

Description

Varying the drop ratio is a simple method to utilize during crystallization screening and optimization. Drop ratios allow one to explore varying levels of initial and final protein and reagent concentration, explore different equilibration paths, and cover a wider range of relative supersaturation. All by simply changing the amount of sample and reagent added to the drop.

Drop ratio is the volume relationship between the sample and reagent in a drop. For example, if a drop is created by adding 200 nl of sample plus 200 nl of reagent, the drop ratio is 200:200, which can be mathematically simplified to 1:1.

Vapor diffusion is the process where solution is exchanged between the drop and the reagent well (reservoir) through the vapor phase. In a hanging or sitting drop experiment, vapor exchange takes place between the drop and the reservoir until the drop and reservoir are in equilibrium. In most experiments, water vapor leaves the drop for the reservoir, thereby increasing the concentration of the sample and chemicals left behind in the drop.

Let's look at a typical hanging drop vapor diffusion experiment X (Figure 1, green line) with a 1:1 drop ratio of 1 μ l of 20 mg/ml protein plus 1 μ l of 2.0 M Salt for the drop against a reservoir of 2.0 M Salt. Immediately after pipetting the drop. At T_0 the drop contains 10 mg/ml protein and 1.0 M Salt. During vapor diffusion, water vapor will leave the drop until the drop and reservoir are in equilibrium T_1 . At equilibrium the drop will contain 20 mg/ml protein and 2.0 M Salt.

One can change the drop ratio to alter the volume relationship between the sample and reagent in the drop. This will affect both the initial and final protein concentration in the drop as well as the initial reagent concentration in the drop. The final reagent concentration in the drop will remain the same because the system will seek equilibrium between the drop and the reservoir.

Now let's vary the drop ratio and look at vapor diffusion experiment Y (Figure 1, red line) with a 2:3 drop ratio of 200 nl of 20 mg/ml protein plus 300 nl of 2.0 M Salt as well as an experiment Z (Figure 1, blue line) with a 3:2 drop ratio of 300 nl of 20 mg/ml protein plus 200 nl of 2.0 M Salt. Initially at T_0 , the protein concentration is 8 mg/ml in Y and 12 mg/ml in Z. At T_0 the salt concentration in Y is 1.2 M and Z is 0.8 M. When the drop and reservoir have equilibrated at T_1 the protein concentration in Y is 13.3 mg/ml and Z is 30 mg/ml.

Figure 1 illustrates the three different initial and final protein and reagent concentrations, as well as the equilibration path explored by drop ratio experiments X, Y and Z. A small change in the drop ratio can produce experiments with not only different initial protein and salt concentrations, as well as different final protein concentrations, but three unique paths through the solubility phase diagram. Utilizing drop ratios, one can explore and evaluate different protein and reagent concentrations as well as equilibration paths and rates and the influence of these variables on the size, number, morphology and quality of crystals.

Figure 1

Exploring drop ratios. Three different drop ratios are plotted to show the different initial and final protein and reagent concentrations, as well as the unique equilibration path.

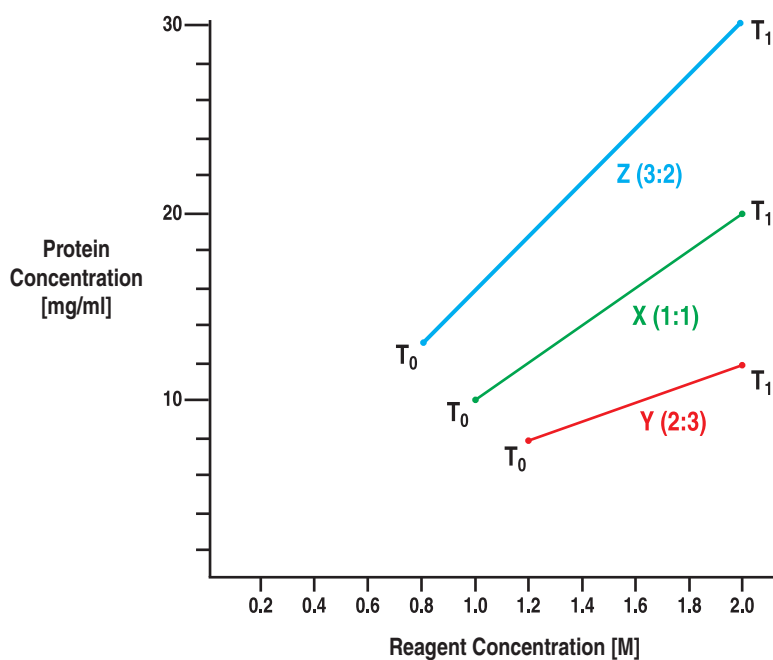


Table 1

Protein (μl)	Reagent (μl)	Drop Ratio	Initial Protein Concentration	Final Protein Concentration	Equilibration Factor	Initial Reagent Concentration	Final Reagent Concentration
1	9	1:9	2.0	2.2	1.1	1.8	2.0
2	8	1:4	4.0	5.0	1.3	1.6	2.0
3	7	3:7	6.0	8.6	1.4	1.4	2.0
4	6	2:3	8.0	13.3	1.7	1.2	2.0
5	5	1:1	10.0	20.0	2.0	1.0	2.0
6	4	3:2	12.0	30.0	2.5	0.8	2.0
7	3	7:3	14.0	46.7	3.3	0.6	2.0
8	2	4:1	16.0	80.0	5.0	0.4	2.0
9	1	9:1	18.0	180.0	10.0	0.2	2.0
Protein (nl)	Reagent (nl)						
200	300	2:3	8.0	13.3	1.7	1.2	2.0
100	300	1:3	5.0	6.7	1.3	1.5	2.0
200	200	1:1	10.0	20.0	2.0	1.0	2.0
300	100	3:1	15.0	60.0	4.0	0.5	2.0
300	200	3:2	12.0	30.0	2.5	0.8	2.0
Protein Concentration			20 mg/ml				
Reagent Concentration			2.0 M				

Table 1 shows the effects of varying the drop ratio of sample and crystallization reagent in a vapor diffusion experiment. The initial protein concentration is 20 mg/ml and the crystallization reagent is 2.0 M Salt. The volume of protein and reagent used to create the drop appear in column 1 and 2, the resulting drop ratio in column 3. Initial and final protein concentrations for the varying drop ratios appear in column 4 and 5, while the initial and final reagent concentrations appear in column 7 and 8. The equilibration factor is column 6.

Setting two or three different drop ratios during screening or optimization can produce two or three different unique crystallization experiments without consuming any more reagent, plates or seals than a single drop experiment.

Multiple drops can be dispensed onto a cover slide for hanging drop vapor diffusion and there are numerous plate options to accommodate multiple drops (1, 2, 3, or 5 drops) for sitting drop vapor diffusion experiments.

References

- Efficient optimization of crystallization conditions by manipulation of drop volume ratio and temperature. Joseph R. Luft, Jennifer R. Wolfley, Meriem I. Said, Raymond M. Nagel, Angela M. Lauricella, Jennifer L. Smith, Max H. Thayer, Christina K. Veatch, Edward H. Snell, Michael G. Malkowski, and George T. DeTitta. *Protein Sci.* 2007 April; 16(4): 715–722. doi: 10.1110/ps.062699707.
- Protein crystallization strategies for structural genomics. Edited by Naomi Chayen. International University Line, 2007, pages 27, 40.
- Rational selection of crystallization techniques. Joseph R. Luft and George T. DeTitta. Pages 29-32, in the book *Protein Crystallization*, Second Edition, Edited by Terese M. Bergfors, International University Line, 2009.

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