Granada Crystallization Box

HAMPTON RESEARCH
Solutions for Crystal Growth

Description

The Granada Crystallization Box (GCB) consists of four elements made of polystyrene:

- A reservoir to introduce the gel
- A guide to hold capillaries
- A cover
- A holder to maintain the boxes

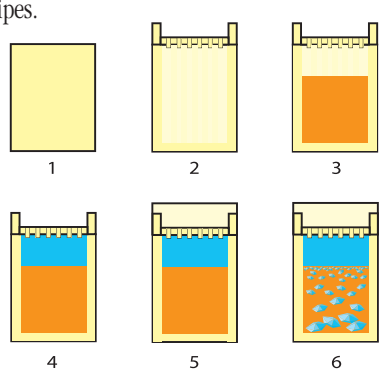
The GCB has been designed to be used in four different ways:

- To grow crystals inside gels under diffusion controlled mass transport.
- To grow protein crystals inside capillaries with un-gelled precipitating agent by the counter-diffusion technique.
- To grow protein crystals inside capillaries with gelled precipitating agent by the counter-diffusion technique.
- To grow protein crystals inside capillaries by the batch method.

How to use the GCB - by Juan Ma. Garcia-Ruiz

A) Growth of crystals inside gels under diffusion controlled mass transport.

The growth of crystals inside gels is very simple. Below you can find three different recipes.



A.1. Crystallization of a slightly soluble compound by means of a chemical reaction provokes in most cases a cloud of small crystallites or amorphous precipitate. The use of gels solves the problem in most cases provided that the reactants are soluble in water. A typical case is calcium tartrate, which can be obtained by reaction of tartaric acid and calcium chloride. Make a gel of silica by mixing aliquots of tartaric acid 1M solution with sodium silicate solution (density = 1.06 g/cc) under continuous stirring. Pour the solution in the GCB and introduce the guide. Wait for one day until the silica gel is set. Then pour on top of the gel layer a solution of calcium chloride (1M).

To harvest the crystals, pull out the guide and the gel will be removed.

A.2. Want to make beautiful Liesegang's rings? It's easy with the GCB. Prepare a 0.05 M solution of potassium iodide gelled with agarose at 1% w/v concentration. While the solution is still hot, pour it into the GCB. Let it cool to room temperature and wait for 20 minutes. While waiting, prepare a lead nitrate Pb(NO3)2 1M solution. Pour onto the already gelled KI solution and close the box. That's all.

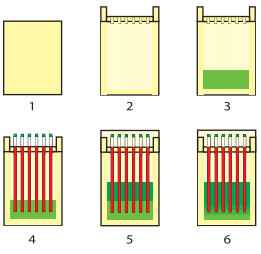
Note that the concentration of lead nitrate was selected to be much higher than the concentration of potassium iodide and therefore lead molecules will invade the gelled iodide solution. As soon as they meet, an amorphous phase of PbI2 forms. The precipitation process continues producing intermittent precipitation. As new bands form they are made by fewer crystals of larger size. This is exactly the foundation of the counter-diffusion technique for protein crystallization. The difference is the type of reaction used to trigger the precipitation. Here a precipitation reaction occurs in which both components (lead and iodide) enter in the crystals. Unlike this, in the case of protein crystallization, precipitation is triggered by solubility reduction. Therefore only the protein concentration decays noticeably.

A.3. Crystallization of a protein by solubility reduction driven by ionic strength. This method is designed to prepare many large high-quality crystals for special purposes but it consumes a larger amount of protein than usual cases. Here I describe the recipe to make tetragonal crystals of the model protein lysozyme.

Make a sol of agarose at 1% by heating the mixture under continuous stirring above the gelling point. Prepare a solution of lysozyme at 40 mg/ml. Let the agarose cool down to a temperature of about 35°C. Keep the agarose sol at this temperature. Mix while stirring one part of agarose sol with four parts of protein solution. Pour the mixture into the GCB and introduce the guide. Finally, pour in a solution of NaCl at 20% w/v.

B) Growth of protein crystals inside capillaries with un-gelled precipitating agent by the counter-diffusion technique.

The growth of biological macromolecules inside capillarieshas many advantages. Among them are:



a) The scanning a large number of crystallization conditions in a single experiment.
b) The avoiding of manipulation of crystals after they are obtained: you can use for X-ray diffraction the same capillaries where the crystals were grown.
c) To grow the crystals under diffusion controlled mass transport.

To perform the experiment with the GCB is very easy. The first thing to do is to prepare the agarose gel. Agarose is a polysaccha ride that does not interact chemically with most proteins (note that in some cases you can also use

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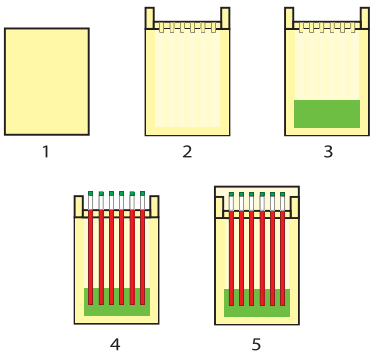
silica gel). To make it, mix the appropriate volume of buffer solution with agarose powder, under continuous stirring, for an final agarose concentration of 1.5 % w/v. Heat the mixture to boiling in order to break the cross-links of the agarose fibers. Note that the agarose solution becomes transparent. Maintain the solution boiling about two minutes under continuous stirring. Poor the agarose sol in the GCB and let it cool at room temperature. The cross-links of the agarose will reform and a gel will be made.

Once the gel is set, fill the capillaries with the protein solution. To do this, introduce one of the ends of the capillary into the protein solution. You will see that the solution flows up by capillarity. Once it reaches a height of five or six centimeters, remove the capillary. You will see that the solution remains inside the capillary. Then seal the upper end of the capillary with a small piece of plasticine or your preferred sealing material. The next step is to punch the capillary in the gel layer (but be sure that the gel is set!!!). Introduce the capillary through one of the holes in the guide of the GCB and push it into the gel about 2-3 mm (just to maintain it straight). Finally, pour the solution of your buffered precipitating agent onto the gel layer and place the cover on the GCB. Pour a volume equal to the volume of the gel layer. Note that you can use up to six capillaries per GCB. This is termed the gel acupuncture method.

C) Growth of protein crystals inside capillaries with gelled precipitating agent by the counter-diffusion technique.

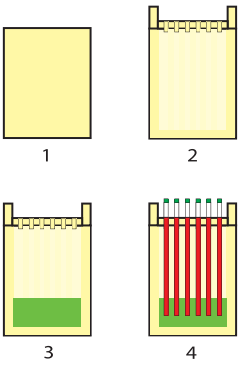
The procedure is basically the same as above. However, here you can take advantage of the possibility to gel some buffers and common precipitating agents. For instance, ammonium sulfate, polyethylene glycol, sodium chloride and others can be incorporated into agarose gels as well as most classical buffers.



To do this, just mix the agarose with your buffered starting precipitating agent solution. In other words, replace the water used to mix the agarose in the above protocol with your precipitating agent solution. Once the gel is set, just punch your capillary containing the protein solution and wait. Note that you can use the protein at any pH value at which it is stable. Because of the large volume of the gelled solution compared with the volume of the protein solution in the capillary, the pH value of the protein solution will change as the buffered precipitating agent moves into the capillary.

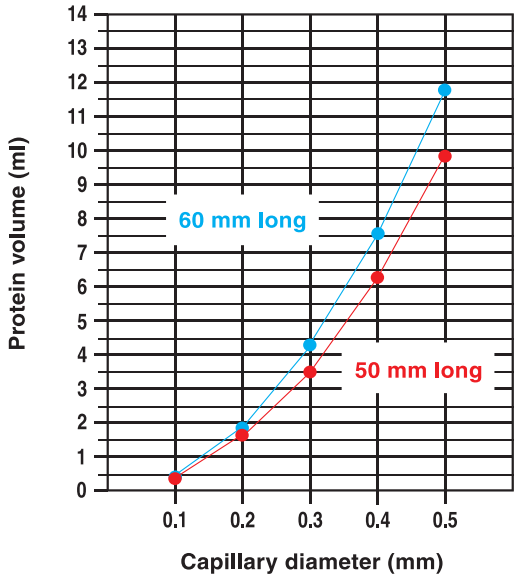
D) Growth of protein crystals inside capillaries by the batch method.

You can also use the GCB to grow crystals by the microbatch method. Obviously you lose all the advantages of the counter-diffusion techniques for screening of crystallization conditions. However, if you want to try this traditional method and to grow crystals ready for Xray diffraction inside the capillaries, you can use the GCB to hold the capillaries, to have an easy optical observation and to transport them.



To do this, prepare your buffered precipitating agent solution and mix it with agarose at 0.1 %. Boil it for 1 minute and then cool it to 35°C. Maintain the sol at this temperature. Mix the appropriate volume of the protein solution with the appropriate volumes of the sol. In other words, proceed as to prepare a drop for microbatch. Then, suck the drop into the capillary by capillarity. Seal both ends of the capillary and hold them in the GCB.

How much protein?



The answer to this important question depends obviously on the diameter of the capillary chosen. We recommend filling the capillaries up to a length of 50 or 60 mm in order to have a wide screening of the phase diagram. The plot above shows the amount of protein solution you need for the experiment as a function of the inner diameter of the capillary. The values are listed in the table below:

Diameter	Length = 50 mm	Length = 60 mm
0.1 mm	0.39 µl	0.47 µl
0.2 mm	1.57 µl	1.88 µl
0.3 mm	3.53 µl	4.24 µl
0.4 mm	6.28 µl	7.53 µl
0.5 mm	9.81 µl	11.78 µl

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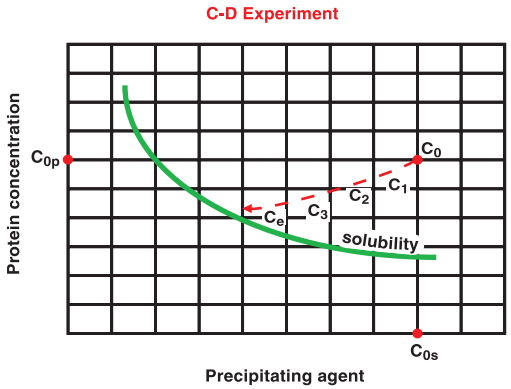
Note that you can perform screening with 0.1 mm capillary using a protein solution volume of less than 500 nanoliters per experiment!!!

How do counter-diffusion techniques work?

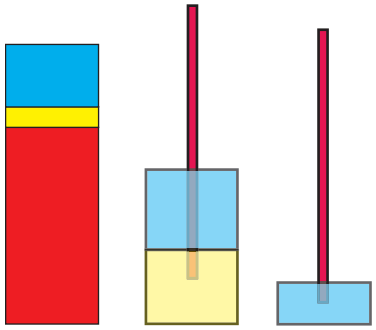


The precipitation of the protein occurs because its solubility varies with, for instance, the ionic strength, i.e., with the concentration of salt (see the ideal phase diagram in the Figure above). Let the starting protein concentration in the capillary be C0p and the concentration of the salt be C0s. When these solutions come into contact near one of the ends of the capillary, the system moves towards the point C0 in the phase diagram. Note that the supersaturation is very high. Therefore, the first precipitate will be an amorphous or ill-defined crystalline phase forming in the lower end of the capillary. Its formation depletes the concentration of protein in the neighboring zones. As the salt continues to diffuse up in the capillary, a new precipitation takes place, this time at lower supersaturation producing microcrystals (location C1 in the phase diagram). Iteration of this process provokes precipitation at lower supersaturation as the precipitation front moves far from the gel towards the upper part of the capillary (C2, C3, ….. Ce). This yields precipitation zones of fewer crystals of larger size and higher quality. Unlike the classical drop and batch methods, the counter-diffusion technique explores a large number of crystallization conditions in one single experiment.

Note that the crystallization pathway evolves always towards equilibrium and therefore the experiment self-searches the optimal crystallization scenario. The experiment is therefore equivalent to making a large number of hanging-drop or batch experiments across the phase diagram. Counter-diffusion techniques make such a search automatically in one single experiment. And in addition you do not need to check the crystals in different drops to look for those of higher quality. In the counter-diffusion experiment the best crystals are always those formed in the upper part of the capillary. If an X-ray capillary is used, you can collect diffraction data directly without touching the crystals.

Do not worry about the starting conditions. Just select a typical concentration for protein solutions to be crystallized, for instance 10 to 20 mg/ml. Then use a very high concentration of precipitating agent to trigger the protein precipitation at high supersaturation as soon as the precipitating agent meets the protein solution. As discussed above the system will evolve itself towards better crystallization conditions.

It is important to note that in any counter-diffusion experiment there are three main parameters (See Figure below):



a) The length of the reservoir where the precipitation will occur (red). This reservoir contains the compound to be crystallized (for instance a protein) or, in the case of crystallizationby chemical reaction, the reactant of smaller diffusivity or at lower concentration.

b) The length of the physical buffer (yelow). This is a gel layer which does not interact chemically in the crystallization process. Its function is to slow down the mixing of the solutions. Note that this buffer layer can exist or not. For instance in the gel acupuncture method, the length of the buffer is double the depth of penetration of the capillary in the gel (before reaching the protein, the precipitating agent must travel the penetration depth down –outside the capillary- and up –inside the capillary-. However, in the case of the method using gelled precipitating agent, there is no such a buffer layer. The precipitant is in direct contact with the protein.

c) The relative values of the volume (in blue) of the precipitating agent (for the case of protein) or the component with lower concentration or smaller diffusivity (for the case of chemical reaction) with respect to the volumes in red and yellow. It is critical to consider the values of these parameters and the initial concentration of the reactants to understand the evolution of the experiment. Depending on the configuration used, the system will evolve differently. With the configuration of the gel acupuncture method, the salt on top of the gel diffuses down through it and after a given time, reaches the punched end of the capillary. Then, it continues diffusing up through the capillary filled with the protein solution, triggering its precipitation. Note that with this configuration, the concentration of the precipitation agent at the end of the experiment will be the initial concentration of precipitating agent divided by:

$$\frac{\text{Volume of the precipitating agent + Volume of the gel layer + Volume of the capillary (negligible)}}{\text{Volume of the precipitating agent}}$$

For instance, if we use a volume of precipitating agent (in blue) equal to the volume of the gel layer (yellow), the final concentration of precipitating agent in the capillary will be half of the initial concentration. Selecting the values of these volumes, we can tune how wide the screening of the phase diagram will be.

Technical Support

Inquiries regarding GCB general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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