Protein Crystallography Application of Acoustic Dispensing by the ATS-100

Objective: Produce high quality crystals for x-ray diffraction using acoustic dispensing.

General Procedure for Crystallography

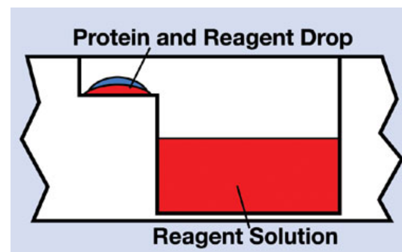
1. Produce an adequate crystal of the material under study. 🡐 ATS-100
   Because of the variability of proteins, each requires a specific set of environmental conditions (pH, temperature, precipitants) in order to successfully crystallize.
2. Test crystals with X-rays and collect data.
3. Data is computationally analyzed and refined to determine crystal structure.

Crystal Production -Vapor Diffusion by Sitting Drop Method

General Procedure:
While there are many different methods to crystallography, the most common ones involve vapor diffusion. This involves mixing the protein with a certain buffer (known pH, temp, precipitants) and placing a small volume (1uL) of this mixture into a well. Within the same well, place a much larger volume (50uL) of the buffer without protein. As the two solutions equilibrate, the properties of the buffer/protein mixture will change and hopefully induce crystallization. Repeat this procedure for 100s of different buffers and over time you will hopefully get one to crystallize correctly. (Companies make standard sets of these buffers in blocks of 96; Nextal, Axygen)

General Procedure using the ATS-100
Plate used: Corning® Crystal*EX*™ (Corning Cat. No. 3785)

1. Using a bulk liquid handler, dispense 15uL of entire 96 well screening block into an ATS-100 acceptable source plate.
2. Pipette 6uL of protein into one of the remaining source plate wells.
3. Using a bulk liquid handler, dispense 20uL of entire 96 well screening block into large reservoirs on crystallography plate. This is your target plate. (The ATS-100 can also be used to fill large reservoirs, depending on the crystallography plate used)
4. Load source and target plates into the ATS-100.
5. Run dispense using pre-designed maps on the ATS-100. Maps will specify 50nL of buffer and 50nL of protein.
6. After dispense, remove target plate and seal.



Benefits Over Other Screening Systems

1. Use less protein and reagents: ATS-100 has been shown to produce crystals with as little as 10nL (the lowest in the field) with potential to go down to 1nL. This allows you to expand your research to previously expression-limited projects.
2. More visible crystals: By using small volumes, the depth of field becomes smaller and the crystals are easier to image.
3. Faster crystal production: With less excess volume to diffuse away, crystallization takes place faster, cutting wait times down by 50-75%.
4. No more clogged tips: By using acoustics, we eliminate all tubes and tips; drops travel through the air directly from the source plate to the target plate.
5. Scalability: The flexibility of the ATS-100 allows you to dispense protein and buffer from 1nL to 10uL allowing you to go from an initial to hit to production size volumes with ease.
6. Fluid diversity: Acoustic dispensing has been shown to dispense hundreds of mother liquors, proteins, detergents, crystal seeds, oils, and other additives.
7. Target plate flexibility: While the sitting drop method is the backbone of crystallography, there are countless plate geometries used to achieve this. This instrument can handle every SBS-standard crystallography plate and allows you to easily switch between many plates.
8. Miniaturization: Most crystallography labs restrict themselves to 48 and 96 well plates, now they can expand to 384 plates and even 1536 plates for microbatch crystallography.

Calibrations for a Variety of Fluids

1. Proteins: Standard media used to carry proteins can be calibrated the same way DMSO is calibrated for compound management and high throughput screening applications. This involves doping the media with fluorescein and creating a standard dilution curve.
2. Oils: Due to the viscosity of oils they require a considerably higher energy to dispense. But ultimately they too will be calibrated like DMSO. Certain oils will not dispense with the ATS system, contact EDC for more information.
3. Crystal seeds: The presence of microscopic crystal seeds in a protein media has not shown to require different calibrations from the media itself.
4. Buffers: Due to the multitude of buffers used in crystal screening, its impractical to calibrate them all in the same manner as proteins and DMSO. Here you have multiple options:
    a. Run them "raw" with constant calibrations. Using simple well drains with "average" buffers we can determine a general energy setting to use. Running all buffers with this calibration will yield CVs across screens of 20%. This has been shown to be extremely effective.
    b. Process several general calibration runs where we dispense large volumes of all fluids. Using the log files produced by the ATS software, we can generate energy maps that vary the energy for each individual well. This will bring CVs down to 10-15%.
    c. Process initial runs to generate focus maps. These maps factor in the variation in speed of sound across screening fluids. Then process the general calibration runs, with focus maps activated, to determine energies to use. CVs should lower to 10% with this additional step.

Current Research and Future

1. Further miniaturization: As the rest of crystallography equipment and instrumentation goes to small volumes, high-density crystallography plates will become standard. The ATS will keep you on the forefront by adapting to new plates with ease.
2. Hanging drop crystallization: has been shown to work with the ATS-100, using volumes comparable to experiments done with other instruments, 500nL minimum protein. While this works and satisfies those who prefer to use this method, it does not take full advantage of acoustic dispensing. With future plate development, hanging drop experiments could potentially be done with 5 or 10nL of protein.
3. Microbatch crystallization: The ATS has been shown to be able to dispense standard oils (like paraffin oil) used in crystallography. For microbatch experiments though, you need a specific drop placement scheme; with oil resting on the top and sides of the protein creating a closed microenvironment. The optimized method for having the ATS produce this arrangement has not been determined at this point.