	10780	D01a	OK: Mounted Clear	18-08-30 15:47:27	00:00:32	CFSLS010	4	-C
51	10780	D01c	OK: Mounted Clear	18-08-30 13:39:15	00:00:47	CFSLS009	5	-C
52	10780	D02a	OK: Mounted Clear	18-08-30 13:40:04	00:00:27	CFSLS009	6	-C
53	10780	D02c	OK: Mounted Clear	18-08-30 15:48:02	00:00:40	CFSLS010	5	-C
54	10780	D03c	OK: Mounted Clear	18-08-30 15:48:44	00:01:00	CFSLS010	6	-C
55	10780	D05a	OK: Mounted Clear	18-09-04 13:01:24	00:00:37	CFSLS003	1	-C
56	10780	D05c	OK: Mounted Clear	18-09-04 13:02:04	00:00:41	CFSLS003	2	-C
57	10780	D06a	OK: Mounted Clear	18-09-04 13:02:47	00:00:31	CFSLS003	3	-C
58	10780	D07a	OK: Mounted Clear	18-09-04 13:03:19	00:00:19	CFSLS003	4	-C
59	10780	D09a	OK: Mounted Clear	18-09-04 13:03:41	00:00:19	CFSLS003	5	-C
60	10780	E01a	OK: Mounted Clear	18-09-04 13:04:02	00:01:21	CFSLS003	6	-C
61	10780	E01c	OK: Mounted Clear	18-09-04 13:05:24	00:00:29	CFSLS003	7	-C

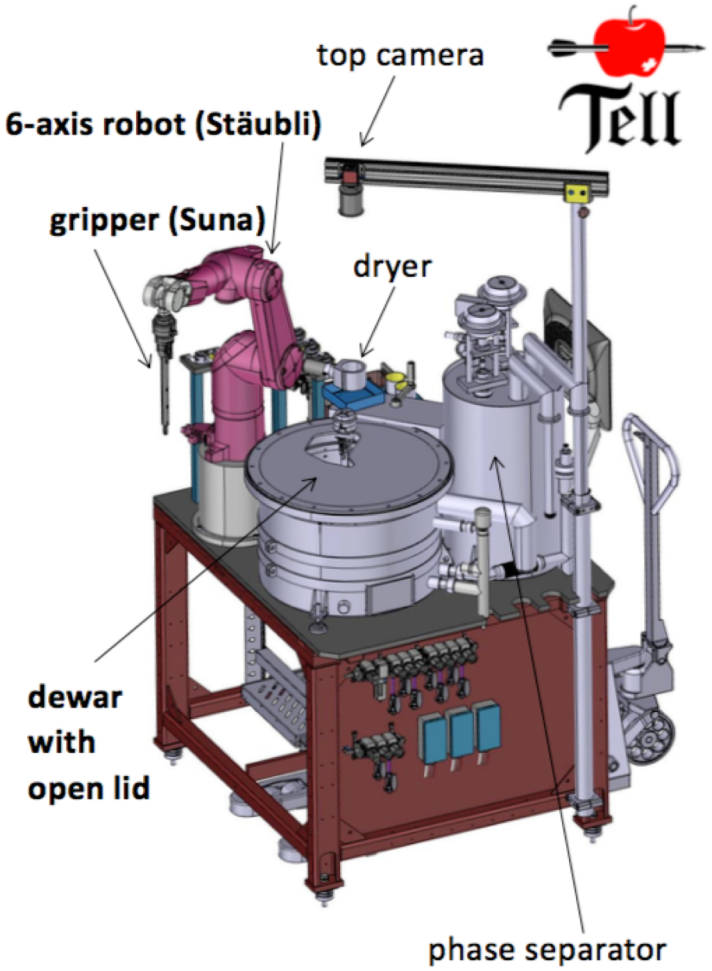


Crystal Growth | Soaking | Harvesting | **Sample Delivery** | **Data Collection** | Data Processing +Refinement | Structures!

Data Collection

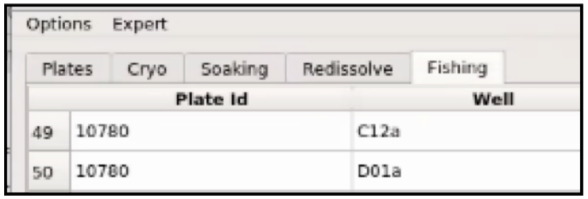
- Crystals delivered to beam using in house designed sample changer robot
- 20-30 samples / hour
- Unattended data collection





Martiel *et al.*, (2020). J. Sync. Rad. 27, 860-863

DMSO free soaking



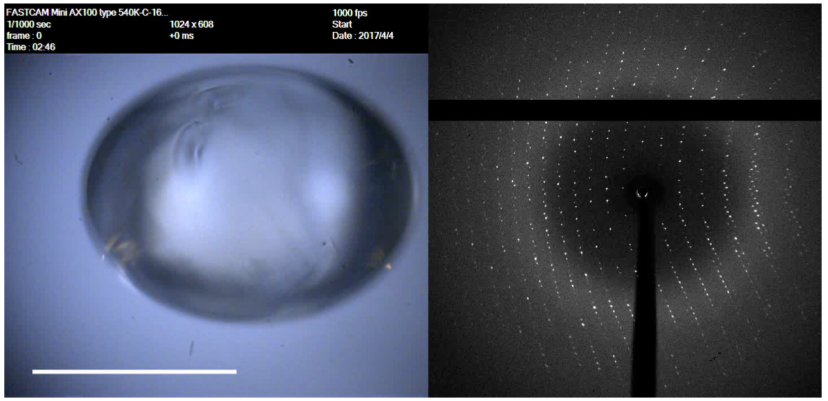
- DMSO can block binding sites and damage crystals
- The pipeline is based around DMSO stocks of compounds
- Can we dispense from DMSO stocks and then get rid of the DMSO?
- Dry fragment onto crystallisation plate, re-suspend in crystal soaking solution
- Transfer crystal to plate ➞ soak ➞ harvest
- Echo used to transfer both fragments and soaking solution
- Shifter expedites transfer of crystals to soaking plate

Bedi *et al.*, (2020). ACS Chem Biol. 15, 618-625

Crystal Growth | Soaking | Harvesting | Sample Delivery | Data Collection | Data Processing +Refinement | Structures!

Acoustic Droplet Levitation for Sample Delivery

- Crystal handling and freezing takes a lot of time and manual effort.
- Medicinal chemistry would benefit from RT data (e.g. RT water structure).
- Crystal delivery by acoustic droplet levitation would transform crystal handling.
- Would enable rapid, automated collection of RT data for fragment screening.



S. Tsujino & T. Tomizaki, Scientific Reports 6, 25558 (2016).

Crystal Growth | Soaking | Harvesting | Sample Delivery | Data Collection | Data Processing +Refinement | Structures!

Acknowledgements

- PSI
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  - Aengus Mac Sweeney
  - Geoffroy Bourquin

Sharpe *et al*, Nippon Kessho Gakkai-Shi (2021) **63**(3) 232

Kaminski *et al*., (2022). Acta Cryst. D**78**, 328-336