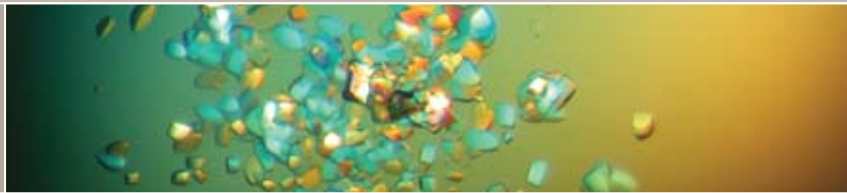


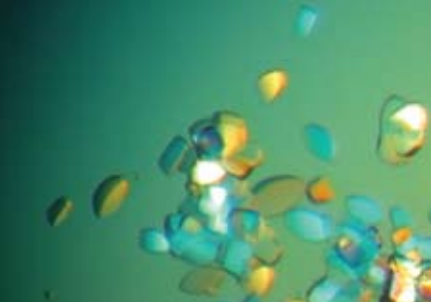
HAMPTON
RESEARCH

TIPS FROM RAMC

Crystals of the Deinococcus radiodurans 50S ribosome subunit.

From the group of Paola Fucini, Max Planck Institute for Molecular Genetics, Berlin, Germany.





Filtering the Protein

Naomi Chayen

To reduce the number of crystals and increase their size, try filtering the protein solution prior to setting up the experiment. Try the following filter sizes: 0.22, 0.1 micron and 300 kD molecular weight cut-off. Try the Millipore centrifugal filters.

Pseudo-Microseeding

Mike Sintchak

When working with crystals that grow fairly rapidly (one day) try the following. Pipette multiple protein drops (2 to 4 works best) onto the cover slip. Using a single pipette tip, get the reservoir solution to mix with the first drop. Now, go back into the reservoir with the same tip to get the reservoir solution for the second drop. Continue for the remaining drops with the same tip. In certain cases, seeding starts very quickly, so by using the same tip one can introduce minute seeds to successive drops. Use the same cover slide with multiple drops to minimize evaporation.

Low Molecular Weight PEGs

Lesley Haire

When screening with low molecular weight PEGs try microbatch. Crystals appear rapidly with PEG 400-2000. To convert to vapor diffusion use 0.2 M buffer in the well and a 1:1 drop ratio. Try using a positive displacement pipette such as the Anachem Microman 1 - 10 μ l. These are much more accurate.

Purest is Not the Best

Michal Harel

A protein which was purified and showed some faint bands of contaminants on a native gel was crystallized and solved successfully. The same protein, purified by HPLC and resulting in a single band native gel did not crystallize.

Cryoprotectant

Elsbeth Garman

When making up cryoprotectant solutions containing glycerol, put a test tube of glycerol in a beaker of warm water. The viscosity falls and it is easier to pipette accurately.

Mass spectrometry

David Leys

We found mass spectrometry like ESMS and MALDI highly efficient in determining impurities and/or microheterogeneities in our protein sample/batch. In most cases it is a simple, straight forward method which requires a minimum amount of sample. In some cases it has shown to detect impurities/microheterogeneities when other techniques did not.

Shape of Drop

Ursula Kamlott, Hoffmann-La Roche: ursula.kamlott@roche.com

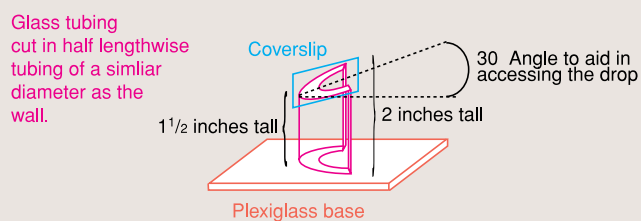
One of my proteins produced only zillions of tiny useless crystals when I mixed the drops the conventional way-mixing well, overlaying, etc. the protein with

precipitant solution. Large, gorgeous crystals were produced when I crossed the drop, creating a gradient within the drop. This worked best, setting up sitting drops with vapor diffusion.

Don't Flip

Dennise Dombroski

When removing crystals from a hanging drop, I sometimes find that the biggest crystals fall against the coverslip and are impossible to resuspend without damage. I had our glass shop make a stand to transfer the coverslip that enabled me to manipulate the crystals more easily.



Extreme Soak

Jirundon Yuvaniyama

For soaking crystals with compounds with limited solubility I have tried two "extreme ways" (although not much- and more experiments should be tried):

- Leave some solid compound in the soaking solution.
- Dissolve some compound in n-octanol and layer the octanol solution on top of the soaking experiment.

These two provide (hopefully) more or less constant concentration of the compound in the soaking solution. The octanol layer may help reduce air oxidation by preventing direct contact of soaking solution to air.

I found that 200 μ l of soaking solution in a well of the 24 well Linbro® plate is a good volume to work with:

—Not too little that possibly causes concentration change due to evaporation (Sealed well) and not too much that the crystals get lost in the solution.

The Glycerol Effect

Anil Mistry

Glycerol has many benefits but also some drawbacks. We found it to be beneficial with one protein we were working with; this protein is a transpeptidase called Mur A. The protein is quite soluble and could concentrate to 20 mg/ml but it lost activity over time when stored at 80°C. We therefore dialysed it into 50% v/v Glycerol to see if the activity could be retained for longer, this would allow us to make a large batch rather than regular smaller batches, for crystallisation. On dialysis we found a significant reduction in the volume of protein, so much so that the protein had concentrated from 5-10 mg/ml to almost 50 mg/ml. Activity was also found to be retained with no significant loss after 6 months at 80°C. This gave us a method for storing large batches of Mur A at 80°C, without losing activity and also resulted in a sample pre-concentrated for crystallisation and containing a cryo-protectant. Other sugars gave similar effects; sucrose, sorbitol, etc., but none were as effective as glycerol in achieving the 50 mg/ml final concentration.

Iodoacetic Acid*Bernie Santarsiero*

Add a small amount of (~ 1%) iodoacetic acid to buffer solutions. This helps prevent aggregation by carboxymethylation of cysteine. Also, iodoacetic acid seems to help form salt bridges and aid in crystallization.

Soaking Crystals to Improve Resolution*Irene Weber*

Try soaking poorly diffracting crystals in higher concentration of precipitant, ammonium sulfate, or PEG. It may take several weeks so test after 1 or 2 months.

Rapid Preliminary Screening of Protein for Aggregation Using Protein Quantities*Tom Zarembinski*

When only small amounts of protein are available, it is not feasible to screen many compounds which promote monodispersity using a dynamic light scattering machine. To detect aggregation, we use a pseudo-native gel approach: 1 l of protein is mixed with 1 l of additive from Hampton Additive or Detergent Screens. These samples are then incubated at room temperature for 20-30 minutes and then placed in 2x sample buffer containing no DTT, no SDS and are not boiled. These samples are run on a standard SDS-PAGE gel. We have screened many additives using this approach and it has given us leads for subsequent optimization of protein buffers.

HPLC Profile*Glenn Dale*

Keep an HPLC (Reverse phase) profile of your protein before crystallization and after crystal formation. It can be used as a quality control and tells you if any modifications have occurred.

Don't Throw Away Without Looking Close*Kalevi Visuri*

Look closely at your old test tubes when cleaning the place. Proteins do crystallize on the walls of the tube when stored in a cold room. I picked up my tubes from the wastebasket and an x-ray was made from an old supersaturated protein tube.

Concentration of Protein without Aggregation*Paul Reichert*

Use centiprep (millipore) for concentrating protein >0.5 ml. Protein concentrating away from membrane. (No micro high concentration, ppt on membrane), works very nicely for a number of proteins.

Preserve Hampton Solutions*Cheryl Janson*

To preserve Hampton solution when you or a co-worker gets a "hit" and suspect the Hampton solution may be "magic" or you cannot reproduce crystals with lab-made solution, make the reservoir solution from lab ingredients and use your homemade solution for reservoirs. Use the Hampton "magic" solution

only for the drops thus using a few µl per experiment rather than 0.5 ml. Saves having a 48 well screening solution set with one tube empty and the rest at 8 ml and still allows the superstitious or in Alex's case, the contaminated solution to reproduce crystals.

Recycle Your Precipitate*Neali Armstrong*

If your protein refolding reaction has a low yield and produces lots of precipitate try collecting the precipitate, resolubilize in GuHCl, and refold again. This material is sometimes more pure than the washed inclusion bodies.

90% Solutions*Anna Stevens*

When optimizing or making solutions for a random scan, omit the buffer (@ final 0.1 M) so that your stock is 90%. Prior to putting your stock in the well, add 100 µl of 1M buffer of your choice. Next, add 900 µl of 90% stock and mix. This reduces the number of tubes for crystallization (precipitant) stocks and allows flexibility in buffer identity and pH range. Be sure to make plenty of (~20 mL) of precipitant, you'll need 900 µl per buffer.

Check Both Liquid Nitrogen + Stream Flash Cooling*Hans Parge*

If your crystal does not freeze well in the cold stream try liquid nitrogen or vice versa.

To Determine the Optimal Concentration of Your Protein for Screen I and Screen II*Jaru Jancarik*

Try (setup) drop no. 6 first (of Screen I). It should produce light precipitation if ppt appears too heavy, reduce the protein concentration by 1/2 and try again. If there is no precipitation in drop 6 try drop 4. If there is no precipitation in either of the drops concentrate the protein 2 fold and try again.

Cryoprotectant Additive*Laura Pelletier*

I tried adding 1-10 mg/ml BSA in the cryoprotectant when soaking crystals that would crack. The one time it was used, the crystal did not crack and froze nicely. I don't know if the BSA was the reason for successful freezing. I was wondering if anyone else has tried this.

Slow Cryosoaks for Improved Crystal Stability*Bryan Prince & Melissa Harris*

In a sitting drop well, cryoprotectant (20% Glycerol in crystallization buffer) should be dribbled down the side of the well.

Crosslinking of Crystals*Clare Stevenson*

When crystals are fragile or you want to transfer them to a different mother liquor e.g. for heavy atom soaking, why not try crosslinking your crystals with



0.1% glutaraldehyde in your mother liquor. This can be done by adding the glutaraldehyde directly to the drop or placing it next to the drop and allowing for vapour diffusion. This crosslinking enabled us to solve Mod A at 1.2 Å resolution.

Know When Enough is Enough!

Brent Segelke

As a graduate student, I spent countless hours and quantities of protein trying to get crystals of a single construct. We never got crystals. Another group got the structure of a homologous protein that was auto-digestive. Had we stopped after 400 trials and altered our construct, perhaps we would have fared better, we couldn't have fared any worse. There is a reasonable statistical argument to demonstrate that 400 trials are a good limit.

Buffer Screening

Anil Mistry

To find the best buffer system which will keep your protein happy, stable, and soluble for concentration prior to crystallisation, setup your protein (at 1-2 mg/ml) in hanging drops over 1 ml well solutions containing a series of buffers at various pHs, with various additives/stabilizers, etc. (but no precipitant!). Checking for drops which are clear will give an idea of which solutions keep the protein happy. In addition, as the system attains equilibrium some in situ concentration of protein will be induced due to the bulk difference between the drop and well volumes, hence an idea of how the protein behaves upon concentration in this solution will also be observed. Modifying a clear or slightly clear drop by adding a higher concentration of buffer to the well may even produce crystals. However, the main piece of information this method can produce is an idea of which buffer system and additives to put your protein into prior to concentration a crystallisation screening.

Soft Crystals

Allan D'Arcy

If you have crystals that are very sensitive to being touched (they break) or stick to the glass or dish, use a pointed strip of parafilm to move them. Otherwise, grow the crystals on parafilm and punch "wells" around the crystals to move it.

Crystal Annealing

Clare Stevenson

As I said in my talk, give it a go. You might be surprised!!

"No More 4°C"

Marie Anderson

Prepare trays for crystallisation, leave at 4°C, and fill polystyrene box or flat container with ice. Imbed a metal plate in the ice and set out cover slips. When you're ready to set out crystallisations place the trays in the bed of the ice and prepare drops, when finished transfer to 4°C. Simple but it works.

Cross Seeding to Generate Crystals of a Related Protein or Protein/Inhibitor Complex

Margaret O'Gara

If your protein or protein complex fails to crystallize try seeding from crystals of the same protein (if it's a protein you want to crystallize) or a related protein for apo protein crystals. Serial micro seeding works best, make sure you look at the drops after seeding to identify any visible crystal seeds in there (i.e. not new crystals).

Advice for the Follically - Challenged Crystallographer

Jonathon Hadden

It has been long accepted that crystallizers with an abundance of facial hair have highly successful careers (Leeds University personal observation). This was thought to stem from the fact that matter found its way from the hair into the trials and acted as a nucleation centre-BUT SERIOUSLY; Addition of small grains of sand to a crystallization drop that is "close to producing" crystals can aid in nucleation and crystal growth.

Raise the DMSO?

Melissa Harris

If higher DMSO does not damage your protein, try higher concentrations of DMSO (10-20%) for crystallization. It can help when dealing with insoluble compounds and is an excellent cryoprotectant. Freeze directly from drop!

TCEP as a Reducing Agent

Barbara Brandhuber

Use TCEP instead of DTT as a reducing agent in your protein solution. It isn't oxidized as quickly as DTT. Be sure to watch the pH of your solutions because it is very acidic.

Temperature Variation

Irene Weber

To grow crystals at different temperatures around room temperature search the lab for spots that are consistently at higher or lower temperatures. A difference of several degrees can be found. Temperature shifts can be easily made by moving crystals to a different place. (Check office shelves too!) [Discovered by Charles Reed in my lab]

Drop Drying Technique

Anil Mistry & Ron Rubin

When setting up drops with a protein which has low solubility a low starting concentration has to be used. Setup larger drops (5-10 µl) and leave them to stand "dry" for 3-5 minutes at room temperature /4°C prior to inverting over a well of a Linbro® plate, this should allow some pre-concentration.

Using DLS to Test for Irreversible Aggregation*Anil Mistry*

Generally, people concentrate a protein to 10 mg/ml or higher, then dilute 2-fold when setting up their hanging drops by doing a 1:1 mix. What if in concentrating to 10 mg/ml aggregation has been induced which is irreversible, such when pipetting your 1:1 mix hanging drop, it already contains aggregates, a bad starting point.

(For monodisperse protein samples)

Using DLS test the limit of concentrated protein, i.e., the maximum concentration that can be achieved before a polydisperse signal is obtained. Then test samples, up to this limit, by concentrating up to this limit and test for irreversible aggregation by diluting a concentrated sample to a number of levels and test for monodispersity. With the sensitivity of current DLS equipment even samples at 10 mg/ml should be measurable. In this way and within the limits of your DLS machine it should be possible to find out whether you will have aggregates when you dilute your concentrated protein 2-fold when setting up a hanging drop.

Pickled Crystals Unusual Additives! (A True Story)*Michael Hickey*

Pickle juice was added as an additive to a mutant form of a crystallized native protein. The mutant could not be crystallized in near similar conditions of the native. By chance, the components of pickle juice were read and found to contain compounds used in crystallization (i.e. glycerol, PEG 400, citric acid, acetic acid, alum, and a few vitamins). This juice (Sweet & Snappy Vlassic brand) was filtered (0.45 μ l and adjusted to neutrality. It was then added to various PEG's (that crystallized the native form) and set up with the mutant. Crystals formed after 1 week! Trying to "add back" single components of the pickle juice to determine which component was responsible gave no crystals, the pickle juice (~1%) was necessary. Hint/ Tip: Commercially available food/ detergent solutions ought not be discounted as additives for crystallization!

Improve Your Crystals in Size, Shape, and Quantity*Nham Nguyen*

After you have crystals, open the coverslip, remove the mother liquid in the droplet, dissolve the crystal with 3 μ l of H₂O or buffer and add 3 μ l of well solution. Close the coverslip. The crystal will appear again. My crystals diffract ~ 4 Å, sometimes I get twins that diffract ~2.5 Å.

No Hits! What Next?*Anil Mistry, Pfizer*

No hits from a screen, what next? You can always set up another screen! Or, you can make a list of all drops that have precipitate and use the precipitate as a potential seed stock. Streak seed from the precipitate into new drops that have been set up at 50% and 75% of the original screening solution. There may be nucleation sites or microscopic seeds in the precipitates that may grow at lower precipitate saturation. Better still, streak seed from the precipitate to a clear zone into the same drop to recycle the drop.

Micro-Seeding With A Cryoloop*Anna Aagaard, AstraZeneca*

For reproducible micro-seeding by hand use a cryoloop to fish out your seed from the seed stock and transfer them to the drop. Use a 0.3-0.4 mm cryoloop.

Reduce To Enlarge*Zhanna Druzina, SGX Pharma*

For bigger crystals try to add 0.5-1 microliter of 14 M beta-mercaptoethanol to the reservoir after the protein drop was set up.

Complex Screening*Annie Hassell, GlaxoSmithKline*

Problem: Can grow crystal but no protein-ligand crystals. Tip: Take the conditions from the apo crystals and develop a focused optimization screen (24 well maximum). Screen complexes using cross seeding and the focused screen and three drop ratios (1:1, 2:1 and 2:3).

Change the Method*Annie Hassell, GlaxoSmithKline*

Problem: Poor crystal quality. Tip: Change tray type or crystallization method. For example, initial screens done in sitting drop tray and crystal quality improved in 96 well hanging drop tray.

Watch Out For Ruts*Brandon Collins, Boehringer-Ingelheim*

Every project, every protein, every construct is unique. Be careful of knowing too much. Just because things did or did not work in the past does not mean things will work that way for the next project.

Heavy Atoms To The Rescue

Problem: Poor diffraction. Tip: Heavy atom soaks to stabilize floppy regions of the protein.

Don't But All Your Eggs In One Basket*Joseph Luft, Hauptman-Woodward Institute*

Don't focus all of your optimization efforts on a single crystallization condition. If you have several different crystallization conditions identified for a sample go after them. Crystals of the same protein produced from different chemical conditions and/or temperatures will have unique physical properties. These properties will determine how easy the crystal can be looped (physical stability), cryoprotected and ultimately how well the crystal diffracts X-rays. Avoid single points of failure, go after several hits.



Insoluble Ligands

Doug Marcotte, Biogen IDEC

If ligands can't be soaked into crystal or co-crystallization is ligand specific try seeding into drops that contain ligand of interest. When soaking crystals with insoluble ligands try adding the cryoprotectant to the soaking solution. This can help solubilize the ligand and also cryoprotect (so less handling). Doesn't work so well when salt is the cryoprotectant, but may well when it's glycerol, DMSO or ethylene glycol.

Getting Away From Clear Drops

Annie Hassell, GlaxoSmithKline

Problem: Drops are all/mostly clear. Tip: Remove stabilizing agents (salt, glycerol, etc) from the protein buffer. Then do crystallization screens.

Avoiding Excessive Precipitation

Vaheh Oganessian, MedImmune

At suboptimal protein concentrations the interface between protein solution and crystallization screen solution may exhibit excessive precipitation. To avoid this, before adding screen, solution add 1 microliter of water. Downside of this is equilibration will take slightly longer. Upside of this is decreased osmotic shock for protein and less precipitation.

Heavy Precipitate Control

Janet Newman, CSIRO

Put one conditions of 40% TCA into your standard screen. This should precipitate out all of your protein, so that you have an idea of what heavy precipitate should look like.

Look At The Big Picture

Edward Snell, Hauptman-Woodward Medical Research Institute

Don't consider a crystallization result in isolation. Look for neighbors in chemical space and use those results to provide chemical directions for optimization. If a cryocooled crystal does not diffract well. You cannot tell if it is the crystal, cryoprotectant or cooling that is causing the problem. Look at room temperature data before moving on.

Poor Nucleation

Mei Xu, Novartis

Consider the case of poor nucleation and seeding did not work. Tip: Mix protein and well solution then use pipette tip to cross the drop into branched shape. Crystal may grow in the branches of the drop.

Seeding Suggestion

Paris Ward, GlaxoSmithKline

To increase you choices of producing more optimal crystal condition or conditions using seeds, try the following. Program a small volume liquid handler to dispense your protein and seed solution directly into 96 well commercial screens and/or an additive screen.

Delete To Succeed

Heidi Schubert, University of Utah

Recent success with loop deletions. Sequence alignments reveal either charged loops and/or loop insertions relative to homologues. I have removed 3 to 34 amino acids and retained high expression soluble protein and novel crystals.

Seeking Stability

Annie Hassell, GlaxoSmithKline

Problem: Unstable protein. Tip: Add 1-5% low molecular weight (200 to 1,000) PEG directly to the protein and then screen.

Doh!

Jim Pflugrath, Rigaku

Start with big crystals! Add at least 5% glycerol to everything.

Stabilize For Cryo

Laura Pelletier, SGX Pharmaceuticals

Stabilize crystals in cryo by adding protein buffer components into the cryo. For example, most commonly I add 100 to 150 mM NaCl plus reservoir components plus cryoprotectant(s).

Ratios

Ayse Sinem Ozyurt, SGX Pharmaceuticals

Play with protein-mother liquor ratio, especially with low solubility proteins. Try different concentration of protein coupled with streak seeding.

Eliminate One Offs

Nancy Bump, Millennium Pharmaceuticals

After looking at the results of initial screening or of additive screen, pick several of the best "hits" and screen in 96 well format, 6 or 8 identical drops of each favorite before scaling up. Helps to eliminate "one offs" and save time.

Complex It!

Paul Reichert, Schering Plough Research Institute

Apo protein is monidisperse but won't crystallize. Complex it! Complex it! Complex it!

Matrix Seeding Tweak

Armando Villasenor, Roche Palo Alto

If your crystal seeds withstand large serial dilutions, try matrix seeding via the reservoir by doing the following. 1) Create crystal seeds as described by Allan D'Arcy. 2) Dispense seed into reservoirs containing reservoir. 3) Aspirate/dispense to mix seed in reservoir. 4) Dispense mother liquor droplet containing seed onto protein drop.

A Little Salt Please*Neil Grodsky, Pfizer Global Research & Development, La Jolla Labs*

If you do not see any crystal growth in several days after set-up (more than 1 to 2 weeks) and the drop are not all clear, add salt (such as ammonium sulfate) to 0.5 M to the drops. Even though ammonium sulfate salt crystals might form, you might actually get protein crystals. This worked for me recently.

Urea To Solubilize*Gloria Borgstahl, Eppley Institute UNMC*

Non denaturing (less than 3 M) concentrations of urea can be helpful to solubilize your protein.

Multivalents*Simon Low, Pfizer, Inc.*

Try stoichiometric levels of multivalents, cations as additives. They may be necessary for crystallization but sometimes the levels found in commercial screens are too high and toxic.

Cooking for Crystals*Liping Wang, GlaxoSmithKline*

Heat treatment of protein complex to obtain diffraction quality crystals. Original complex has no initial crystal hits. Heat treat protein complex (25 to 80 degrees Celsius) for various times (5 to 30 minutes). Centrifuge to get rid of aggregated protein and screen again.

Toothpick Tip*Paul Reinfelds, Illinois Institute of Technology*

When using pre greased trays, take a toothpick and remove a bit of grease from each well. Now as you push down on your cover slip, you turn it a few degrees. This will allow the air to escape and the turn will form an airtight seal over each well.

Thermofluor To The Rescue*Jackie Day, Pfizer St Louis*

Thermostability. Monitor the effect of additives, buffers, ligands, etc. on melt temp of your protein. We have seen in multiple cases that the most thermostable construct, buffers, additive yields the best or only crystals. How? We use Bio-Rad's iQ5 iCycler (a PCR instrument) as it has 5 sets of filters for excitational emission and hydrophobic dyes that fluoresce upon binding (protein unfolding).

Getting Out Of A Sticky Situation*Michael Wiener, University of Virginia*

Problem: Crystals adhering to plastic of sitting drop plate, and mechanical dislodging (by cryoloop, tool, etc) does not work. Solution: Stan a fine gauge syringe needle into the plastic, near but not into the crystal. This often distorts/disrupts the plastic near the crystal and breaks the seal.

Liquid Bridge*Margarete Neu, GSK Stevenage, UK*

Getting bigger crystal by low tech / low cost counter diffusion. If you are faced with either no nucleation or showers of crystals and the usual tricks including seeding do not work, try this: On a cover slide, set the protein and precipitant drop (example 1 microliter plus 1 microliter) separate, but very close to each other. Then, with a whisker or pipette tip streak through the drops to form a connecting bridge between the protein and precipitant solution. Invert cover slide and place over well. Crystals will form along the gradient and "self screen" for best conditions.

Low Concentration Complexing*Elizabeth Fry, Abbott Laboratories*

When working with compounds for co-crystallization, if the compounds are highly insoluble in protein buffer (50 micromolar or less) we often employ low concentration complexing. We dilute the protein and then add in diluted compound, so that the compound is added close, or at least closer to a concentration where it is soluble and the content of DMSO in the protein sample remains less than 2%. The protein-compound complex is then concentrated for crystallization trials. This has helped us with several projects with highly insoluble compounds.

Ionic Liquids*Christopher Bonagura, Exelixis, Inc.*

In experiments using model proteins we found that Ionic Liquids (IL's) specifically 1-Butyl-2-methyl imidazolium chloride gave increased numbers of crystallization outcomes compared to the IL controls. A large number of the crystals obtained had precipitated outcomes in the IL controls. In many other cases the IL and crystal had an improved morphology (needles to plates, plates to 3D crystals) over the IL controls. Tip: Using an IL such as 1-Butyl-2-methyl imidazolium chloride as an additive to improve chances of getting a crystal from conditions which otherwise would give precipitate. Marc Pusey, MI Research, Inc. Our favorite cryoprotectant. 1x UCP (Ultimate Cryo Protectant) 8% Glycerol, 8% Ethylene glycol, 9% Sucrose, 2% Glucose. We make a 2x solution. Generally add this 1:1 with reservoir. The ratio can be modified, for example 1.2 microliter 2x UCP : 0.8 microliter reservoir, or 1.4 microliter 2x UCP : 0.6 microliter reservoir, or 0.6 microliter 2x UCP : 1.4 microliter reservoir, etc. I believe this has been successful in cryoprotecting some 70% of all of our systems, resulting in more than 50 solutions of these targets regardless of previous cryogenic treatments. Author in unknown to me, but the credit is published in a singled Hencrickson paper. I was tipped off 6 years ago.



Protect The Bridge

Shirley Robert, York Structural Biology Laboratory

If you can't get your Se-met protein to crystallize try leaving out the DTT/TCEP during purification and crystallization. Sometimes there are disulfide bridges near crystallization contacts that need preserving for crystallization to take place. Collected the Se edge is still possible.

DMSO For Cryo

Rich Romero, SGX Pharmaceuticals

Try 20 to 30% DMSO as cryo. This has worked well in a number of cases for me and I've added this to a very short list of cryos that I personally use. Note: If your mother solution contains a high salt concentration the DMSO will cause it to precipitate out of solution. So beware!

No Fog

Beat Blattmann, University of Zurich

4 degrees Celsius crystallization plates prepared at room temperature always have a condensation problem on the plate seal. To avoid condensation cover the finished plates with two lids and plate it in the cold room for 20 minutes or on top of a cold metal block, This will reduce the temperature of the reservoir solution while the 2 lids delay the temperature change from the top long enough to avoid condensation.

Anti Slip Tip

Barbra Pagarigan, Celgene

Ever been manually sealing a plate only have it slip out from under you? The result is usually death for hanging drop plates, and with sitting drop plates your best bet is hoping the drop is not splashed onto the seal above. To ensure the plate stays put when applying pressure, we use a "grip pad" in our lab. Simply place the grip pad onto the bench, set plate on top and seal as usual. The grip pad prevents the plate from slipping out from under the compression tool, usually a brayer, used for ensuring the plate seal is applied correctly. The grip pad can be cut from the material commercially available for lining tool shop drawers. In addition, we created a fixed plate holder that encloses the entire plate to guarantee the brayer does not slip off the plate when sealing, a common occurrence when manually sealing many plates. Our plate holder is custom cut from a hard rubber to fit both the 24 well and 96 well plates. The plates sit slightly above the platform to ensure both ends are sealed and for easy removal. The sides of the platform are rounded to ensure the brayer has a smooth path of travel.