

# Microdialysis Crystallization

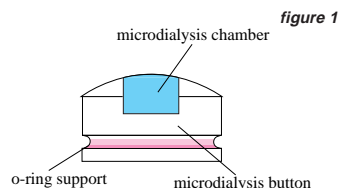
## Crystal Growth 101

### Method

Crystallization by dialysis is an easy variation to the typical vapor diffusion method used to grow crystals. In the microdialysis method the sample in question is separated from the “precipitant” by a semi-permeable membrane which allows small molecules such as ions, additives, buffers, and, salts to pass but prevent biological macromolecules from crossing the membrane. Equilibration kinetics depend upon the molecular weight cut-off of the dialysis membrane, the precipitant, the ratio of the volume, the concentration of the components inside and outside of the microdialysis cell, and the geometry of the cell.

### Description

The microdialysis buttons offered by Hampton Research are machined from transparent perspex. The button has a chamber which varies from 5 to 350 microliters depending upon which size button one chooses to use. The sample is placed in this chamber so as to create a slight dome of liquid at the top edge of the button. A dialysis membrane (having the appropriate molecular weight cut-off) is placed over the top of the button/sample and is held in place with an O-ring. The O-ring is held in place by a groove in the dialysis button.



Dialysis buttons are notoriously tricky to set up since beginners often trap air bubbles between the sample solution and the membrane which impedes dialysis. With a little practice using a “Golf Tee Applicator” or a “CRE Applicator” one can master the technique. Microdialysis buttons from Hampton Research are supplied with O-rings and a Golf Tee Applicator. The package of microdialysis buttons does not include dialysis membranes. The CRE Applicator is available separately from the dialysis buttons and is used to apply the O-ring to the dialysis buttons.

### Using the Microdialysis Button

A typical dialysis experiment is used to take the sample from the presence of a high ionic strength solution to a lower ionic strength solution (however, the technique can just as easily be used to proceed from low ionic strength to a higher ionic strength). This is accomplished by placing the sample in high ionic strength in the dialysis button, sealing the button with a dialysis membrane and placing the sealed button in a solution of ionic strength lower than that inside the button. Salts, ligands, and compounds smaller than the pore size of the dialysis membrane will leave the button as long as their concentration is lower on the opposite side of the membrane. Once the concentration of the diffusible species is the same on both sides of the membrane, the system is in equilibrium.

### Cleaning

The microdialysis buttons can be cleaned with soap and deionized water. Do not clean the buttons with organic solvents as this may turn the perspex opaque.

### Practical Example

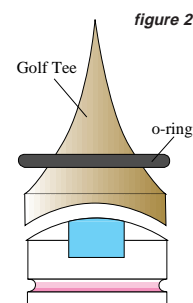
The following two practicals offer examples of how to set up a dialysis experiment.

#### Practical 1 - Carboxypeptidase A

1. Using Carboxypeptidase A (*Sigma CO386 or CO261*), make an 8 to 20 mg/ml solution of the Carboxypeptidase A in 20 mM Tris HCl pH 7.5, 15 M LiCl.
2. Place 100  $\mu$ l of 10 mg/ml carboxypeptidase in 20 mM Tris HCl, 15 M LiCl, pH 7.5 in

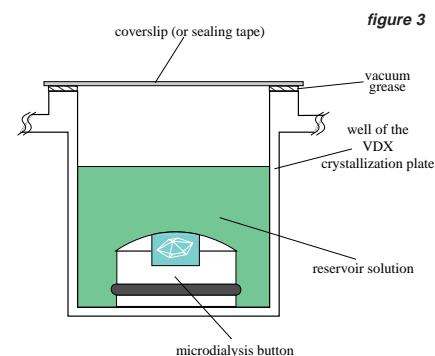
a 100  $\mu$ l dialysis button. The droplet should have a slight dome shape following the hemispheric edge of the top of the dialysis button.

3. Seal the button with dialysis membrane which has equilibrated in water, place the membrane over the top of the button. Place an inverted golf tee on top of the membrane and button. Roll the O-ring down the golf tee until the O-ring rolls off the edge of the button. Roll the O-ring into the machined groove on the edge of the button. Remove the golf tee. There should be no bubbles between the membrane and the sample inside the button. Bubbles will prevent dialysis. Some researchers prefer to use the rubber tipped plunger of a syringe with a modified syringe body to apply the O-ring. Others prefer to use the CRE O-ring Applicator. Try each and see which method works best.



4. Place 0.9 ml of 20 mM Tris HCl, pH 7.5 in the reservoir of a VDX plate (or Linbro plate, or Costar plate, or Q Plate, or small chamber which can be sealed).

5. Place the dialysis button in the well, membrane side up. Be sure the reservoir solution covers the top of the membrane/button. Seal the VDX plate using grease and a cover slide.



6. Observe under a microscope. Crystals will appear within 2 to 3 days. Final concentration of LiCl will be 0.15M.

*Reference for the above protocol: Dr. Jim Pflugrath and Dr. Gary Gilliland, Cold Spring Harbor Laboratory Protein Crystallography Workshop.*

#### Practical 2 - Lysozyme

1. Prepare 10 mg/ml lysozyme in 50 mM sodium acetate pH 4.5. Filter the solution using a 0.2 micron filter.
2. Fill a 100 microliter dialysis button with 100 microliters of the lysozyme solution as described for the carboxypeptidase practical.
3. Pipet 1 milliliter of 50 mM sodium acetate buffer into a small (5 ml) beaker.
4. Place the filled button, membrane side up in the beaker.
5. Pipet a small amount of concentrated sodium chloride into the beaker such that the final concentration of sodium chloride in the beaker is 0.2 M. Seal the beaker with parafilm and store at room temperature.
6. Increase the concentration of sodium chloride each day by 0.2M. Repeat until crystals are observed in the button.

*Reference for the above protocol: Crystallization of nucleic acids and proteins, a practical approach. Edited by A. Ducruix and R. Giegé, Oxford University Press, 1992. Pages 95-96.*

### Considerations

Just as in a vapor diffusion experiment, the path is often as important as the endpoint in a microdialysis experiment. The path is the equilibration course which the

solution inside and outside the button take towards achieving equilibrium. This course can be changed by manipulating the following:

- Ratio of button volume/reservoir volume
- Button and Reservoir Components & Concentration
- \* Molecular Weight Cut Off of Dialysis Membrane
- Viscosity of Solutions
- Plus the usual assortment of crystallization variables including pH, sample concentration, temperature, etc...

## Variations of Microdialysis

### *Macro-dialysis*

The sample is loaded into dialysis tubing of the appropriate molecular weight cut-off and is dialyzed against the appropriate reservoir solution. This method typically requires at least 100 microliters of sample and can be performed with liters of sample in large dialysis tubing.

### *Zeppenauer Cells*

Capillary tubes are closed with dialysis tubing or gel plugs. See Zeppenauer, M. 1971, *Methods In Enzymology*, 22, 253.

### *Microcap Dialysis*

The sample is placed in a glass capillary with one end sealed with wax, the other with dialysis membrane. The tube is placed in a microcap/small centrifuge tube filled with the appropriate reservoir. See *Crystallization of nucleic acids and proteins, a practical approach*, Edited by A. Ducruix and R. Giege, Oxford University Press, 1992.

### *Double Dialysis*

This method reduces the rate of equilibration and can provide enhanced control over the crystallization of the sample. Simply put, a dialysis button is prepared and placed inside a reservoir sealed with a dialysis membrane, which is in turn placed inside another reservoir. Confused? See Thomas, D.H., et al., 1989, *Protein Engineering*, 2, 489.

## References and Readings

1. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giege, *The Practical Approach Series*, Oxford Univ. Press, 1992.
2. Preparation and analysis of protein crystals. McPherson, A. *Eur. J. Biochem.* 189, 1-23, 1990.
3. Zeppenauer, M. et al., Crystal. of horse liver alcohol dehydrogenase complexes from alcohol solutions. *Acta Chem Scan*, 21, 1099, 1967.
4. Christopher Bunick, A.C.T. North, and Gerald Stubbs., Evaporative microdialysis: an effective improvement in an established method of protein crystallization. *Acta Cryst.* D56, 1430-1431, 2000.

## Technical Support

Inquiries regarding the microdialysis crystallization, interpretation of screen results, optimization strategies and 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 5:00 p.m. USA Pacific Standard Time.

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