Delivery of compounds in crystallization plates:
A new practical approach for hit validation and fragment-based screening by X-ray analysis

J.-M. Rondeau[1], E. Bourgier[1], S. Lehmann[1], H. Mees[2], D. Baeschlin[2]

Novartis Institutes for BioMedical Research, Basel; [1] CPC/SB; [2] CPC/LFP

Introduction

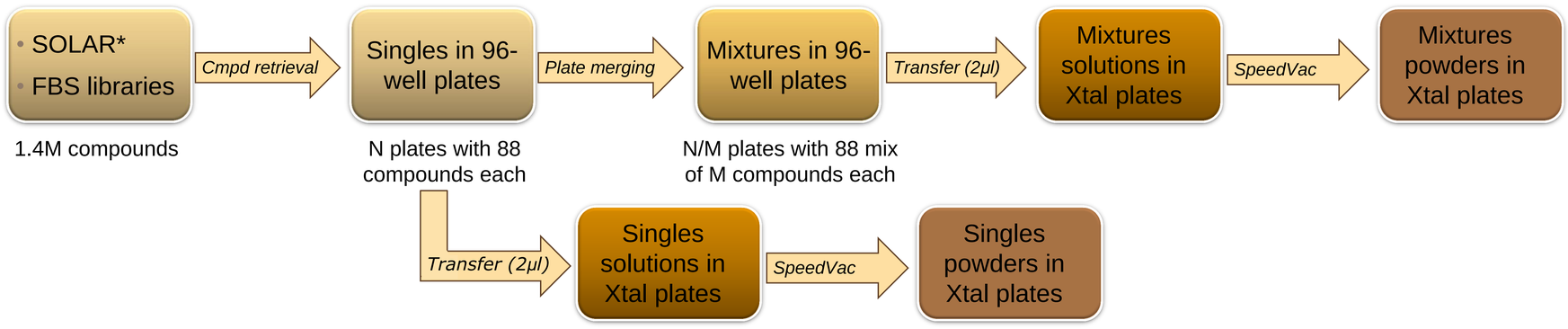
Protein crystallography plays a central role in fragment-based drug discovery as well as in the validation of hits identified by high- or medium-throughput screening. Recent advances in crystallographic technologies and methods have turned X-ray analysis into a straightforward and highly automated procedure once a robust crystallization protocol has been established and a first structure solved.

This progress opens up the possibility of investigating relatively large sets of compounds (100-1000) by protein crystallography, provided, however, that compound handling can be streamlined.

Traditionally, powder samples are ordered individually, and, because of the very high compound concentration required for X-ray analysis, 50-100mM stock solutions are prepared manually. We show here that compound powder can instead be delivered by the Novartis Compound Bank directly into crystallization plates, using the already available logistics and robotic systems.

This approach enables the testing of several hundreds of compounds in co-crystallization experiments, with minimal effort and hands-on time. It is highly flexible and applicable to compound mixtures as well.

Process for Compound Delivery in Crystallization Plates



* Novartis solution archive

Concentration of compound achieved in the crystallization experiment(*)

	Singles	Mix of 5	Mix of 8
Solar (old) 2mM	5mM	1mM	0.62mM
Solar (new) 10mM	25mM	5mM	3.1mM
FRAG100 25mM	62.5mM	12.5mM	7.8mM
FBS library 50mM	125mM	25mM	15.6mM

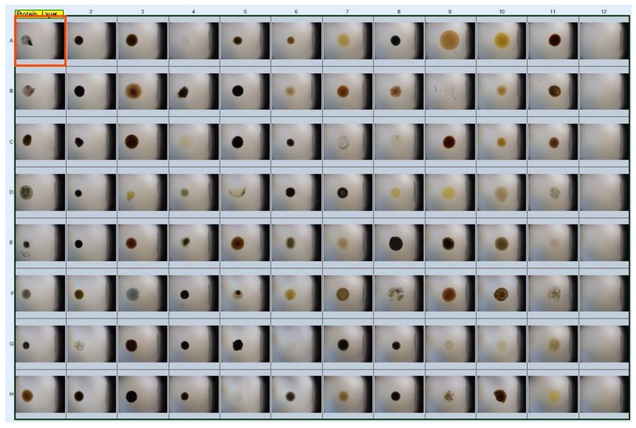
(*) Assuming crystallization drops of 0.8µl (total volume)

Pilot Study

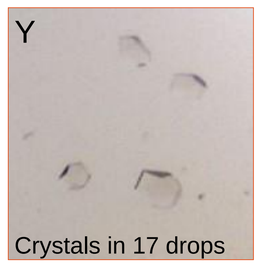
Study Design

- 88 x 5 = 440 compounds selected from Solar (reference compounds as positive controls, random selection of 2nd and 3rd generation FBS libraries, and 176 compounds from FRAG100 library)
- 2µl of mixtures of 5 dispensed in protein well followed by solvent evaporation (SpeedVac)
- Co-crystallization experiments set up with test proteins: 0.8µl of protein/crystallization buffer mix dispensed in one pass with Phoenix robot
- Crystals were X-rayed and analysed

Rockimager view of crystallization plate (Intelli-plate) delivered with compound powders



Some crystals



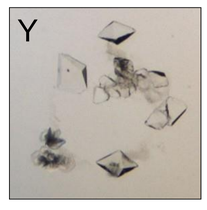
➢ Co-crystallization success rate: 20-36%

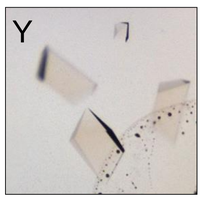
Some interesting findings: 12 structures from 66 data sets, success rate: 18%

❖ *reference compounds were observed*

❖ *novel hits were found*

❖ *2 new crystal forms for Y protein*





Discussion and outlook

➢ **We have demonstrated that compounds can be delivered directly in crystallization plates for crystallographic work**

➢ **This new approach enables high-throughput co-crystallization with large number of compounds ...**

➢ Co-crystallization has several advantages:

✓ best approach for large ligands and/or ligands inducing conformational changes

✓ allows change of crystal symmetry/packing

➢ **... but it requires a robust crystallization system**

- in our pilot, crystals were obtained only in 1/5 to 1/3 of the crystallization drops
- it may be possible to increase the crystallization success rate by:
  - reducing the size of the mixtures
  - setting up experiments in duplicates / triplicates

➢ Alternatively, the plates can be used for soaking experiments when the crystallization is not robust enough. *However, in that case the throughput is reduced due to the manual handling of crystals*

➢ **The delivery of compounds in crystallization plates offers several advantages:**

✓ It frees crystallographers from all tedious compound handling tasks

✓ It gives access to all compounds present in the Solar archive, *including those which are no longer available as powder samples*

✓ Compound consumption is dramatically reduced (2-4µl of SOLAR solution instead of 1-3mgs of powder)

✓ Storage and book-keeping of multiple stock solutions in individual labs is avoided.

➢ **Moreover, this new approach is highly flexible and can be used for a variety of applications:**

✓ **Hit validation:** (compounds delivered as singles)

✓ rapid identification of the “low hanging fruits”

✓ fast generation of structural data on multiple chemotypes, no biased selection of compounds

✓ **Lead optimization:** (compounds delivered as singles)

✓ co-crystallization of multiple analogues rather than a selected few, best crystals used for structure analysis

✓ **Fragment-based screening by X-ray:** (compounds delivered as mixtures)

- established fragment libraries / mixtures
- ad hoc compound set with customized mix design

✓ **“Silver bullet screening”:** (singles or mixtures)

- identification of new crystal forms or crystals with improved diffraction properties