Temperature as a Crystallization Variable

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Temperature and Crystallization

Temperature can be a significant variable in the crystallization of biological macromolecules (proteins).[1,5] Temperature often influences nucleation and crystal growth by manipulating the solubility and supersaturation of the sample. Temperature has also been shown to be an important variable with phase separation in detergent solutions during membrane protein crystallization.[7]

Control and manipulation of temperature during the screening, optimization and production of crystals is a prerequisite for successful and reproducible crystal growth of proteins with temperature dependent solubility. Christopher et al., testing 30 randomly chosen proteins, found 86% demonstrated a temperature dependent solubility and suggested that temperature induced crystallization could be a generally useful technique.[5] Temperature was shown to affect quantity, size, and quality of the crystals as well as sample solubility and preliminary crystallization data.

One advantage of temperature is that temperature provides precise, quick, and reversible control of relative supersaturation. Using temperature in addition to standard crystallization variables such as sample concentration, reagent composition and concentration, as well as pH can increase the probability of producing crystals as well as uncover new crystallization conditions for a sample. Additional crystallization conditions may uncover reagent formulations more amicable to heavy atom derivatization, cryoprotection, and optimization or at least offer options. Temperature is amenable to control and can be used to carefully manipulate crystal nucleation and growth. This control can also be used to etch or partially dissolve then grow back the crystal in an attempt to improve crystal size, morphology, and quality or assist with seeding. Temperature control is noninvasive and can manipulate sample solubility and crystallization with altering reagent formulation.

Traditionally, crystallization screens and experiments are performed at room temperature and sometimes 4 degrees Celsius. A reasonable range of temperature to screen and optimize for protein crystallization is 4 to 45 degrees Celsius and some proteins have been crystallized at 60 (glucagon and choriomammotropin) degrees Celsius. A practical strategy would be to screen at 10, 20 (or room temperature) and 30 degrees Celsius when the sample volume permits. Temperature incubations above room temperature should be monitored closely for evaporation from the drop and reservoir. A 2 microliter hanging drop vapor diffusion experiment at 37 degrees Celsius can evaporate in as little as 48 hours depending upon the plate, quality of seal. Microbatch under Paraffin Oil can minimize evaporation problems. In the case of room temperature incubations, temperature control and stability are often minimal since the experiments may be left in the open room. In an open room, temperature fluctuations may be significant, especially over a 24 hour period and on weekends when thermostatic control of the room environment can fluctuate 10 degrees or more. Incubation at 4 degrees Celsius and other temperatures are often more stable since the incubation is performed in some type of incubator. Another source of temperature fluctuation occurs while viewing experiments. The light microscope is a heat source and extended viewing can significantly alter the temperature of small drops. Quick efficient viewing can minimize temperature changes. Also, remember to turn off the light source when leaving plates on the stage in one position for more than a few seconds.

While controlled temperature can be important for consistent results, temperature fluctuation can be useful in obtaining high quality crystals by screening a larger range of crystallization conditions since for a sample with temperature dependent solubility changes in temperature can equate to changes in a crystallization reagent condition.[8] Hence, a sparse matrix screen takes on a new dimension when screened at multiple temperatures, or ramped over several different temperatures over a period of time.

How does one test for the effect of temperature and temperature dependent solubility without consuming a lot of sample? One solution is to set a single crystallization screen at one temperature, allow the experiment to incubate for a week, record the results and then move the plate to another temperature. Allow the experiment to incubate for a week at the new temperature and record the results. If one notices changes in solubility (i.e. clear drop turning to precipitate, or precipitate turning to clear drops) between the two temperatures, then the sample has temperature dependent solubility and temperature should be explored as a crystallization variable.

Temperature gradients can be used for screening and optimization of proteins with temperature dependent solubility. For screening, set the experiment at one temperature, allow the experiment to equilibrate and then slowly change the temperature to a second temperature. In general, ramp the temperature so that the sample is exposed to an increase in relative supersaturation as the temperature changes over time. In other words, ramp from high to low temperature if the sample is more soluble at high than low temperatures. This can be accomplished using a programmable temperature incubator. A temperature gradient or ramp, allows one to slowly approach temperatures where a sample may have a decrease in solubility with a corresponding increase in relative supersaturation. Published examples of temperature gradient or temperature ramp crystallization include elastase (25 to 20 degrees Celsius gradient), alpha-amylase (25 to 12 degrees Celsius gradient), and insulin (50 to 25 degrees Celsius gradient).[9,10,11]

To demonstrate how screening temperature could affect and enhance the results obtained from a preliminary crystallization screen, a programmable temperature incubator was used to screen 4 different temperatures. Using Glucose Isomerase and Crystal Screen, sitting drop vapor diffusion experiments were set using Cryschem plates at 4, 15, 25, and 37 degrees Celsius. Drops were observed daily and the results were quite interesting. Glucose Isomerase crystallized in 19 conditions at 25 degrees Celsius, 23 conditions at 15 degrees Celsius, 28 conditions at 4 degrees Celsius, and 12 conditions at 37 degrees Celsius. A similar approach with Trypsin, yielded crystals in 8 conditions at 15 degrees Celsius, 4 conditions at 25 degrees Celsius, and 7 conditions at 32 degrees Celsius. In the case of Trypsin, a single set of Cryschem plates were set and the plates simply moved from one temperature to another over a period of a weeks time, scoring results before each temperature change.

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Temperature Tips

• For proteins with "normal" solubility, in high salt the protein will be more soluble at cold than at warm temperatures.

• For proteins with "normal" solubility, in low salt the protein will be more soluble at warm than at cold temperatures.

• Proteins with "normal" solubility will precipitate or crystallize from lower concentration of PEG, MPD, or organic solvent more slowly at low than at high temperatures.

• Diffusion rates are less and equilibration occurs more slowly at low than at high temperatures. Crystallization may occur more slowly at low than at high temperatures.

• Temperature effects can be more pronounced at low ionic strength reagent conditions.

• Do not use the appearance or non-appearance of crystals at various temperatures to gauge the effectiveness of temperature as a crystallization variable. Rather, use the difference in the solubility at different temperatures to gauge the effect temperature has on sample solubility. If an effect is observed, explore temperature as a crystallization variable.

• Temperature can effect different crystal forms and growth mechanisms.[12]

• When incubating experiments below and above room temperature and viewing experiments at room temperature, condensation can be a problem. To minimize and avoid condensation with vapor diffusion experiments, stack a "Dummy Plate" with reservoir filled with water and sealed, at the bottom and top of the stack of plates. This will slow the temperature change in the sandwiched plates and minimize condensation.

• The Microbatch method works well for temperature exploration. In a traditional Microbatch experiment, the relative supersaturation of the system does not change since, in theory there is no vapor diffusion. However, if the sample exhibits temperature dependent solubility, temperature can be used to manipulate sample solubility in a Microbatch experiment. Another plus of using Microbatch is the lack of condensation while viewing the experiment.

• Condensation with a hanging drop can mean alteration of your drop with the when the condensation mixes with the drop. Condensation with a sitting drop can mean there will be no mixing of the condensation with your drop, unless the condensation falls into the drop. Moral, sitting drop has less change for mixing with condensation.

• To dry up condensation, add a small amount of concentrated salt solution to the reservoir. Keep in mind this might also dry your drop a bit.

• Nucleic acid temperature stability allows one to examine temperatures between 4 and 35 degrees Celsius.

• Ideally, one should set the experiment at the eventual incubation temperature and all reagents, samples, and plates should be equilibrated to the incubation temperature. This is a reality for room temperature setups and 4 degrees Celsius setups for those of us with cold rooms. For the rest of us, we can set the experiment at room temp and then toss it into the incubator. Or, for 4 degrees Celsius set ups, one can cheat. Simply incubate the reagents, sample, plates and slides in the refrigerator before set up. During the set up, place materials in a tray full of ice. Maintain the plates on ice during the set up. Seal and move smartly to the 4 degrees Celsius incubator.

• Increasing temperature increases the disorder of reagent molecules. Varying the temperature of a crystallization experiment can manipulate sample-sample as well as sample-reagent and reagent-reagent interactions. Such manipulations may have an impact on interactions which control nucleation and crystal growth. In addition, such interactions may have an impact on crystal packing as well as the termination of crystal growth. Hence, temperature can impact nucleation, growth, packing, and termination.

• Temperature can be a habit modifier and change the crystal lattice. For example, at temperatures below 25 degrees Celsius and in the presence of sodium chloride and acidic pH, the tetragonal form of lysozyme is favored. Under similar reagent conditions above 25 degrees Celsius, the orthorhombic form is favored.[13]

• The preparation of heavy atom isomorphous derivatives can depend upon the temperature of the experiment. In most cases, it seems the soak temperature is the same as the crystallization temperature.

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