

**I grew crystals of my protein by the hanging drop method and I would like to improve their quality. The crystallization conditions I used are the following:  
In the drop: 6 mg/ml protein and 12% ammonium sulfate;  
In the well: 24 mg/ml ammonium sulfate.  
How can I extrapolate these conditions to the counter-diffusion technique?**

I guess you have your protein at 12 mg/ml concentration and then, when you mix equal volumes of the protein solution and the  $(\text{NH}_4)_2(\text{SO}_4)$  you got the actual concentration in the drop. Let us plot your drop evolution in the phase diagram. The starting situation in the drop in red is C

0  
. The starting concentration in the well, in blue is unlabeled. The concentration in the drop changes because it evaporates. Therefore, as the water molecules move from the drop towards the well the concentration changes along the dash line starting in the origin of the plot and passing through the starting conditions of the drop. If the well drop system is completely closed, the evaporation of the drop is driven by the difference in vapor pressure in the drop and in the well. Therefore, the maximum achievable concentration of protein in the drop is C

max  
, obviously twice the starting protein concentration. If you got crystals in the drop, certainly that maximum concentration was never achieved. Also, if you got crystals in the drop it means that the location of the super-solubility curve is between C

0  
and C

max

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You can use your starting protein and precipitating agent solution (those you use to make the drop). In your particular case, you should fill the capillary with your buffered protein at concentration 12 mg/ml. Then prepare the gel of agarose with the same buffer. Finally, punch the capillary into the gel and pour onto the gel layer the ammonium sulfate solution at 24%.

